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VEGETABLE GROWING

The variability analyses of characters at carrot variety Ceahlău

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Keywords: Variability, standard breach, dispersion, correlation, carrot Ceahlău, authenticities, genotype.

ABSTRACT

The experiments were accomplished at VRDS Bacau during a period of 3 years, 2005-2007, and sustained through plants development phase's observations and barometric determination over the main characters of authenticity and varietal typicality. The calculation and analyze of variability proved that the variety has little variability for the characters:

- low variability of characters:
  - root length (cm) (CV = 5,74%);
  - diameter at colet (cm) (CV = 8,071%);
  - seedy plant's height (cm). (CV = 7,25%);
- medium variability:
  - diameter of central cylinder (cm) (CV =10,066%);
  - root's weight (g) (CV = 10,075%);
  - quantity of seeds/plant (g) (CV =13,66%);
  - number of floral cane/plant (CV = 18,45%)

The phenotypic correlation coefficients of the main characters at carrot seedy plants, were:

- the root's weight is positively correlated with the quantity of seeds/plant (r1 = 0,808; r2 = 0,810) and with the number of inflorescences/plant (r1 = 0,771; r2 = 0,812);
- the number of seedy stems is very significant positive correlated with the quantity of seeds/plant (r1 = 0,842; r2 = 0,862)

INTRODUCTION

The genetic diversity and the influence of environmental factors over the variability of some characters of carrots varieties were underlined by Kuckuck (1979). His studies showed that the observed variability was determined by the interaction of many genes with discrete effects named polygenes.

The variability on the correlation of quantitative characters at this specie was observed also by Choi and colab. (1994), also by Sazanana (1977).

The genotype, interaction between environmental factors and binding allogamy of carrots plants, impose the variety of this specie to manifest as populations with a high uniformity degree (Banga, 1963).

The methods of correlations utilized, in the analyze of characters variability, by Kellner and Varga (1964) and Chira (1995), revealed interesting results in what concerns the resistance and direction of interactions between genotype and environment.

The annual analyze of characters variability at carrot varieties target toward avoiding the modification of genetic constitution and valuable features as well as the degeneration of varieties in time.

MATERIALS AND METHODS

The analysis of variability of the main characters at carrot variety Ceahlău was accomplished at Vegetable Research and Development Station Bacău during 2005-2007 years.

The study of variability was focused on samples extracted from statistic population analyzing the following characters:

- **mother plants**: root's length (cm), diameter at colet (cm), diameter of central cylinder (cm), root's weight (g);
- **seedy plants**: plant's height (cm), number of floral cane/plant, number of inflorescences/plant, quantity of seeds/plant.

The character variability was estimated with the variation range and of histograms (Potlog and Velican, 1971).

Based on the establishment of variability's coefficients and the variation's limits calculated for each character within the links from the selection scheme, the choice of the biological material was made in each year, in order to maintain the Ceahlău variety in the specificity and authenticity limits.

RESULTS AND DISCISSIONS

The results that were obtained are presented in Table 1-3.

The analyses of observations regarding the behaviours over the main phenophases proved that the variety Ceahlău is middle early with a vegetation period of 90-100 days from springing till consumption maturity. The study of characters variability (Table 2) expressed in the population control field of descendents (mother plants and seedy plants) revealed that:

- variety Ceahlău presents low variability for characters:
  - root length (cm);
  - diameter at colet (cm);
  - seedy plant's height (cm). (CV < 10%);
- middle variability:
  - diameter of central cylinder (cm) (CV =10,066%);
  - root's weight (g) (CV = 10,075%);
  - quantity of seeds/plant (g) (CV =13,66%);
  - number of floral cane/plant (CV = 18,45%)

The variability of the main characters at carrot variety Ceahlău (Table 2) is at is follows:

- low variability was recorded at characters:
  - root length (cm);
  - diameter at colet (cm);
  - seedy plant's height (cm). (CV < 10%);
- middle variability:
  - diameter of central cylinder (cm) (CV =10,066%);
  - root's weight (g) (CV = 10,075%);
  - quantity of seeds/plant (g) (CV =13,66%);
  - number of floral cane/plant (CV = 18,45%)

The coefficients of phenotypic correlation of the main characters at seedy plants of carrot are presented in Table 3. As you can see the root's weight is weight is positively correlated with the quantity of seeds/plant (r1 = 0,808; r2 = 0,810) and with the number of inflorescences/plant (r1 = 0,771; r2 = 0,812);

The number of seedy stems is very significant positive correlated with the quantity of seeds/plant (r1 = 0,842; r2 = 0,862)

The habit of seedy plants influence the quantity and quality of seeds and this is due the fact that a grow type non-axial, suppose more equivalents stems, respectively more inflorescences of first range/plant, with simultaneous blossom at the begging of blossom phenofases, followed by the maturation of seeds in the same rhythm.

CONCLUSIONS

The correct and efficient application of selection, in the process of seeds production of carrot variety Ceahlau involved the accomplishment of a large number of observation and determinations over the main characters of families that compose the population.

In order to have more accurate dates, was necessary to have a large number of individuals from each family.

The modification of genes frequency and population genotypes determine the modification of characters average and as a result of variety features. In order to accomplish this goal the structure of variety must be known and after each selection cycle to be compared with the initial structure.

The calculation and analyses of variability of this genotype revealed:

- low variability was recorded at characters:
  - root length (cm);
  - diameter at colet (cm);
  - seedy plant's height (cm). (CV < 10%);
- middle variability:
  - diameter of central cylinder (cm) (CV =10,066%);
  - root's weight (g) (CV = 10,075%);
  - quantity of seeds/plant (g) (CV =13,66%);
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The coefficients of phenotypic correlation of the main characters at seedy plants of carrot were:

- the root's weight is weight is positively correlated with the quantity of seeds/plant (r1 = 0,808; r2 = 0,810) and with the number of inflorescences/plant (r1 = 0,771; r2 = 0,812);
- the number of seedy stems is very significant positive correlated with the quantity of seeds/plant (r1 = 0,842; r2 = 0,862)

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Tables

Table 1. The vegetation phenofases at carrot variety Ceahlău (2005-2007)

Nr. crt.	Phenofases	Phenofases duration - days -	Sum of temperature degree (^{0}C)	Total precipitations -mm-
	Mother plants			
1.	Sowing - springing	14	328,7	50,8
2.	Springing – root's thicken	19	330,9	47,6
3.	root's thicken –technological maturity	67	1680,0	204,0
	Seedy plants			
4.	Planting – initiation of vegetation	12	142,5	8,0
5.	initiation of vegetation – rising of floral cane	50	874,4	100,1
6.	rising of floral cane –large scale blossom	20	370,4	40,9
7.	large scale blossom -ripen	25	680,7	68,0
8.	ripen -harvest	20	507,8	7,0

Table 2. The variability of main characters at carrot variety Ceahlau – average on 3 years

Nr. crt.	Character	Average	Standard breach	Variability coeff.	Dispersion degree
	Mother plants				
1.	root length (cm)	12,74	0,73	5,74	11,0-14,6
2.	diameter at colet (cm)	4,24	0,34	8,071	3,4-5,0
3.	seedy plant's height (cm)	3,0	0,30	10,066	2,2-3,6
4.	Root weight (g)	129,56	13,05	10,075	85-150
	Seedy plants				
5.	Plant's height (cm)	94,10	6,050	7,25	80-110
6.	quantity of seeds/plant (g)	14,28	7,090	13,066	10-18
7.	number of floral cane/plant	7	2	18,45	4-10

Table 3. The coefficients of phenotypic correlation of the main characters at carrot seedy plants Ceahlău

Correlated characters		The value of correlation coeff	Significance
Root's weight Mother plants	No of seedy branches	0,895	***
Root's weight Mother plants	Plant's height	0,069	
Root's weight Mother plants	Quantity of seeds/plant	0,810	***
Root's weight Mother plants	No of inflorescences/plant	0,812	***
Root's weight Mother plants	M.M.B.	0,852	***
No of seedy branches	Plant's height	0,031	
No of seedy branches	No of inflorescences/plant	0,862	***
No of seedy branches	Quantity of seeds/plant	0,843	***

Researches regarding the influence of protection complexes used to control downy mildew *Peronospora destructor* (Berk.) Casp., on onion bulbs, onion neck rot -*Botrytis allii Munn*. and onion maggot - *Delia antiqua* Meig. affecting mean weight of onion bulbs

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Keywords: protection complex, bulbs, means weight

ABSTRACT

Onion's crops are devastated by numerous diseases and pests that severely diminish bulb yields in climate conditions favourable for their development with direct impact on bulb weight and implicitly on production. The healthy maintenance of entire leaf system influences directly bulb weight with positive responses on onion yields level. In the present paper, it has been studied the influence of protection complexes on mean weight of onion bulbs

INTRODUCTION

Onion occupies an important place in the vegetable range and the forth position as world's most cultivated vegetable species. In our country, shallot onion is cultivated on small areas, the largest surfaces being registered in the Western Europe, where it is widely requested by the consumers.

The size of onion bulbs represents a variety specific character as well as bulb shape and colour. Bulb size mainly depends on number of metamorphosed leaves whilst the last is tightly correlated with number of leaves per plant (Nedelea, G., Madoşă, E. 2004).

The maintenance in health conditions of leaf number has direct influence on bulb weight and consequently on yield level of onion bulbs.

The number of applied treatments and effectiveness of protection complexes strongly influence the attained yield by maintaining leaves free of specific onion diseases such as downy mildew (*Peronospora destructor* (Berk.) Casp.), onion neck rot (*Botrytis allii* Munn.) and pests like onion maggots which may cause major crop damages.

MATERIALS AND METHODS

The studies carried out under the pedo-climatic conditions from Albina, the district of Timiş, have been accomplished in three years (2003-2005). The onion crop has been founded with scallion.

In this experience, we have applied all works of soil preparation, crop maintenance, and prophylaxis and integrated therapy of diseases and pests stipulated within scallion-based onion crop technology, under conditions of natural infection.

We have applied four treatments at warning, in which we have used, in the 8 variants, the following protection complexes: V_1 – Trichodex 25 WP 0.2% + Victenon 50 WP 0.05%; V_2 – Bravo 500 SC 0.15% + Actara 25 WG 0.01%; V_3 – Previcur 607 SL 0.15% + Confidor 70WG 0.02%; V_4 – Folpan 80 WDG 0.15% + Karate Zeon 0.02%; V_5 – Ridomil Gold MZ 68 0.25% + Victenon 50 WP 0.075%; V_6 – Ridomil Gold Plus 42.5

WP 0.3% + Mospilan 20 SP 0.025%; V_7 – Dithane M45 0.2% + Fastac 10EC 0.02%;V_8 – Untreated control variant.

The experiment was performed in the collection field, the experimental data being obtained from observations and biometrical measurements.

The weights of onion bulbs registered on variants and replications were statistically processed and compared with Mt – untreated control variant, $M\bar{x}$ - experience mean and $M\bar{x}cp$ – mean of protection complexes (excluding mean weight of bulbs registered for V_8 – untreated control variant) under the influence of protection complexes.

RESULTS AND DISCUSSIONS

The mean weight of bulbs attained in the experimental period 2003-2005 as well as the significance of weight differences comparing with Mt, $M\bar{x}$ and $M\bar{x}cp$ is summarized in table 1 and it enables to draw conclusions referring to graduation of influences exerted by protection complexes (V_1 -V_7) on bulb weight.

Variability has been observed for mean weight of onion bulbs comprised between 78,03 g/bulb in V_8 (untreated control variant) and 122,73 g/bulb V_6 (Ridomil Gold Plus 42,5 WP 0,3% + Mospilan 20 SP 0,025%). In case of six of the studied variants, mean weight of bulbs registered over 90,0 g/bulb (V_1-V_6) while in case of V_7, this was of 83,98 g/bulb and for V_8 – untreated control variant of 78,03 g/bulb.

The significance of weight differences comparing untreated control variant was positively very significant under the influence of protection complexes of V_2 –V_6, positively distinct significant for V_1 (Trichodex 25 WP 0,2% + Victenon 50 WP 0,05%) variant and insignificant for V_7 (Dithane M45 0,2% + Fastac 10EC 0,02%). It could be noticed that in case of V_7 variant where, diseases and pest attack was pronounced, bulb weight was smaller due to the influence of health state of foliar system on number of metamorphosed leaves.

Continuing the analysis concerning the significance of weight differences and comparing with experience mean ($M\bar{x}$), it has been noticed relevant differences in terms of variants for significance degree and only in case of two variants (V_6 and V_5) the significance of differences was positively very significant.

When assessment of significance of differences concerning bulb weight was performed only in terms of bulb weight mean, excluding V_8 – untreated control variant ($M\bar{x}cp$), the situation of significance graduation has changed.

Thus, in case of protection complexes for V_6 (Ridomil Gold MZ 68 0,25% + Victenon 50 WP 0,075%) and V_5 (Ridomil Gold Plus 42,5 WP 0,3% + Mospilan 20 SP 0,025%) variants, the significance of differences comparing with $M\bar{x}_{cp}$ remained unchanged (positively very significant) while for V_3 (Previcur 607 SL 0,015% + Confidor 70WG 0,02%) it was positively significant. In case of V_2 (Bravo 500 SC 0,15% + Actara 25 WG 0,01%) and V_4 (Folpan 80 WDG 0,15% + Karate Zeon 0,02%) variants no significance has been observed while for V_7 (Dithane M45 0,2% + Fastac 10EC 0,02%) and V_1 (Trichodex 25 WP 0,2% + Victenon 50 WP 0,05%), the significance of weight differences was negatively very significant and negatively distinct significant, respectively.

When differences regarding bulb weight were diversely presented (comparing with Mt, $M\bar{x}$ or $M\bar{x}cp$), the significance of differences ranged from positively very significant (for instance, protection complex from V_3 - Previcur 607 SL 0,015% + Confidor 70WG 0,02%) or from positively very significant to insignificant (V_2 - Bravo 500 SC 0,15% + Actara 25 WG 0,01% and V_4 - Folpan 80 WDG 0,15% + Karate Zeon 0,02%) or from insignificant to negatively very significant (V_7- Dithane M45 0,2% + Fastac 10EC 0,02%) or maintained the same significance (V_5 - Ridomil Gold MZ 68 0,25% + Victenon 50 WP 0,075% and V_6 - Ridomil Gold Plus 42,5 WP 0,3% + Mospilan 20 SP 0,025%).

CONCLUSIONS

1. Protection complexes used for V_6 - Ridomil Gold Plus 42,5 WP 0,3% + Mospilan 20 SP 0,025% and V_5 - Ridomil Gold MZ 68 0,25% + Victenon 50 WP 0,075% insured the best effectiveness regarding the control of two most important diseases (downy mildew caused by *Peronospora destructor* (Berk Casp..) and onion neck rot caused by *Botrytis allii* Munn.) and pests (onion maggot– *Delia antiqua* Meig.), this fact being demonstrated by mean weight of bulbs and by significance of differences regarding bulb weight.

2. The most reduced effect regarding bulb weight under direct influence of control measures for both diseases and pests was registered using protection complex from V_7 - Dithane M45 0,2% + Fastac 10EC 0,02% because the significance of weight differences comparing with $M\bar{x}$ and $M\bar{x}cp$ was negatively very significant while for Mt proved to be insignificant.

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Figures

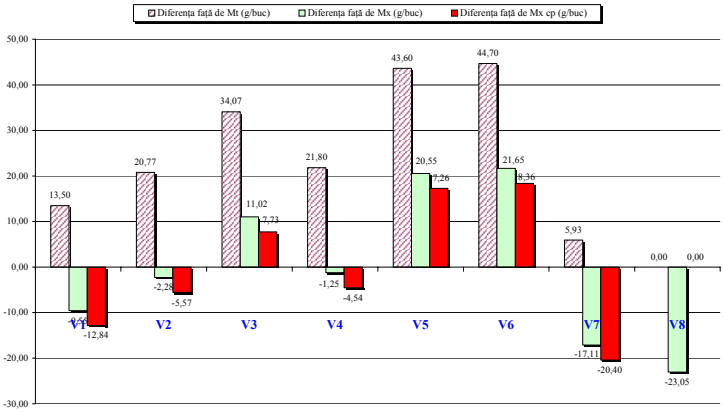

Fig. 1. Differences compared to Mt, $M\bar{x}$ and $M\bar{x}_{cp}$ of the onion bulbs weight in experimental period 2003-2005

Tables

Table 1. Average bulb weight achieved and significance of weight differences compared to the untreated control variant Mt
(experimental mean $M\bar{x}$ and protection complex mean $M\bar{x}$ during 2003-2005)

Statistical analysis elements \ Protection complex			V_1 Trichodex 25 WP 0,2% + Victenon 50 WP 0,05%	V_2 Bravo 500 SC 0,15% + Actara 25 WG 0,01%	V_3 Previcur 607 SL 0,015% + Confidor 70 WG 0,02%	V_4 Folpan 80 WDG 0,15% + Karate Zeon 0,02%	V_5 Ridomil Gold MZ 68 WP 0,25% + Victenon 50 WP 0,075%	V_6 Ridomil Gold Plus 42,5 WP 0,3% + Mospilan 20 SP 0,025%	V_7 Dithane M 45 0,2% + Fastac 10 EC 0,02%	V_8 Control variant
Mean weight (g/bulb.)			91,53	98,80	112,10	99,83	121,63	122,73	83,97	78,03
Relative weight (%)		Mt	117,30	126,61	143,66	127,94	155,87	157,28	107,60	100,00
		$M\bar{X}$	90,56	97,75	110,90	98,77	120,33	121,42	83,07	77,20
		$M\bar{X}_{cp}$	87,70	94,66	107,40	95,65	116,54	117,59	80,45	-
Weight difference (g/bulb.)	Mt	78,03	13,50	20,77	34,07	21,80	43,60	44,70	5,93	-
	$M\bar{X}$	101,08	-9,55	-2,28	11,02	-1,25	20,55	21,65	-17,11	-23,05
	$M\bar{X}_{cp}$	104,37	-12,84	-5,57	7,73	-4,54	17,26	18,36	-20,40	-
Significance of differences		Mt	**	***	***	***	***	***	-	Mt
		$M\bar{X}$	0	-	**	-	***	***	000	000
		$M\bar{X}_{cp}$	00	-	*	-	***	***	000	

	DL 5%	DL 1%	DL 0,1%
DL Mt	7,84	10,87	15,10
DL $M\bar{X}$	7,23	9,96	13,72
DL $M\bar{X}_{cp}$	7,48	10,36	14,40

The influence of the variety and moment of consumption on the quality of tomatoes resulting from crops grown in unheated green houses

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Keywords: tomatoes for consumption when they are fresh, the quality of the tomato

ABSTRACT

In the period of the months of June and July in the past years, the market of fresh tomatoes meant for consumption was characterized by a supply which has constantly exceeded the demand. In these circumstances, it was necessary to introduce recent hybrids, whose fruit should be characterized as well by the maintenance of the commercial qualities for a longer period of time. This paper is providing a presentation of the dynamics of the biochemical composition of the fruit produced by new tomato hybrids, coming from crops grown in unheated green houses.

INTRODUCTION

The occurrence on the Romanian market in the past years of new hybrids meant for being grown in solariums and unheated green houses has changed substantially the point of view of many growers. The growers, giving up the short growth cycle, have increased the surfaces cultivated with tomatoes with extended growth cycle, which provide very good crops with fruit that are similar as size and weight to the ones coming from summer-autumn crops, grown in the fields.

Thus, the tomato production in solariums and unheated green houses continues to be competitive to the summer-autumn crops.

The study provides data resulting from a research survey related to the physical and biochemical characteristics of the tomatoes harvested from extended cycle cultures, both fresh and after a short and medium storage period.

MATERIALS AND METHODS

In order to perform the experience, there have been used the following cultivars as experimental variants: Tamaris F1 – originating from France (Clause); Electra F1 - originating from Israel (Hazera); Abellus F1 and RZ 73-490 - originating from Holland (Rjik Zwaan). The 4 hybrids are recommended for being grown in solariums and unheated green houses, mainly with extended growing cycle.

Research goals:

- determining biochemical and agro-chemical characteristics of the fresh vegetables, immediately after being harvested;
- the dynamic of the characteristics, after a short period of preservation (one week) and after a medium period of storage (2 weeks), at room temperature (24-27^{o}C) and in a refrigerator (4 ^{o}C)

The main parameters and works imported from the growing technology applied within the experiment:

- location: individual, unheated green house
- plantation: March 28th, with seedlings that are 53 days old
- plantation distance: 80/40 cm
- density: 3.12 plants/sqm
- basic fertilization: 5 kg/1mm sqm of Agroblen (complex granulated stripped fertilizer) with release of the macro elements within 5 months;

- phase fertilization: from the occurrence of the first tomato by fertirrigation; 1 g of complex entirely soluble fertilizer is applied weekly, for each plant (Universal – manufactured by Scotts's); weekly, there has been performed a fertilization procedure with calcium nitrate since the complex soluble fertilizer Universal doesn't contain any calcium;
- phytosanitary treatment for combating pestilent agents: the green house whitefly (*Trialeurodes vaporariorum*), the leafminer (*Liriomyza brioniae*), etc.
- controlling the temperature to maintain it within the interval of the optimal values; during the warm summer periods, there have been temperatures of more than 40 ^{o}C, which could not be countered
- hanging the plants, with rope, on a 2 meter high frame
- thinning out of plants
-defoliation of the plant base
- top removal of the plants every 6 inflorescences
- harvesting the tomatoes that are mature from the physiological point of view

Comments and tests:

- the recording of the data related to the occurrence and the manifestation of the main phenophases
- the dynamics of the vegetative growth and of the fructification
- lab tests related to the quality of the fresh tomatoes and of the stored ones, at time intervals mentioned within the presentation of the variants.

RESULTS AND DISCUSSIONS

The results related to the total production have indicated values which may be assessed as good in the conditions provided by the material base (unheated individual green house, more than 50 years old). The obtained production is of 78-82 t/ha, with minor variations between the 4 cultivars that were used, which is justified by the fact that between these cultivars, such as indicated by the previous experiences, there have not been great productivity variations.

From the point of view of the average weight of the fruit, the cultivar Electra F1 stood out.

For the performed lab tests in order to respond to the content of the variants, the following procedure was performed:

- for each series of tests, 30 tomatoes were harvested from each hybrid, which were used as follows:
  - 6 tomatoes for the tests performed on the harvest date
  - 6 tomatoes for each variant of storage for tests performed in 7 days after the harvesting
  - 6 tomatoes for both variants of storage for tests performed in 14 days from the harvesting

The data resulted after the tests (Table 1) outline the following aspect:

- the acidity of the fruit of the four cultivars is different in the fresh tomatoes, varying from a maximum of 0.370% in Electra F1 to the minimum of 0.,310% in RZ-73-490;
- during the storage, the acidity drops progressively, with no significant variations between the fruit of the same hybrid kept at room temperature or in the refrigerator;
- the carbohydrate content is increasing during the storage; large increases were determined in all the four cultivars, in the variants which were refrigerated;

- the most interesting results were obtained after the analysis of the content of $N\text{-}NO_3$. A low content of nitrates was obtained in the fruit of the hybrid Abellus F1. For this test, the variation in time of the content is relatively small, with a slightly different dynamic in all cultivars;
- the content of P of the tomatoes is lower in the RZ-73-490 (92-96 ppm) as compared to the one determined in the fruit of the hybrids Abellus F1 (175-185 ppm) and Tamaris F1 (174-186 ppm). The values of the potassium content are very similar in the 4 hybrids, and during the storage, no significant variations were recorded.

CONCLUSIONS AND RECOMMENDATIONS

1. The content of biochemical and agrochemical elements of the fresh tomatoes is different in the 4 hybrids used during the experiment, with higher values of the Vitamin C and of the carbohydrates in the hybrids Abellus F1, RZ-73-490F1 and Tamaris F1.
2. The storage in various temperature and duration conditions causes the significant reduction of the acidity and of the Vitamin C content of the tomatoes. The storage at a temperature of 4^oC assures, from this point of view, lower losses than the situation in which they are kept at room temperature (at least $24\text{-}26^oC$).
3. The slow rhythm of modification of the biochemical composition in the four hybrids indicates the fact that they have an outstanding storage capacity, which allows maintaining the commercial quality of the tomatoes for at least 2 weeks, in force major situations.
4. The sampling of different quantities of $N\text{-}NO_3$ in the tomatoes allows the presumptive formulation of the use of recent hybrids, for the purpose of obtaining productions meant for the consumption of fresh tomatoes, with a low pollution degree.

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Tables

Table 1. The dynamics of the biochemical and agrochemical characteristics in the tomatoes in unheated green houses – USAMV BUCHAREST, 2007

No.	Test data	Obs.	Acidity %	Vit. C mg/100g	Carbohidrates %	N-NO3 (ppm)	P (ppm)	K (ppm)
Tamaris F1								
1	06.07.07	Keeping room	0,350	10,39	5,05	127	186,8	2060
2	13.07.07	Keeping room	0,267	6,93	5,25	116	175,1	2015
3	13.07.07	Keeping 4°C	0,246	7,87	5,15	120	180,2	2035
4	20.07.07	Keeping room	0,256	6,85	5,30	115	174,0	1890
5	20.07.07	Keeping 4°C	0,250	7,60	5,20	122	178,0	1920

Table 2. The dynamics of the biochemical and agrochemical characteristics in the tomatoes in unheated green houses – USAMV BUCHAREST, 2007

No.	Test data	Obs.	Acidity %	Vit. C mg/100g	Carbohidrates %	N-NO3 (pmm)	P (ppm)	K (ppm)
Electra F1								
1	06.07.07	Keeping room	0,370	8,500	5,190	126,0	141,2	2220
2	13.07.07	Keeping room	0,296	7,875	5,270	121,0	135,1	1995
3	13.07.07	Keeping 4°C	0,315	8,190	5,100	124,0	136,5	2200
4	20.07.07	Keeping room	0,278	7,800	5,300	119,0	136,0	1970
5	20.07.07	Keeping 4°C	0,282	8,050	5,250	120,0	134,0	1935
Abellus F1								
1	06.07.07	Keeping room	0,330	11,340	5,056	98,0	185,2	2140
2	13.07.07	Keeping room	0,231	8,190	5,150	99,0	178,0	2015
3	13.07.07	Keeping 4°C	0,310	8,820	5,110	97,0	181,3	2100
4	20.07.07	Keeping room	0,220	8,100	5,250	98,0	175,0	1955
5	20.07.07	Keeping 4°C	0,290	8,700	5,300	95,0	178,0	1975
RZ 73-490 F1								
1	06.07.07	Keeping room	0,310	7,87	5,119	142,0	94,0	2020
2	13.07.07	Keeping room	0,289	7,51	5,200	132,0	96,0	1985
3	13.07.07	Keeping 4°C	0,300	7,62	5,170	135,0	94,0	2005
4	20.07.07	Keeping room	0,260	7,38	5,250	130,0	94,0	1875
5	20.07.07	Keeping 4°C	0,287	7,50	5,350	135,0	92,0	1925

Researches regarding the influence of varieties and plants density, relates with the number of fruit per plant, on eggplant seeds production

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Keywords: cropping, *Lucia*, *Contesa*, fructification standards, fruit limitation

ABSTRACT

The experiments were effectuated in the year 2007, on the leguminous sector of vegetable gardening department. They followed the varieties and plant's density influence, relates with the number of fruit on plant, about eggplant's seeds production.

Lucia and *Contesa* varieties were studied, on the following densities:

- 70/20cm with 71.428 pl/ha restricted to 2 fruits on each plant
- 70/30cm with 47.619 pl/ha restricted to 3 fruits on each plant
- 70/40cm with 35.714 pl/ha restricted to 4 fruits on each plant
- 70/45cm with 31.746 pl/ha restricted to 5 fruits on each plant
- 70/35cm with 40.816 pl/ha unrestricted number of fruit on plants (control)

Seeding was achieved on March 17th, springing happens on March 23rd and transplantation was performed on April 8th, 2007. At planting date, the seedlings had 61 days.

Planting on the experimental field was achieved on May 23rd and mature fruits harvest was performed at October 15th, 2007 to *Lucia* variety and September 24th, 2007 to *Contesa* variety.

Plant's seed production was swelling with growth number of fruit on plant. On both varieties, the eldest production of seeds were recorded to 71.428 plants per hectare density and limited to 2 fruits on each plant. The average seeds production was 222,85kg to *Lucia* variety and 164,99kg to *Contesa* variety. Seed production obtained using described procedure is significant bigger than the one presented in the literature.

INTRODUCTION

The eggplants (*Solanum Meloncena L. var Esculentum*) are parts from the leguminous assortment of our country; eggplant fruits are prepared in a large range of food assortments (Indrea, 2007).

Seed technology for producing the pure species is well enough subedited (Dumitrescu, 1977), some specific problems are mentioned also for hybrid seeds production (Ciofu, 2003).

In the horticulture literature is mention a certain density (Dumitrescu, 1977) and the fruits limitation number on plants (Popescu, 2000 and Butnariu 1992).

Actual study is focusing on seeds production quality and quantity; researches are done on two new eggplant species, cultivated to different plantation densities, relate with a limited number of fruits on each plant.

MATERIALS AND METHODS

The research was made on the experimental field of Vegetal Department of Horticulture Faculty of The University of Agriculture Science and Veterinary Medicine Bucharest.

All researches are done on two new eggplant species: *Lucia* variety (figure 1) and *Contesa* variety (figure 2). Each of both varieties was cultivated on different plantation densities, as is reveled on following description.

Experimental variants factors ware:

A factor: a1 – *Lucia*
a2 – *Contesa*

B factor: b1 – 70/20cm – 71.428pl/ha limited to 2 fruits on plant
b1 – 70/30cm – 47.619pl/ha limited to 3 fruits on plant
b3 – 70/40cm – 35.714pl/ha limited to 4 fruits on plant
b4 – 70/45cm – 31.746pl/ha limited to 5 fruits on plant
b5 – 70/35cm – 40.816pl/ha unlimited number of fruits (control)

Mixing above factors, the experiments ware performed on following variants:

V_1 – *Lucia*, planting on 70/20cm – 71.428pl/ha, limited to 2 fruits on plant
V_2 – *Lucia*, planting on 70/30cm – 47.619pl/ha, limited la 3 fruits on plant
V_3 – *Lucia*, planting on 70/40cm – 35.714pl/ha, limited la 4 fruits on plant
V_4 – *Lucia*, planting on 70/45cm – 31.746pl/ha, limited la 5 fruits on plant
V_{5CTL} – *Lucia*, planting on 70/35cm – 40.816pl/ha, unlimited fruits (control)
V_6 – *Contesa*, planting on 70/20cm – 71.428pl/ha, limited la 2 fruits on plant
V_7 – *Contesa*, planting on 70/30cm – 47.619pl/ha, limited la 3 fruits on plant
V_8 – *Contesa*, planting on 70/40cm – 35.714pl/ha, limited la 4 fruits on plant
V_9 – *Contesa*, planting on 70/45cm – 31.746pl/ha, limited la 5 fruits on plant
V_{10CTL} – *Contesa*, planting on 70/35cm – 40.816pl/ha, unlimited fruits (control)

The saplings were produced in multiplier greenhouse. Seeding was performed on March 17th, in rectangle pots, using a mixture of grounds between earth of leaf, earth of celery, peat and rotted manure in equal proportions.

Transplantation was done into small pots, having 6cm in diameter and using the same ground mixture, on April 8th, 2007. Usual attendance work was applied, starting from the seedling phase: driving vegetation factors, disease struggle and pest prevention and removal. Planting on the experimental field was achieved on May 23rd, according to experimental technique standards (Dimancea S. and Berca M., 1976)

Upon period of vegetation, usual attendance works was applied, limiting the number of fruit on each plant on every variant according with experiment description above, got observations concerning plants dynamic growth in height, leaves number on each plant, production of fruits and seeds production.

For eliminating eventually possible errors and peculiarities of earth, all the observations and statistical calculus ware accomplished on 4 groups of repetition, to each experimental variant, disposed in Latin rectangles, as described in experimental techniques (Dimancea S. and Berca M., 1976).

RESULTS AND DISCUSSIONS

Deployment of main phenologic phases to study varieties is rendered in table 1.

Noticed that springing happens on March 23rd 2007, after except 7 days from seeding. The prick-out was performed after 23 days after seeding and field plantation was done after 61 days from springing (that is 68 after seeding). Eggplant fruits arrived at the physiological maturity after 207 days for the *Lucia* variety and after 185 days for *Contesa*.

The features seedling, to plantar time, is reveled in the table 2. The height was 10,74cm to *Lucia* type and the number of mature leafs was of 5,12 leaf/plant. For to *Contesa* type, the height was 15,64cm and the number of leafs was 4,3 leaf/plant.

In table 3 is render growth plants dynamic for each variant. Analyzing both eggplant types, is noticeable that *Lucia* variety has a slow rhythm of growing, touching on August 6th, the heights between 57,2 - 64,7cm, and *Contesa* variety demonstrated a quick rhythm of growing, touching on the same date heights between 68,5 - 85, 3cm.

Looking to the same studied eggplant variety, don't notice to big differences, that means that the limiting number of fruit on plant do not influenced significantly the growth rhythm of the plants, as is can be noticed also from figure 3.

The production of seed is quantizing in table 4 and chart disposed in figure 4. For both varieties, the biggest production of seeds on each plant, was registered to the density of 31.746 pl/ha and limited to 5 fruit on plant (b4). But on both varieties, the biggest seeds production on surface field unit, was registered to the density of 71.428 pl/ha and limited to 2 fruit on plant (b1). At the same density was obtained the highest productivity of seeds to the kilogram of harvest fruit. Thus for the *Lucia* variety was obtained a value of 221,15 kg fruit/1 kg seeds and for to the *Contesa* is necessary 147,16 kg fruit/1 kg seeds.

Analyzing production of seeds per hectare, as is emphasizes also in figure 4, the difference between species are consistent: 222,85 Kg/ha for the *Lucia* type and 164,99 kg/ha for the *Contesa* type.

The seeds production obtained in those experiments are significantly bigger comparing with ones specified in the literature (Dumitrescu, 1977; Ciofu, 2003) and those differences are dew to cultivation improvements added in this experiment.

CONCLUSIONS

1. *Lucia* variety has a slow rhythm of growing comparing with *Contesa* variety; around 61 days old the *Lucia* plants height was between 57,2 - 64,7cm, and for *Contesa* plants the height was between 68,5 - 85, 3cm.
2. Fruits limitation on plants do not influenced significantly the plant growth rhythm.
3. Seed productivity per plant is rising with the number or fruits per plant
4. Seed productivity per hectare is rising with the plants density per hectare
5. At *Lucia* variety, the biggest hectare productivity was obtained for V_1 variant with density of 71.428 pl/ha and with 2 fruits limitation on plant and it is 222,85kg/ha
6. At *Contesa* type, the biggest hectare productivity was obtained for V_6 variant with density of 71.428 pl/ha and with 2 fruits limitation on plant and it is 164,99kg/ha
7. Seeds productions from experiments are bigger, comparing with science specific literature quotation.

ACKNOWLEDGMENTS

Thanks to my phd. coordinator, professor doctor Victor POPESCU for all advices and hints during those experiments and results interpretations.

Thanks to I.C.D.L.F–Vidra for biological materials, scientific advices.

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Tables

Table 1. Main phenological activities on *Lucia* and *Contesa* variants

Variety	Seed (date)	Spring		Transplantation		Field Plantation		Harvest (date)
		date	Number of days from seed	date	Number of days from seed	date	No. of days from seed	
Lucia	March 17	March 23	7	April 8	23	May 23	68	October 15
Contesa	March 17	March 23	7	April 8	23	May 23	68	September 24

Table 2. Morphological eggplant seedling characteristics

Variety	Seedling age (days)	Seedling height - airy part - (cm)	Length of seedling, including root part (cm)	Number of leafs
Lucia	68	10,74	13,98	5,12
Contesa	68	15,64	14,6	4,3

Table 3. Height growing of plants on different evaluation dates

Var.	Variety	Observation date							Differences between fist and last measurement
		May 12	May 23	June 12	June 23	July 12	July 26	August 6	
		Height of the plant (cm)							
V_1	*Lucia*	7,8	9,09	11,4	16,7	22,2	46,1	58,1	50,2
V_2	*Lucia*	8,5	12	17,2	19,2	22,4	45,9	61,4	52,9
V_3	*Lucia*	9,8	13,8	18,5	21	24,5	36,7	60,6	50,8
V_4	*Lucia*	8,3	9,2	11,4	23,6	32,2	47,3	64,7	56,4
V_{5CTL}	*Lucia*	6,4	9,6	11,9	15,8	25	47,5	57,2	50,8
V_6	*Contesa*	13,6	16,6	21,5	28,2	49,5	68,5	70,8	57,2
V_7	*Contesa*	12,2	16,2	26,2	28,9	32,7	58,6	85,2	73
V_8	*Contesa*	14,8	18,2	25,5	27,8	48,5	78,5	85.3	70,5
V_9	*Contesa*	10,4	12,4	22,6	28,2	29,5	48,6	68,5	58,1
V_{10CTL}	*Contesa*	11,4	14,8	24,5	36,2	45,8	58,2	71,2	59,8

Table 4. Seeds production results on variants for both varieties

Var.	Plants per hectare	Number of fruits per plant	Fruits weight per plant (g)	Seeds weight per plant (g)	Fruits needed to obtain "1 kg" of seeds (kg)	Kilograms of seeds per hectare (kg)
V_1	71.428	2	690	3,12	**221,15**	**222,85**
V_2	47.619	3	1.110	4,03	275,43	191,90
V_3	35.714	4	1.380	4,50	306,66	160,71
V_4	31.746	5	2.480	**4,97**	498,99	157,77
V_{5CTL}	40.816	unlimited	1.160	3,80	305,26	155,10
V_6	71.428	2	340	2,31	**147,18**	**164,99**
V_7	47.619	3	840	2,86	293,70	136,19
V_8	35.714	4	1.100	3,64	302,19	129,99
V_9	31.746	5	1.625	**4,21**	385,98	133,65
V_{10CTL}	40.816	unlimited	830	3,26	254,60	134,06

Figures

Fig. 1. *Lucia* variety

Fig. 2. *Contesa* variety

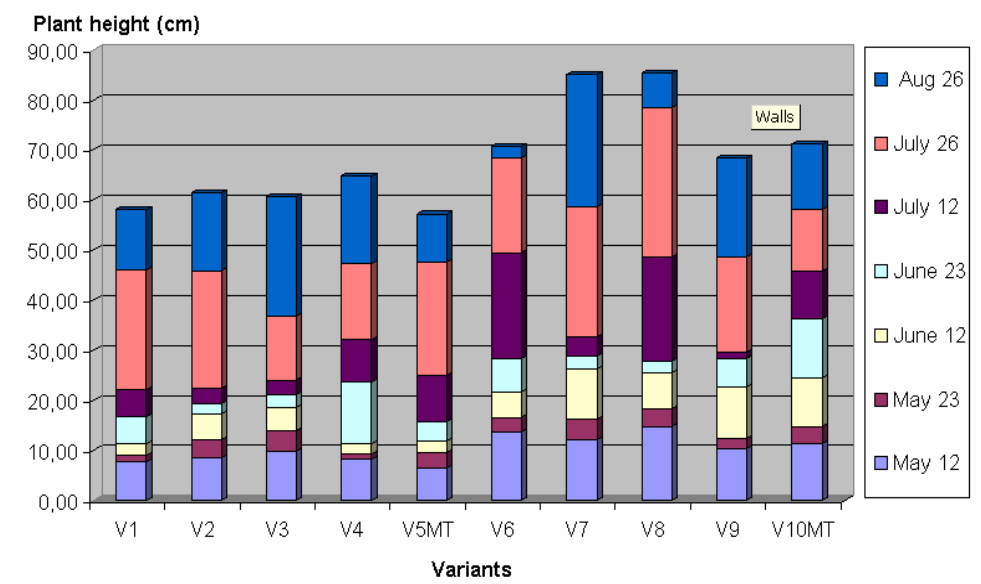

Fig. 3. Height growing dynamics on different evaluation dates for each variant

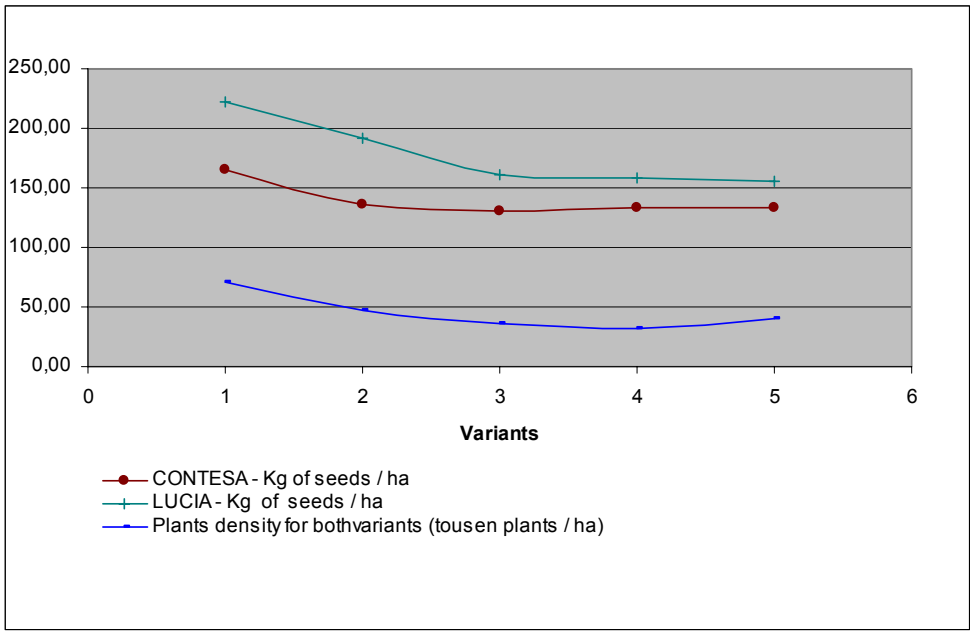

Fig. 4. Dependency between plants density and seed production

Researches regarding chemical and biochemical components existed in different tomato hybrids

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Keywords: tomato hybrids, solarium, quality, chemical elements

ABSTRACT

This research was due to characterize some hybrids from the point of view of the quality of crop in solarium culture so to recommend of them to the growers in our country.

Results of that culture present the different accumulation of chemical elements, nitrates during the period of harvest, also the accumulation of phosphorus and potassium and gustative characteristics as glucoses, acidity, vitamin C.

INTRODUCTION

Tomato cultivated in solarium could be easy revaluating if the fruits have some qualitative and quantitative characteristics corresponding standard foresights. Choosing of a productive assortment in respect of qualitative characteristics, regarding a normal content of nutritive elements and a low content of nitrates from the list of so many hybrids is a big problem when there are insufficient tested in the culture of our country [2].

This research was due to characterize some hybrids from the point of view of the quality of crop in solarium culture so to recommend of them to the growers in our country.

Results of that culture present the different accumulation of chemical elements, nitrates during the period of harvest, also the accumulation of phosphorus and potassium and gustative characteristics as glucoses, acidity, vitamin C [1, 3].

MATERIAL AND METHODS

The experiments were realized in a farm from Ploieşti Region in 2006 year. Researches were made on four hybrids from Holland. Variants cultivated were Marisa F1, Vemone F1, Cristal F1, Fortara F1.From every hybrid were transplanted 25 plants at 80 cm distance between rows and 40cm between plants on the same row. During the vegetation time was registered the crop and were made qualitative analyses regarding the content of tomato fruits in some chemical and biochemical components.

For the analyses, it was used standard methods like: for nitrates, phosphorus and potassium it was used the unmethobolised forms and total forms obtained with humid mineralization with H_2SO_4 and H_2O_2. Determination of phosphorus and nitrates with colorimetric method and for potassium phlamphotometric method, for glucoses method Abbe, for acidity was used the titration with NaOH 0.1n and for vitamin C titration with 2,6 – diclorfenolindofenol.

RESULTS AND DISCUSSION

The elements which are analyzed from tomato are presented in table 1. Nitrogen varies from 2.15 and 2.4% from dry matter and represents a good supply of tomato in that element. The best supply is presented at Vemone hybrid which attends a value of 2.4%. Phosphorum attended values of 0.30% and in general that element was

represented in high values in tomato fruit. Also potassium an important element of quality of fruits was high absorbed in tomato fruit at values between 2.1 and 2.2%.

Calcium an element necessary for the quality of fruits for consumption was absorbed in high quantity in the tomato hybrids with the exception of Cristal F1.

Another element which could affect the nutritive aspects of tomato is ferum which had high values at Vemone and Cristal hybrids.

From the analyses of the results of the tomato elements all of them are in the limits presented by the scientific literature and not one of that presented a toxic value.

During the harvest period, there were analyses the tomato from the point of view of the content of nitrates, phosphorus and potassium. The results are presented in table 2.

The data regarding the tomato quality from the point of view of the nitrogen, phosphorus and potassium contents present normal value of that elements and compounds.

The nitrates were analysed at three period of analyse, respectively at the beginning, at the middle and at the finish of harvest (figure 1). The nitrates values are low comparison with the 150 ppm value which is the admissible content for the tomato used in the human consume presented by the Ministry of Health from our country also the gustative analyses recommended all the hybrids for the culture in our country.

The value of P and K presents no problem for the crop quality.

Biochemical analyses of tomato fruits from experimental hybrids are presented in table 3.

Glucides from tomato hybrids are between 4.980 and 5.187% and represents tomato with a lot of sweet and respectively good taste.

Vitamin C another characteristic of tomato was accumulated in low quantity at Marisa F1 respectively 11.34 mg/100g and high quantity at Cristal F1 a value of 18.75 mg/100 g.

In balance with vitamin C, acidity is another gustative quality for tomato. From the results of those characteristics it could be seen that Marisa F1 had the highest acidity from all hybrids and Fortara presented the lowest acidity of all analysed tomato hybrids.

CONCLUSIONS

The results regarding the quality of tomato fruits show:

1. Nitrogen, phosphorus and potassium, macro elements which determine the quality of tomato are in normal limits comparison with the scientific literature;
2. Calcium an element necessary for the quality of fruits for consumption was absorbed in high quantity in the tomato hybrids with the exception of Cristal F1;
3. Another element which could affect the nutritive aspects of tomato is ferum which had high values at Vemone and Cristal hybrids;
4. The content of nitrates at all hybrids are under the admissible content of that compound presented by the scientific literature;
5. The content of other chemical elements are in normal limits for this fruit;
6. The values for nitrates were under the maximum admissible limits presented by the Ministry of Health from our country also the gustative analyses recommended all the hybrids for the culture in our country.

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Tables

Table 1. The contents of different chemical elements in tomato hybrids

Element	Marisa	Vemone	Cristal	Fortara	Limits for different elements presented by scientific literature
Nt,%	2.3	2.4	2.3	2.15	**2.3 – 2.5% from d.m.**
Pt.%	0.29	0.24	0.27	0.30	**0.20 – 0.33% from d.m.**
Kt.%	2.2	2.3	2.1	2.15	**1.8 – 2.6% from d.m.**
Cat.%	4.2	4.05	3.34	4.52	**4.6% from d.m.**
Mgt.%	0.48	0.46	0.44	0.43	**0.4 – 0.43% from d.m.**
Fet. ppm	178	268	276	186	**31 – 300ppm from d.m.**
Bt. ppm	22	26	28	65	**31-90ppm from d.m.**
Mnt. ppm	65.2	52.0	66.0	75.3	**50 – 150ppm from d.m.**
Cut. ppm	7.5	9.3	15.5	10.7	**12 – 15ppm from d.m.**

Table 2. The agrochemical characeristics of tomato hybrids

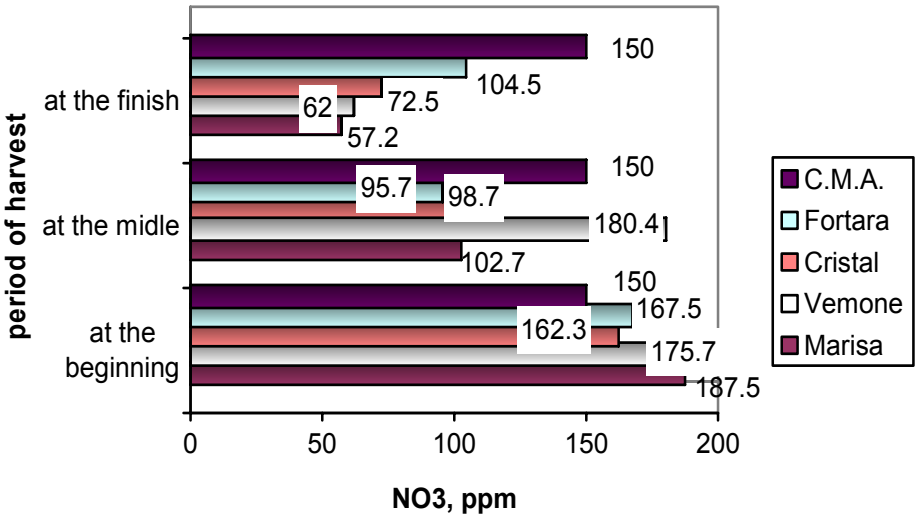
No	Variant	Content. ppm		
		$N\text{-}NO_3$	**$P\text{-}PO_4^{3-}$**	**K^+**
1	**Marisa F1**	57.2	155.25	204
2	**Vemone F1**	62	141.0	242
3	**Cristal F1**	72.5	141.75	342
4	**Fortara F1**	104.5	182	342

Table 3. Results regarding biochemical analyses in tomato hybrids

Biochemical characteristic	Marisa	Vemone	Cristal	Fortara	Limits for different elements presented by scientific literature
Glucides.%	5.056	5.187	5.087	4.980	**5.20**
Vitamin C. mg/100g	11.34	17.24	18.75	16.45	**20.85**
Acidity.%	0.33	0.25	0.21	0.39	**0.44**
Dry matter.%	5.21	4.98	5.02	4.87	**5.90**

Figures

Fig.1. Nitrates content at three period of analyse



Researches regarding the quality and quantity of some tomato hybrids used in our country in solarium culture

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Keywords: *Lycopersicon esculentum*, solarium, hybrid, quality, quantity

ABSTRACT

To introduce tomato hybrids in solarium culture must determine the potential of them from the point of view of their quality and quantity. The quality of tomato hybrids is obtained from the studies of the dimensions and weight of tomato fruits. The quantity of tomato hybrids is to study the earlier crop and total crop.

INTRODUCTION

Tomato cultivated in solarium in our country could be revaluated if qualitative and quantitative characteristics are in correspondence with the standards. To recommend a productive assortment which respects the characteristics of quality and quantity obtained in the climate conditions of our country.

The aim of this research was to characterize some tomato hybrids in solarium condition from the point of view of the quantity and quality of crop.

MATERIAL AND METHODS

Researches were made in the solarium of a farm from Ploiesti in the period of 2006 year. The hybrids tested were Marisa F1, Vemone F1, Cristal F1, Fortara F1.

The transplant was made in the condition recommended by the technology from our country. At the start of culture, the tomato transplants have 70 days of age. From every hybrid, there were transplanted 25 plants at 80 cm distance between the rows and 40 cm between the plants in the same row. The transplants were planted after the soil was fertilized with complex chemical fertilizer in a quantum of 100 kg/ha active substance. The fertilization was necessary because the agrochemical analyze of soil revealed small contents of nutritive elements.

During the vegetation period the crops were registered and also there were measured every tomato from the point of view of their dimension and weight.

RESULTS AND DISCUSSIONS

Tomato from Marisa F1 had big dimensions with the weight of 120-170g/fruit in a significant 61% percent. Fruits with the weight 100-170g represent 20% and small tomato with the weight under the 100g/fruit had a proportion of 3%. Tomato with the weight upon the 200g/fruit is in a proportion of 7%

The Marisa F1 tomato crop is presented in the figure 1, and if it analyse the dimension of tomato it can be seen that the highest percent of tomato fruits were between 120 and 170g/tomato.

In the case of Vemone hybrid the harvest made in time revealed that the weight of tomato between 170 and 200g a percent of 53%. The other part of crop with a weight between 120 and 170 g/tomato represents a percent of 34%. The weights of tomato fruit are presented in figure 2.

From the point of the crop the weights of tomato fruits were in predominant percent of 87% between 120 and 170g/tomato.

The harvests made at Cristal F1 are presented in figure 3.

The quality of tomato obtained at hybrid Cristal F1 revealed that the main crop was with the weight between 70 and 120g/tomato, in a percent of 81%. So tomato with the weight between 101 and 120g represent a 46% of the number of tomato crop and fruits between 70 and 100g/tomato represent a percent of 36%. In the case of Fortara F1 the majority of the fruits are between 120 and 1700 g/tomato with a percent of 61%. The other weights represent percent between 3 and 11%.

From the data presented it could be seen that the best hybrids which had bigger fruits with the weights between 120 and 170 g/tomato are Fortara F1, Vemone and Marisa F1 with a percent of 61%, but only Vemone realized a lot of fruits with a higher weight of 120-200g/tomato in a cumulate percent under the of 87%.

Analysing total tomato crop obtained at four hybrids it could be seen that the highest total crop was at Vemone with 12.556 kg/m^2 and the lowest was at Marisa with 8.485 kg/m^2.

The highest earlier crop was obtained at Fortara (5,647 kg/m^2) with a percent of 58% comparison with total tomato crop and so this hybrid is the best for the solarium production. The smallest earlier tomato crop was at Vemone hybrid (1,00 kg/m^2) with a percent of 8% comparison with total crop (Fig. 5.).

CONCLUSIONS

Research made at some hybrids to certify the quality the introduction of them in the culture of the in solarium. The results show that:

1. The best hybrids which had bigger fruits with the weights between 120 and 170 g/tomato are Fortara F1, Vemone and Marisa F1 with a percent of 61%
2. Vemone realized many fruits with a higher weight of 120-200g/tomato a cumulate percent under the 87%.
3. The highest earlier crop was obtained at Fortara (5,647 kg/m^2) with a percent of 58% comparison with total tomato crop and so this hybrid is the best for the solarium production

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Figures

Fig.1.Tomato weights from Marisa hybrid

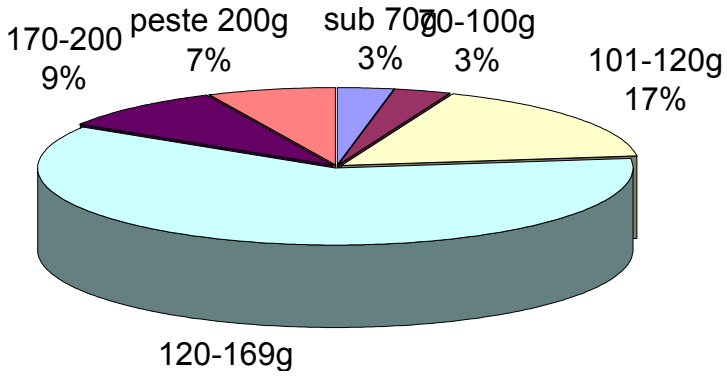


Fig.2. Tomato fruits weight from Vemone F1

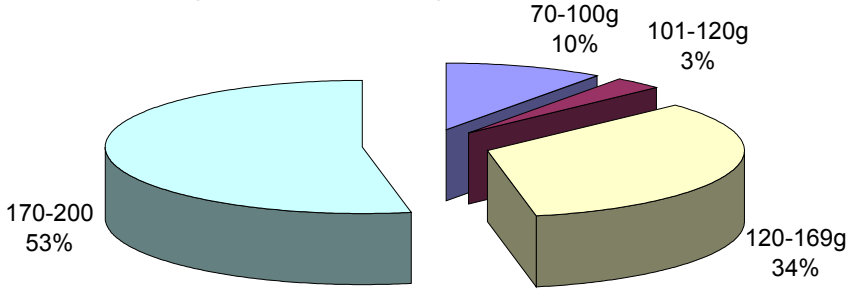


Fig.3.Tomato fruits weight of the Cristal F1

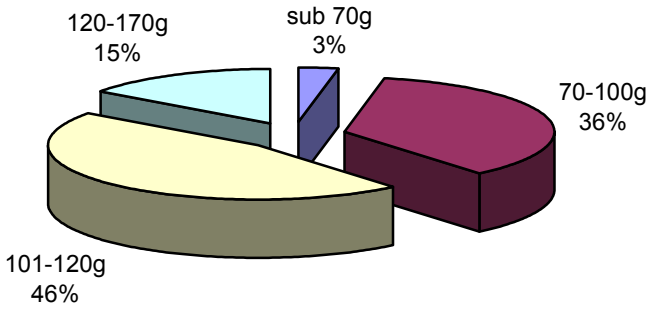


Fig.4.Tomato fruits weights from Fortara F1

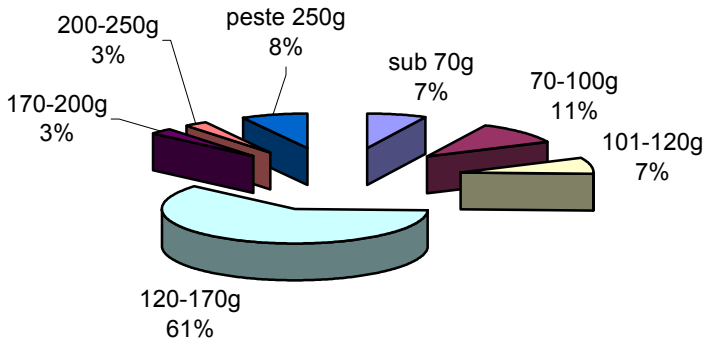
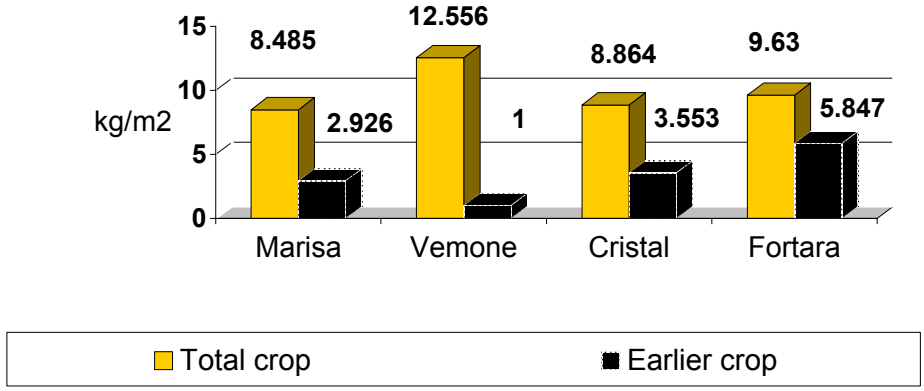


Fig.5.Total and earlier tomato crop



Epidemiology and control of the pathogen *Fusarium oxysporum* Schl. f. sp. *radicis lycopersici* (Jarvis and Shoemaker) on tomatoes

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Keywords: *Fusarium* crown rot, epidemiology aspects, variety resistance

ABSTRACT

The purpose of this research was to study the influence of temperature and soil humidity on apparition and evolution of the pathogen *Fusarium oxysporum* f. sp. *radicis– lycopersici* attack on tomatoes, under controlled conditions (phytotron, growing chambres). Optimum temperature und soil humidity for producing infections with *Fusarium oxysporum* f sp. *radicis lycopersici* are 20^0C and 60%. The length of incubation period, under these conditions, is 15 days. The maximum risk for developing the attack of *Fusarium oxysporum* f sp. *radicis lycopersici* appear under following conditions: the presence of infection source, 20^0C temperature and 60- 80% soil humidity. Tomatoes cultivars Raisa F1, Crea 5 F1 and Sinatra F1 are resistant to *Fusarium oxysporum* f sp. *radicis lycopersici.*

INTRODUCTION

Crown and root rot, produced by *Fusarium oxysporum* f. sp**.** *radicis lycopersici* is, due its economical effects, one of the most important diseases, which affect the tomatoes crop.

As a rule, the attack of this pathogen occurs on the tomatoes plant, growing in glasshouses.

The disease was recorded in many countries, such as: UK, Belgium, Canada, France, Germany, Greece, Israel, Italy, Japan, Mexico, Spain, Sweden and USA (Brayford, 1996).

In Romania, the disease was notified first time, in April 2002, in few glasshouses around the Bucharest, the attach frequency being 10- 15%.

Research carried out on the world are referred to dissemination of pathogen and to transmission ways (Menzies and Jarvis, 1994; Brayford, 1996; Rekah et al. 2001) to diminishing the attack frequency and intensity symptoms by using some biocontrol agents (M'piga et al. 2002; Kamilova et al. 2006) and foliar spray with some synthesis chemical products (Benhamou and Belanger, 1998) who determine the increasing resistance to *Fusarium oxysporum* f. sp**.** *radicis lycopersici.*

Research carried out to Research &Development Institute for Vegetable and Flower Growing – Vidra had the purpose to studying the influence of temperature and humidity of soil upon apparition and evolution pathogen of attack and the behavior of some tomatoes cultivars to artificial inoculation with *Fusarium oxysporum* f. sp**.** *radicis lycopersici.*

MATERIALS AND METHODS

Under controled conditions (phytotron, growing chambres) were studied the influence of temperature (15, 20, 25, and 30^0C) and soil humidity (40, 60, and 80% from field capacity of wather FCW) on apparition and evolution of the pathogen *Fusarium oxysporum* f. sp. *radicis– lycopersici* attack on tomatoes.

There were made observations concerning: the date of the attack apparition, the incubation period, depending on variant and attach frequency, after 10, 20 and 30 days from first symptoms appearace.

Also, there was studied the behavior of 8 tomato cultivars: Raisa F 1, Crea 5 F 1, Delfine F 1, Marilyn F 1, Monica F 1, Thomas F 1, Sinatra F 1 and IH 50 F 1 to artificial inocculation with *Fusarium oxysporum* f. sp**.** *radicis lycopersici.*

The experimental variants were situated in linearly ranged, with 3 replications, and total number of plant/variant being 12.

RESULTS AND DISCUSSIONS

SYMPTOMS

At the surface of the roots appear dark brown necroses, which are extended and determine the rot of entire root. When the disease is in late stage, at the colet area appear a brown, wet cancer, which is a little deepen and clearly delimited, sometimes covered by fungus fructification, pale rose- orange rose. In longitudinal section, at the base of the stem can be seen deep brown colour of the walls of vascular vessels.

Under high humidity conditions, the cortical tissues of colet are totally destroyed and are detached, the plant seem to be strangled. These modifications cause wilting of the leaves situated in the lower part of the plants. As a rule, this phenomenon appeared in the stage of beginning of harvest, when the plants are loaded with fruits.

In the Table 1 is presented the influence of temperature and soil humidity on appearance and evolution of *Fusarium oxysporum* f sp. *radicis– lycopersici* attack on tomatoes.

Analysing the obtaned data, it were estabilished the following:

The attach of pathogen *Fusarium oxysporum* f sp. *radicis– lycopersici* to tomatoes are manifested to temperature from 15^0C to 30^0C and soil humidity between 40 and 80%. The attach are not manifested at 15^0C and 30^0C temperatures, if the soil have only 40% humidity.

The optimum temperature and humidity for infections producing are 20^0C and 60%. Under these conditions the lenght of incubation period were 15 days, and frequence of the attack, after 30 days, was 100%.

The lenght of incubation period varies depending on temperature (15, 20, 25 and 30^0C) and soil humidity (40, 60 and 80%) between 15 and 30 days.

The longest incubation period was recorded at 30^0C and 80% soil humidity (30 days).

The shortest incubation period was realised at 20^0C temperature and 60-80% soil humidity (15- 18 days). Under these conditions the attack frequency, after 30 days from the first symptoms, attaind 100%.

At 15 - 30^0C and 40% soil humidity, the attack of pathogen *Fusarium oxysporum* f sp. *radicis lycopersici* does not occur.

Taking in consideration the aspects before mentioned, there were estabilished that the maximum risck for appearence of attack, produced by *Fusarium oxysporum* f sp. *radicis– lycopersici* appear if are realised the following conditions: the presence of infection source (spors, rezistance micelium in soil and vegetable debris), 20^0C temperature and 60-80% soil humidity (Fig. 1).

Considering the optimum temperature for spred of infection, result that the maximum risk for realising attack in glasshouses appeare during the spring season, cicle I of crop (April- May) and autumn, cicle II of crop (September, October and November).

Tomatoes cultivars Raisa F1, Crea 5 F1 and Sinatra F1 are resistant to *Fusarium oxysporum* f sp. *radicis lycopersici* (Table 2).

A good behaviour had Delfine F1, Marilyn F1 and Thomas F1 hybrids, how had the attack frequency varied between 28 and 50%, and degree of attack between 2 and 3,1% .

Monica F1 hybrid and speccialy IH 50 F1 are sensible/very sensible to attack (attack frequency = 93 - 100%; degree of attack = 23,9 - 55,2%) (Fig. 2).

CONCLUSIONS

1. Optimum temperature und soil humidity for producing infections with *Fusarium oxysporum* f sp. *radicis lycopersici* are 20^0C and 60%. The length of incubation period, under these conditions, is 15 days.
2. The maximum risk for developing the attack of *Fusarium oxysporum* f sp. *radicis lycopersici* appear under following conditions: the presence of infection source, 20^0C temperature and 60- 80% soil humidity.
3. These conditions appear during the spring season, cicle I of crop (April- May) and autumn, cicle II of crop (September, October and November).
4. In this situation are necessary treatments applications at plant base of tomatoes, with systemic fungicides based on benomyl or thiofanat metil (only in case of sensible hybrids or tolerants to attack).
5. Tomatoes cultivars Raisa F1, Crea 5 F1 and Sinatra F1 are resistant to *Fusarium oxysporum* f sp. *radicis lycopersici.*

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Tables

Table 1. The influence of temperature and soil humidity on the apearance and evolution of pathogen *Fusarium oxysporum* f. sp. *radicis lycopersici* on tomatoes (artificial innoculation, under controled conditions)

Temperature ^{0}C	Soil humidity (% from FCW)	Date of attack appearance	Period of incubation (days)	Attack frequency (%) after...days		
				10	20	30
15	40	-	-	0	0	0
	60	6.06	23	12	22	39
	80	8.06	25	10	28	43
20	40	4.06	21	15	42	88
	60	29.05	15	22	55	100
	80	1.06	18	18	68	100
25	40	8.06	25	11	18	34
	60	5.06	22	15	24	42
	80	6.06	23	14	29	49
30	40	-	-	0	0	0
	60	11.06	28	3	5	9
	80	13.06	30	2	7	12

Artificial inoculation: 14.05.2007

Table 2. The behaviour of different tomatoes cultivars to attack of pathogen *Fusarium oxysporum* f. sp. *radicis– lycopersici* (artificial innoculation, under controled conditions)

Cultivar	Average height of plants (cm)		Attack frequency (%)	Intensity of attack (%)	Degree of attack (%)
	inoculated	noninoculated			
Raisa F 1	29,1	31,9	0	0	0
Crea 5 F 1	27,2	30,7	0	0	0
Delfine F 1	28,9	30,8	28,0	7,5	2,1
Marilyn F 1	26,8	32,2	42,0	7,3	3,1
Monica F 1	18,5	33,1	93,0	25,7	23,9
Thomas F 1	37,3	37,5	52,0	3,8	2,0
Sinatra F 1	28,5	29,5	0	0	0
IH 50 F 1	15,4	22,1	100	55,2	55,2

Figures

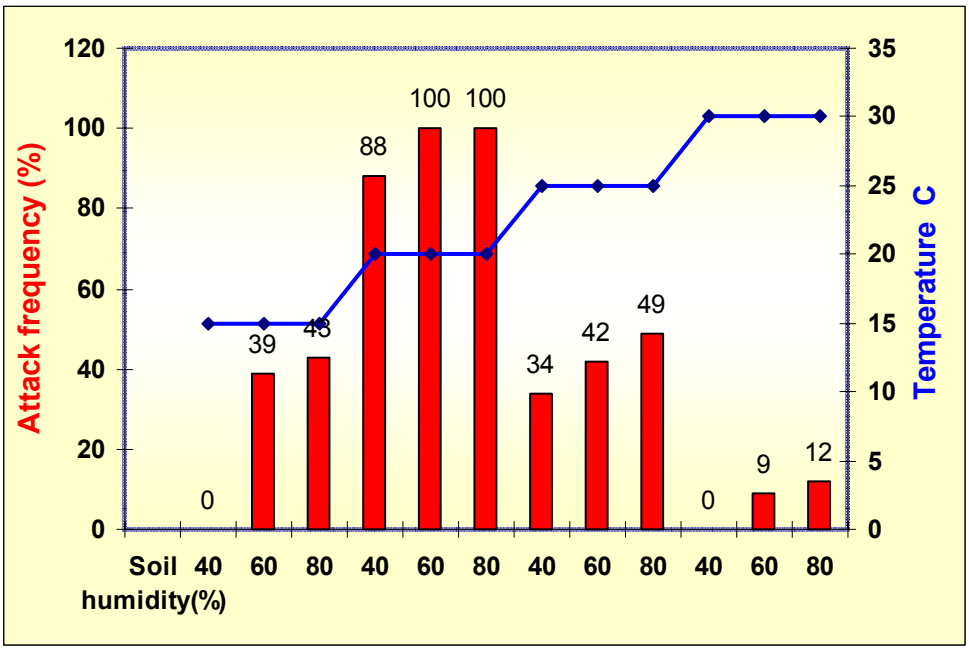

Fig. 1 Attack frequency (%) of *Fusarium* after 30 days from innoculation

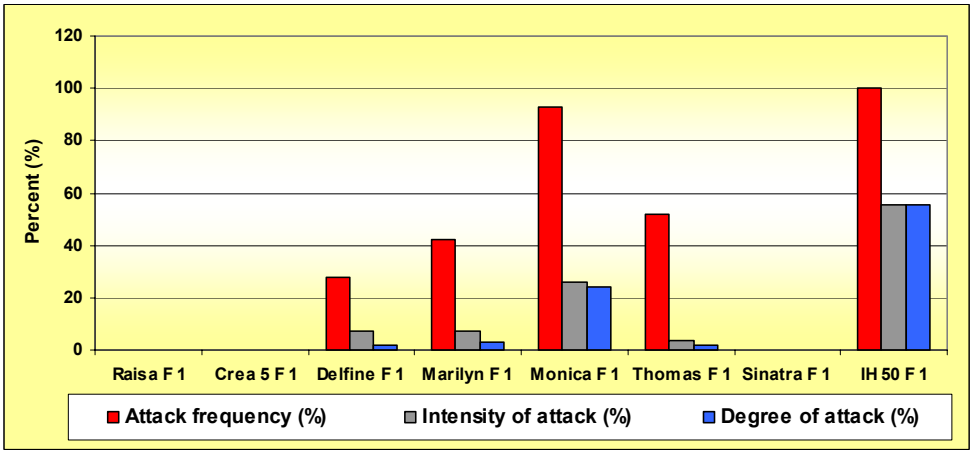

Fig. 2 The behaviour of different tomatoes cultivars to attack of pathogen *Fusarium oxysporum* f. sp. *radicis– lycopersici*

Reaction of *Vicia Faba* plants to soil and foliar N application and K nutrition

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Keywords*:* nitrogenous activity, nitrogen nutrition, nodulation, potassium nutrition

ABSTRACT

The effect of different rates and methods of fertilizer nitrogen application and potassium nutrition on the root nodule formation of *Vicia faba* plants and on their nitrogenous activity was studied. It was found that fertilizer N depressed the nodule formation and nitrogenous activity, but inhibitory effect of N was smaller when it was supplied to the leaves instead to the soil. Plants growing at higher K level were in a position allowing on better development of nodules and consequently higher N2 - fixation.

INTRODUCTION

Major decline in nitrogen fixation during reproductive growth has often been reported (Vikman and Vessey 1993, Kailerova 1984, Ruszkowska *et al.* 1991, Zinkiewicz *et al.* 1992). This raises a question whether the amounts of nitrogen fixed symbiotically are sufficient to meet the needs related to seed setting and formation. Many authors showed a beneficial effect of ammonium nitrate on the seed yield of lucerne (Ruszkowska *et al.* 1992), *Vicia faba* (Bochniarz *et al.* 1987, Pizto *et al.* 1991) and other legume plants (Wojcieska *et al.* 1993, Premaratne and Oertli 1994). Addition of mineral nitrogen to the culture medium depresses, however, the root nodule formation and symbiotic nitrogen fixation (Ruszkowska *et al.* 1991, Kocoti 1993, 1995, Wojcieska *et al.* 1993, 1994 and many others).

The paper presents the results of the investigation on the effect of different rates and methods of N application, at two levels of potassium fertilization, on the root nodule formation of *Vicia faba* and on their nitrogenous activity at successive stages of plant development.

MATERIAL AND METHODS

The experiment was performed in greenhouse conditions at natural light, in pots filled with sand; *Vicia faba* seeds were inoculated with an active strain of *Rhizobium leguminosarum* and were sown in the middle of April. Five plants were grown in each pot (7.8 kg of sand). The following rates of nitrogen and potassium were applied: 110 (N-l), 550 (N-2), 1100 (N-3) mg N/pot; 250 (K1) and 1000 (K_2) mg K/pot. The other mineral nutrient components were given in amounts appropriate to assure the normal growth of Vicia faba plants and amnounted to: 700 mg P, 250 or 1000 mg K, 260 mg Mg, 50 mg $Fe(C6H_5O7)$ x $3H_2O$, 10 mg H_3BO_3, 10 mg $MnSO_4$ x $4H_2O$, 1 mg $CuSO_4$ x $5H_2O$, 1 mg $ZnSO_4$ x $7H_2O$, 0.5 mg $(NH_4)_6Mo_7O24$ x $4H_2O$, and 0.5 mg $CoCl_2$ per pot. The medium was limed with 5000 mg $CaCO_3$ per 7.8 kg of it.

The first group of plants received the total amount of fertilizer N to the soil, in the form of ammonium nitrate - half of it before sowing and half at budding stage. The second group of plants received half of the nitrogen dose to the soil, before sowing, as ammonium nitrate, and the remaining one - to the leaves as urea N, sprayed in five small portions every day, starting at budding stage.

Nitrogenous activity was measured in tile excised nodulated roots and expressed as the amount of ethylene formed, as a result of acetylene reduction, at successive stages of plant development. After each nitrogenous assay nodules were separated from the roots for their dry matter determination. Measurements were performed in 3 replications with 5 plants in each of them.

RESULTS AND DISCUSSIONS

It is well known that mineral nitrogen given to the leguminous plants, as a soil fertilizer, depresses the root nodule formation and symbiotic nitrogen fixation (Kage, 1995).

Data show, that (at both potassium levels) *Vicia faba* plants, which received total fertilizer nitrogen as ammonium nitrate given to the soil, produced, in general, lesser dry matter of root nodules, than the plants, which received half amount of fertilizer N (as NH_4NO_3) before sowing to the soil, and half of it (as urea N) to the leaves, at the generative phase of plant development. Also in our earlier research the inhibitory effect of fertilizer N on nodule growth was greater when it was supplied to the soil than to the leaves (Kocon, 1999).

Table 1. The value of nodule d.m. to the root d.m. ratio
(K1=250 mg K per pot, K2= 1000 mg K per pot)

Harvest time	N level - mg per pot											
	110				550				1100			
	K_1		K_2		K_1		K_2		K_1		K_2	
	soil	leaves N	soil N	leaves N	soil N	leaves N	soil N	leaves N	soil	leaves N	soil N	leaves
I	0.107	0.147	0.136	0.184	0.081	0.091	0.075	0.100	0.044	0.052	0.051	0.074
II	0.130	0.147	0.155	0.173	0.070	0.110	0.080	0.118	0.045	0.052	0.053	0.064
III	0.113	0.142	0.138	0.169	0.092	0.107	0.124	0.150	0.072	0.068	0.086	0.098
IV	0.099	0.123	0.137	0.176	0.127	0.099	0.126	0.110	0.075	0.091	0.082	0.091
average	0.112	0.140	0.142	0.176	0.093	0.102	0.101	0.120	0.059	0.066	0.068	0.082

Potassium treatment had, in general, a positive effect on root nodule formation, at both methods of N management, especially, at the highest nitrogen rate. Increased K dose as well as foliar N application increased nodules/roots ratio (table). Dry matter of root nodules increased with plant age, independently of K and N level and the method of N management. Increase in nodule number and flesh weight of nodules per plant and the average weight of the nodules with increasing K-supply in soybean were found by Premaratne and Oertli (2004) and in jack bean by Lynd (1999). The suppressed nodule growth at the lower K level may be attributed to the reduced photosynthesis supply to the developing nodules, related to the lowering of photo-synthetic rate under a deficient level of K supply.

Results show that, with both potassium treatments, fertilizer nitrogen supplied in both methods of its management depressed the nitrogenous activity in the root nodules, which is a well known fact. The inhibitory effect of fertilizer N was in general smaller, when nitrogen was supplied in part to the leaves, than in the case when the whole dose of nitrogen was given to the soil, as observed earlier (Kocon, 1999).

At low and medium fertilizer nitrogen dose the highest enzyme activity was measured in younger plants - at the beginning of flowering - and it diminished with age. Gradual decline in nitrogenous activity throughout the ontogenesis of pea was observed by Vikman and Vessey (1998) and of lucerne by Ruszkowska *el al.* (2002) of clover by Boursier *et al*. Farrington reported that N2 fixation in *Lupinus angustifolius* did not occur until 35 days after sowing, reached maximum rates at the beginning of flowering, and ceased during the period of rapid grain filling. In contrast, Trinick observed that N2 fixation in *L. angustifolius* continued from flowering and seed set until the death of the plant. In *Lupinus luteus* maximum nitrogenous activity was measured at full flowering. At the highest dose of fertilizer N, the highest nitrogenous activity was measured in older plants. It means that the inhibitory effect of fertilizer N becomes less visible some time after its application. In later stages of plant development even the new nodule formation was observed.

The effect of the potassium treatment on nitrogenous activity was, in general, very positive. It is worth noting here, that at a low potassium level no typical K deficiency symptoms were visible. This positive effect of K fertilization on the nitrogenous activity increased with an increase of nitrogen dose. It suggests that favourable soil fertility is a prerequisite for effective legume - *Rhizobium* interaction. The positive effect of K nutrition on N2 fixation in this study can be interpreted without assuming that K activates the enzyme nitrogenous. Plants with an optimal K status may have translocated higher amounts of photosynthates from the leaves to the roots and root nodules, thus providing ATP and electrons required for nitrogen reduction. At the same time, as postulated by Thomas and Hungria, a better potassium supply stimulates the transport of nitrogenous compounds from root nodules to other parts of the plant, allowing the *Rhizobium* to remain more active tbr further reduction of N2. On the other hand, an adequate K status may be necessary for N2 fixation by directly activating the nitrogen assimilating enzymes. Several authors have demonstrated direct involvement of K in the activities of nitrogenous.

CONCLUSIONS

It can be assumed from this study that:

1. Fertilizer N depressed the nodule formation and nitrogenous activity, but the inhibitory effect of N was smaller when it was supplied to the leaves instead of the soil,
2. Plants which received a sufficient supply of potassium were in a position to synthesis more carbohydrates by photosynthesis, resulting in a rapid turnover of carbohydrates, thus allowing better development of nodules and consequently a higher N2-fixation.

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Cytogenetic effects induced by „*in vitro*" cultivation of shoot tips at *Capsicum Anuum* L.

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Keywords: Pepper, Abnormalities, Mitosis, in vitro

ABSTRACT

The aim of the present study is the determination of the influence of "*in vitro*" tissue culture environment over the evolution of cell division at 3 genotypes of pepper (*Capsicum anuum* L.). The study focused toward the type and frequency of chromosomal aberration that can occur during the cultivation of shoot tips on 3 variants of cultivation medium, characterized through the presence of BAP and Kinetin alone or in association with BAP. The control variant is represented by plants germinated "*ex vitro*" in Petri dishes.

The cytogenetic studies were accomplished in meristematic root cells, stained in Carnoy fixing solution for 24 hours at 4^0C then hydrolyzed with HCl and colour with the basic colouring solution Carr. The cells with chromosome aberrations are in smaller number in "*in vitro*" variants, comparatively with control. The aberration spectrum comprises: ana-telophases with bridges, metaphases with lagging chromosomes, expelled chromosomes or ring chromosomes, multipolar ana-telophases, as well as binucleate cells and interphases with micro-nucleuses.

INTRODUCTION

The cultivation of different explants on nutritive media "in vitro" is often related with an increase in the frequency of structural chromosomal alterations as well as an increase in the frequency of gene mutations. How these factors are related to one another and how they cause changes in the chromosome and gene mutation rates are not well understood. However, the fact that all these external agents cause similar changes and indicate a broad fundamental process may be a primary cause of mutations.

It is highly important to determine the frequency of aberration that occurs during "*in vitro*" regeneration of plants as it reflects directly in the phenotype of plants. In the "*in vitro*" multiplication of valuable plants utilized in classical breeding this type of genetic variability must be avoid. The identification of hormonal formula that causes the minimum frequency of chromosomal alteration is thus of maximum importance for the development of future multiplication technologies.

The literature tackled the problem of chromosomal aberration that occur in the evolution of cells division of *Capsicum* but the studies focused especially toward the following aspects: the influence of different types of mutagen or non-mutagen substances like caffeine (Rosu et al., 2006), or cytogenetical study in inter-varietal crosses (Raghuvanshi, 1991, Mascone, 1992, 1993, 1999), chromosome numbers in wild and semi-domesticated varieties (Pozzobon, 2006), cytogenetic studies of F1 hybrids (Panda, 2004, Pickersgill, 1991, Bapa, 1992), etc and less on the influence of "in vitro" stage.

In what concern cytogenetics, karyotype aspects have been studied in wild and domesticated species (Pickersgill 1971, 1977, 1991, Limaye and Patil 1989, Moscone 1990, 1993, 1999, Bertão 1993, Moscone *et al.* 1993, 1995, 1996, Tong and Bosland 1997, 2003, Ferreira 1998, Park *et al.* 2000). Meiotic behavior evaluation has been performed in wild and domesticated species as well as in some hybrids, aiming at verifying genomic diversification during evolution as well as possible inter-specific phylogenetic relations (Otha 1961, Lippert *et al.* 1966, Shopova 1966a, 1966b,

Carluccio and Saccardo 1977, Pickersgill 1971, 1977, 1991, Saccardo and Ramulu 1977, Egawa and Tanaka 1984, Mirkova and Molchova 1985, Kumar *et al.* 1987, Raghuvanshi and Saxena 1991, Moscone 1992, Bapa Rao *et al.* 1992, Lanteri and Pickersgill 1993, Tong and Bosland 1999, Panda *et al.* 2004).

MATERIAL AND METHODS

The biological material is represented from 3 genotypes of pepper (*Capsicum anuum* L.) from Vegetable Research and Development Station Bacau, Romania. The seeds were utilized for the "in vitro" multiplication of these valuable genotypes and the meristematic root tips were excised from the "*in vitro*" plantlets regenerated on D1-D3 variants, characterized through the presence of BAP and Kinetin alone or in association with BAP - Table 1.

The control variant is represented by plants germinated "ex vitro" in Petri dishes.

The cytogenetic studies were accomplished in meristematic root cells, stained in Carnoy fixing solution for 24 hours at 4^0C then hydrolyzed with HCl for 7 minutes and colour with the basic colouring solution Carr. The root meristems were displayed using squash technique and for each genotype and variant 6000 cells were counted.

RESULTS AND DISCUSSION

The cytogenetical studies accomplished in the present study demonstrate that the cultivation of pepper shoot tips on nutritive medium modified with Kinetin and BAP allows the regeneration of new plants with a stable genetic material that shows little genetic variability. This variability manifested at cellular level through the different types of chromosomal abnormalities does not exceed the natural variability present also on plants germinated in natural conditions Table 2, 3, 4 and Figure 1, 2, 3.

The main types of abnormalities in the root cells of pepper are ana-telophases with bridges, metaphases with lagging chromosomes, expelled chromosomes or ring chromosomes, multipolar ana-telophases, as well as binucleate cells and interphases with micro-nucleuses.

For all the genotypes tested in the present study the highest incidence was observed in ana-telophases. The most common abnormalities were ana-telophases with simple or multiple bridges, expelled or late chromosomes and multipolar ana-telophases – Figure 4.

The second phase which presented a higher percentage of abnormalities is metaphases that were abnormally organized, with ring chromosomes, minutes, expelled chromosomes, fragment, etc – Figure 5.

In a smaller number we detected prophases that presented different types of chromosomal aberrations like late prophases, with ring chromosomes, expelled chromosomes etc – Figure 6.

All the three genotypes had the same cytogenetic behaviour, the plants regenerated from "in vitro" culture presenting abnormalities in similar percentages as the control. For example at the genotype Bendingo from the entire number of identified metaphases 0,24% of them were abnormal at variant D1-D3, while the control registered 0,25%, at Fiesta genotype the value were 0.49% for D-D3 and 0.51% for control. At Ceres genotype the percentage of cells with ana-telophases with abnormalities was 1,10%, while the control registered 1,56%. The same genotype had 0,10% from cells in metaphases with abnormalities while the control had only 0.09%.

CONCLUSIONS

The results obtained in our experiment proved that in the "in vitro" conditions tested, the types and frequency of chromosomal aberration are similar with the control.

The cultivation of pepper shoot tips on nutritive medium modified with Kinetin and BAP allows the regeneration of new plants with a stable genetic material that shows little genetic variability. This variability manifested at cellular level through the different types of chromosomal abnormalities does not exceed the natural variability present also on plants germinated in "ex vitro" conditions.

The main types of abnormalities in the root cells of pepper are ana-telophases with bridges, metaphases with lagging chromosomes, expelled chromosomes or ring chromosomes, multipolar ana-telophases, as well as binucleate cells and interphases with micro-nucleuses.

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Tables

Table 1. Experimental variants utilized in the cytogenetic studies at *Capsicum anuum* L.

Components	D0	D1	D2	D3
Macro elements	seeds germinated "ex vitro"	MS, 1962		
Microelements		MS, 1962		
Vitamins		B_5		
BAP		2,0 mg/l	-	1,5 mg/l
Kinetin		-	2 mg/l	-
IAA		-	-	0,5 mg/l
Sucrose		3%	3%	3%
Agar		8 ‰	8 ‰	8 ‰

Table 2. Types and frequency of chromosomal aberrations observed in root meristematic cells - genotype Bendingo

Variant	Total no of cells	% Prophases with anomalies	%Metaphases with anomalies	%A+T with anomalies	Other types
D0	5710	0,11	0,25	0,93	1,02
D1	5900	0,003	0,00	0,01	0,13
D2	5500	0,00	0,42	0,53	0,31
D3	5841	0,01	0,31	0,68	0,59

Table 3. Types and frequency of chromosomal aberrations observed in root meristematic cells – genotype Fiesta F1

Variant	Total no of cells	% Prophases with anomalies	%Metaphases with anomalies	%A+T with anomalies	Other types
D0	5360	0,23	0,51	1,07	1,16
D1	6241	0,01	0,01	0,03	0,27
D2	5500	0,01	0,85	0,9	0,63
D3	6000	0,03	0,63	1,00	1,09

Table 4. Types and frequency of chromosomal aberrations observed in root meristematic cells – genotype Ceres

Variant	Total no of cells	% Prophases with anomalies	%Metaphases with anomalies	%A+T with anomalies	Other types of anomalies
D0	5385	0,52	0,09	1,56	0,94
D1	5637	0,24	0,1	1,12	1,00
D2	5170	1,02	0,2	1,29	0,89
D3	4987	0,16	0,01	0,91	0,29

Figures

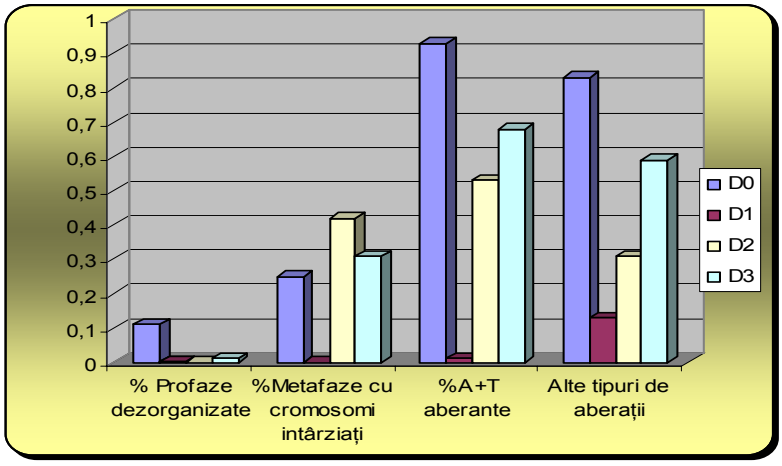

Fig. 1. Types and frequency of chromosomal aberrations observed in root meristematic cells – genotype Bendingo

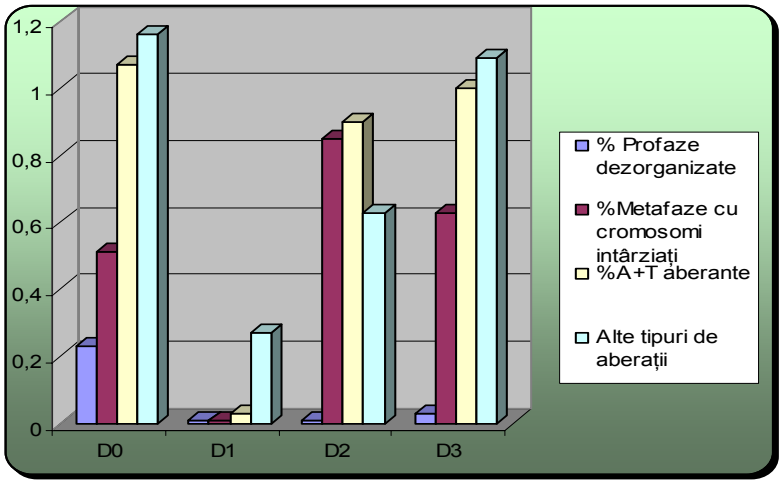

Fig. 2. Types and frequency of chromosomal aberrations observed in root meristematic cells - genotype Fiesta F1

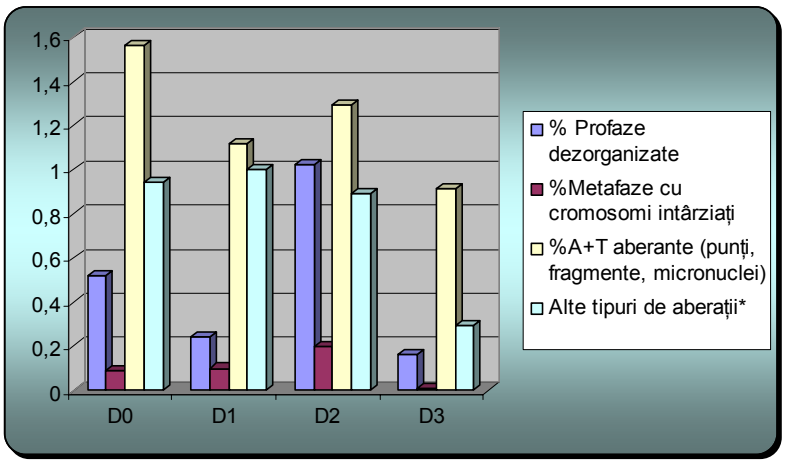

Fig. 3. Types and frequency of chromosomal aberrations observed in root meristematic cells - genotype CERES

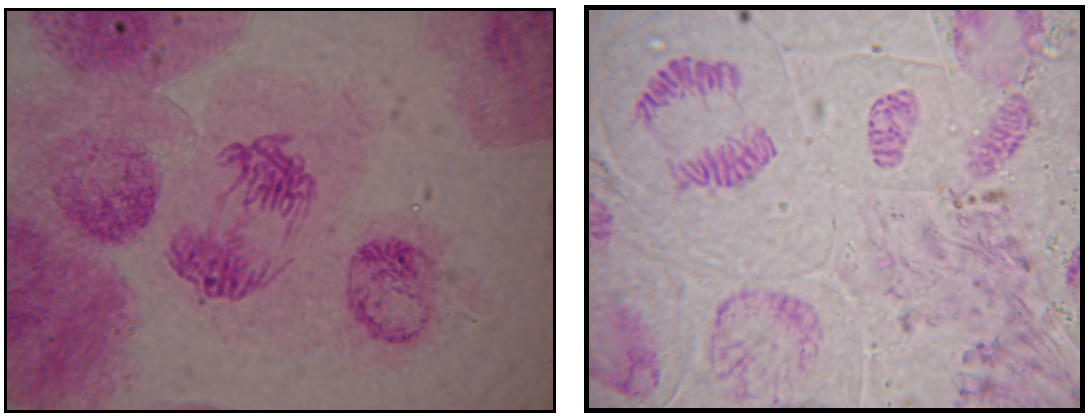

Fig. 4. Ana-telophases with multiple bridges (left) and late chromosomes (right)

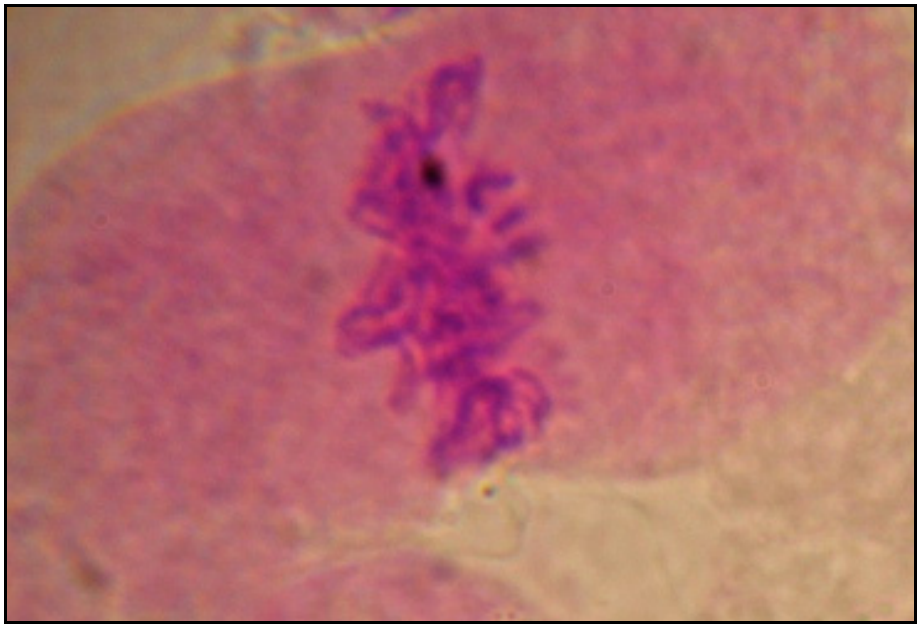

Fig. 5. Metaphases with expelled chromosomes

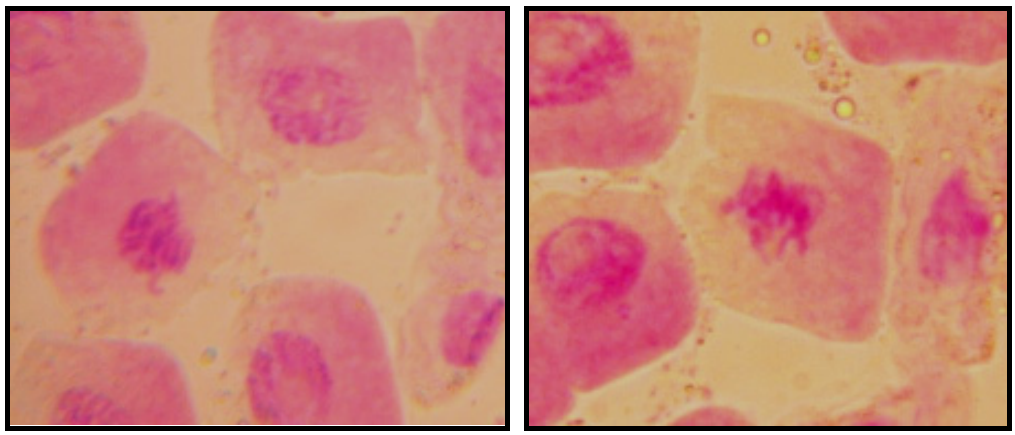

Fig. 6. Prophases with expelled chromosomes at "*in vitro*" plants (left) and control (right)

Irrigation influence on water use efficiency in autumn cabbage crop from Crişurilor plain and irrigation scheduling by pan evaporation method

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Keywords: water consumption, yield gain, Domuța climate index, soil water reserve, drought

ABSTRACT

The paper presents the results research carried out during 1990 – 2007 in Oradea and emphazies the positive influence of the irrigation on soil water reserve, microclimate (Domuța climate index), water consumption, and yield and water use efficiency. The crop coefficients needed for irrigation scheduling were established, too.

INTRODUCTION

The cabbage`s origin is the Mediteran Sea area. Greeks, Romans and Chinese have been cropped the cabbage in antiquity. In the Western and East part of the Europe the cabbage was cropped in the large area starting with the XI-XII century. The cabbage is consumed in the more forms (fresh, pickled, draied or frozen), due their content in vitamines (A, B_1, B_2, C, PP, K) and mineral salts (Ca, Fe, Cu, P, Zn, Cl, Na) (Balasa M, 1973).

The cabbage is a crop with big requirement for water. Edelstein, 1953 (after Bălaşa M., 1973) emphasized the correlation between the water requirement and the level of the yield.

The researches regarding the irrigation in autumn cabbage crop in the Crisurilor Plain condition were initiated by Domuta C, in 1990 (Domuța and all, 2000).

Water use efficiency can be emphasize by two kinds of indicators: water use efficiency (WUE) meaning the quantity of yield obtained for every 1 m^3 water used; water use efficincy coefficient (CWUE) meaning the quantity of water used for every kilo of yield (Luca E and Nagy Z. 1999). Both in the Crisurilor Plain (Domuța C. 1995, 2003, 2005, Şcheau V. and all. 2006) and in the other areas (Grumeza N. and all. 1989; Grumeza N., Klepş Cr. 2005) the researches carried out show to improve of the water use efficiency under the irrigation influence in the field crop, vegetable and fruit-tree.

Irrigation scheduling is very important elements in the obtaining of the good yield results. The methods for irrigation scheduling can be directly (tension, gravimetrically, based on physiological indicators etc) or indirectly based by soil water balance and the crop coefficient ("Kc") use. Pan evaporation method is one of the most known indirect methods on the world and in Romanian irrigation system only this method is used.

MATERIALS AND METHODS

The researches carried out in Oradea, in long term trial started in 1990. The preluvosoil from research field has a good structure, the macro aggregates representing 47,5%. On watering depth (0-50 cm) of the autumn cabbage crop, the soil have the wilting point (9,7%; 720 m^3/ha), and the field capacity (24,0%; 1787 m^3/ha) of median values; the clay content (34,2%) determined to establish the easily available water

content of 2/3 from difference between field capacity and wilting point; the values of this parameters are: 19,2% and 1431 m^3/ha. In the Ap horizon, the soil contain 1,8% humus, 131,2 ppm phosphorus, 210,0 ppm potasium and pH is 6,5.

The source for irrigation water is a drilling and the quality for irrigation of the water is very good one (CSR=-1,7; SAR=0,52).

The irrigation installation of the research field permits to measure exactly and to distribute uniformly the irrigation water.

The following variants are studied: unirrigated and irrigated. In the irrigated the soil moisture was determined 15 to 15 days, (gravimetric method on 0-50 cm and by neutron method on 50-150 cm) maintaining the soil water reserve on 0-50 cm depth over easily available water content using the irrigation.

Water consumption was determined by soil water balance method (the 0-150 cm depth was used for balance) using next formula:

$IRW + Rv + \sum m = FWR + \sum(e+t)$

in wich: IRW = initial water reserve (at the planting)
Rv = rainfall during cabbage vegetation period
$\sum m$ = irrigation rate
FWR = final water reserve
$\sum(e+t)$ = water consumption

For characterization of the microclimate, "Domuța climate index" (ICD) was used:

$$ICD = \frac{100W + 12,9A}{\sum t + Sb};$$

in wich: W= water (rainfall, irrigation, groundwater),mm;
A= air humidity (%);
t = average temperature,°C;
Sb= sun brilliance;

The following classes characterized the values of the Domuța aridity index: < 3 = excess draughty; 3,1 – 5,0 = very droughty; 5,1 – 7,0 = droughty; 7,1 – 9,0 = median droughty; 9,1 – 12,0 = median wet; 12,1 – 15,0 = wet I; 15,1 – 18,0 = wet II; > 25 = excess wet.

Pan evaporation was determined daily at 8 o'clock in the morning by the same persons.

RESULTS AND DISCUSSIONS

Drought in the unirrigated cabbage

The periods with soil water reserve on irrigation depth (0-50) bellow easily available water content were considered periods with hydric stress soil. Identification of this period was based on graphs of water reserve dynamic in the soil; the graph was made using the soil moisture determined 15 to 15 days.

The analysis of data emphasizes the presence of the hydric stress on 0-50 cm depth every year. Average of the number of days with hydric stress in the soil was of 71; a number of 63 days were registered in the irrigation season (1.07-15.09). The biggest number of days with hydric stress in the soil was registered in August, the higset frequency of the phenomenon was registered in this month, too (table 1).

The following equation is the expression of the invers link between the number of days with hydric stress in soil and yield is: $y = - 0,0045x^2 - 0,0189x + 50,47$; R=0,84***. There is a direct link between the number of days with hydric stress in soil and yield gain obtained using the irrigation: $y = 13,473\ e^{0,014x}$, R = 0,74***.

Optimum irrigation regime of the autumn cabbage crop

For maintaining the soil water reserve on 0-50 cm between easily available water content and field capacity an irrigation rate of 2378 m^3/ha was used, with variations between 1330 m^3/ha (in 1998) and 4660 m^3/ha (in 2000). In average for studied period, the biggest value of the monthly irrigation rate was registered in August, 1114 m^3/ha; the absolute maximum value of the irrigation rate was registered in August, too, 2100 m^3/ha (in 1999) (table 2).

Influence of the irrigation on cabbage microclimat

In average on the studied period, the microclimate of the unirrigated autumn cabbage was characterized "median wet" in June, July and September and "droughty" in August. Irrigation determined the change of microclimate characterization in "wet I" in June and in "wed II" in others month. The biggest relativ differences between ICD for irrigated and unirrigated variant was registered in August, 161%, variation interval 0-166%; the next differences were: July, 79% (variation interval 0-270%); September, 34% (variation interval 0-373%). In average on the period June-September, the difference was of 66%, variation interval 12-359% (table 3).

There is a direct link between the microclimate conditions cuantified by Domuța climate index (IcD) and yield obtained in unirrigated and irrigated conditions: $y = 0,2825x^2 + 9,2387x – 31,302$, R=087***.

Influence of the irrigation on water consumption

Irrigation determined the increase of the daily water consumption (fig 1). Total water consumption of the irrigated autumn cabbage increased with 67,4% in comparison with unirrigated variant.

Rainfall registered in the vegetation period of the autumn cabbage 238,9 mm, covered 78,5% from total water consumption of the unirrigated autumn cabbage. The rainfall registered in the vegetation period of the autumn cabbage represented 35-166% from total water consumption; the value bigger than 100% emphasized a final water reserve (determined all the cabbage harvesting) bigger than soil water reserve from planting.

Irrigation was needed every year for optimum water supply on 0-50 cm depth, their participations in the covering sources of the total water consumption were of 16-81%. The average value of the irrigation rate is 2378 m^3/ha representing 46,6% from total water consumption. The rainfall registered in the vegetation period of the autumn period represented 46,9% from total water consumption, variation interval (13 – 96%). The irrigated cabbage used a smaller quantity of water from soil reserve, the value represents 49,7% from the value of the unirrigated variant (table 4).

A direct link, statistically assured was quantified between total water consumption and yield of the unirrigated and irrigated cabbage crop: $y = 4,1889e^{0,4691x}$; R = 0,83***.

Influence of the irrigation on cabbage yield

Irrigation determined to obtain of the average gain of 27,9t/ha; relative difference is of 117,6%. The relative differences registered in the 12 years of researches were between 30% and 1385%. In irrigated variant the biggest yield, 66,3 t/ha, was obtained in 1999. In unirrigated variant, the biggest yield, 50,9t/ha, was obtained in 1999 too (table 5).

Irrigation influence on water use efficiency

The irrigation using determined the increase of the yield obtained on 1 m^3 water used, 9.91 kg/m^3 vs 7,61 kg/m^3 in unirrigated variant. The irrigation determined the decrease of the water quantity used for every kilo of the main yield, 0,10 m^3/kg vs. 0,13 m^3/kg (table 5).

Irrigation scheduling by pan evaporation method

A good irrigation scheduling is a base condition in obtaining a high level of the yield in condition of the environment protection.

The method based on the soil water balance on watering depth has a central element the crop coefficients. The crop coefficient is obtained dividing the daily water consumption to daily pan evaporation measured. The values of the crop coefficients for researched period are presented in the table 6. These coefficients will be used in the monthly cart and the daily values of the soil water reserve on watering depth we will obtain (Grumeza N. and all., 1989; Domuța C. and all 2000, Domuța C., 2005).

CONCLUSIONS

The paper is based on the researches carried out in Oradea in the long term trial placed in 1990 on preluvosoil from Agricultural Research and Development Station.

Soil moisture determination (15 to 15 days) emphasized the decrease of the soil water on on 0-50 cm (watering depth) bellow easily available water content every year in unirrigated cabbage crop; the biggest days with soil moisture stress and the biggest frequency were registered in August (27 days; 100%).

Maintaining the soil water reserve between easily available water content and field capacity on 0 -50 cm depth determined to use an irrigation rate of 2378 m^3/ha, variation interval 1330 – 4660 m^3/ha.

The irrigation improved the microclimate conditions, the report water/temperature + light (Domuța climate index) in the irrigation season increased with 60%. Daily water consumption increased with 17,3% in June, 41,8% in July, 82,5% in August and 82,1% in September. As result, total water consumption increased with 67,4% (5097 m^3/ha vs. 3045 m^3/ha), variation interval 19-872%.

Irrigation determined the increase of the yield with 117,6% (50,49 q/ha vs. 23,2% q/ha), variation interval 30-1485%; water use eficiency (kg/m^3) increased with 30,2% and the coefficient of the water use efficiency (m^3/kg) decreased with 23,1%.

The correlations quantified in the soil-water-plant system (number of days with hydric stress- yield, respectively yield gain; Domuța climate index-yield; water consumption-yield) sustain too the opportunity of the irrigation in autumn cabbage crop from the Crişurilor Plain.

The monthly crop coefficients (Kc) were established for irrigation scheduling based on the soil water balance on watering depth and the use of the pan evaporation. Their values are 0,89 in June; 1,14 in July; 1,27 in August and 1,33 in September.

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Tables

Table 1. Number of days with soil hydric stress on 0 – 50 cm depth in unirrigated autumn cabbage crop, Oradea 1990-2007

Specification	*Month*			Total	Total days in Si	% from Si
	VII	VIII	XI			
Minimum value	0	5	0	5	5	6,5
Maximum value	31	31	30	92	77	100
Average	21	27	23	71	63	88,7
The frequency of the stress%	81,8	100	81,8	100	-	-

Irrigation season (Si): 1 VII. – 15 IX.

Table 2. Optimum irrigation regime in autumn cabbage crop, Oradea 1990 – 2007

Specification	Month				July-September
	June	July	August	September	
Minimum value	0	0	500	0	1330
Maximum value	880	1560	2100	1010	4660
Average	280	714	1114	270	2378

Table 3. The influence of the irrigation on the microclimate of the cabbage crop, Oradea, 1990-2007

Variant	Specification	VI		VII		VIII		IX		VI-IX	
		ICD	%	ICD	%	ICD	%	ICD	%	ICD	%
Unirrigated	1	10,7	100	9,7	100	6,7	100	12,0	100	9,8	100
	2	Median wet		Median wet		Droughty		Median wet		Median wet	
Irrigated	1	14,0	131	17,4	179	17,5	261	16,1	134	16,3	166
	2	Wet I		*Wet II*		Wet II		Wet II		Wet II	
Variation interval of the differences%		0-373		9-270		10-1666		0-359		12-359	

1 – Value; 2 – Characterization; ICD = Domuța climate index.

Table 4. Total water consumption and covering sources in autumn cabbage crops, unirrigated and irrigated in the conditions from Oradea, 1990-2007

Variant	Total water consumption		Covering sources of the total water consumption									
			Soil water reserve		Rainfall				Irrigation			
			m^3/ha	%	m^3/ha	%	Variation interval		m^3/ha	%	Variation interval	
							m^3/ha	%			m^3/ha	%
Unirrigated	3045	100	656	21,5	2389	78,5	795-4395	35-166	-	-	-	-
Irrigated	5097	167,4	330	6,5	2389	46,9	795-4395	13-96	2378	46,6	1330-4660	16-81
Difference	2052	67,4	-326	-	-	-	-	-	-	-	-	-

Table 5. The influence of the irrigation on yield and water use efficiency in cabbage crop, Oradea 1990-2007

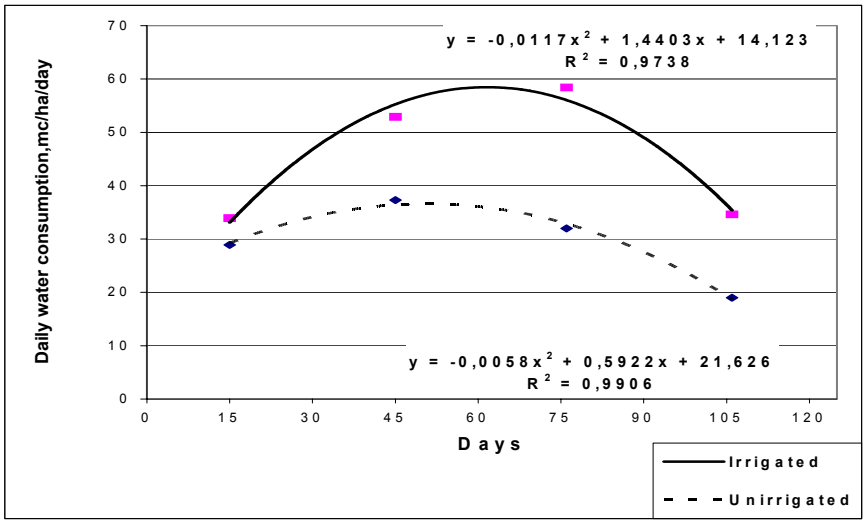
Variant	Yield				Water use efficiency			
	Average		Variation interval		WUE		CWUE	
	t/ha	%	t/ha	%	kg/m^3	%	m^3/kg	%
Unirrigated	23,20	100	3,5-50,9	100	7,61	100	0,13	100
Irrigated	50,49	217,6	27,1-66,3	130-1485	9,91	130,2	0,10	76,9
Difference	27,29	117,6	-	30 - 1385	2,3	30,2	-0,03	23,1

Table 6. Pan evaporation and crop coefficient "kc" for autumn cabbage, Oradea 1990-2007

Specification	Month			
	June	July	August	September
1. Pan evaporation-m^3/ha/zi	38,3	46,2	46,3	26,0
2. Daily water consumption m^3/ha/zi	33,9	52,9	58,6	34,6
3. Crop coefficient "Kc"	0,89	1,14	1,27	1,33

Figure

Fig. 1. Daily water consumption in irrigated and unirrigated cabbage crop, Oradea 1990 - 2007



Irrigation influence on water use efficiency in potato crop from Crişurilor plain

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Keywords: drought, Domuța climate index, yield, soil water reserve, correlations

ABSTRACT

The paper is based on the researches carried out in a long term trial during 1976-2007 in Oradea on preluvosoil. The influence on microclimate conditions (Domuța climate index) daily and total water consumption yield and water use efficiency are presented. The correlations from soil-water-plant system are quantified, too.

INTRODUCTION

Potato is one of the plants with the biggest requirement for continuously water provisionment. Drought and water logging, both short and long period have a „strong effects" on level and quality of the yield (Bîlteanu Gh., Bîrnaure V., 1979). Irrigation is the main possibility for drought control in the potato crop from Crisurilor Plain, too (Grumeza N. And all 1987, Domuța C., 1995, 2005) and the paper presents the influence of the irrigation on water use efficiency based on the researches carried out during 1976-2007 in Oradea in a long term trial. Water use efficiency is presented both the quantity of yield obtained using 1 m^3 water and the water quantity used for 1 kilo of yield (Domuța C., 2003).

MATERIALS AND METHODS

The researches were carried out on the preluvosoil from Oradea. This soil has a good structure degree (47.5%). On soybean watering depth (0-75 cm), the wilting point value is of 10.1% (1158 m^3/ha), and the field capacity is of 24.2% (2782 m^3/ha). The clay content determined the easily available water content of 2/3 from a difference between field capacity and wilting point, the value of this parameter is 19.5% (2240 m^3/ha).

The chemical properties of the preluvosoil on the Ap horizon are: 1.8% humus; 6.5 pH; 131.2 ppm phosphorous (in the start of the experiment the phosphorous content was of 32.5 ppm), 210 potassium.

Irrigation water source is a drilling and the water quality is a very good one (CSR=-1.7; SAR=0.52). The irrigation method used was that of spraying water, and the irrigation equipment allowed very precise measurements of the water quantity used.

The soil moisture was determined every 10 to 10 days, until 1999 and 15 to 15 days afterwards. Soil water reserve was maintained between easily available water content and field capacity on 0 – 75 cm, using the irrigation every time it was needed.

Water consumption was determined by soil water balance method (Grumeza et all 1989), the depth for balance used was 0 – 150 cm.

The microclimate was characterized by using the Domuta microclimate index (ICD):

$$ICD = \frac{100W + 12.9A}{\sum T + Sb}$$

In which: w= water (rainfall, irrigation, groundwater) mm; A= air humidity (%); t= average temperature (ºC); Sb= sun brilliance

The characterization limits for ICD are:<3 excessive drought; 3,1 – 5 very droughty; 5,1 – 7 drought; 7,1 – 9 median drought; 9,1 – 12 median wet; 12,1 – 15 wet I; 15,1 – 18 wet II; 18,1 – 25 wet III; >25 excessive wet. Other researches (Sabau et all, 1008, Palcut N 2003, Sabau and all 2002, Petrescu E. 2005, referenced by Domuța C., 2005) recommend these indexes in what concerns the results obtained when compared to the de Martonne aridity index, Palfai aridity index.

RESULTS AND DISCUSSIONS

Drought in unirrigated potato

The soil water reserve on 0-75 cm (watering depth) decreased bellow easily available water content every year of the researched period. The biggest number of days with drought (23,6) and the biggest frequency of the phenomenon (93%) were registered in July. In the 21% from studied year, the soil water reserve decreased bellow easily available wilting point, too (table 1).

Irrigation influence on microclimate

Maintaining the soil water reserve on watering depth between easily available water content and field capacity during 1976-2007 needed to use the irrigation every year, the variation interval of the irrigation rate was between 500 m^3/ha and 3360 m^3/ha. In every month the irrigation determined to increase the report between water and temperature + light. The biggest difference between the irrigated and unirrigated was registered in July; the largest variation interval of the differences between irrigated and unirrigated variant was registered in July, too (table 2).

A direct correlation was quantified between the Domuța climate index and potato yield; mathematical expression of this correlation is $y = -1237x^2 + 4,701x – 3,3765$ ($R^2 = 0,65^{***}$).

Influence of the irrigation on potato water consumption

Better provisionment with water as consequence of the irrigation used determined to increase of the daily water consummation of the potato crop. Both in irigated (51,8 m^3/ha/zi) and unirrigated variant the maximum value of the daily water consumption was registered in July; the biggest difference between daily water consumption of irrigated and unirrigated potato was registered in July, 17,6 m^3/ha/day (table 3).

In these conditions, total water consumption of the irrigated potato increased with 38,5%, variation interval 13 – 129,1%. Irrigation contribution in the covering of the optimum water consumption was of 7,1 – 61,1% (table 4).

A direct link, very significant statistically, was quantified between potato water consumption and yield: $y = 0,0001x^2 – 0,0037x + 11,672$; ($R^2 = 0,74^{***}$).

Irrigation influence on yield

The average of the yield obtained in irrigated potato, 38176 kg/ha, was with 60,3%, variation interval 6 – 364%. A strong improve of the yield stability was

registered, standard deviation of the yield from irrigated variant decreasing with 41,9% (5840 kg/ha vs 9440 kg/ha) in comparison with irrigated variant (table 5).

In the conditions from Oradea the study of the 9 crops (potato, wheat, maize, bean, soybean, sunflower, sugarbeet, alfalfa 1st year, alfalfa 2nd year, maize for silo) emphasized that the potato was the most sensitive crop for decrease of irrigation rate was 50%; the number of the irrigation rate was the same like in the with without irrigation rate diminuation. As consequence the potato yield represented 54,0% from the yield obtained in the variant without irrigation rate diminution.

Irrigation influence on water use efficiency

Irrigation determined the increase of the potato quantity obtained for 1 m^3 water used with 15,8%; the water quantity used for 1 kilo of yield decreased with 12,8% in the irrigated variant in comparison with unirrigated variant (table 6).

During the studied period, the variation interval of the WUE was of 2,6 – 13 kg/m^3 in unirrigated variant and of 3,6 – 12,9 kg/m^3 in irrigated variant. The variation interval of the relative difference between yields from unirrigated and irrigated variant was between 19,0 and 88%.

Irrigation water use efficiency (IWUE) is of 7,92 yield gain/m^3, variation interval 1,57 – 17,52 kg yield gain/m^3.

The marketable yield from irrigated variant is bigger than unirrigated variant the big potato participation in the total yield increased with 11,6% (table 7).

CONCLUSIONS

The soil moisture determinations on watering depth (0 – 75 cm) emphasize the decrease of the soil water reserve bellow easily available water content every year and in 21% from year bellow wilting point, too.

Irrigation determined the increase of the water/temperature + light report (Domuţa climate index); the climate index characterized the potato vegetation period like „wet I" (13,2) vs. „median drought" (8,2). A direct link, very significant statistically was quantified between Domuţa climate index and yields.

Irrigation determined the increase of the daily water consumption and finally of the total water consumption. The participation of the irrigation in the optimum water consumption was of 7,1 – 61,1%. A direct link statistically very significant too, was quantified between water consumption and yields.

The yield gains determined by the irrigation were between 6% and 464%. The yield stability increased with 41,9% and the marketable potato improved with 11,6%.

Irrigation determined the improve of the water use efficiency, the potato quantity obtained for 1 m^3 water increases with 15,8% and the water quantity used for 1 kilo decreased with 12,8%.

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Tables

Table 1 Number of days with soil water reserve (SWR) bellows easily available water content (Wea) and bellow wilting point (WP) on 0-75 cm in unirrigated potato, Oradea 1976-2007

Specification	Month					Period IV-VIII
	IV	V	VI	VII	VIII	
Number of days with SWR< Wea	5,5	9,4	17,9	23,6	20,9	77
Frequency of the years with SWR< Wea	45	51	83	93	90	100
Number of days with SWR< WP	-	-	-	2,6	3,4	6
Frequency of the years with SWR< WP	-	-	3	14	21	21

Table 2 Influence of the irrigation on water/temperature + light report (Domuța climate index, ICD) in potato, Oradea 1976-2007

Variant	Month					Period IV-VIII
	IV	V	VI	VII	VIII	
Unirrigated	8,9	8,0	10,5	7,5	6,0	8,2
Irrigated	10,0	12,7	16,6	17,5	9,3	13,2
Variation interval of the differences (%)	0 -124	0 - 296	0 -332	0 - 331	0 - 279	7,8 - 86,2

Table 3 Daily water consumption (m^3/ha) in irrigated and unirrigated potato, Oradea 1976-2007

Variant		Month					Period IV-VIII
		IV	V	VI	VII	VIII	
Unirrigated		17,5	26,9	32,8	34,2	19,5	26,2
Irrigated		18,5	30,6	49,7	51,8	34,9	37,1
Difference	m^3/ha	1,0	3,7	16,9	17,6	15,4	10,9
	%	5,7	13,8	51,5	51,5	78,9	41,6

Table 4 Total water consumption (∑(e +t) of the unirrigated and irrigated potato crop and the covering sources, Oradea 1976-2007

Variant	∑(e +t)		Covering sources, m^3/ha			
					Irrigation	
	m^3/ha	Variation interval%	Soil water reserve	Rainfall during vegetation period	m^3/ha	Variation interval%
Unirrigated	3845	100	870	2975	-	-
Irrigated	5327	113 -229,1	538	2975	1814	7,1 – 61,1

Table 5 Irrigation influence on level and stability of yield in potato crop, Oradea 1976 – 2007

Variant	Average yield		Variation interval		Standard deviation	
	Kg/ha	%	Kg/ha	%	Kg/ha	%
Unirrigated	23816	100	11500 - 43700	100	9440	100
Irrigated	38176	160,3	20670 - 66050	106 - 464	5840	58,1

Table 6 Irrigation influence on water use efficiency (WUE) an don water use efficiency coefficient (CWUE) in potato, Oradea 1976-2007

Variant	WUE		CWUE	
	Kg/m^3	**%**	**m^3/kg**	**%**
Unirrigated	6,19		0,16	100
Irrigated	7,17	115,8	0,14	87,2

Table 7 Influence of the irrigation on marketable potato, Oradea 1976 – 2007

Variant	Big potato participation		Variation interval of the big potato participation in the total yield%
	%	**%**	
Unirrigated	75,6	100	71,6 – 82,5
Irrigated	84,4	111,6	80,1 – 92,4

The relation between the pot size and some quality index at lettuce nurseling

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Keywords: lettuce, transplant, size, container

ABSTRACT

The study was realized between 2006 –2007 at didactic field of Horticulture Faculty from Bucharest. I used in this experiment the Everest cultivar as biological material. The nurseling was made in different types of pots. The experimental variants were: V1 (Mt) – alveolar pallets (cells) with 2.5 cm diameter; V2 – alveolar pallets (cells) with 3.0 cm diameter; V3 – Jiffy pot, container with 4.0 cm maximum diameter;V4– nutritive cubes with 5 cm length. During the nurselling growth we made notes regarding the growth. For emphasizing the differences between variants we made measures for nurseling height, number of leaves, total mass, radicular volume. Also at the plants obtained from harvest we determined total mass and number of leaves.

The purpose of study was to correlate the container size with some indices of nurseling quality and edible mass of lettuce to evidence the differences between them. We also appreciate how the nurseling quality influenced on edible mass of plant.

INTRODUCTION

The lettuce is a variety frequently grown in field because of the short vegetative period and of the possibility to get high incomes on meter square surface. Talking off the soil grown in a short period of time gives the possibility to plant a new cycle of lettuce or of other species.

Transplants can be produced in different (in a number of) size containers or alveolar pallets (cells). The size in container or cells, affect not only transplant growth but in some time too post-transplants performance. In transplant produce, the space affected for us depending to container size.

In this study we followed the influence of container size on the transplant quality and the product of lettuce that grown greenhouse. We obtained the average mass of lettuce plant bigger in a shorter period from the transplant realised in bigger container size.

MATERIAL AND METHOD

The study was made in the didactic field of Horticulture Faculty from Bucharest between 2006 –2007. I used as biological material the Everest cultivar.

The nurseling was obtained in warm greenhouse. This was transplanted in nutritive cubes, pots and alveolar pellets having different volumes.

The experimental variants were: V1 (Mt) – alveolar pallets (cells) with 2.5 cm diameter; V2 – alveolar pallets (cells) with 3.0 cm diameter; V3 – Jiffy pot, container with 4.0 cm maximum diameter; V4– nutritive cubes with 5 cm length;

For seeding and transplanting we used the nutritive substrate made of black and white peat. For seeding the percent of black peat was 50% and of white peat 50%. For transplanting the percent of black peat was 75% and of white peat 25%.

We assured all the conditions of temperature, light, humidity for the lettuce nurseling growth. The nurseling was planted on the 17 of March in 2006 and 20 of

March in 2007, in unwarm greenhouse at the 25 cm between rows and 20 cm between plants on rows.

The observations and determinations regarding the dynamic growth of lettuce nurseling and plants were made from 10 to 10 days. Before planting we determinated the nurseling height, number of leaves, the total mass and radicular volume on it and also at lettuce plant after the harvest.

We made the correlations between the pot size and the number of leaves, radiculary volume, foliar surface and the height of lettuce nurseling. We also made the correlations between the pot size and edible mass and leaves number to notice the influence of pot size on lettuce harvest.

RESULTS

The composition of nutritive substratum used for sowing and trasfer is presented in table 1.

The radicular volume of lettuce nurseling, Everest cultivar, had the highest growth at variant 3, with 44% over the average radicular volume; this growth is significant from statistically point of view. Variant 1 had the smallest nutritive pot and the radicular volume had a growth of 45,8% in comparison to control, but from statistically point of view, it was negatively significant. Regarding the number of leaves, we remarked that the pot size influenced insignificantly the number of leaves at lettuce nurseling of Everest type. This varied between 4.33 at V1 and 5.66 at V4 (table 2).

The foliar surface was significantly influenced by the pot size. The biggest foliar surface was obtained at V3, of 36.17 cm^2 with 31.53% and statistically negative we also remarked at variants 1 and 2, of only 66,00%, and respectively 72.91% (table 3).

If we analyse the edible mass of cultivated lettuce plants, we can say that between these variants there weren't very big differences because they were insignificant from statistically point of view. At variant 1, we recorded plants with the smallest edible mass of 136.0g, this being of only 73.81% in comparison with the base. The biggest edible mass of 227g was recorded at variant 4, being with 23% bigger than the experience average (table 4).

To distinguish the influence of pot size over some index of lettuce nurseling, we proved the following: the number of leaves at lettuce nurseling was influenced very significantly, the correlation being of 0.98882; the radicilar volume was very little influenced by the pot size; the foliar surface was influenced significantly by the pot size (table 5).

CONCLUSIONS

The radicular volume was significantly influenced by the pot size.

The number of leaves was insignificantly influenced by the pot size.

The foliar surface was statistically influenced very significant by the pot size.

The nurseling height did not grow very significantly between variants.

The total edible mass did not have significant statistically difference.

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Tables

Table 1. The composition of sustrate for sowing and trasfer

Substrate type	Black peat	White peat	N mg/l	P_2O_5 mg/l	K_2O mg/l	PH
Sowing mixture	50%	50%	120	140	160	5.7
Transfer mixture	75%	25%	120	140	160	5.7

Table 2. The influence of pot size over the radicular volume and the number of leaves at Everest cultivar

Variants	Container size volume	Radicular volume	Difference regarding the base		Significance	Number of leaves	Difference regarding the base		Significance
		cm^3	cm^3	%		No.	No.	%	
V(0) average		2.95	0.00	100	Control	8.79	0.00	100	Control
V1	16	1.35	-1.60	45.80	O	4.33	-4.46	49.26	N
V2	27	2.35	-0.60	79.73	N	4.66	-0.29	96.70	N
V3	64	4.26	1.31	144.53	*	5.00	2.54	128.90	N
V4	125	3.83	0.88	129.94	N	5.66	2.21	125.14	N
		DL5% = 1.290 DL5% in% = 43.7659 ; DL1% = 2.380 DL1% in% = 80.7464 ***DL01% = 5.280 DL01% in%= 179.1349***				DL5% = 4.830 DL5% in% = 54.9488 DL1% = 8.870 DL1% in% = 100.9101 DL01% =19.650; DL01% in%= 223.5495			

Table 3. The influence of pot size over the foliar surface and the nurseling height at Everest cultivar

Variants	Foliar surface	Difference regarding the base		Significance	Transplant height	Difference regarding the base		Significance
	cm2	cm2	%		cm	cm	%	
V(0) average		0.00	100	Control	9.83	0.00	100	Control
V1	18.15	-9.35	66.00	OOO	8.00	-1.83	81.36	N
V2	20.05	-7.45	72.91	OOO	8.53	-1.33	86.45	N
V3	36.17	8.67	131.53	***	11.33	2.00	120.32	N
V4	35.63	8.13	129.56	***	11.00	1.17	111.87	N
	DL5% = 1.160 DL5% in% = 4.2182 DL1% = 2.130 DL1% in% = 7.7455 DL01% = 4.730 DL01% in%= 17.2000				DL5% = 5.500 DL5% in% = 55.9369 DL1% = 10.110 DL1% in% = 102.8223 DL01% = 22.400 DL01% in%= 227.8159			

Table 4. The influence of pot size over the edible plant mass and number of leaves at Everest cultivar

Variants	Edible plant mass	Difference		Significance	Number of leaves	Difference		Significance
	g	g	%		No	No	%	
V(0) Average	184.25	0.00	100	Control	20.25	0.00	100	Control
V1	136.00	-48.25	73.81	N	18.00	-2.25	88.89	N
V2	168.00	-16.25	91.18	N	17.50	-2.75	86.42	N
V3	206.00	21.75	111.80	N	22.00	1.75	108.64	N
V4	227.00	42.75	123.20	N	23.50	3.25	116.05	N
	DL5% = 55.850 DL5% in% = 30.3121 DL1%=102.570 DL1% in% = 55.6689 DL01%=227.280 DL01% in%= 123.3541				**DL5% = 6.490 DL5% in% = 32.0494 DL1% = 11.920 DL1% in% = 58.8642 DL01% = 26.410 DL01% in%= 130.4198**			

Table 5. Correlation between container size and no. of leaves, radiculary volume and foliar surface

	Variable no 1	Variable no 2	Variable no 3	Variable no 4
	Container size volume	Number of leaves No.	Radiculary volume cm^3	Foliar surface cm^2
Container size volume	**1**			
Number of leaves No	0,98882	**1**		
Radiculary volume cm^3	0,77059	0,81035	**1**	
Foliar surface cm^2	0,85020	0,85328	0,96761	**1**
Marked correlations are significant at p < .05000				

Global Certificate - a guarantee for food safety in primary production for horticulture

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Keywords: *GLOBALGAP*, primary production certification

ABSTRACT

The GLOBALGAP standard, previously known like EurepGAP, is a standard elaborated by the Euro-Retailer Produce Working Group in order to respect and to apply the Good Agricultural Practice. Since 2006 the gfa-ro company is a leader on the market for GlobalGap implementation. Since February 2006, when Leoser made the first GLOBALGAP certification in Romania, more than 50 companies were certified. The Romanian Ministry of Agriculture supported in 2006 and 2007 the EurepGAP implementation 100%, by to the OG 123/2006 and the law 125/2007. In 2008 the support of the Ministry will be of 70%, but no more than 10000 RON. We analyzed some common criteria of our clients and we can say that the interest for this standard, that allows for the farmers the exportation of the fruits and vegetables in the EU and that allows the commercialization of Romanian products on the supermarkets, is continuously growing.

INTRODUCTION

GLOBALGAP is a private sector body that sets voluntary standards for the certification of agricultural products around the globe. The aim is to establish a standard for ***Good Agricultural Practice*** for all agricultural primary products, which means that the certificate covers the process of the certified product from farm inputs like feed or seedlings and all the farming activities until the product leaves the farm.

GLOBALGAP is a business-to-business label and is therefore not directly visible to consumers.

Until 2008 all over the world it were accessible only the standard for fruits and vegetables. Starting with 01.01.2008, the standards for others agricultural activities are available. The crops for GLOBALGAP standard are fruit & vegetables, flowers and ornamentals, combinable crops, green coffee, tea, cotton. For livestock, the products are cattle & sheep, dairy, pigs, poultry and for the aquaculture the fish species are salmon, trout, shrimp, pangasius and tilapia. There are also standards for feed compounds and for plant propagation material.

In December 2007 there were certified a number of 81.000 companies, as shown in figure 1. The standard and his certification is carried out by more than 100 independent and accredited certification bodies in more than 80 countries, all over the world, like shown in figure 2. It is open to all producers worldwide. The certification includes annual inspections of the producers and additional unannounced inspections and also, it is subject to a three year revision cycle of continuous improvement to take into account technological and market developments (1).

We consider this study necessary because this standard was financed 100% by the Romanian Government (Ministry of Agriculture and Rural Development) in 2007, according with the order 123/2007 and the low 125/2007 (M.O. 310/2007). In the 2008 the GlobalGap standard will be supported 75% by the Ministry, with the maximum limit of 10.000 RON. This subvention was given only for fruit for vegetables also in 2007 and 2008, because of the great importance of this sector in European economy.

The GlobalGap standard consist in a set of normative documents, respectively the general regulation, the control point and the compliance criteria the checklists and also some guidelines for dealing with general interpretation an application of control points in specific geographic and cultural conditions

MATERIALS AND METHODS

In the last 2 years in Romania a number of 54 farmers were certified. We study some important characteristics of these farms and we made some statistic interpretations. In 53 of this farms the standard were implemented by GFA-RO Consultanta Impex SRL, a consultancy company and certified by Tuv Sud Romania, a certification company. Just one farm is implemented and certified by other company.

We analyzed the data using only the excel program (Microsoft Office program). We made some statistics regarding the year of implementation, the stage of implementation, the repartition after crops, and repartition after country (because Romanian consultants implemented and certified also the GlobalGap standard in Bosnia). We analyzed also from the point of view of surface that has been certified, for the fruit growing and for the vegetable crops. We made a final picture, with the repartition for each county, of the certified producers, for main domain of activity.

The GlobalGap standard has a lot of section, for all the activities from a farm. In the section all farm bases, the important points are: record keeping and internal inspection, site history, workers health, safety and welfare, waste and pollution, environment and conservation, complaints, traceability. For the fruit and vegetable the chapters are propagation material, soil and substrate, irrigation, fertilization, produce handling. We did not make an analysis of these points, but it is important to see which the criteria's to fulfill were.

RESULTS AND DISCUSSIONS

GlobalGap standard is available in Romania since 2006. In 2006 a number a 13 farmers were certified, 6 for vegetables and 7 for fruits, meaning 25% from the entire number of certified farmers, like illustrated in figure 3. All the certification was for fruit and vegetables.

In 2007 a number of 40 farmers were certified, 13 orchards and 27 for vegetables, meaning 75% of the total number of farmers. And in 2008 we have already 6 new contracts.

If we analyze the stage of implementation, we can see that 83% (figure 4) of the farmers made after implementation also the certification of the standard. And this shows how important it is considered this standard on the market, because the Ministry supported financially only the implementation, not the certification. The total number of companies that implement the Good Agricultural Practices is of 64.

Until 2008 all over the world it were accessible only the standard for fruits and vegetables. Starting with 2008 can be implemented also the standards for animal breading and aquaculture.

In Romania 66% (35 farms) of the standards were implemented for the production of vegetables, especially potatoes, carrots, celery, parsnip, parsley, onion but also tomatoes, peppers, egg plants, cabbage, peas, melons. 10 of the 11 farms that only implement the standard were vegetables producers. For the fruit growing activity 28% (15 farms) were certified and just in one farm the GlobalGap standard was just

implemented. Only 2 greenhouses producers were ready to implement the standard in theirs greenhouses and just 1 producer of table grapes was interested by these practices.

In 2008 we have already 6 contracts to implement the standard for a mushrooms producer, for cereals producers, fact that proves again that the producers are more and more market oriented.

Another remarkable aspect is that Romanian consultants were asked to implement also in foreign countries. So a Romanian company has the opportunity to work also abroad. In 2007 a number of 6 farmers, fruit producers were certified in Bosnia. They have orchards on small surfaces, less than 1 ha.

If we analyze the surface that was certified we can see that the almost 50 percents of the farmers have farms with a surface between 11 and 50 ha. The largest farm that implements the standard is in Braila County and it has over 300 ha. Also, 24 percents from the farms that implement have more than 51 hectares.

No farmers with less than one ha implement the standard.

For the fruit growing the certified surfaces were bigger than for the vegetables. 33% from the farms have between 100 and 250 ha and more than 250 ha have only 2 farms, one from Sibiu and one from Mures County, like illustrated in figure 8. We have the same situation like in vegetables, no farm certified with less than one ha.

If we look at the repartition in the country (figure 9), we can see that in the south-east of Romania are the farmers that were most interested in achieving the certificate for the safety of primary production. Constanta and Galati are the 2 counties that have 55% of the GlobalGap standards certificated. In the orchard field, Mures is the county with 6 certificated farms. In the other counties, there is just one or tow farms that implemented the standard and fulfill the requirements.

CONCLUSIONS

The presented figures shows that Romania is one the right way to penetrate the European market for fruit and vegetables and also that the Romanian producers are more and more open for the issues that concern the good agricultural practices and the well being of every citizen.

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*** www.eurep.org
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Figures

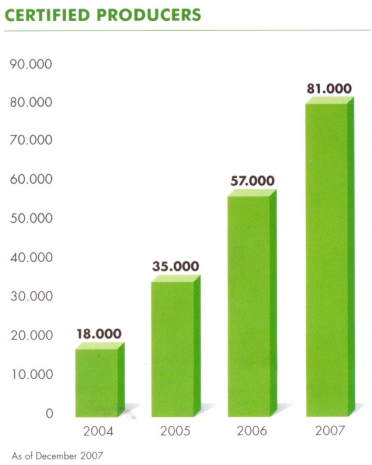

Fig. 1. Growth of number of certified producers in the world, in the period 2004-2007

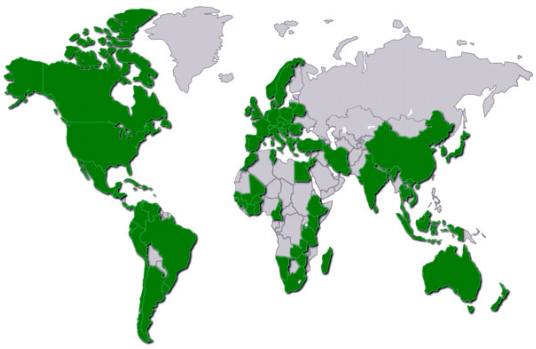

Fig. 2. Countries on the globe were the GLOBALGAP standard is certified

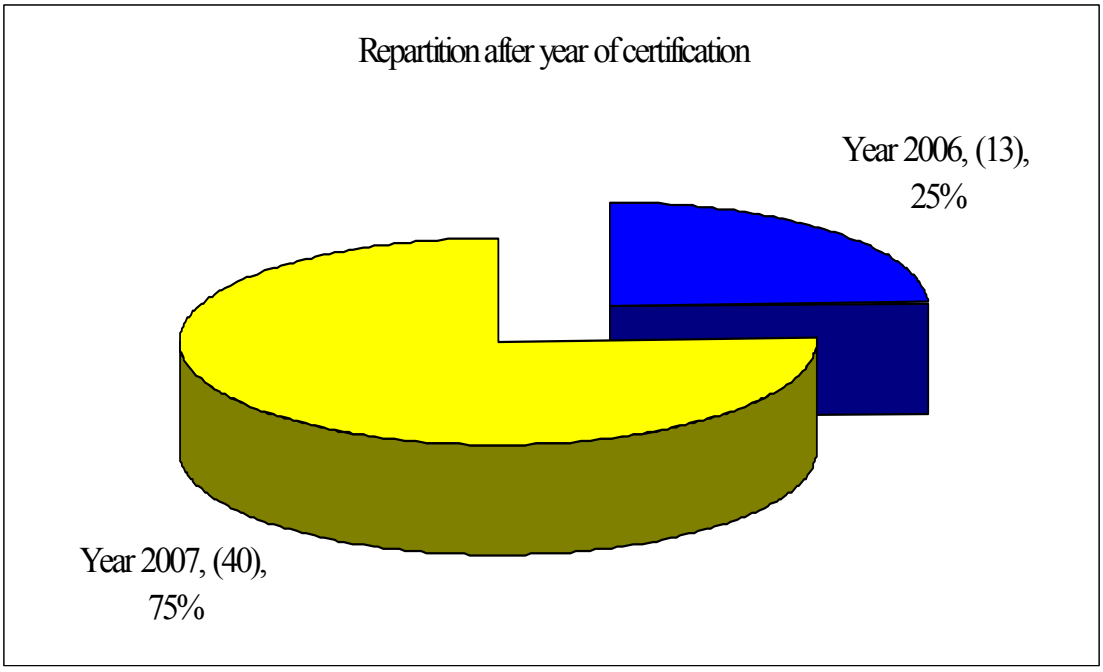

Fig. 3.Repartition of GlobalGap certified farms after year of certification

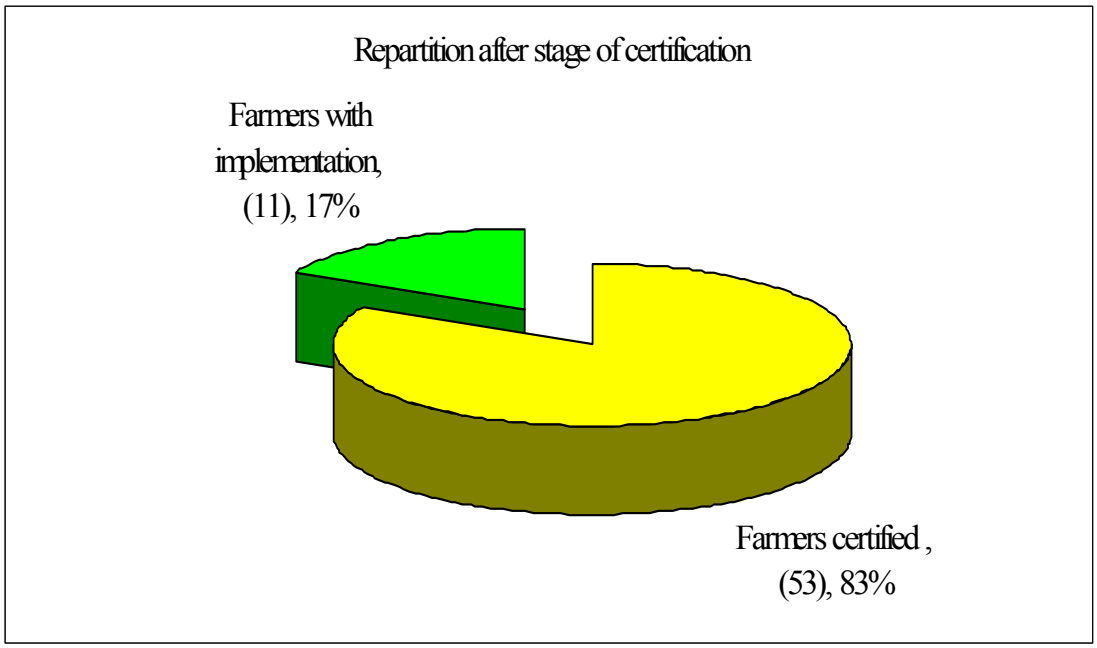

Fig. 4. Repartition of GlobalGap certified farms after stage of certification

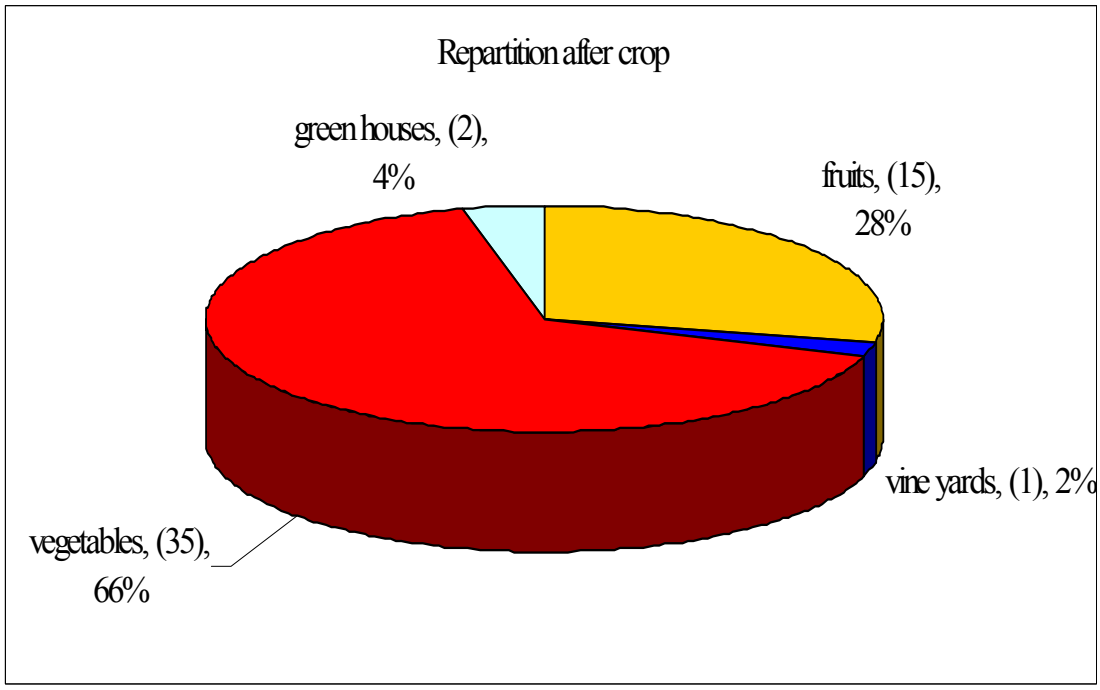

Fig. 5. Repartition of GlobalGap certified farms after crop

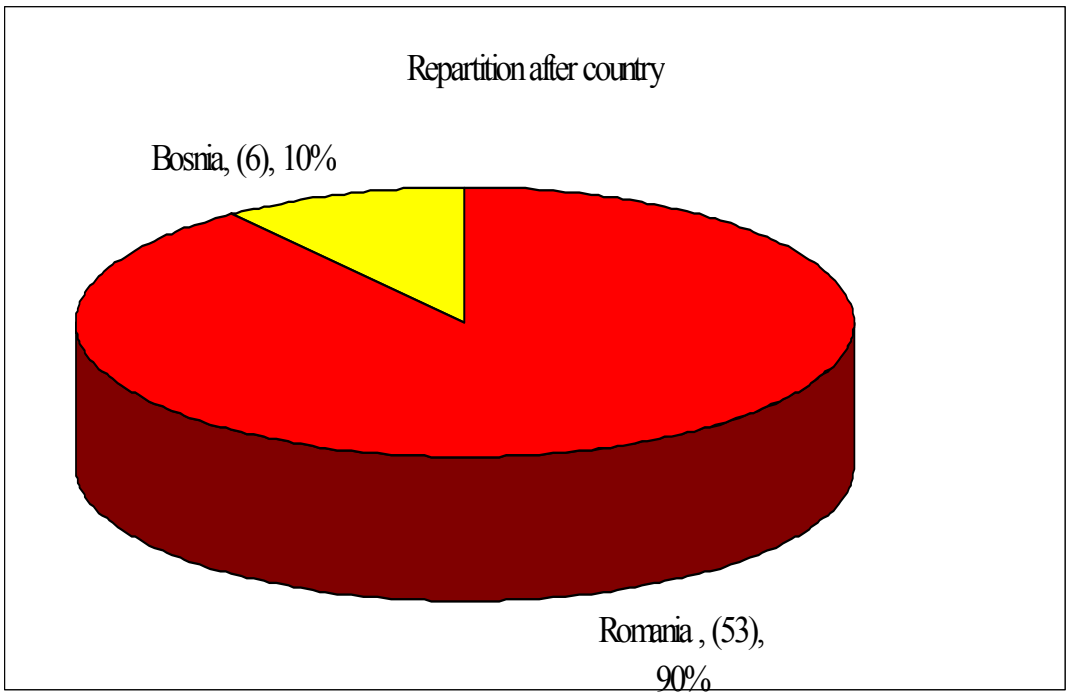

Fig. 6. Repartition of GlobalGap certified farms after country

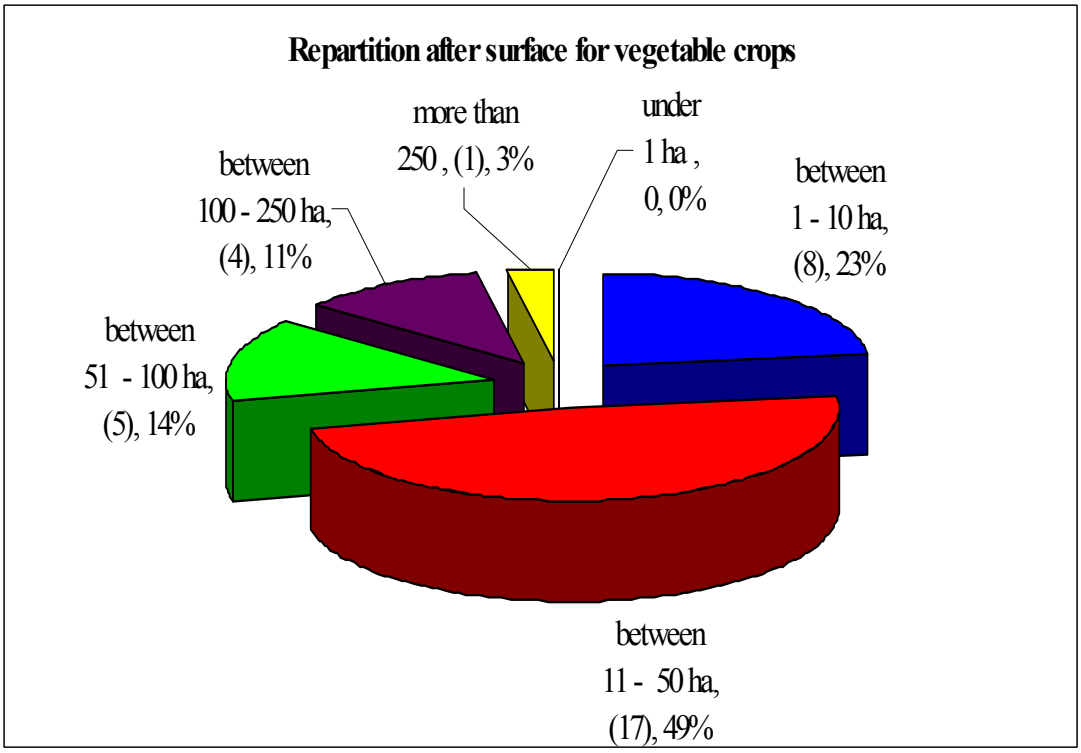

Fig. 7. Repartition of GlobalGap certified farms after surgace for vegetable crops

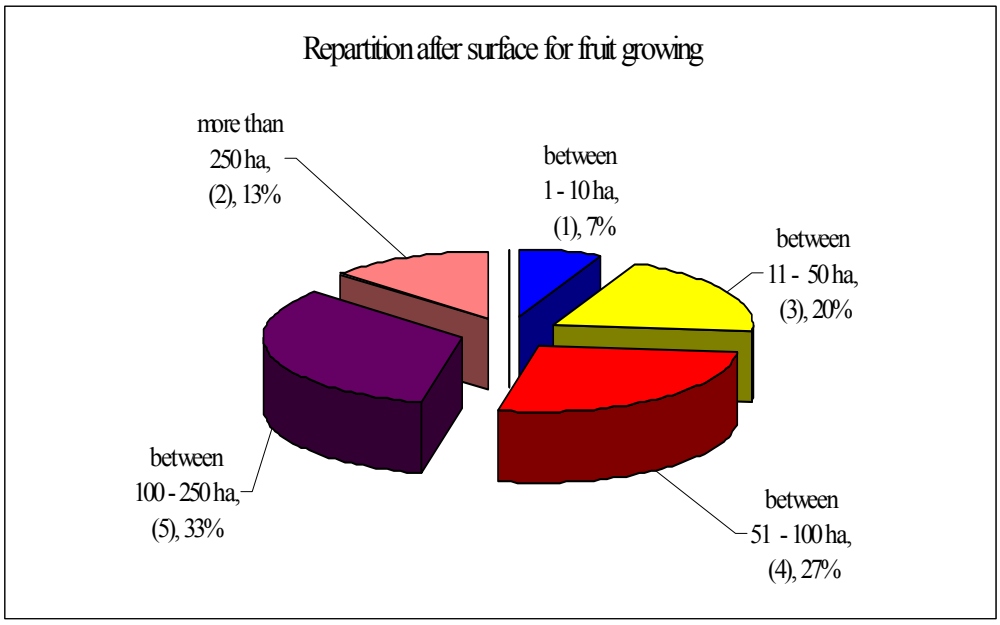

Fig. 8. Repartition of GlobalGap certified farms after surgace for fruit growing

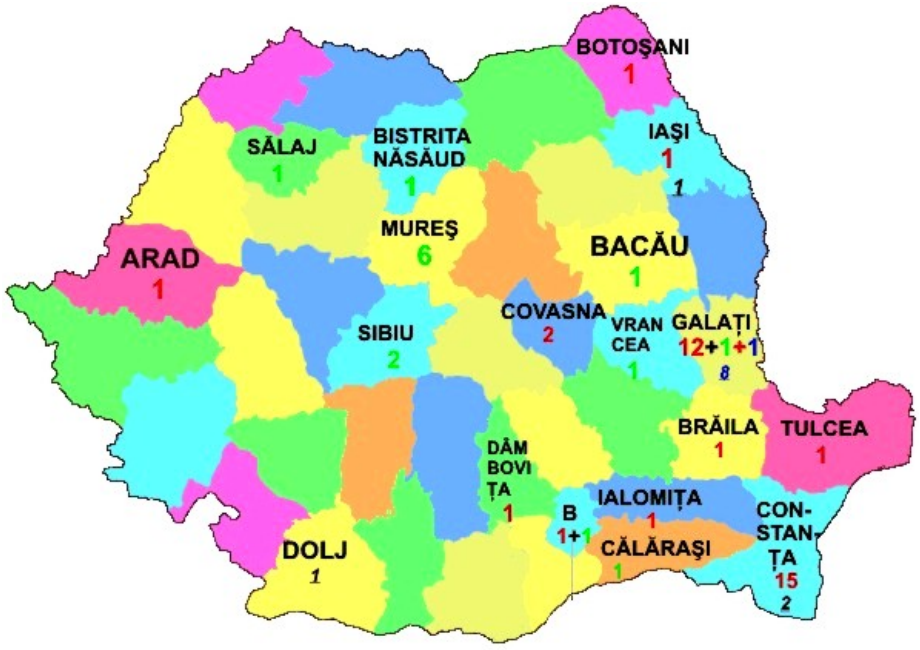

Fig. 9. Repartition of GlobalGap certified farms after

The study of „*re-incubation*", operation specific for modern technologies regarding cultivation of mushrooms *Pleurotus ostreatus*, on expression of yield potential of HK-35, P-80 and K-12 hybrids

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Keywords: cellulose nutritive substrate, hybrid, incubation, „*re-incubation*", yield efficiency.

ABSTRACT

Incubation of mycelium as a technological operation of *Pleurotus sp.,* growing process aims the overall spawn of cellulose nutritive substrate by inseminated mycelium, its quality being crucial for obtaining high and upper quality yields.

In this paper, it is presented a new concept regarding incubation, as an important step of modern crop technology known also as "*re-incubation*" which is performed after obtaining first mushrooms corresponding to first harvest. The role of "*re-incubation*" consists in reactivation of mycelium spawning within the cellulose cultivation substrate and thus increasing yield efficiency of cultivated hybrids.

INTRODUCTION

The incubation of mycelium into the cellulose nutritive substrate corresponds to spawning process that generates the forming of a white down that can be seen beneath the polyethylene sheet when plastic bags are used as cultivation recipients or other types of recipients such as parallelepiped bags made of PE foil. Incubation may be defined as the period between mycelium insemination and forming of first fruit bodies.

Temperature is constantly maintained at 20-22^{0}C for *Pleurotus ostreatus* and 24-26^{0}C for *P. florida, P. cornucopia and P. sajor-caju*, respectively. The optimal incubation period at high temperatures is approximately 20 days for *P. florida* and 25-30 days for *P. ostreatus,* and is completed when cellulose nutritive substrate is compact and white and this state being known as *"briquette"*.

At temperatures of 26^{0}C, substrate fermentation occurs and it causes rapid increase of temperature to the levels that totally compromises mushroom cultures.

These considerations have been made on the ground of cultivation technology known in our country and for which lower incubation temperatures are recommended comparing with new technologies applied for *P. ostreatus* hybrids and indicated by Sylvan firm. As an example, in case of H-3000, it is recommended an incubation temperature of mycelium into the cultivation substrate of 25-30^{0}C for a two-week period. K-12 hybrid incubates rapidly in order to obtain 24-25^{0}C within cultivation substrate but without exceeding 30^{0}C. Incubation in this case requires between 15 to 20 days. Considering other hybrids (HK-35, P-80), incubation temperatures of 34-36^{0}C are required, this demonstrating that newly bred hybrids may present completely different traits associated with the resistance of mycelium to cultivation substrate temperatures. Most of the studied hybrids registered two and even three fructification stages. After incubation, the first mushrooms can be harvested and after temperature conditions are created in order to stimulate a new fructification, this being known as *"re-incubation"* at high temperatures but without exceeding the critical threshold of 30^{0}C.

In the present paper, it is presented a new concept related with incubation, as an important step of modern growing technologies and that is applied after first harvest of mushrooms. The role of *"re-incubation"* consists in reactivating complete mushroom spawn and thus to increase yield efficiency of cultivated hybrids.

In terms of growth-development-harvesting phases, cultivation cycle requires the following number of days: 15-24 days of incubation; 10 days of fructification and growth of pinheads or primordia; 6-8 days first harvesting; 2 days disinfection and drying after first harvest is completed, 14-21 days incubation; 10 days fructification and growth of pinheads or primordia; 6 days second harvest**,** 2 days disinfection of cultivation room for a new cycle.

MATERIALS AND METHODS

Researches regarding the study of *re-incubation* as a new technological step, specific for modern technologies for growing mushrooms *Pleurotus ostreatus* were developed in a mushroom profiled farm S.C. Biosolaria S.R.L Curtici. In this regard, it was set up an experience with two experimental factors: A Factor – mycelium incubation method in cellulose nutritive substrate; a_1 – classical incubation method; a_2 – incubation method followed by *re-incubation* (after harvesting first mushrooms) B Factor – cultivated hybrid: b_1 –HK hybrid – 35; b_2 –P hybrid – 80; b_3 –K-12 hybrids.

Cellulose nutritive substrate was imported from S.C. Pilze Nagy KST Hungary and was in the second preparation stage for cultivation, represented by „*cellulose nutritive substrate inseminated with mycelium*" which required incubation.

The recipients are made of PE parallelepiped-shaped sheet: L=60 cm, l=45 cm and h=35 cm, with capacity of 25 kg/piece cellulose substrate. The adopted crop system was intensive, with briquettes placed on the floor inside cultivation rooms. The cultivation place is represented by a greenhouse L=32 m, l= 8 m, h= 4,5 m with a total surface of 250 m^2, divided in three compartments, 83,3 m^2 each, covered with two polyethylene sheets filled with mineral cotton with insulation properties. The capacity of each compartment is of 220 briquettes placed exclusively on the floor. In each compartment, there are briquettes in different stages of cultivation (incubation, re-incubation, first or second harvesting stage).

Crop cycle required 72-75 days between 1.01.-15.03. 2008, of which 15-24 days of incubation, 6-8 days of first harvesting depending on hybrid (6-7 harvests), minimum 14 days of re-incubation in terms of temperature (30^0C within the nutritive substrate and 20-22^0C in the cultivation room) and 14-15 days of second harvesting (4-5 harvests).

RESULTS AND DISCUSSIONS

In table 1, there are summarized the experimental data regarding the obtained yields for the following mushroom hybrids: *P. ostreatus,* HK-35, P-80 and K-12 as a result of applying both incubation methods.

There are comparatively presented mushroom yields of all three studied hybrids as well as yield components such as: number of clusters/briquette, number of mushrooms/briquette and their average weight.

The analysis of data included in the tables allowed to conclude that: yields obtained as a result of *re-incubation*($a_{2.2}$), operation specific for the second harvest, were more reduced that those obtained after initial incubation ($a_{2.1}$), specific for the first harvest, the yields obtained for a_2 regarding all three hybrids registered values

between 28 and 36 kg/100 kg s.n.c. were higher than those obtained for a_1 (20-28,4 kg/100 kg s.n.c.) with 23,5%; mean weight of mushroom clusters for $a_{2.2}$ are smaller comparing with values obtained for $a_{2.1}$ with differences of 5-29,3 g/cluster.

The analysis of data from table 2, where yield results are synthesized in terms of influence of the two experimental factors, showed the incontestably superior yields in case of a_2. It has been observed a differentiated manifestation of yield potential under the influence of both incubation methods, with yields varying between 7,6-8,0 kg/100 kg s.n.c. The manifestation differences concerning yield potential of hybrids a_2 are not as conspicuous as for a_1. For instance, K-12 hybrid attained exceeding yields in a_1 with 42,4% comparing with HK-35, while for a_2 the registered value was of only 28,6%, considering that in a_2 yield efficiency was of 36 kg/100 kg s.n.c., and for a_1 it registered only 28,4 kg/100 kg s.n.c.

In order to draw conclusions, it was necessary to perform a detailed analysis of data from table 3 rising from statistical results concerning variance analysis and considering obtained experimental results. It has been observed from the table that single influences and the interaction between the two experimental factors (A and B), were influences reflected in the significance of yield differences considering each factor or their combination effect.

Analyzing the influences of experimental factors on yield, it has been observed that: yields obtained under the influence of a_2 factor were statistically covered, the significance of yield differences comparing a1 was positively high significant (point 1); yields obtained in case of b_2 (P-80) and b_3 (K-12) hybrids are also statistically covered, the significance of yield differences comparing b_1 (HK-35) being positively distinct significant and high significant (point 2);. The yield obtained for b_3 (K-12) hybrid was not statistically covered without significance of yield differences comparing b_2 (P-80).

The detailed analysis of table 3 referring to the influence of interaction between the two considered experimental factors in the binomial equations, allowed to conclude that: attained yields under the influence of interaction between different incubation methods considering the same (a_1 and a_2) or different ($b_1 - b_3$) hybrids in the comparative binomial equation ($a_2b_1 - a_1b_1$ or $a_2b_2 - a_1b_1$), are statistically covered in all cases, the significance of yield differences being very significantly positive (point 3); In addition, yields obtained under the influence of interaction between the same incubation method of mycelium ($a_1 - a_2$) and different hybrids ($b_1 - b_3$) are statistically covered only in some cases, the significance of yield differences being positive significant and distinct positive significant ($a_2b_1 - a_1b_1 \rightarrow *$; $a_2b_3 - a_2b_1 \rightarrow **$, etc.) (point 4).

CONCLUSIONS

1. Modern incubation method of *P. ostreatus* mycelium proved to be an efficient method for obtaining maximum yield potential and mushrooms with superior quality
2. Regarding the manifestation of yield potential under the influence of this method (a_2), it has been obtained in the given conditions a yield exceed of 30,8%, comparing with the yield obtained following classical incubation method (a_1) (32,3 kg/100 kg s.n.c. comparing with 24,7 kg/100 kg s.n.c.).
3. Relating to quality, in case of modern incubation method ($a_{2.1}$), the mean weight of mushroom clusters is higher comparing with classical incubation method (a_1) and moreover the mean weight of mushroom caps is higher due to *re-incubation ($a_{2.2}$)*.
4. P-80 and K-12 hybrids proved to be valuable both for attained yields and quality of mushrooms comparing with HK-35 hybrid. The mean weight of mushrooms clusters

at first harvest (incubation $a_{2.1}$) for K -12 hybrid was double comparing with HK-35 and also mean weight of mushrooms after *re-incubation* ($a_{2.2}$- second harvest) was higher with approximately 10g/piece.

5. It is recommended to continue the researches for other cultivation cycles considering different year periods and thus to define completely the results of performed studies.

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Tables

Table 4. Single and interaction influences between experimental factors

Variant	Mean yield (kg/ha)		Relative yield (%)	Difference (± t/ha)	Significance of difference
1. Influence of mycelium incubation method in the cellulose nutritive substrate					
a2-a1	32,33	24,67	131,08	7,67	***
DL 5% = 0,74	DL 1% = 1,12		DL 0,1%=1,80		
2. Influence of cultivated hybrid					
b2-b1	29,30	24,00	122,08	5,30	**
b3-b1	32,20	24,00	134,17	8,20	***
b3-b2	32,20	29,30	109,90	2,90	-
DL 5% = 3,16	DL 1% = 4,35		DL 0,1% = 5,99		
3.Influence of interaction between incubation methods and same or different hybrids					
a2b1-a1b1	28,00	20,00	140,00	8,00	***
a2b2-a1b2	33,00	25,60	128,91	7,40	***
a2b3-a1b3	36,00	28,40	126,76	7,60	***
a2b2-a1b1	33,00	20,00	165,00	13,00	***
DL 5% = 3,72	DL 1% = 5,14		DL 0,1% = 7,13		
4. Influence of interaction between the same incubation method and different hybrids					
a1b2 –a1b1	25,60	20,00	128,00	5,60	*
a1b3–a1b1	28,40	20,00	142,00	8,40	**
a1b3–a1b2	28,40	25,60	110,94	2,80	-
a2b2–a2b1	33,00	28,00	117,86	5,00	*
a2b3–a2b1	36,00	28,00	128,57	8,00	**
a2b3–a2b2	36,00	33,00	109,09	3,00	-
DL 5% = 4,47	DL 1% = 6,16		DL 0,1% = 8,47		

Table 1. Experimental results concerning the obtained yield of mushrooms *Pleurotus ostreatus* for HK – 35, P – 80 and K-12 hybrids cultivated in mono-regional intensive system and applying "*re-incubation*"

Incubation method (A Factor)	Hybrid (B Factor)	No. of clusters/briquette (pieces./briquette)	Mean no. of mushrooms in cluster (pieces./cluster)	Numberof mushrooms/briquette (pieces./briquette)	Mean weight		Obtained yield			Mean yield for A factor			
					cluster (g/ piece)	Mush-room (g/ piece)	kg/briquette	kg/100 kg s.n.c.* or%	kg/m^2 local cultivated area	No of mushrooms/briquette (piece./ briquette	Obtained mean yield		
											kg/briquette	kg/100 kg s.n.c.* or%	kg/m^2 local cultivated area
a_1- classical incubation	b_1 – HK-35	6,63	10,8	71,6	753,8	69,8	5,0	20,0	13,2	65,5	6,2	24,7	16,3
	b_2 – P-80	5,53	11,4	63,0	1153,7	101,2	6,4	25,6	16,9				
	b_3 – K-12	5,54	11,2	62,0	1291,4	115,3	7,1	28,4	18,8				
a_2- total incubation + re-incubation	b_1 – HK-35	11,18	8,9	103,2	629,4	68,2	7,0	28,0	18,5	85,8	8,1	32,3	21,4
	b_2 – P-80	9,40	10,7	80,3	1103,9	103,3	8,3	33,0	21,9				
	b_3 – K-12	7,81	9,6	73,9	1165,1	121,7	9,0	36,0	23,8				
$a_{2.1}$ - incubation followed by- re-incubation	b_1 – HK-35	5,63	13,4	75,4	805,5	60,2	4,5	18,0	11,9	57,2	5,0	20,0	13,2
	b_2 – P-80	5,00	14,1	50,0	1410,9	100,0	5,0	20,0	13,2				
	b_3 – K-12	3,44	13,4	46,1	1615,1	120,3	5,5	22,0	14,5				
$a_{2.2}$ - re-incubation	b_1 – HK-35	5,55	5,0	27,8	450,8	89,5	2,5	10,0	6,6	28,6	3,1	12,3	8,2
	b_2 – P-80	4,40	6,8	29,9	755,1	110,3	3,3	13,0	8,7				
	b_3 – K-12	4,37	6,5	28,0	810,9	125,0	3,5	14,0	9,2				

* cellulose nutritive substrate

Table 2. Influence of experimental factors on mushroom yield of *Pleurotus ostreatus* for HK – 35, P – 80 and K-12 hybrids cultivated in mono-regional intensive system and applying "*re-incubation*"

A Factor (incubation method)	B Factor (hybrid)	Obtained yield				Mean yield obtained for A factor				Mean number of mushrooms for A factor per:		Mean weight for A factor	
		kg/briquette	kg/100 kg s.n.c.* or%	kg/m^2 local cultivated area	%	kg/briquette	kg/100 kg s.n.c.* or%	kg/m^2 local cultivated area	%	briquette (piece)	cluster (piece)	cluster (g/ piece)	mushroom (g/piece)
a_1- classical incubation	b_1 – HK –35	5,0	20,0	13,2	100,0	6,2	24,7	16,3	76,5	65,5	11,1	1047,0	94,2
	b_2 – P-80	6,4	25,6	16,9	128,0								
	b_3 – K-12	7,1	28,4	18,8	142,4								
a_2- total incubation+ re-incubation	b_1 – HK-35	7,0	28,0	18,5	100,0	8,1	32,3	21,4	100,0	85,8	9,7	965,9	97,7
	b_2 – P-80	8,3	33,0	21,9	118,4								
	b_3 – K-12	9,0	36,0	23,8	128,6								

* cellulose nutritive substrate

Researches regarding the influence of the "*fazial*" fertilization on the growth and fructification of tomatoes

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Keywords*:* tomatoes, fertilisation, plant growth

ABSTRACT

The tomato culture is one of the most important ones and this is the main reason why it has been chosen to be the subject of this experiment. For it were used individually or combined two fertilizers, Cropmax 0,15% and Agroleaf 0,5%, applied in five stages of growing of plants. The best results were obtained by using them individually, fact proved by the capacity of fructification of plants. Regarding the biochemical composition, the fruits fertilized had superior features to the ones treated with control; they had a higher content of C vitamin, mineral substances, soluble dry substance, etc. Another proof of the positive influence of fertilizers on the tomato culture is the fruit production, placed between 5,6 kg/m^2 and 6,4 kg/m^2. Compared with 4,7 kg/m^2 at control, the effect of fertilizers is obvious.

INTRODUCTION

The 'foliar' fertilization is a very important technological stage from the culture technology of plants. The main reason for which the 'foliar' fertilization is used is to assure the food supply for plants and to eliminate the lack of it. A good supply with nutritive elements conducts to a better resistance while facing stress factors and to an appropriate growth and fructification.

MATERIAL AND METHOD

The researches were effectuated in the didactic field of Horticulture Faculty from Bucharest, in 2006-2007, in solarium culture. The biological material used was the 1600 Buzau variety, a kind with endless growth, big, strong and breaking proof fruits.

The fertilizers were provided by Holland Farming, who also gave the fertilizing plan for tomato culture and the fertilizers combinations.

The culture was founded by planting the nursery which was produced in greenhouse throughout direct seeding in "turba' pots 7 Jiffy type. The seeding work was done in the first decade of February. During nursery production, specific works were done in order to obtain proper nurseries.

The planting in solarium was effectuated on the 17-19 oh April, because of the unfavourable weather conditions at night, which might have affected the tomato culture. The seeds were planted at equal distances of 70/30cm, resulting a density of 4,8 plants per square meter.

During the vegetation period were conducted specific works for the tomato culture. Regarding the 'foliar' fertilization, many products of different concentration were used, resulting many experimental variants, each of it being subjected to many treatments.

The experimental scheme:

- **V1** - unfertilized control,
- **V2** - treatment 1 – fertilization with Cropmax 0,15%;
  - treatment 2 – fertilization with Cropmax 0,15% +Agroleaf P 0,5%;
  - treatment 3 – fertilization with Cropmax 0,15% +Agroleaf T 0,5%;
  - treatment 4 – fertilization with Cropmax 0,15% +Agroleaf T 0,5%;
  - treatment 5 – fertilization with Cropmax 0,15% +Agroleaf K 0,5%;

- **V3** - treatment 1 – fertilization with Agroleaf P 0,5%;
  - treatment 2 – fertilization with Agroleaf P 0,5%;
  - treatment 3 – fertilization with Agroleaf T 0,5%;
  - treatment 4 – fertilization with Agroleaf T 0,5%;
  - treatment 5 - fertilization with Agroleaf K 0,5%;
- **V4** - treatment 1 – fertilization with Cropmax 0,15%;
  - treatment 2 – fertilization with Cropmax 0,15%;
  - treatment 3 – fertilization with Cropmax 0,15%;
  - treatment 4 – fertilization with Cropmax 0,15%;
  - treatment 5 – fertilization with Cropmax 0,15%.

During researches, observations and measurements were effectuated regarding the dynamic of vegetative growth and fructification capacity of plants. As follows, it was determined the number of flowers in each flower growth, the number of tied fruits, the percentage of tied fruits, the average weight of fruits, the average production of fruits per plant and m^2. Also, biochemical works were effectuated regarding the biochemical composition of the fruits obtained in this system of fertilization (the resolution, the content of soluble dry substance, the total dry substance, the acidity, the content of C vitamin and minerals).

RESULTS AND DISCUSSIONS

During the experimental period, observations showed that the fertilizers used influenced positively the growth and fructification of tomato fruits. The plants had an increasing growth from the first to the last determination, but the best results had the variant fertilized with Cropmax 0,15% and Cropmax 0,15%+Agroleaf 0,5% (Table 1).

The treatments applied influenced positively the capacity of fructification of plants. Regarding the average number of flowers per plant, the observations show that at all variants was higher than at control, which proves the influence of fertilizers on the plants flourishing dynamic. It what regards the number of flowers between variants, the best results were obtained at V2 at the first 3 flower growing, using 2 combined products. At V4, better results were obtained after using Cropmax 0,15% (Table 2).

The average number of flowers formed in flower growing was influenced by the fertilizers used. So, control registered the smallest number of tied fruits. The best effect had Cropmax and Agroleaf or Agrolef alone. Similar effect had Cropmax alone, the registered data having slightly smaller values (Table 3).

The percentage of tied fruits is very important and it was influenced by fertilizers, fact proved by the following results: 61,5% at control and 66,5% at V3. Better and similar results were obtained after using the two products separately, but the combination of the two of them also conducted to satisfactory results: 63,5% (Table 4).

The production capacity of 1600 Buzau variety was positively influenced by Cropmax and Agroleaf, increasing the percentage of tied fruits and their weight. As consequence, the tomato fruits registered an average weight of 140 g at control and aproximatively 200 g at V2, which represents a significant influence on the fruit growth. The influence of fertilizers is also proved by the production of fruits per plant: 1kg/plant at control and 1,34kg/plant at V2 (Table 5).

Due to fertilizers, the biochemical composition of fruits was richer than at control. At every variant, the fruits had a highly content of mineral substances (0,25-0,28%), soluble dry substance (4,95-5,3%) and C vitamin (57,84-59,74 mg/100g). The fruits were stronger, especially at variant 3 and 4 (Table 6).

CONCLUSIONS

From the researches made regarding the influence of some 'foliar' fertilizers on the tomato culture, the following conclusions can be stated:

1. the fertilizers used positively influenced the vegetative growth of plants, especially at V2 and V4;
2. the flower growing was influenced by Cropmax and Agroleaf, two fertilizers used individually;
3. the variants 3 and 4 had the biggest number of flowers, which conducted to the biggest number of tied flowers; this process was also influenced by using a combination of the two fertilizers;
4. the fertilizers improved the biochemical composition of fruits by increasing the quantity of C vitamin, soluble dry substance, mineral substances, etc.;
5. the tomato production of the variants treated with fertilizers was superior to the one treated with control.

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Tables

Table 1. The height dynamic of plants (cm) 2006 - 2007

Variant	The date of measurements					
	26-05	07-06	13-06	20-06	28-06	04-07
V1	420	600	790	830	970	1020
V2	490	690	830	970	1100	1230
V3	480	670	780	890	1010	1160
V4	490	680	790	930	1070	1230

Table 2. The average number of flowers formed in flower growing 2006-2007

Variant	The flower growing			
	I	II	III	IV
V1	4.25	4,4	4,45	4,35
V2	5,35	5,3	5,05	4,7
V3	5,15	5,25	4,7	5,1
V4	5,2	4,8	4,95	5,0

Table 3. The average number of fruits in the flower growing 2006-2007

Variant	The flower growing			
	I	II	III	IV
V1	2,9	2,8	2,8	3,3
V2	3,6	3,3	3,3	3,95
V3	3,9	3,2	3, 2	3,95
V4	3,25	3,1	3, 1	3,8

Table 4. The average percentage of tied fruits in flower growing 2006-2007

Variant	The flower growing			
	I	II	III	IV
V1	61	54,5	60,5	61,5
V2	66,5	57,0	65,0	63,5
V3	66,5	60,5	64,5	66,5
V4	65,5	59,5	63,5	66,0

Table 5. The capacity of production of tomato fruits 2006-2007

Variant	Average weight of fruit (g)	Production/ plant (kg)	Semnification	Production/m^2 (kg)
V1	140	0,98	mt	4,7
V2	200	1,34	***	6,4
V3	190	1,27	**	6,1
V4	190	1,17	**	5,6

DL 5% - 0,080 kg
DL 1% - 0,160 kg
DL0,1% - 0,360 kg

Table 6. The biochemical composition of tomato fruits

Item	V_1	V_2	V_3	V_4
Fermity (kgf/cm^2)	1.15	1.17	1.95	1.70
Soluble dry substance (%)	4.60	5.30	5.10	4.95
Acidity (acid oxalic) (%)	0.234	0.330	0.270	0.262
C Vitamin (mg/100)	50.96	58.08	59.74	57.84
Total dry substance (%)	7.51	7.43	7.54	6.26
Mineral substances (%)	0.15	0.25	0.26	0.28

Researches regarding the influence of some ecological fertilizers on the growth and fruit forming of tomatoes

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Keywords: tomatoes, ecological fertilizer, plant growth,

ABSTRACT

The researches were made in 2006-2007, studying the tomatoes in field, using 2 ecological products, Cropmax and Kelpak with 0,2% concentration. These products are aimed to stimulate the growth and fruit forming processes, they improve the metabolism of plants and help them in stress conditions. The products were used in 3 sprinkles in mixture with other products for protection as it follows: once at seedling and 2 times at the culture. The first sprinkle was made when the first flowers appeared and the second one 15 days later. The best results were obtained with Cropmax which gave more flowers per plant registering a growth of 33,4 flowers, one of 30,9 formed fruits, a percentage of 92,04 formed fruits and a production of 10,1 kg/mp. Kelpak gave lower results, the number of flowers per plant was of 32,1, the number of fruits 27, 8, the percentage of formed fruits 87,4% and a production of 8,4 kg/mp.

INTRODUCTION

The production of vegetables throughout nonpolluting technologies has become a national and international interest because food needs to have a high quality. These techniques help obtaining healthy vegetables favorable for the consumers' health and for the environment. Using nonpolluting paths of work, the quality is improved, the soil is protected the pollution of air and water is reduced, etc.

MATERIAL AND METHOD

The researches were made in 2006-2007, at tomato, in the field of Horticulture Faculty from Bucharest.

The biological material used was the Cristal hybrid, which has big, strong fruits, aimed to be consumed in fresh condition.

The culture was founded on a field formerly used for cultures of Bruxelles cabbage and pea (2005) and for autumn cabbage (2006), by planting the nurseries at a distance of 80cm/40cm. During the vegetation period there were made specific works for the culture, excepting the conventional fertilization. It was made throughout 2 ecological products, Cropmax and Kelpak. The variants resulted are the following:

V1 – unfertilized control
V2 – fertilization with Kelpak 0,2%
V3 – fertilization with Cropmax 0,2%

The 3 fertilizations were made at the nursery faze and in culture. The 2 ones in culture were made when the firs flowers appeared and 15 days after.

Measurements were done regarding the dynamic of the vegetative growth of plants, the formation of flowers and fruits, the average weight of fruits, the estimated production, etc.

The products used have the following features:

Kelpak

Is a stimulator of growth based on auxines and citocinines, pure concentrate of marine alga (Echlonia maxima). It contains macro elements, micro elements and aminoacids and it can be used for the majority of culture plants. It is recognized as an ecological product by many organizations, it is not toxic; it increases the resistance of plants at drawn, stress, pests and water excess. It contributes to obtaining large productions with superior quality.

Cropmax

The chemical composition of this product is complex, containing macro elements, micro elements, organic aminoacids and growth stimulators, vitamins, enzymes obtained throughout natural extracts. This fact explains the favorable influence on the metabolism of plants, ended with a growth in quantity and quality of production. The use on plants during the vegetation period can be done throughout specific fertilization works, but also with the works of maintaining the culture and treatments against pests and diseases because it is compatible with the majority of products based on Cu. It increases the resistance of plants at diseases and pests, at drawn, because it develops the radicular system of plants.

RESULTS AND DISCUSSIONS

The evolution of the nurseries growth was studied during 42 days. The highest growth was registered at V3. The period when the nurseries had the highest growth was between day 14 and day 21, respectively from 9,7 cm to 14,8 cm. the lowest growth was registered at control (table 1).

In culture, the plants registered a good vegetative growth until the fifth inflorescence, reaching a height of 1,6 m at control, 1,71 m at Kelpak and 1,87 m at Cropmax.

The number of leaves until the first inflorescence was different: 8,9 at control, 8,2 at V2 and 7,9 at V3. Also, the distance between leaves until the first inflorescence and the average number of leaves between inflorescences was influenced by the products used until (table 2).

The capacity of fruit forming of plants was influenced by the products used. The average number of flowers in inflorescence (fig.1) was higher at the fertilized variants in comparison with control and progressive from the first inflorescence to the last one, excepting the fifth one.

The number of fruits in inflorescence was influenced by the environment conditions during flourishing. The high temperature influenced negatively the process, especially at the fifth inflorescence. Also, the fertilizers used had different effects on the number of formed fruits (fig. 2).

The percentage of formed fruits (fig. 3) registered a progressive growth, excepting the fifth inflorescence, having the same evolution as formed flowers or formed fruits.

Using the growth stimulators influence the capacity of fruit forming of Cristal hybrid, giving important increases in production (table 3, fig. 4). The best results were obtained using Cropmax (an increase of 60%), followed by Kelpak (an increase of 33).

These products also had a benefic effect on the size of fruits. The values were of 97 g and 105g at Kelpak and Cropmax and 80g at control.

CONCLUSIONS

From the researches regarding the effect of some ecological fertilizers on the growth and fruit forming of tomatoes, the following ideas can be concluded:

1. The possibility of using the products with simple technology and in mixture with other products aimed for protection;
2. The products assure a very good vegetative growth which represents the base of an optimum fruit forming;
3. The percentage of fruit forming grew from 82,4% at control to 92,04% at the variant fertilized with Cropmax;
4. The estimated production grew from 2,03 kg/pl at control to 3,2 kg/pl at the variant fertilized with Cropmax.

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Tables

Table 1. The dynamic in growth of nurseries after transplant

Variant	7 days		14 days		21 days		28 days		35 days		42 days	
	H (cm)	No. lvs	H (cm)	No. lvs	H (cm)	No. lvs	H (cm)	No. lvs	H (cm)	No. lvs	H (cm)	No. lvs
V1- control	5,2	3,8	8,5	5,7	12,5	6,8	14,7	7,4	17,3	7,9	19,5	8,1
V2	5,6	4,1	9,3	5,8	13,6	7,2	15,8	7,8	18,3	8,1	20,9	8,3
V3	5,8	4,3	9,7	6,3	14,8	7,7	15,9	8,1	18,8	8,8	21,8	9,3

Table 2. Biometrical indicators

Variant	Average number of leaves until the first inflorescence	Average distance until the first inflorescence	Average number of leaves between inflorescences
V1- control	8,9	18,4	3,4
V2	8,2	17,3	3,2
V3	7,9	16,8	3,0

Table 3. The estimated production of fruits

Variant	Production/plant			Production/mp		
	Kg/pl	The difference from control		Kg/mp	The difference from control	
V1-control	2,03	-	100	6,3	-	100
V2	2,7	+0,7	133	8,4	+2,1	133
V3	3,2	+1,2	160	10,1	+3,8	160

Figures

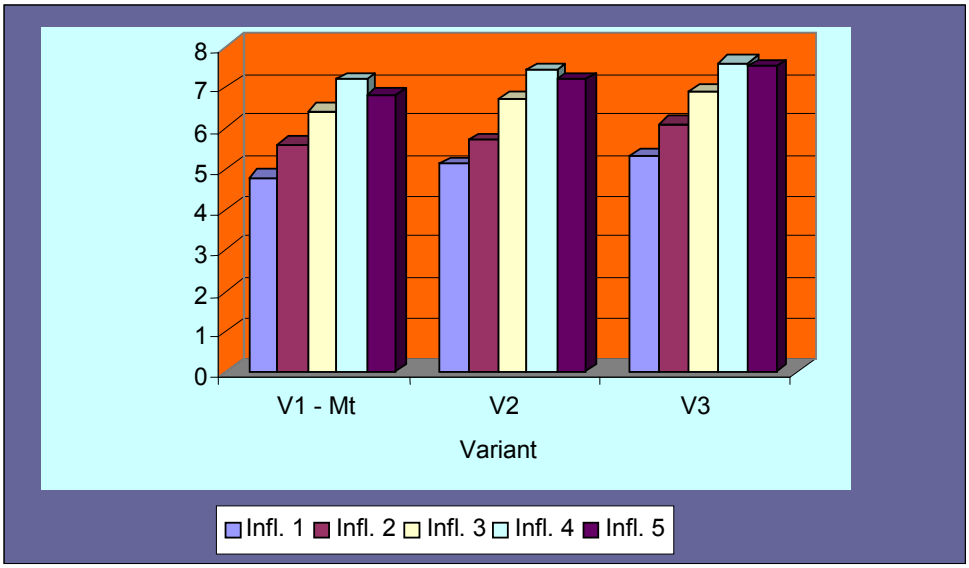

Fig. 1. Mean flowers numbers on inflorescence

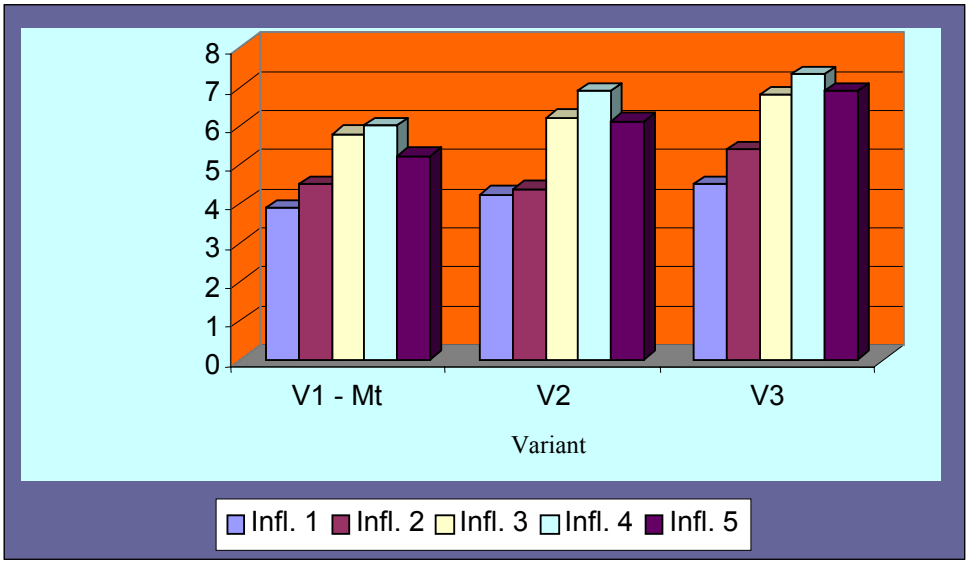

Fig. 2. Mean fruits number

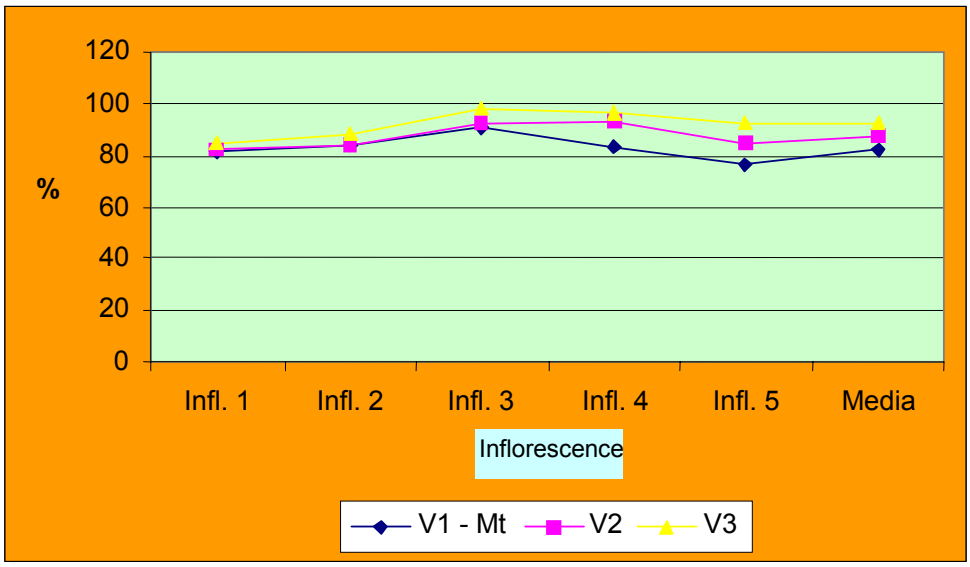

Fig. 3. Percent of the formed fruits

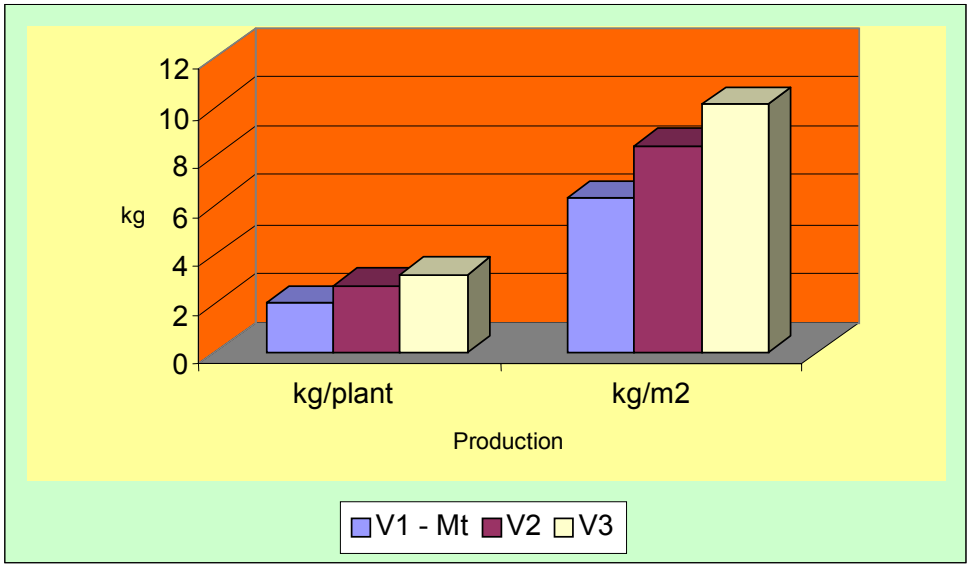

Fig. 4. Production capacity

Effect of the irrigation with saline water on the behavior of 2 soil enzymes urease and saccharase, soil respiration and soil humidity

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Keywords: soil moisture, soil respiration, urease activity and saccharase activity

ABSTRACT

The aim of this study is to show the effect of soil salinity on the soil moisture and the soil respiration and for some soil enzymes specially urease and saccharase. Three vegetable crops were grown in 4 compartments irrigated with water, which contains different salt concentration respectively 0.37 ms/cm for the control V_1, 1.5 ms/cm as V_2, 2.5ms/cm as V_3, 4 ms/cm as V_4. Some soil sample was taken at the depth of 10 cm from each compartment. The results obtained are the following: we had an increasing of the soil moisture with the increasing of the soil salinity. We obtain a decreasing respectively for the soil respiration, the urease and saccharase activity with the increasing of the soil salinity.

INTRODUCTION

Salinity stress, which usually occurs in arid and semiarid regions, is a major environmental constraint to crop productivity. Low rainfall, high evaporation, native rocks, saline irrigation water and poor water managements can cause salinity problems in agricultural areas. The majority of crop plants is susceptible and cannot survive under conditions of high salinity or can survive only with decreased yields. Plants are stressed in three ways in saline soils; low water potential of the root medium leads water deficit (R.Munns, *et al.,* 1986), the toxic effects of the ions mainly Na and Cl (A.Lauchli et al., 1986), nutrient imbalance by depression in uptake and/or shoot transport (H. Marschner *et al.,* 1995). To alleviate the deleterious effects of salinity some rehabilitations such as reclamation of salinized lands, improving of irrigation saline water and special cultural techniques are applied.

MATERIAL AND METHODS

The experiment has been carried out in 2006, in the glass house of the department of Horticulture in the University of Agronomical Science and veterinary medicine Bucharest, three variety of vegetable crop were grown *Lycopersicum esculentum*, *Raphanus sativus*, *Spinacia oleracea* and has the following objectives: the effect of the irrigation with saline water on the soil respiration and the soil humidity.

The effect of the irrigation with saline water on the potential activity of some soil enzymes: Urease using the calorimetric way with the Nesseler reactiv, Saccharase using the spectrophotometrical determination of saccharase activity by the dinitrosalycilic acid reagent.

The soil samples were taken in 14 December 2007, at the depth of 8-10 cm.

In this experiment we used 4 variants:

- V_1- Control irrigated with fresh water 0.37 ms/cm (Control)
- V_2- Plant irrigated with saline water 1.5 ms/cm
- V_3- Plant irrigated with saline water 2.5 ms/cm
- V_4- Plant irrigated with saline water 4 ms/cm

Each variant had three repetitions.

RESULTS AND DISCUSSION

Soil moisture

We conclude that in the soil irrigated with saline water had a much higher humidity that the control and that is due to the increasing of the osmotic pressure in plant root cells.

We had an increasing in the soil moisture for about 6% between the control and the treatment irrigated with a highest salinity concentration.

Soil respiration

Soil respiration is one of the major carbon (C) fluxes between terrestrial ecosystems and the atmosphere and plays an important role in regulating the responses of ecosystem and global C cycling to natural and anthropogenic perturbations.(Wenhua Xu, Shiqiang Wan, 2008).

Soil water content at 0–10 cm depth was a major limited factor of soil respiration in semi-arid grassland, accounting for 76.4% of the variation.(Bingrui Jia, *et al.,* 2006).

In this study we can conclude that the soil respiration increase with the increasing of the soil salinity concentration. We found 37.34 in the case of the control and this value increased to reach 25.63 mg/100g of soil in the case of the treatment with the highest salt concentration. We have an increasing of 31.36%.

In the second part of the discussion we will discuss the behavior of some soil enzymes Saccharase and Urease.

Saccharase

The figure 3 shows that the amount of Saccharase decreases with the increasing of salinity concentration. In case of the control we had a value of Saccharase 475.55 mg/100g soil and for the variant irrigated with saline water of 4ms/cm is 385.79 mg/100g soil.

Urease

Urease enzyme is responsible for the hydrolysis of urea fertiliser applied to the soil into NH3 and CO2 with the concomitant rise in soil pH (Andrews *et al.,* 1989; Byrnes and Amberger, 1989). This, in turn, results in a rapid N loss to the atmosphere through NH3 volatilisation (Fillery *et al.,* 1984; Simpson *et al.,* 1984, 1985; Simpson and Freney, 1988). Due to this role, urease activities in soils have received a lot of attention since it was first reported by Rotini (1935), a process considered vital in the regulation of N supply to plants after urea fertilisation. The figure 2 shows that urease activity decrease with the increasing of the soil saline concentration. For the control we obtain a value of urease 55,81 mg/100g soil with an (r^2 0,9505) and for V4 we had 41,94 mg/100g soil.

CONCLUSIONS

We conclude that the soil moisture increase with the increasing of the soil salinity. In the control we had 24,215% and the treatment with 4 ms/cm 31,913%. The soil respiration decreases with the increasing of soil salinity. Regarding the soil enzymes urease and Saccharase we can see that in both case we had a decreasing of their activity with the increasing of the soil salinity.

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Figures

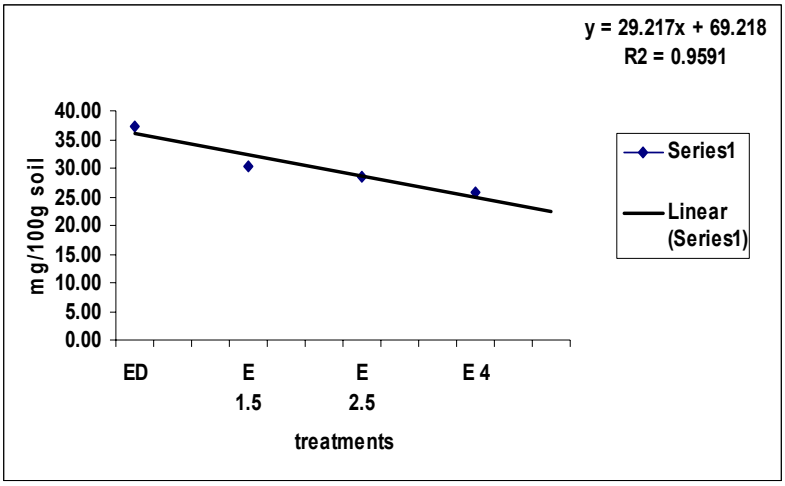

Fig. 1. Soil respiration for the 4 variants

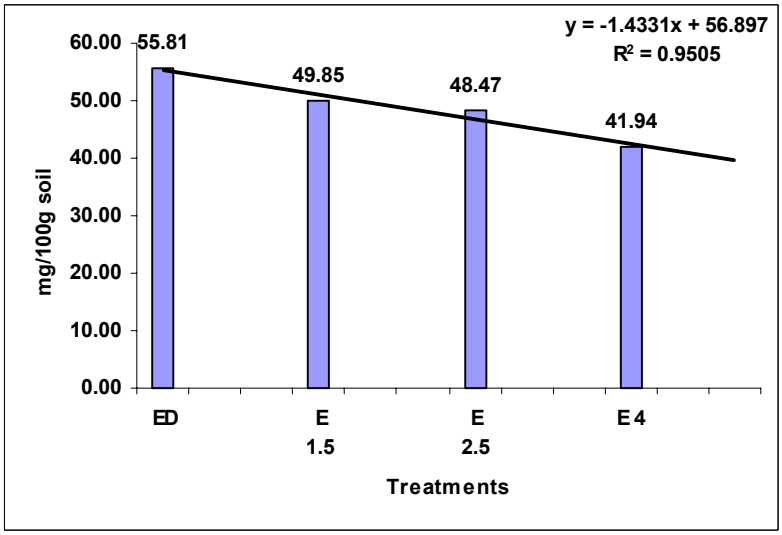

Fig. 2. Urease for the 4 variants

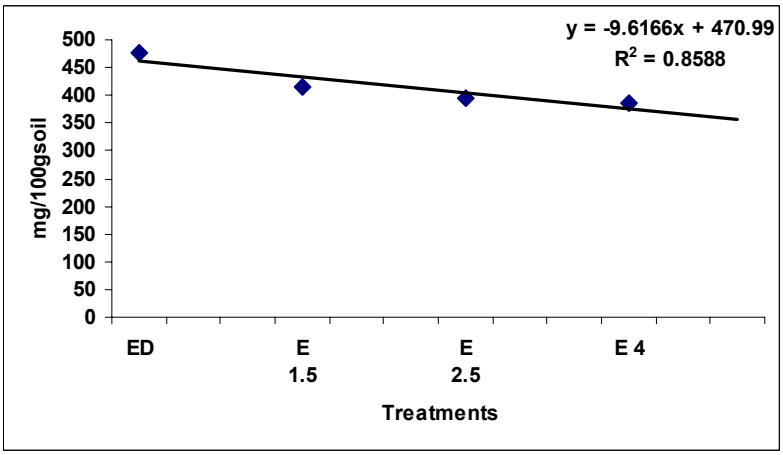

Fig. 3. Saccharase obtained in the 4 variants studied

Researches concerning the influence of some Romanian photoselective films on the productivity and quality of lettuce and tomato

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Keywords: *Lycopersicon esculentum, Latuca sativa,* high tunnel, photoselective films.

ABSTRACT

The paper present results concerning the influence of some Romanian photoselective films on the productivity and quality of lettuce and tomato plants. Five film types were studied which had different colors and additives for protection against ultraviolet light, infrared light, and water condensation. These films were used for plant protection in high tunnel. As a result of the microclimate changes under the photoselective films the plant growth, yield and produce quality were improved. Remarkable results were obtained concerning the produce quality under the pink and green films for lettuce, and under pink and yellow films for tomato.

INTRODUCTION

The vegetable species react differently when being covered with colored photoselective plastic films. The major influences of the photoselective covering films on plants' environment are the modification of light quantity and quality. These changes in the light environment induce physiological responses that affect plant growth and development, yield, and productivity (1, 2).

The influence of light on stem growth, leaf expansion and growth is a dynamic process governed by specialised photoreceptors. In red light the phytochromes are the primary photoreceptors controlling hypocotyl elongation. Cryptochromes are the blue light receptors which control elongation and leaf expansion; moreover the blue spectrum is known to promote plant growth, especially green leafy growth (4).

Important phototropic dependent effects on plant growth were reported under low photosynthetically active radiation in the natural environment (6). Red and blue light was shown to have the greatest impact on plant growth while green light was least effective. While blue light is primarily responsible for vegetative leaf growth, red light, when combined with blue light, increases flowering.

Our previous results showed that plant cultivation under photo-selective films may positively influence lettuce and tomato seedling growth, root absorption and other physiological processes (3, 5).

The objectives of the present study were to evaluate the response of lettuce and tomato plants cultivated in a high tunnel covered with novel monolayer LDPE photoselective films (PROINTERMED SA, Pitesti, Romania) containing in its matrix special chemical additives.

MATERIALS AND METHODS

The experiment was organised in the experimental field of the Vegetables Crops Department at the University of Agronomical Sciences and Veterinary Medicine, in high tunnel covered with different polyethylene -PE photoselective films and compared with a control – an ordinary PE film.

The experimental films were produced from LDPE (Low Density Polyethylene), by blow extrusion. The special chemical additives incorporated into the polymer matrix

were: pigments, ultraviolet (UV) absorber, UV and infrared barrier and antifogging. The characteristic properties of the films are the following: 180 μm thick; colour - yellow, pink, white, blue, green; light transmittance in the range of 380-700 nm; increased mechanical and weathering resistance; anti-condensation and anti-oxidative properties.

The crops were lettuce ('Ilona') and tomato ('Cindel'). The experiment was set up by covering the s before lettuce plant cultivation, using a linear random block design with 4 replicates of 30m^2 per unit.

The tomato culture was interlaid with the lettuce culture. Lettuce plants were planted at 30 cm between rows and 20 cm between plants and tomato plants were planted as interlaid culture at 70 cm between rows and 35 cm between plants. The production practices were in accordance with the extension recommendations for lettuce and tomato cultivation and were identical for all treatments and replicates.

The treatments were:

V1 control – PE film transparent, with no additives;
V2 – transparent film, with additives;
V3 – yellow film, with additives;
V4 – pink film, with additives;
V5 – green film, with additives;
V6 – blue film, with additives.

Measurements were made of rosette diameter and weight in lettuce, and number of developed fruit, average fruit weight, nitrate concentration in mature fruit, and fruit yield in tomato. 12 plants were measured for each replicate. The statistical analyses consisted of ANOVA, Tukey-Kramer and Dunnett tests which were performed using the JMP 5.0 software.

RESULTS AND DISCUSSIONS

Lettuce

The photoselective films influenced lettuce plant growth, head formation, yield, and quality. Improved values of weight were found for plants grown under transparent (ordinary and with additives) films, yellow and pink, which were greater by 3-7% compared with the averaged weight for this variety. The weight of control plants was exceeded by that of plants grown under pink film. Under another variant the plants had a reduced weight compared with the plants from the ordinary film (Table 1).

The statistical analyses of the yield (Table 2) shows that for the hight tunnel covered with photoselective films the yields were not significantly smaller than for the control plants, except the significantly reduced yield which was obtained for plants under blue film (a reduction of 19% i.e. 7.17 t/ha) compared with the control. Blue film showed a significant yield decrease compared with the other films with the exception of the green film. In plants under pink film the yield was of 0.21 t/ha greater compared to that of plants grown under the control and the blue film (Table 2).

The Romanian legislation considers the rosette diameter as a production quality attribute. A plant with a rosette diameter over 30cm is referred to as "Extra." Rosette diameter was modified by film type. After 45 days from planting, significant differences were observed between treatments and the characteristic average value for the Ilona variety. Compared with the control and the average value for the Ilona variety, the pink and green films increased the rosette diameter, while the yellow and transparent films decreased the diameter. The rosette diameter under the ordinary PE film was similar

compared to the mean variety diameter value. The best results were obtained for the green and pink films, for which the rosette diameter was increased by 8% and 6%, respectively, compared with the control and the variety value, which suggests the possibility for an early harvest in these films.

The harvest under the yellow and transparent films which were treated with additives was delayed showing significantly smaller rosette diameters compared with the rest of the plants (Table 3 and 4). The green film significantly increased the rosette diameter compared with the control, the yellow and transparent with additives films (table 4). Under the transparent with additives film the rosettes were significantly smaller compared with all treatment except the yellow film. Compared with control (Dunnett test, table 4), the rosette diameter was significantly influenced only for lettuce plants under the green and transparent with additives films.

For the treatments yielding large rosettes (under green and blue films), for which the head formation was delayed, smaller weight by 12-18% compared with control and by 7-14% compared with the average value were obtained. One exception was the pink films treatments under which the rosette diameters were greater by 6-7% compared with the mean variety characteristic values (table 4).

Tomato

The photoselective films influenced the fructification process (the ratio between the number of flowers and fruits per inflorescence). Compared with the control, fructification was increased by 11% under the pink film and 6% under the yellow film and decreased by 3% – 9% under the blue film, green film and the transparent film with additives. (Figure 1). The average number of fruits per plant was 11.6, being exceeded only by the plants under the pink and yellow films, with 3.8 and 2.2 fruits/plant, respectively.

In the control the number of fruits was close to the average value, while for the other treatments there was a decrease with the lowest value being for the blue film (Figure 2). The highest fruit number per plant was obtained by plants under pink film.

Yields were highest for the pink and lowest for the blue film (Table 5). Very significant statistical differences (9.76t/ha) were obtained between the control and the pink film, while no significant differences (2.61t/ha) were found between the yellow film and the control. For the blue and green films there was a very significant yield decrease by 25-42%, compared to the control films. The transparent film with additives induced a significant yield decrease by 5.26 t/ha too. These results may be explained by the fact that the films considered “warm” induced an adequate thermic environment promoting tomato fructification, as it is well known that tomato plants are thermophilic.

In general, all the photoselective films decreased fruit weight compared with the control. The smallest differences were found for the pink and transparent films, while the largest were for the blue film (aprox. 15g/fruit) (Figure 4).

Nitrate metabolism is modified by environmental conditions which also influence photosynthesis. Fruits from plants grown under pink films (for which the largest yield was obtained) had a nitrate concentration of 235 ppm.

This value was under the maximum admissible European limit (MAL, 300 ppm) and the national limit. The fruits grown under the two transparent films (normal and with additives) also had nitrates concentrations under the MAL. Plants under the green, yellow and blue films had values of nitrate concentration above the MAL. We may

speculate that under these films a less intense nitrate metabolization was observed (Fig. 5).

CONCLUSIONS

1. Plant growth, yield and quality in tomato and lettuce were influenced by photoselective films used for covering the high tunnel.
2. The pink film treatment induced an increase of rosette weight and the yield for the lettuce plants compared with the control while for the rest of the treatments the weight significantly decreased especially for the blue film treatment.
3. The green and pink films increased the lettuce rosette diameter, while the yellow and transparent films decreased it compared with the control.
4. The number of tomato fruits per plant increased under the pink and yellow photoselective films, while the blue film inhibited the fructification.
5. The mean weight of the fruits was decreased by all films compared with the ordinary polyethylene film.
6. Tomato yield was significantly increased in plants grown under the pink film, while the blue and green films induced a very significant yield decrease.
7. Nitrate metabolism in tomato fruits was more intense for plants grown under the pink film as shown by their smaller values of nitrate concentration.
8. In contrast, fruits developed under green, yellow and blue films had fruit with increased nitrate concentration.
9. Pink and yellow photoselective films are recommended for high tunnel lettuce and tomato production to reduce environmental impact on plants.

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Tables

Table 1. The influence of films type on lettuce rosette weight

Treatment	Rosette weight (g)	Differences from:			
		control		averaged values for the variety	
		g	%	g	%
V1 –Mt – PE ordinary	319.3	-	-	17.5	**106**
V2 – transparent with additives	311.9	-7.4	98	10.1	**103**
V3 - yellow	315.6	-3.7	99	13.8	**104**
V4 - pink	322.4	3.1	**101**	20.6	**107**
V5 - green	281.2	-38.2	88	-20.7	93
V6 - blue	260.6	-58.8	81	-41.3	86
Mean for the variety	301.8	-17.5	94	-	100

Table 2. The influence of photoselective films on lettuce yield

Treatment	Rosette weight		
	t/ha	Tukey-Kramer *	Dunnett** (comparing with control)
V4 - pink	38.6	A	A
Control -V1 –Mt – PE ordinary	38.4	A	A
V3 yellow	37.9	A	A
V2 – transparent with additives	37.4	A	A
V5 - green	33.7	AB	A
V6 - blue	**31.3**	B	B

*Tukey-Kramer HSD □ = 0.05; $q^* = 2.93510$ Levels not connected by same letter are significantly different

**Dunnett □= 0.05; d = 2.57590

Table 3. The influence of films type on lettuce rosette diameter

Treatments	Rosette diameter (cm)	Differences from:			
		Control		Averaged values for the variety	
		(cm)	(%)	(cm)	(%)
V1 –Mt – PE ordinary	31.6	-	100	0.04	100
V2 – transparent with additives	28.7	-2.9	91	-2.88	91
V3 - yellow	29.6	-2.0	94	-1.96	94
V4 - pink	33.3	**1.8**	106	**1.79**	106
V5 - green	34.2	**2.6**	108	**2.62**	108
V6 - blue	31.9	0.3	101	0.37	101
Mean	31.5	-0.1	100	-	100

Table 4. Statistical interpretation of the results concerning the influence of films on rosette diameter

Treatment	Rosette diameter		
	cm	Tukey-Kramer*	Dunnett** (Comparing treatments with control)
V5 - green	34.2	A	B
V4 - pink	33.3	AB	A
V6 - blue	31.9	ABC	A
Control- V1 –Mt – PE ordinary	31.6	BC	A
V3 - yellow	29.6	CD	A
V2 - transparent with additives	28.7	D	B

*Takey-Kramer HSD; □= 0.05; Levels not connected by same letter are significantly different
**Dunnett - □ = 0.05;

Table 5. The statistical analyses on the tomato yield from high tunnel

Treatment	Yield (t/ha)	Differences from				significance
		mean		control		
		(t/ha)	%	(t/ha)	%	
V1 control	36.36	2.89	108	-	100	-
V2 transparent treated	31.10	-2.37	93	-5.26	85	o
V3 yellow	38.97	5.50	116	2.61	107	N
V4 pink	46.12	12.65	138	9.76	127	***
V5 green	27.34	-6.13	81	-9.02	75	ooo
V6 blue	21.22	-12.25	63	-15.14	58	ooo
Mean value	33.47	-	100	2.89	92	N

DL 5% = 3.08 t/ha; DL 1% = 5.45 t/ha; DL 0.1%= 8.20 t/ha

Figures

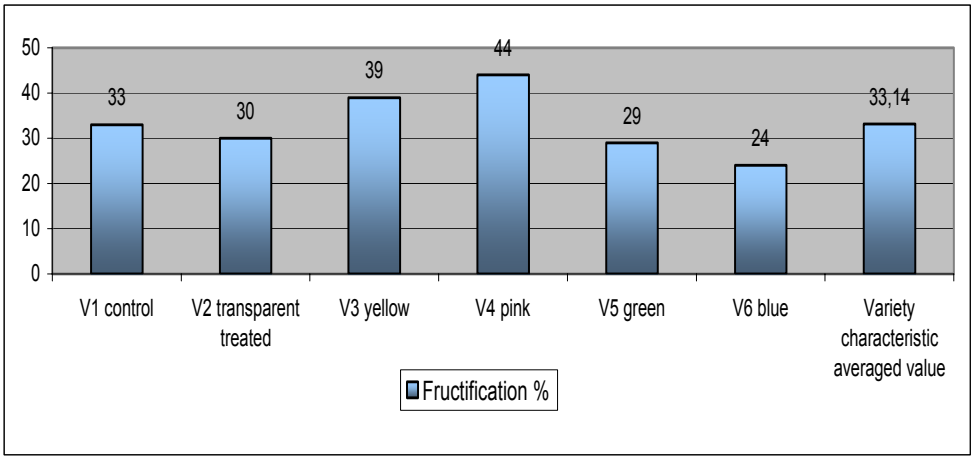

Fig. 1. The influence of photoselective films on the fructification of the tomato plants cultivated in high tunnel

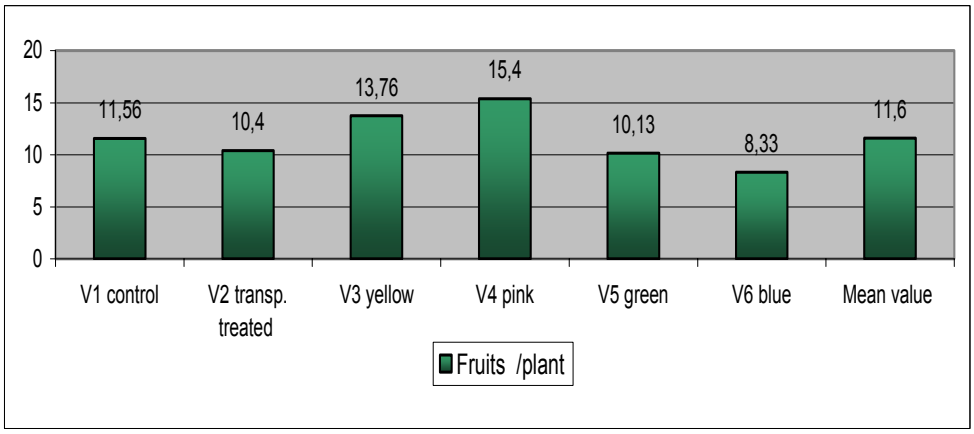

Fig. 2. The influence of photoselective films on the number of fruits

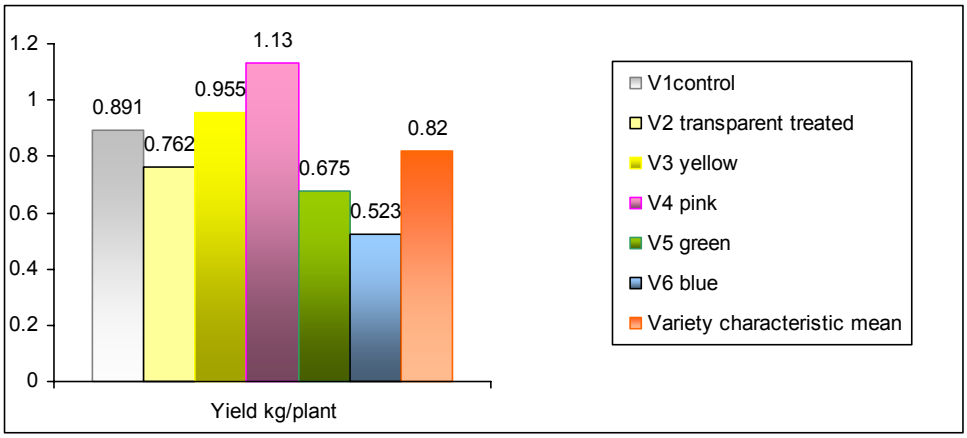

Fig. 3. The influence of photoselective films on the tomato plants yield

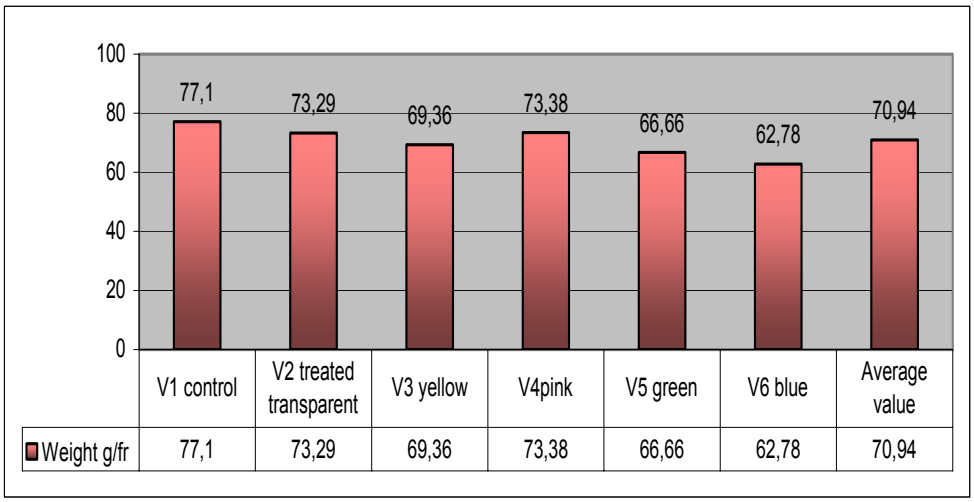

Fig. 4. The influence of photoselective films protection on mean weight of tomato fruits

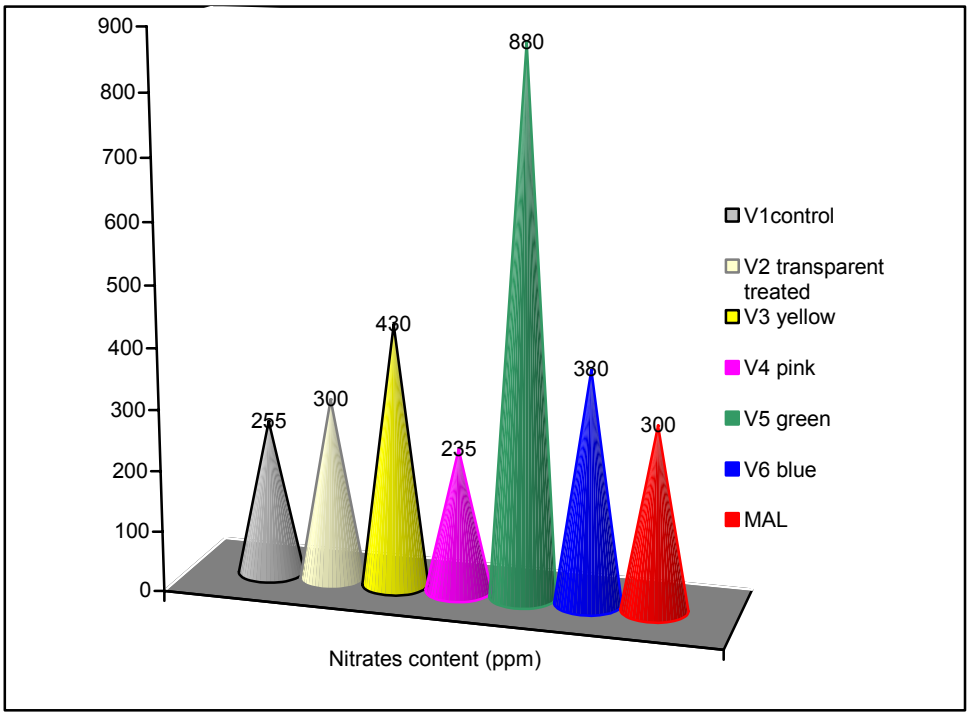

Fig. 5. The influence of photoselective films protection on the nitrates content of tomatoes fruits at harvest period

Behaviour of some green pepper lines (C*apsicum annuum* l. Var. *Grossum* Sendt) tested in the comparative plots for evaluation

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Keywords: pepper, lines, early yield, total yield.

ABSTRACT

During the 2004-2006 period in the frame of the experimental field of RDIVFG VIDRA 5 green pepper lines created at the Plant Breeding Department of the Institute were investigated under comparative plots for evaluation As control the green pepper variety Export was used. Several observations and morphological determinations were carried out aiming the following characteristics: early yield, total yield, number of fruits per plant, their shape and color. The results emphasized that the best behaviour had the lines L 250 by comparison with the control variety, yielding 10-12 fruits per plant of large size (150-170g) and yellowish green color. This line revealed a total yield higher than the control variety (Export) while its early yield had a weight of over 75% from the total yield.

INTRODUCTION

Green pepper represent one of the vegetable species of great importance for the mankind nutrition due to the high nutritional value of the fruit, namely by its high content in sugars and vitamins, especially in ascorbic acid (200-250mg/100 g fresh product)

Owing to its fruits quality, this species has a great importance both for the fresh consumption and processing for obtaining pf tinned food.

On the world scale, there were and there are a great number of varieties and hybrids in green pepper assortment bread in the different parts of the world. But their differ it reaction of behaviour in our local condition of our country and their introduction in our growing in a wise way, taking into account their ecological plasticity lead to the conclusion that it was and still remains the necessity for the development of the breeding works in green pepper in order to assure a national assortment specific for our country.

Green pepper breeding nowadays aims mainly to obtain high productivity green pepper varieties and F_1 hybrids having multiple diserse resistance characterized by fruits of medium or big size of light color at the maturity for consumption-according to the consumer preferences from intense green color to ivorine white color .

This paper aims to emphasize our achievements in the field of green pepper breeding carried out RDIVFG Vidra during the last years.

MATERIAL AND METODS

The research works carried out at RDIVFG Vidra during the 2004-2006 periods in the frame of program green pepper breeding. Five green pepper lines which were developed in the frame of the Institute were studied in the comparative plots for their behaviour using as a control variety the cultivar Export.

The variants were set up in randomized blocks placed in 4 replications and during the growing season several observations and determination were performed according to U.P.O.V. file.

The germoplasm resources used in the frame of green pepper breeding process in order obtain of this material consisted in native and/or local populations as well as varieties and hybrids brought from Holland, France and Hungary.

As breeding methods bulk positive selection yearly resumed in the valuable populations, intraspecific pollination among valuable varieties and lines followed by the pedigree selection and bulk positive selection yearly resumed in the advanced populations were used.

RESULTS AND DISCUSSION

By comparison of the data regarding the total yield of the green pepper lines under investigation (average of the 2004-2006 periods) one could notice that all the 5 lines assured a higher total yield to the control variety Export.

The values for total yield ranged between 47.8 t/ha in the line 32 and 49.5 t/ha in the line L 250 by comparison with control variety Export that gave 29.5 t/ha.

The differences of the yield among green pepper lines by the comparison with the variety Export were computed as statistically sure, they being characterized as very significant (table 1).

Early crop harvested up to the 31 of July recorded a higher value by comparison with Export.(20.2 t/ha in the line 12; 31.5 t/ha in the line 259 white the check variety reached an early crop of 17.17 t/ha).All the 5 green pepper lines assured positive early crops, statistically assured, for example distinct significant in the line 12 and very significant for the other lines (table 2).

On could notice that the weight of the early crop in over 50%from the total yield in the case of the two variants, line 259 and line 32 (figure 1). From the table 3 one could conclude that regarding the variability of the main morphological traits of the fruit, the diameter of the fruit recorded a low variability coefficient for all the variants ranging between 5.87% and 9.07% while the trait fruit length varied between 6.52% and 12.08% but the pericarp width varied from 8.07% to 14.4%.

CONCLUSIONS

By this study carried out in 2004-2006 in the frame of a comparative crop plot for evaluation, regarding several green pepper lines developed at RIDVFG Vidra, one could conclude that all the lines recorded absolute values whitch were higher for the early and total yield than the control variety Export, and on the other hand, the yield differences had a statistical significance. Regarding early crop, one could emphasize the line 259 which gave a yield of 75% from its whale yield (31.5 t/ha up to the 1st of August, so it surpassing the check variety with 2 tone (29.5 t). Also, one could emphasize the line 141 having an early yield of 26.5 whichmeans 90% from the total yield of the control variety Export. Regarding the totalyield, one could emphasize the lines 250 and 141 which gave a yield gain of 20 and 19.4 t respectively by the comparison with the control variety Export. Considering the shape and fruit colour one could render evident the lines 259 and 141 having fruit of medium size (average weight 110 g) characterized by light colours, well appreciated by consumers, of yellowish – white and yiellowish green respectively. The line 250 having large fruit of 230 g in light green colour without pistil concavity is also remarkable.

ACKNOWLEDGEMENTS

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Table 1. Total yielding of some green pepper cultivars (comparative culture plot during the 2004-2006 period)

VAR.	Specification	Absolute total yield (t/ha)	Relative total yield(%) (t/ha)	Crop gain against ct(t/ha)	Significance
1	EXPORT (Mt)	29.5	100.0	-	-
2	L250	49.5	167.79	+ 20.0	***
3	L12	40.9	138.64	+ 11.4	***
4	L259	42.0	142.37	+12.5	***
5	L141	48.9	16.57	+ 19.4	***
6	L32	47.8	162.03	+18.3	***

DL 5% = 5.9 t/ha
DL1% = 7.8 t/ha
DL 0.1% = 10.1 t/ha

Table 2. Early yielding of some green pepper cultivars (comparative culture plot during the 2004-2006 period)

VAR.	Specification	Absolute total yield (t/ha)	Relative total yield(%) (t/ha)	Crop gain against ct(t/ha)	Significance
1	EXPORT (Mt)	17.7	100	-	-
2	L 250	21.13	119.37	+3.43	***
3	L 12	20.2	117.64	+2.5	***
4	L 259	31.5	177.96	+13.8	***
5	L 141	26.5	149.7	+8.8	***
6	L 32	24.5	134.41	+6.8	***

DL 5% -1.2 t/ha
Dl 1% - 2.3 t/ha
DL 0.1% -3.9 t/ha

The main morphological characteristics of the fruit

V	Specification	Fruit shape	Topshape	Fruit lenght (cm))			Fruit diameter (cm)			Pericarp width (mm)			Number of seed rooms	Fruit place on the plant	Fruit color In	
				X	S	S%	X	S	S%	X	S	S%			Technological stage	Physiological stage
1	Export	prismatic	deepened	9.52	0.7	7.35	6.24	0.37	5.92	5.66	0.81	14.4	3-4	erectă	Green yellowish	red
2	L250	pyramidal	sharpened	10.89	0.82	8.11	7.29	0.58	8.05	6.64	0.73	11.3	3-4	pendulă	Green yellowish	red
3	L12	conical	sharpened	10.2	0.8	8.6	6.23	0.36	5.87	6.69	0.85	12.7	3-4	erectă	White yellowish	red
4	L259	conical	sharpened	9.9	1.11	11.2	7.3	0.58	7.94	7.8	0.63	8.07	3-4	erectă	White yellowish	red
5	L141	conical	sharpened	9.5	0.62	6.52	6.15	0.46	7.47	8.3	0.82	9.87	3-4	pendulă	Yellow greenish	red
6	L32	pyramidal	sharpened	9.04	0.49	5.42	6.20	0.57	9.07	7.1	0.66	9.29	3-4	erectă	Green yellowish	red

The influence of nitrogen and sulfur complex on the spreading of *Ceutorhynchus assimilis* (Paykull) in canola crops

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Keywords: cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull), canola crops, nitrogen concentration, sulfur concentration, spatial distribution.

ABSTRACT

The incidence of a pest in a particular crop is not an isolated event and must be studied in relation to a larger area. The study investigates the influence of nitrogen concentration and sulfur concentration context on cabbage seedpod weevil (*Ceutorhynchus assimilis*) density in canola crops and how does this relationship can affect the pest density. The goal of the study is to establish the correlation between leaf tissue nutrients concentrations and cabbage seedpod weevil dispersion and to construct a predictive model of spread of cabbage seedpod weevil in canola crops. The research was conducted in a canola crop (*Brassica rapa*) before the mating period of cabbage seedpod weevil in June. The density of cabbage seedpod weevil was found to be in a strong relationship with the concentration of these two nutrients. The findings emphasize the strong link between nitrogen concentration pattern and insects and a week relationship between sulfur and insect density.

INTRODUCTION

Ceutorhynchus assimilis is one of the most significant insect pests of canola and rapeseed in Europe and North America (Cárcamo *et* al. 2001; Dosdall *et* al. 2002). The present techniques used for controlling the level of cabbage seedpod weevil in canola crops need to be improved by increasing the accuracy of prediction in weevil populations and their dynamics in crop during the mating period.

The mating takes place during end of May to the beginning of June. Females chew in pods holes in which oviposit the eggs (Kalischuk and Dosdall, 2004). After oviposing the female extends the ovipositor and deterring a pheromone (Kozlowsky *et* al. 1983). Foraging behavior showed that the insects may be able to locate the plant patches more easily than single plants. Host plant specific stimuli are more pronounced from patches, and therefore, these plants are more conspicuous for foraging and mating insects.

It was establish that the soil nutrients or fertilization can increase or decrease insect populations on the plants. Plant chemical defense systems have an impact on specialist vs. generalist insect herbivores (McLeod, 1962). The glucosinolate-myrosinase system protects plants from herbivore damage (Haughn *et* al., 1991). Glucosinolates and their hydrolyzing agent, myrosinase, are spatially separated within plant cells. When the cell is disrupted, myrosinase cleaves the sugar from the glucosinolate, and a series of toxic compounds are released. These toxins include nitriles, isothiocyanates, oxozaladines, and epithioalkanes. Glucosinolates were demonstrated to be feeding stimulants for specialist herbivores (Thorsteinson, 1953). The glucosinolates are a sink for nutrients like nitrogen and sulphur (Haughn *et* al., 1991). Due to this feature a large quantity of nitrogen or sulfur indicate a large resource

for plants for secreting glucosinolates as a response to feeding behavior of insect pest and, in the same time, an increasing level of foraging stimuli.

The study tested the relationship between the local nitrogen concentration and sulfur concentration context and pest densities. The connection tested was used as a predictor for weevil distribution and density level in the crop.

The different concentrations of nitrogen and sulfur and the physiological mechanisms in plants imposed different densities of insects among the crop.

This study addressed whether the local occurrence of pest insects can be predicted from data on nutrients in leaf tissue. The model predicted that a different gradient in nutrients concentration is the main cause of gradient density in weevil population.

MATERIAL AND METHODS

The study area was in one commercial crop of *Brassica rapa* was selected for testing. The average temperature for June was 25ºC and was not significant precipitation. The study area was situated within the crop with the two edges of the grid bordering a grass ditch and the other two inside the crop. The extend was 80 m on 110 m and area cover is about 8800 m^2 for each crop. An 8 x 11 grid was laid out in crop. A sampling unit was placed at the center of every unit within the grid pattern for a total of 88 sampling units within crop. Each sampling unit consisted of a bowl trap, a bracket, and a metal post. In each unit of the grid were recorded the abundance of weevils in traps and leaf tissue nutrients (nitrogen and sulfur). The traps were installed in June and were removed after a week. In the study area, 88 points were sampled. The data obtained for nitrogen and sulfur concentrations were measured based on laboratory analyses of leaf tissue plants.

The analysis selected had few enough parameters to be precise but enough to avoid bias, and that were scientifically acceptable and supported by the data. To test the correlation between cabbage seedpod weevil density and nitrogen and sulfur concentration in plant leaf tissue, it was used Pearson correlation coefficient.

It was used semivariograms to assess the spatial continuity of data. The extensive semivariogram modeling subsystem was used as an integrated data analysis tool for assisting in selecting the appropriate model for gridding with the kriging algorithm.

The GIS ArcMap 9.1 (Environmental Systems Research Institute) was used to create point maps of weevil density, N and S concentrations. These point maps were used to create maps of density, N and S concentrations. For building the predictive map of density of cabbage seedpod weevil in crop was used the Ordinary Kriging as geostatistical method and Prediction Map option. The trend in data was removed.

For semivariogram modeling was used the model Hole Effect and consider an isotropic model. The isotropic model was chose because it was considered that wasn't a directional influence that affects the points. The movement of individuals was considered to be without a preferred orientation. To provide an accurate prediction was performed the cross validation tool to test the robustness of the model used.

RESULTS AND DISCUSSIONS

Ceuthorhyncus assimilis was widespread among the crop. Averaged after a week, adults per sample (mean ± SE = 17,78 ± 11,44) was higher in the center of the study area (*max* = 66).

The nitrogen and sulfur showed different patterns. The quantity (g/100g leaves dry mass) of nitrogen and sulfur indicated a different spatial distribution among the crop. The highest concentration of N in leaves tissue was 4,950 (g/100g leaves dry mass) (mean ± SE = 3,205 ± 0,546) while the minimum was 2,400 (g/100g leaves dry mass). The higher concentrations were in the center and in the eastern side of the crop. The sulfur had the highest concentrations in different locations of the crop comparing with nitrogen. The highest concentration of sulfur in leaves tissue was 2,910 (g/100g leaves dry mass) (mean ± SE = 2,025 ± 0,634) while the minimum was 1,100 (g/100g leaves dry mass).

Pearson correlation was measured between weevil density and nitrogen and between weevil density and sulfur. The results of data field analysis from June, before mating period, displayed two main trends. The results indicated a negative correlation between the S concentration in leaves and weevil density in crop and a positive correlation between N concentration and weevil density. Pearson correlation coefficient performed between S concentration and weevil density was (r = -0,526, df = 6, α = 0,05) (Fig. 1) and for N concentration and weevil was (r = 0,672, df = 9, α = 0,05) (Fig. 2). The nitrogen and sulfur were negative correlated (r = - 0,792, df = 6, α = 0,05) showing a different trend. The overall trend apparent in the semivariogram for weevil density (Fig. 3) was driven primarily by north - south variation, as supported by the 90□directional semivariogram, in which the variation among points increases almost with distance from the center to the border (direction: 0.0; number of lags: 10; lag distance: 10; lag tolerance: 5; angular tolerance: 90,00)

The semivariogram of weevil density indicated that large-scale spatial trend was driven primarily by the north - south gradient like the nitrogen trend (Fig. 4).

The semivariogram for sulfur suggested a presence of additional spatial structure not related to the north-south gradient. For spatial data, the semivariogram increased with distance until it converges at a "sill," which corresponds to the population variance.

The distance at which the sill was reached and data were no longer autocorrelated corresponded to 50 m distance in the crop for weevil density and 50 to 60 m for N concentration. This range may be interpreted as a *maximum* autocorrelation distance, while autocorrelation is generally strongest at the distance for which the semivariogram slope is steepest (Robertson, 1987).

In zonal anisotropy the form covariance structure has to be different in different directions. In particular, the sill has to be different for different directions. Results indicated that there was a variation of the sill for different directions indicating that the model was slightly anisotropic.

The species exhibited distinct large-scale spatial trends that appear to follow the nitrogen concentration trend. This trend occurred in the north – south direction and in the east-west direction as suggested by their distribution maps (Fig. 5A).

The investigation of spatial structure in weevil density based on visual inspection of maps indicated that the species appeared to vary from center to borders. The nitrogen concentration map showed the highest concentration in the center of the map and the second high concentration level from the center to the west side. The juxtaposition of the weevil density and N concentration map displayed the same pattern.The sulfur map indicated a high variability of concentration among the crop with two locations with high S level: in the southern part of the map and in the center – west. There were also average concentrations in the center and on north-south direction.

The spots with lower S level were displayed in an area with a high weevil density (Fig. 5B).

CONCLUSIONS

The weevil density, in canola crop, examined in this study demonstrated significant population responses to nitrogen concentration at small spatial scales, indicating that their abundances may be affected by changes in nitrogen concentration in plant tissues.

The weevil population exhibited spatial trend in their abundance, coinciding with the north-south nitrogen gradient. The sulfur concentration was negative correlated with weevil density and displayed a different spatial pattern.

The negative correlation between nitrogen and sulfur concentration in leaves tissues could be explained by the oxidative process that occurred in cells. The higher concentration of nitrogen indicates a higher quantity of glucosinolates in plants and consequently a higher level of feeding stimuli. This is associated by cabbage seedpod weevil with a high quality of food.

Spatial autocorrelation can be interpreted as indication of the ecological neighborhoods for a species that is, the "regions of activity or influence during periods of time appropriate to particular ecological processes" (Addicott *et* al., 1987).

Also other potential explanatory variables could certainly be measured. The small-scale autocorrelation, which has a large stochastic component, is difficult to predict. Thus, in the absence of some variables, the use of spatial model can ensure that conclusions are valid for the explanatory factors examined (Wiens, 1989).

This study provides an example of how spatial methods can be used to examine spatial structure in analyzing relationships between spatially distributed variables.

The method used helped confirm previous conclusions regarding the influence of nutrients on weevil abundance, while also providing further insight into the spatial structure of the pest population examined.

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Figures

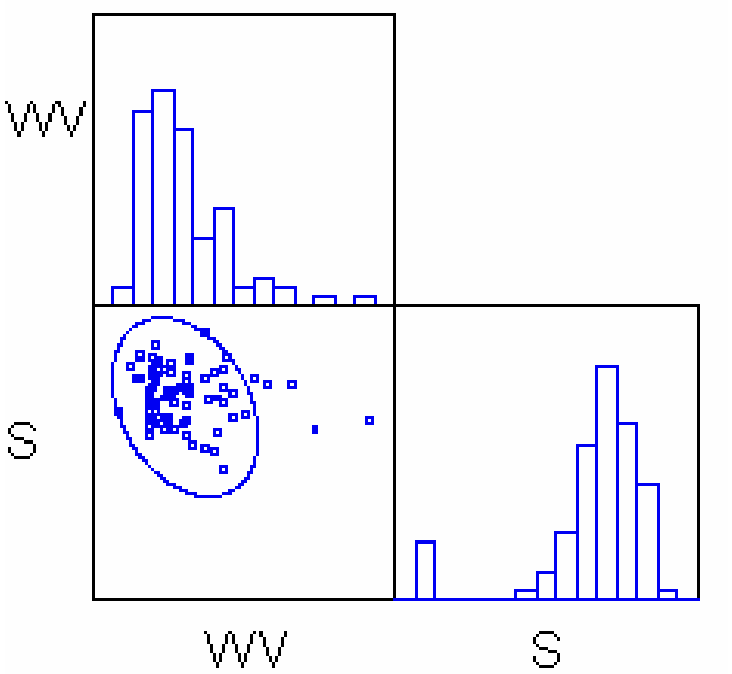


Fig. 1. The correlation between the sulfur (S) concentration in leaves and weevil density (WV)

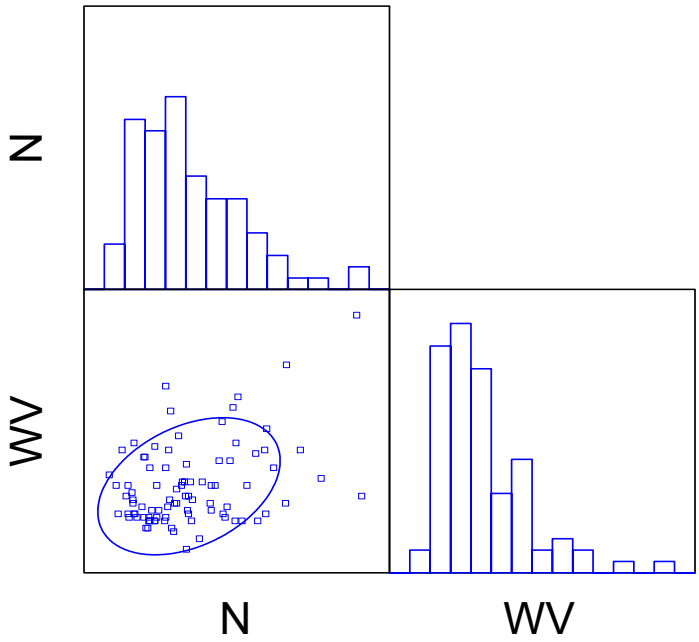


Fig. 2. The correlation between the nitrogen (N) concentration in leaves and weevil density (WV)

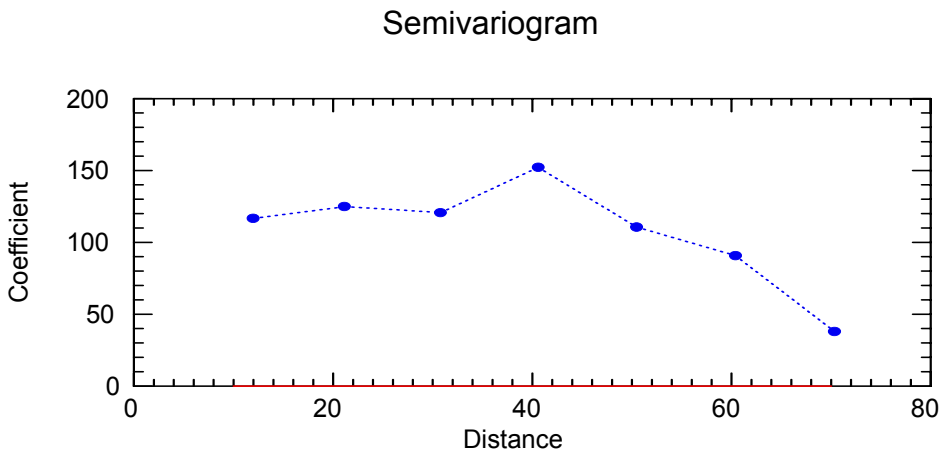


Fig. 3. Semivariogram for weevil density in crop in June

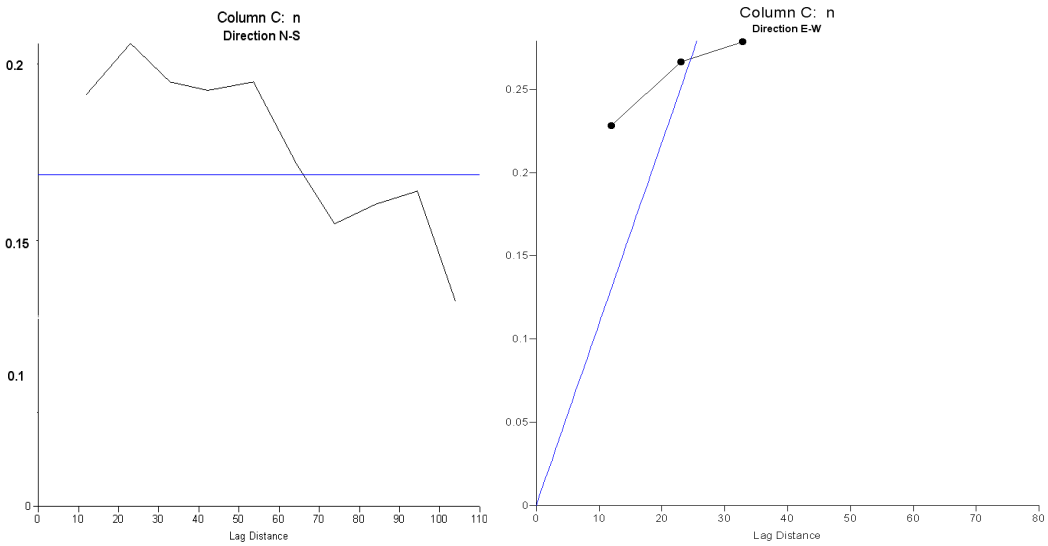
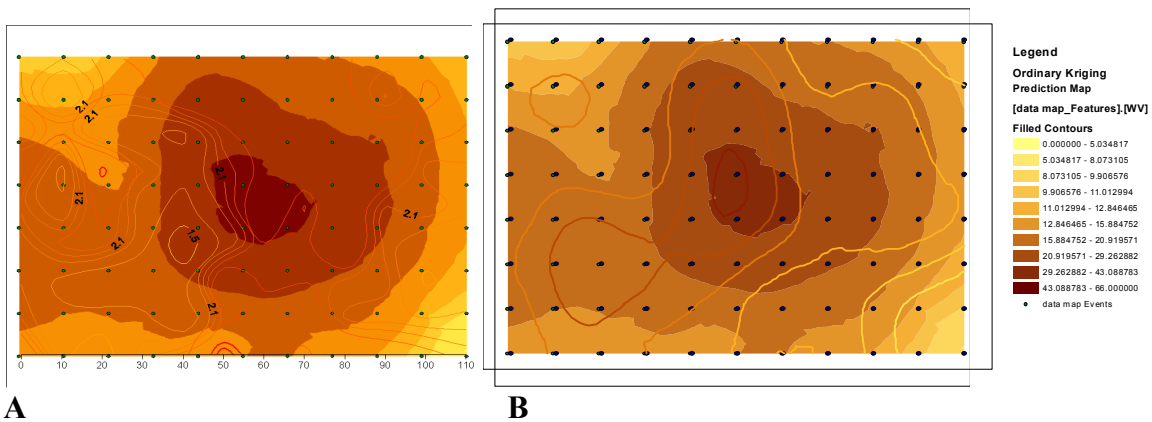


Fig. 4. Directional Semivariogram for nitrogen concentration after N-S and E-W direction



A **B**

Fig. 5. Weevil density map (solid colour) and sulfur and nitrogen concentration map (the higher S and N concentrations the red isoclines)

Larval development and vegetal biomass consumption of *Trichoplusia ni* (Hübner) under temperature influence

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Keywords: *Trichoplusia ni* (Hübner), temperature, food selection, larval survival, *Brassica napus*, *Brassica rapa*, *Pisum sativum.*

ABSTRACT

The study investigated the role of temperature as one of the principal factors in delimitating survival and feeding of larval stages of *Trichoplusia ni* (Hübner). The preference and selection of food were tested on three host plants *Brassica napus, Brassica rapa* and *Pisum sativum* under the high and low level temperature. It was performed the calculation of daily leaves consumption for each Petri dishes per larva. The data of development and survival was used for plotting the survival rate for each temperature and type of food. All three plant species supported the development from first instar to the fifth instar and the beginning of pupation. ANOVA results indicated a level of statistical significance between three type of food on No-choice and Multi-choice feeding in high temperature and in a low temperature.

INTRODUCTION

The cabbage looper - *Trichoplusia ni* (Hübner) is a major pest with a large distribution among cruciferous crops. The cabbage looper feeds on a wide variety of cultivated plants and weeds. It feeds readily on crucifers, and has been reported damaging broccoli, cabbage, cauliflower, Chinese cabbage, mustard, radish, and turnip (Shorey, 1963). Not all hosts are equivalent for larval development and survival (Shorey *et* al., 1962). Soo Hoo *et* al. (1984) conducted one of the most complete studies of relative suitability, and reported that only about one-third of the plants tested were suitable for complete development of larvae.

Larval development may be completed in two weeks if weather is favorable, and the cabbage looper can have three or more generations per year in the northern (Shorey, 1963). With warm temperatures, development of all cabbage looper stages - from egg to adult - takes about 18 to 25 days. Looper activity (egg laying and larval feeding) will decrease as cool weather (13˚C) becomes common. There are three to five generations per year and activity continues through most of October in most years (Shorey, 1963).

Temperature is one of the principal factors delimitating survival and reproduction of insects. The presence of temperature stress has resulted in a wealth of physiological and behavioral adaptations that have evolved to ameliorate or avoid the impact of low or high temperature (Bale, 1987). Physiologically insects prepare for temperature variation in temperate climates by increasing the concentration of some specific proteins in hemolymph and arresting development at a certain tolerant stage (Bones and Rossiter, 2006) series of proteins produced in response to extreme temperatures increase the tolerance of organism to further stress.

MATERIALS AND METHODS

The individuals were reared in the lab colony. During the experiment the larvae were reared in small plastic dishes. One of the problems was to retain enough moisture, but to avoid allowing the early instars to escape or suffocating later instars. The dishes were ones with a loosely-fitted lid. For the first instar the lid was kept tight. When the larvae had completed several molts, the lids were untied. The small dishes permitted to make observations through.

The specimens were held in a laboratory incubator at a temperature of 14 ± 0.5 ºC and 26 ± 0.5 ºC. The relative humidity (r.h.) at these temperatures was 65% for high temperature and low temperature lot. Additional humidity conditions in the Petri dishes were achieved by placing moist filter paper. Inside each dish was used a circular filter paper with several drops of water to prevent the desiccation of vegetal material. Periodically the filter papers were removed and replaced with new other.

There has been no noticeable loss of vigor during the period of this study. The moths were reared at ambient temperature and humidity on a 24 hr without daylight cycle.

The artificial diets were provided for first instar after hatching for three days. This diet was used for rearing the stock culture.

During the experiment was used natural diet for *Trichoplusia ni* larvae. The individuals received periodically fresh leaves. The diet consisted of *Brassica napus, Brassica rapa* and *Pisum sativum.*

The experiment was No-choice feeding and Multi-choice feeding experiment.

In each Petri plate were placed 5 individuals in first instar. In incubators were placed 2 replicates for each type of food for No-choice feeding and 2 replicates for Multi-choice feeding in incubators at a temperature of 14 ± 0.5 ºC and respectively at 26 ± 0.5 ºC. In addition four Petri plates were placed with vegetal material for desiccation control at high temperature and four at low temperature (Table 1).

For each Petri plate were recorded the mass of leafs consumed periodically and the survival rate and development. In control lots the leaves were weight periodically to establish the desiccation rate.

Weigh leaves data from control was recorded and used to establish the rate of desiccation during 24h for each temperature lot and for each plant species.

Weigh leaves data from each temperature lot with both No-choice feeding and Multi-choice feeding was recorded and was subjected to transformation by subtracting the specific percent of desiccation for each plate.

Was performed the calculation of daily leaves consumption for each Petri dishes per larva. The data of development and survival was used for plotting the survival rate for each temperature and type of food.

The data of leafs consumption from high and low temperature and No-choice and Multi-choice feeding were used in ANOVA two factor analyses for comparison.

RESULTS AND DISCUSSIONS

The desiccation rate varies in small proportion from a species to other: *B. rapa* > *B. napus* > *P. sativum* and from a plate to other (± 1.5%). The desiccation was higher in high temperature lot with 2.7%. The values of desiccation were subtracted from the total quantity of leaves consumed in each plate.

The results showed an increasing consumption according with the developmental rate. The total leaves consumption was higher in high temperature lot

being stimulated by the higher metabolic rate of individuals (Figure 2). In low temperature lot, No-choice feeding experiment, the larvae from the *B. napus* and *B. rapa* diet consumed a higher quantity of leaves (Figure 1A). In Multi-choice feeding the larvae consumed a higher quantity of leaves than in No-Choice diet and selected in principal *B. napus* and *B. rapa* (Figure 1B). The weight leaves data indicated a constant consumption of *P. sativum* even the species is not a frequent food in nature for *Trichoplusia ni* (Figure 1).

The average consumption of leaves per larva in 24h in Multi-choice feeding had an increasing trend earlier than in No-choice feeding indicating a difference in rate of development of larvae. The complex physiological response activates the selection of food in Multi-choice feeding being emphasized by three peaks in the different species leaves consumption (Figure 1B). The No-choice feeding rate had a similar trend for all three plant species in accordance with developmental rate.

The high temperature lot consumed in the same period of time about 8 times more food than the low temperature lot. The high temperature lot indicated an increasing similar consumption of plant leaves for both No-choice and Multi-choice feeding (Figure 2A and 2B). The average consumption per larva in 24h increased earlier that in low temperature lot. In high temperature lot, in both No-choice and Multi-choice feeding experiment, the larvae from the *B. napus* and *B. rapa* diet consumed a higher quantity of leaves (Figure 2). The overall difference in leaves consumption for all three plant species was very small. The weight leaves data indicated a constant consumption of *P. sativum* like in low temperature lot. The high temperature increased the metabolically rate of individuals masking or inhibited the food selection mechanism.

Larvae of *Trichoplusia ni* developed from first instar to fifth instar on all three plant species. The first and second instar had a normal molting and development with a very small mortality rate in both high and low temperature conditions. The high temperature lot has a higher rate of survival through all larval development period with a small mortality in forth and fifth instar but they failed to begin the pupation.

The mortality in low temperature lot was higher but 10% of larvae started pupation. The pupation was unsuccessful.

The rate of survival of specimens in low temperature lot in No-choice feeding and Multi-choice feeding conditions from the first instar to pupation indicated a higher survival rate on Multi-choice feeding (Figure 3) while the rate of survival for high temperature lot indicated the same trend for both No-choice and Multi-choice conditions. Survival of larvae from low temperature indicated a lower mortality on *B. napus* and *B. rapa* from second to fifth instar but a higher proportion of larvae that started pupation on Multi-choice feeding. The *P. sativum* showed a higher mortality than other diets and a small proportion of individuals that started to pupate.

Individuals reached pupation significantly faster on Multi-choice feeding and on *B. napus* in No-choice feeding. There was a little difference in developmental time through the first, second and third instar on all diets. The forth and fifth instar began earlier on Multi-choice feeding and *B. napus*.

ANOVA results indicated a high level of statistical significance between three type of food on No-choice and Multi-choice feeding in high temperature (Table 2) and in a low temperature (Figure 3). The leaves consumption pattern was not different in high temperature lot between No-choice and Multi-choice feeding (S = 0.331166 R-Sq = 0.27% R-Sq(adj) = 0.00%) among the diets (Table 2). The biomass consumption indicated no difference between types of feeding in high temperature lot. There was a

difference between different diets supported by the small quantity of *P. sativum* leaves consumed (Table 2). In low temperature lot the leaves consumption pattern was significantly different between No-choice and Multi-choice feeding among the diets (S = 0.0603310 R-Sq = 11.28% R-Sq(adj) = 7.37%) (Table 3).

CONCLUSIONS

While *Trichoplusia ni* has not been reported to feed on *P. sativum* in the wild, it is not unreasonable to think that it would do so if given the opportunity. As a polyphagous insect herbivore that has been introduced in many parts of the world, cabbage looper feeds on many species that were not its original hosts (Vail *et* all, 1991). The feeding behavior of cabbage looper on *P. sativum* was similar to that on the natural host, *Brassica sp*.

The cabbage looper showed a strong preference for the *B. napus* in both No-choice and Multi-choice plant feeding experiments and there was a significant difference between *B. napus*, B rapa and *P. sativum* in leaf consumption.

Even the survival rate and pupation on *P. sativum* were smaller in comparison with *B. napus*, and *B. rapa* (Soo Hoo *et* al, 1984). The selection of *B. napus* was an active process during the experiment period while the lack of polyunsaturated fat in *P. sativum* decrease the survival rate of individuals comparing with the survival rate on *B. napus* and *B. rapa* on both No-choice and Multi-choice feeding. This selective behavior could be correlated with a physiological response. Insects have a complex behavioral and physiological response to counter the effects of temperature variations and the selection of both *Brassica* species could be an effective way to compensate a lack of chemical compounds necessary for pupation.

The total quantity of leaves consumed was higher in Multi-choice feeding conditions in both low and high temperature lots supporting the polyphagous characteristic of species. Data from laboratory gave the evidence of this characteristic but all the plants accepted as food were not capable to support entire development (Soo Hoo *et* al., 1984).

In field conditions older larvae could easily migrate and disperse from host plant to other capable to sustain the end period of growth and development. A choice experiment with leaf was made leaves showed that cabbage looper larvae exhibited a significant preference for the older leaves (Vail *et* all, 1991).

Due to the trophic features of species, the Multi-choice feeding provided a higher rate of survival. The average daily consumption also indicated that the food selection in Multi-choice feeding was present only in low temperature while in high temperature conditions the higher metabolic rate masked or inhibited the selection mechanism.

The cabbage looper therefore proved to be an active physiological and behavioral mechanism to temperature variations.

Due to the fact that almost all phytophagous insects respond to cold and heat by regulating the level of polyols, sugars, stress and thermal hysteresis proteins the future experiments of food selection have to consider a broader range of host plants based on chemical composition of vegetal tissues.

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Tables

Table 1. The experimental design used for rearing *Trichoplusia ni* larvae

	Low Temperature				**High Temperature**			
	No-choice feeding			**Multichoice feeding**	**No-choice feeding**			**Multichoice feeding**
Control	*B.napus*	*B. rapa*	*P.sativum*	Mixed	*B.napus*	*B. rapa*	*P.sativum*	Mixed
R I	*B.napus*	*B. rapa*	*P.sativum*	Mixed	*B.napus*	*B. rapa*	*P.sativum*	Mixed
R II	*B.napus*	*B. rapa*	*P.sativum*	Mixed	*B.napus*	*B. rapa*	*P.sativum*	Mixed

Table 2. The analyze of variance for high temperature lot with two factors (No-choice and Multi-choice feeding)

ANOVA						
Source of Variation	**Seq SS**	**df**	**Adj SS**	**Adj MS**	**T**	**P**
Temperature	0.0037	1	0.0037	0.0037	0.03	0.856
Diets	0.0057	2	0.0057	0.0028	0.03	0.974
Error	3.5095	32	3.5095	0.1097		
Total	3.5188	35				

Table 3. The analyze of variance for low temperature lot with two factors (No-choice and Multi-choice feeding)

ANOVA						
Source of Variation	**Seq SS**	**df**	**Adj SS**	**Adj MS**	**T**	**P**
Type of feeding	0.01293	1	0.01293	0.01293	3.55	0.064
Diets	0.01853	2	0.01853	0.00926	2.55	0.086
Error	0.24750	68	0.24750			
Total	0.27898	72				

Figures

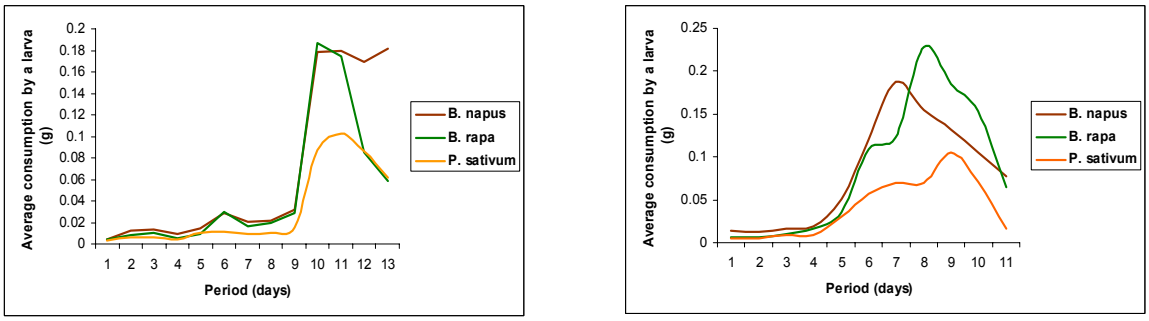

Fig. 1. The average consumption of leafs by a larva from a No-choice feeding (A) and from Multi-choice feeding (B) lot in low temperature

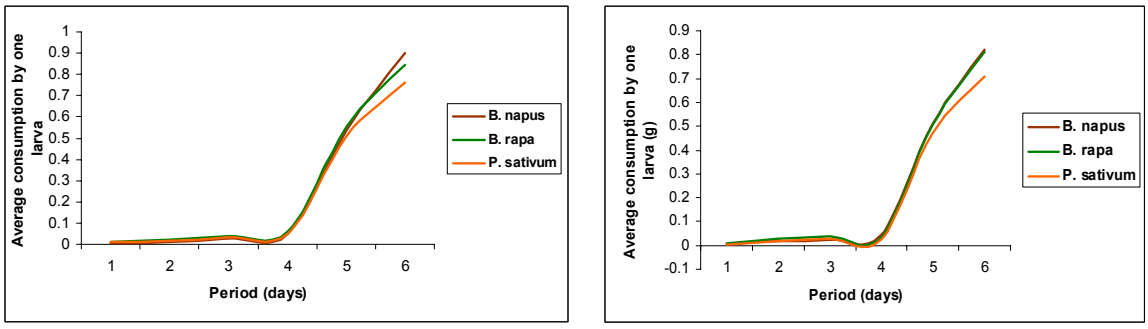

Fig. 2. The average consumption of leafs by a larva from a No-choice feeding (A) and from Multi-choice feeding (B) lot in high temperature

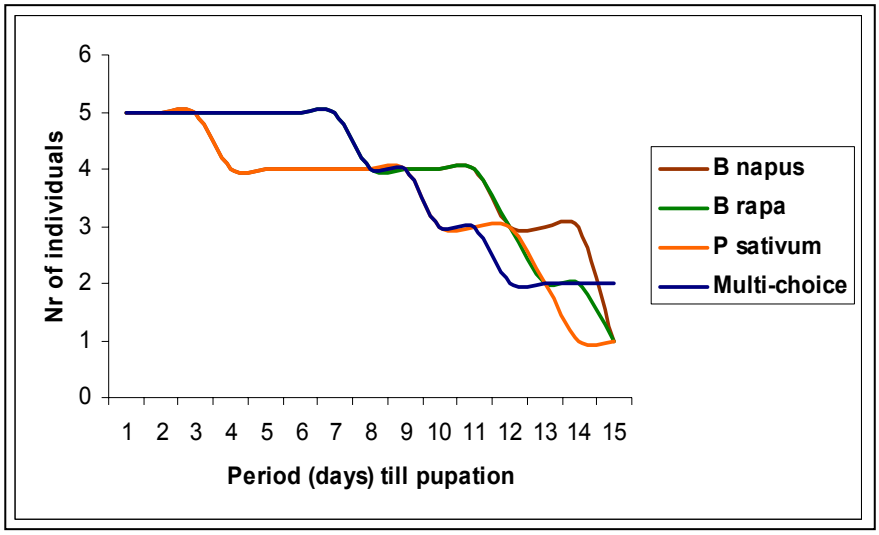

Fig. 3. The rate of survival of specimens in low temperature lot in No-choice feeding and Multi-choice feeding

The influence of fertilization with green manure and husks of grapes compost in organic garden bean crop

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Keywords: agrochemical characteristics of the soil, yield and quality

ABSTRACT

In order to maintain an optimum condition regarding the level of nutritive supplies of the soil in the organic vegetable technology, the fertilization basis is the organic fertilizers. In this context, at the Banu Mărăcine Research Station of the University of Craiova, they studied the influence of the soybean (*Glycine max*) + fodder radish (*Raphanus sativus oleiformis*) as green manure (the control) and of the husks of grapes compost (15 t/ha and 25 t/ha) on the agrochemical characteristics of the soil and also on the yield and the quality of the garden bean crops. The experience was laid out on a field cultivated in the last two years using organic technologies (2005-2006). The green manure, used as the only fertilizer or together with the husks of grapes compost, improves the soil fertility, by increasing the contain in humus from 2.50% to 3.16-4.00%, in total nitrogen from 0.131% to 0.216-0.259%, in mobile phosphorus from 68 ppm to 86.4-92.0 ppm and in mobile potassium, from 205 to 252-404 ppm. The 25 t/ha husks of grapes compost doses, applied together with green manure, determines the highest yield level, 14.8 t/ha. The positive yield difference, by 3.1 t/ha (26.5%), compared with the control, is significant from statistical point of view. Concerning the nitrate accumulation, the level of 128-195 ppm is situated under the maximum accepted concentration (200 ppm).

INTRODUCTION

The promoting of the vegetable crop organic technologies provides the sustainability of the soil fertility by recirculation of the nutritive elements from the organic material that is incorporated in the soil, and also by applying the green manure and the composts. Using those actions it is ensured the decreasing of the environment pollution.

Among the green manure, the fodder radish (*Raphanus sativus oleiformis,* fam. *Brassicaceae*) presents multiple effects on the soil: the increasing of the organic material containing, respectively humus, biological disinfectant by releasing the sulphurous compounds, worm destructive action and others. Together with fodder radish, the soy bean plant (*Glycine max* L*,* fam. *Fabaceae*) is characterized by a heavy vegetative growing that ensures high quantities of nutritive elements in general, and because of the nitrification bacteria of the gnarls of the roots, this species releases in the soil between 80 and 120 kg/ha nitrogen (Bâlteanu Ghe., 1989).

The husks of grapes, a secondary product obtained from the grapes processing, that represents about 4.5-4.9 m^3 residual material/ha, is mineralized by composting and becomes a fertilizer, ensuring in that way the waste recirculation.

MATERIALS AND METHODS

In order to maintain an optimum condition regarding the level of nutritive supplies of the soil in the organic vegetable crop, the fertilization basis is constituted by the organic fertilizers. The aim of the researches of the Banu Mărăcine R.S. of the University of Craiova, was to establish the influence of an organic fertilizer assortment, the green manure formed of soy bean + fodder radish and husks of grapes compost on the yield and quality of the garden been crops (Maxidor cultivar). The experience was a

lay-out on a field cultivated in the last two years using organic technologies (2005-2006). The experimental model was made up of three variants, replaced in randomized blocks, in three repetitions, the area of a variant-repetition being of 15 m^2, with a number of 200 plants/variant-repetition:

V_1 – green manure (control);

V_2 – green manure + 15 t compost/ha;

V_3 – green manure + 25 t compost/ha.

The control was constituted by the green manure (fodder radish+soy bean), that were sowed in the summer of 2006 as a successive crop. The fresh vegetal material was incorporated in the autumn, once with the current main soil works. The husks of grapes compost was applied before the garden bean planting season, on the preparation of the germination soil layer. In table 1 there are shown, for comparison, the agrochemical characteristics of the mineralized husks of grapes compost and of the sheep mineralized manure. The husks of grapes compost has the advantage that it doesn't contain weeds seeds and diseases.

During the experimentation period, there was applied maintenance work according to organic technologies: row irrigation; fertilization with Cropmax 0.1%, organic certified fertilizer, that has the next agrochemical composition: N=0.2%; P=0.4%; K=0.02%; Fe=220 mg; Mg=500 mg; Zn=49 mg; Cu=30 mg; Mn=54 mg; B; Cu; Mo; Co; Ni; growing vegetal stimulants; the diseases management by using specific preventive treatments with organic products: $CuSO_4$ 0.5-1%, Champion 0.4%, onion extract, aluminium sulphate 2%; mulching with peanuts hull.

The main agrochemical characteristics of the soil were determined before sowing the green manure, and also after the harvest of garden bean: the humus, nitrogen, phosphorus, potassium, nitrates and nitrites content, and pH.

The data regarding the yield efficiency of the garden bean crop statistically were processed according to mathematical models applied on the randomized blocks. Also the yield was analyzed regarding the biochemical composition: SDS, TDS, acidity, sugar, C vitamin, nitrates and nitrites.

The climatic date from the research period, are shown in table 2.

RESULTS AND DISCUSSIONS

The agrochemical characteristics of the soil of the Banu Mărăcine R.S. after two years of conversion are shown in table 3. In this way, in the 0-29 cm soil layer, the content in humus is 2.50%, in total nitrogen 0.131%, in phosphorus 68 ppm, in potassium 205 ppm, pH is 6 and alkali saturation level (V) is 82.1. This date characterise the soil as being moderate mezzo-basic.

In the spring of 2007, at the preparation of the germination soil layer and at the end of the garden bean harvest, there were made soil analyses in order to monitories the nutritive elements supplies level depending of the experimental variants (Table 4, Graphic 1). From the recorded data at 02.04.2007, it can be seen a small improvement of the agrochemical characteristics of the soil on all three fertilised variants: the contain in humus is situated between 3.16-4.00%, in nitrogen between 0.182-0.220%, in mobile phosphorus 86.4- 92.0 ppm and in mobile potassium 252- 404 ppm, pH being of 6.9-7.6.

At 09.09.2007, after ending the garden bean crop, the nutritive elements from the soil, excepting the mobile phosphorus, present an increasing of the values: 3.74% (V_2) – 3.91% (V_1) humus; 0.194% (V_1)-0.223% (V_3) total nitrogen; 337 ppm (V_1) – 429 ppm (V_3) mobile potassium.

The average yields of the garden bean, obtained from the organic crop, respectively without mineral fertilization and only by applying biologic health treatments are situated between 11.7 t/ha and 14.8 t/ha. By using husks of grapes compost on a found of green manure, there can be observed yields increasing by 10.2-26.5%, respectively 1.2-3.1 t/ha. The most favourable result, by 14.8t/ha, was on the fertilization with 25 t/ha husks of grapes compost (V_3), surpassing significant the control, with 3.1 t/ha (Table 5).

Regarding the biochemical composition of the yield, obtained by using the organic technology, one can se that the main nutrient components are situated in the optimum parameters of the bean garden (Table 6, Graphic 4.). In this way, the content in TDS is 8.07-8.92%, in SDS is 6.17-6.93%, in sugar is 5.09-5.73% and in C vitamin is 24.20 -32.15 mg/100g f.m. Regarding the nitrates accumulation level, the values by 128-195 ppm are situated under the maximum accepted concentration (200 ppm).

CONCLUSIONS

Based on the results concerning the influence of the green manure (soy bean +fodder radish) and of the husks of grapes compost on the agrochemical characteristics of the soil and on the yield and the quality of the garden bean, here are the conclusions:

1. the green manure, used as the only fertilizer or together with the husks of grapes compost, improves the soil fertility, increasing the contain in humus from 2.50% to 3.16-4.00%, in total nitrogen from 0.131% to 0.216-0.259%, the mobile phosphor from 68 ppm to 86.4-92.0 ppm and in mobile potassium, from 205 ppm to 252-404 ppm;
2. the 25t/ha husks of grapes compost doses, applied together with green manure, determines the highest yield level, by 14.8 t/ha, with an significant increasing of 3.1 t/ha (26.5%) compared with the control;
3. the biochemical value is considered as being superior: 8.07-8.92% TDS, 6.17-6.93% sugar, 24.20-32.15 mg/100g f.m. C vitamin;
4. the nitrates accumulation is low, 128-195 ppm, on all fertilized variants, being situated under the maximum accepted concentration (200 ppm).

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Tables

Table 1. The agrochemical characteristics of the husks of grapes compost and of the sheep mineralized manure

The product	pH	$CaCO_3$ (%)	N total (%)	P total (%)	P mobile (ppm)	K total (%)	K mobile (ppm)	Humus (%)
Husks of grapes compost	7.6	0.2	1.90	0.83	3630	0.93	7720	31.56
Sheep mineralized manure	8.5	0.5	1.48	1.31	5710	0.77	6380	32.80

Table 2. Climatic data recorded during the experimentation period – 2007–

Month	Average temperature (^oC)		Rain fall (mm)		ARH (%)
	Month average	60 years average	Month average	60 years average	
March	5.5	4.8	54.0	35.0	69
April	12.7	11.4	0	42.8	52
May	19.6	16.8	121.0	61.7	59
June	20.8	20.9	36.0	63.8	-
Julie	29.2	22.1	6.0	54.6	-
August	25.3	22.0	171.0	43.6	63.1
September	17.4	17.5	73	38.0	68.2

Table 3. The agrochemical characteristics of the soil – 04.06. 2006 –

Soil Layer	Deep (cm)	Humus (%)	tN (%)	P	K	pH	Ah	SB	T	V (%)
				(ppm)			me/100g sol			
Ao	0-29	2.50*	0.131*	68*	205*	6.9	3.6	13.8	16.8	82.1
A/B	29-42	1.52	0.056	109	85	6.4	3.3	14.4	17.7	81.3

Table 4. The monitoring of the agrochemical characteristics of the soil

Variants	Data	Humus (%)	pH	Total N (%)	NO_3 ppm	Mobile P ppm	Mobile K ppm
V_1	2.04	3.16*	6.9	0.182**	225	86.4**	252*
	9.09.	3.91*	7.3	0.194**	108	52.5*	337**
V_2	2.04.	3.59*	7.5	0.220**	216	92.0**	385**
	9.09.	3.74*	7.1	0.189**	190	69.9*	370**
V_3	2.04	4.00*	7.6	0.203**	259	90.7**	404***
	9.09	3.88*	7.6	0.223**	144	99.3**	429***

The supply level for the vegetable crops (ICPA 1981): Humus: *- medium. Total N: *-poor; **- medium. Mobile P: *- poor; **- medium. Mobile K: *- medium; ** - high; ***- very high.

Table 5. The influence of the organic fertilization on the garden bean yield

Variants	The yield		± Difference (t/ha)	Signification
	(t/ha)	(%)		
V_1 (green manure)-Control	11.7	100	-	-
V_2 (green manure +15 t/ha compost)	12.9	110.2	1.2	-
V_3 (green manure +25 t/ha compost)	14.8	126.5	3.1	x

LD 5% = 2,0 t/ha LD 1% = 3,2 t/ha LD 0,1% = 6,0 t/ha

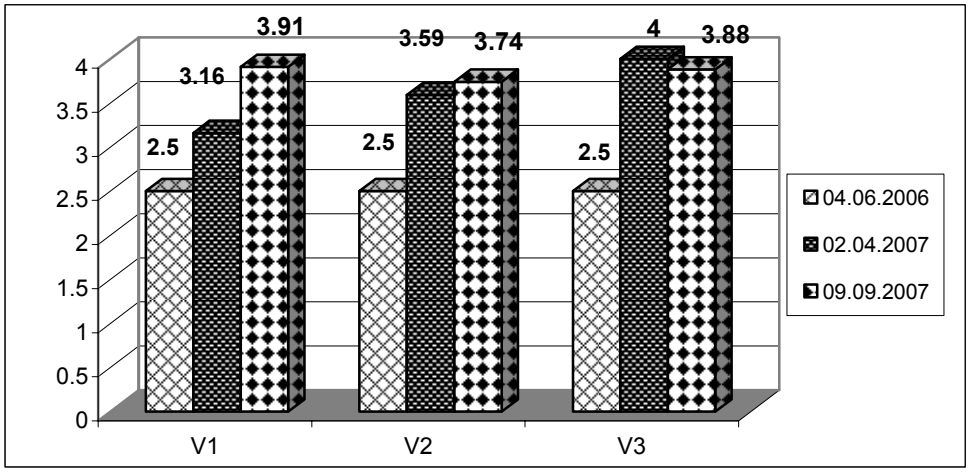
Table 6. The biochemical composition of the organic cultivated garden been

Variants	TDS	SDS	Sugar	C Vitamin (mg/100 g f.m.)	NO_3^* (ppm)
	(% f.m.)				
V_1 (green manure)-Control	8.88	6.38	5.73	32.15	173
V_2 (green manure +15 t/ha compost)	8.92	6.17	5.09	24.20	128
V_3 (green manure +25 t/ha compost)	8.07	6.93	5.41	27.46	195

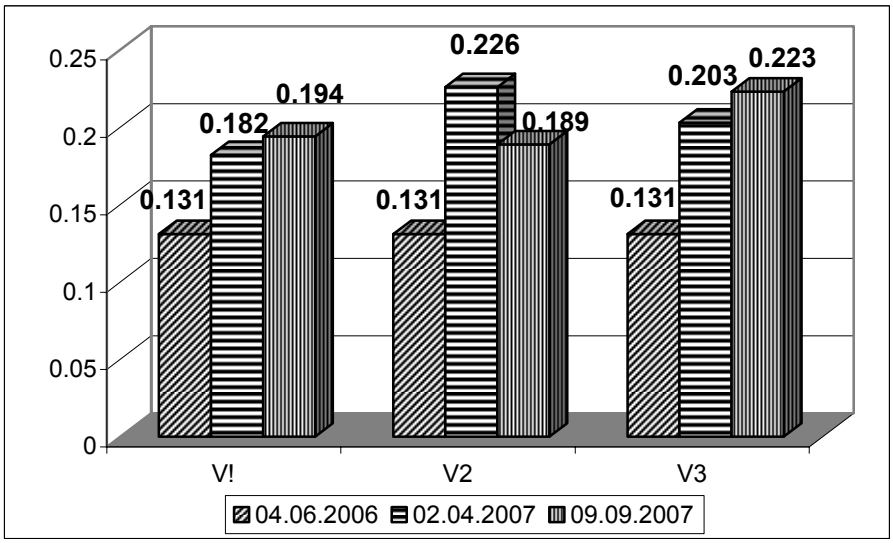
*-MAC NO_3-200 ppm (V. Lăcătuş. 1997).

Figures

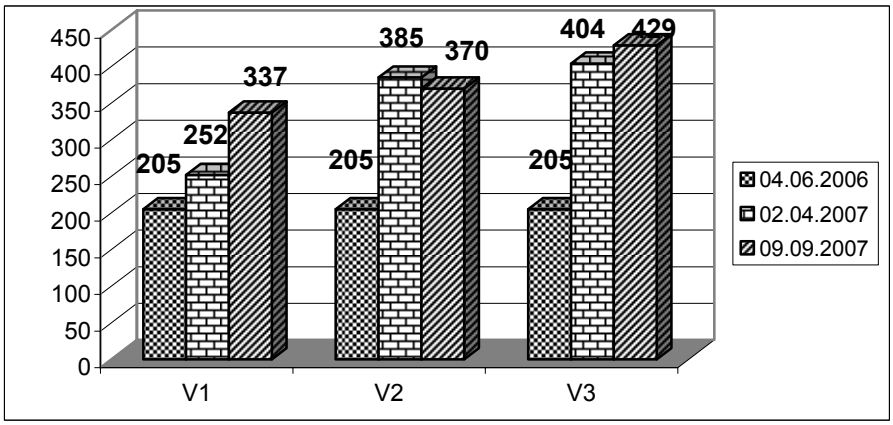
Graphic 1. The humus content evolution (%) according to fertilisation variant



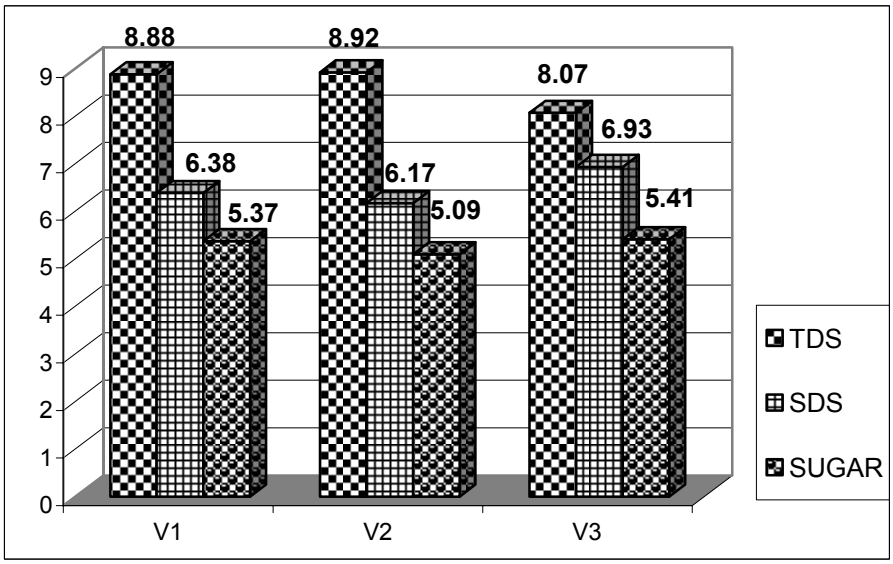
Graphic 2. The monitoring of the total nitrogen (%) content



Graphic 3. The monitoring of the mobile potassium (ppm) content



Graphic 4. The TDS. SDS and sugar content (%)
on the organic cultivated garden been



The monitoring of the nitrates content for an organic and non-organic vegetables assortment cultivated in the S-W of Romania

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Keywords: conventionally and ecologically, NO_3, NO_2, green manure

ABSTRACT

The discretionary and in large quantities utilization of organic fertilizers, but especially of chemical stimulants on a basis of nitrogen, determines the accumulation in vegetables of nitrates and nitrites that exceed alimentary safety limits. The research carried out has had for purpose the monitoring of the content of nitrates and nitrites in tomatoes, lettuce, cabbage, carrots, onion and green beans grown conventionally and ecologically, the vegetable farms having been placed on different types of soil, such as: cernisoil, luvisoil and psamosoil. In the ecological system, on all the studied species, the content of nitrates is situated far bellow the maximum allowed concentrations and the nitrates have not been identified. In what concerns the conventional crops, excepting tomatoes, on all other vegetables, the quantities of NO_3 surpass the maximum allowed concentration, the values being higher in the case of luvisoils. The species carrot and lettuce have registered the highest accumulation of nitrates, of 884-2406 ppm, respectively 3537-4029 ppm, the MAC for carrots being 300 ppm and for lettuce 2000 ppm.

INTRODUCTION

In Romania, conventional vegetable farms are predominant, exploited intensively through the utilization of uncontrolled doses of chemical fertilizers, especially those containing nitrogen, which have a toxic effect on the micro-flora located in the soil, the eutrophication and accumulations in plants above alimentary safety limits. In this concern, nitrates and nitrites present important toxicological implications, which reside in the cumulative effect that they have on the human and animal organism and in the possibility of formation of nitrosamines.

By comparison, the cultivation in an ecological system reduces this phenomenon, by using exclusively organic fertilizers. But, in this situation also, a surplus of nitrogen compounds can appear, the directives of the E. U. rule the doses of nitrogen of organic origin allowed. Nevertheless, according to the studies carried out by Henkens and Van Keulen (2001), almost 25% of the organic farms use more nitrogen than allowed by European norms, respectively 170 kg/ha, coming from organic fertilizers. Almost 12% of vegetable-producing farms exceed the standard by 20 kg/ha, the high test incidence being signaled in the case of small vegetable-producing farms, with vulnerable soils.

MATERIAL AND METHODS

The researches carried out had for purpose the monitoring of the content of nitrates and nitrites in an assortment of vegetables cultivated in conventional and ecological system.

The ecological crop growing system has been placed on a field in its third year of organic use within S.D Banu Maracine, belonging to the University of Craiova, recording the content of nitrates and nitrites in tomatoes, cabbage, onion, carrots and green beans. The crops have been fertilized with green manure and husks of grapes compost.

The green manure, fodder radish and soybean, incorporated in the soil in the fall during basic fertilization and the husks of grapes compost has been used in preparing the soil in the spring, at a dose of 15 t/ha.

Within the experiment, maintenance actions characteristic to ecological crops have been resorted to.

In what concerns the conventional cultivation system, used in vegetable-producing basins with tradition in the area of Oltenia, when establishing the monitored localities, there has been taken into account the position in groups of different soils, given the fact that the levigation of nitrates in the fertilizers is also determined by the type of soil:

▪ cernisoils have a well formed clay-humic complex and a high capacity of retaining nitrates; localities - Izbiceni and Motatei;

▪ luvisoils present a high content of argil, a well formed argilo-humic complex, with the capacity of retaining nitrates into the soil: locality - Islamite;

▪ psamosoils, with light, sandy soils, which have an clay-humus complex very poorly formed and a low capacity of retaining nitrates: localities - Tamburesti and Teasc.

Within each locality, the samples have been taken from 3 farms, thus constituting an average samples, which has been analyzed to determine the content of nitrates and nitrites in the vegetables.

The level of NO_3 and NO_2 in the vegetables has been determined with a refractometer, model RQ Flex Plus. The preparation of the vegetal matter has been executed with an extract of acetic acid, at a ratio of 2%.

RESULTS AND DISCUSSION

The accumulation of nitrates in vegetables is found under the incidence of certain factors, like applied agro techniques, and especially the supplements used for basic fertilizations, vegetable species and distinctive biochemical and physiological characteristics of genotypes, vegetation phase, harvesting time, etc.

In the case of the ecological crop growing system, where only organic fertilizers have been used (green manure and husks of grapes compost), the recorded data shows that on all studied species the level of accumulated NO_3 is below maximum allowed limit.

In what conventional crops are concerned, with the exception of tomatoes, on all other vegetables the content of nitrates, excepting that of nitrites, exceeds the maximum allowed concentrations (Table 1).

For every species, the situation is the following:

▪ tomatoes: in the ecologic system the content of nitrates was 82 ppm, while in the conventional system it was situated between 56 ppm (Tamburesti) and 152 ppm (Motatei), values inferior to MAC (150 ppm). Equally, nitrites have also accumulated below the maximum allowed level (Graphic 1).

▪ white cabbage: it presents a content of 206 ppm NO_3 for the ecological crop and 637 ppm (Tamburesti) - 821 ppm (Motatei), MAC being 500 mg/kg s.p. The level of nitrites is within the maximum allowed limits (Graphic 2);

▪ lettuce: at a maximum allowed concentration of 2000 ppm, the crop fertilized exclusively with organic substances registers 1852 ppm, while by the use of conventional technology , the accumulation of nitrates exceeds by a lot this limit, situating itself between 3537 ppm (Teasc) and 4029 ppm (Isalnita). The locality

Tamburesti makes an exception (1986 ppm). In what the nitrites are concerned, at Motatei, Isalnita and Teasc there have been determined values slightly above the MAC (Graphic 3);

▪ carrot: for the ecological crop there has been an accumulation of nitrates of 202 ppm NO_3, MAL being 300 ppm. In the monitored localities the content has increased 4 to 8 times, being situated between 884 ppm (Tamburesti) and 2406 ppm (Isalnita). The content of nitrites does not show values over the MAC (Graphic 4).

▪ garden beans: in the case of the ecological crop a level of 128 ppm NO_3 has been determined and for the conventional system 167 ppm (Tamburesti) – 420 ppm (Isalnita), the maximum allowed limit being 200 ppm. Concerning the nitrites, the accumulations of 0.1-0.8 are below the MAC (Graphic 5);

▪ onion: the quantity of 49 NO_3 ppm, registered in the case of the ecological crop is situated below the average monitored values within the 5 localities: 99 ppm (Motatei) and 129 ppm (Teasc), these values exceeding the maximum limit allowed (80 ppm). The nitrites (0.4-0.7 ppm) are situated below the MAL (1 ppm) (Graphic 6).

CONCLUSIONS

On the basis of the results regarding the accumulation of nitrates and nitrites in the edible organs of some vegetable species of which have been grown in both ecological and conventional systems, we can formulate the following conclusions:

1. Through the use of ecological technology on vegetable crops, the content of nitrates presents values far below the maximum allowed concentrations and the nitrites have not been identified.
2. With the exception of tomatoes, for the other species of vegetables grown in a conventional system, the quantities of NO_3 exceed the MAC, also presenting an accumulation of nitrites, but 1 ppm under (MAC).
3. The lowest quantities of nitrates and nitrites have been recorded on all the species grown on psamosoils (Tamburesti, Teasc) and the highest for the cultures on luvisoils (Isalnita).
4. From the monitored vegetables, the species carrot and lettuce, grown conventionally, have registered the highest content of nitrates, surpassing the MAC by 194% (Tamburesti) – 702% (Isalnita), respectively, 77% (Teasc) – 102% (Isalnita).
5. These date confirm the hypothesis that in the case of conventional vegetable farms, the fertilizers, especially the chemical ones containing nitrogen, are used discretionarily, in exaggerated amounts that pollute the entire trophic chain: alimentary products, soil, water, etc.

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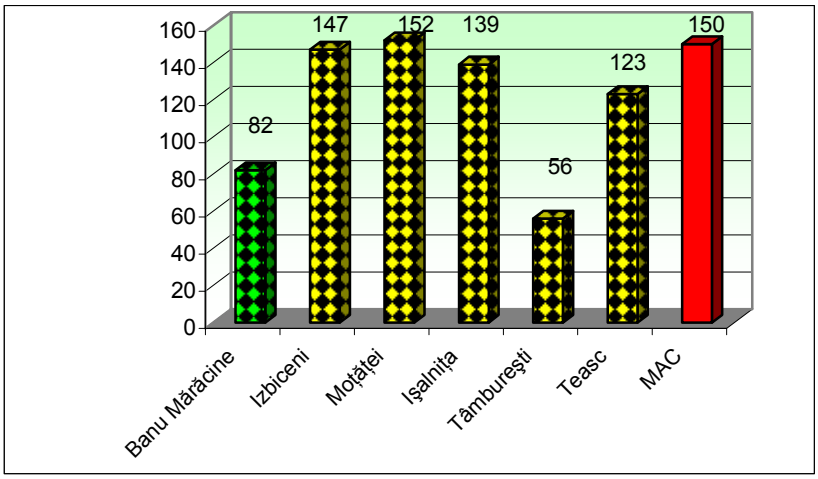
Table 1. The nitrates and nitrites contain (ppm) of the organic and non-organic vegetables yield

The crop	**The contain in:**	**Crop technology**						**MAC ***
		Organic	**Non-organic**					
		Banu Mărăcine	**Izbiceni**	**Motatai**	**Isalnita**	**Tamburesti**	**Teasc**	
Tomatoes	NO_3	82	147	152	139	56	123	150
	NO_2	-	0.3	0.5	0.3	-	0.5	1
White cabbage	NO_3	206	780	821	726	637	703	500
	NO_2	-	1.0	0.9	0.7	0.9	1.1	1
Lettuce	NO_3	1852	3543	3740	4029	1986	3537	2000
	NO_2	-	0.9	1.1	1.1	0.3	1.1	1
Carrot	NO_3	202	1891	2103	2406	884	1269	300
	NO_2	-	0.7	0.8	0.7	-	0.7	1
Garden beans	NO_3	128	406	368	420	167	381	200
	NO_2	-	0.8	0.8	0.8	0.1	0.7	1
Onion	NO_3	49	103	99	127	114	129	80
	NO_2	-	0.4	0.6	0.6	0.7	0.4	1

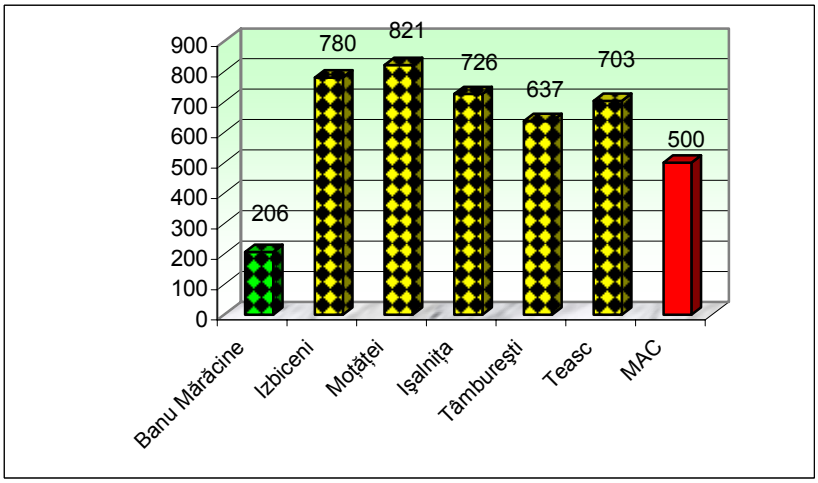
*MAC-The maximum accepted concentration (V. Lacatus, 1997)

Figures

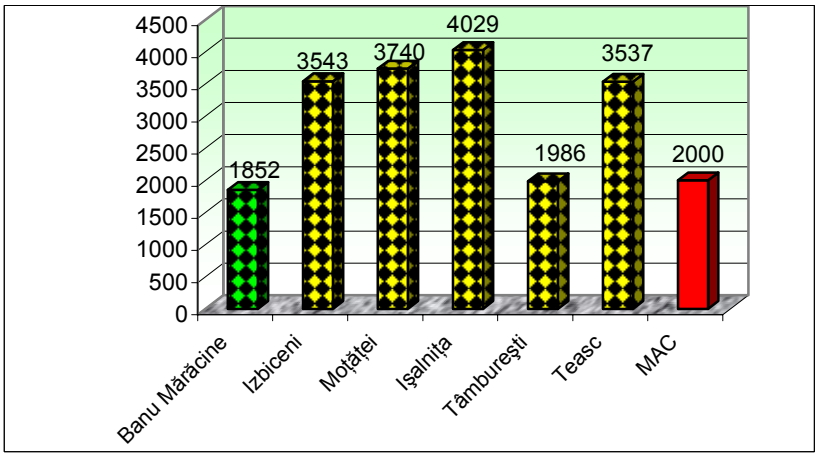
Graphic 1. The nitrates contain (ppm) of the organic and non-organic tomatoes



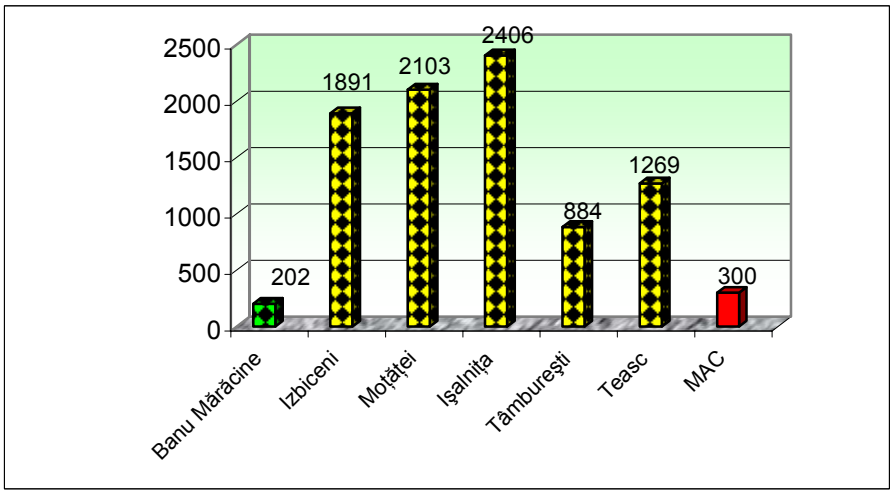
Graphic 2. The nitrates contain (ppm) of the organic and non-organic white cabbage



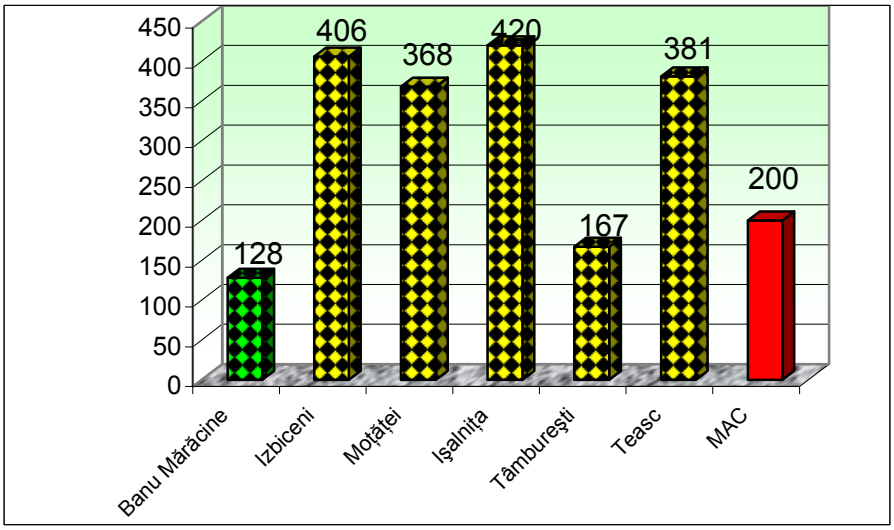
Graphic 3. The nitrates contain (ppm) of the organic and non-organic lettuce



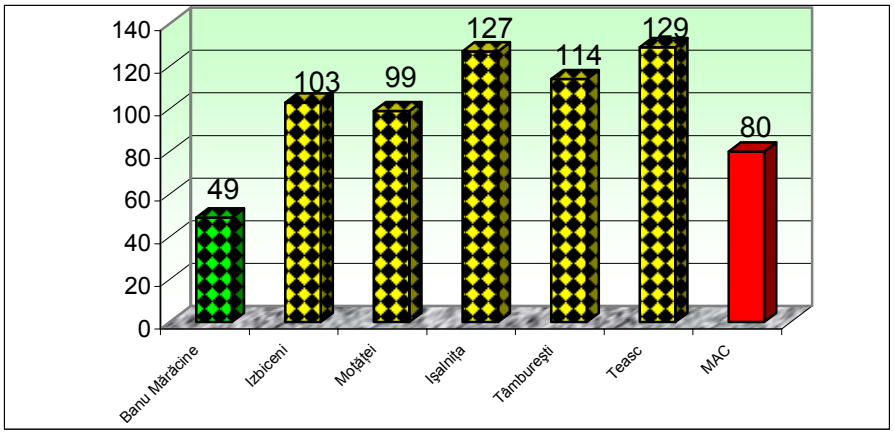
Graphic 4. The nitrates contain (ppm) of the organic and non-organic carrot



Graphic 5. The nitrates contain (ppm) of the garden bean



Graphic 6. The nitrates contain (ppm) of the onion



Researches concerning the particularities to seed plants of the chive onion (Kind) variety Liliana

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Keywords: *Allium caepa*, phenotypic, vegetative and generative

ABSTRACT

In this work is presented a little part from the researches which were made in the sight of the settlement of genetic determinism of "the number of floral draw bars" to the onion of chive.

The floral draw bars are emitted by the developed and vernalized buds from the plants mother.

INTRODUCTION

The improvement and the conservative selection of the onion cultivation enforces as a must a study upon the biologic peculiarities of the plants concerning the phenophase period, the period of vegetation, the breed and development (vegetative and generative) of plants, aso.

To the Allium caepa L species the pronouncedly alogamy causes in an outsize matter the phenotypic combinations which answering differently under the influence of the average factors.

The IIIrd year represents for the triennial onion the last year from the cycle production of "from seed to seed". The significant fact of this biologic sequence consists in the fact that is in progress the change of the biologic category. It is imperativeness the assurance of isolation space.

MATERIALS AND METHODS

The studies were accomplished to ICDLF Vidra in the 1994- 1999 period.

It was used as study resources the biologic issues from the links of conservative selection of the onion kind Liliana (L1)

The experiences were accomplished in the field, in different climatic conditions.

The placements of the variants were accomplished in a geometric drawings way, in superposed blocks.

RESULTS AND DISCUSSIONS

In the 1994-1999 period was studied to ICDLF Vidra on purpose of follow up, the phenotypic manifestation of seed plants to the onion from chive kind: the breed and the development (vegetative and generative), the flourishing and fructification …

Table 1. The phenophase to seed plants of onion

Year	Date				
	Sowed	Rise	Emission of stem	Flourishing	Physiologic maturity
1996	24.04	5.05	17.05	20.06	20.07
1999	4.03	30.03	15.05	18.06	8.08
1999	7.04	17.04	24.05	14.06	10.08

Table 2. The phenophase duration and the period of vegetation

Year	Phenophase period (days)			
	Sowed- Rise	Rise – Emission of stem	Rise- Flourishing	Rise- physiologic maturity
1996	11	12	46	66
1999	26	46	80	131
1999	10	37	58	115

I. The breed and the development of plant from the seed plant varieties were given by the components which were phonotypical revealed and influences the period of vegetation phase, of vegetation phase in different average conditions (table 1, table 2).

In the 1995 year the seed plants have emitted 1-3 floral draw bars having the weight the plants with 1-2 floral draw(83,4%) which formed even 1-2 onion/plant (8,7% have formed even 3 onions/plant).

In the 1996 year it was studied the vegetative and generative development of seed plants with different provenance:

- from develop chive (with the transversal diameter across 22 mm);
- from mother plants (bulbs) with the weight of 50- 80 g/bulb
- from mother plants (bulbs) with the weight of 20- 30 g/bulb

From chive, 19, 3% plants passed from the vegetative phase to the generative one and have formed 1-3 floral draw bars/plant (17, 8% have emitted a single draw bars/plant), and the remainder of 80, 7% plants have formed bulbs.

The descendent seed plant form the onion bulbs with a weight of 20- 30 g/bud (table 3) have emitted floral draw bars, in interest of 79, 7% and 20, 3% have not passed in the generative phase and have formed just buds. Have been recorded 16 phenotypes regarding the vegetative generative development of the plants.

The descendent of the mother plants (of the buds) with the weight of 50- 80 g/bud have characterized through plants which have emitted floral draw bars in the interest of 98,4% and 1, 6% from plants which have not emitted floral draw bars (table 3). It has registered 19 phenotypes regarding the vegetative and generative development of plants.

In the condition of the 1997 year, had studied the vegetative and generative development of the descendent seed plants of the mother plants from different biologic categories: CA mp (choose field mother plants), CS .II. mp (field of the IInd study of the descendent mother plants and from yielded buds from superelite seed plants SES). Have been recorded 69 phenotypes concerning the vegetative and generative development of plants.

For the cutback of biologic production loop to the triennial onion seed have studied also the possibility of the production seed from the developt chive (with the

diameter across 22 mm). So (in the 1998 year) had studied two varieties: A (in which had used the chive with the diameter between 22 and 25 mm) and B (in which had used the chive with a diameter of 25 mm). In the table 5 is rendered the vegetative and generative development of derived plants from developt chive (over 22mm), from where follows:

- In the A variant: 38, 2% have emitted the floral draw bars, 35, 95% of plants have emitted two floral draw bars/plant, 13, 48% plants have emitted three floral draw bars/plant, 2, 25% plants have emitted four floral draw bars/plant, 18, 99% plants have emitted 2-4 floral draw bars/plant and 15, 19% of plants have not emitted floral draw bars.

Table 3. The number of develop buds and the emission of floral stem, in the 1996 year

Origin	Buds		Floral stem	
	Number of develop buds/plant	The total number of plants	Number of floral stem/plant	Total number of plant
Mother plants (50-80) g/bud	1	232	1t	232
	2	701	1t	117
			2t	571
	3	410	1t	17
			2t	75
			3t	304
	4	83	1t	4C
			2t	6
			3t	28
			4t	44
	5	10	2t	1
			3t	2
			4t	1
	6	2	5t	6
			6t	2
Mother plants (20-30) g/bulb	1	268	1t	213
	2	287	1t	67
			2t	158
	3	99	1t	16
			2t	16
			3t	49
	4	23	1t	3
			2t	2
			3t	8
			4t	7
	5	4	3t	2
			5t	1

Table 4. The number of mature buds and the emission of floral draw bars in the 1997 year

Origin	Buds		Floral stem	
	Number of develop buds/plant	**The total number of plants**	**Number of floral stem/plant**	**Total number of plant**
CApl.m	1	9	1t	6
	2	188	1t	23
			2t	157
	3	339	1t	7
			2t	47
			3t	270
	4	127	2t	17
			3t	39
			4t	69
	5	58	2t	2
			3t	19
			4t	12
			5t	27
CS.II.pl.m	1	1	1t	46
	2	664	1t	91
			2t	552
	3	614	1t	29
			2t	119
			3t	456
	4	273	1t	4
			2t	96
			3t	72
			4t	89
	5	96	1t	2
			2t	13
			3t	52
			4t	12
			5t	22
	6	58	3t	38
			4t	7
			5t	5
			6t	7
	7	3	3t	1
			4t	2
	8	6	4t	4
			5t	1
	9	1	9t	1

Origin	Buds		Floral stem	
	Number of develop buds/plant	The total number of plants	Number of floral stem/plant	Total number of plant
SEs	1	97	1t	81
	2	438	1t	71
			2t	352
	3	430	1t	17
			2t	89
			3t	314
	4	132	1t	4
			2t	23
			3t	35
			4t	60
	5	42	2t	2
			3t	19
			4t	5
	6	24	3t	20
			4t	4
	7	3	3t	1
			4t	1
			7t	1

Table 5. The vegetative and generative development of descendent seed plants from the developt chive (over 22 mm)

Variant	Phenotype	The relative number of plants	
A	1t	13,48	38,2
	1t+1c	19,10	
	1t+2c	5,62	
	2t	29,22	35,95
	2t+1c	5,61	
	2t+2c	1,12	
	3t	11,24	13,48
	3t+1c	1,12	
	3t+2c	1,12	
	4t	2,25	
	1c	1,12	
	2c	7,87	
	3c	1,12	
B	1t	43,03	65,82
	1t+1c	22,79	
	2t	13,92	18,99
	3t	3,80	
	4t	1,27	
	1c	12,66	15,19
	2c	2,53	

CONCLUSIONS

I studied the seed plants to the onion of chive, obtained differently as like source from the biologic material:

a) the biologic material achieved in the Ist year, meaning the sizeable chive (cross diameter > 22- 25 mm);

b) the biologic material achieved in the IInd year, meaning onion bulbs;

c) from develop slumber bulbs of the seed plants- IIIrd year.

The amount of achieved seed on plant and to the above - ground unit is conditioned y the characteristics of the biologic material used in the seed plant fields and the influence of the average factors.

In the 1995 year the seed plants have emitted 1-3 floral draw bars having the weight the plants with 1-2 floral draw(83, 4%) which formed even 1-2 onion/plant (8,7% have formed even 3 onions/plant).

In the 1996 year it was studied the vegetative and generative development of seed plants with different provenance:

From chive, 19, 3% plants passed from the vegetative phase to the generative one and have formed 1-3 floral draw bars/plant (17, 8% have emitted a single draw bars/plant), and the remainder of 80, 7% plants have formed bulbs.

The descendent seed plant form the onion bulbs with a weight of 20- 30 g/bud (table 3) have emitted floral draw bars, in interest of 79, 7% and 20, 3% have not passed in the generative phase and have formed just buds. Have been recorded 16 phenotypes regarding the vegetative generative development of the plants.

The descendent of the mother plants (of the buds) with the weight of 50-80 g/bud have characterized through plants which have emitted floral draw bars in the interest of 98,4% and 1,6% from plants which have not emitted floral draw bars (table 3). It has registered 19 phenotypes regarding the vegetative and generative development of plants.

In the condition of the 1997 year, had studied the vegetative and generative development of the descendent seed plants of the mother plants from different biologic categories: CA mp (choose field mother plants), CS .II. mp (field of the IInd study of the descendent mother plants and from yielded buds from superelite seed plants SES). Have been recorded 69 phenotypes concerning the vegetative and generative development of plants.

The study of technological elements in the process of red orache (*Atriplex hortensis f. rubra*) seed production

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Keywords: crooked and uncrooked plants, bi factorial experiences, rehearsals, graduations

ABSTRACT

The studies analyzed the bi factorial experiences (A and B) with two (A_1 – variants with uncrooked plants, A_2 – variants with crooked plants) and also six graduations (B_1 : 10 cm between plants/row, meaning 133 333 plants/he; B_2 : 20 cm between plants/row, meaning 66 666 plants/he; B_3 : 30 cm between plants/he, meaning 44 444 plants/he; B_4 : 40 cm between plants/row, meaning 33 333 plants/he; B_5 : 50 cm between plants/row, meaning 26 666 plants/he; B_6 : 60 cm between plants/row, meaning 22 222 plants/he).

INTRODUCTION

In the process of seed production of red orache you faced with a lot of issues, at a level of harvesting plants in the physiologic mature phase regarding/concerning the manually cutting pf plants or threshing them because in the case of low densities the haulm form a hard wooden fibro issues.

Instead, as much as the density of plants is growing as much the wooden fibro tissue is easier to be cut (haulm) both manually as technical.

By turns, this condition enforces as a must the study of plants density of red orache, in the aim of growing seed productions to the above ground unit/low possible efforts.

Simultaneously was followed the influence of the peak of growth limitation of the plants.

MATERIALS AND METHODS

The researches were accomplished to ICDLF Vidra.

As biologic material was used the descending plants, from the pre based seed from the red orache kind.

The placement of the experience was accomplished after the type of bi factorial experiences with subdivided lots of the four rehearsals:

- The A factor, with 2 graduation:

A_1: with uncrooked plants,

A_2: with crooked plants;

- The B factor, with 6 graduations:

B_1: 10 cm between plants/row (133 333 plants/he.)

B_2: 20 cm between plants/row (66 666 plants/he.)

B_3: 30 cm between plants/row (44 444 plants/he.)

B_4: 40 cm between plants/row (33 333 plants/he.)

B_5: 50 cm between plants/row (26 666 plants/he.)

B_6: 60 cm between plants/row (22 222 plants/he.)

Excepting the factors, from our study, which were takes under consideration, the other technologic elements were suitable to the in force technology at ICDLF Vidra.

The results have been interpreted by analyzing the variant.

RESULTS AND DISCUSSIONS

In the tables number 1- 10 are revealed the amount of seed on plant, the seed production to hectare, the signification of the differences among variants, the influence of every factor, as well as the interaction among the studied factors.

I. The weight of seed on plant

Table 1. The variant analyses (the weight of seed/plant)

The variability cause	SP	GL	S^2	F
Sizable lots	260	7		
Repetitions	18	3		
Technologic (A)	196	1	196	12,8(10,13;34,12)
Error (a)	46	3	15,3	
Small lots	9916	47		
Densities (B)	10807	5	2161,4	10,7(2,53;3,70)
Interaction (AxB)	4899	5	979,8	4,8(2,53;3,70)
Error (b)	6050	30	201,7	

Table 2. The influence of A factor (the weight of seed/plant)

The factor	The weight of seed/plant	The relative weight (%)	The difference	The significance
A_1	37,79	112	4,04	*
A_2	33,75	100	-	

DL. 5% = 3, 59
DL. 1% = 6, 60
DL. 0, 1% = 14, 62

The weight of seed on plant has demonstrated a significant positive difference of the A_1 factor (uncrooked plants) against the factor A_2 (crooked plants).
The multiple comparisons of the B factor (densities) from the analysis of the production to hectare revealed very significant positive differences in the case of the low densities against the high densities to "weight of seed/plant" features. So (table 3):

Table 3. Multiples comparators of B factor (the weight of seed/plant)

Classification	The weight of seed/plant(g)	The difference from the variant situated on the:				
		VI place	V place	IVth place	IIIrd place	II nd place
I-B_6	54,3	40,03***	33,18***	23,20**	11,55	11,25
II- B_4	43,05	28,77***	21,92**	3,95	0,27	-
III- B_5	42,75	28,50***	21,65**	3,67	-	-
IV- B_3	39,10	24,82**	17,97*	-	-	-
V- B_2	21,12	6,85	-	-	-	-
VI B_1	14,27	-	-	-	-	-

DL.5%=14, 48
DL.1%=19, 52
DL. 0, 1% = 25, 91

B_6 demonstrate differences:
- Very significant from B_1 and B_2,
- Distinctly significant from B_3,

B_5 demonstrate differences:
- Very significant from B_1,
- Distinctly significant from B_2,

B_4 demonstrate differences:
- Very significant from B_1,
- Distinctly significant from B2,

B_3 demonstrate differences:
- Very significant from B_1,
- Distinctly significant from B_2,

Between the B_1 and B_2 factors don't exist significant differences analyzing the multiples comparisons of the B factors to the same graduate level of the A factor (table 4).

Table 4. Multiples comparisons of the B factor to the same graduate level of the A factor (weight of seed/plant)

Classification		**the weight of seed/plant(g)**	**The difference from the variant situated on the ….place:**				
			VI-B_1	**V-B_2**	**IV-B_3**	**III-B_5**	**II-B_4**
A_1	I-B_6	56,75	37,15***	32,45**	17,05	16,95	10,15
	II- B_4	46,60	27,00*	22,30*	6,90	6,80	-
	III- B_5	39,81	20,20	15,50	0,10	-	-
	IV- B_3	39,70	20,10	15,40	-	-	-
	V- B_2	24,30	4,70	-	-	-	-
	VI B_1	19,60	-	-	-	-	-
A_2			VI-B_1	V-B_2	IV-B_3	III-B_4	II-B_5
	I-B_6	51,85	42,90***	33,90**	13,35	12,35	6,10
	II- B_5	47,75	36,80***	27,80**	7,25	6,25	-
	III- B4	39,50	30,55**	21,50*	1,00	-	-
	IV- B_3	28,50	29,55**	20,55*	-	-	-
	V- B_2	17,95	9,00	-	-	-	-
	VI B_1	8,95	-	-	-	-	-

DL. 5%= 20,48
DL. 1%= 27,61
DL. 0,1%= 36,65

Are noticed to:

A_1 (variants with uncrooked plants)

B_6 demonstrate differences:
- Very significant from B_1,
- Distinctly significant from B_2,

B_4 demonstrate differences:
- Distinctly significant from B_2 and B_1

A2 (variants with crooked plants)

B_6 (the same as A_1) demonstrate differences:

- Very significant from B_1,
- Distinctly significant from B_2 ,

B_5 demonstrate differences:

- Very significant from B_1,
- Distinctly significant from B2,

(at A_1 the significations are only significantly)

B_3 demonstrate differences:

- Very significant from B_1,
- Distinctly significant from B2,

(at A_1 the significations don't exist considerable differences).

To the same graduation of B factor has been found to the both average of A factor, as convenient to the variants with uncrooked plants (table 5, graphic 1).

Table 5. The comparisons of two A average to the same or different graduate level of the B factor (the weight of seed/plant)

The factor	The weight of seed on plant	The difference from the variant situated on the … place:										
		XII	XI	X	IX	VIII	VII	VI	V	IV	III	II
I B_6A_1	56,75	47,80***	38,80***	37,10***	33,30	18,25	17,20	17,40	16,80	11,00	10,10	4,90
II B_6A^2	51,85	42,90***	33,90**	32,25**	27,55**	13,35	12,30	12,10	11,90	6,15	5,25	-
III B_4A_1	46,60	37,65***	28,65**	27,00**	22,30*	8,10	7,10	6,90	6,70	0,90	-	-
IV B_5A_2	45,70	36,75***	27,75**	26,10**	21,40*	7,20	6,20	6,00	5,80	-	-	-
V B_5A_1	39,81	30,90**	21,86*	20,21*	15,51	1,31	0,31	0,10	-	-	-	-
VI B_3A_1	39,71	30,76**	21,76*	20,11*	15,41	1,21	0,20	-	-	-	-	-
VII B_4A_2	39,50	30,55**	21,55*	19,90*	15,20	1,00	-	-	-	-	-	-
VIII B_3A_2	38,50	29,55**	20,55*	18,90	14,20	-	-	-	-	-	-	-
IX B_2A_1	24,30	15,35	6,35	4,70	-	-	-	-	-	-	-	-
X B_1A_1	19,60	10,65	1,65	-	-	-	-	-	-	-	-	-
XI B_2A_2	17,95	9,00	-	-	-	-	-	-	-	-	-	-
XII B_1A_2	8,95	-	-	-	-	-	-	-	-	-	--	-

DL 5% = 19,03
DL 1% = 25,87
DL 0,1% = 35,02

Analyzing the average of A factor to the different graduate of B factor has been revealed that the weight of seed/plant has been inverse proportionate with "the density of plants/row" to the both average of the A factor.

II. The production of seed to hectare

The accomplished study about the production of seed to hectare has emphasized the significantly distinctive difference among the both two graduation of the A factor in the favour of A_1 variants (table 6, table 7).

Table 6. The analysis of variant (seed production/hectars)

The variability cause	SP	GL	S^2	F
Sizable lots	1,29	7		
Repetition	0,01	3		
Technologic(A)	1,19	1	1,190	44,1(10,13;34,12)
Error (a)	0,08	3	0,027	
Reduced lot	8,91	47		
Densities (B)	3,69	5	0,738	33,5(2,53;3,7)
Interaction (AxB)	3,28	5	0,656	29,8(2,53;3,7)
Error (b)	0,65	30	0,022	

Table 7. The influence of A factor (seed production/hectare)

The factor	The weight of seed/hectare	Of relative the weight (%)	The difference	The significance
A_1	1,61	124	0,31	**
A_2	1,30	100		

The multiple comparisons of the B factor (densities) from the analysis of the production to hectare revealed very significant differences between the low densities and the high densities (between plants by rows and the above- ground unit). In the table 8 is presented the signification of the differences between the variants of B factor, where:

B_1 demonstrate differences:
- Very significant from the B_5 and B_6, B_4 and B_2
- Significantly from B_3;

B_3 demonstrate differences:
- Very significant from the B_5 and B_6, B_4 and B_2

B_2 demonstrate differences:
- Very significant from B_5,
- Distinctly significant from B_6,

B_4 demonstrate differences:
- Significantly from B_5;

Table 8. Multiples comparisons of the B factor (seed production/hectar)

Classification	The weight of seed (q/ha)	The difference from the variant situated on the ….place:				
		VI-B_5	**V-B_6**	**IV-B_4**	**III-B_2**	**II-B_3**
I-B_1	1,90	0,76***	0,69***	0,57***	0,49***	0,16*
II- B_3	1,74	0,60***	0,53***	0,41***	0,27***	-
III- B_2	1,41	0,27***	0,20**	0,08	-	-
IV- B_4	1,33	0,19	0,12	-	-	-
V- B_6	1,21	0,07	-	-	-	-
VI B_5	1,14	-	-	-	-	-

DL. 5%= 0,15
DL. 1%= 0,20
DL. 0,1%= 0,27

The analysis of multiple comparisons of B factor variants at the same graduation of A factor looked for the feature of "the seed production to hectare" emphasizes a different dependency among "the weight of seed on plant" feature and the density of the plants.

From the analysis of the B factor as part as the A1 factor graduation (variants with uncrooked plants) reveals that the B variant achieves the biggest production/he (2, 61 q) then the variants: B3- 1,76 q; B2- 1,62 q; B4- 1,35 q; B6- 1,26 q; B5- 1,06 q (these were in order).

As part as the A_2 graduation of the A factor the multiple comparisons of variants of the B factor emphasizes the B_3 variant (1,71g) which achieves the highest production/ha and the other variants being in descending order: B_4 (1,32g), B_5 (1,22g), B_2 (1,20g), B_1 (1,19g), B_6 (1,15g).

Table 9. Multiples comparisons of the B factor to the same graduate level of the A factor (weight of seed/hectar)

Classification		the weight of seed/he (q)	The difference from the variant situated on the ….place:				
			VI-B_5	**V-B_6**	**IV-B_4**	**III-B_2**	**II-B_3**
	I-B_1	2,61	1,55	1,35	1,26	0,99	0,85
	II- B_2	1,76	0,70	0,50	0,41	0,14	-
	III- B_3	1,62	0,56	0,36	0,27	-	-
	IV- B_4	1,35	0,29	0,09	-	-	-
	V- B_6	1,26	0,20	-	-	-	-
	VI B_5	1,06	-	-	-	-	-
			VI-B_6	**V-B_1**	**IV-B_2**	**III-B_5**	**II-B_4**
	I-B_3	1,71	0,56	0,52	0,51	0,49	0,39
	II- B_4	1,32	0,17	0,13	0,12	0,10	-
	III- B_5	1,22	0,07	0,03	0,02	-	-
	IV- B_2	1,20	0,05	0,01	-	-	-
	V- B_1	1,19	0,04	-	-	-	-
	VI B_6	1,15	-	-	-	-	-

Table 10. The comparisons of the two A average to the same or different graduate level of the B factor (seed production/ha)

The factor	Prod./ha (to)	The difference from the variant situated on the ….place:										
		XII	XI	X	IX	VIII	VII	VI	V	IV	III	II
I A_1B_1	2,61	1,55***	1,46***	1,42***	1,41***	1,39***	1,35***	1,29***	1,26***	0,99***	0,90***	0,85***
II A_1B_2	1,76	0,70***	0,61***	0,57***	0,56***	0,54**	0,50**	0,44**	0,41**	0,14	0,05	-
III A_2B_3	1,71	0,65***	0,56***	0,52**	0,51**	0,49**	0,45**	0,39**	0,36**	0,09	-	-
IV A_1B_2	1,62	0,56***	0,47**	0,43**	0,42**	0,40**	0,36**	0,30*	0,27*	-	-	-
V A_1B_4	1,35	0,29*	0,20	0,16	0,15	0,13	0,09	0,03	-	-	-	-
VI A_2B_4	1,32	0,26*	0,17	0,13	0,12	0,10	0,06	-	-	-	-	-
VII A_1B_6	1,26	0,20	0,11	0,07	0,06	0,04	-	-	-	-	-	-
VIII A_2B_5	1,22	0,16	0,07	0,03	0,02	-	-	-	-	-	-	-
IX A_2B_2	1,20	0,14	0,05	0,01	-	-	-	-	-	-	-	-
X A_2B_1	1,19	0,13	0,04	-	-	-	-	-	-	-	-	-
XI A_2B_6	1,15	0,19	-	-	-	-	-	-	-	-	-	-
XII A_1B_5	1,06	-	-	-	-	-	-	-	-	-	-	-

DL 5% = 0,219
DL 1% = 0,336
DL 0,1% = 0,54

Analyzing the comparisons of the two average of the A factor to the some graduation of B factor regarding the seed production/ha, results that the variants: are significantly positive

CONCLUSIONS

The most favorable results were recorded to the A_1B_1 (2, 61 q/he) variants and A_2B_2 (1, 71 q/he) variants.

The researches have revealed a direct negative correlation between the distance of plant/row (to all variants has maintaining the same distance between rows) and the wooden grade of the seed plants stem. The variants with the most sizeable density to plants were very easy to be cutting in the time of seed plants yield, as result of the stems which were grassier. The more the distance between plants/row were lowest and implies a diminish density of plants on the area unit the more the cutter process of seed was more difficult because of the accentuate developt of the wooden tissue. In such situation was obtained that to the density of 22 222 plants/he the cutting plants (from the harvest process) to be possible only by hatchet.

The most sizeable seed production/he were realized to the variants with the densities of 133 333 plants/he with uncrooked plants.

It is recommended the crops with seed Orache plants having the density of 133 333 plants/he, after the selection scheme 75 cm/10 cm (75 between rows and 10 between plants on row).

Study concerning the properties of some diazotrophic rizobacteria stains for their utility in sustainable agriculture technologies

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Keywords: *Rhizobium sp, Bradyrhizobium* sp**,** survival capacity, edaphic actinomycetes

ABSTRACT

The edaphic diazotrophic bacteria have the possibility to obtain the necessary nitrogen for protein synthesis from the huge gaseous reservation, respectively from atmosphere. The greatest potential of nitrogen fixation is possessed by symbiotic genera like Rhizobium and Bradyrhizobium. It was tested the survival capacity of 16 stains, in relationship with actinomycetes representants. The symbiotic bacteria was *Bradyrhizobium japonicum*, *B. lupini, Rhizobium phaseoly*, *R. leguminosarum* var *cicer ,* stains wits specificity for *Glycine max , Phaseolus vulgaris, Lupinus albus* si *Cicer arietinum*. The obtained results shown intra and interspeciffical differences concerning the resistance to antibiotics produced by edaphic actynomycetes.

INTRODUCTION

The symbiotic nitrogen bacteria, with specificity for Legumes species have a great ecological and agronomical potential that consists in the securement of their use in sustainable agriculture practices and environmental management. For guarantying the symbiosis with performing stains is necessary to isolate and characterize the rhizobacteria stains over the survival capacity in the edaphic microflora, tolerance of prescribed pesticides to the Legumes crops, high efficiency on nitrogen fixation, high competitiveness in nodule formation in the presence of other stains, tolerance to high temperature and low soil moisture (Brockwell, J et al, 1982).

The increasing interest on international level for the use of legumes inoculated with more efficient stains of rhizobia does not resume just at assuring proteic necesaaire through production growth. Maybe most important benefit of simbiotic diazothroph bacteria consists in the sustenability of agriculture technologies as viable alternative in the conservation of soils fertility (Freire et al, 1988). In any cultural systems such as principal culture, intercropping, crop rotation, allied crops through utilization of performant symbiotic stains is assured the improvement of total nitrogen content in soil and is increased the sequestration of carbon dioxide from atmosphere and its incorporation into soil organic matter, which may be an useful way of reducing the greenhouse effect (Freire, J. and Saccol de Sa E. L., 2006).

MATERIAL AND METHODS

There were tested 16 stains of rhizobia, 4 for each species of *Bradyrhizobium japonicum*, B. *lupini, Rhizobium phaseoly*, *R. leguminosarum* var *cicer,* stains with specificity for *Glycine max, Phaseolus vulgaris, Lupinus albus* si *Cicer arietinum.* For the determination of the stains' survival capacity in soil we use the in vitro determination of antibiotic sensitivity test for lyncomycin, neomycin, gentamycin, kanamycin, eritromycin, chloramphenicol and tetracyclyn. The antibiograma test was used for ascertaining of the sensitivity levels. This method assumed obtaining the stationary cultures through „in turf" inoculations and distributing the antibiotic ingrained discson the medium surface after a slight dry. Three repetitions were used for

each stain. The results were read after 48 hours of incubation at 27^0C with measuring the diameter of inhibition area in around of the antibiotic disc.

RESULTS AND DISCUSSION

Regarding the *Bradyrhizobium japonicum* sensitivity, the results obtained show in figure 1, a general high sensibility on chloramphenicol, the values of the inhibition area ranging between 37-41 mm. To lyncomycin the sensitivity is low, the inhibition area ranging between 8-0,5 mm

The reaction to antibiotics of the *Rhizobium phaseoli* stains, figura 2, we remark a high sensibility to kanamycin, the results ranging between 38-40 mm. The lowest sensibility of these stains is remarked to lyncomycin (10-12 mm)

In case of *Rhizobium leguminosarum var cicer* we have determined a high sensitivity on cloramphenicol but this sensitivity is remark only on Nt3 stain, the rest of stains have a lower sensitivity. This illustrates a high survival capacity of these stains in the concurential relationships in soil microflora. The same stain has the lowest sensitivity on lincomicin, the value of inhibition area is 2 mm.

The *Bradyrhizobium lupinii* stains have proven the highest sensitivity on chloramphenicol (34-24 mm), next eritomycin, kanamycin, tetracyclin, neomycin, gentamycin and finaly lyncomycin. We remark on figure 4, the LP 83 stain that has not sensitivity on lincomycin.

Resuming the intraspecific response of stains, represent in fig 5, show a high sensitivity on chloramphenicol of *Bradyrhizobium japonicum* stains, that proves a low competition capacity on *Streptomyces venezuelae*. The high sensibility on kanamicin of *Rhizobium phaseoli* stains reflects a low competition capacity of these rhizobia on *Streptomyces kanamyceticus*. *Rhizobium leguminosarum var cicer* has a high sensitivity on cloramphenicol and tetracyclyne while it is tolerant to the presence of gentamycin and lyncimycin. On *Bradyrhizobium lupinii* stains we found sensitivity on chloramphenicol produced by *Streptomyces venezuelae*, followed decreasingly by tetracyclin and kanamycin. It is also remarked the highest tolerance on lyncomycin.

CONCLUSIONS

The obtained results show that the diazothrophic bacteria have a high surviving capacity in soil on *Streptomyces licolnensis* species. The presence of *Steptomices venezualae* and *Streptomyces erythreus* in soil has an inhibitor effect on rhizobia, a fact that is worth considering at ascertaining the quantity used for legumes seed bacterization. The in vitro estimation of the surviving capacity of rizobia shows an advantage for selecting the valuable genotipes with high competitional capacity.

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Figures

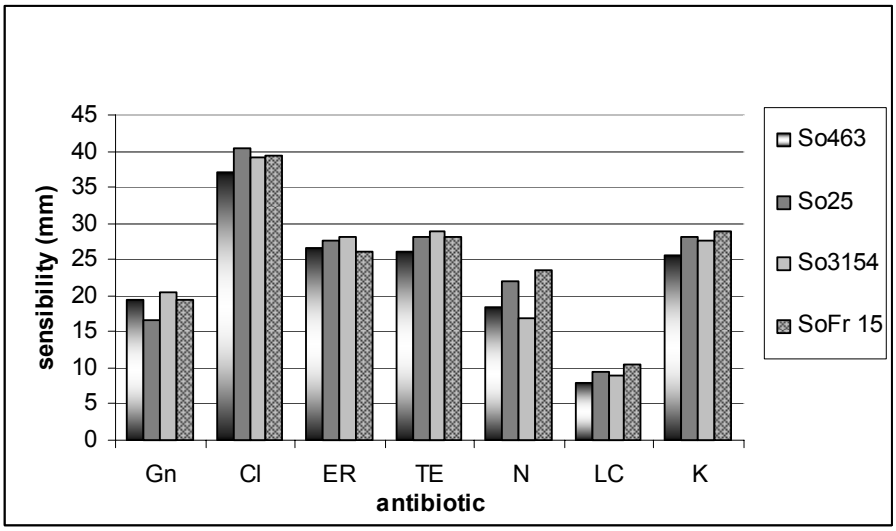

Fig. 1. The sensitivity of *Bradyrhizobiom japonicum* stains

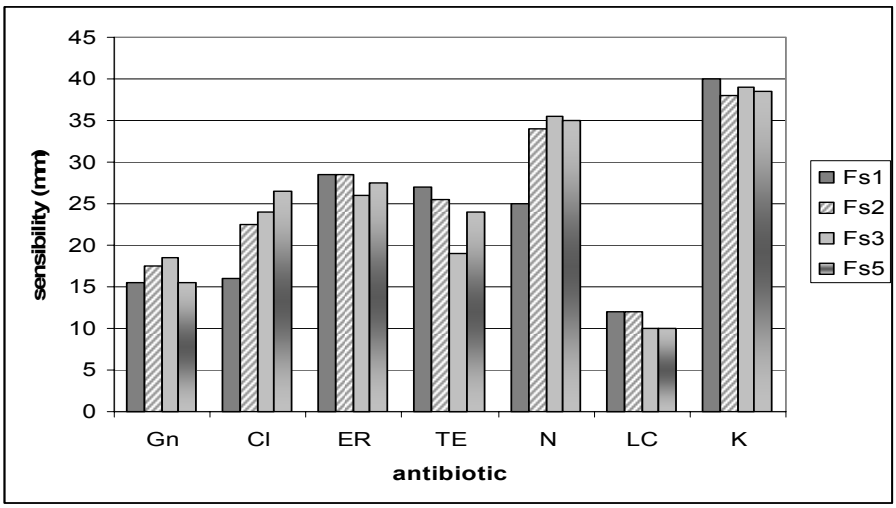

Fig. 2. The sensitivity of *Rhizobium phaseoli* stains

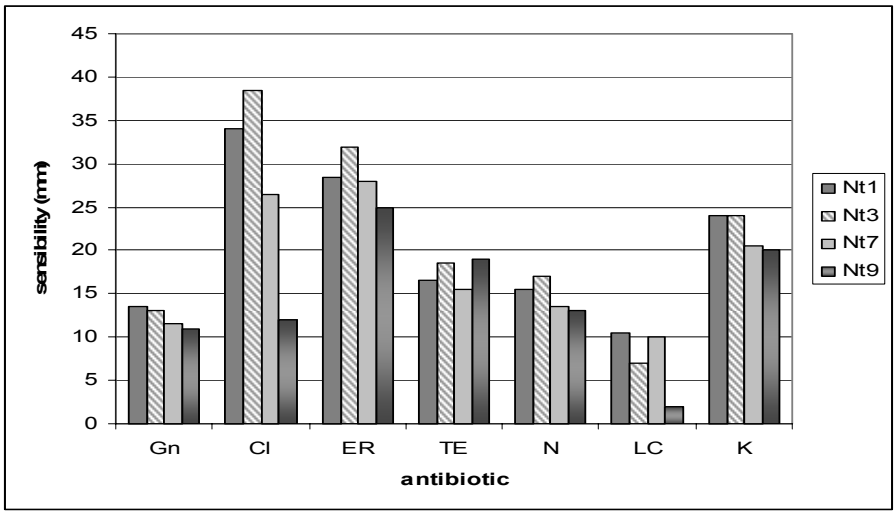

Fig. 3. The sensitivity of Rhizobium *leguminosarum cicer biovar* stains

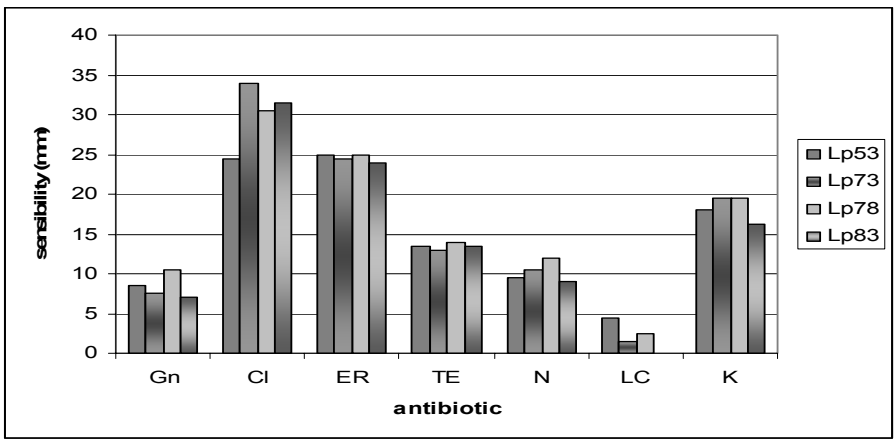

Fig. 4. The sensitivity of *Bradyrhizobium lupinii* stains

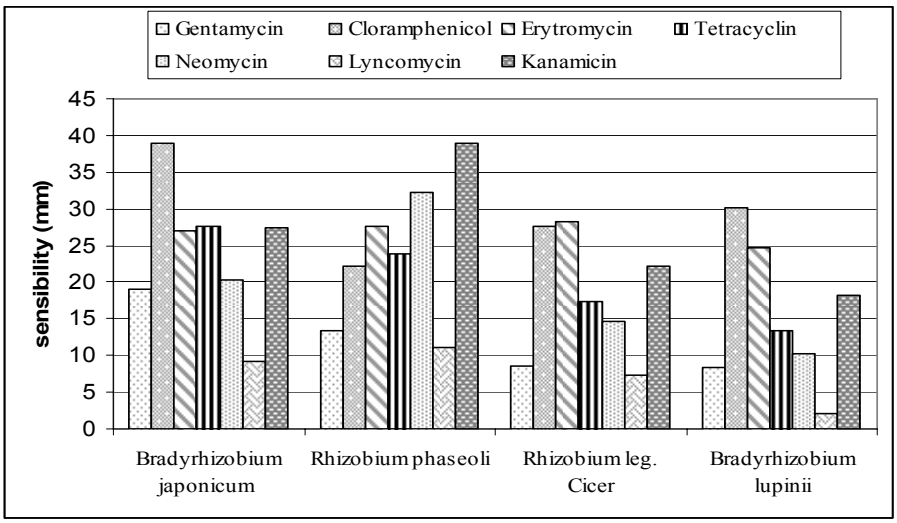

Fig. 5. The sensitivity of rizobacteria diazotrophic species

Yielding capacity of some *Pleurotus ostreatus* mycelia originating from spores

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Keywords: multispore cultures, monokaryons, fertile dikaryons, primordia, sporophores

ABSTRACT

Fertile *Pleurotus ostreatus* mycelia obtained by sporal cultures method have been cultivated on lignocellulosic substrates in order to fructify. They were tested for the yield capacity, together with other two commercial strains and one strain isolated from nature (wild-type).

Two variants of substrate have been used: wheat straws only (S1) and wheat straws mixed with corn cobs (S2). The best results were obtained with the mixture of straw and corn cobs. When cultivated on both types of substrate, one of the multispore cultures (421/I) yielded significantly higher than the commercial strain (P80) used as control.

INTRODUCTION

The mycelial cultures obtained by germination of the basidiospores are often used in the selection and breeding programs of the edible mushrooms (Delmas, 1989; Zervakis et al., 2004). Multispore cultures proved to be very useful for obtaining new valuable commercial strains (Elliott, 1985) and for rejuvenation of some old strains (Fritsche, 1991).

During the former experiments we obtained *Pleurotus ostreatus* mycelia from basidiospores. They were studied and characterized concerning their morphology and growth onto agar plates. The study of mycelium grown onto labs media represents a very useful working instrument for the selection and breeding programs of *Pleurotus sp*. strains (Wu, 1996; Zagrean si Mateescu, 1989).

This works shaws the results of fruiting trials of some mycelia that have been obtained in the initial experiments. Only the strains that proved a significant fruiting capacity in the early experiments have been considered.

MATERIALS AND METHODS

For the fruiting trials three strains of *Pleurotus ostreatus* and some of their multispore cultures were tested: 7bis, 7bis/I, 7bis/II, 426s, 426/I. 426/II, 421, 421/I, 421/II, 421 x 421/I, 421/I x 421/II. A fertile dikaryon derived from individual matings of monokaryons (421/I/8 x 426/I/10) was added.

Granulated spawn was prepared from these mycelial cultures in order to cultivate them on lignocellulosic substrates. Growth of mycelial colonies was recorded after 14 days at 24°C (average of 2 x 10 test tubes) and growth rate was expressed in mm/day. The same was done for the mycelial growth rates on the specific substrate of culture, prepared in two variants: wheat straws (S1) and wheat straws + corn cobs, in equal parts (S2).

In order to evaluate yielding capacity the following *Pleurotus ostreatus* strains were tested: T1) 421 – commercial strain, ICDLF Vidra; T2) 421/I – multispore culture; T3) 426s – tissue culture isolated from a sporophore collected in nature; T4) 426/I - multispore culture; T5) 421/I/8 x 426/I/10 - dikaryon obtained from two monokaryons; T6) control - P80 - commercial strain, Italspawn.

All the variants representing strains were tested in four replicates - bags with 12 kg of substrate, each. The polietilene bags filled with lignocellulosic substrate were spawned with 0,3 kg granulated spawn/bag (2,5%).

The two variants of substrate used as basic materials: S1) wheat straws; S2) wheat straws + corn cobs, in equal parts. Four replicates (bags with 12 kg substrate) were used for each variant, a total of 48 kg of substrate/variant.

The trial was performed in a mushroom house and the spawned bags were randomized on racks with two levels. After spawning and incubation periode (21 days at 24-26°), the environment for fruiting was established: 15-19°C air temperature, 80-85% R.H., 8-10 air exchanges/hour. The mushroom yield was harvested and registered along 42 days (three flushes).

REZULTS AND DISCUSSIONS

The assortment of *Pleurotus ostreatus* strains and their descendents (spore cultures) were checked for the mycelia growth on agar plates, wheat grains, and lignocellulosic substrates.

The comercial strain 421 and the multispore cultures derived from it showed the most rapid growth rates on agar plates and wheat grain (over 8.00 mm/day). The strains 7bis/II, 426/II si 426s (wild-type) showed a slower mycelial growth rate (under 6.00 mm/day).

A special comment should be made on the behaviour of T5 strain (421/I/8 x 426/I/10) - a fertile dikaryon obtained from two monokaryons. Although the two monokaryons showed very slow growth rates (under 2.00 mm/day), the dikaryotic mycelium derived from their mating showed faster growth rates on agar (6.25 mm/day) and wheat grain (6.88 mm/day), respectively. This was the reason for filling it into the list with the strains destinated for fruiting trials.

Growth of mycelia on lignocellulosic substrates for the reproductive stage and fruiting, was checked for nine of the experimental strains/isolates. The results are shown in Figure 1.

The graphic showes that all the mycelia grew faster on the mixture straws + corn cobs (S2) than on the wheat straws only (S1). Multispore cultures 421/I and 421/I x 421/II registered the most rapid growth rates: over 9.40 mm/day on S1 and 9.70 mm/day on S2, respectively.

In the frame of the fruiting trials for yielding capacity of six *Pleurotus ostreatus* strains/isolates, fruitbodies (sporophores) were harvested during the first three flushes, along (42 days). The earliest strain was P80 – a commercial strain (Italspawn) used as control : the primordia were noticed 18 days after spawning. The other strains fallowed it: 421/I (19 days), 421 (20 days), 421/I/x 426/I/10 (25 days), 426s si 426/I (27 days). Mycelia with faster growing rates also fructified earlier. The wild-type isolates – 426s and 426/I – fructified the latest. This could be explained by the fact that *P. ostreatus* needs for fructify of an important decreasing of temperature (a negative thermal shock), but in our trials this condition was not accomplished.

If the combined influence of substrate and strain on the yield of fresh mushrooms is checked, it should be noticed that all the strains yielded better on straws + corn cobs (S2) than on straws only (S1). Multispore culture 421/I (T2) showed the biggest production (27.38 kg/100 kg substrate S2), with a very significant higher yield (+4.04 kg/100 kg substrate S2) than the control strain P80 (T6).

Due to its high yield and earliness, multispore culture 421/I became important for the assortment of *Pleurotus ostreatus* strains cultivated in our country.

The other strains showed inferior levels of production by comparison with the control strain, with very significant negative differences, as following: - 12,95 kg/100 kg substrate (426/I), -12,62 kg/100 kg substrate (426s), - 9,75 kg/100 kg substrate (421/I/8 x 426/I/10) and – 3,77 kg/100 kg substrate (421), respectively.

CONCLUSIONS

The growth of *P. ostreatus* mycelium on laboratoire media (malt extract - agar and wheat grains) was faster - over 8.00 mm/day – in the case of the comercial strain 421 and its descendent multispore cultures. The strains 7bis/II, 426/II si 426s (wild-type) showed a slower mycelial growth rate (under 6.00 mm/day).

The fertile dikaryotic strain obtained from two monokaryons (421/I/8 x 426/I/10) showed mycelial growth rates of 6.25 mm/day (agar) and 6.88 mm/day (wheat grain), higher than growth rates of the two monokaryons (under 2.00 mm/day).

The mycelia of the strains tested for yield capacity colonized faster the mixture straws + corn cobs (S2) than the wheat straws only (S1). Multispore cultures 421/I and 421/I x 421/II registered the most rapid growth rates: over 9.40 mm/day on S1 and 9.70 mm/day on S2, respectively.

First primordia appeared onto the bags cultivated with strain P80 (control), after 18 days from spawning and onto the bags spawned with 421/I (19 days). The strains of wild-type 426s and 426/I fructified the latest, after 27 days from spawning.

Multispore culture 421/I showed the biggest production, 27.38 kg/100 kg substrate S2, with 4.04 kg more than the control strain P80. Due to its high yield and earliness, multispore culture 421/I became important for the assortment of *Pleurotus ostreatus* strains cultivated in our country.

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Tables

Table 1. Combined influence of substrate and strain on the yield of *P. ostreatus*

Var.	S1: wheat straws				S2: wheat straws + corn cobs			
	Yield			Semnific.	Yield			Semnific.
	Kg/100 kg substrate	Dif. +/-	%		Kg/100 kg substrate	Dif. +/-	%	
T1	19.3256	-3.8734	83.30	ooo	19.5755	-3.7693	83.85	ooo
T2	25.5939	2.3949	110.32	***	27.3848	4.0400	117.30	***
T3	10.3500	-12.8490	44.61	ooo	10.7248	-12.6200	45.94	ooo
T4	11.2038	-11.9952	48.29	ooo	10.3912	-12.9536	44.51	ooo
T5	12.5366	-10.6624	54.04	ooo	13.5987	-9.7461	58.25	ooo
T6 (Mt)	23.1990	-	100.00		23.3448	-	100.00	-

DL 5% = 1.3446 DL 5% = 1.2752
DL 1% = 1.7883 DL 1% = 1.7689
DL 0,1% = 2.3261 DL 0,1% = 2.4544

Figures

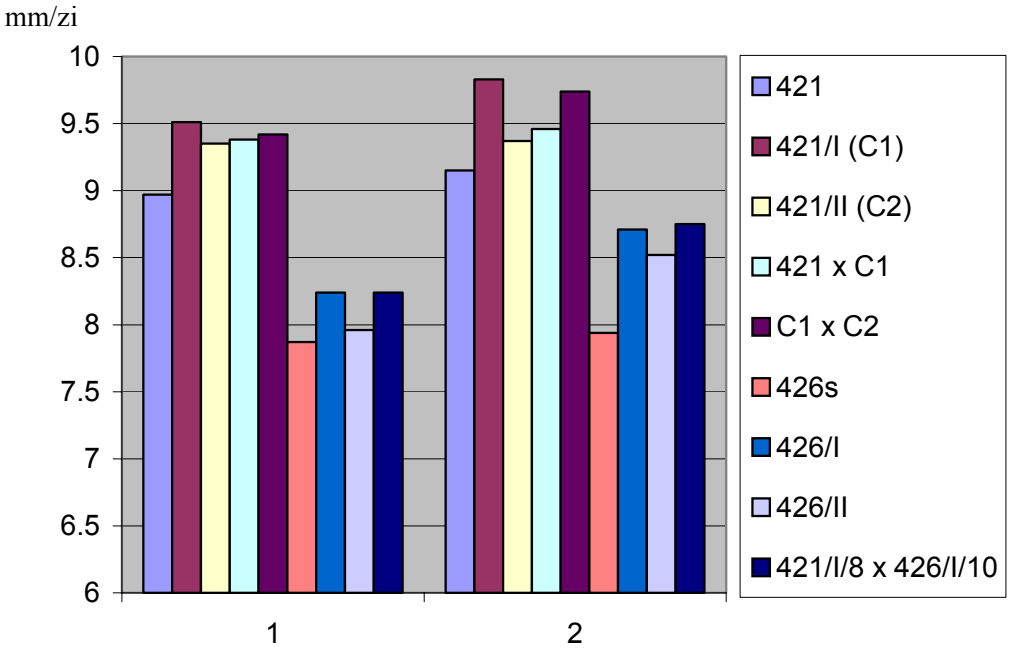

Fig. 1. Growth of *Pleurotus ostreatus* mycelium on lignocellulosic substrates (1 = wheat straws ; 2 = wheat straws + corn cobs)

Researches on the use of the spore cultures for obtaining *Pleurotus ostreatus* commercial spawn

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Keywords: pure cultures, basidiospores, monokaryons, electrophoresis, isozymes.

ABSTRACT

Pure cultures of *Pleurotus ostreatus* isolates with various origins have been examined collection stock-cultures and wild–type isolates (new strains from natural soils). Mature fruit bodies were obtained from these pure cultures during fruiting trials. Their basidiospores were used in order to obtain monospore and multispore cultures. These spore cultures were studied for their growth characteristics on agar plates. The possibility to obtain fertile dikaryons by mating pairs of compatible monokaryons (controlled hybridization) or by mixing multispore cultures (random hybridization), has been tested too. In order to characterize the hybrids as well as the parental strains, electrophoretic pattern of total proteins or of peroxidase isozymes was examined. The results obtained allowed the selection of some strains that presented increased performances, useful for commercial purposes.

INTRODUCTION

Pleurotus ostreatus is an edible fungus of great practical interest, not only for its ability to grow on various agricultural residues to produce mushrooms of high organoleptic quality, but also for their secondary metabolites with pharmaceutical applications and for some proteins of industrial use (Marino et al., 2003).

Mycelium pure cultures are traditionally obtained by using of four basic methods: mycelia transfer, monospore cultures, multispore cultures and tissue cultures (Petersen and Hughes, 1997). The mycelia transfer (subculture) is the usual method for the manipulation and propagation of the fungi pure cultures (Zagrean and Mateescu,1992). The tissue cultures are indicated for obtaining fertile isolates from wild sporophores collected from nature. The spore cultures are utilized for the selection and breeding programs of the edible mushrooms (Zervakis et al., 2004).

Many valuable commercial strains originate from the multispore cultures that have been created in the breeders laboratories. More, rejuvenation of some ”old” strains is often done by subculturing multispore isolates (Fritsche,1991). The monospore cultures of heterothallic species - e.g. *Pleurotus sp.* – are important elements for obtaining intra- and interspecific hybrids (Perberdy et al, 1993).

The study of mycelium cultures grown on agar media represents a very useful working instrument for the identification and classification of *Pleurotus* species/strains and for their breeding programs (Zagrean and Mateescu,1989; Iracabal et al,1991). The application of some electrophoresis techniques, e.g. detection of the isozyme activities, adds valuable information to the knowledge obtained by conventional methods, providing valuable data about the genetic structure of the studied mushrooms.

The objective of this work was to examine the possibility to obtain *Pleurotus sp*. commercial mycelium originating from mono- and multispore cultures. This study includes both morphological aspects and growth characteristics of the mycelia colonies and checks up for the possibilities to put into practice some electrophoresis techniques in order to distinguish different isolates.

MATERIALS AND METHODS

Mushroom strains: 1) strains from the collection - *Pleurotus ostreatus* 421, 7 bis, *P. florida* 308, 362, *P. sajor-caju* 392, *P. cornucopiae* 386, *Agaricus bisporus* 460/I (M1), 460/II (M2)**,** 462/II (O1), 462/3 (O2), 465/I (R1); 2) wild – type isolates collected from nature - *P. ostreatus* 426 s, 515 s; 3) basidiospores (spore prints) – collected from some sporophores of the *Pleurotus* strains/isolates used in the experiments.

„In vitro"cultures: Mycelium cultures were grown on malt extract (2%) – agar (2%) plates (Petri dishes), pH 6.5-6.0 and sterilized 25 min/121°C. To get monospore cultures the method of dilutions (10^{0},10^{-1},10^{-2},10^{-3}) in sterile distilled water was used: 0,1 ml of strong spore diluted suspensions were seeded on agar surface and incubated in darkness at 24-26°C, 4 replicates per isolate. To get multispore cultures the basidiospores were "in mass" seeded on agar surface and incubated in darkness at 24-26°C, 4 replicates per isolate. Then, the mono- and multispore cultures were subcultured on fresh media (two successive transfers) for the determination of their colony characters. In order to obtain intra-/interspecific hybrids of *Pleurotus sp*., some mating of "side by side" type between different mycelia fragments on Petri dishes were realized.

Enzyme extraction and polyacrylamide gel electrophoresis . For the production of mycelial cultures, fungal strains were grown in 10 ml liquid medium (20% malt extract). Mycelium was harvested, and stored at -20^{o}C for 24 h in extraction buffer (50 mM Tris HCl, pH 6,8). Frozen mycelium was crushed in a mortar, then centrifuged and supernatant was collected and used for electrophoresis. Proteins were separated by 10% polyacrylamide gel electrophoresis and peroxidase activity was detected using benzidine solution (1mg/ml benzidine in acetic acid/sodium acetate buffer) and hydrogen peroxide as substrate.

RESULTS AND DISCUSSIONS

The "in mass" seeded basidiospores germinated without any stimulation and viable mycelium colonies emerged on the surface of agar plates. Spore germination and growth of the resulting multispore cultures were faster in case of *Pleurotus* strains (3-7 days incubation) than in *Agaricus bisporus* (8-12 days), at 24-26^{o}C. Contrary, at temperatures under or above these values the time necessary for mycelium development increased significantly. There were no significant differences regarding the germination time between the basidiospores originated from several sporophores of the same strain.

The colonies morphology remains unchanged during repeated transfers done once at every 2-4 months. The distinctive type of *Agaricus bisporus* mycelium is a uniform feltry net of grey-white colour with medium aerial development and density. The mycelium of *Pleurotus sp.* strains has a snow white fluffy-aerial semblance with a quite uniform and dense net - unless *P. cornucopiae* – and it frequently shows concentric growth strips.

The use of morphological and growth characteristics of the mycelia colonies for their selection in subcultured phase constitutes a quite efficient method for the preservation and propagation of cultivated mushrooms strains. This method is very useful in the frame of some conventional breeding programs, too.

On agar plates, *Pleurotus sp.* multispore cultures showed faster growing rates (4.42 – 8.64 mm/day) than *Agaricus bisporus* (2,01 - 3,27 mm/day). The most rapid growth was recorded with *P. ostreatus* strains C1 and C1 x C2, both descending from

the commercial strain 421. At the opposite side there were situated *P. cornucopiae* strains 386 and 386/I, which showed comparatively the slowest growth (fig.1).

Pleurotus sp. single-spore cultures originated from 3 sporal prints: *P.ostreatus* 426/I (sporophore collected from natural environment), *P. ostreatus* 421/I and *P.florida* 362/III (collection strains). From these spore prints, 98 putative monokaryotic isolates (single spore isolates) have been studied: 21 isolates from original 426/I strain, 21 isolates from 421/I strain and 56 isolates from 362/III.

Morphological and growth differences between some monospore cultures which are originating from the same spore print and grown in identical conditions were observed. These differences indicate putative monokaryons (fig.2).

These data are interrelated with those obtained by studying the same biological material with electrophoresis methods. Electrophoretic pattern of the total proteins extracted from different *Pleurotus* species and visualized after CBB staining shown an increased interspecific polymorphism (fig.3). Similar aspects were observed when peroxidase activity was detected in polyacrylamide gel: the isozymes profile was different among *Pleurotus* species. Moreover, surprisingly, enzymatic activities, detected after electrophoretic separation of proteins extracted from *P.ostreatus* 421 and various descendents from monospore cultures were related to different protein bands, the pattern being polymorphic. For example, parental strain presented only two proteins with enzymatic activity, the descendent 20 presented 6 enzymatic bands and the other descendents contain among 1 and 3 bands with peroxidase activity (fig.4). Similar results were observed in *P.ostreatus* by different authors, and suggest an increased intraspecific variability.

CONCLUSIONS

1. Basidiospores collected from *Pleurotus sp*. sporophores germinate and form mycelial colonies in a shorter period of time (3-7 days incubation) than those from *Agaricus bisporus* (8-12 days).
2. The mycelium of *Pleurotus sp.* strains has a snow white fluffy-aerial semblance with a quite uniform and dense net and it frequently shows concentric growth strips.
3. The morphological and growth differences between the monospore cultures originating from the same spore print and grown in identical conditions constitute valuable indications for the identification of the monokarions derived from heterothallic specia, e.g. *Pleurotus*.
4. The most rapid growth on agar plates was recorded with *P. ostreatus* strains C1 and C1 x C2 (hybrid), both descending from the commercial strain 421.
5. Electrophoretic separation of proteins extracted from various fungal strains and peroxidase activity detected in PA gels could offer supplementary information concerning the intra- and interspecific variability, and could be useful for a more complete characterisation of the new isolates.

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Figures

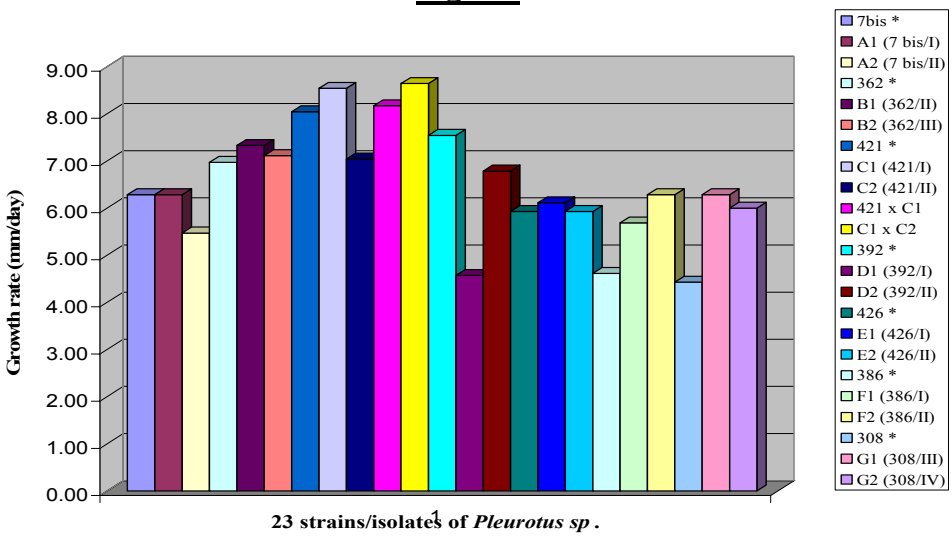

Fig.1. Multispore cultures of *Pleurotus sp*. grown on malt extract – agar plates

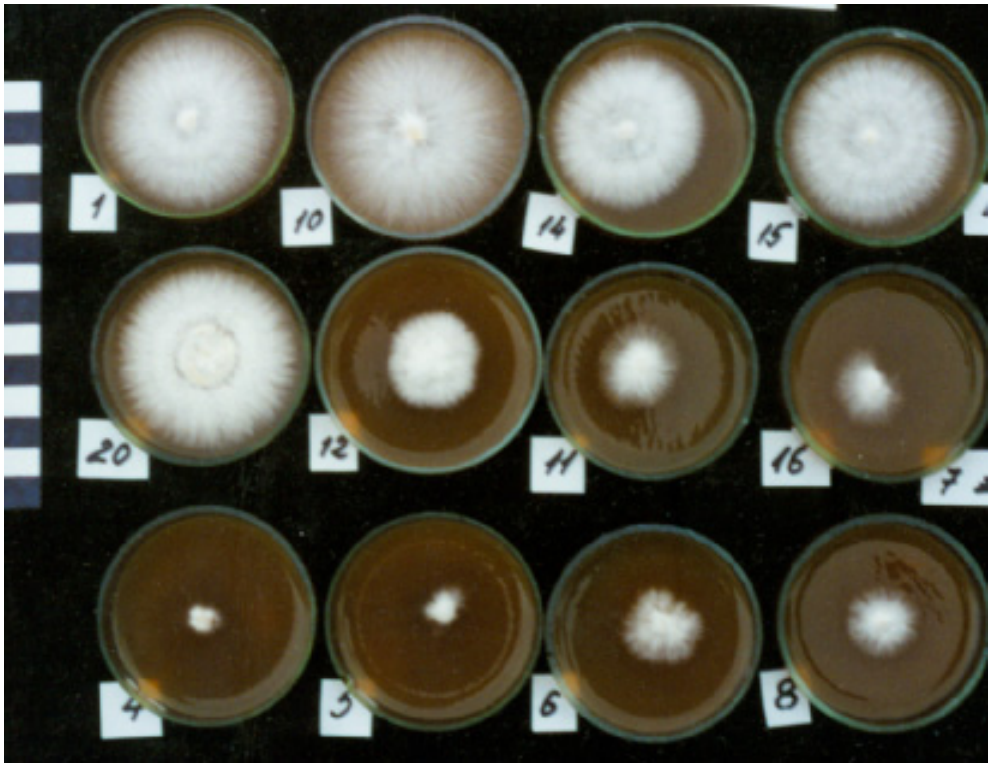

Fig.2. Monospore cultures of *Pleurotus ostreatus* – originated from 421/I obtained on malt extract – agar plates, after 7 days incubation

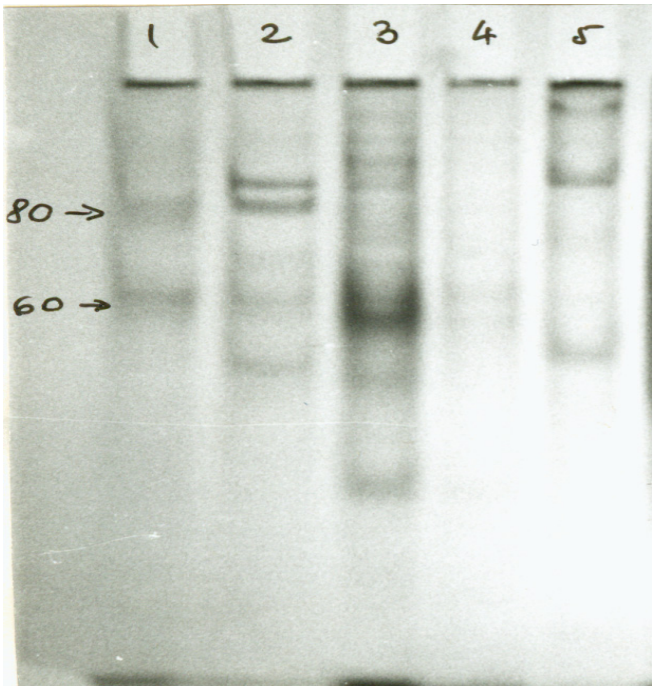

Fig. 3. Electrophoretic pattern of proteins extracted from different *Pleurotus* species: 1) *P. florida* 308; 2) *P. sajor - caju* 392; 3) *P. ostreatus* 421; 4) *P. ostreatus* 426s; 5) *P. cornucopiae* 386;

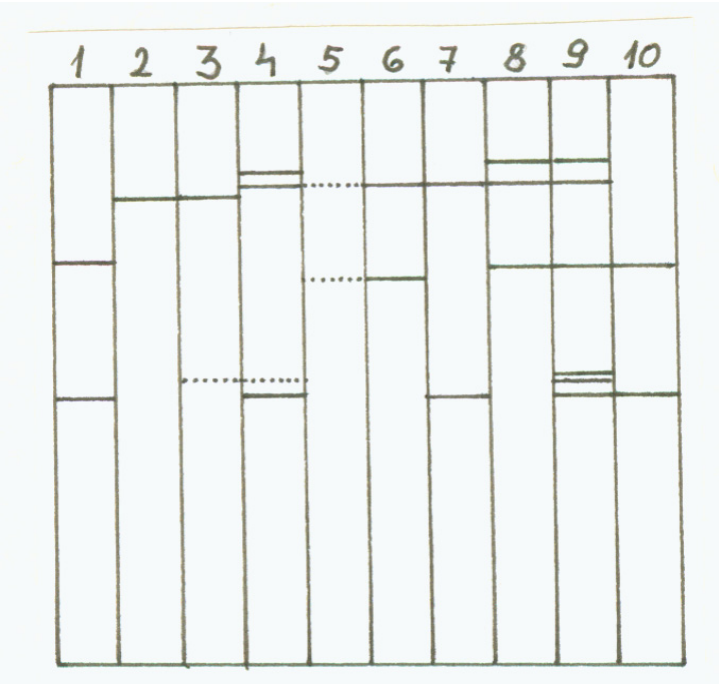

Fig. 4. Schematic representation of proteins with enzymatic activity detected after separation in polyacrylamide gels and benzidine treatment. 1) *P. ostreatus* 421; 2) 421/I/1; 3) 421/I/5; 4) 421/I/6; 5) 421/I/10; 6) 421/I/12; 7) 421/I/14; 8) 421/I/15; 9) 421/I/20; 10) *P. ostreatus* 421

Studies about the influence of the hybrid and the density in the fall crop of broccoli

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Keywords: *Brassica oleracea, convar. botrytis var. italica* Plench (1808), autumn crop, nutrition space, hybrids, production

ABSTRACT

In Romania, broccoli is a relatively new vegetable, being cultivated on limited surfaces. These species tend to have a bigger and bigger presence among the vegetables, especially because of the therapeutic effect recently discovered of protection against the cancer. The purpose of the researches was to outline the influence of the density in the development in the crop of three hybrids of broccoli, in order to obtain a better technology and an improvement of the production. As a biological material, there were used the following hybrids: Chevalier (Asgrow-USA), Milady (Asgrow-USA), and Clx3501-Ms (Clause-France). There were studiet four densities: 3,7 (the control variant); 4,1; 5,7; 6,6 plants/m^2. The biggest mean main production was obtained at a large density 6,6 plants/m^2 (36,39t/ha at Chevalier, at Milady 29,26t/ha and at Clx-3501Ms 24,39t/ha), but the inflorescences were smaller in this case (0,546 kg/plant at Chevalier, at Milady 0,439 kg/plant and 0,366 kg/plant at Clx-3501Ms). At a small density (3,7 plants/m^2), there were obtained the biggest inflorescences (0,760 kg/plant at Chevalier, at Milady 0,558 kg/plant and at Clx-3501Ms 0,490 kg/plant), but the production was smaller (28,14t/ha at Chevalier, at Milady 20,66t/ha and 18,14t/ha at Clx-3501Ms).

INTRODUCTION

It isn't completly clear how the broccoli plant reponds at the changes of the shadowing with respect to the plant density, eventhough the effects of the density upon the commercial characteristics are well determined (Francescangeli et al., 2006).

As with most crops, yield per unit area increases with number of plants, until a plateau is reached (Cutcliffe, 1975 a). Although head size at maturity decreases as the plant population increases, consumers accept a range of head sizes (Wien H. and Wurr D., 1997).

In addition to high yields, close spacing has additional advantages: plants have a lower incidence of hollow stem (Zink and Akana, 1951), they produce few sideshoots, and the main shoot has fewer leaves, reducing trimming waste at harvest (Thomson and Taylor, 1976).

The purpose of the researches was to study in comparison the adaptation of three hybrids of broccoli in the fall crop, as well as the may in which the plants responded at different plant densities.

The development of broccoli cultures in our country will permit not only a diversification of the vegetables, and some additional incomes, but also the improvement of the nutritive share in a balanced alimentation.

The studies made by specialists (Ciofu Ruxandra and Dobrin Elena, 2003) have demonstrated that this species can have good results even in the climatical conditions from Romania, having the possibility of being cultivated in several crop systems.

These kinds of experiments with different hybrids and plant densities were applied in the case of early crop, highlighting the importance of the nutrition space and of the hybrid which is being cultivated (Zăvoianu R. and Popescu V., 2006).

MATERIAL AND METHODS

The research was made at the Departament of Vegetable Growing of Horticulture Faculty belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest, in 2006. It was organized (table 1) a bifactorial experiment in subdivised lots with three repetitions: The A Factor (Hybrid), with three gradations: a1=Chevalier F1; a2=Milady F1; a3=Clx-3501Ms, and the B Factor (Density), with four gradations: b1=70/25cm, 5,7plants/m²; b2 = 60/25cm, 6,6 plants/m²; b3=70/35cm, 4,1 plants/ha; b4=60/45cm, 3,7 plants/ha (the control variant). Densities of 5,7plants/m² and 4,1plants/m² are recommended in the literature (Popescu V., and Atanasiu N., 2000).

Taking into account the importance of this species, the crop started with the planting of pricked aut transplant. The seeding (table 2) was made in boxes at 19th June, and the emergence of the plants happened after four days (23rd June). In the phase with 1-2 real leaves, the transplants were pricked out in plastic flower-pots, differently coloured. The planting was made on 27th July (the Chevalier and Milady hybrids) and 31st July (the Clx3501-Ms hybrid). During the vegetation period there were applied the specific operations of maintenance: the completion of the empty spaces, digging in order to destroy the grass, two phasale fertilizations with Complex III, the rebutment of the harmful insects, the irrigation of the crop in moderate phases, but more often than usual because of the high temperatures.

The harvest was made in the most propitious moment, being obtained good inflorescences, with floral buds completely closed, compact and having an uniform colour, specific for the hybrid, without any deficiencies. During the research there were made comparative determinations between the hybrids and the used densities.

The data conditioning of the mean main production (t/ha) were obtained using the method of the analysis of the variant, and correlations between the mean main production (t/ha) and plant density (plants/ha), and between mean weight of the main inflorescences (kg/plant) and density (plants/ha) have been calculated.

It was also analysed the content of pigment (mg/100g) of the broccoli inflorescences, the total dry matter (%) and dry soluble substance (%), the acidity (mg/100g) and the water content (%).

RESULTS AND DISCUSSIONS

The emergence rate (table 2) was very good, respectively 93,8% at the Chevalier hybrid, 91,4% at the Milady hybrid, and 90% at Clx-3501Ms. The most propitious conditions of light and heat have determined the speeding up of the growing rhythm of the plants, so the plants had reached the best parametres for planting in a shorter period of time (35-38 days). Before planting, the transplants were equally developed, having a height of 15,2 cm and 6,3 leaves at the Chevalier hybrid, 13,9 cm and 6,1 leaves at Milady, and 12,9 cm and 5,8 leaves at Clx3501- Ms. Observing the growth of the broccoli plants (figure 1) after their planting in the field, it was noticed the influence of the hybrid and of the nutritional space. The decrease of the plants density is directly related with the plants height, but has a good influence on the stem's diameter, which grows.

The stages of the phenophases after the planting of the transplant are showed in tabel 3.

It can be noticed that the appearance the secondary sprouts took place between August 11th and August 15th, while the appearance of the main inflorescences took place between 12th and 18th September.

The harvest of the main inflorescences took place between October 5th (Chevalier and Milady) and October 12th (Clx-3501Ms). The secondary inflorescences were harvested between 26th and 27th of October. The number of days between planting and harvesting varied between 71 and 74 days, depending on the type of hybrid cultivated.

During the period of inflorescence formation (September), there were recorded propitious temperatures for their growth, so the minimum mean temperature was of 10,7°C, and the maximum mean temperature of 24,9°C (figure 2).

With analyze of the inflorescence during the harvest, it was noticed that there are some differences determined by the applied density. The mean weight of the main inflorescence is smaller at large densities and grows direct proportionally with the growth of the nutritional space. The dates presented at table 4 refering at the main production of broccoli shows that in the variants with large densities, big broccoli productions are obtained at all kinds of hybrids. These results are in accordance with those obtained by other authors (Wien and Wurr, 1997; Cutcliffe, 1975). Because of the big number of harvested inflorescences, the mean main production at big density (6,6 plants/m²) is of 36,39t/ha in the case of Chevalier hybrid, 29,26 t/ha in the case of Milady, and 24,39t/ha in the case of Clx3501-Ms. A smaller production is obtained by the variants where the density is smaller, so at 3,7 plants/m², the mean main production at V4controlChevalier is of 28,14t/ha, at V8control Milady 20,66t/ha, and 18,14t/ha at V12controlCLx3501-Ms.

From the total production, the main production was the biggest (88,06-93,71% at Chevalier hybrid, at Milady 88,02-90% and at Clx-3501Ms 87,03-88,19%), while the secondary production oscillated between 6,28%- 11,93% at Chevalier hybrid, 10-11,97% at Milady hybrid, and between 11,80%-12,96% at Clx3501-Ms hybrid.

The statistic interpretation of the results of the production can be seen in table 5.

At the Chevalier hybrid, at the variants with large plant densities (5,7 – 6,6 plant/m2), the differences with regard to the control are very significant. At V3, the difference is significant in a negative way. At Milady and Clx-3501Ms hybrids, at all the variants with larger plant density than that of the control, the differences are very significant.

There is a very significant positive correlation (figure 3) between the mean main production (t/ha) and the density (plants/ha), and also a very significant negative correlation (figure 4) between the mean weight of the main inflorescences (kg/plants) and the plant density (plants/ha).

The largest content of total chlorophyll (table 6) was found at the hybrid Chevalier 65,13mg/100g, at Milady of 40,44mg/100g and Clx-3501Ms 61,48mg/100g. The ratio Total Chlorophill/Carotene which gives information about the value of the green shade had the biggest value at the Milady hybrid 3,84mg/100g followed by Chevalier 2,53mg/100g and Clx-3501Ms with 1,10mg/100g.

The content of total dry matter of the inflorescences had the biggest value at the Clx-3501Ms hybrid (14,95%), while at the other two hybrids the values were similar (15,3%). There were no differences between hybrids with respect to acidity, which had

the value of 1,8mg/100g. The results obtained are in accordance with those from the literature (Burzo I. et. al., 2005).

Some aspects of the variants studied are presented in the figures 5, 6, 7, 8, 9, 10.

CONCLUSIONS

The broccoli transplants for the fall crop can be easily obtained in glasshouse or in could substratum in 35-38 days.

Knowing the length of the period between planting and harvesting, which differs depending on the hybrid (71 days for Chevalier, 72 days for Milady, and 74 days for Clx-3501 Ms), in can be made a schedule of the harvest.

In the fall crop, the studied broccoli hybrids gave good results, the biggest mean total production was obtained by the Chevalier hybrid 41,33/ha, followed by Milady with 33,19t/ha, and Clx3501-Ms, with 27,66t/ha.

At all the hybrids, the biggest mean main production was obtained by the density of 6,6 plants/m² (36,39t/ha-Chevalier, 29,26t/ha-Milady şi 24,39t/ha-Clx-3501Ms).

There is a very significant positive correlation between the weight of the inflorescences and the nutrition space, at all the hybrids studied.

Tha ratio total chlorophyll/caroten, which gives information about the green nuance of the inflorescences had the biggest value at the Milady hybrid 3,84 mg/100g, followed by Chevalier 2,53 mg/100 g and Clx-3501 Ms with 1,10 mg/100g.

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Tables

Table 1. Experimental variants

Hybrid	Experiences – plant density				
	Variant	Distances (cm)	Nutrition space (cm^2)	Number of plants/m^2	Number of plants/ha
Chevalier	V1	70/25	0,175	5,7	57142
	V2	60/25	0,150	6,6	66666
	V3	70/35	0,245	4,1	40816
	V4 Control	60/45	0,270	3,7	37037
Milady	V5	70/25	0,175	5,7	57142
	V6	60/25	0,150	6,6	66666
	V7	70/35	0,245	4,1	40816
	V8 Control	60/45	0,270	3,7	37037
Clx-3501Ms	V9	70/25	0,175	5,7	57142
	V10	60/25	0,150	6,6	66666
	V11	70/35	0,245	4,0	40816
	V12 Control	60/45	0,270	3,7	37037

Table 2. The results regarding the unfolding of the phenophases at the production of the broccoli transplants for the establishment of the fall crop

Specification		Hibryd		
		CHEVALIER	MILADY	CLx-3501-MS
Production of the transplants	Seeding	19th June	19th June	19th June
	Emergence	23rd June	23rd June	23rd June
		93,8%	91,4%%	90%
	Pricking out	3rd July	3rd July	5th July
	Age of the transplant (days)	35	35	38
	Height (cm)	15,2	13,9	12,9
	Number of leaves	6,3	6,1	5,8
Planting (date)		27th July	27th July	31th July

Table 3. The phenophases at broccoli from the planting to the harvesting

Specification		Hybrid		
		CHEVALIER	MILADY	CLX-3501MS
Vegetation	Appearance of the secondary sprouts/plant	11th August	11th August	15th August
	*Appearance of the main inflorescences	12th September	12th September	18th September
Harvesting	The main inflorescences	5th Octomber	6th Octomber	12th Octomber
	The secondary inflorescences	26th Octomber	26th Octomber	27th Octomber
Number of days between planting and harvesting		71	72	74

* easy to see

Table 4. Production indicators at the broccoli in the fall crop

Variant	Mean weight of the main inflorescences*	Mean main production		Mean weight of the secondary inflorescences*	Mean secondary production		Mean total weight of the inflorescences*	Mean total production	
	(kg/plant)	(t/ha)	(%)	(kg/plant)	(t/ha)	(%)	(kg/plant)	(t/ha)	(%)
The Chevalier hybrid									
V1	0,575	32,856	92,00	0,050	2,857	8,00	0,625	35,713	100
V2	0,546	36,399	88,06	0,074	4,933	11,93	0,620	41,332	100
V3	0,667	27,224	93,28	0,048	1,959	6,71	0,715	29,183	100
V4cont.	0,760	28,148	93,71	0,051	1,888	6,28	0,811	30,037	100
The Milady hybrid									
V5	0,500	28,571	88,02	0,068	3,885	11,97	0,568	32,456	100
V6	0,439	29,266	88,15	0,059	3,933	11,84	0,498	33,199	100
V7	0,527	21,510	88,13	0,071	2,897	11,87	0,598	24,407	100
V8cont.	0,558	20,666	90,00	0,062	2,296	10,0	0,620	22,962	100
The Clx-3501Ms hybrid									
V9	0,380	21,713	88,16	0,051	2,914	11,83	0,431	24,628	100
V10	0,366	24,399	88,19	0,049	3,266	11,80	0,415	27,666	100
V11	0,469	19,142	87,17	0,069	2,816	12,82	0,538	21,959	100
V12cont.	0,490	18,148	87,03	0,073	2,703	12,96	0,563	20,851	100

* Commercial inflorescence: botanical inflorescence + stem (10cm)

Table 5. The statistic interpretation of the results of the production

Place	Variant/ (plants/m²)	Mean main production (t/ha)	The difference (t/ha) between it and the variant from the following places:			
			IV	III	II	I
Hybrid Chevalier						
I	V1 - 5,7	32,856	4,708***	5,632***	-3,543°°°	-
II	V2 - 6,6	36,399	8,251***	9,175***	-	3,543***
III	V3 - 4,1	27,224	-0,924°°°	-	-9,175°°°	-5,632°°°
IV	V4Mt-3,7	28,148	-	0,924***	-8,251°°°	-4,708°°°
Hybrid Milady						
I	V5 - 5,7	28,571	7,905***	7,061***	-0,695°°°	-
II	V6 - 6,6	29,266	8,6***	7,756***	-	0,695***
III	V7 - 4,1	21,510	0,844***	-	-7,756°°°	-7,061°°°
IV	V8Mt - 3,7	20,666	-	-0,844°°°	-8,6°°°	-7,905°°°
Hybrid CLX3501-Ms						
I	V9 - 5,7	21,713	3,565***	2,571***	-2,686°°°	-
II	V10 - 6,6	24,399	6,251***	5,257***	-	2,686***
III	V11 - 4,1	19,142	0,994***	-	-5,257°°°	-2,571°°°
IV	V12Mt - 3,7	18,148	-	-0,994°°°	-6,251°°°	-3,565°°°

DL $_{5\%}$=0,186 t/ha; DL $_{1\%}$=0,255 t/ha; DL $_{0,1\%}$= 0,347 t/ha;

Table 6. The content of pigment from the broccoli inflorescences

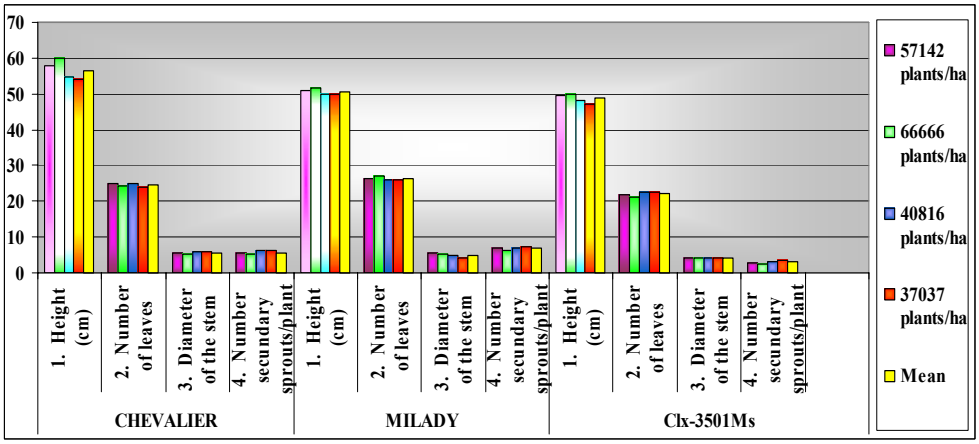
Hybrid	(mg/100g)					
	Chloro-phyll-a	Chloro-phyll-b	Total chloro-phyll	a:b Chloro--phyll ratio	Carote-nes	Total chlorophyll: carotenes ratio
Chevalier	41,99	23,13	65,13	1,86	35,75	2,53
Milady	26,02	14,41	40,44	1,82	10,55	3,84
Clx-3501Ms	39,03	22,95	61,48	1,73	56,61	1,10

Table 7. The content of broccoli inflorescences

Hybrid	Water (%)	Mineral substances (%)	Total dry substance (%)	Dry soluble substance (%)	Acidity (mg/100g)
Chevalier	84,65	1,26	15,34	6,45	1,8
Milady	84,60	1,25	15,39	6,12	1,8
Clx-3501Ms	85,04	1,29	14,95	5,8	1,8

Figures

Fig. 1. The influence of the density in the growing of broccoli plants



1. Total mean height (cm)
2. Total mean number of leaves
3. Mean diameter of the stem (cm)
4. Mean number of secundary sprouts/plant

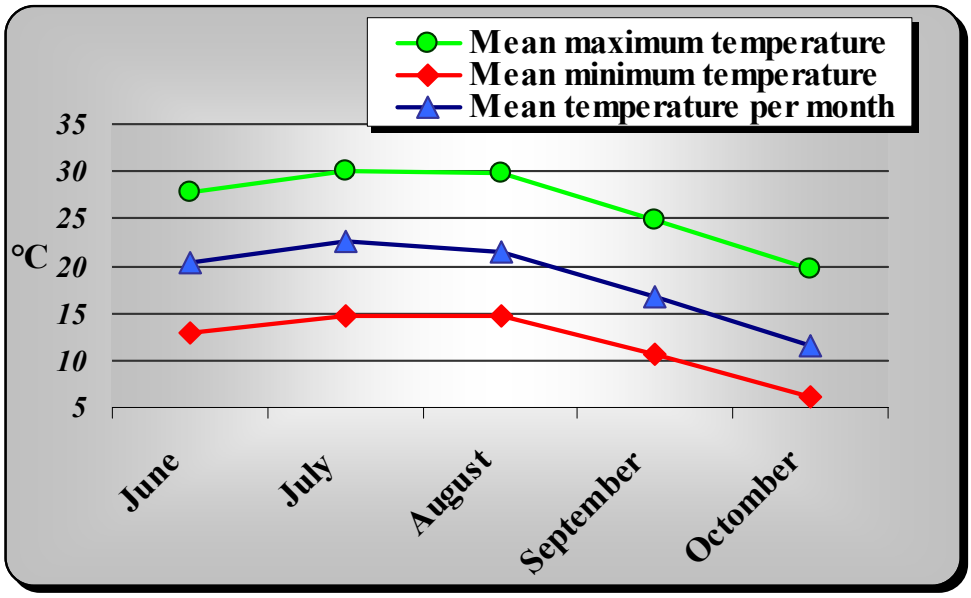


Fig. 2. The evolution of temperature during the experiment

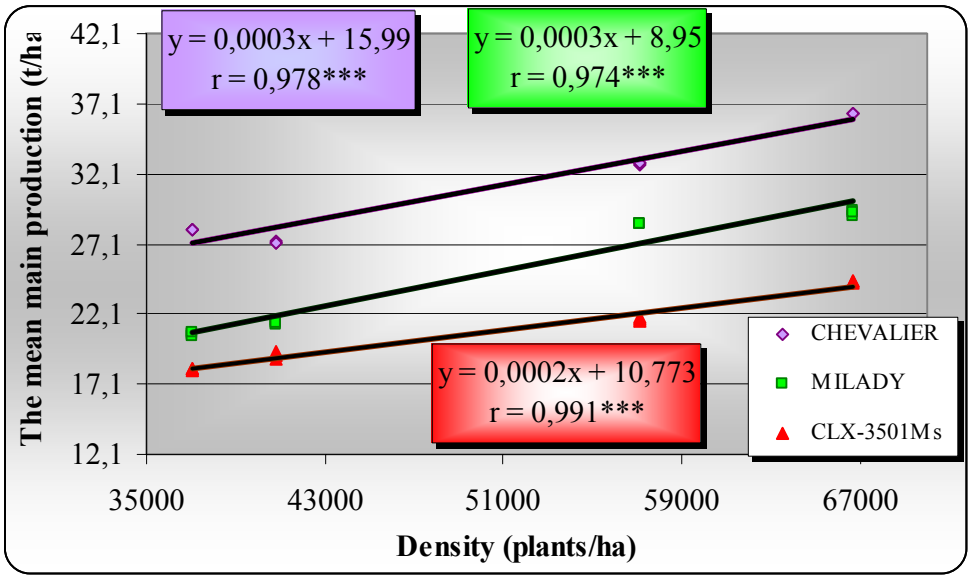


Fig. 3. The positive correlation between the mean main production (t/ha) and the density (plants/ha)

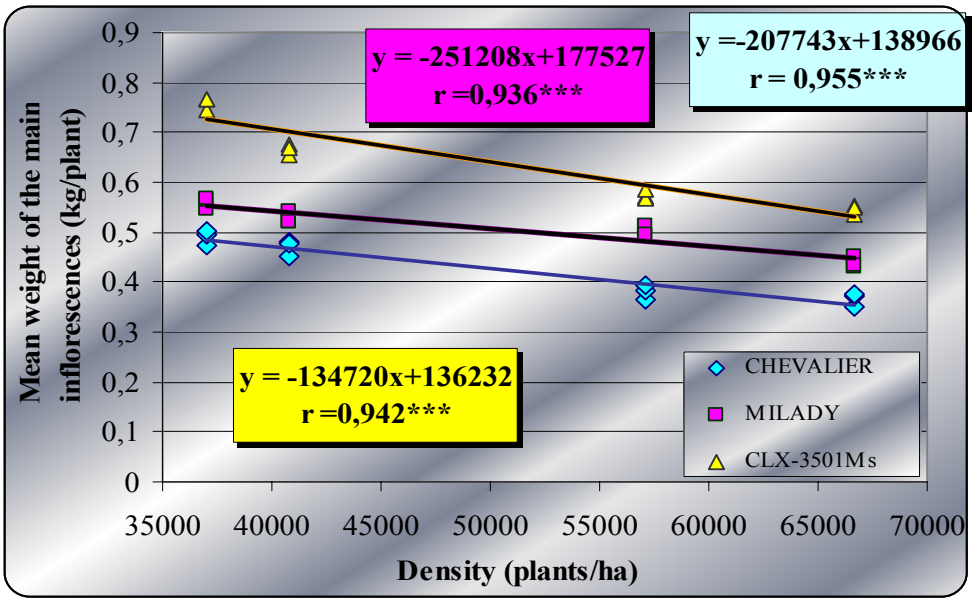


Fig. 4. The negative correlation between the mean weight of the main inflorescences (kg/plant) and the density (plants/ha)

Fig. 5.

Fig. 6.

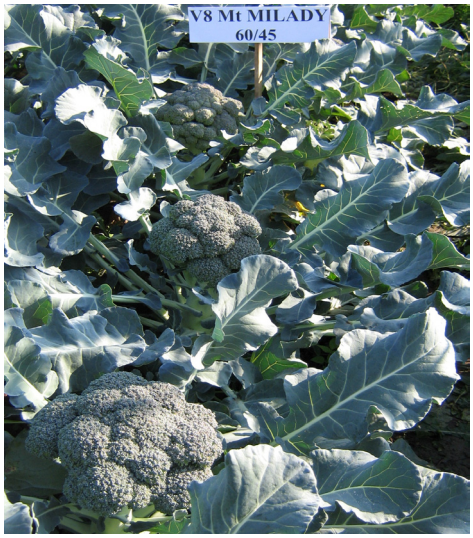


Fig. 7.

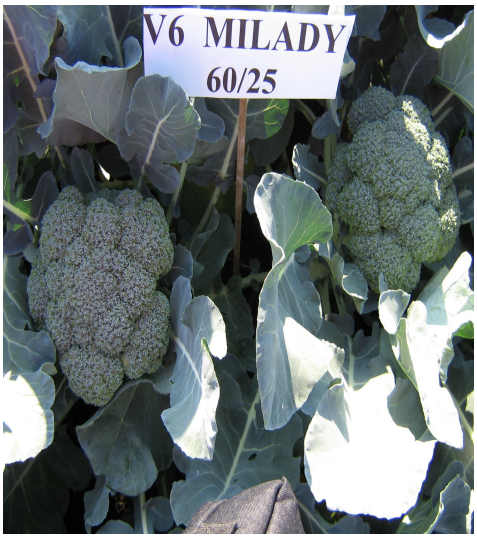


Fig. 8.

Fig. 9.

Fig. 10.

ORNAMENTAL PLANT & LANDSCAPE ARCHITECTURE

Preliminary results concerning the micropropagation "*in vitro*" of *Jasminum tortuosum* Willd. and *Murraya exotica* L.

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Keywords: differentiation, explants, culture medium, meristems

ABSTRACT

Jasminum tortuosum Willd. and *Murraya exotica* L. are usually propagated by classic methods. The researches aim the *in vitro* propagation possibilities of both flower species, trying to avoid the classical propagation methods inconvenient. In this paper is treated the differentiation stage. There have been tested four culture mediums, the bud positions on the shoot and the prelevation time of the meristems, and established them influence in the differentiation phase.

INTRODUCTION

Murraya exotica L. is flower specie with bushy growth habit which decorate through all of the plant parts (stem color, foliage, flowers and fruits). The propagation is mainly made in generative way, using maturated red fruits. *Jasminum tortuosum* Willd. is a specie with voluble growth which decorate mainly through flowers and them special and fine scent. The jasmine propagation is easily done by cuttings. So, both flower species could be propagated in classic manner (using fruits or cuttings) but with restrictions given by the certain periods of the year and the long duration used for obtaining rooted plants. In this way, *Murraya exotica* L. is conditioned by the presence of the fruits on the plant and the number of the future new plants by the available number of fruits per plant. For jasmine, the number of the future plants is correlated with the possible number of cuttings that could be obtained from the mother-plant and on the other hand, depending on the cutting period, the rooting time is different and often too large. Thus, in order to eliminate these inconvenient of the classic propagation methods, the present study is made in order to emphasize the potential of these flower species for in vitro micropropagation.

MATERIALS AND METHODS

The biological material used in the experiment was represented by two flower species: *Jasminum tortuosum* Willd. and *Murraya exotica* L, respectively shoots of 10-15 cm length used for meristems prelevation in different vegetation phase of the mother plants. For each flower, it were preleved 6 explants for initiation of the in vitro culture. After explants inoculation, the vessels were moved to the growing rooms and put them in thermal and illumination specific conditions depending on the culture phase and experimental variants (light - photoperiodism 16/8, 2000 lux and 24^0C temperature).

The bottles were laid on the shelves fixed on metallic stands at convenient distances, in order to offer a suitable light for in vitro cultures.

In the differentiation stage, it was established more variants aiming:

1. the influence of the medium culture upon the explants differentiation degree;
2. the influence of the buds position on the shoots upon the differentiation;
3. the influence of the prelevation moment upon the explants differentiation degree.

Because the basic culture mediums (Murashige&Skoog – simple and modified, Lepoivre, Fosard and Miller) contain only macroelements, microelements and vitamins, these mediums were supplied the next components: saccharose 20 g/l; agar 7-10 g/l; IBA 0,1 ml/l; Na Fe EDTA 3,2 ml/l, pH 5,5-5,7. These stimulators were added in the same concentrations, in all basic culture mediums (table 1). The same culture mediums were also used in each explants prelevation moment.

Another studied factor in the explants differentiation stage was the buds positions on shoots. Thus, it was preleved 12 buds from the superior third, median third and basal third of the shoots.

Knowing the specific of the flower species bloom in the summer season with natural high light and temperature, it were made prelevations as follows: in April – before the flower buds formation, in June-July – the bloom wave, in October – when flower blooming is stopped because of the days shortness and lower illumination and temperature conditions.

The biological material sterilization was realized by introducing the material for 10 minutes in absolute ethylic alcohol next by holding in calcium hypochlorite 6% for 20 minutes. After that, the vegetal material was 3 times rinsed in bidistilated water and maintained in the last swilling water till the prelevation moment. During all the work operations it was assured a high aseptic condition.

RESULTS AND DISCUSSIONS

Regarding the influence of the culture mediums correlated with the prelevation moment upon the explants differentiation degree.

From table 2, it can be observed that the October period (after the bloom wave) is the best time for buds prelevation for both flowers (*Jasminum tortuosum* Willd. and *Murraya exotica* L.). The formed buds in the start of the summer were 90% flowering buds. That's the main reason of the poor differentiation. At the end of the blooming time, the nutritive substances were leaded to support the plants bloom and the formed buds till this moment were 60-70% vegetative. We believe that is the reason for the easier prelevation.

As culture mediums, it remarks the modified M&S medium (with nutritive substances added). *Murraya exotica* L. recorded 100% differentiated plants and *Jasminum tortuosum* Willd. 83,33%.

Regarding the influence of the buds positions on shoots upon the differentiation

To point out this issue, it was preleved from different positions (the superior, median and basal third of the shoots) meristems and put on the modified M&S culture medium. For each position it was preleved 12 explants.

From table 3, it can be observed that *Murraya exotica* L. presents flower buds as the most of the buds on the superior third of the shoot. The best results obtained for this specie are registered by the basal and median buds. *Jasminum tortuosum* Willd. record

differentiated explants from all the bud positions but the best results were obtained also by the buds preleved from the basal and median third of the shoots.

CONCLUSIONS

Regarding the influence of the culture mediums correlated with the prelevation moment upon the explants differentiation degree, it was observed that the October period is the best time for buds prelevation for both flowers.

The modified Murashige&Skoog medium (with nutritive substances added) proved to be the best culture medium for this micropropagation stage

Regarding the influence of the buds positions on shoots upon the differentiation, it was remarked that the best results were obtained by the buds preleved from the basal and median third of the shoots.

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Tables

Table 1. The culture mediums composition for in vitro explants differentiation

The culture mediums composition	Murashige&Skoog (1962) (mg/l)		Lepoivre (1977) (mg/l)	Fosard (1977) (mg/l)	Miller
	simple	modified			
Macroelements					
NH4NO3	1650	1650	400	800	100
KNO2	1900	1900	1800	1011	-
CaCl2*2H2O	440	440	-	330	-
MgSO4*7H2O	370	370	360	370	715
KH2PO4	170	170	270	-	120
K2SO4	-	-	-	-	-
Ca(NO3)2*4H2O	-	-	1200	-	500
NaH2PO4	-	-	-	138	300
Microelements					
FeSO4*7H2O	27,9	27,9	-	10,7	-
MnSO4*4H2O	22,3	22,3	0,75	8,45	14,0
ZnSO4*7H2O	8,6	8,6	8,6	5,75	3,8
H3BO3	6,2	6,2	12,0	3,09	1,6
CuSO4*5H2O	0,025	0,025	0,025	0,024	0,025
Na2MoO4*2H2O	0,25	0,25	0,25	0,024	0,1
CoCl2*6H2O	0,025	0,025	0,025	0,118	-
Kl	0,83	0,83	0,08	0,415	-
Na2EDTA	-	-	-	18,61	1,32
Na2SO4	-	-	-	144,99	-
Vitamins					
Inozitol	100	-	100	54,048	100
Tiamina HCl	0,1	0,5	0,4	0,674	0,1
Nicotinic acid	0,5	-	-	2,462	0,5
Piridoxina HCl	0,5	0,2	-	0,616	0,1
Glycina	2,0	-	-	-	2,0
Colin	-	-	-	0,104	-
Biotin	-	-	-	0,048	-
Calcium Panthetonat	-	-	-	0,476	-
Riboflavin	-	0,2	-	0,374	-
Ascorbic acid	-	0,5	-	0,176	-
Folic acid	-	0,2	-	-	-

Table 2. The influence of the culture mediums correlated with the prelevation moment upon the explants differentiation degree

Flower specie	Prelevation moment	Basic medium Murashige&Skoog simple		Murashige&Skoog modified		Fossard		Lepoivre		Miller	
		Diff. pl	%	Diff. pl	%	Diff. pl	%	Diff. pl	%	Diff. pl	%
Murraya exotica L.	April	0[1]/6[2]	0	2/6	33,3	0/6	0	0/6	0	2/6	33,3
	June-July	0/6	0	0/6	0	0/6	0	0/6	0	0/6	0
	October	3/6	50	6/6	100	2/6	33,3	3/6	50	2/6	33,3
Jasminum tortuosum Willd.	April	0/6	0	2/6	33,3	2/6	33,3	0/6	0	1/6	16,6
	June-July	0/6	0	2/6	33,3	1/6	16,6	0/6	0	2/6	33,3
	October	1/6	16,6	5/6	83,3	3/6	50	4/6	66,6	2/6	33,3

[1] Number of differentiated explants
[2] Number of preleved explants

Table 3. The influence of the bud position on the explants differentiation degree

Specie	Bud position on shoot	Differentiated explants		
		rosette	flowering	necrosed
Murraya exotica L.	superior third	1/12	7/12	4/12
	median third	6/12	1/12	2/12
	basal third	7/12	-	-
Jasminum tortuosum Willd.	superior third	5/12	4/12	3/12
	median third	7/12	-	-
	basal third	6/12	-	5/12

Figures

Fig. 1. Differentiated explants of *Murraya exotica* L. and *Jasminum tortuosum* Willd.

Fig. 2. Explants from preleved meristems depending on shoot bud position

Research on the growing and the quality of *Euodia hupehensis* Dode (*Rutaceae*) seedlings

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Keywords: magnetic water, generative propagation, variation, stratification, quality

ABSTRACT

The presowing treatments at *Evodia hupehensis* seedlings are very important. In this research we establish the best method to obtain in first year the best yield of saplings at the best quality. The goal is to determine the influence of the presowing treatments upon the biometrical parameters and seedlings production, using native seeds of *Evodia hupehensis* which is an exotic tree, with high ornamental value.

INTRODUCTION

In the PhD thesis "Research regarding the possibilities of turning to account of some woody species from Romanian flora, for introduction in the ornamental assortment", some exotic trees were tested, among others the *Euodia hupehensis*. It is a tree with a great ornamental value and rare in nurseries and landscape planning.

MATERIALS AND METHODS

Euodia hupehensis is a medium sized tree, with unequally-pinnate leaves. The leaflets are oblong-elliptic, with entire or crenate margins especially at young plants. The tree produces many flowers in June. The flowers are little and white, in clusters. It is a melliferous tree. The fruits are reddish shiny capsules, with annual maturation. For the sapling production at this species, we used the generative propagation.

The seeds were obtained in October from U.SA.M.V. Cluj park. The experience was realised in the greenhouses of Vegetable and Floriculture Department and Botanical Garden of U.S.A.M.V. Bucharest.

In the experience were choosed three presowing treatments:

V1 – seeds kept at room temperature and sown in greenhouse in early spring.

V2 – seeds kept at room temperature, treated with magnetic water and sown in greenhouse in early spring.

The magnetic water was obtained from the town supply and kept in a special vessel. The vessel is made up of a ceramic pot with double walls. The pot is rounded with magnets. The water is stored in the vessel during 24 hours.

The seeds were soaked in the magnetic water during 12 – 14 hours and sowed immediately.

V3 – seeds stratified at a temperature of 1 – 5^{o}C, in moist sand, sown in early spring.

In each variant were established three replications. The seedlings were realised in greenhouse, in boxes, in February. In the first decade we determined the plantlet number and the emergence percentage. In May, the boxes with seedlings were taken out from the greenhouse. In October we made the inventory of viable saplings. In November, after end of vegetation, the saplings were taken out from boxes and we made biometrical measurements: the stem length, the root length, the collar diameter, the radicular volume and the saplings variability.

The saplings yield (%) was obtained using the inventory of viable saplings in October

The collar diameter (mm) was measured with the sliding callipers.

The root length (mm) was measured with the ruler, between the collar and the apex of the longest root ramification. The stem length (mm) was measured with the ruler, between the collar and the terminal bud.

The radicular volumes (cm3) were determined with a scaled vessel, by immersing the roots in water.

The saplings variability was determined using the amplitude of sapling parameters deviations.

The statistical analysis was made using ANOVA test, with single variation factor, based upon the biometrical parameters, the emergence percentage and the saplings yield.

RESULTS AND DISCUSSIONS

Regarding the emergence percentage and the saplings yield (fig. 1,2) we observe that se variant V3 (seeds stratified at a temperature of 1 – 5^{o}C, in moist sand, sown in early spring) have the highest values. The variant V1 (seeds kept at room temperature and sown in greenhouse in early spring) have the lowest values. It results that the dry medium and the relative, high temperature (18-22^{o}C), are a bad influence upon the storage of *Evodia hupehensis* seeds, until the spring sowing.

The seed sand stratification has the best effect upon emergence percentage, because the cooler (4-7^{o}C) wet medium has the tegument soaking effect and probably it stimulate the metabolization of biochemical inhibitors.

The average values of the others parameters (table 2) show that the most vigorous saplings were obtained at V2 variant (seeds kept at room temperature, treated with magnetic water and sown in greenhouse in early spring).

The vigour of the root system is much influenced by the culture conditions such as plants density and the limited volume of the substrate in the boxes. We observe that the radicular volumes riches the highest average values at V1 variant and the lowest average values at V3 variant. From this dates (tables 1, 2) reveals that the radicular volume vary in reverse with the saplings yield and the emergence percentage.

The root length varies from one variant to another, reaching the maximum values at V3 variant and the minimum values at V2 variant.

The variability (table 2, fig 2) reveals the sapling quality in each variant. The goal of this experiment is to establish the best presowing treatment, which gives the most uniform saplings and the maximum saplings yield. We observe that the most uniform saplings were obtained at V3 variant with the best yield.

The root length and the radicular volume variability are related. The saplings with the most uniform root system are in V2 variant.

From statistical data from year 2007 (tables 3 – 6), we observe that the presowing treatments have an irrelevant influence (NS), upon *Evodia hupehensis* sapling parameters. Also we reveal a significant influence (*) (table 7, 8), upon the emergence percentage and the saplings yield. The V3 variant has a significant positive difference towards the other variants, regarding the emergence percentage and the saplings yield.

CONCLUSIONS

The graphics and the statistical analysis reveals that the presowing treatments of *Evodia hupehensis* seeds influence only the emergence percentage and the saplings yield. The quality parameters the stem length, the root length, the collar diameter, the radicular volume were influenced directly by cultural conditions such as the light, the edaphic volume and plant density.

The presowing treatments may influence indirectly the saplings quality, by the viable saplings number which leads at certain plant densities of seedlings and obvious at differences of light and edaphic space.

Thus the V3 variant (seeds stratified at a temperature of 1-5 ^{o}C, in moist sand, sown in early spring) gives the best results regarding the emergence percentage and the saplings yield in first year. The quality is determined directly by the cultural conditions.

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Tables

Table 1. The emergence percentage and the saplings yield of *Euodia hupehensis* seedlings

	The emergence percentage 07.V.2007			**The saplings yield (%)19.X.2007**		
Variants	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
R1	23,33	40	66,66	13,33	40	56,66
R2	30	30	46,66	23,33	30	43,33
R3	13,33	26,66	56,66	10	23,33	50
Average	22,22	32,22	56,66	15,55	31,11	50

Table 2. Biometrical parameters and variation of *Euodia hupehensis* saplings

Variants		**V1**	**V2**	**V3**
The average collar diameter (mm)		5,9	6,95	5,28
The average root length (mm)		234,44	183,21	246,92
The average stem length (mm)		339,77	407,5	298,84
The average root volume(cm^3)		56.94	28.07	19.46
The saplings variability	The collar diameter (mm)	8,3	11	8,8
	The root length (mm)	410	225	370
	The stem length (mm)	520	595	370
	The root volume(cm^3)	115.5	93	107.5

Table 3. The statistical analysis of the collar diameter of *Euodia hupehensis* saplings

The collar diameter	**The average**	**The differences**						
V2	7,05	1,753333	1,44	NS	Restrictive differences	2,797074	5%	6,84444
V1	5,61	0,313333	0				1%	10,36875
V3	5,296667	0					0,10%	16,66776

Table 4. The statistical analysis of the stem length of *Euodia hupehensis* saplings

The stem length	**The average**	**The differences**						
V2	410,5	112,4167	91,63333	NS	Restrictive differences	144,9388	5%	354,6652
V1	318,8667	20,78333	0				1%	537,2881
V3	298,0833	0					0,10%	863,6902

Table 5. The statistical analysis of the root length of *Euodia hupehensis* saplings

The root length	**The average**	**The differences**						
V3	244,5833	60,91667	21,41667	NS	Restrictive differences	118,0284	5%	288,8155
V1	223,1667	39,5	0				1%	437,5314
V2	183,6667	0					0,10%	703,3314

Table 6. The statistical analysis of the radicular volume of *Euodia hupehensis* saplings

The radicular volume	The average	The differences						
V1	38.31667	17.83333	10.8	NS	Restrictive differences	45.34371	5%	110.956
V2	27.51667	7.033333	0				1%	168.0891
V3	20.48333	0					0.10%	270.2031

Table 7. The statistical analysis of emergence percentage of *Euodia hupehensis* saplings

The emergence percentage	The average	The differences			
V3	56.66	34.44	*	24.44	NS
V2	32.22	10	NS	0	
V1	22.22	0			
Restrictive differences	12.0715		5%	29.53895	
			1%	44.74903	
			0.10%	71.93404	

Table 8. The statistical analysis of the viability of *Euodia hupehensis* saplings

The viable saplings	The average	The differences			
V3	15	10.33333333	*	5.666667	NS
V2	9.333333	4.666666667	NS	0	
V1	4.666667	0			
Restrictive differences	3.126944		5%	7.651632	
			1%	11.59158	
			0.10%	18.63346	

Figures

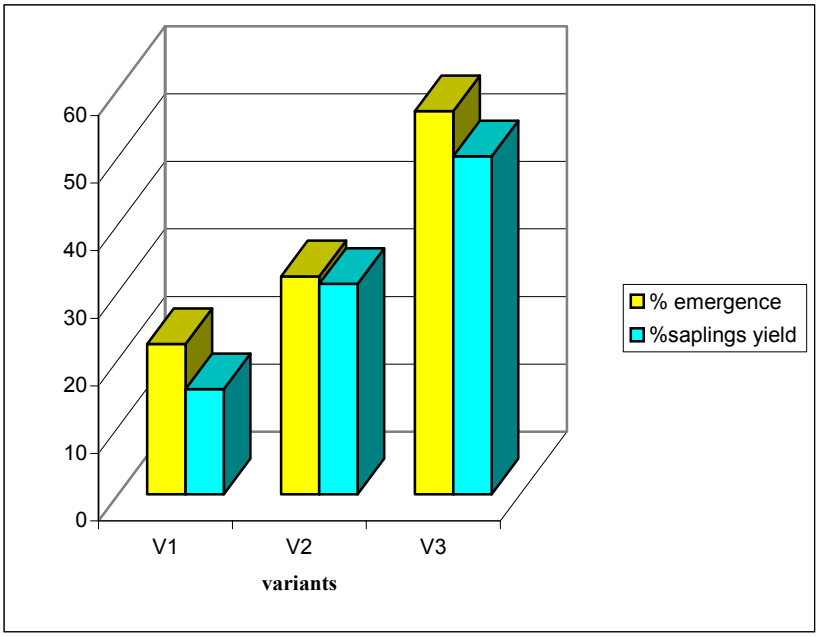

Fig. 1. The emergence percentage (7V) and the saplings yield (19 X)

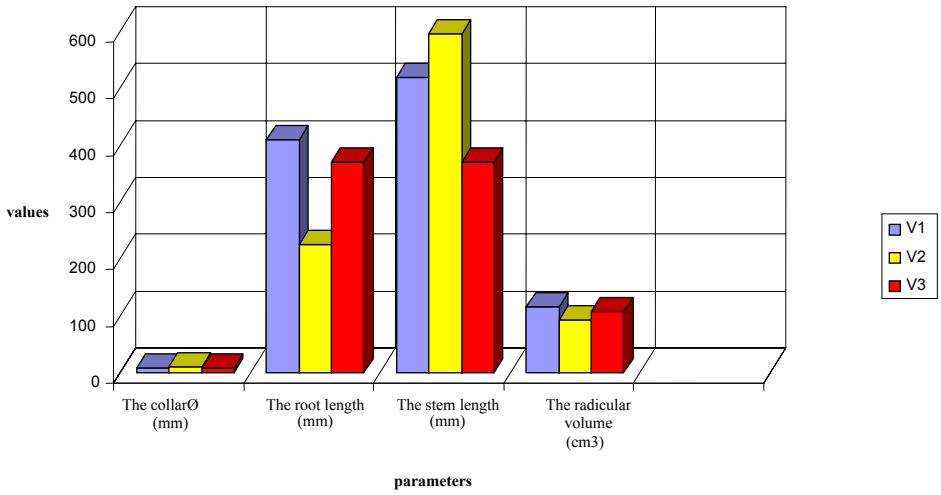

Fig. 2. The variability of biometrical parameters of *Evodia hupehensis saplings*

Researches concerning the influence of organic and mineral fertilizations upon the growth and flowering of *Euphorbia pulcherrima* Willd. ex Klotzsch potted plants

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Keywords: Euphorbia pulcherrima, mineral fertilization, organic fertilization

ABSTRACT

Our researches intended to assess the influence of organic and mineral fertilizations upon the growth and flowering of ***Euphorbia pulcherrima*** plants**.** The experiment was conducted over a two-year period, 2006 and 2007, and included eight experimental variants: a control variant, which received no fertilization, four mineral fertilization variants and three organic stage fertilization variants (table 1). The plants' growth and flourishing were studied with the help of the following biometric observations: the number of shoots that have already started to grow after the cuttings' pinching, the plant height, the number of nodes for each shoot, the bracts number during flowering and the bracts rosette diameter. The obtained results highlight the fact that complex mineral nutrients fertilization (which include both macro and microelements) supplied in a liquid form has the most favorable impact on Poinsettia plants growth and flourishing.

INTRODUCTION

Euphorbia pulcherrima is one of the decorative plants that request large amounts of nutritive elements in order to have a harmonious growth and flourishing. The increased consume is justified by the substratum's quick and powerful exhaustion, due to plants increased consumption and to frequent washing determined by repeated watering.

For Poinsettia, fertilization strategy implies:

- to supply all the essential nutrients;
- to provide these nutrients in a balanced ratio;
- to assure the necessary nutrients according to cultivar, age and vegetation level, growth rate, decorative parts, the plantation season, the substratum's physical and chemical particularities etc.

The need for nutrients is also assessed according to their degree of being soluble and to the plants possibilities of using certain nutrients.

When the fertilization system is conceived, the following are taken into consideration: nutrients choosing, the amount of nutrient, the NPK proportion, the way the nutrient is administered.

Ecke (2004) says that thirteen mineral nutrients are required for Poinsettia growth and flourishing: six macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) and seven micronutrients (iron, manganese, zinc, boron, copper, molybdenum, and chlorine).

MATERIALS AND METHODS

The experiments have been conducted in the Greenhouse sector of Floriculture Department of the University of Agronomic Sciences and Veterinary Medicine from Bucharest, over a period of two years, 2006 and 2007.

The most representative plants of *Euphorbia pulcherrima* from the instructional collection were used as biological material for the cuttings drawing; they were chosen during the flowering period.

When the resting period was over, the plants were reintroduced into the growth circuit, after their ramifications have been previously cut back to two or three nodes and planted in fresh soil mixture.

The planting material used to start a Poinsettia culture in pots was obtained by cuttings rooting in different substratum variants that contained red peat, perlite and sand. The rooting duration was of six weeks.

At August 10, 2006 and August 24, 2007, we planted the rooted cuttings into panhead pots with the upper diameter of 8 cm and a depth of 6 cm. In the same time with their planting it was also executed the pinching of the shoots tips.

The substratum used for planting the cuttings was composed from peat moss, manure, leaves ground and sand in the following proportion: 2:2:2:1 for the control variant V_1 and peat moss for the rest of the variants.

The control variant received no fertilizer, the rest of seven variants followed the fertilization diagram showed in Table 1.

The first stage fertilization was made at 3 weeks after the planting, with mineral and organic fertilizing solutions, applying 150-200 ml fertilizing solution for each pot.

Monthly were conducted the following biometric observations: number of shoots that have started to grow after the pinching of the cuttings, the plant height, the number of nodes for each shoot, the bracts number during flowering and the bracts rosette diameter.

At October 16, 2006 and October 30, 2007 - first color appeared on the bracts (the bracts show the first transition from green to their final color) (figure 1)

At December 18, 2006 and December 22, 2007, the observations and measurements regarding the bracts number around the inflorescence and rosette diameter were made (figure 2).

RESULTS AND DISCUSSIONS

The performed observations and measurements highlight the fact that the most favorable fertilization variants for Poinsettia are V_4, Vitaflora, 0,3% solution (the producer's recommended doze and concentration) and V_5 variant, complex fertilizer NPK 15 :15 :15, 10g/l followed by V_3 variant, which consists in Vilmorin nutritional sticks.

In figure 3 we may observe an S-shaped growth pattern at the Poinsettia plant. This S-shaped has three distinct growth phases.

The first one, lag phase (the first two weeks after pinching) has as a distinctive mark a slow growth. This is the period when lateral buds of the cutting will begin to grow, forming the future shoots of the plant.

The second phase (linear phase) is one of linear growth, during which an intensive stem elongation appear to the Euphorbia pulcherimma plants. This is the period of irreversible quantitative changes, finalized by the increase in the cells number, in those volume and mass, based on the meristematic tissues activity.

The third growth phase is a plateau one (plateau phase) and represents the period when the growth is diminished in favor to the flourishing process, when the quality changes are predominant. This is the time when the inflorescences are formed and the modified leaves appear.

The highest growing rates during 2006 compared to control variant V_1 (100%) were obtained to V_5 variant - 164%, V_4 variant – 156%, followed by V_3 variant with

123% . The lowest growth rates compared to control variant were noticed to the ones fertilized with organic nutrients and with Osmocote (table 2).

The number of shoots that started to grow was small, waving between two and three shoots for each plant. The mineral fertilized variants had the best values, excepting the V_5 variant, where a complex fertilizer was used: NPK 15:15:15. (table 3)

The average length of the internodes, determined by the proportion between the internodes number and the shoot length, has the lowest values at the V_7 variant (5.2 cm) and V_8 variant (5.8 cm), the highest ones being register in the case of V_4 variant (9 cm) and V_5 variant (8 cm) (figure 4)

Both the bracts number from a rosette (figure 5) as its diameter (figure 6) has maximum values in the case of the variants fertilized with Vitaflora and NPK Complex 15:15:15 .

In 2007, the average values obtained were smaller than the ones from 2006, when the cuttings were planted with two weeks early, the growing period being longer.

CONCLUSIONS

The results of our researches that have been conducted over the two years of study period show that the best fertilizer for Poinsettia was Vitaflora 0,3%, in weekly administration. This is the variant that covers the biggest proportion from the micro and macro elements need for Poinsettia.

Similar results were also obtained when the complex fertilizer NPK 15:15:15 was used regarding to the plant growth. As far as the plants' aesthetical characteristics are concerned, the analyzed parameters (the bracts number from a rosette and its diameter) had lower values than the variant that have been fertilized with Vitaflora.

The basic mineral fertilization variants had closely related values to the control variant, which received no fertilization, but in which substratum could be found the manure obtained from bovine muck.

The organic fertilized variants were characterized by weak growths, decorative elements situated below the standards, late bracts coloration.

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Tables

Table 1. The fertilization diagram

Variant	Substratum	Fertilizer	Fertilizer composition		Administration frequency	Notes
			Macro elements	Microelements		
V_1 - M	Manure:peat moss:leaves ground:sand 2:2:2:1	-	-	-	-	Without fertilization
V_2	Peat	Osmocote	N:P:K 18:6:12		-	Basic fertilization
V_3	Peat	Vilmorin Nutritional sticks	10% N, 6% P_2O_5, 7% K_2O, 3% MgO	0,13% Fe EDTA	Every two months	Basic and stage fertilization
V_4	Peat	Vitaflora 0,3% solution	13%N, 4,5% P_2O_5, 6,5% K_2O, 100mg/kg Mg	mg/kg: Fe-200, Zn-100, Mn-100, Cu-100, B-200, Mo-10	Weekly	Stage fertilization
V_5	Peat	NPK complex 10g/1l water	N:P:K 15:15:15		Every two weeks	Stage fertilization
V_6	Peat	Bovine muck 0,1% solution	N, P, K, Ca, Mg, S		Every three weeks	Stage fertilization
V_7	Peat	Equine muck 0,1% solution	N, P, K, Ca, Mg, S		Every three weeks	Stage fertilization
V_8	Peat	Poultry muck 0,05% solution	N, P, K, Ca, Mg, S		Every three weeks	Stage fertilization

Table 2. Growth rates compared to control variant 2006

Variants	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
Length (cm)	17.2	19.2	21.2	26.8	28.2	17.4	18.2	18.8
% Growth	100	112	123	156	164	101	106	109

Table 3. Organic and mineral fertilization influence upon the shoots number of each plant

Variants	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
Average shoots number/plant 2006	2.8	2.4	2.4	2.4	2.8	2.8	2.8	2.8
Average shoots number/plant 2007	2.4	2.4	2.8	2.8	2.8	2.8	2.4	2.8

Figures

Fig. 1. First color appearing on the bracts

Fig. 2. December 18, 2006

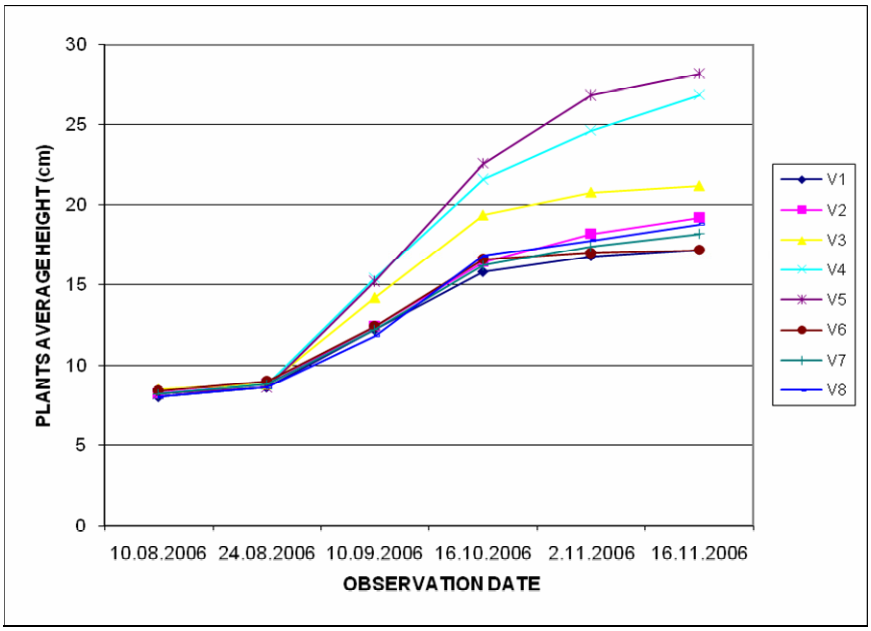

Fig. 3. Fertilization influence on the plant height

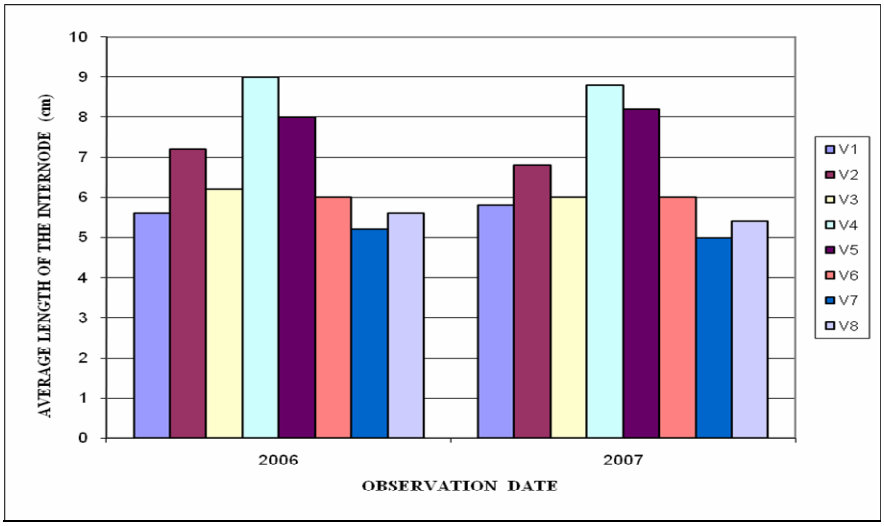

Fig. 4. Organic and mineral fertilization influence on the average length of the internodes

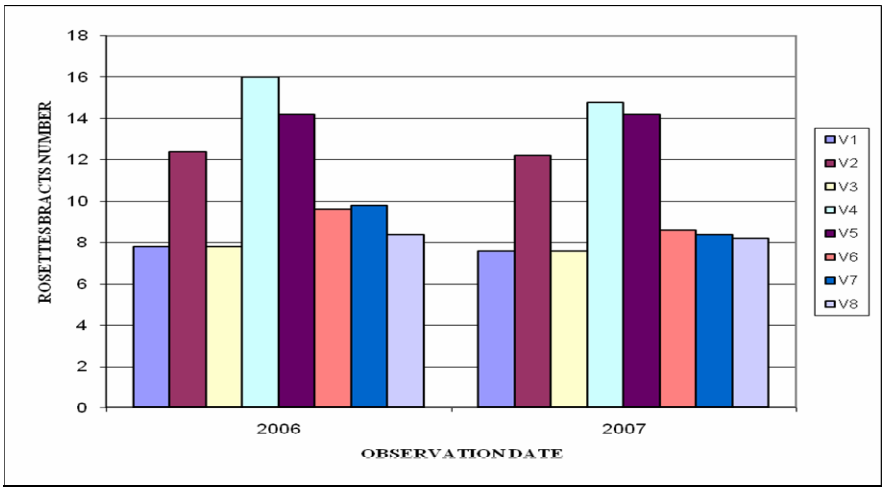

Fig. 5. Organic and mineral fertilization influence on the bracts number during the flowering period

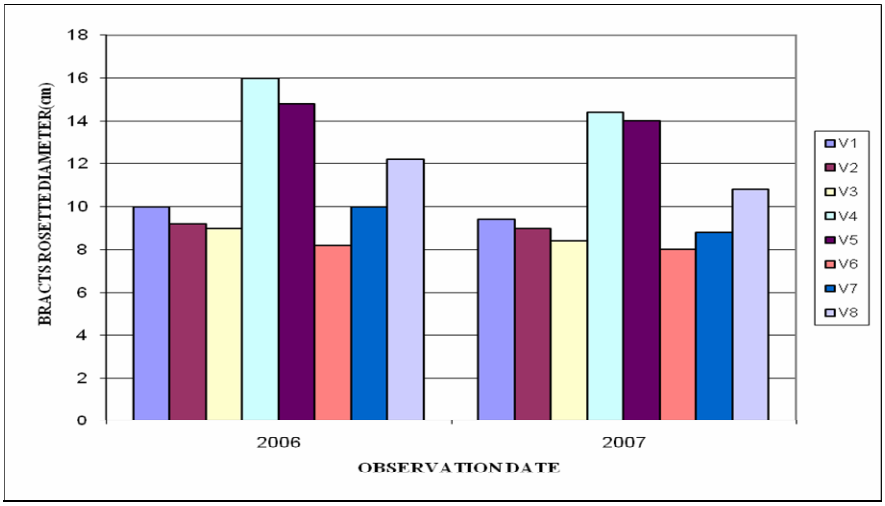

Fig 6. Organic and mineral fertilization influence on bracts rosette diameter

Researches concerning the influence of the rooting media on the cuttings' rhizogenesis of *Euphorbia pulcherrima* Willd. ex Klotzsch

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Keywords: *Euphorbia pulcherrima, cuttings, rooting media, rhizogenesis*

ABSTRACT

Our researches intended to assess the influence of the rooting media on the cuttings' rhizogenesis of ***Euphorbia pulcherrima***. Six rhizogenic rooting media were studied, four of them being simple and two of them mixed, over a period of two years, 2006 and 2007. The cuttings rooting degree was expressed by the roots' volume formed at each cutting's basis. Observations and measuring were made also on the cuttings' length and leaves number. The obtained results highlight the fact that the most favorable rooting media are the perlite and peat pellets.

INTRODUCTION

Euphorbia pulcherrima is well known all over the world as the Christmas plant. Native to Mexico, Poinsettias were first introduced into the United States at the beginning of the XIX-th century by Joel Poinsett, the U.S. Ambassador to Mexico at that time. This is the reason why *Euphorbia pulcherrima* is also named Poinsettia.

Although it is considered a flowering plant, the top leaves colored in red, pink, yellow and white, known as bracts, distributed as a star around the inflorescence, represent the decorative part. The true flower is small, yellow, without sepals and petals, being represented only by the cup shape androecium (Selaru, 2005).

Euphorbia pulcherrima is a perennial plant that needs an approximate two months resting period after the flowering stage. It needs to be cut in order to control its growing. It flowers during wintertime. Taking into consideration the natural conditions of our country, its cultivation rest over a 5 to 7 months period from the cuttings propagation and until its commercialization. In order to obtain the flowering and the coloration of the bracts, the plant needs about two months of short days (Selaru, 2005).

The cuttings propagation is used for the plant production. The most beautiful mother plants, with well-developed bracts, the ones that are homogeneous and with floral stems without branching growth habit are chosen during the flowering period.

MATERIALS AND METHODS

The experiments have been conducted in the Greenhouse sector of Floriculture Department of the University of Agronomic Sciences and Veterinary Medicine from Bucharest, over a period of two years, 2006 and 2007.

The most representative plants of *Euphorbia pulcherrima* from the instructional collection were used as biological material for the cuttings drawing; they were chosen during the flowering period.

When the resting period was over, the plants were reintroduced into the growth circuit, after their ramifications have been previously cut back to two or three nodes and planted in fresh soil mixture.

Six rhizogenic media were used for the cuttings rooting, four of them being simple and two of them mixed (see table 1). For each experimental variant 50 cuttings were planted into each type of rooting media.

Cuttings from the top of the shoots were taken on June 20, 2006 and July 13, 2007 from the shoots' tip growth that emerge due to the previous year ramifications cut back.

The phases of the cuttings' obtaining technical process were:
- trimming to 5 to 7 cm (with 3 nodes), by a transversal cut directly through the base node;
- removing the leaves from the cutting base;
- reducing the foliar surface, by cutting of 1/3 to 2/3 of the foliar limb;
- arranging the cuttings with the base in warm water, in order to avoid the leading vessels' obstruction by the latex coagulation.

The cuttings were planted at a depth of 1-1,5 cm, except the V_4 variant (perlite), where the depth was of 2 - 2,5 cm due to the stability lack.

The cuttings were planted into ceramic pots (figure 1), at 2cm/2cm distances (2006), in alveolar trays (figure 2) with panhead alveolus of 36 ml volume (2007) and in peat pellets (figure 3) in 2006 and 2007.

It was provided a medium temperature of 25 – 28 °C at the substratum level and 22 – 25 °C in the atmosphere; in order to maintain a relative high humidity the cuttings were frequently dusted and maintained covered with a pinched foil tunnel.

The observations over the rooting were made at 6 weeks after the cuttings were planted by checking the tearing resistance, and in the case of peat pellets at the moment of the roots appearance at the exterior (figure 4).

The cuttings rooting degree was expressed by the roots' volume formed at each cutting's basis.

RESULTS AND DISCUSSIONS

The obtained results (table 2) highlight the fact that the most favorable rooting media are the perlite (V_4) and peat pellets (V_2), followed by the red peat (V_1) and the peat:perlite 1:1 variant (V_6).

Considering the observations and measurements conducted on August 1, 2006 and August 24, 2007 of the roots volume, cuttings length and leaves number, important differences between the variants were founded.

So, we could observed that the roots volume varies between 0,8 cm^3 for the V_3 variant and 1,8 cm^3 for the V_4 variant in 2006 and between 0,7 cm^3 for the V_3 variant and 1,8 cm^3 for the V_4 variant in 2007 (figure 5).

The length of the cuttings varies between 9 cm for the V_3 variant and 14,5 cm for the V_1 variant in 2006 and between 9 cm for the V_2 and V_3 variants and 12,25 cm for the V_1 variant in 2007 (figure 6).

The average of the leaves number for each cutting varies between 5 for the V_3 and V_5 variants and 6,6 for the V_1 variant in 2006 and between 5 for the V_3 variant and 6,25 for the V_1 variant (figure 7).

As far as the rooting percentage is concerned we could observed that varies between 68% for the V_3 variant and 88% for the V_4 variant in 2006 and between 64% for the V_3 variant and 86% for the V_4 variant in 2007 (figure 8).

The lower rooting percents from 2007 are determined by the highly heat conditions during cuttings propagation period, when many consecutive canicular days were recorded, with higher than 40°C temperatures.

Another cause of the small number of rooted cuttings from that year was the presence of the *Bradysia sp*. (fungus gnats) larvae, their attack being encouraged by the

extremely high temperature and atmospheric humidity. The callus formed at the cutting base and the young roots represent those larvae food (figure 9).

CONCLUSIONS

The results of our researches that have been conducted over the two years of study period show that the best rooting media for the Poinsettia cuttings was the perlite (an 88 percent of successful rooting).

Similar results were also obtained in the case of the usage of peat pellets (86% rooted cuttings). Considering the fact that the cuttings produced in peat pellets are less vulnerable to pots plantation we could say that practically speaking this is the best variant for producing rooted cuttings of Poinsettia.

The rooting media variants that have contained sand recorded the smaller rooting percentage, those substrata being colder and having a less favorable rooting granulometry.

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Tables

Table 1. Rooting media

Variant	Rooting media	Components share
V_1	Red peat	1
V_2	Peat pellets (Jiffy-7)	1
V_3	Sand	1
V_4	Perlite	1
V_5	Red peat : Sand	1:1
V_6	Red peat : Perlite	1:1

Table 2. The Poinsettia cuttings rooting over the rooting media influence results synthesis

Variant	Rooting media	Rooted cuttings in %			
V_1	Red peat	2006	80	2007	78
V_2	Peat pellets (Jiffy-7)		86		84
V_3	Sand		68		64
V_4	Perlite		88		86
V_5	Red peat : Sand 1:1		72		68
V_6	Red peat : Perlite 1:1		76		72

Figures

Fig. 1. Cuttings planted in ceramic pots in 2006

Fig. 2. Cuttings planted in alveolar trays in 2007

Fig. 3. Cuttings planted in peat pellets

Fig. 4. Roots appearance

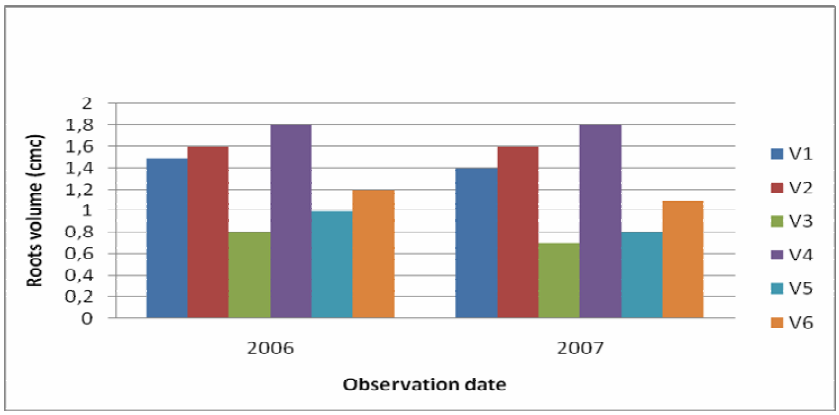

Fig. 5. The cuttings rooting degree

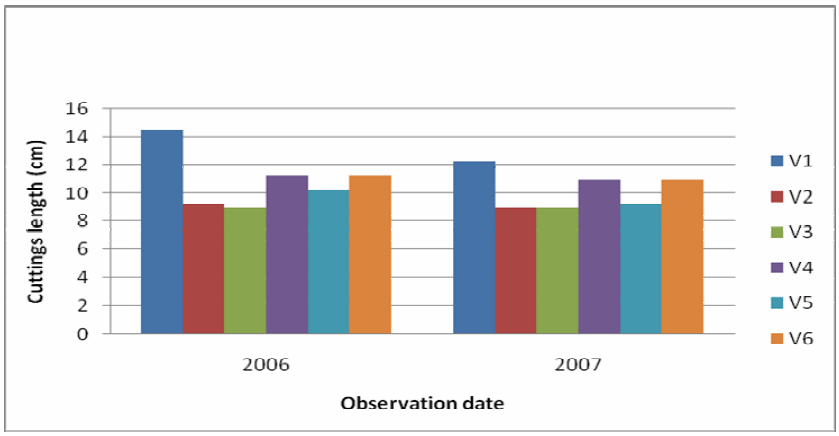

Fig. 6. The cuttings length variation

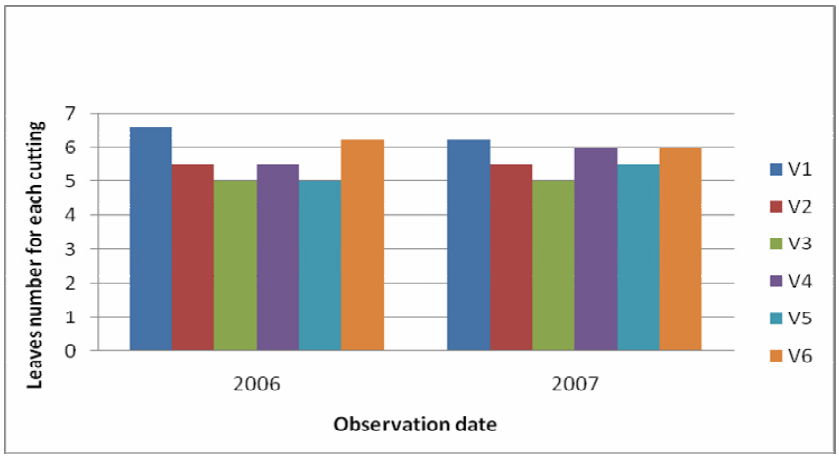

Fig. 7. The average of the leaves number variation for each cutting

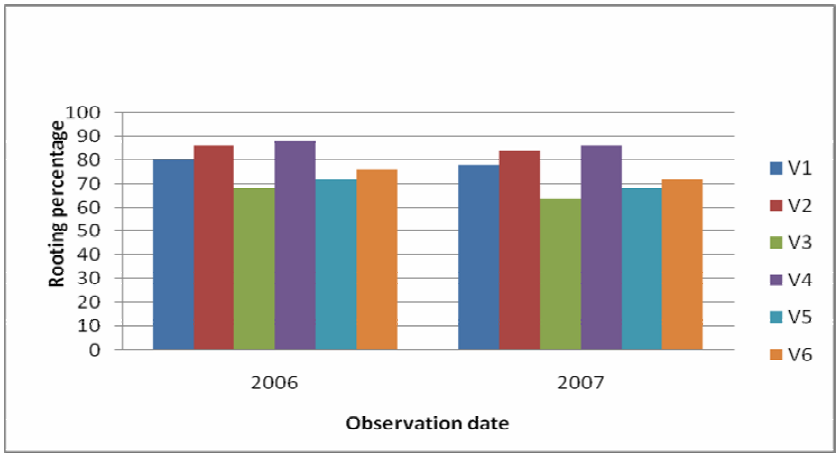

Fig. 8. The rooting percentage variation

Fig. 9. The Bradysia spp. larvae attack

Studies for Improve the Vegetative Propagation of *Pelargonium* spp.

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Keywords: stem cutting, rooting, *Pelargoniun spp.*, vegetative propagation

ABSTRACT

Pelargonium sp. belongs in the family *Geraniaceae*, has approximately 11 genera and 800 species in subtropical and temperate regions of the world. There are approximately 270 species of *Pelargonium* which occur in S-E and NE Africa, Australia and New Zeeland. Their mass propagation is performed using cuttings or seeds, (Mithila et al. 2001). In the last period in vitro propagation it is more efficient method. Petioles gave significant yield difference over 6 cm vine length and generally showed high potential for vegetative propagation in terms of rooting ability and survival rate (Lewu, Grierson and Afolayan, 2006). In our researches a study was conducted to determine the best method for vegetative propagation of three species of genus *Pelargonium* (*P. zonale, P. peltatum, P. grandiflorum*). A study was conducted in two separate experiments. In the first experiment, three stem tip cuttings (5, 8 and 12 cm) of the species were rooted in substrate experiment in a green house. In the second experiment, three substrate tips were used for rooting.

Data were collected and analyzed on various growth and development indices. In the first experiment, the 8 cm shoot length exhibited best result for different parameters measured. The substrate perlite + peat (1:1) gave the best results for all species.

INTRODUCTION

Geraniums (*Pelargonium* spp.) are traditional ornamental plants largely cultivated in Europe and northern America and also in Romania. *Pelargonium* geraniums make attractive house plants. They can be grown indoors for their ornamental value, as well as to maintain them for re-use in the landscape the following spring when are planted directly in the ground; in hanging baskets or window boxes; or in containers on decks, patios or entrances.

Paul Herman of Holland was probably the first botanist that collected pelargonium in 1672 in South of Africa and sent the seeds Jacob Breyne, who made illustrations species in 1678. The exchange of plants and knowledge between the English and Holland's botanists were intense at that time, and some species begun to be planted in short time at Oxford and Chelsea.

One of most important papers on taxonomy was published in France by Charles-Luis L'Heritier, who described 90 species of pelargonium, published after he died in 1789 in Hortus Kewensis. Robert Sweet begun work on his (plantlets) in 1819 and later he published the monumental work in five volumes about *Geraniaceae*.

The German botanist R. Knuth wrote in 1912 the last taxonomy revision, before that the Van der Walt grout in Cape town begun their large work on taxonomy, that resulted with the publication of first volume about the *Pelargonium* in South Africa in 1977, followed by other 2 volumes.

Vegetative propagation by means of stem cuttings is the method most widely used. Cuttings may be taken in autumn or summer from a parent plant that is strong and healthy. The method most used for Geraniums is taking cuttings from stem.

In the last time the species *Pelargonium* is very appreciate and cultivated in Romania, and that prompted use to work on some aspects of rooting technology of vegetative multiplication of this species.

MATERIALS AND METHODS

From 2006 to 2007 three species of genus *Pelargonium* (*Pelargonium zonale, Pelargonium grandiflorum and Pelargonium peltatum*) were investigated at the USAMV Cluj-Napoca, Department Floriculture, concerning there vegetative propagation.

We study the different methods in which the cuttings of different length, respectively 5, 8 and 12 cm long, from same species rooted in different culture substrate, in green house. From each species were prepared 30 stem cuttings. In the second experiment the rooting substrate had the following variants:

1) Perlite
2) Perlite + peat in 50/50 percent
3) Mixture of culture soil, Perlite, Peat + Perlite, Compost with 2:1:1:1 proportion.

Stem cuttings were made on 10.27.2006 and on 01.04.2007 were put in pots. The data was processed statistically each specie being profiled by variant method (a method allows to test the significance of variation with the help of DL).

RESULTS AND DISCUSSIONS

Analyzing Table 1 regarding the rooting of (cuttings), we can see that the average rooting was 19 out of 30 and the variability function of variant range between 16.0 (V_2) and 23.8 (V_9).

The percentage of rooting within each variant was between 53.3%(V_2) and 79.3%(V_9) an average of 63.3%.

The difference in variants can only be explained by the influence of two factors studied. To be sure by this fact, we statistically process the results, establishing the variants, the limits of influence and the influence of each factor, as well as the interactions of the factors over the rooting of stem cuttings as follows.

To determine the cuttings length over the rooting capabilities, we determine the average number of cuttings rooted per established length category (table 2, 3, 4).

Looking at the results we note that cuttings length influence over the rooting capacities of *P. zonale* and *P. grandiflorum* result significance differences between variants while for *P. peltatum* is not significance difference.

Reported to long cuttings Mt_1, the best rooting capability results were obtained for variant medium cuttings with average length for 4.6 units for *P. zonale* and 4.3 units for *P. grandiflorum* and that represent an increase of 26.9% and respective 25.1%. This difference is significant compared with DL 1%.

Comparing the experimental results for Mt_2 (the average experiment), we notice a significant negative influence for long cuttings and for *P. zonale* species, we notice a positive influence for average length, and for *P. grandiflorum* a negative influence for long and medium cuttings.

Analyzing the unique influence of substrate culture substrate over the rooting capabilities (Table 5) we notice that comparing with Mt_1 (perlite) the statistical results indicate for variant perlite + peat a positive increase of 1.9 units over the witness sample, and that represents 10.3 %. Comparing with the Mt_2 (experiment average), the results are negative for variants that use perlite and mixed substrate. A positive variation is noted for perlite + peat but there is no statistical assurance for this result.

CONCLUSIONS

The following conclusions can be made from experiments on *Pelargonium,* species *P. zonale, P. grandiflorum* and *P. peltatum*:

1. The stem cuttings rooting capabilities were influence by each analyzed factors and by the interaction between length of the cuttings and culture substrate.
2. The best results were obtained for medium length cuttings. The differences are positive and statistically significant and distinct significant.
3. The best results were obtained using planting substrate made of perlite + peat 1:1 that show a positive difference versus witness sample.
4. The interaction between the length of cuttings and the planting substrate cause significant differences for the same class of length cuttings.

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Tables

Table 1. Results regarding the rooting of the *Pelargonium* cuttings

Var.	Specie	No. cuttings	Length of cuttings (cm)	No. of cuttings rooted	
				No.	%
V_1	*P. zonale* peat + perlite	30	7,2	16.8	56.0
V_2	*P. zonale* perlite	30	6.4	16.0	53.3
V_3	*P. zonale* mixed soil	30	7.1	18.5	61.7
V_4	*P. grandiflorum* peat + perlite	30	8.3	17.8	59.3
V_5	*P. grandiflorum* perlite	30	8.9	17.5	58.3
V_6	*P. grandiflorum* mixed soil	30	8.8	19.0	63.3
V_7	*P. peltatum* peat + perlite	30	8.8	21.0	70.0
V_8	*P. peltatum* perlite	30	6.4	20.3	67.7
V_9	*P. peltatum* mixed soil	30	6.8	23.8	79.3
V_M	**Average**	**30**	**7.6**	**19.0**	**63.3**

Table 2. Summary of cuttings length influence over the rooting capacities of *Pelargonium zonale*

Cuttings length	Rooted cuttings		% of rooted over the average	±d over Mt_1	Significant differences	% of rooted over the Mt_2	±d over Mt_2	Significant differences
	No.	%						
a1 – long cuttings (Mt_1)	17.1	57.0	100	-	-	90.2	-1.8	o
a2 – medium cuttings	21.7	72.3	119.9	4.6	**	114.4	2.8	*
a3 - short cuttings	18.1	60.3	95.4	1.0	-	95.4	0.8	-
Average (Mt_2)	19.0	63.2	-	-	-	100.0	-	-

DL 5% = 1.8 DL 1% = 4.3 DL 0.1% = 5.8

Table 3. Summary of the cuttings length influence over the rooting capacities of *Pelargonium grandiflorum*

Cuttings length	Rooted cuttings		% of rooted over the average	±d over Mt_1	Significant differences	% of rooted over the Mt_2	±d over Mt_2	Significant differences
	No.	%						
a1 – long cuttings (Mt_1)	18.2	60.7	100	-	-	96.1	-0.7	oo
a2 – medium cuttings	22.5	75.0	139.8	4.3	**	118.8	3.6	*
a3 - short cuttings	16.1	53.7	85.0	-2.1	0	85.6	-2.8	oo
Average (Mt_2)	18.9	63.1	-	-	-	100.0	-	-

DL 5% = 1.7 DL 1% = 4.1 DL 0.1% = 5.6

Table 4. Summary of the cuttings length influence over the rooting capacities of *Pelargonium peltatum*

Cuttings length	Rooted cuttings		% of rooted over the average	±d over Mt_1	Significance differences	% of rooted over the Mt_2	±d over Mt_2	Significance differences
	No.	%						
a1 – long cuttings (Mt_1)	17.6	58.7	100	-	-	101.1	0.4	-
a2 – medium cuttings	18.5	61.7	114.9	0.9	-	106.3	0.5	-
a3 - short cuttings	17.8	59.3	99.1	0.2	-	99.1	0.2	-
Average (Mt_2)	18.0	59.9	-	-	-	100.0	-	-

DL 5% = 1.5 DL 1% = 4.4 DL 0.1% = 5.4

Table 5. Summary of the planting substrate influence over the rooting capacities of *Pelargonium*

Rotted substrate	Rooted cuttings		% of rooted over the average	±d over Mt_1	Significant differences	% of rooted over the Mt_2	±d over Mt_2	Significant differences
	No	%						
Perlite (Mt_1)	18.5	61.7	100.0	-	-	97.9	-0.4	-
Perlite + peat	20.4	68.0	110.3	1.9	*	107.9	1.5	-
Mixed substrate	17.9	59.7	96.8	-0.6	-	94.7	-1.0	-
Average (Mt_2)	18.9	63.3	-	-	-	100.0	-	-

DL 5% = 1.8 DL 1% = 4.3 DL 0.1% = 5.8

The influence of BAP and TDZ upon multiplication rate in *Rosa sp.*

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Keywords: Rosa sp., in vitro, multiplication rate, BAP, TDZ

ABSTRACT

This paper presents aspects regarding multiplication rate in Rosa sp., by using, as growthregulators, 6-benzylaminopurine (BAP) and thidiazuron (TDZ) at various concentrations. Threerose varieties created at SCDP Cluj were studied: Simina, Rosalinda and Rusticana. For cultivarRosalinda the multiplication rate was established on four variants of media (the basal mediumused was Murashige-Skoog 1962 (V_1 – MS+ 0.7 mg/l BAP, V_2- MS + 0.1 mg/l TDZ, V_3- MS + 0.2mg/l TDZ and V_4- MS + 0.7 mg/l BAP+0.2 mg/l TDZ. The highest multiplication rate in cultivar Rosalinda was obtained on variant V1, due to which multiplication rate was tested also in cultivars Simina and Rusticana on the same variant.

INTRODUCTION

The colour generosity and the diversity of growth type (shrub, climbing, ground cover, dwarf, miniature) places the rose in the top of the most widespread ornamentals for parks, gardens, terraces, so that research regarding the creation of new cultivars as well as developing propagation techniques is in continuous expansion. Due to its numerous advantages, in vitro propagation has been applied by many researchers for this species, also (Al-Khalifah et al., 2005, Hameed N. et al. 2006, Khosh-Khui M. and Jabbarzadeh Z. 2007, Maior, C. M . et al. 2007, Ozel C. A. and Arslan O. 2006, Senapati S. K. and Rout G. R. 2008). As basal medium, diverse variants of MS medium were generally used, and as growth regulators for multiplication BAP, kinetin and TDZ were used.Having in view the tradition of SCDP Cluj in creating new rose cultivars (40 cultivars created and homologated), the reaction of the newly created cultivars to this type of propagation was tested in the in vitro culture laboratory.

MATERIALS AND METHODS

The rose cultivars Rosalinda (1994, author Stefan Wagner), Simina (1996, author Stefan Wagner) and Rusticana (2000, author Stefan Wagner) were studied.

The experiment is made up of 4 variants of media (Table 1), all the variants have as basal medium Murashige-Skoog medium(1962) and various concentrations of plant hormones: V_1- MS + 0.7 mg/l BAP, V_2- MS + 0.1 mg/l TDZ, V_3- MS + 0.2 mg/l TDZ and V_4- MS + 0.7 mg/l BAP+0.2 mg/l TDZ.

The nutritive media were dispensed into Magenta GA7 vessels (cca 50 ml medium/vessel), using the standard procedure for preparing the media. The plant material used for establishing multiplication rate came from in vitro cultures initiated in the in vitro culture laboratory at "Babes-Bolyai" University Cluj-Napoca on MS medium containing 4 mg/l BAP, 0.2 mg/l kinetin, 1 mg/l GA_3 and 30 g/l sucrose. Five inoculi were planted into each vessel. Explants growth was done in controlled environmental conditions, at the temperature of 24-26 ºC and 2500-3000 lux light intensity, with 16-hour photoperiod. Establishing multiplication rate was done 8 weeks after inoculation, when the newly formed plantlets were transferred to a hormone-free medium (Table 2) for rooting.

RESULTS AND DISCUSSIONS

In order to establish multiplication rate in cultivar Rosalinda the four variants of media were tested (Table 1), from each variant 5 Magenta GA_7 vessels were taken, with 5 inoculi/vessel (Fig. 1). The results obtained 8 weeks after inoculation show that variant V_1 - MS+ 0.7 mg/l BAP offers the best multiplication rate, 12.4 respectively and it offers an average number of 62 inoculi/vessel (Fig. 2). The inoculi were 1-1.5 cm long shoots or shoot fragments with 2-3 nodes. In the case of variant V1 vigorous plants were obtained, with normally developed shoots and leaves. In the case of variants V2, V3 and V4, although multiplication rate is quite close to variant V1, deformed plants were obtained, with callus at the base, short, thickened shoots, deformed leaves, many of which were vitrified. In the second phase the multiplication rates of the three cultivars were followed, comparatively, on variant V1. The number of inoculi resulted/vessel was of 62 in cultivar Rosalinda, 35.5 in cultivar Rusticana and 53.6 in cultivar Simina. The multiplication rates in the three cultivars were 12.4 in cultivar Rosalinda, 7.1 in cultivar Rusticana and 10.72 in cultivar Simina (Fig. 3). The results obtained show that multiplication rate differs for each cultivar, the greatest multiplication rate being that of cultivar Rosalinda. The lowest multiplication rate was calculated for cultivar Rusticana, but in which the shoots were longer and more robust (Fig. 4). We mention that all the cultivars are part of the Floribunda group.

In order to see whether the number of initial inoculi/vessel influences multiplication rate, 2 experimental variants with cultivars Rusticana and Simina were compared, using as nutritive medium variant V_1- MS + 0.7 mg/l BAP, the one that proved to be optimal in the preceding experiment.The two variants consisted in two experimental batches, one with 5 inoculi/vessel and one with 9 inoculi/vessel.On the variant with 9 inoculi/vessel a multiplication rate of 6.07 was obtained for cultivar Simina and 5.02 for cultivar Rusticana, respectively an average number of 55.4 inoculi/vessel for cultivar Simina and 45.2 inoculi/vessel for cultivar Rusticana were obtained. In the variant with 5 inoculi/vessel a multiplication rate of 10.72 was obtained for cultivar Simina and 7.1 for cultivar Rusticana was obtained, respectively an average number of 53.6 inoculi/vessel for cultivar Simina and 35.5 for cultivar Rusticana were obtained (Fig. 5).

The shoots obtained on medium MS + 0,7 mg/l BAP were transfered to hormone Free medium (Table2), in order to watch their growth and rooting. After four weeks in culture, rooted plantlets are obtained, suitable for acclimation (Fig. 6). Acclimation was done in perlite, in covered plastic trays, in about four weeks. The acclimated plantlets were transplanted to pots, obtaining self-rooted plants (Fig.7).

CONCLUSIONS

Among the three variants of media tested, variant V_1 - MS + 0.7 mg/l BAP proved to be the most effective, ensuring the highest multiplication rate and normally developed plantlets for all the three cultivars.

On the variants containing TDZ, deformed and vitrified plantlets resulted. Increasing the Concentration of TDZ lead to diminishing multiplication rate.

The variants with 5 inoculi/vessel proved to be optimal, ensuring multiplication rates superior to those with 9 inoculi/vessel in all three cultivars.

Eight weeks after inoculation the shoots resulted on the variant with 0.7 mg/l BAP can be transferred to hormone-free MS medium for rooting. After 4 weeks rooted plantlets are obtained, which can be acclimated ex-vitro in perlite.

In vitro micropropagation is an effective method for propagating roses, by which self-rooted plants can be obtained, eliminating the necessity of grafting.

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Tables

Table 1. Variants of media used for multiplication

Components	Variant 1	Variant 2	Variant 3	Variant 4
Microelements	MS*	MS	MS	MS
Macroelements	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS
BAP	0,7 mg/l			0,7 mg/l
TDZ		0,1 mg/l	0,2 mg/l	0,2 mg/l
B1	1 mg/l	1 mg/l	1 mg/l	1 mg/l
Sugar	30 g/l	30 g/l	30 g/l	30 g/l
Agar	5 g/l	5 g/l	5 g/l	5 g/l
pH	5,8	5,8	5,8	5,8
FeNa EDTA	36,7 mg/l	36,7 mg/l	36,7 mg/l	36,7 mg/l

* Murashige& Skoog

Table 2. The media used for rooting

Components	Concentration
Microelements	MS*
Macroelements	MS
Vitamins	MS
B1	1 mg/l
Sugar	30 g/l
Agar	5 g/l
pH	5,8
FeNa EDTA	36,7 mg/l

* Murashige& Skoog

Figures

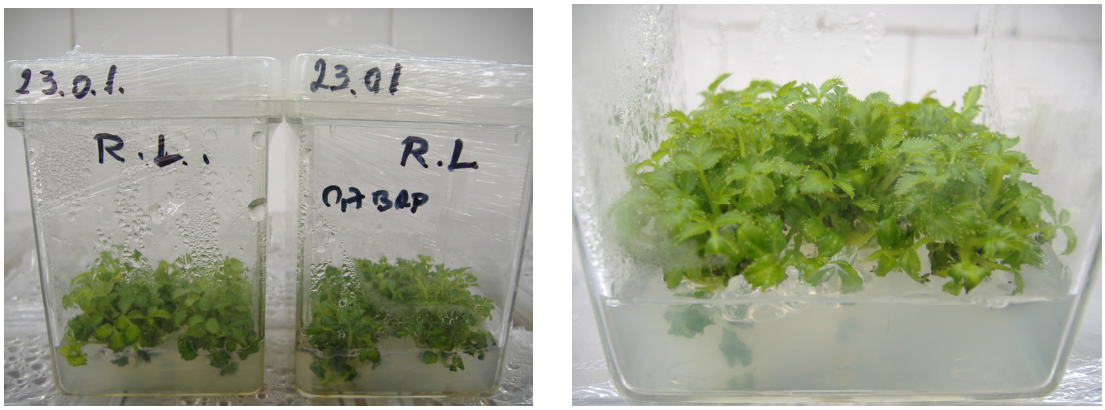

Fig. 1. Cultivar Rosalinda on medium MS+ 0,7 mg/l BAP

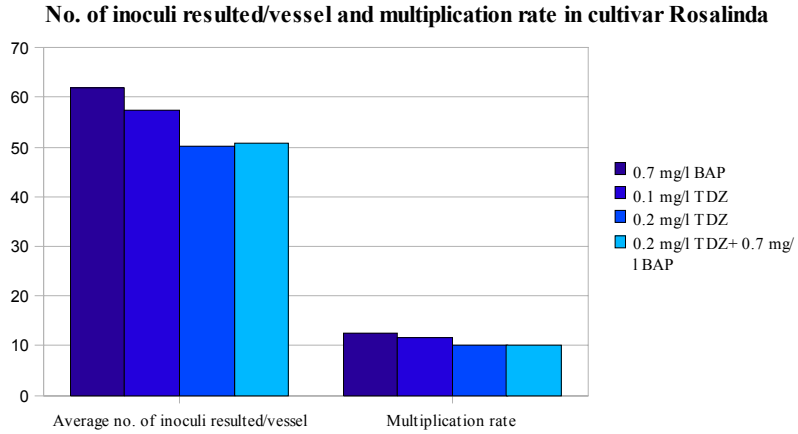

Fig. 2. The number of inoculi resulted/vessel and multiplication rate in cultivar Rosalinda

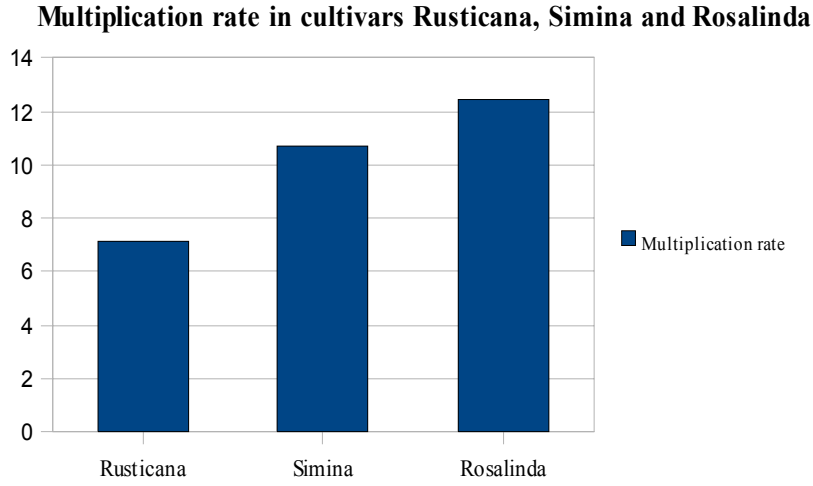

Fig. 3. Multiplication rate in cultivars Rusticana, Simina and Rosalinda using 0.7 mg/l BAP as growth regulator

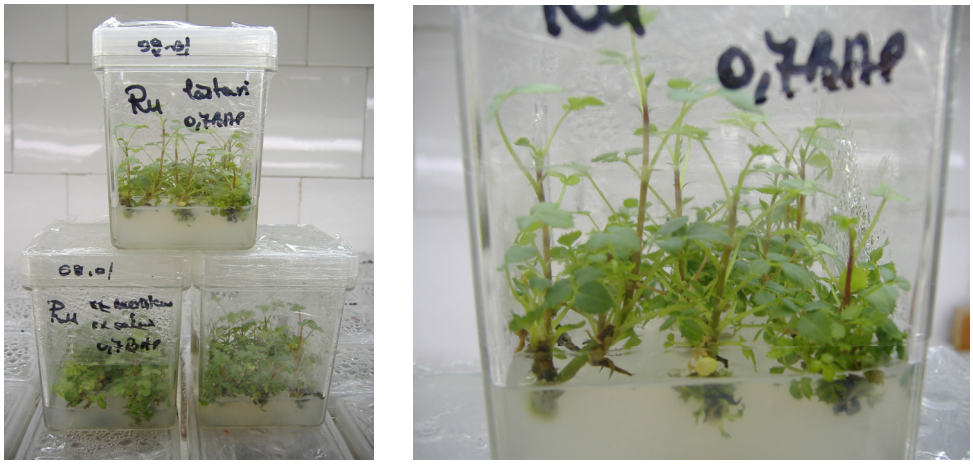

Fig. 4. Cultivar Rusticana

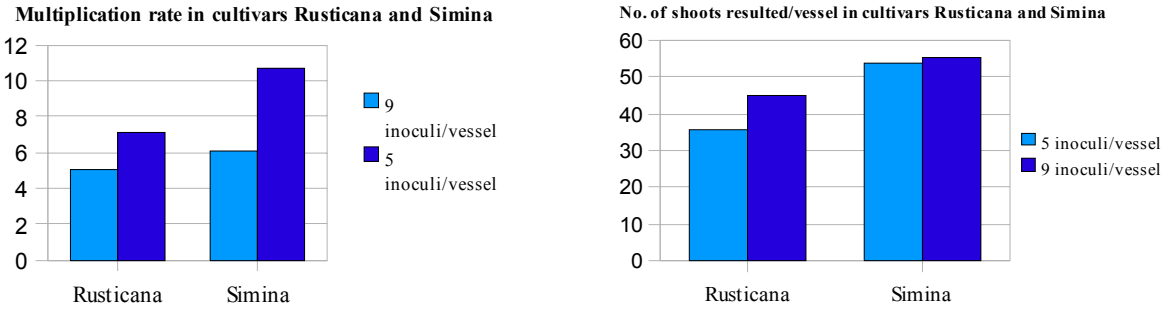

Fig. 5. The influence of the number of inoculi upon multiplication rate

Fig. 6. Shoots rooted on hormone-free MS medium

Fig. 7. Self-rooted rose plants obtained in vitro

The role and the evolution of urban green structures and the possibility of developing an ecological Bucharest.

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Keywords: urban planning, industrial wasteland, spatial resources, urban policy

ABSTRACT

In time, especially after World War II - in the context of industrial development and the ecological imbalances caused by it - urban green structures have become, one of the foremost aspects concerning the strategies for urban and land development. Nevertheless, Bucharest is yet to develop such a strategy, despite the fact that the deindustrialization has generated in its wake important resources that can be used for this precise purpose.

INTRODUCTION

There is a vast diversity of both theoretical and practical models for the development of urban green structures, whose shape, drive and efficiency have varied through time. In the following paper we will try to introduce a series of concepts, developed since the middle of the nineteenth century until present day, in order to create a picture as complete as possible of the solutions found by landscape planners in response to the issues concerning urban development. This paper's goal is to achieve a better understanding of the dynamics of urban development and of the different models for regional planning, in order to formulate a realistic solution for the planning and development of the City of Bucharest, in the context of the serious environmental problems it is facing.

THEORETICAL BASIS

As the theoretical basis for our study, we will present a short summary of the way visions for green belts and green urban and metropolitan structures have evolved:

Parkland[3]- The parkland belt represents a fundamental concept for colonial settlement planning. All entrances to this rectangular city would be through a peripheral parkbelt, 1-2 miles wide, save those sides washed by lakes or rivers. The benefits of such a reservation would be the following: "to contribute to the health and pleasure of the inhabitants, render the surrounding proprieties beautiful, and to bestow a magnificent appearance to the town." [4] From a morphological perspective, these settlements were divided into three distinct areas: town, parkland, and suburban land. As government-owned land, the parkland acted as a provider for public and recreational uses.

Garden city – Ebenezer Howard's concept of a city surrounded by an agricultural belt, with clearly defined urban and rural areas, has determined an extraordinary literature. In Howard's vision, the ideal town was of a limited size, with well defined rural boundaries preventing lateral spread. The principal of growth for the Garden City was that of always preserving a rural belt around the cities. The significance of rural space in regards to regional planning has decreased in favor of towns and suburbs. Countryside problems have become separated from urban ones and

[3] Free, un-built areas, that surrounded colonial settlements.
[4] Robert Freestone–From Garden City to Green City, The Johns Hopkins Univ. Press, London, 2002, p. 70

were reassigned a secondary status, and the agricultural belt turned into a buffer of green.

Parkbelts[5]- Howard's concept can be applied in the case of new cities but not for the already existing ones. Therefore, it was adapted to a simple geometrical model of organic spatial order, being stripped, during this process, of its social content. Thus, the belt's main purpose became that of defining and separating urban communities surrounding metropolitan centers. In time, the concept of a parkbelt type of greenbelt became associated with the satellite city and suburban concept. The theoretical basis of this idea was structured by the planner-architect Raymond Unwin[6]. His parkbelts take different shapes, including narrow stripes of woodland, avenues of trees, agricultural lands and sports areas. An environmental approach and the idea of natural reservations are introduced for the first time.

Green Girdle – The green girdle is a form of parkbelt, as a narrow, circumferential, discontinuous chain of green spaces, at the extremity of large cities. The purpose is that of establishing certain logic of the urban sprawl, of providing a "green lung" for residents, and of promoting recreational activities. Raymond Unwin was again the key theorist, using as inspiration the landscape possibilities of the spaces created by the demolition of the fortification rings around continental towns. Examples of this concept can been seen in planning concepts for the city of London, during the decades leading to Patrick Abercombie's greenbelt scheme in the 1940s.

Parkways[7] *and Greenwebs* – The idea of separating and connecting different districts through open spaces was born as part of the American park movement. Planned green spaces could bring nature into the town, represented a source of oxygen in the middle of dense living areas, enhanced recreational opportunities and provided certain logic to the urban space. By the 1940s the idea of using greenwebs to define neighborhood boundaries within the urban tissue was already part of the planning process, and it endures into present day.

Green Blackcloth – To deal with the issue of urban sprawl, various planning models have been promoted: from linear to ring cities. The concept, termed by Hall „the green blackcloth", involves independent communities, of limited size, and surrounded by a large, *non aedificandi* area, which represents the fusion of several greenbelts. This model was adopted, adapted and imposed in countless regions throughout the twentieth century, and it was deemed to be a model of dispersal and recentralization.

Greenbelt Cities- The greenbelt concept represents a planning ideal used into the 1950s. In order to accomplish the decentralization of population and economic activity, planners used a greenbelt defined as a "broad cordon sanitaire separating the threatened countryside from the threatening town."[8] Its integration along complementary concepts such as satellite cities, central city redevelopment and freeway networks has contributed to the standardization of the international vocabulary for regional planning during the post-World War II period. Behind greenbelts integration lays a series of precise objectives, whose importance and interdependence has fluctuated through time, in response to the pace and nature of the urbanization process, the importance of country versus town in planning policies, the changes in the political climate, and the development of an ecological agenda. Its objectives include controlling the urban sprawl, reducing land speculation, encouraging urban infill, protecting agricultural

[5] green boundary, separating independent communities
[6] Town Planning in Practice – 1909, Nothing Gained by Overcrowding – 1912
[7] planned green strips, interlinked so as to create a route within city limits
[8] Robert Freestone–From Garden City to Green City, The Johns Hopkins Univ. Press, London, 2002, p. 77

areas, promoting tourist and scenic resources, promoting recreational opportunities, maintaining the towns cultural identity, conserving flora and fauna.

Green Wedges and Corridors – Green wedges represent un-built areas that separate radial growth corridors. Charles Reade, an important figure of the movement for reevaluating the green-agricultural belt concept from the interwar period, started supporting the idea of a staged growth along corridors specified in town extension plans. Also, George Pepler proposed a plan of the ideal city, which included wedge parks and parkways within "an inviolable Green Belt of open country"[9].

The regional City – The regional city represents a modern and sophisticated adaptation of Howard's "Social City". This consists of a "galaxy" of small communities (not satellite cities) with well defined functions, connected through modern means of transportation, but separated by un-built spaces: "for farmland, recreational area, or natural woodland." Clarence Stein's regional city is an alternative to the problem of „limitless agglomeration". This is Stein's city, ironically called „green city", where large, un-built spaces are left open to provide „the surest method of preparing for flexible growth".

Greenways – The origins of the modern greenway movement lie in the practical responses to urban environmental issues in different communities, partly stimulated by the ecological approach in landscape planning from the 1960s. This model takes the form of linear greenways that protect natural areas and rehabilitated spaces and ecosystems. The ecological side tends to prevail, aesthetical values are preserved and unconventional recreational opportunities are prioritized. The shapes and functions of greenways vary greatly, although, most of them, include both cultural and natural values. Charles Little identifies five main types: urban riverbeds, recreational, ecologically significant, scenic and historic, and comprehensive natural systems. The greenways movement developed since the 1980s, a mid-1990s survey identifying 500 associations in the USA.

Green Zones – A contemporary form of greenbelt modifies Howard's agricultural belt so it includes a great variety of open spaces: wetlands, parks, rural areas, golf courses, waterfalls, retention basins, natural habitats, natural parks or conservation areas, which mark the outer limit for urban growth. The urban growth boundary is an officially designated line dividing land to be developed for urban purposes over a long period, from land to be protected for natural and rural uses.

The Ecological City – The reality of the twentieth century mega-urbanization defies the existing spatial formulas. Using the north European example, K.R. Kunzman shows how space use in metropolitan areas is becoming increasingly fragmented and specialized, with new spatial categories emerging: out-of-town shopping complexes, warehouses, theme parks, airports, etc. In this new urban pattern, rural spaces from around the cities are transformed into residential areas for the „urbanites" (middle class). It is Peter Calthorpe belief that, "a well-designed city is walkable".[10]Thus, he forwards the concept of transit orientated development (TOD) that aims to relieve traffic and to improve pedestrian travel. TODs require a new urban structure, consisting of mixed-use communities and an above-average density. The British model touches the sensitive subject involving the need for combining a greenbelt policy with a transport and land-use responsible policy.

[9] Robert Freestone–From Garden City to Green City, The Johns Hopkins Univ. Press, London, 2002, p. 84
[10]Scott London- The City of Tomorrow : An Interview with Peter Calthorpe, http://www.scottlondon.com/interviews/calthorpe.html

We can thus understand the evolution of the greenbelt concept or of a structure parallel with that of urban structures, these concepts representing answers to issues caused by the rapid urban growth during the last century. From an initial green, linear belt, used as a boundary for colonial settlements, to the system of independent communities, surrounded by vast, un-built territories, and, finally, to the integration within ecological cities, the purposes of green structures have diversified, addressing an ever growing array problems. An important aspect of this evolution is the transition from a "greenbelt" type of vision, based on town-country segregation, to a network type of vision, based on a connection between the center and the outer part of a city and on a quasi-continuity of the green structures, which will allow a inner-outer communication, from an ecological perspective.

PRACTICAL APPLICATION AND STUDY CASE ON BUCHAREST

We shall not insist on Bucharest current, disastrous, situation, especially from an ecological perspective. There are many analysis of this situation. Another starting point would be the manner in which Bucharest's Greenbelt project was conceived. A greenbelt cut away from the urban structure, unconnected with the inner open spaces, and that, for all these reasons, would bring no real ecological contribution to the urban territory. In this context our goal is to draw up a possible vision for the development of a green structure meant to rectify this situation, vision based on the city's current structure and on the development opportunities of several land resources ignored so far.

A first category is made up of areas adjoining the water flows and the natural areas developed in their vicinity, as well as of natural peri urban regions. However, we shall not further elaborate on this subject, as it has already been studied at large and it presents obvious qualities and opportunities.

A second category of land resources consists of the city's industrial areas that are presently in decline. Bucharest's industrial areas have developed during to main stages: at the end of the nineteenth century and during the interwar period – thus generating a ring of industrial spaces at the edge of the historical center – and, the second industrialization stage, started during the communist regime, which marked the urban structure in a completely different manner. The new industrial platforms developed from the inside outward, following the railways built during the nineteenth century, thus creating a structure of wedges inside the urban tissue, and forming a type of „ridges" between the great residential ensembles developed during the same period. The deindustrialization process started after 1989 involved the desertion of many such sites and the displacement of production outside Bucharest's belt. This process left in its wake many abandoned buildings, structures and wastelands, without an apparent functionality or use. And still... these seeming holes in the urban tissue have their own life and special dynamic. Some of the former industrial spaces are being rented and rehabilitated by small enterprises, garages, workshops, etc. Yet, while buildings can find such users/new uses, the huge spaces in between, once filled with trucks, equipment and people become true no-man's lands. An outcome of the desertion of these sites is their devaluation (both from an economical and a social perspective).

On the other hand, they represent new resources. First of all, we can consider these spaces as being a „social" resource, for their role as extensions of the apartment buildings neighborhoods – playgrounds for children, improvised sport fields, spaces for rising carpets, cars or... on the down side – for disposing of garbage. Secondly, these sites have evolved into an ecological reserve with an important influence on a city level

because these industrial wastelands have been gradually pervaded by various plant species. We are facing a complete ecological cycle. The deserted areas soon take in pioneer species, characterized by a fast cycle, which quickly disappear in favor of more stabile species. This replacement cycle is carried on until a state of equilibrium is achieved. This fast appearance of pioneer species, and afterwards their disappearance in favor of stabile species represents a phenomenon distinctive for wastelands: pioneer plants can only settle down on an empty soil, devoid of competition. The flora of the deserted lands does not consist exclusively of natural indigenous species, but it integrates all the pioneer exotic plants compatible with the environment. The ruderal flora is a particular category included in the anthropogenic flora. It is the result of human activity and consists of species with a great ability to adapt to the urban environment: pollution, wear, poor soils, etc. Therefore, these sites are all the more valuable for our capital, as they are home for a vast diversity of plant species adapted to „city life". This fact increases the environmental potential and importance of these places, adding to their contribution towards adjusting the local micro-climate, regenerating the soil, adjusting the temperature, etc. Because they grow on deserted lands, ruderal plants from the urban environment are associated with wastelands and seen by most as weeds. Even some of the specialists who interact with such spaces fail to recognize these plant's singular features, of adapting to the urban environment, and consider them to be harmful, or (absurdly) "unaesthetic". The paradox lies at the intersection with the eternal natural model so thought after by man, especially in the age of flamboyant ecology: how is it possible that today we find ourselves incapable to wisely assimilate these open spaces and this spontaneous vegetation, when they probably represent the sole „complete" expression of the ecological philosophy? Why is it that in Bucharest post-industrial wastelands are still black holes on the development plans? The easiest answer would probably be the lack of a coherent and global development strategy, a strategy that would define not only land-uses but also a general vision for a Bucharest pertaining firstly to its inhabitants, and secondly to big phantom investors. In today's urban "policies", these open spaces are devoted to a type of short term development, meant to answer certain private financial interests and punctual issues, and that fails to take into consideration their connection with other urban areas, or, most of all, the long-term consequences of such interventions on an urban level. These lands are subjected to ever increasing land speculations, and, having small chances faced with the simplistic financial reason, they stand the risk of disappearing in the future, regardless of their potential or of the consequences such loss will have on the city and its development.

This reality becomes even more absurd and sever as all the cities (that don't benefit from such territorial resources), are making extreme efforts in order to create a green network to connect the inner-city parks with the outer-city open spaces. Earlier we emphasized the way in witch Bucharest's industrial spaces were structured into wedges and rings: therefore, post-industrial wastelands follow a pattern extremely favorable for the development of a well balanced and quasi-continuous green network. Moreover, most of these spaces afferent to the railway and industrial networks are state-owned (ministries, administrations ...), which supports the idea of a mixed development (public-private; habitat, activities, recreation, transport...). Taking into account these aspects, we should strongly consider the issue of protecting an including these territories into a city scale green system. This is not to be understood as a „civilization" of wasteland, or the „planting of daisies", but as the incorporation of the existing nature

and the spontaneous social uses in a series of projects based on the functional and social diversity, which will put forth a balanced urban development.

There are also plenty of studies in this direction as well, amongst which we could mention here the USAMV landscape students' final presentations (graduates 2007), or Andrei Fufezan's study, presented at the 2007 Architecture Annual (Anuala de Arhitectură). Those studies discus o possible development of a R.E.R type of public transportation network along these sites, doubled by bicycle tracks, urban walkways, etc. Therefore, we are not talking of green spaces for the sake of green spaces, but of the development o ecological networks and public spaces, which could revitalize the neighboring apartment buildings ensembles. While waiting for new development strategies for Bucharest, these areas will continue to be used as extemporary public spaces (that is in the happy alternative that they won't disappear, casualties of financial interests) and the landscape will exist only in the eyes of those able to acknowledge its huge aesthetic and ecological potential.

CONCLUSIONS

The goal of this article is to prove that, despite the bleak statistics, there is still a real possibility of at least partially restoring to "the region of Bucharest the character of islands of buildings amid a sea of green, instead of that of islands of green amid a sea of buildings". And to this end, the greatest resources are represented by these industrial areas, which seem to have been left with no apparent purpose. All it takes is political support.

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Figures

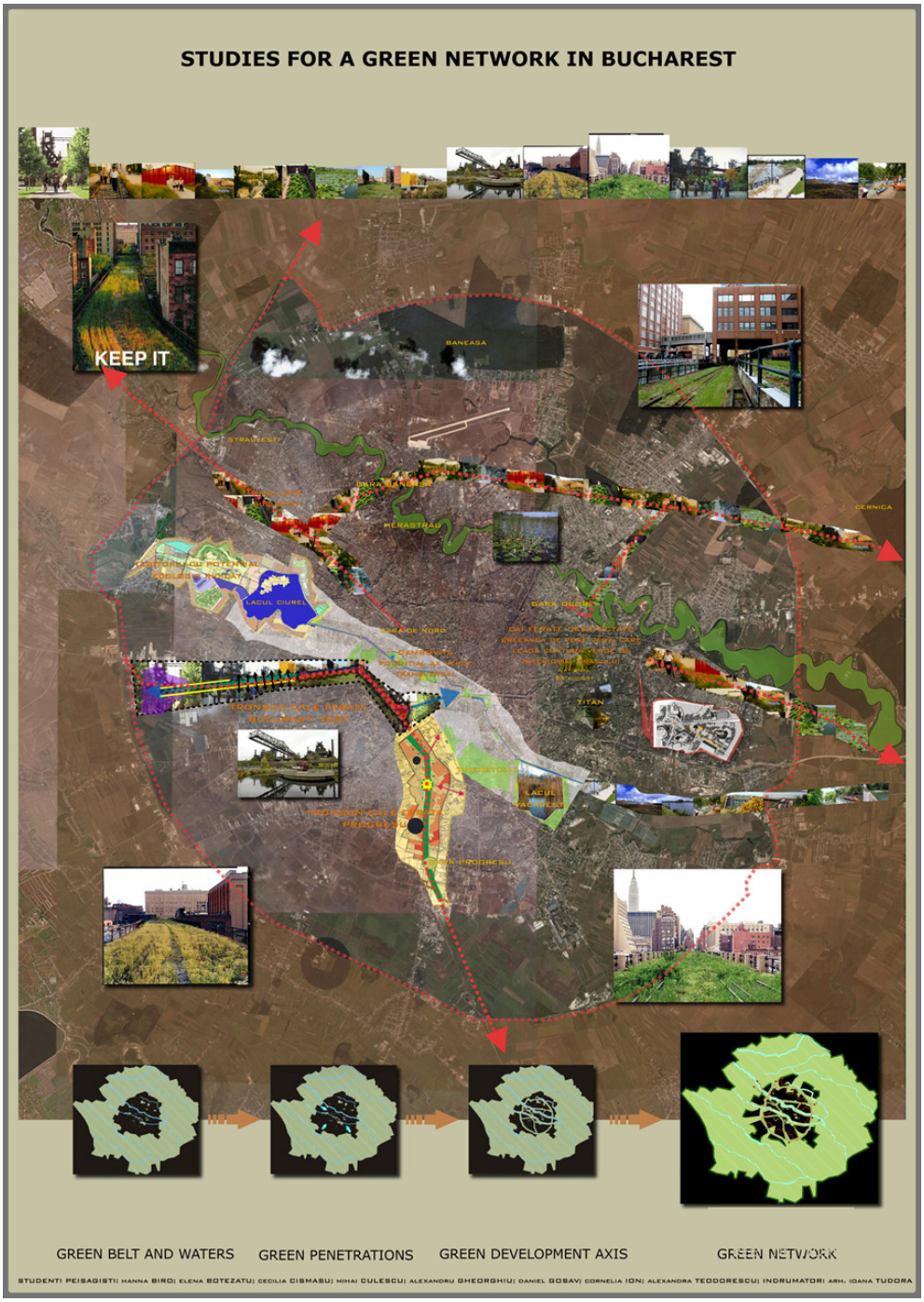

Fig. 1. Bucharest plan including impact and resources studies of a green network development

The park of the Cantacuzino Palace – study on the valuation of the historic landscaping monument

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Keywords: restoration, revitalization, protection, analysis, performing of inventory, degradation, interventions.

ABSTRACT

The valuation of historic gardens and parks refers countrywide traditionally to the physical condition thereof. Due to the shortages in maintenance, management, financing, as well as due to the absence of a national strategy on preservation, amelioration and capitalization of historic monuments of landscaping interest, we currently notice the accelerated degradation of the few landmarks of the national landscaping. On the list of historic monuments from Romania is also the estate of the Cantacuzino family from Floresti, located on the left bank of the Prahova River, sheltered by the Northern Sub-Carpathian hills. The landscaping study of the park of the Cantacuzinilor Palace aims at identifying the proposals to intervene in the restoration of the historic monument further to a more rigorous inspection of the objectives performed at regular time intervals. In the valuation shall be analyzed systematically the two material components: vegetal elements and mineral elements in historic-stylistic and aesthetic-functional view. Further to quantifying the results, the value of the emergency degree of the intervention in the restoration of the historic landscaping monument shall be established.

INTRODUCTION

The perception of gardens and parks as monuments is rather new in Romania, as the green space is deemed adjacent and subordinate to architecture. For such purpose, the classification of the historic parks and gardens, but also a mapping thereof with the valuation of their current condition appears as a necessity.

An up-to-date approach in view of the latest international conventions upon the cultural patrimony, landscape, monuments and sites shows the significance they have in the cultural heritage of a community. The salvation of landscaping architectural monuments supposes the performance of their differentiated inventory and specialized interventions for their maintenance, preservation and restoration and the obtaining of an as high as possible authenticity degree.

The scope of the study is that of commencing long-term researches in order to identify and perform the inventory of historic landscaping monuments, whether subordinate to an architectural monument or as public parks and gardens. This enables the drafting of the List of Historic Landscaping Monuments, distinct and complementary to the one wherein are inventoried historic architectural monuments (where they are treated to a minimum). The analysis and valuation of historic parks and gardens is a study in progress, a similar paper on the valuation of the park of the Mogosoaia Palace being the début paper of the study that has been already presented.

MATERIALS AND METHODS

The valuation method of historic parks in view of their restoration and revitalization was drafted by the landscaping engineer Florin Teodosiu, as a member of the National Commission for Historic Monuments – Historic Parks.

The analysis criteria shall underpin estimation operations quantified in a unique scoring system from 1 to 5 (where 1 is the lowest value). This method aims firstly to record all indicators and then they are agreed upon, so that each criterion should be

independently valuated. At the end of the analysis, a global aggregation is operated on basis of which are valuated the emergency degrees of interventions according to the results of the three specific general indicators:
- the global value of the historic site
- the value of the objective's degradation
- the value of vulnerability (aggressiveness degree of external factors).

A higher general value determines an urgent intervention.

The method of valuating historic parks and gardens:

1. Historic-stylistic value (VI)

VI = (0.2 x VCS) + (0.4 x VA) + (0.2 x VR) + (0.2 x PS)

where:
- VCS - monumental value of constructions;
- VA - artistic value: VA = 0.4 Ps + 0.3 Es + 0.2 As + 0.1 Sf, where
- Ps - stylistic conspicuousness;
- Es - stylistic expressiveness;
- As - stylistic authenticity;
- Sf - physical condition;
- VR - restorative value – percentages from the historic area with suffice documents for restoration;
- PS - stylistic weighting - percentages from the area of parks dominant in view of a lay-out style.

2. Aesthetic – functional value (VEF)

VEF = (0.4 x VC)+(0.3 x VV)+(0.1 x VCR)+(0.1 x VD)+(0.05 x VE)+(0.05 x VU)

where:

VC- lay-out value

VC = (0.3xVu)+(0.2xVd)+(0.2xVe)+(0.1xVp)+(0.1xVf)+(0.1xVa)
- Vu - value of the unit
- Vd - value of the diversity
- Ve - value of the volumetric and chromatic balance
- Vp - value of the depth of sights
- Vf - value of the focuses
- Va - value of the amplitude

VV - value of the vegetation

VV = (0.3x Vsf) + (0.3xVm) + (0.1xVa) + (0.1xVf) + (0.2xVu)
- Vsf - value of the physical condition (exceeding of longevity);
- Vm - value of the tree group;
- Va - value of the shrubs;
- Vf - value of the flower plantations;
- Vu - value of the shadowing of alleys;

VCR - value of the circulations

VCR = (0.6 x Vfl) + (0.3 x Vcp) + (0.1 x Vth).
- Vfl - value of the fluency;
- Vcp - value of the capacity;
- Vth - technical value – physical condition.

VD - value of the outfit

VD = (0.4x Vdv)+(0.3xVcp)+(0.2xVes)+(0.1xVth)
- Vdv - value of the diversity;
- Vcp - value of the capacity;

- Ves - aesthetical value;
- Vth - technical value – physical condition.

VE - value of the electrical equipment

VE = (0.7 x Vcp) + (0.3 x Ves)

- Vcp - value of the capacity and weighting within the territory;
- Ves - aesthetical value.

VU - value of the watering facilities:

VU = (0.7 x Vcp) + (0.3 x Vth)

- Vcp - value of the capacity and weighting within the territory;
- Vth - technical value – physical condition

3. Global value (VG)

VG = (0.5 x VI) + (0.5 x VEF)

- VI - historic – stylistic value.
- VEF - aesthetical-functional value.

4. Value of the emergency degree of interventions (VU)

VU = (0.4 x VG) + (0.2 x VD) + (0.4 x VV)

-VG - global value;
-VD - degradation value;
-VV - vulnerability value.

RESULTS AND DISCUSSION

The first buildings of the estate from Floresti were commenced by Minister of Justice Grigore Cantacuzino (1800-1849). The first edifice was the church built between the years 1826 – 1830, whereto was added between 1840 – 1842 a mansion composed of several pavilions (sheltering nowadays a tuberculosis sanatorium) that by their placement within a square were forming a small park decorated with an artesian well.

Besides the buildings of the mansion, George Grigore Cantacuzino, the son of Grigore Cantacuzino, commenced in 1911 the construction of a sumptuous palace under the guidance of the architect Ioan D. Berindey. Inspiring himself from the architecture of the Petit and Grand Trianon, the latter managed to accomplish an architectural synthesis between the two edifices, with a pool situated in front of the Palace that could be admired from the terrace.

The park of the castle accomplished in romantic manner was very rich in rare species brought at great expense by Grigore G. Cantacuzino. Besides trees and shrubs, there were numerous animals in the park, a pond, artesian wells, even a sculpted bridge of reinforced concrete. George Grigore Cantacuzino died in the year 1913, leaving the construction of the Palace unfinished; only the inner designs were missing. The Palace was inherited by succession by the third of his sons, Prince Mihail G. Cantacuzino, a very influent man of the time.

Unfortunately, during World War I, the German took the copper roof of the Palace and the inner design was thus exposed to accelerated degradation. Due to inexplicable reasons, Mihail G. Cantacuzino, the new owner of the estate failed to continue after 1914 the works on arranging the inside of the Palace.

In 1948 the family was banished from the estate and the Palace was robbed by the local people. During communist times, the Palace was in turns: I.A.S. (Agricultural State Enterprise), School for Canine Training, tuberculosis sanatorium, military unit.

There were certain restoration projects during the governance of the Communist Party and the proposal of President N. Ceausescu was that of changing it into a park for

presidential hunting. Another restoration project drafted by architect Calin Hoinarescu proposed the changing of the Palace into a hotel and its inclusion in a tourist circuit. Finally they chose to preserve the Palace in its current condition.

The building and the park are currently subject to an accentuated degradation process.

The research to restore the historic monument from Floresti – Prahova imposed the division of the entire zone into 3 areas of separate features:

- Area I - Palace area
- Area II - area of the English park
- Area III - area of the sanatorium

Each area was valuated according to the previously drafted analysis and valuation method of historic parks and gardens.

- Area I - Palace area

1. Historic-stylistic value VI = 2.60

VI = (0.2x2)+(0.4x3.5)+(0.2x1)+(0.2x3).

2. Aesthetic-functional value VEF = 2.74

VEF = (0.4x3.8) + (0.3x3.3) + (0.1x0.5) + (0.1x1) + (0.05x1) + (0.05x0.5).

3. Global value VG = 2.67

VG = (0.5x2.60) + (0.5x 2.74)

4. Value of the emergency degree of interventions VU = 4.42

VU = (0.4x2.67) + (0.2x5) + (0.4x5)

- Area II- area of the English park

1. Historic-stylistic value VI = 2.60

VI = (0.2x2)+(0.4x3,5)+(0.2x1)+(0.2x3).

2. Aesthetic-functional value VEF = 3.10

VEF = (0.4x4.5) + (0.3x3.5) + (0.1x1) + (0.1x1) + (0.05x1) + (0.05x0).

3. Global value VG = 2.85

VG = (0.5x2.60) + (0.5x 3.10)

4. Value of the emergency degree of interventions VU = 4.14

VU = (0.4x2.85) + (0.2x5) + (0.4x5)

- Area III. – area of the sanatorium.

1. Historic-stylistic value VI = 2.10

VI = (0.2x3)+(0.4x2,5)+(0.2x1.5)+(0.2x1).

2. Aesthetic-functional value VEF = 1.92

VEF = (0.4x2.5) + (0.3x2.5) + (0.1x2) + (0.1x1) + (0.05x 1) + (0.05x0).

3. Global value VG = 2.01

VG = (0.5x2.10) + (0.5x1,92)

4. Value of the emergency degree of interventions VU = 3.80

VU = (0.4x2.01) + (0.2x5) + (0.4x5)

Diagnosis:

- for area I - value of the emergency degree of interventions 4.42
- for area II - value of the emergency degree of interventions 4.14
- for area III - value of the emergency degree of interventions 3.80

A sensitive difference can be noticed between the three areas further to the analysis of the quantified values of historic-stylistic and aesthetic-functional indexes. This is transposed by the approximate level of degradations that comprise the entire estate, a fact that can be easily noticed even at a first analysis of the historic monument.

However, due to the presence of representative elements within certain areas, a hierarchy of the emergency degrees of interventions can be performed, as follows:
1. Area I - Palace area
2. Area II - area of the English park
3. Area III - area of the sanatorium

At a future restoration, the Palace area could be jointly treated with the park area, basing ourselves on the power to amplify the value of the monument by the presence of the accompanying green element. Landscaping elements that rendered once the charm of the estate: the lake with a bridge artistically made in concrete, decorative pools, sculptural pieces, brooks and waterfalls, terraces are sadly awaiting nowadays, hoping that they would be now highlighted again.

CONCLUSIONS

This research should be completed with a critical analysis upon the manner of accomplishing prior restoration whereof the entire assembly benefited. According to the principles underpinning the restoration of monuments, the restored work should not receive anything in excess besides what it disposed of at the moment of the initial execution. The documents regarding the successive stages that benchmarked changes in the structure or image of the monument should be also studied very carefully. If such were in place, the research should evidence in what context decisions were taken that led to the alteration of the value of the historic monument by the performed supplementations or modifications.

The estate from Floreşti was built as a nobiliary residence and surrounded by a superb park. Much too large as to be managed by the inheritors of the great boyar George Grigore Cantacuzino, this was deserted. The imposing castle that remained unfinished had the same fate. The research task of the restorer is here a much easier one, as there have been no previous restorations, but the monumental value of the components of the domain should be also considered. Such components should be thoroughly analyzed, in order to enable the rendering of the initial appearance of the monument.

It is recommended that further to the final researches, the restorer team should draft guiding lines to be followed in the work of the restoring landscape artist, guiding lines that should indicate varied types of approach in the restoration of historic landscaping monuments, according to the components of the assembly and of their historic and aesthetic analysis. In order to restore and protect the historic parks and gardens, the performance of a rigorous inventory and a hierarchy of the intervention priorities is necessary. Thus, the financing of such works may finalize the restoration or preservation process, in accordance with the degradation degree and the cultural significance of the objective. As early as the moment of the incipient expertise can be tracked major dysfunctions that impose mandatory and urgent measures and further to a criteria-based and exhaustive analysis the intervention priorities may be revealed and set in a hierarchy.

It is recommended to continue this study on the valuation of landscape architecture monuments by approaching in turns the most significant memorial sites.

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Figures

Palatal Cantacuzino – zona 1

Interior palat

Palatal Cantacuzino – zona 1

Bazinul palatuli Cantcuzino –zona 1

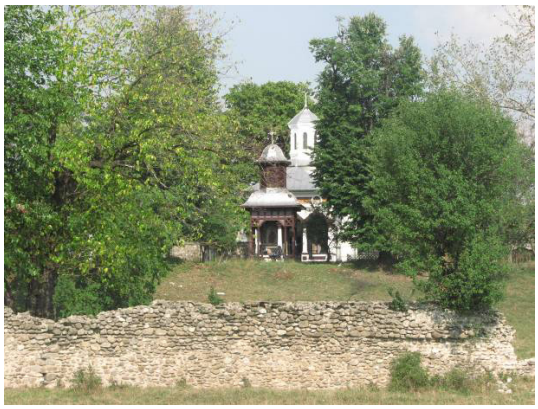
Parcul englez – zona 2

Parcul englez – zona 2

Parcul englez – zona 2

Parcul englez – zona 2

Parcul englez – zona 2



Biserica domeniului – zona 3

Zona sanatoriului – zona 3

Zona sanatoriului – zona 3

Rediscovering a forgotten garden - research upon a monument garden, part of the historic assembly of the Ottetelişanu Mansion

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Keywords: Patrimonial value, forestry association, evolution, transformation, restoration.

ABSTRACT

This study approaches the present state of a monument-garden, which for approximately 158 years has inconstantly changed concurrently with the changes from the Romanian society. This case is not a singular one in Romania, but here it seems that events led to a paradoxical situation: on one side, in legal view, the fact that the garden has never had the status of "public garden" protected it to a certain extent from the flow of human masses and from the decision to adapt the space to irreversible rehabilitation (the case of the Cişmigiu Garden), helping thus up to now the possibility of the initial restoration. On the other side, the idea of a professionally-fitted garden – one of the first gardens fitted out in this manner in Walachia – included in a patrimony monument-assembly, a museological benchmark for the future generations was "forgotten". The own evolution of the garden in this case is interesting. The natural elements participated in a survival battle and nature entered its role where it was only desired to suggest it. The rehabilitation attempts in the past led to the unsuccessful transformation of the built elements, leaving much too obvious marks upon the romantic fit-out style. In exchange, the vegetation has developed according to own rules and many specimens resisted in time to the aggressions and are now grandiose samples, natural monuments. The observance and determination of the species on-site disclosed an extraordinary conduct: exotic samples brought among the first into the country, have coexisted with the native ones, adapting themselves perfectly to the conditions of the site. The atmosphere has gradually become that of a forest and characteristic herbaceous vegetation has developed. Thus, the current forestry association of the Ottetelişanu Garden was classified in the geo-botanical category of the historic area, of waterside silvosteppe.

INTRODUCTION

The Garden of the Ottetelişanu Mansion represents one of the few works of the Austrian landscape artist Carl Friederich Wilhelm Meyer still in place in Romania. He arrived in Walachia in the year 1843 together with his assistant Franz Horer further to a royal decree to the Royal Court from Vienna. They were employed to get involved in the creation of the first fit-out public spaces from Bucharest.

The first garden created according to the norms of the public fit-out green spaces in Europe was the Kiseleff Garden and then the Cişmigiu Garden that managed to resist up to now. As he was in the circle of high society, besides these public gardens, Meyer had the opportunity to fit out also private gardens, among which also the Garden of the Ottetelişanu Mansion, the only one of its kind that was preserved. Besides the Cişmigiu Garden, it had the advantage of not incurring in time major stylistic interventions, disclosing quite easily the own manner of the artist to fit out the space.

There are extremely few data about the Ottetelişanu Garden, the greatest part of them referring to the owners or administrators of the assembly, the personalities that visited the site or the atmosphere of the garden. About the execution of the garden, we currently have no clues. However, after studying other works about which we have documentation, such as the Cişmigiu or Kiseleff Garden, we were able to make a comparison versus the working method at Ottetelişanu.

As we already know, Meyer was the adept of the landscape style, practicing the planting of spaces in their greatest part with native species – supporting mainly the principle of adaptability – as the vegetal samples were taken from the nearby forest.

Also, as they were many times floodable lands, "washed" by nutrients, it enriched the soil with forest land. Meyer concurrently brought here, as well as in Cişmigiu, rare exotic species from overseas. The mastery he proved in the aesthetic combination of the vegetation and especially the skill with which he designed the matching and cohabitation in time of different species enabled the physical and aesthetical survival of this historic garden, a fact wherefore we currently acknowledge his fit-out style.

The scope of this research is that of bringing the Ottetelişanu Garden again to the attention of habilitated estates, besides a field that is new in Romania – that of landscaping, "rediscovering" it in professional view and fulfilling the formalities in view of its restoration and preservation, in order to gain a real status of historic monument. The subject is also part of a complex research in view of drafting a doctor's thesis.

MATERIALS AND METHODS

As materials, we used the study of historical documents, historical and current plans, books of famous Romanian historians and chronologists, and also of sociologists who were approaching fashionable events of the time and the behavior of the Romanian society participating in the new influences, inclusively in the creation and usage of public gardens. Also, we used data storing devices: cameras to shoot films and make photos.

The method comprised documentation; comparing of plans and historic and current data; analysis and valuation on-site; observation and determination.

RESULTS AND DISCUSSIONS

At the middle of the 19th century, the Ottetelişanu family was representing the Romanian high society. Ioan Ottetelişanu became a Minister of Justice in 1845 and then one of the ministries of Alexandru Ioan Cuza. He held an estate with a mansion in Măgurele, near the capital city. The place of the future garden was included in the site of the estate, besides the mansion.

On the land of the garden there was already a pond supplied from underground springs on the site, according to the historical testimonies. Also, besides the mansion – called in certain documentations also a castle – there was a "culă", a shelter built somewhere in the 17th century by the villagers on the place where according to the tradition, Mihai Viteazul (Mihai the Brave) would have stopped with his armies during the battles from Călugăreni. The "cula" was remade around 1850 (?) by the boyar Iancu Ottetelişanu and made part of the park of his mansion; above it, Meyer placed an elegant wooden kiosk with a beautiful belvedere upon the garden.

According to the valuation of the vegetation on site, it is possible that certain samples of trees would have already been on site at the moment of Meyer's fit-out and which he kept, including them in the general composition.

In the new fit-out, Meyer remade the shape of the lake, created islands and a culvert – as there was the issue of flooding, leveled the land forming smooth slopes, fitted the garden with kiosks, a summer kiosk, bridge, winding pebble alleys with gutters on the sides for the draining of water, as they did at the time in the West, creating a "jewel" of the era, a creation of landscape art, a completely new phenomenon for those times in Walachia, as the Cişmigiu and Kiseleff Gardens.

The site is currently situated in the locality Măgurele, in the Ilfov County, approximately 15 km South-West of Bucharest. The historic assembly that was

composed of the Mansion with its Garden and the Church "Sfinţii Împăraţi Constantin şi Elena" (Holy Emperors Constantin and Helen, remade within the period 1851-1853 by Iancu Ottetelişanu and painted by Gheorghe Tatarascu) was divided in administrative and visual view, and the Castle besides the Gardens was included in the premises of the Nuclear Physics Institute Horia Hulubei and the church pertains to the administration of the locality.

The garden, as well as the mansion suffered several rehabilitation attempts in time, but unfortunate ones to the greatest extent. The most aggressive intervention was inflicted upon the lake that in the absence of a maintenance science became insalubrious and it was decided to reduce the area, to fill up the natural springs and concrete the bottom, as well as to lift the edges to the extreme, as well as the surrounding land. The effect was a lasting, but unaesthetic one and further to the absence of maintenance for a long period of time, rainwater collected here, as well as a significant layer of mud. The other fit-outs had also to suffer. By breaking the protection wall, the garden obtained a public, unofficial usage, with destructive effects upon its patrimonial value.

A significant part of the younger tree vegetation was disturbed by different factors, but many monument trees managed however to survive and develop in a spectacular manner. The evolution of the vegetation became somehow independent from the rest of the site and an extraordinary association was formed here between the native and the exotic species. The biologic variety grew further to the slow decomposition of the secular trees knocked down by the wind and the site gained an atmosphere that contradicts a non-anthropic space with zonal geo-botanical features.

The sub-area of the silvosteppe (steppe with forests):

- Precipitations 500-600 mm annually or even over 600 mm within the South-Western region of Walachia.
- The average temperature is of 10-11^{o}C.
- The relief is plane or uneven with an altitude between 0 and 200 m.
- The widest spread soil is the levigated chernozem with numerous intra-zonal soils.
- The wood vegetation in the forests of *Quercus pedunculiflora* from Eastern Walachia on strongly degraded chernozem with a sub-layer of loess is still composed of *Acer tataricum, Ulmus carpinifolia, Quercus pubescens, Quercus frainetto, Crataegus monogyna, Acer campestre,* etc.

Further to the plantations, in the old park of the mansion Ottetelişanu from the locality Măgurele, we can distinguish two large groups of dendrological species:
1. Exotic species: *Sophora japonica* L. – Japanese acacia; *Acer negundo* L. – American maple; *Robinia pseudacacia* L. – acacia; *Platanus* × *acerifolia* Willd. – plane tree; *Aesculus hippocastanum* L. – chestnut; *Vinca major* L. – vinca; *Celtis occidentalis* L. – nettle tree; *Morus alba* L. – mulberry tree.
2. Native species: *Quercus robur* L. – oak tree; *Populus alba* L. –white poplar;*Alnus glutinosa* (L.) – common alder; *Fraxinus excelsior* L. – ash tree; *Hedera helix* L. – ivy.

Many species appearing in the documentation of the time have disappeared from the fit-out due to varied reasons: concreting of the lake, competition among species, lack of maintenance, etc. (*salix alba* – willow tree, *Rosa sp*. – rose, etc.).

The current forest association on the territory of the Ottetelişanu Park (Măgurele, Ilfov County) can be characterized in geo-botanical view as a forest from the waterside silvosteppe area. In its structure, we meet several species of trees, characteristic to causeways of low, damp plain: *Ulmus laevis, Quercus robur, Quecus pedunculiflora, Fraxinus excelsior, Acer campestre, Prunus cerasifera, Malus*

sylvestris. On the level of shrubs, we can see the following samples *Sambucus nigra, Viburnum opulus, Ligustrum vulgare, Euonymus europaea, Rosa canina, Rubus caesius*. As lianas, we meet *Hedera helix, Humulus lupulus* and *Clematis vitalba*.

The herbaceous vegetation layer is much diversified, preserving the structure characteristic to plain causeways with influences from the steppe. One can meet meadowlands of *Allium ursinum* şi *Ranunculus ficaria*, numerous samples of *Arum maculatum*, *Chelidonium majus, Gallium sp., Anemone ranunculoides, Corydalis solida Geranium sp.* and sporadically species of xerophyite steppe, such as *Arcticum lappa, Sambucus ebulus, Potentila sp.,* etc.

CONCLUSIONS

The Ottetelişanu Mansion and Garden are declared historic monuments according to the List of Historic Monuments from 2004, position 626, code IF-II-a-B-15294, dating back from the second half of the 19th century.

The garden was accomplished in romantic style specific to the era, according to the manner characteristic to the landscape artist Meyer. Today it is as the castle undergoing an advanced degradation condition. The vegetation evolves according to the rules of nature and the compositional qualities are noticed less and less.

In view of the forest biocenosis, the forest cluster within the studied area is aging. This can be noticed in the high number of trees knocked down by the wind and with withered branches. A large percentage of knocked down trees can be noticed at the level of the population of *Fraxinus excelsior*. Further to the fit-outs it can be noticed that *Acer negundo* became an invasive species through the large number of saplings with ages spanning between 1 and 5 years at the level of shrubs, besides *Sambucus nigra*. Sporadically are also seen saplings of *Aesculus hippocastanum* and *Celtis occidentalis*. Samples of *Populus alba* planted in the park present in their vicinity numerous saplings originating from root suckers. Near the concreted edge of the lake are also present saplings originating from seeds. In the cracks of the concrete space appeared saplings of *Morus alba.* The samples of *Platanus acerifolia* are in a relatively good vegetation condition, but two samples are undergoing irreversible decline.

As anthropic influences, we can distinguish two large categories:

1. The anthropogeny by successive fit-out works of the park, by erecting specific constructions, hydro-technical fit-out works (pond) and especially by planting wooden species.
2. The anthropogeny by the influence of the local community and current management of the Ottetelişanu complex.

In order to rehabilitate the historic site, it is necessary to subject it to a complex restoration process. The absence of a reaction in this view would probably lead to the losing of this monument of priceless historic-artistic and landscaping value, a cultural asset of future generations.

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Figures

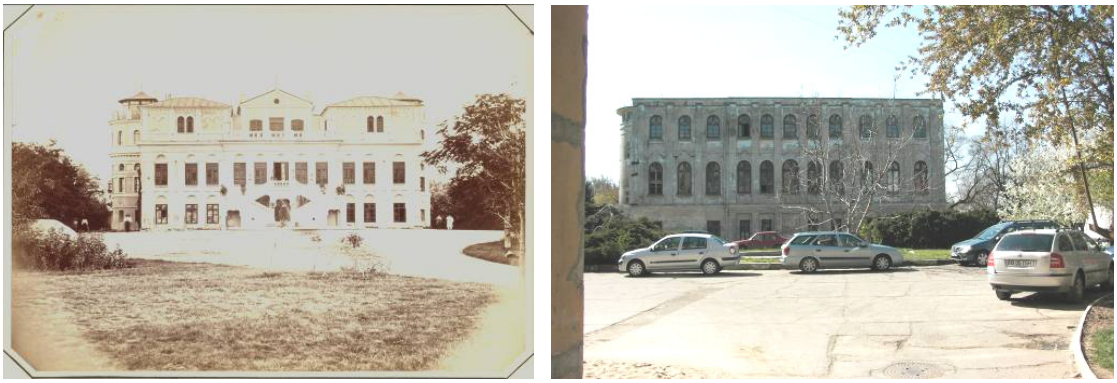

Fig.1. The front of the Ottetelişanu Castle– Then and now
(1863-foto Franz Duschek – the father; 2008)

Fig.2. View of the Ottetelişanu Garden – Then and now
(1863 - foto Franz Duschek – the father; 2008)

Fig.3. Then and now – the lake with the wooden bridge; the "cula" with kiosk; the summer brick kiosk (up – fotos by Franz Duschek – the father – 1863).

Fig.4. The vegetation of Ottetelişanu Garden in present.

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Composition structures in creating historic gardens. The Cişmigiu Garden

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Keywords: Concept, geometrical construction, art, landscape project, restauration data.

ABSTRACT

In this paper we analyzed the manner in which the landscape artist Carl Friederich Wilhelm Meyer designed the project of the Cişmigiu Garden, in the first half of the 19th century. Meyer created this garden according to well-known composition principles, obtaining a unitary, harmonious space, well-proportioned within the urban site. The composition quality defines an artistic creation in aesthetic view. The application of the composition norms by the creator upon his projects placed them among works with aesthetical qualities. Being familiar with it, we can estimate the quality of a landscape work. In case of a historic accomplishment, the deciphering of the composition structure and of the structuring manner in space, according to a certain general style, can bring us closer to the knowing of a personal fit-out manner of the landscape artist.

INTRODUCTION

The "composition" concept appeared for the first time in studies of architecture and art. This involves an aggregate view of the future creation, the division of a space into sub-spaces, being proportional among them, having harmonious and balanced relations and the final result is the idea of "unity in diversity" or the association of the shapes into one single family.

The composition of space preoccupied the creators of the "artificial landscape" as early as the Antiquity and they were familiar with the artistic science. The study of the composition took another boost within the period of the Italian Renaissance, developing further the artistic science of Ancient Greece and the result was a decisive one for the future of architecture and plastic arts. In the field of landscape architecture, the top creations were accomplished on the same composition principles.

The scope of this study is that of following a method to decipher and draw again a composition structure used by the artist in accomplishing his creation. This basic "skeleton" is of special significance in reproducing the vision of the creator upon the space. In regard to the plan drawing for a historic garden where the documentation was lost in time, such a method partially supplements the unknown factors related to the original project, surpassing thus easier the inaccuracies gained during time by the historic plans caused by degradation, successive scale transformations or processing of survey data with the drawing in the shortage plan. The determination of the composition model can also lead us to discover expression forms characteristic to the artist. All this information is anyway useful in accomplishing a restoration project when the establishing of the initial original plan of the historic site matters.

The study is part of a more complex investigation in order to draft a doctor's thesis.

MATERIALS AND METHODS

In this work, I used the study of historical documents, historical and current plans, books of Romanian historians and art books. The method supposed the

overlapping and scales the old plans of the garden and analysis on the used composition structures.

As materials, I itemize: old plans, art books, old photos, drawing materials.

RESULTS AND DISCUSSIONS

The analysis of a compositional model was applied several times to old works of plastic art in view of studying the geometrical quality of the picture or of the manner of the artist in reproducing harmony and balance within the representation space. In approaching a landscape work, differences are not great, provided the concerned work realistically represents an artistic creation accomplished according to the basic compositional principles, such as: the geometry of space, the proportion of shapes, the perspective benchmarks, etc., and in this case, the same norms apply not only to the shapes in the plan, but also to the elements from the 3-dimensional space.

The lanscape artist Carl Friederich Wilhelm Meyer was the creator of the Cişmigiu Garden in the midlle of 19th century. In his project, Meyer used the geometrical form of the site as mark for the future compositional schema. Also, he included the points of the future entrances and placed the upcoming garden in connection with the town's streets. After he finished the compositional structure (schema), he concentrated on the focus points and realised the general form-composition. Until that moment, the composition was pure geometrical and, in order to final stage, the artist aplicated the caracteristic design of the romantic style, mixed it with a monumental axis.

CONCLUSIONS

1. The historical plans of Cişmigiu Garden (drawn by: Eng. Boroczin – 1850; Eng. Knechtel – 1883) are differed one from the other.
2. Meyer represented the Cişmigiu Garden prevalently in romantic style, he based on the rigorously geometrical structure, in accordance with the artistic canons.
3. The composition of space isn't fortuitous. There is a strong connection between plan forms and the compositional structure of the general shape of the site.
4. Meyer used the compositional structure until the space division and the general forms composition.
5. The Cişmigiu Garden is a work based on art principles.

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Figures

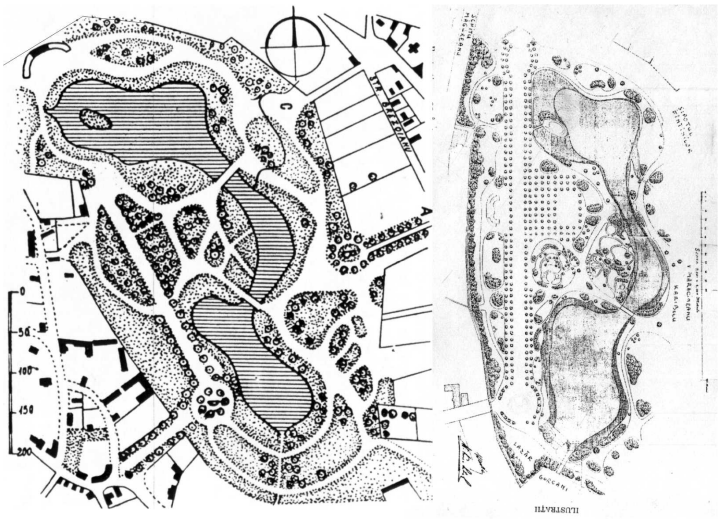

Fig. 1. The Cişmigiu Garden's plan:
- Left: the plan drawn by: Eng. Boroczin – 1850 (source: Marcus R.);
- Right: the plan drawn by Eng. Knechtel – 1883 (source: Toma D.).

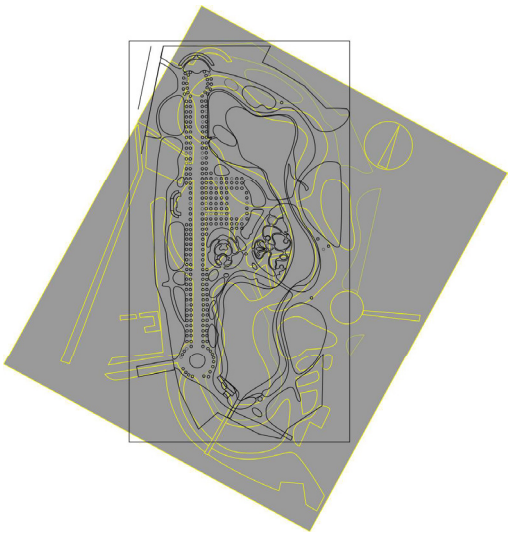

Fig. 2. The overlapping of the two historical plans (vectorisated)

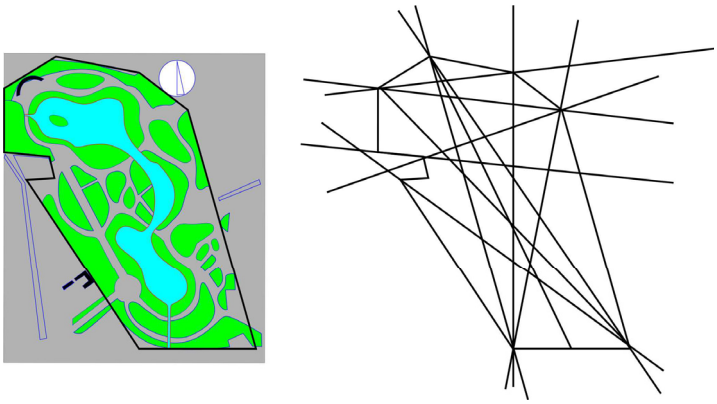

Fig. 3. Left: The general shape of the site; Right: The compositional schema.

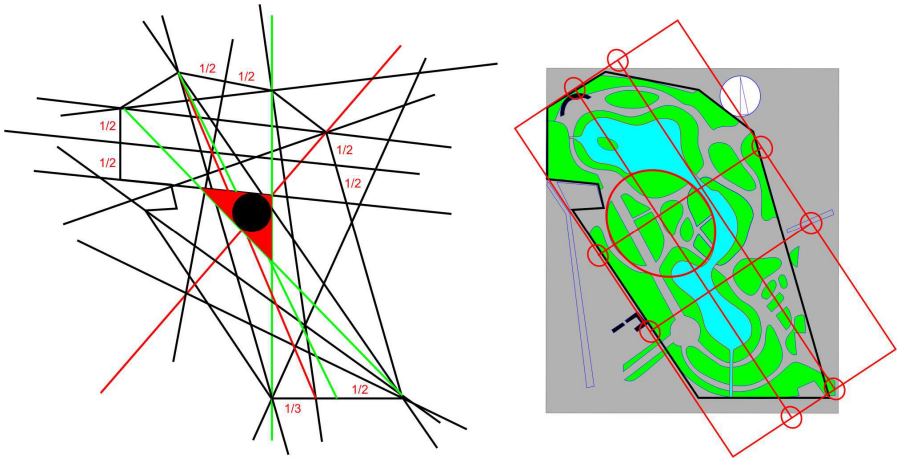

Fig. 4. Compositional structuresŞ
Left: Compositional centre; Right: The structure of the golden sections.

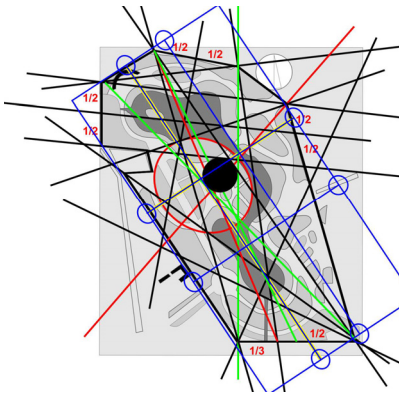

Fig. 5. Overlapping the stages above

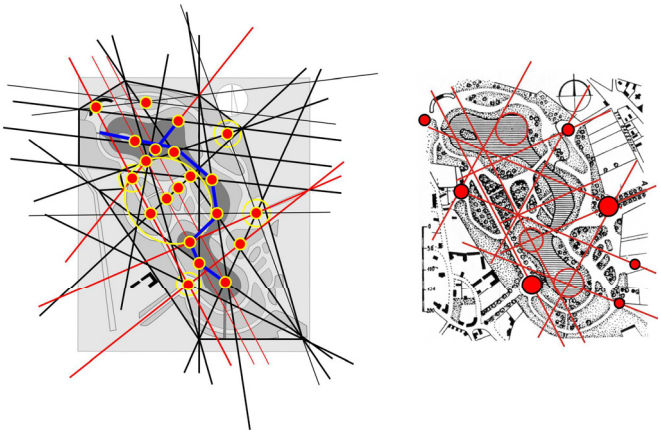

Fig. 6. Left: Focus points and force lines;
Right: The compositional schema in accordance with the acceses - streets trama.

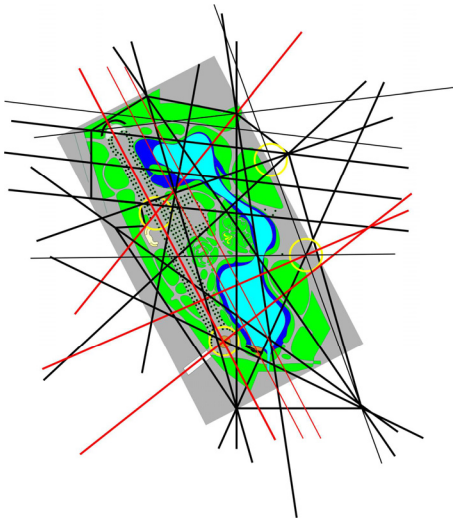

Fig. 7. The plan drawn by Eng. Knechtel – 1883 (vectorised);
the overlapping with the compositional schema.

Ten years of Landscape Architecture education at the Bucharest Faculty of Horticulture

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Keywords: anniversary, development, students, achievements, cooperation

The 60th Anniversary of the Faculty of Horticulture is also a decade of Landscape Department's celebration and, as we hope, will further add a new dimension of faculty's national and international prestige.

Ten years ago a Government Act decided on the establishment of the first landscape architecture school in Romania within the Faculty of Horticulture of the Bucharest University of Agronomic Sciences and Veterinary Medicine (USAMVB).

Through this debut the Romanian school has answered the need to provide skilled specialists in landscape creation, change, restoration, protection and management. Moreover, it has followed the example of the studies in European Union member countries where landscape architecture takes an important place within academic subject areas. This follows from its mission statement as defined by the EFLA (European Foundation of Landscape Architecture): the conservation and development of all kinds of landscape with all their related values (environmental, social, cultural, esthetical, historical and economical) that both present and future generations should enjoy.

The profession of landscape architect in Europe and worldwide has gathered weight in the last few decades as a consequence of the serious environmental changes, the damage and even destruction of natural and manmade landscapes subsequent to the unprecedented development of industrialization, urbanization, exploitation of natural resources and mutations in land use.

In this context and at special moment when our school celebrates ten years of existence and has already provided five generations of graduates, we are proud that it has been the one to open the road for this new profession to emerge in Romania. One more reason to be content is the fact that other faculties of horticulture countrywide - in Timisoara, Cluj and Iasi - have followed our example. They have enjoyed our assistance in that we have provided them with school curricula developed along the lines of European schools. Furthermore, this subject area has been established later on within universities of architecture as well, which have been stimulated by the success of our initiative.

Today's celebration is also a right time to review the past and contemplate the future. Notably, in the USAMVB the efforts of the competent staff devoted to the mission of training landscape architects resulted in successes of the school. Therefore, following the 2007 quality and performance assessment by ARACIS (Romanian Agency for Quality Assurance in Higher Education), landscaping studies were accredited by the Ministry of Education and Research. This success has not come easy. It has involved constant work over the years to improve all analytical programs as well as several amendments to the school curricula that sought both to adapt it to the European schools of landscape architecture and to comply with the Ministry's requirements to reduce the number of subject areas and the courses per week and later

on to bring the length of the degree program down from 5 to 4 years (the Bologna Convention on higher education).

We have paid special heed to seeking and co-opting teaching staff from other institutions for those subjects that cannot be covered by the teachers and professors of our university.

We have developed annual activities to attract and train candidates for admittance which has provided a head start for those admitted, especially in the basic subject of project drawing. Additionally, we have sought to enrich the available teaching materials and create our own library that would include specialized books and magazines acquired in Romania and abroad. Current1y, our library includes more then 300 original copies and photocopies.

The University and the Faculty have helped organize and equip drawing and projection rooms, a computer room and also buy other equipment. The logistical basis of the department has been developed from its own contributions as well - research and projection contracts, sponsorship got by our teaching staff.

To broaden the expertise of our teaching staff is another goal that is achieved through doctoral programs, scientific meetings, internal and international conferences, and exchanges of expertise and fellowship programs with several universities in Europe as well as by recruiting some of our best graduates to the department.

The cooperation with European universities has played an important role in providing good training to our students in landscape architecture at the Bucharest Faculty of Horticulture. We have attended the activities of the ECLAS (European Council of Landscape Architecture Schools) with one single participant at first (the department coordinator - Ph.D. Professor Ana-Felicia Iliescu) and then with the school as a full-member (2001). This has greatly helped implement the expertise of the universities from countries with a long tradition in landscape architecture.

According to the requirements of the European Landscape Convention and the Bologna Convention on the quality and efficiency of higher education, our school has joined the ERASMUS program LE NOTRE (Landscape Education: New Opportunities for Teaching and Research in Europe) initiated by the ECLAS. This project that includes several stages and spans from 2003 until 2012 currently brings together more than 120 universities with interactive connections via an especially designed internet program and meetings in workshops and regular conferences.

Thus, our school has tried to take on the recommendations that follow from the cooperation with sister universities on improving the quality of teaching and research in order to provide the graduates with better expertise in practicing their profession.

Another aspect of the relations with European schools is the cooperation with Haute Ecole Charlemegne in Liege (Belgium) - again through an ERASMUS program (2003-2013) based on student and teacher exchange programs that helps broaden the knowledge in relevant subject areas and improve teaching/learning methods.

The results of this cooperation and of the committed work of our teaching staff would not have been possible but for the interest and the enthusiasm of the young people that have chosen to become landscape architects. Candidate selection through preliminary tests on specific drawing, followed by the development of intellectual and professional skills during studies, it has brought good and very good results not only in study but also in off-curriculum activities. The workshop hours, field studies and research trips, attending conferences, writing papers and essays, the projects, individual study and field applications, team work - all these are a great amount of work for the

students in landscaping that shape the skills and competence necessary to practice their profession.

In order to gain expertise, the future landscape architects attend practical programs with specialized companies working both to draw out projects and put them into practice.

Under the direct guidance of the teaching staff, the students are involved in contract and agreement-based research and project activities. Here we can provide several examples of successful research and projects that have been delivered to our customers: the island in the Brancusi Park in Tg. Jiu, the Cemetery of the German Heroes in Cisnadioara; the Romanescu House (the House of the University) in Craiova; the courtyard of the Ministry for Agriculture, Food and Forests in Bucharest; the premises of the Philip Morris Company in Bucharest; Public Landscape in Bucharest Sector 1 (a contract with the Bucharest Sector 1 City Hall); the participation in the National Council for Scientific Research in Higher Education (CNCSIS) research contract of the A.C.U.M. Consortium with specialized analysis of numerous sites countrywide. Furthermore, several students have submitted their projects to the “Parks of the Future” program initiated by Petrom; in competition with architecture students and young architects, one of our students took II place at the 2007 edition.

On several occasions - the scientific sessions of the faculty, the "Days of Bucharest Horticulture" under the auspices of the XXI Horticulture Association, the exhibitions organized by Bucharest sector city halls (Herastrau Park, Revolution Square, the headquarters of the Bucharest Sector 1 City Hall) - our students have featured their own projects, drawings and artistic photographs and even a land art original demonstrations staged in the USAMVB park.

Other important achievements that have contributed to the prestige of our young school include student and teacher participation in international workshops organized by: The Socrates Intensive Program (Bucharest), the International Summer University in Sibiu, Transylvania Trust in Bontida (the restoration of the Banffy Palace gardens), the participation of our school alongside the French Cultural Center (CCF) and the Association of Urban Transition (ATU) in international workshops in Iasi (2006) and the international project exhibitions in Iasi, Bucharest, Alba Iulia and Lille (France).

These activities are already recommending our school, but the most important test of knowledge and skills acquired during studies is the graduation exam. The specific, extent and difficulty of the subjects tackled by degree papers and the exigency in the final examination are benchmarks for the quality of landscape architecture education.

In our school the diploma examination has been organized by inviting prominent figures in landscaping from Romania and Belgium (Haute Ecole Charlemagne) whose opinions and appreciations have contributed to increasing the level of this exam. While the professors have been very demanding, 92.3% of the graduates of the first four generations have enter their names for the diploma examination and 95.8% of them have successfully taken the exam, while 82.6% of them have got grades between 8 and 10 (according to the 2007 Self-Assessment Report).

All these results of our students mirror the quality of education and the efforts of the teaching staff to motivate and train them on the way to perfection. All teachers have joined this effort, i.e. teachers of all disciplines both from the Faculty of Horticulture and the University.

The school stays in touch with its former students, taking interest in their career development and providing help whenever necessary. Some of the graduates of the first four generations (approximately 17%) attend or have attended post-graduate studies, i.e. Master's and Doctor's Degrees, and most of them work in the field of landscape architecture (around 64%). Four of them have become teachers at the Landscape Architecture department and other leading graduates will join our staff.

The assertion at the national and European level of the landscape education promoted by the Bucharest Faculty of Horticulture contributed to the establishment of the Association of the Romanian Landscape Architects and to its admission as temporary member of the European Federation of Landscape Architects in 2007.

It is an achievement that makes us proud and urges us to work to meet the requirements of all European Landscape Architecture schools in order to have the full recognition of this forum. This is the only way the Romanian landscape architects will get the right to work abroad regardless of the university – agronomical or of architecture – they graduate from.

At its 10^{th} anniversary, our staff's stands by its commitment to bring the landscaping studies at the Bucharest Faculty of Horticulture to a higher level that would render it competitive on a national and European scale. We hope that we shall have the support of the faculty and university in this effort to set landscape architecture apart as an independent subject area within our university that already comprises several different education fields apart from the agricultural field.

Figures

Fig. 1. Open air studio – first year's students in the Village Museum Bucharest

Fig. 2. Field studies – landscape planning

Fig. 3. Landscape design studio

Fig. 4. Setting-up a garden project

Fig. 5. Realizing a temporary garden - Land art

Fig. 6. International summer school at Bontida, Cluj

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The Kiseleff road and garden as identity marks

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Keywords: historical green areas, urban landscape, cultural identity, *genius loci*, green oasis

ABSTRACT

Historical parks and gardens of Bucharest seems to be destined for today to an unfair neglect, thanks to all the changes produced in common mentality by the new throwaway society, but more, because of greats economic interests which tend to monopolize every green centimeter, converting in this way, the urban oasis in unfailing money factories. Old places for social practice of leisure, green areas with heritage value are situated near current center of the city. That's why the high prices of these lands create an ascending pressure to decrease of free building spaces from central areas. The link between culture, environment and community seems to be stronger if the people are conscious about the historical significance of living place, urban landscape being a powerful element of identity.

INTRODUCTION

This study tries to relieve cultural aspects of the Kiseleff Road and Garden, area designed for public destination, in the first intentions for urban modernization period, opened by the Organic Regulation in 1831.

The danger of these areas destruction could be appreciated from their inner multiple values:

- Ecological value – through the filter role for urban intense pollution
- Historical – architectural value – through the age of establishment, but more through artistic qualities and cultural bond with similar spaces and times from european Occident
- Social value – by specific way of use, community coagulation role and mark of appurtenance to a certain type of civilization.

MATERIALS AND METHODS

In the study about cultural and historical function of Kiseleff area, were used the old plans of Bucharest – Boroczyn plan from 1846, 1852, military and topographic plan from 1899, topographic plan of 1911, Sâmboteanu - Moldoveanu plan from 1938, touristic guides from 1923, 1943, 1962 - illustrations and proofs from old periods which are included in many works of the Bucharest historiography (Celebi, 1976, Bilciurescu, 1945, Giurescu, 1966, Kunisch, 2000, Pappasoglu, 1891, Potra, 1985, Sfinţescu, 1933,Vătămanu, 1973) and personal images with contemporary situation of the sites.

Equally, were studied writings about historical parks and gardens with their esthetical and social sides (Marcus, 1958, Toma, 2001). The comparison and the superposition of site plans, the assembling of the historical dates, and the exams of dendrological specimens which are still alive were lead to a new appreciation of today value of this site - a landscape urban value that sustains the identity of Bucharest culture, and which needs promotion and financial support.

RESULTS AND DISCUSSION

1. Bucharest – identity elements in urban landscape

Bucharest, plain town situated in the Vlăsia's forest, on a more or less marsh land, crossing by "romantic" river Dâmbovița, appears in documents like a place owned by strange souls, a composite of superposed cultures and opposite mentalities. The establishment of the city as territorial strategy of antiotoman resistance marked the evolution of a civilization at the Orient-Occident confluence. For the Balkan's city, the reference model was Islamic structure: the lack of political autonomy, scission in ethnic and religious communities articulated around the sacred areas, the lack of institutions and civic spaces – public life evolving in commercial neighborhoods. Street tissue evolves free, functional determined, open spaces sequel to road crossings extension have in many cases, finality in blind alleys that form irregular urban shapes. Opposite to "inside culture" specific for mediterranean and oriental spaces, local cities are open to nature and include it in composition system. The building don't fragmentizes the residential area in patios, yards (front yard, backyard, door yard etc.) like in western typology, this feature contributing to rural image of balcanic sites (Fig. 1).

The initial development of the town around mansion houses, churches and monasteries imprint "a certain discontinuity to the urban structure" (Harhoiu, 2001), image perpetuated in time; punctuated monumentality of churches in a huge garden embody Bucharest until the XIX century (Fig. 2). Here, the existence of green areas inside the city structure is generated less because of spiritual aesthetical needs, but more by an extension necessity for the living space, by pragmatic land use (agriculture, orchards), playful practice in an sacred time (green spaces around churches are used for play and party in time of religious feast days) or simply for leisure.

If Boroczyn 1852 plan, shows us great urban surfaces with cultivate lands, the 1899 relives the existence of private and public gardens, sustaining in this way the memory of an arcadian city with 'green oasis' pointed up throw multiples statements of foreigner travellers. Moreover, the gardens are arranged in european manner, coexisting in this oriental proximate space, architectural gardens with french specificity and english style romantic areas (Fig. 3).

What make our uniqueness; also close us with European cultural context of XIX modernity century. As identity elements, we observe on the one hand, real and/or imaginary, the urban morphology that abound of vegetal spaces, and on the other hand we remark the specific difference of use way for these areas.

2. Public promenade and livable landscape

From historical testimonies of those who knew Bucharest, even for little time, the pure visual culture of landscape is not specific to local inhabitants – with a character less dreamer, open air walking being more a sensorial pleasure then an aesthetical one, that's why the real sense of walking is party or feast... The walking, the immersed landscape, transcends the looking in living, proves the vernacular taste, less subtle but more expressive, alive, organic.

The design of public spaces according to the 1831 Organic Regulation, for "public walking" was made in "spaces constituted and frequented *per se*" (Toma, 2001) with much more time before the organizing work.

To establish a public walking was enough to be "a planted place", the term signifying an orchard or a vineyard or a flowered place, natural or wilding vegetated areas. A document of Prince Mihai Șuțu in 1792 speaks about beautiful garden of

Mavrogheni and about its destination "to embellish and pleasure of public outlook". Less contemplative, the beggarly of Bucharest look for social delight, a touch of untouchable, a voluptuously ingress in the forbidden world of elite. But also the high society needs to see and to be seen, this vanity prolonging the ball room into the public garden.

3. A historical fact: the promenade on the Road

In march 1830, when was established the comision for "city's betterment and embellishment" the first care of mayor board was to plant trees on the road edge to make alleys and to create three public plazas for people's walking, one of them being "the big alley from the Mogosoaia Bridge end". The works began in 1832 when was made the first lime plantations, they continued slowly until 1843 when prince G. Bibescu required to german landscaper C.F.W. Meyer to make a public park in this area. The Kiseleff Road, "the big alley", was then an old road through a muddy slum with little houses and famous Neculce's hostel, a way to Baneasa Forest where the peoples feasted away in holidays.

The General City Assembly had the intention to honor the organizing and administrating principality work with building a statue to general Kiseleff, but he refused and proposed to use this money for better public aims (Vatamanu, 1973). Consequently, Meyer drafted a plan for a garden on the both sides of the road (Fig. 4). With F. Horer gardener, he accomplished the biggest Romanian promenade from the middle XIX century, and a garden in the most appreciated style of the time. No other work for public reasons being treated with such benevolence in the Bucharest history (Toma, 2001). In spring of 1844, Meyer asked 29.350 trees from indigenous species, at the end of the year asked another 42.367, and the next season 16.050 trees! The Kiseleff Garden continued to be enriched until the year of premature disappearance of landscape artist in 1852. All his efforts finalized with special views, beautiful scenes, sculptures, water pieces, exotic species, in one word an edenic place where local people enjoyed to live, walk and party.

The official promenade opening on July 29, 1844, before Meyer to achieve unless a part from what he proposed, was made with blare, and in September 1846 took place a new celebration with fountains bering occasion.

Richard Kunisch described in 1861 the three sections of the road, between barrier and fountain, and spoke about those who frequented the promenade which used to get over for a few times, up and down the street, with luxury carriage and by foot at some certain hours of the day: "there, and only there we could walk" (Potra, 1985).

Romanian epicurean practice beside the nature is stimulated in the next years, in 1864 beginning an extension of the garden to Calea Victoriei, but the works slowdown the rhythm, such as twenty years later it still working on the area transformation.

Roberto Fava an italian journalist who visit Bucharest in 1894, mentioned Kiseleff Road as a promenade place that could rivaling with most famous european places, and Jean Loverdo was impressed in 1897 of "wide and straight boulevard, bordered with lime trees, openings, bushes, floral parterres, fountains, villas and summer houses, Champs Elysees and Bois de Boulogne from Bucharest". Kiseleff promenade was not abandoned after 1900, it continues to be used and subject of perpetual reparation and renovation works. Even he cannot bring quality pluses to Meyer achievement, Rebhun, the landscape architect who made after 1910 most important restoration interventions for Bucharest green areas, enlarged the alleys, added

furniture and introduce a playground. Modifying the curve line of alleys, neglecting water features and vegetation scenes, he diminished romantic look of original creation, limiting the value of a leisure and feast place to one of transit which connect two big circulated routes (Fig. 5). In 1932 I.T.Radulescu said: "Kiseleff Road is what americans mean a parkway – a road which has on one side and another planted stripes sufficiently wide to have the appearance of a park, or the opposed – a strait park crossed on the middle by a road".

CONCLUSIONS

Kiseleff area is today an urban public space that tells us a story of a century. A space that generated and kept urban landscapes, written and rewritten in time by man and nature together, which is now in a conflictual state determined by the discrepancy between political economic interests and historical character and identity. Kiseleff Road between Ion Mincu Street and Victoriei Plaza, with the garden made with a century and a half ago on both of its sides, determined a social habituation with special significance and relates a reference to landscape reflecting a distinctive tradition for Dambovita river area.

What's happening today on Kiseleff Road? Military parades on December 1, or sometimes old cars parades and from time to time are robed some Triumphal Arch flags… The Bucharest habitants forgot about wonderful parties and the promenade on The Road, preferring now to crowd in overpopulated Herastrau or Cismigiu also subject of general indifference, and also 'restored' by inexistent principles.

Although Kiseleff Garden, beside other public spaces, are protected at least formally by their inclusion on the Historical Monuments List from 2004, urban administration doesn't hesitate to approve the aggressive and unqualified interference in so called landscape remodeling (Fig. 6). Professional rehabilitation, temporary restrictions of motor circulation in a space with profound significance in public conscience could contribute to find the lost identity, to urban restructuration through establishment of a green network. Cultural happenings, ephemeral installations with social impact could create resonances with common place of Bucharest souls.

A restored identity, rediscovered, rebuild through receptivity for community needs could active implicate the society in an organic weaving of urban fragments.

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Figures

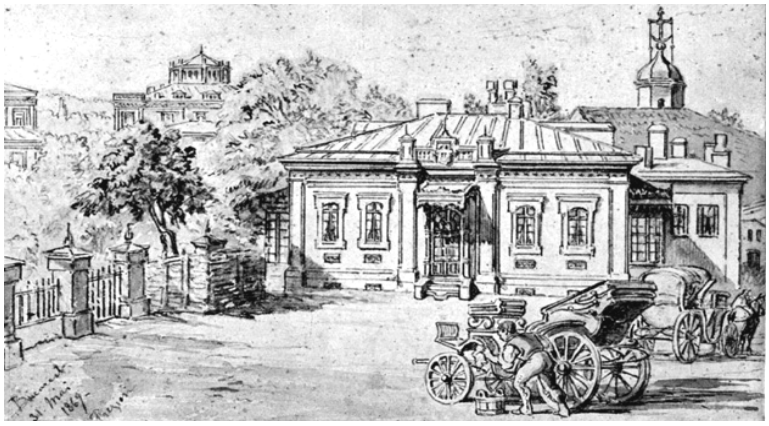

Fig. 1. Bucharest 1869, aquarela Preziosi

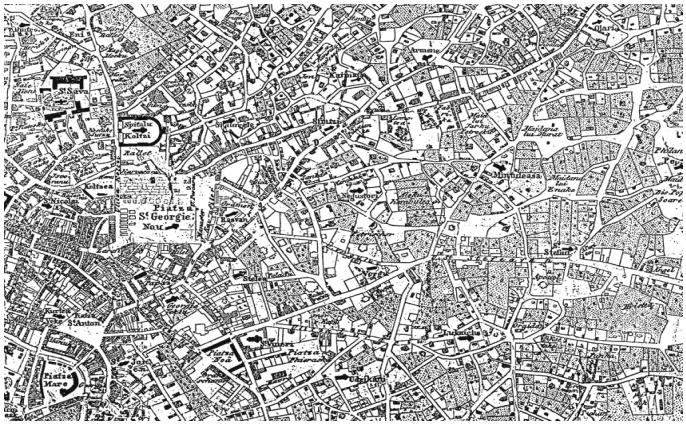

Fig. 2. Fragment of Boroczyn Plan 1852 – cultivated lands (gray)

Fig. 3. Bucharest Plan 1899 – two types of gardens: picturesque and regular.

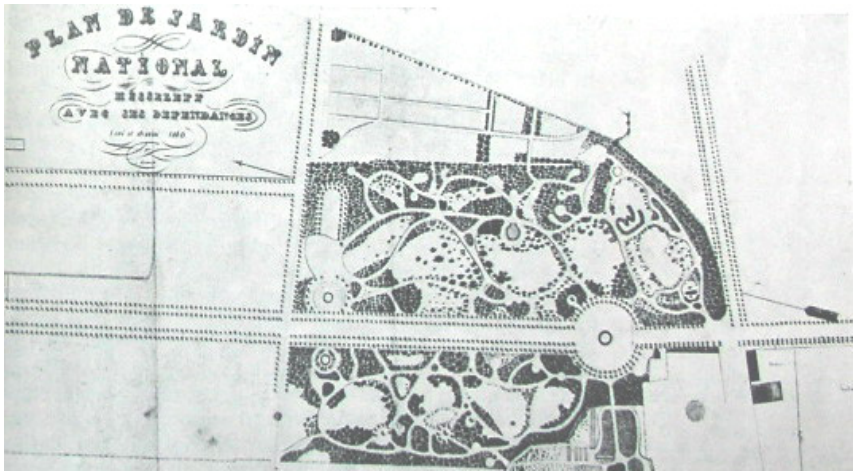

Fig. 4. Kiseleff Garden Plan 1860 (Marcus, 1958)

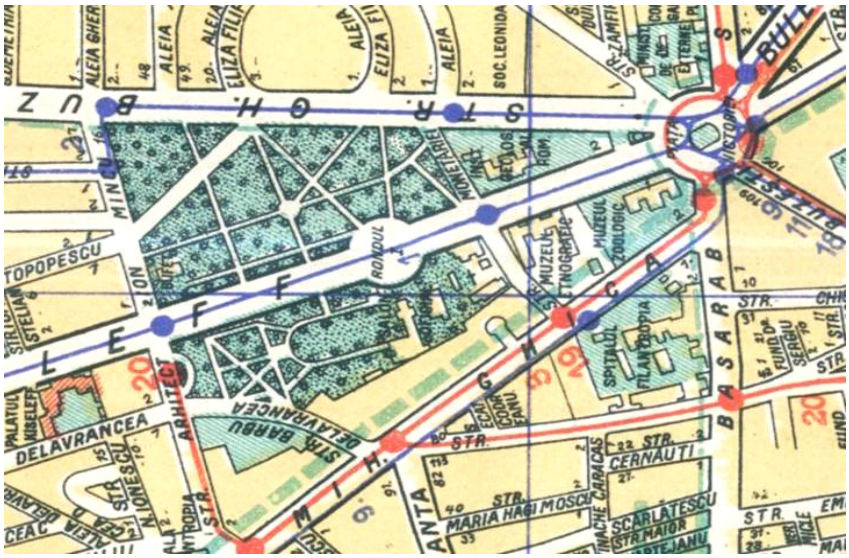

Fig. 5. Fredi Wahnig Guide 1934 – Grădina Kiseleff rearranged by Rebhun.

Fig. 6. Bucharest, Kiseleff Garden „restored” in 2006-2007

Preliminary results regarding the fertilization field culture of *Gladiolus gandavensis*

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Keywords: field culture, *Gladiolus Gandavensi*, fertilization system

ABSTRACT

Gladiolus specie is one of the most cultivated flowers around the world. It has about 250 species from which *Gladiolus Hybrid* is the most use specie in culture. The experience was made in a private farm from 1 Decembre village, Giurgiu County. The experiment had 6 variant and 4 repetitions. The variants of fertilization had different rate of nitrogen, phosphorus and potassium. The applications of fertilizers were at three periods of vegetation:- at the planting of tuberobulbs at 29 April 2006;– at the stage of 3-4 leaves respectively at 12 June 2006;– at 5-6 leaves, respectively at the 3 July 2006. The fertilizers used were NH_4NO_3, 34%N, superphosphat, 17% P_2O_5, K_2SO_4, 52%K_2O. The analyses of total forms of nutritive elements from the *Gladiolus* leaves showed that the fertilization with $N_{200}P_{100}K_{150}$ and cu $N_{100}P_{200}K_{150}$ determined a rapid absorption of nitrogen and phosphorus at the first period of analyze and at the second period of analyze the values of these elements were lower; The potassium analyze of leaves showed that the absorption of that element was lower at the first period of analyze and than the absorption was accelerated. The highest values of potassium in leaves were registered at V5 fertilized with $N_{100}P_{100}K_{200}$ and variant 6 fertilized with $N_{200}P_{200}K_{200}$.

INTRODUCTION

Gladiolus specie is one of the most cultivated flowers around the world. It has about 250 species from which *Gladiolus Hybrid* is the most use specie in culture. The culture of *Gladiolus* is use to produce cutter flowers and to decorate gardens and parks. That specie is cultivated in our country and especially near the Bucharest town where there are a large number of producers who assures the flower markets and flower shops of that town.

The aim of that paper was the improvement of that specie culture technology, the system of fertilization to obtain quality and crop.

MATERIALS AND METHODS

The experience was made in a private farm from 1 Decembre village, Giurgiu County, on 36 square meters. The soil was marked out and was make protection strips between variants so the experimental technique was respect.

The distances for planting were 25cm/15cm and the number of plants on every variant was 132 plants. Biologic material used for the experiment was OSCAR specie. OSCAR is specie of *Gladiolus* appreciated for its red and big flowers. Plants had a period of vegetation of 95/105 days and a blossom period for flowers of 18 days. The data of tuberobulbs planting was 29 April 2006 and the rising date was 15 May 2006 [1, 2, 3]

The experiment had 6 variant and 4 repetitions. The variants of fertilization had different rate of nitrogen, phosphorus and potassium presented in table 1.

Table 1. The scheme of experimental variants 2006

No.	Variant	Quantity of macroelements applied, kg a. s./ha			The equilibrium rate of fertilization N:P:K
		N	P	K	
1	Mt (V1, control)	0	0	0	0
2	V2	100	100	150	1:1:1.5
3	V3	200	100	150	2:1:1.5
4	V4	100	200	150	1:2:1.5
5	V5	100	100	200	1:1:2
6	V6	200	200	200	2:2:2

The applications of fertilizers were at three periods of vegetation:
-The first period – at the planting of tuberobulbs at 29 April 2006
- The second period – at the stage of 3-4 leaves respectively at 12 June 2006
- The third period – at 5-6 leaves, respectively at the 3 July 2006.

The fertilizers used were NH_4NO_3, 34%N, superphosphat,17% P_2O_5, K_2SO_4, 52%K_2O.

Before every period of fertilization were pick up soil samples for analyze and after vegetation start there were pick up plants samples, respectively leaves.
The analyses made at soil were: pH, total soluble salts content, macroelement contents – soluble forms. The methods used for analyses were standard ones:
- pH extraction in distillate water, extraction ratio was 1:2.5 and the method was potentiometer one;
- total content of soluble salts, water extraction in 1:5 ratio and the determination was with conductometric method
- determination of macroelements respectively N in nitric and ammonium form, P, K, extraction in distillate water, in ratio 1:5 and photocolorimetric method for reading in the case of nitrogen and phosphorus and in phlamphotometric method for potassium.

At plants were determined the dry matter content and macroelements contents.

During the vegetation period at same key phases, there were make biometrical measurements regarding the height of plants and same morphological qualities of flowers respectively the number of inflorescence and the medium diameter of flowers.

RESULTS AND DISCUTIONS

Before *Gladiolus* tuberobulbs planting it was make agrochemical analyze of soils from experimental variants. The results are present in table 2:

Table 2. The agrochemical characteristics of soil at the beginning of the experiment

No	Variants	pH	Soluble salts %	Content, ppm			
				$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$P\text{-}PO_4^{3-}$	K
27.04.2006							
1	V1	6.71	0.0173	32.5	1.25	8.6	50
2	V2	7.29	0.0132	32.5	4.75	11.5	40
3	V3	7.37	0.0187	34.0	2.75	8.4	45
4	V4	7.20	0.0173	27.5	3.75	7.5	35
5	V5	7.12	0.0144	22.5	2.25	11.1	40
6	V6	6.88	0.023	7.75	1.75	24.3	90

The soil used in the experiment shows a pH basic with the values between 6.71 and 7.37 and a low content of soluble salts. Nitrogen analyzes (ammonium and nitrate) show a high content of ammonium nitrogen and low content of nitrate nitrogen at all variants.

The ammonium nitrogen is high because in April the temperatures are low, nitrification process are low because the soil microorganisms that transform ammonium nitrogen in nitrate nitrogen there are not activated. Phosphorus analyze shows a variable content from a low supply from variant one until five to a middle supply at variant 6. The potassium content shows a soil with a middle supply content at variant 1 to 5 and high content at variant 6.

The plant analyses made regards the contents of dry matter, nitrogen, phosphorus and potassium, total forms. The results are present in table 3.

The nitrogen from soil was absorbed in *Gladiolus* leave (table 3) in high quantities at the first stage of analyze (15.06.2006) with values which varies from 2.98 and 3.45%. After this period respectively at 6-7 leaves stage (20.07.2006) total nitrogen values varies between 2.61% and 3.19%.

Analyzing experimental variants it can be show that at the first period of analyze the highest value of total nitrogen of 4.449% was registered at variant 4 fertilized with $N_{100}P_{200}K_{150}$ and the lowest value was of 2.9795% registered at V6 fertilized with $N_{200}P_{200}K_{200}$.

Table 3. The analysis of Gladiolus leaves Oscar specie

15.06.2006

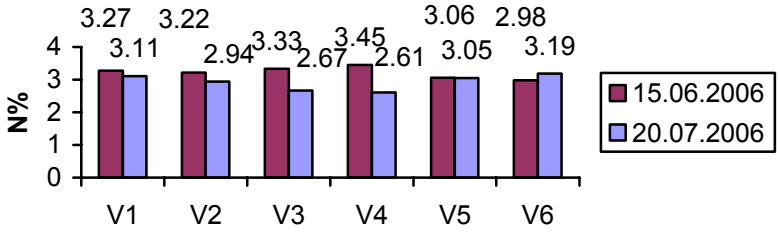
No	Variants		Dry matter, %	Content, %		
				N	P	K
1	V1	$N_0P_0K_0$	33.18	3.2712	0.362	0.9
2	V2	$N_{100}P_{100}K_{150}$	20.53	3.2242	0.403	1.25
3	V3	$N_{200}P_{100}K_{150}$	18.69	3.3276	0.450	1.45
4	V4	$N_{100}P_{200}K_{150}$	18.45	3.4498	0.453	1.8
5	V5	$N_{100}P_{100}K_{200}$	15.14	3.0644	0.416	1.5
6	V6	$N_{200}P_{200}K_{200}$	18.82	2.9798	0.336	1.8

20.07.2006

No	Variants		Dry matter, %	Content, %		
				N	P	K
1	V1	$N_0P_0K_0$	25.45	3.1114	0.307	1.25
2	V2	$N_{100}P_{100}K_{150}$	22.29	2.9422	0.301	1.5
3	V3	$N_{200}P_{100}K_{150}$	20.92	2.6696	0.284	1.45
4	V4	$N_{100}P_{200}K_{150}$	21.53	2.6132	0.234	1.3
5	V5	$N_{100}P_{100}K_{200}$	22.56	3.055	0.321	2.1
6	V6	$N_{200}P_{200}K_{200}$	21.37	3.1866	0.335	1.95

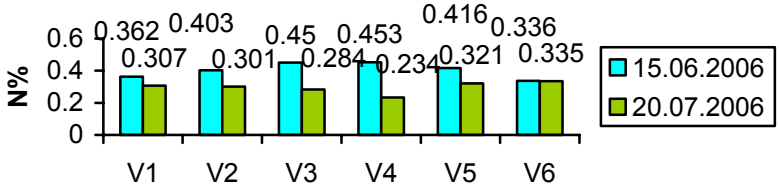
At the second period of analyze, variant 6 recovered the absorption of nitrogen registered the highest value of 3.186% and variant 4 presented the lowest value from all variants.

Fig. 1. The variation of nitrogen in Gladiolus leaves at the two periods of harvest



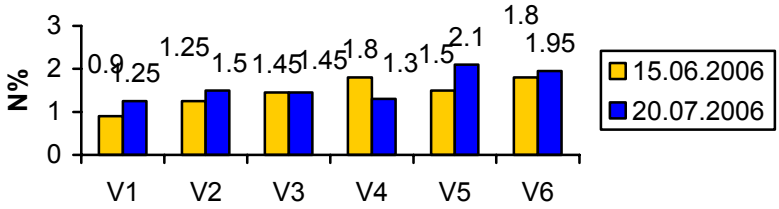
The absorption of phosphorus (figure 2) in *Gladiolus* plants at the two periods of analyses shows different values. The absorption from the first period of analyze to the second period lowering. If initial phosphorus had values between 0.453% and 0.336%, at the seco0nd period of analyze the lowest value was at variant 6 with a value of 0.335%.The values of phosphorus registered at the second period of were between 0.234% and 0.335%.

Fig.2.The variation of leaves phosphorus values at the two periods of harvest in experimental variants



Initially phosphorus registered the highest values at variants V3 ($N_{200}P_{100}K_{150}$) with 0.45% and V4 ($N_{100}P_{200}K_{150}$) with 0.453% value, at last the same variants presented the lowest values of that element respectively 0.234% at V4 and 0.284% at V3.

Fig. 3. The variation of leaves potassium values at the two periods of harvest



So the fertilization with $N_{200}P_{100}K_{150}$ and with $N_{100}P_{200}K_{150}$ determined the rapid absorption of nitrogen and phosphorus at the first period of analyze and registered the lowest values of that elements at the second period of analyze.

Potassium from the *Gladiolus* leaves was different absorbed comparatively with the other elements analyzed. The highest values of absorption were at variant V5 fertilized with $N_{100}P_{100}K_{200}$ with the value 2.1% and V6 fertilized with $N_{200}P_{200}K_{200}$ with the value 1.95%.

The absorption of potassium at the first period of analyze 15.06.2007 were the lowest values respectively 0.9 and 1.8%. Some times after, at potassium come an acceleration of the absorption so that at the second period of analyze 20.07.2006, the potassium values were between 1.25 and 2.1%. The highest values of potassium in leaves were registered at variant 5 fertilized with $N_{100}P_{100}K_{200}$ and variant 6 fertilized with $N_{200}P_{200}K_{200}$.

Biometric and morphologic measurements

Biometrical measurements made at *Gladiolus* on the vegetation periods respectively at 3-4 leaves stage, 5-6 leaves stage and at harvest, periods after these were made the fertilization of culture. The results were presented in table 4.

Comparing the plant heights from experimental variants it could be observed that at stage 3-4 leaves, variant 1, control, unfertilized presented the heights flower stems with a height of 67.5cm and the smallest stems were in variant 5 ($N_{100}P_{100}K_{200}$) with a 47.5cm height.

At the second period of measurement respectively at 5-6 leaves stage the effect of fertilization action at all variants. Therefore, it can be observed that the fertilization with $N_{200}P_{200}K_{200}$ (V6) determined the biggest difference of growing of +13.5cm, and than V5 with $N_{100}P_{100}K_{200}$ influence the growing of flower stem with 12.5cm.

Table 4. The heights of *Gladiolus* plants,
Oscar specie during the vegetation period 2006

Variant		Medium heights, cm					
		12 June 2006	**3 July 2006**	**Dif +/- between 12 June**	**At harvest**	**Dif +/- between 12 June**	**Dif +/- between 3 July**
V1	$N_0P_0K_0$	**67.5**	**72.5**	5.5	142.2	74.7	69.7
V2	$N_{100}P_{100}K_{150}$	64	**74**	10.0	143.8	**79.8**	**69.8**
V3	$N_{200}P_{100}K_{150}$	59	69.5	10.5	143.8	77.1	66.6
V4	$N_{100}P_{200}K_{150}$	65	72	7.0	146.0	**81.0**	**74.0**
V5	$N_{100}P_{100}K_{200}$	52	64.5	**12.5**	118.0	66.0	53.5
V6	$N_{200}P_{200}K_{200}$	47.5	61	**13.5**	100.0	52.5	39.0

At harvest, the biggest differences of growing comparison with the first period of measurements were registered at variant 4 fertilized with $N_{100}P_{200}K_{150}$, which one was of 81 cm and at variant 2 fertilized with $N_{100}P_{100}K_{150}$with the difference of 79.8 cm. In that case, the fertilization with $N_{200}P_{200}K_{200}$ had the smallest stem with the difference of 52.5 cm.

Analyzing the growth of plants, the heights of flower stem presented the optimum variants V2 ($N_{100}P_{100}K_{150}$) and variant 4 ($N_{100}P_{200}K_{150}$) which growth was slow during the vegetation period but with good final results.

The fertilization from variant 5 fertilized with $N_{100}P_{100}K_{200}$ and variant 6 fertilized with $N_{200}P_{200}K_{200}$ assured a rapid growth of stems in initial stage because of a rapid absorption of nutritive elements but at final stage that had the shortest stem and the smallest growth, the heights were under the control value.

Table 5. The morphological behavior of *Gladiolus* flower 2006

Variant	Length stem, cm	No of inflorescence in a flower	No of flower simultaneously open	The medium diametre of inflorescence, cm
V1	119	18	7	8.2
V2	121	18	8	9.8
V3	**122.7**	**20**	**10**	**11.8**
V4	**122.64**	**20**	**10**	**12.2**
V5	99	17	7	8.7
V6	84	17	5	7.5

Morphological data analyze of *Gladiolus* flowers were presented in table 5 showed the fallow:

- The lengths of flower stem is higher in variants 3 and 4 fertilized with $N_{200}P_{100}K_{150}$ and $N_{100}P_{200}K_{150}$ in comparison with control;
- The number of flowers on the inflorescence was bigger in variants 3 and 4, the value is 20;
- The number of flower simultaneously open on the inflorescence were 10 in variant 3 and 4 comparison with 5-8 in the other variants;
- The diameter of the inflorescence was of 11.8cm at variant 3 and 12.2cm at variant 4 and there were the biggest diameter from all experimental variants.

CONCLUSIONS

Analyzing the growth of plants, the heights of flower stem presented the optimum variants V2 ($N_{100}P_{100}K_{150}$) and variant 4 ($N_{100}P_{200}K_{150}$) which growth was slow during the vegetation period but with good final results.

1. The fertilization from variant 5 fertilized with $N_{100}P_{100}K_{200}$ and variant 6 fertilized with $N_{200}P_{200}K_{200}$ assured a rapid growth of stems in initial faze because of a rapid absorption of nutritive elements but at final faze that had the shortest stem and the smallest growth, the heights were under the control value.
2. The analyze of morphological data of *Gladiolus* flowers showed that the lengths of flower stem, number of flowers on the inflorescence, the number of flowers open simultaneously and the diameter of flowers were higher in variants 3 and 4 fertilized with $N_{200}P_{100}K_{150}$ and $N_{100}P_{200}K_{150}$ in comparison with control;
3. The analyses of total forms of nutritive elements from the *Gladiolus* leaves showed that the fertilization with $N_{200}P_{100}K_{150}$ and cu $N_{100}P_{200}K_{150}$ determined a rapid absorption of nitrogen and phosphorus at the first period of analyze and at the second period of analyze the values of these elements were lower;
4. The potassium analyze of leaves showed that the absorption of that element was lower at the first period of analyze and than the absorption was accelerated. The highest values of potassium in leaves were registered at V5 fertilized with $N_{100}P_{100}K_{200}$ and variant 6 fertilized with $N_{200}P_{200}K_{200}$.

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New trends in public urban parks – The Public Hanging Park

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Keywords: green space, urban Public Park, urban flagstone

ABSTRACT

In this paper, I will briefly present the new trend in urban public parks in the context of a reduction of the space reserved to it, being the hanging urban park, the descendant of the renowned hanging gardens of the Semiramis. The hanging system, which has been developed in the '70s, is based on the construction of a fully artificial soil on a waterproof concrete cover supported on structural pillars, which altogether make up a complex that needs a draining system. This way, these green spaces can overhang traffic paths, parkings, or underground commercial or cultural areas, etc. The first urban hanging green systems were built in the French Capital and enjoyed considerable success. There is the Planted Promenade (*La Promenade Plantée*), which is a green promenade which floats at the level of the second storey and crosses an important section of the city – arrondissement XII – and the Atlantic Garden (*Le Jardin Atlantique*), which is a hanging public garden over the Montparnasse high-speed railway station.

INTRODUCTION

This work's purpose is to shortly present a new trend in the design of public urban parks, being the hanging public park – a trend which was born following the reduction of the city spaces aimed for this function.

MATERIALS AND METHODS

The methods I used in studying/analysing urban flagstone park are:
- visits and analyses of sites;
- study of documents: books, reviews, internet sites, images;
- systemisation of analyses.

RESULTS AND DISCUSSIONS

The public urban park has been developed in time as a typology. Following the constant reduction during the recent decades of the spaces aimed for that function, the hanging green space has been developed as a continuation of the renowned hanging gardens of the Semiramis.

The hanging system, which has been developed in the '70s, is based on the construction of a fully artificial soil on a waterproof concrete cover supported on structural pillars, which altogether make up a complex that needs a draining system. Yet hanging plantations impose important technical constraints, including security issues, limited soil space for the development of the tree roots, and a proper deployment of certain ventilation or safety outlets for the underneath layers. This way, these public green spaces can overhang traffic paths, parkings, underground commercial or cultural areas etc

The first hanging urban green spaces were built in the French Capital and enjoyed considerable success. There is the Planted Promenade (*La Promenade Plantée*), which is a green promenade which floats at the level of the second storey and crosses an important section of the city – arrondissement XII – and the Atlantic Garden (*Le Jardin*

Atlantique), which is a hanging public garden over the Montparnasse high-speed railway station.

Planted Promenade

The Planted Promenade is an above the ground green space, completely isolated from the car traffic, 4.5 km long, located in the 12th arrondissement, starting from the Bastille Opera up to the eastern limits of Paris and almost reaching the Bois de Vincennes. It has been constructed over an abandoned railway viaduct dating from 19th century, which has been deserted in 1969.

Landscaper Jacques Vergely and architect Philippe Mathieux have designed this space with an area of 9.8 ha, including 6.5 ha for the promenade. The works have been built between 1988 and 2000. Here, the passengers enjoy a garden environment for an upper walk, wherefrom they can discover the architecture from an unusual angle, while the bicycle racers enjoy a ground-level runway which meets the boardwalk close to the Bois de Vincennes. Through its multiple access points from the streets, the promenade also plays the role of a connection between heterogeneous neighborhoods.

In the first section of the project, the promenade reaches above a viaduct section whose arcades have been turned into arts and crafts, thus becoming the renowned Viaduct of Arts (Fig. 1, 2). Furthermore, the promenade runs above a new viaduct built on an earthwork. The composition features a central alley with lateral vegetal massifs dominated by lime trees. Pockets, largos, closed and open spaces, shades and lights and sequences made by a double lateral and longitudinal symmetry are breaking the monotony of that straight path. Open, wide areas, which invite to relaxation, are alternating with more intimate, protected and narrow spaces. Rectangular arcs of steel with shrubs provide a rhythm to the promenade, alongside with the closed gardens with a central composition constructed into the alley expansions, which are by excellence conviviality spaces.

Thereafter, we reach the Reuilly garden (Fig. 4) with a large central lawn which is fit for open-air rest or games, crossed by a bent high-tech catwalk. Thus the promenade reached the street level and then crossed under a tunnel and runs into a forest environment (Fig. 3, 5).

The promenade features a very interesting idea of recycling: “an old infrastructure is turned into a luxury garden” (Brunon and Mosser, 2006). The new plantations are in the spirit of existing spontaneous vegetation (such as *Robinia Pseudoacacia* and *Ailanthus Altissima*) which support and provide it with a wild characteristic that is contrasting the more sophisticated species and the urban environment where the promenade lies.

Atlantic Garden

The Atlantic Garden has been designed by landscapers François Brun and Michel Péna overhanging the high-speed train runway of the Montparnasse Station in Paris. The works lasted from 1992 to 1994. Fully surrounded by high buildings which make up a rectangular area of 3,4 ha, the system could support bigger loads on the laterals only, on 5-meter wide strips close to the buildings, where the designers have located high vegetation blocks and left the central area for grass lawns and solitary trees (Fig. 6, 7, 8, 11).

The project suggests the ocean metaphor – a travel toward the ocean – and underlines the relationship between the railway station, neighborhood, garden and the

Atlantic. The garden is a successful détente place, with tennis courts, fountains where you can have a bath, wooden solarium and pier, which provides for a visual innuendo to waves (Fig. 10).

The plantation is designed in three layers: gramineaes and crawling plants, curvy plants and ferns in the "moisture hall" are making up the first layer. The second layer consists in trees and lianas, while the third one is made up of tall trees that form a protection curtain.

The overall project is an oceanic innuendo. On both sides of the main alley, pair trees of the same kind were planted – European species on one side and American species on the other one.

CONCLUSIONS

Hanging green areas represent a very good solution for the today's city, where the space for parks is smaller and smaller, while the needs for green areas is constantly increasing.

The Planted Promenade and the Atlantic Garden in Paris are two very successful examples. Such projects have never been accomplished, but there are plans for a similar promenade in the Chelsea neighborhood in Manhattan and in Chicago.

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Figures

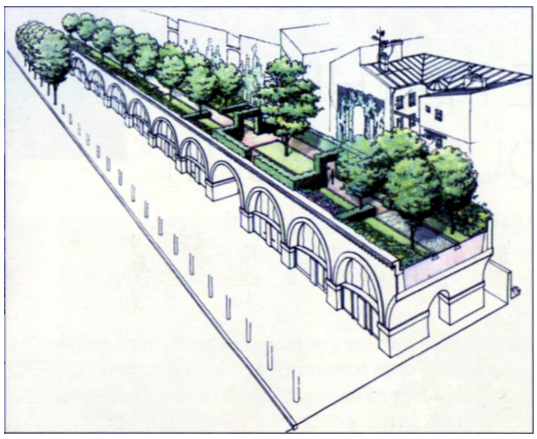

Fig. 1. Planted Promenade, Viaduct of Arts (Brunon and Mosser, 2006)

Fig. 2. Planted Promenade, Viaduct of Arts

Fig. 3. Planted Promenade, near Bois de Vincennes

Fig. 4. Planted Promenade, Reuilly garden

Fig. 5. Planted Promenade, near Bois de Vincennes

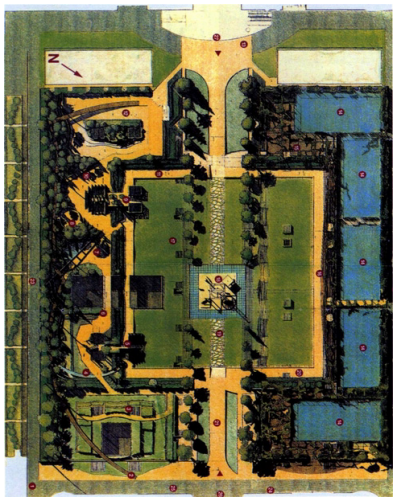

Fig. 6. Atlantic Garden, Plan (Cortesi, 2000)

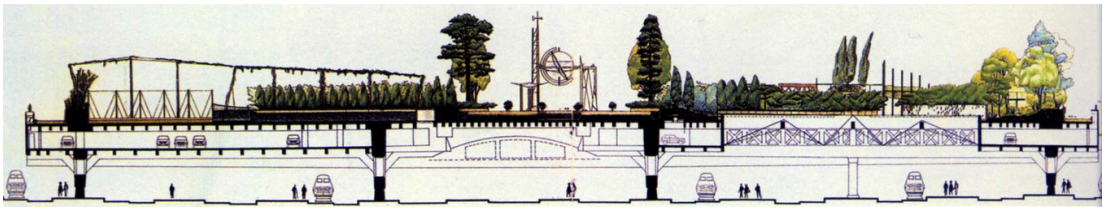

Fig. 7. Atlantic Garden, Section (Cortesi, 2000)

Fig. 8. Atlantic Garden (Brunon and Mosser, 2006)

Fig. 9. Atlantic Garden, wooden solarium

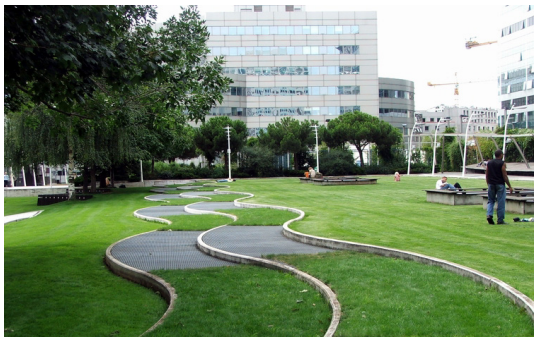

Fig. 10. Atlantic Garden, terraces

Fig. 11. Atlantic Garden, central element

Behaviour in the multiplication process a some ornamental species/varieties coniferous with high ornamental value

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Keywords: ornamental species/varieties, multiplication, cuttings, rooting biostimulators

ABSTRACT

The ornamental species and varieties of coniferous are of great decorative interest being utilized in landscape arrangements as simple samples or in together with others. The propagation of these ornamental varieties is usually difficult due to their specific biological features. The commercial extension of these ornamental species/varieties was done into a less extent due to a low rate of propagation, although these was a great demand as well as their difficult propagation on studies will be focused on: behavior of the some ornamental species/varieties: *Tujopsis dolobrata, Tsuga canadensis, Juniperus chinensis „Aureospicata", Juniperus chinensis „Blaauw's Varietat"* şi *Picea abies „Albertiana Conica"*during the initial multiplication process. The studies carried out at the Research Institute for Fruit Growing have had in view the response of five ornamantal species/varieties of coniferous to propagation by softwood cuttings, employing Radistim 1and Radistim 2 under artificial mist. *Thujopsis dolobrata* and *Tsuga canadensis* varieties showed the highest rooting percentage in all treatments (42,8-95,4% and 41,2-91,4%) and the little rooting percentage (10,4-51,2 %) of the *Juniperus chinensis „Blaauw Varietat"*. Application of the biostimulators (Radistim 1) has obviously improved the rooting yield versus the untreated control, treatment.

INTRODUCTION

The interest for the coniferous culture in the ornamental purpose is lately special; they have an important role in the nursery production. The breeding of the coniferous species with high decorative value, which were brought from import in the last two decades, is very hard and pretentious.

The promotion of these conifers in the production nurseries and by it to the breeders, supposes the establishment of the technological links of fast and efficient breeding which will insure to the breeders a profitable and efficient activity.

The achieved researches had as objective the testing of the vegetative multiplication capacity of the following ornamental species/varieties of conifers: *Tujopsis dolobrata, Tsuga canadensis, Juniperus chinensis "Aureospicata", Juniperus chinensis "Blaauw's Varietat" and Picea abies "Albertiana Conica"*.

The experiments were placed in solariums which had hotbeds with equipments which produced artificial fog, existent at ICDP Maracineni, in 2005 – 2007.

The greatest rootedness efficiencies were registered with the Thujopsis dolobrata and the Tsuga canadensis species (values between 42.8-95.4% and 41.2-91.4%).

The ornamental variety *Juniperus chinensis "Blaauw's Varietat"* had the lowest rootedness percentage, with values between 10.4-51.2%, in all the study years.

MATERIALS AND METHODS

Knowing the importance of the of the ornamental conifers in the landscape arrangements and the fact that the breeding of the ornamental varieties of *Juniperus, Picea, Tsuga and Thujopsis* is generally difficult due to each one's specific biological characteristics, we have proposed the following objectives:

- the comparative behavior of the ornamental varieties of: *Juniperus, Picea, Tsuga and Thujopsis* when they are bread by semi-wooden cuttings, in the solarium, under artificial fog;
- the testing of the rootedness capacity of the studied ornamental varieties when they are treated with different rootedness bio-stimulators;
- the effects of the rootedness bio-stimulators upon the radicular system and of the aerial part of the rooted cuttings;

The experiments were placed in the breeding places (solariums), in 2005 – 2007.

Biological material was used from 2 ornamental varieties of *Juniperus*, an ornamental variety of *Picea* and two species: *Tujopsis dolobrata, Tsuga Canadensis*.

The biologic material was represented by cuttings with *"heel"*, in the physiological semi-wooding state at the *"green"* cutting. The cuttings were taken from mother plants, of over 10 years old, which are in the park-collection of ICDP Pitesti-Maracineni.

The *"green"* cutting epoch has coincided with the calendar period of the 15th of July – the 10th of August, which is the growth period for sprouts (cuttings in the semi-wooding phase).

After producing the cuttings and removing the foils on the portion which was introduced in the planting orifice, the cuttings having the dimensions between 10.5-16.5 cm (according to each specie/variety), were treated at the base with the following rootedness bio-stimulators, as powder and solution:

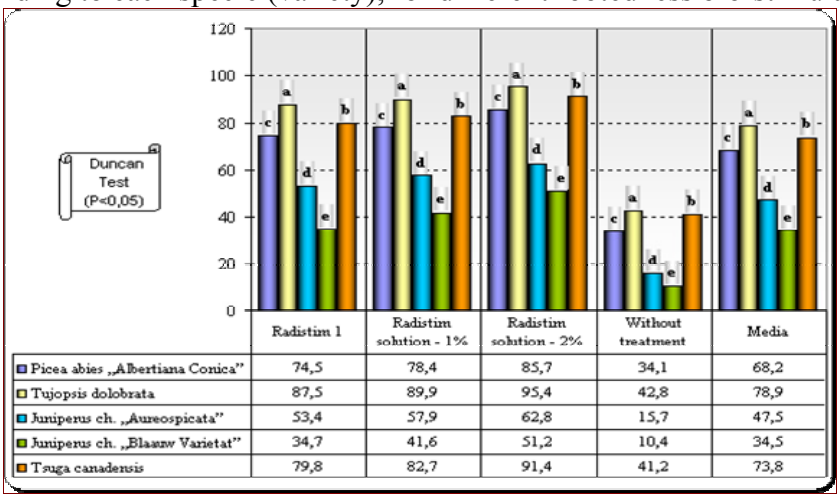
- as powder: Radistim powder – a commercial product of the BIOS Chemistry Institute from Cluj Napoca;
- as solution: Rdistim solution of 1% and 2% – a commercial product of the BIOS Chemistry Institute from Cluj Napoca, (the immersion period of the cuttings being of 1 minute).

The planting of the cuttings in the rootedness layers made of pearlite was performed in prior open orifices with the help of a planter. The plating distances were of: 7 cm between rows and 5 cm between the cuttings of a row, and the planting depth was of about 2/3 cm of the cutting's length, in order to insure its vertical position.

When the cuttings were planted, the layer was sufficiently moist. After the planting, the artificial intermittent fog equipment was turned on, which was automatically released, at time periods of 5-10-20 minutes. The spraying period was of 5-10 seconds, according to the exterior temperature, so that the tumidity state of the cuttings was maintained, stopping them from dehydrating or rotten.

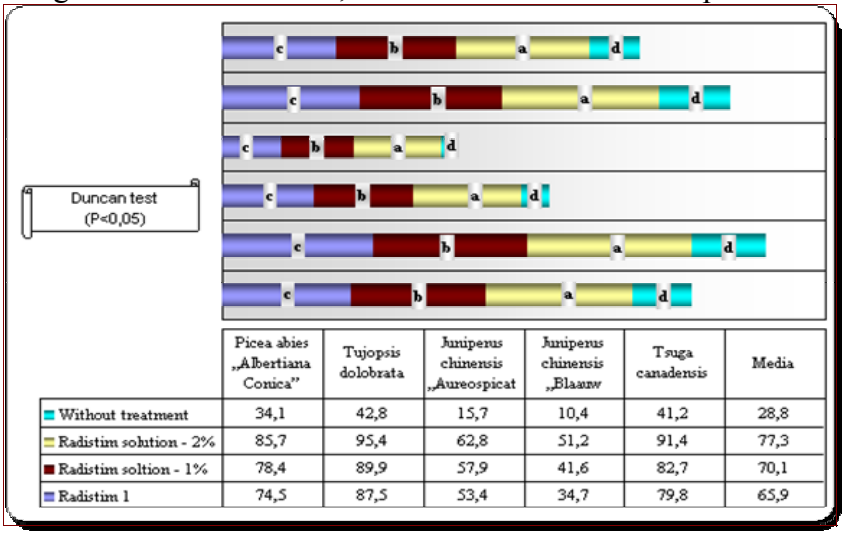
RESULTS AND DISCUSSIONS

Charter 1 – The variation of the rootedness efficiency (%) of the semi-wooden cuttings according to each specie (variety), for different rootedness bio-stimulators



	Radistim 1	Radistim solution - 1%	Radistim solution - 2%	Without treatment	Media
Picea abies „Albertiana Conica"	74,5	78,4	85,7	34,1	68,2
Tujopsis dolobrata	87,5	89,9	95,4	42,8	78,9
Juniperus ch. „Aureospicata"	53,4	57,9	62,8	15,7	47,5
Juniperus ch. „Blaauw Varietat"	34,7	41,6	51,2	10,4	34,5
Tsuga canadensis	79,8	82,7	91,4	41,2	73,8

Charter 2 – The variation of the rootedness efficiency (%) of the semi-wooden cuttings according to the bio-stimulator, for the studied coniferous species/varieties



	Picea abies „Albertiana Conica"	Tujopsis dolobrata	Juniperus chinensis „Aureospicat	Juniperus chinensis „Blaauw	Tsuga canadensis	Media
Without treatment	34,1	42,8	15,7	10,4	41,2	28,8
Radistim solution - 2%	85,7	95,4	62,8	51,2	91,4	77,3
Radistim soltion - 1%	78,4	89,9	57,9	41,6	82,7	70,1
Radistim 1	74,5	87,5	53,4	34,7	79,8	65,9

CONCLUSIONS

The researches regarding the breeding by "green" cuttings under artificial fog of the *Thujopsis dolobrata* and *Tsuga canadensis* species and the *Juniperus chinensis "Aureospicata", Juniperus chinensis "Blaauw's Varietat" and Picea abies "Albertiana Conica"* varieties, achieved in the 2005 – 2007 period, in the Breeding and Dendrology laboratory of I.C.D.P. Maracineni, Arges, allow us to express some conclusions and recommendations which are useful for research and education, especially for the state and private production units.

1. In all the study years great efficiencies were emphasized for the *Thujopsis dolobrata* and *Tsuga canadensis* species (values between 42.8-95.4% and 41.2-91.4%).
2. The *Juniperus chinensis "Blaauw's Varietat"* ornamental variety had the lowest rootedness percentage, with values between 10.4-51.2% in all the study years.

3. By using rootedness bio-stimulators, the rootedness efficiency was visibly improved, as well as the quality of the planting material, obtaining a radicular rich system, comparative to the untreated specimen.
4. Of the used rootedness bio-stimulators, Radistim solution –2% proved to be the best, for the rootedness of the cuttings, as well as for the architectonics of the radicular system and of the aerial part of the coniferous cuttings studied during the three years, followed by Radistim solution –1%, comparative to the untreated specimen.

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Research on behaviour of *Magnolia soulangiana* in the multiplication stage of "*in vitro*" culture

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Keywords: phytohormones, micropropagation, explant, culture media, microcuttings

ABSTRACT

This article presents the realizations of the tehnology of producing biological material with rapidly clonal multiplication with reference at the phase of *in vitro* multiplication. Magnolia has a different behaviour in the micropropagation phase; the results were influenced by the composition of culture media. For the magnolia multiplication proved to be efficient the concentration of 5 mg/l 2iP when the rate of multiplication reached 5 microcuttings/explant.

INTRODUCTION

Magnolia soulangiana belongs to the Family Magnoliaceae which includes approximately 80 species of trees and tall bushes with persistent or falling leaves and which bloom before or after had grown foliage.

In Romania, during the last period, the Magnolia species were planted mainly for ornamental and medical purposes.

Considering the disadvantages of traditional reproduction by seed, marcottage, grafting, summer cutting propagation induction of genetic variability, passing on viral and micoplasmatic diseases as well as the success scored during the last years by the *in vitro* tissue culture propagation of ornamental plants, we took the initiative of *in vitro* propagation of *Magnolia soulangiana* species in order to obtain rejuvenated planting material, in good phytosanitary condition.

MATERIALS AND METHODS

For the initiation of the experiments in the *in vitro* multiplication phase the biological material was represented by explants who were obtained in the initiation phase on the next culture media: macro and microelements Murashige - Skoog (1962), vitamins Linsmaier - Skoog (1965), 0,1 mg/l gibberellic acid, 1 mg/l naphthalenacetic acid, 0,7 mg/l benzylaminopurine, 5 mg/l ascorbic acid, 32 mg/l NaFeEDTA, 40g/l glucose, 7 g/l agar.

The explants transfer on the multiplication culture media was made in a sterile room, on a hood with laminar air flow (fig.1).

The surgical type instruments used (tweezers, scalpel with the single use blade) were sterilized in the drying stove, at 120^{o}C temperature for 2 hours and in the working time were sterilized after every utilization. For avoiding the cultures contamination, the operator carried mask and the hands were desinfected by washing with soap and utilising the desinfectants gels (ex. Hexigel).

The culture media used in the micropropagation phase are complex composition with differentes types and concentrations of phytohormones (tab. 1). Out of this variantes we tasted also the initiation culture media with and without active charcoal (300 mg/l).

In order to avoid the weighing errors, the macroelements were used like 10x more concentrates solutions while the microelements and the vitamins for 100x more concentrates solutions. We used phytohormones like dilutions of 100 mg/l (10^{-4}).

The pH registered in a culture medium was adjusted to 5,6-5,8 before autoclaving.

The sterilisating of culture media was realized by autoclaving at 120^{o}C temperature for 20 minutes. During the micropropagation phase, in the growing room (fig. 1) we have ensured controled conditions (photoperiod of 16 hours, temperature between 22-24^{o}C).

The observations were realized weekly and the explants were passed on fresh culture media when appearing the vitrification phenomenon or oxidatives processes.

RESULTS AND DISCUSSIONS

The *Magnolia soulangiana* explants were affected by the oxidative processes who determined their brunification and it was necessary to pass the explants on fresh cultures media after only 14 days of culture. As early as explants growing phase we tried stopping oxidation of phenolics compounds by adding in culture medium the active charcoal in 0,3 g/l concentration. On this culture medium the microcuttings were mantained 4 week, the report 0,2 mg/l IBA/1mg/l BAP determined the evidentiation of axillary buds.

In purpose of the organogenous enlargement capacity and microcuttings elongation , in the culture media were utilised much more complex vitamins (Miller, 1982A) and we tested different types and concentrations of cytokinines.

From the tested culture media, a positive influence over the caulogenesis and the microcuttings growing was 2iP in 5 mg/l concentration when we have obtained 5 microcuttings/explant multiplication rate. The formed microcuttings have 1-2 cm and they can be easy individualized for realizing a new subculture (fig.2).

The microcuttings were normal; they didn't presented vitrification and the brunification which is characteristic of oxidative phenomenons.

On the culture medium with 2 mg/l BAP (V.1.) was obtained only 3 microcuttings/explant, but these didn't elongate too much and for this cause the individualization was realized with difficulty and after transfer many were necrosed.

In the variants with kinetines (4 mg/l) we didn't obtain micropropagation, but the explants were longer. The culture medium V.2. may be important if is necessary the raising phase before *in vitro* rooting.

CONCLUSIONS

Magnolia has a different behaviour in the micropropagation phase; the results were influenced by the composition of culture media. For the magnolia multiplication it proved to be efficiente the concentration of 5 mg/l 2iP when the rate of multiplication reached 5 microcuttings/explant.

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Tables

Table1. The components of culture media used for the micropropagation explants of *Magnolia soulangiana*

Components (mg/l)	V.1	V.2	V.3
Macroelements	MS	MS	MS
Microelements	MS	MS	MS
Vitamins	M	M	M
Naphthalenacetic acid	0,1	0,1	0,1
Benzylaminopurine	2	-	-
Kinetine	-	4	-
2iP	-	-	5
NaFeEDTA	32	32	32
Glucose	40.000	40.000	40.000
Agar	7.000	7.000	7.000

Legend: MS = Murashige - Skoog (1962);
M = Miller şi colab. (1982)A;
2iP = N ^{6}- (2 - isopentyl) adenine.

Figures

Figure 1. Aspect from the sterile room (left) and the growing room (right)

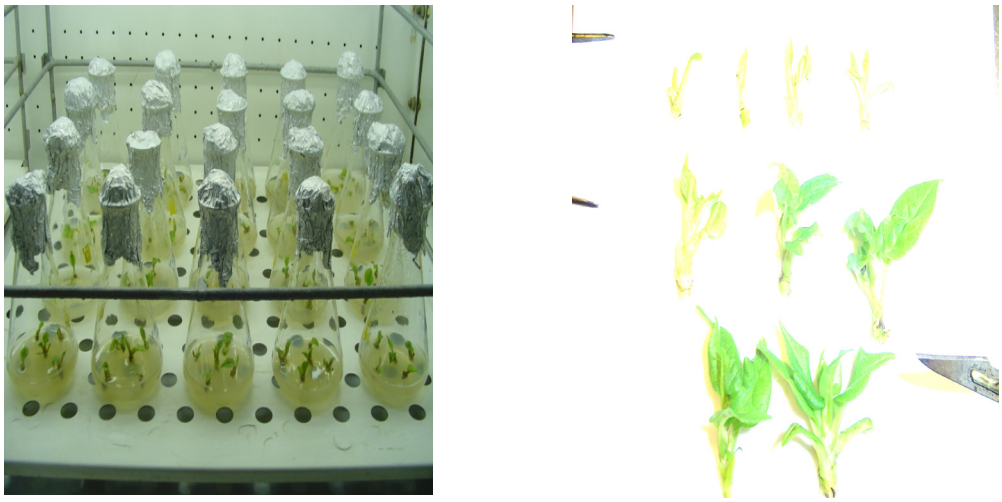

Figure 2. Microcuttings obtained on the culture medium with 2iP

Figure 3. *In vitro* multiplication of magnolia

The optimization of the quality of the public green space system in District IV- Bucharest

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Keywords: optimization, spatial configuration, functional-ecological quality

ABSTRACT

A possible model of optimization of the quality of the urban green space system ensemble, takes into consideration the three principle criteria of amelioration of the quality of the urban green: spatial-urban configuration, functionality and ecological aspects. These criteria generate proposals of optimization of the ensemble quality of the public green space and apply to different approach bearings: general bearing (the green system in its ensemble), zonal bearing ("the green pen") and the local bearing (green entity).

INTRODUCTION

The current state of the green spaces from district IV-Bucharest is characterized through a series of dysfunctions both in the system ensemble and in zonal or local level. The principle dysfunctions come from the high rate of human interference with the territory and from the lack arrangements of corresponding planted spaces, to which there is added the microclimate conditions and urban pollution. They can be resumed in this way: air pollution, phonic pollution, presence of stagnant waters which come from the groundwater layer (in the Văcăreşti area), unfit management of the waste, dysfunctions in the ruttier traffic, the state of the existing vegetation, lack of functions for the loisir which correspond to the actual needs of the population. Fro these considerents there is necessary an elaboration of amelioration proposal of the green spaces quality for the three important bearings of spatial-urban organization: general bearing (green system), zonal bearing (green pen) and local bearing (green entity).

MATERIALS AND METHODS

The proposals for optimizing the quality of the urban green spaces have in consideration the three principle criteria: spatial configuration, functionality and ecological quality. These criteria apply to each bearing of urban structuring of the green spaces system: general bearing, zonal bearing and local bearing.

General bearing- general system of green spaces

The proposals for optimizing the spatial configuration take into account:

- **completing the green space system** with the green "pen" from the north-west of the district through **arranging an urban park on the Văcăreşti territory**, which would mean an expand of the current total surface (174 ha) with approximately 100%of public green spaces in the district. The ensemble quality of the urban green is so improved by the **extensive-quantity expand.**
- **the decentralization of the current spatial configuration and realization of a plasmatic configuration** on the district ensemble, by arranging gardens of proximity in the free space between blocks of flats (fig. 1) having in mind their actual reduced number and the total reduced surface of only 12 ha.

By this proposal of decentralization of the actual spatial configuration there was kept in mind the realization of new unities of public green space disposed plasmatic on district's territory, **outside the dominant nuclei composed of the green "pen".**

Optimizing the functionality has in mind the fact that urban functions from the territory of district IV are dominated by the residential function; it is obvious **the scope-function of the optimization solution** which is satisfying the need for loisir; all the more as in the vicinity of residences ensemble or in their interior, this function is insufficiently represented by proximity gardens and lack of a complex multifunctional unity of green space with unlimited access (urban park). It is about the residential ensemble Oltenița where there can be especially observed this functional deficiency.

The optimization of the ecological quality comes from the amelioration of the spatial configuration and of the general functionality. By plasmatic spatial configuration and the decentralization of the actual spatial configuration as well as the functional diversification **as a result of the optimization of the spatial and functional quality:**

- the effects of the optimization of the ecological quality become obvious;
- the global indicator Ig/Iv becomes favorable to the green infrastructure;
- areas of favorable ecological influence are distributed more uniformly on the territory of the district, determining **direct and indirect ecological effects**:
  - reduction of pollution level;
  - bacterial depuration and dust fixation;
  - noise reduction;
  - regulation of the termo-higrometric regime;
  - diminution of the negative effects of the urban heat isles;
  - atmospheric ionization;
  - stimulation of the air exchanges;

This increase of the ecological quality leads to the heightening of the comfort grade and of the life quality.

Zonal bearing - green "pen"

The optimization proposals have in mind completing the existent green zone, which has a strong configurative-spatial impact on the green ensemble in the district, by completing the existent park surfaces (Carol, Tineretului, and Copiilor) with a new park arranged on the Văcăreşti territory as a public green space with unlimited access. In this way, the new spatial configuration of the green "pane" (fig.2) will influence through the extended contour not only the quality of the spatial configuration of the urban green in district IV, but in all the southern half of the city through the urban and ecological influence, and the influence aria of this positive impact becomes much larger (fig.2).

The solution of functional optimization for this bearing has the objective of completing the existent functions in the already arranged parks that do not cover in total the need for loisir of the users (ex. the Tineretului Park) - especially on different age categories. In this sense, by realizing a wider green zone, **the quality of the spatial configuration (fig.2) sustains the functional quality** by adding in the existent green "pen" functions which are either missing in the present, or have a low grade of complexity, such as: educational functions, cultural functions (represented by endowments such as libraries in open air, expositions, pavilions with flexible cultural usage), recreational functions, type active or passive, functions of daily promenade, sportive functions.

The optimization solution stands in creating multiple functioning of the green space spatially reconfigured.

The optimization of the ecological quality has as an objective the widening of the aria of zonal influence (fig.2) sustained by direct ecological influences resulted from expanding the current green "pen", practically by doubling the existing park surface.

We can also propose **the street alignments and the creating of ecological protection plantations** as **objectives of zonal ecological optimization.**

Local bearing - green entity

The optimization of the configurative-spatial quality on this bearing takes into account the Văcăreşti territory, space which is now presented as an amorphous space from an urban point of view, with an important landscape potential which is not valued. The spatial-volumetric reconfiguration confers this territory, by arranging an urban park, **direct quality special, functional and ecological valences** which result from the general compositional organization in which there are included: vegetal volumetry, functions, circulation or water surface. These could be disposed in percent-type relations which respect the already known proportions for urban parks:

- waters 8-10%
- circulations 12-15%
- constructions (endowments + arrangements) 5%
- plantations 67-70%

The proposal for realizing a park on the Văcăreşti territory constitutes the mere essence of the functional optimization solution. The urban park, as a complex spatial-functional unity, is the category of public green space that can assure the functional profiles' diversity, necessary as a solving of the valuing the usage of urban territory need. In the same time the park becomes a pole of social attraction by facilitating social contacts and obviously has through functional complexity, high social efficiency.

The optimization of the ecological quality on this bearing has in mind a scope-function which aims for improving quality by setting up the park specific plantation; it will produce direct quality-ecological effects in sanogen and microclimatic plan.

RESULTS AND DISCUSSIONS

Taking into account the fact that for an urban park the planting average on 1 ha is of 200 arbors and 1000 bushes there can be estimated some of the quality-ecological effects of this planting on a surface of approximately 170 ha.

Equaling 10 bushes to one arbor, there results a number of 300 arbors/ha, of which 200 leafy arbors (70%) and 100 cone arbors (30%). The total number of arbors on a territory can be of approximately over 50000 which equals approximately 80 ha of forest (20 ha of cone arbors and 60 ha of leafy arbors).

- **80 ha of forest fix 4000t of dust/year** (80x50t/year=4000t/year)
- **20 ha of cone trees fix 100t of carbon/year** (20x5000kg/year=100000kg/year)
- **60 ha of leafy trees fix 288t of carbon/year** (60x4800 kg/year=288000kg/year)
- **80 ha of forest eliminate 5184 t of oxygen/year** (80x180 kg/ha day x 360 days=5,184,000 kg/year)
- **80 ha of forest absorb 7,200t of carbon dioxide/year** (80x 250 kg/day x 360 days= 7,200,000kg/year)
- **70 ha of grass lawn fix 70t of carbon/year** (70x1000kg/year)

CONCLUSIONS

The proposals for optimizing the quality of the urban green space system from district IV- Bucharest were elaborated for each of the three urban-spatial bearings of the green space: general, zonal and local; they had as foundation the principle three criteria: spatial configuration, functionality and ecological quality. These proposals aim especially for the greatening of the green spaces by creating the Văcăreşti Park as a green entity. In this way the public green space surface is widened (it practically doubles), fact which leads to the obvious enhancement of spatial-functional and ecological quality of the system of green spaces in its ensemble.

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Figures

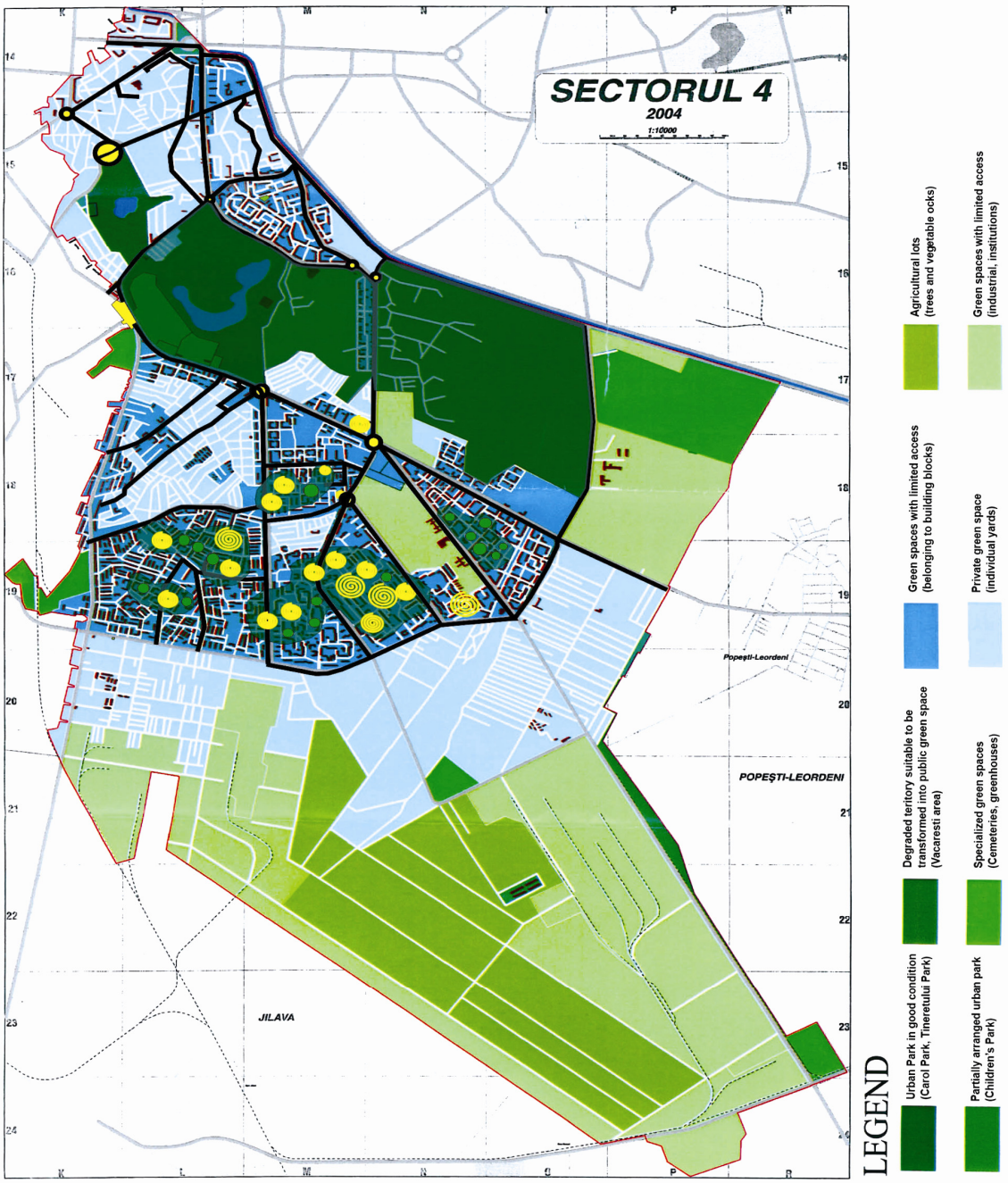

fig. 1 Quality optimisation proposal through plasmatic spatial configuration

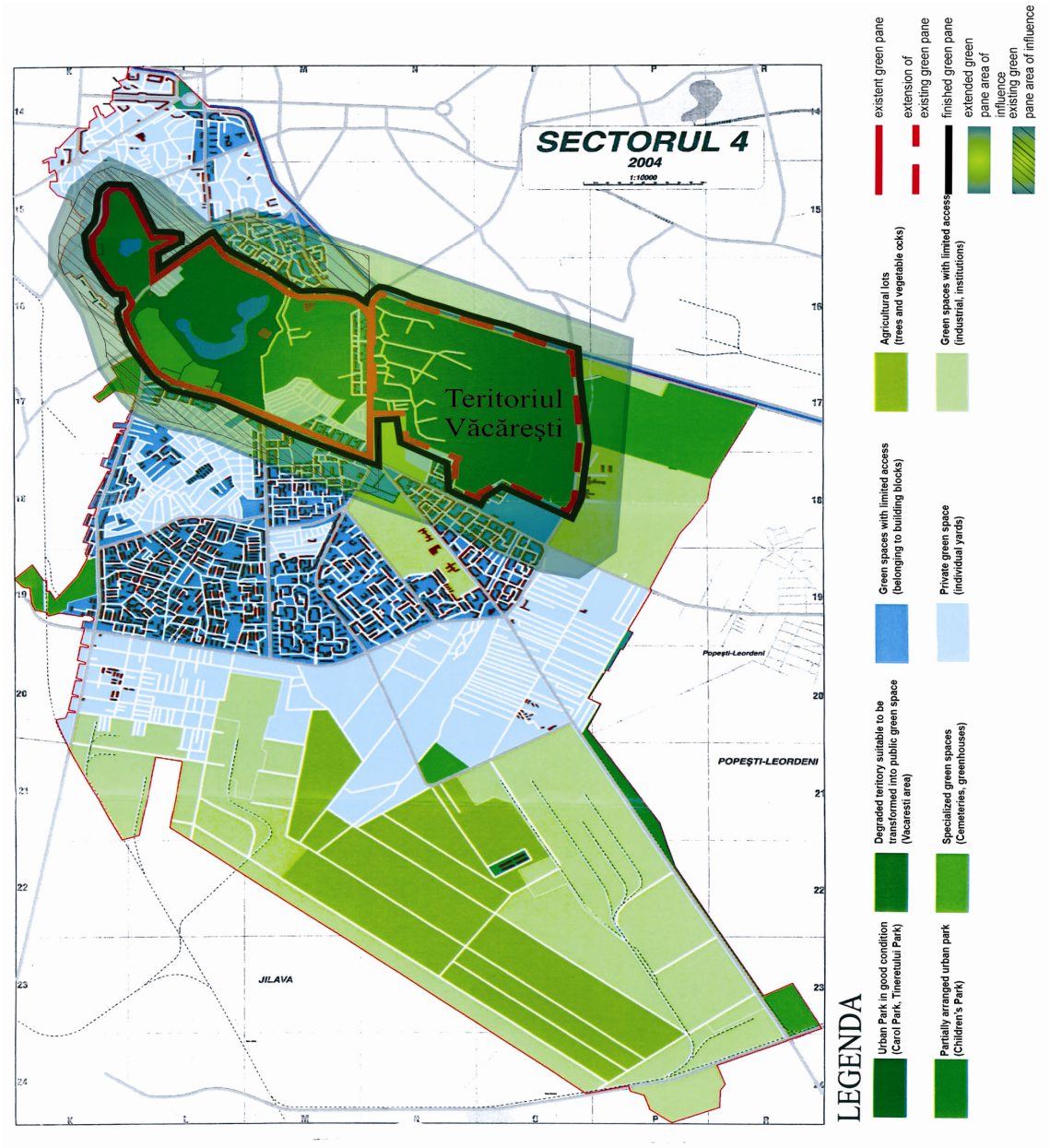

fig. 2 Urban and ecological influence area derived from the spatial reconfiguration of the green space made from the parks Carol, Tineretului, Copiilor and Vacaresti

Research as regard to the biologically and ornamentally valuation of a fifteen gladioli cultivars assortment

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Keywords: gladioli, phenology, growth, development

ABSTRACT

Gladioli occupy a main position in the summer Romanian cut flowers assortment. Flowers's esthetical quality, between which it can be mentioned a slender aspect of the floral stalk and the wealth of the distinguished colors, as well as plant flexibility at different technological variants is trumps by which it manages consumers and producers requirements. Because cultivars behavior is different in function of the ecologically conditions and the applied technology, sometimes their reaction tends to compromise decorative features or even gradually disappearance by inadaptability. In the present work there was performed an ornamentally and biologically valuation of fifteen Romanian and foreign cultivars, cultivated in the naturally conditions of the South Romanian area.

MATERIAL AND METHODS

The studied biological material comprised 6 Rumanian creations realized at ICDLF Vidra (Ramona, Denisa, Gabriela, H 9604-4, Corona, Alexandra) and 9 from the more foreign cultivated cultivars (Priscila, Nova Lux, Ice Cream etc.). The experimentally cultures have been performed in the years 2005 and 2006, on a brown - reddish soil, in the specifically climatically conditions for the North-Vest plain area of Bucharest city (Table 1). Corms plantation was done on April 24 - 2005 and April 15 – 2006. Irrigation was necessary almost all the time during the vegetation period. There were applied three fertilizations with Nutrilife, one treatment with Atomic (5 mL/10L H_2O) and current take care works. Cultivars valuation has been carried out based on the phenology observations and by registering plants growth and development indicators.

RESULTS AND DISCUSSIONS

Appearing and flowering phenology is presented in Tables 2 and 3. Plants apparition began during the periods: 05 – 19.05.05 or 27.04- 9.05.06 with duration of 6-9 days, respectively 4-6 days. Ramona, Denisa and Gabriela cvs. were emphasized the most earlier. Excepting Ice Cream cv., all foreign cultivars are more tardy and with 3-4 days longer plants apparition period. Flowering began on 15.07.05 (Ramona) and only on 25.07.05 (Nova Lux). These cultivars have had the same behavior in 2006 too, marking the beginning flowering spell. Flowering duration was only 7-10 days in the year 2005 and extended between 11-27 days in 2006. From this viewpoint interesting are Plumtart and Wing Song cvs., which had the flowering period concentrated on the shortest period. Flowers of one inflorescence open in about 6-7 days for the majority cvs. As concerning culture duration, since corms plantation till flowering, these was 81-91 days in 2005 and 72-103 days in 2006. All this phases were carried on under obvious influence of the temperature and precipitation level, during the two years culture. So, raining and cold periods altering with hot days conducted to the shortening of flowering duration and disturbed flowers quality during the year 2005.

As regard as floral stalk growth and development (Tables 4 and 5), majority of the studied cultivars are able to form 2-3 floral stalks. Priscila cv. is the most prolific one (3-4 floral stalks in 2006), Plumtart and Her Majesty rarely present 2 floral stalks.

Floral stalk length depends by its position on a plant and also by the plant life conditions. These more oscillate from one to another cultivars, being a genetically feature, but in large limits also from one to another year, under the culture conditions influence. Indifferent of the position state, it corresponds to the market requirements. Concerning the inflorescence length, differences between cultivars and from one to another year are relatively large. Some cultivars emphasized a better stability, as against the influencing factors (Ramona, Gabriela, Butterfley Pink). There is a positive relation between floral stalk length and those of the inflorescence for the majority cvs. Interesting are data referring to the simultaneously open flowers: Gabriela, Priscila, Deciso and Ice Cream emphasized the maximum digit -8, that is an important quality indicator. Also, there were noticed flower buds that did not opened. From this viewpoint it can be appreciated Alexandra, Deciso, Butterfly pink, White prosperity and Ice Cream cvs. which all floral buds are able to open.

As regard as the flowers color, the choused assortment presents a large color variety, from white to red, and from tawny to violet or mauve, each of them being well expressed in the local conditions were the cultures have been done.

CONCLUSIONS

The climatically conditions from the area where the experiment has been carried out correspond to gladioli exigencies, excepting very raining years, when flowers quality is more disturbed.

The chouse assortment assures flowers production valuation on a period up to 45 days, in the presented research conditions.

Romanian creation (Ramona, Denisa, Gabriela) are remarked by being earlier and stabile at the climatically factors fluctuations.

Decorative characteristics feature are remarkable at Gabriela, Alexandra, Priscila, Butterfley Pink and Ice Cream cvs.

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Tables

Table 1. Dynamics of the main climatically factors

Month	Medium temperatures (^{0}C)		Medium precipitation (L m^{-2})		Medium duration of sunshine (hours)	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
January	0.8	-3.6	45.5	40.8	125.4	87.3
February	-2.2	-1.2	54.7	20.7	72.4	106.6
March	3.5	4.7	24.6	60.5	185.0	138.6
April	10.5	11.8	50.2	50.6	192.7	204.4
May	17.4	16.4	72.4	46.4	257.0	269.5
June	18.9	20.2	121.7	45.2	268.5	252.6
July	21.6	21.7	149.2	42.8	272.9	311.2
August	21.0	21.2	163.8	100.8	257.8	296.5
September	16.6	17.0	199.6	87.8	183.0	201.3
October	10.7	-	61.4	-	184.9	-
November	4.5	-	47.6	-	109.9	-
December	1.2	-	37.9	-	68.5	-
Absolute minimum	-21.0 06.12.2005	-	-	-	-	-
Absolute maximum	36.3 21.07.2005	35.5 20.08.2006	55.4 20.09.2005	-	13.3 9.08.2005	13.4 19.07.2006

Table 2. Growth and flowering phases (year 2005)

Nr.	Cultivar	Emergence beginning	Emergence end	Emergence duration (days)	Flowering debut	Flowering end	Flowering duration (days)	One inflorescence flowers opening duration	Days number since planting till flowering
1	Ramona	5.05	9.05	5	15.07	26.07	11	6	81
2	Denisa	6.05	11.05	6	18.07	26.07	9	6	84
3	Gabriela	7.05	14.05	6	17.07	25.07	9	6	83
4	9604-4	10.05	15.05	6	23.07	3.08	10	9	89
5	Corona	15.05	20.05	6	25.07	2.08	8	7	91
6	Alexandra	8.05	14.05	7	16.07	26.07	11	6-7	82
7	Plumtart	18.05	25.05	8	23.07	30.07	8	5	89
8	Priscila	10.05	16.05	7	17.07	25.07	9	6-7	83
9	Deciso	11.05	18.05	9	20.07	27.07	8	6	86
10	Nova Lux	15.05	22.05	8	25.07	30.07	6	4-5	91
11	Butterfley Pink	8.05	14.05	7	17.07	25.07	9	6	83
12	Wind Song	18.05	26.05	8	24.07	1.08	7	4-5	90
13	Her Magesty	15.05	23.05	9	20.07	28.07	9	5-6	86
14	White Prosperity	7.05	12.05	6	22.07	2.08	11	5-6	88
15	Ice Cream	8.05	14.05	7	21.07	29.07	9	4-5	87

Table 3. Growth and flowering phases (2006)

Nr.	Cultivar	Emergence beginning	Emergence end	Emergence duration (days)	Flowering debut	Flowering end	Flowering duration (days)	One inflorescence flowers opening duration	Days number since planting till flowering
1	Ramona	27.04	30.04	4	30.06	25.07	26	5-6	75
2	Denisa	29.04	2.05	5	27.06	24.07	27	5-6	72
3	Gabriela	30.04	5.05	6	3.07	25.07	22	-	78
4	9604-4	-	-	-	-	-	-	4-5	-
5	Corona	7.05	12.05	5	23.07	10.08	17	7-8	98
6	Alexandra	29.04	6.05	8	8.07	28.07	20	5-6	83
7	Plumtart	10.05	17.05	8	20.07	31.07	11	7-8	95
8	Priscila	2.05	10.05	9	9.07	31.07	22	5-6	84
9	Deciso	10.05	20.05	10	20.07	29.07	10	6-7	95
10	Nova Lux	9.05	16.05	8	19.07	11.08	22	6-7	94
11	Butterfley Pink	2.05	9.05	8	2.07	22.07	20	5-6	77
12	Wind Song	11.05	16.05	6	28.07	11.08	13	5-6	103
13	Her Magesty	5.05	12.05	8	12.07	28.07	16	5-6	87
14	White Prosperity	28.04	1.05	5	1.07	23.07	23	9-10	76
15	Ice Cream	29.04	3.05	6	15.07	1.08	16	9-10	90

Table 4. Floral stalks and inflorescences characteristics feature (year 2005)

Nr.	Cultivar	Nr. floral stalks /plant	Floral stalk length (cm)			Leaves number/ Floral stalk			Inflorescence length			Buds number on one inflorescence			Simultaneously Opened flowers (number)			Buds number that are not opened		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	Ramona	2-3	134	131	127	6	5	5	66	62	65	18	17	16	5	5	4	1	1	1
2	Denisa	1-3	130	121	115	6	6	5	58	48	39	18	16	16	6	5	5	2	1	1
3	Gabriela	2-3	128	121	99	6	6	5	56	48	42	18	18	16	8	7	5	2	1	1
4	9604-4	1-3	145	-	-	7	-	-	65	-	-	22	-	-	6	-	-	-	-	-
5	Corona	1-2	90	87	-	6	6	-	62	58	-	20	18	-	5	5	-	1	1	-
6	Alexandra	1-2	110	108	-	7	6	-	70	66	-	20	18	-	7	6	-	-	-	-
7	Plumtart	1	103	-	-	6	-	-	52	-	-	20	-	-	6	-	-	2	-	-
8	Priscila	3-4	128	118	110	7	6	6	65	59	56	22	20	18	8	7	5	2	1	1
9	Deciso	1-3	124	119	116	6	6	6	40	38	32	20	18	16	8	6	4	-	-	-
10	Nova Lux	1-2	154	142	-	6	6	-	52	48	-	18	18	-	6	4	-	2	1	1
11	Butterfley Pink	2-3	122	121	118	6	6	6	61	54	50	20	20	18	6	6	4	-	-	-
12	Wind Song	1-2	152	148	-	7	6	-	64	50	-	20	18	-	7	5	-	2	1	-
13	Her Magesty	1	131	-	-	6	-	-	60	-	-	20	-	-	6	-	-	1	-	-
14	White Prosperity	1-2	161	156	-	10	9	-	80	79	-	22	22	-	6	5	-	-	-	-
15	Ice Cream	1-2	162	160	-	7	7	-	80	78	-	22	22	-	8	6	-	-	-	-

Table 5. Floral stalks and inflorescences characteristics feature (year 2006)

Nr.	Cultivar	Nr. floral stalks /plant	Floral stalk length (cm)			Leaves number/ Floral stalk			Inflorescence length			Buds number on one inflorescence			Simultaneously opened flowers (number)			Buds number that are not opened		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	Ramona	2-3	139	135	129	7	6	6	68	67	54	18	17	16	4	4	3	2	2	1
2	Denisa	2-3	120	115	112	5	5	4	40	38	36	12	12	11	4	3	3	2	1	1
3	Gabriela	2-3	150	140	128	6	6	6	50	44	40	17	16	15	5	4	3	2	2	1
4	9604-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Corona	1-2	115	100	-	-	-	-	55	53	-	20	18	-	5	4	-	2	1	-
6	Alexandra	2	129	108	-	6	6	5	80	76	-	23	17	-	7	6	-	2	1	-
7	Plumtart	1-2	138	112	-	6	6	-	60	56	-	20	-	-	4	-	-	2	-	-
8	Priscila	2-3	140	121	119	6		6	66		58	22	18	16	5	5	4	-	-	-
9	Deciso	1-2	112	118	-	7	6	-	50		-	18	16	-	6	5	-	1	1	-
10	Nova Lux	2-3	138	129	120	6	6	5	50	60	44	17	16	14	5	5	4	2	1	1
11	Butterfley Pink	2-3	132	128	120	6	6	6	62	46	55	20	18	16	6	6	5	1	1	1
12	Wind Song	1-2	130	125	-	6	6	-	50	48	-	18	18	-	4	4	-	2	2	-
13	Her Magesty	1-2	121	118	-	6	6	-	50	60	-	18	16	-	4	4	-	1	-	-
14	White Prosperity	2	159	148	-	7	6	-	74	48	-	20	18	-	6	5	-	-	-	-
15	Ice Cream	2-3	160	155	148	7	6	6	68	46	60	20	18	18	6	6	4	-	-	9

Table 6. Dynamics of the main floral stalk growth and development

Nr.	Cultivar	Floral stalk length (cm)			Inflorescence length (cm)			Buds numbers on an inflorescence		
		2005	2006	Medium	2005	2006	Medium	2005	2006	Medium
1	Ramona	134	139	137	66	68	67	18	18	18
2	Denisa	130	120	125	58	40	49	18	12	15
3	Gabriela	128	150	139	56	50	53	18	17	18
4	9604-4	145	-	145	65	-	65	22	-	22
5	Corona	90	115	103	62	55	59	20	20	20
6	Alexandra	110	129	120	70	80	75	20	23	22
7	Plumtart	103	138	121	52	60	56	20	20	20
8	Priscila	128	140	134	65	65	66	22	20	21
9	Deciso	124	112	118	40	50	45	20	18	19
10	Nova Lux	154	138	146	52	50	51	18	17	18
11	Butterfley Pink	122	132	127	61	62	62	20	20	20
12	Wind Song	152	130	141	64	50	57	20	18	19
13	Her Magesty	131	121	126	60	50	55	20	18	19
14	White Prosperity	161	159	160	80	74	77	22	20	20
15	Ice Cream	162	160	161	80	68	74	22	20	21

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Beginnings of alcohol distillation at world level and in the Romanian principalities

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Keywords: fermentations, alembic, elixir, brandies, calvados.

ABSTRACT

At the beginning the production was sporadic, reduced and unequal. The phenomenon was not understood, they talked about spiritus vini (geist in German), «the spirit» that was believed to come from the distillation heat or fire (vinum adustum, Branntwein - brandy). The Salerno school (in the south of Italy) takes over through the Arabian channel the practical methods to obtain the alcoholic distillates for medical purposes. Arnauld de Villeneuve/Villanueva (1240-1311), a famous doctor and alchemist from Catalonia and his disciple Raymond Lulle study the distillation preparing the first medicinal beverages (that often evolved into liquors).The distillation of alcohol has been mentioned in writing since the Arabian Middle Ages. Some authors consider that the distillation of alcohol, before being spread by Arabs, would have existed in empirical variants in China and Tibet. Other hypotheses make the alchemists from Alexandria (Egypt) responsible for the development of the first tools necessary for distillation, the vases called "ambix", from which comes the term "al-ambix = alembic". Zosimos of Panopolis (3rd century-the beginning of the 4th century A.D.) and Hypathia of Alexandria (350-415 A.D.) wrote important papers on distillation. The Persian doctor and alchemist Rhazes (Abu Bakr Mohammad Ibn Zakariya al Razi, 865-925 A.D.), improved the distillation methods obtaining ethylic alcohol and sulphuric acid, substances non-separated up to him in pure distinct state.

For half a millennium, alcohol will be used only to produce remedies, macerates of medicinal plants (el-ixir, liquor). Words like alcohol, alembic and araq are of Arabian origin.

INTRODUCTION

Though distillation as a procedure has been known from early antiquity (3500 B.C.) there was not any written information before the Islamic civilization on the alcohol distillation. In the alchemists' labs they kept the secret that did not allow the transmission of some information related to the eventual successful or unsuccessful essays. There is a possible failure to obtain some quality products when the necessary conditions lack (installations were primitive, the raw materials used were often improper).

MATERIALS AND METHODS

We used a vast bibliographical material to point out the beginnings of alcohol distillation. The alcoholic distillates start being produced initially by the preparation of medicines, and being traded at first only by chemists. The doctors and chemist used to prepare alcoholic elixirs and beverages using a large number of species of plants, spontaneous or cultivated, European or from import, with medicinal and aromatic properties. A technical progress in distillation emerges only in the 16th century. Paracelsus (1493-1541) studies distillation and consecrates the terminology used. Basilius Valentin (Germany, the 15th century) invents the cooling of refrigerant through water. Gianbattista della Porta (1534 – 1615) builds the first practical alembic in 1535.

Andreas Libau (Libavius, 1550- 1616) creates the zigzag refrigerant. As fruit distillates, in the Orient they obtained the araq (araq at tamr or dates perspiration), calvados (1553, from cider in Normandy), „eaux de vie" (specific to France), kirschwasser (from cherries in Alsace and Schwartzwald) and other wasser, and in the Eastern Europe diverse brandies from plums or other fruits. The researchers related to the problem of fermentations are more and more important in the 17th century due to the increasing processing industry. The Dutch traders and naval transporters opened the way for commerce and consumption of alcoholic distillates on a larger scale. In 1621 there were only in London and Westminster (England) more than 200 distilleries producing „brandies, aqua – vitae and spirits" from inferior wines, cider and cereals. The demand was high, the wine being an import article and beer a too common alternative that did not satisfy all tastes.

RESULTS AND DISCUSSIONS

At the beginning of the 19th century, the processing industry starts to evolve. There appear the first continuous distillation installations (E. Adam, 1801; Cellier Blumenthal – 1808, Derosnes and Cail in 1818, Pistorius in Germany and especially the famous Coffey in Ireland in1830). The lab studies dedicated to fermentations, first of all to the alcoholic and acetic ones, are approached by great scientists. Antoine L. Lavoisier (1743-1794) establishes the quantitative aspects for the alcoholic fermentation. In the practical field the papers of J. A. Chaptal de Chanteloup (distillation, concentration) had important applications. The continuous improvement of the distillation installations pursued the effectiveness of the contact liquid-vapors, the easiness of making and installation as well as the reliability in functioning. The most performing were the French ones (Savalle, Egrot, Barbet etc). The steam boilers, the installations and materials for pressure, filtration or transport evolved too. Since 1850, distillation became in numerous countries a distinct industry. After 1860 the price of distillates registered a real increase due to the generalization of some diseases and pests to the vine (mildew, phylloxera). Bălan, Şt. et al. (1985) and Godea, I. (2005) affirm that the beginning of the production of alcoholic distillates on the territories inhabited by Romanians would situate about the end of the 14th century and the beginning of the 15th century, arguing indirectly this information since there is no written information. The vassal town of Bistrița supplied the ruler Petru Rareş and his successors in reign with „vino sublimato", a wine distillate (1545-1585). On February 22, 1545, Chr. Baumgartner, a secretary of the Council of Bistrița, wrote down in his register „Provino sublimatum, Petri Waywodae misso 56 dinari". The ruler Petru Rareş, a suzerain of Bistrița, in his second reign (February 1541 – September 1546), had probably known the alcoholic distillates during his refuge in Ardeal between 1538-1541. There appears for five times the observation that they sent „vino sublimato". The gifts in alcoholic distillate from Bistrița continued also for the successors of Petru Rareş: „sublimato vino" (14.10.1580), „vino sublimato" (13.10.1581), „sublimato vino" (22.09.1584, a scund) and „vinoque sublimato" (28.10.1585). In the 16th century appeared the first information or evidence on the production of alcoholic distillates in the Romanian countries. In Transylvania this craft was taken over from the west of the continent (specific term plum brandy –Slovakia) or north (sugar beet brandy, distiller – Galicia), and in Țara Românească, from the south of Danube (marc brandy, distillery, strong plum brandy, malt brandy, lees). From the Orient comes the term „brandy", and through the French language we have the words "alembic" and "alcohol" of Arabic origin. At Iaşi they discovered a boiler that processed plums, cherries and grapes marc brandy, dating from the 16th century. At Cluj, they mention for the first time, in the municipality

register, the purchase of brandy in amount of 6 dinars on 30.10.1585, for the lunch of a Moldavian messenger. The vinegar for the same lunch cost also 6 dinars. Town Cluj shipped during the year 1599 a total of 11 barrels and 1910 liters of brandy at Carei, Oradea, Satu Mare and Sălaj. The commerce of Braşov with Ţara Românească and Moldavia, reflected in the trade registers and the mail of that time included brandy as purchases or gifts. The trade of brandy makers is more frequent attested through documents in the 17th century as practiced in towns (Bucharest, Târgovişte, Râmnicul Sărat, Roman), on the monastery territories (Horezu, Dealul, Neamţ, Suceava) or the boyars properties (Oravița and Bolboşani in Mehedinți, Jiblea in Vâlcea).

The information from the Romanian principalities attests the existence of "brandy makers" who deployed their activity in distilleries. There are references related to quality, price, consumption or trade with distillates in all the Romanian counties. Between 1660-1664 Evlia Celebi, a Muslim traveler writes down several interesting aspects. In Iaşi, in 1659, "they also have several brandies … the best of them being called hurelka, ...rye water (čavadar suyu), strong water (hadd suyu), and that is why all inhabitants get drunk until they get dizzy and walk staggeringly without a work to do" (Guboglu, M. and colab., 1963). However Dimitrie Cantemir said that: „Brandy is loved only by soldiers, the others only drink one small glass before lunch" (1714). Nicolae Iorga (1925, 1927) said that: „Vodă Brâncoveanu (1688-1714) shared vodka and orelcă, the sugar beet brandy from the monastery alms of that time". About Iaşi, Evlia Celebi also said: „their brandies of all kinds and white bread are famous". At Timişoara (1660) he mentioned: "among the famous drinks they recommend the sour cherry brandy and hydromel". The Muslin traveler also mentions the brandies from the vine area Cohalm (Odorhei, 1662). At Bucharest in 1664 he says that "under stores there are caves where inhabitants keep different types of wine of the ruby colour as well as other alcoholic drinks such as horelca and mead" (Guboglu, M. and colab., 1963). Information on technologies and methods applied description of some installations. The obtaining of the fruits brandy became in the 18th century a spread activity. In Ţara Românească they registered between 1730-1740 more than 70 "distilleries", only in monasteries, the number of brandy makers being important in town like Bucharest, Craiova or Buzău. In Moldavia (1743) the number of boilers was less but their sizes were bigger. The distilleries from Târgu Neamţ of the Neamţ monastery, from Gura Tazlăului of the high steward's wife Maria, numbered up to 6 buckets. Brandy makers were also in Iaşi. The boilers or buckets were made of brass, with wooden or metal lids, with one or several pipes passed though a cooler to condensate the alcohol vapors. The price of a boiler was several zloty or Austrian silver coins. Peasants produced fruit brandy or from the viticulture sub products, only for their won consumption in village installations (mentioned at Oreavița and Bolboşani – Mehedinți, Jiblea – Vâlcea, Oprişeni – Neamţ etc). Dimitrie Cantemir also mentions the name of "lembic, a bucket with which they pull out brandy or flower water". In the paper of N. Iorga "O gospodărie moldovenească la 1777" they mention the manner how they supplied the boyars' houses of that time. The cave of the house (Iaşi) had "wine barrels, brandy barrels and brine cabbage". N. Iorga (1925) reminds us of the gipsy makers of the brandy boilers (documents from 1711). There is the description of some installations. A distillery (Moldavia) or a still (Ţara Românească) had one or several buckets (big) or boilers (small) with one or several pipes each. Monastery Strehaia had a boiler with 3 pipes. Ilinca's boiler, a tradeswoman from Hurezi (1736), had 2 pipes and weighed 22 ocale (66 lbs). The brandy was kept in small barrels (bătloage in Craiova, the beginning of the 18th century) or poloboace

(1741, Kishinev). The end of the 18th century brings, once wit the change of the force ratio in the Eastern Europe, a period of political, economic and commercial reorientation. In such circumstances we assist to an important increase of the number of distillery installations, distilleries, vodka installations, distilleries and stills, especially in the wine-growing areas: Drăgăşani, Topoloveni, Ciocăneşti, Văleni, Valea Călugărească, Negovani (Țara Românească) and Focşani, Nicoreşti, Coteşti, Huşi, Cotnari, Bucium (Moldavia).

In Moldavia, from 151 distilleries in 1776 (24 at Hârlău, 20 at Iaşi, 16 at Suceava, 14 at Neamț, 7 at Dorohoi, 2 at Vaslui, 2 at Fălciu etc.), in 1785 the number increases to 800, surpassing Țara Românească. They also processed larger quantities of cereals. In Bucharest, Craiova, Piteşti, Târgovişte, they mention lots of distilleries and in Iaşi, Huşi, Odobeşti and Soroca, many stills. In 1783 the clergy and boyars of Moldavia decides to stop the importation of brandy from Poland "for here in our region they make a brandy better than sugar beet brandy because it is made from prunes, plums, marc brandy, the wine lees and the stale wine". In Țara Românească, Alexandru Ipsilante (1774-1782) also stopped this importation and in 1793 Alexandru Moruzi took steps against the inobservance of this princely order. The Romanian chemist Constantin Istrati (1850-1918) is one of the pioneers of chemistry of alcoholic drinks from Romania, who published a series of original papers in the filed. His activity was continued by valuable specialists like V. Cârnu Munteanu (1858-1913), C. Roman (1860-1919) and I. Enescu from the Central Agricultural Station set up in 1887 under the Central School of Agriculture, and at the University of Iasi by dr. C. Şumuleanu. The agricultural monographs made up by Ion Ionescu de la Brad for the counties Dorohoi (1866) Mehedinți (1868) and Putna (1869) are a proof of the potential, diversity and importance of the wine-growing-fruit-growing sector, gardening that was practiced as well as of the products obtained. The author also describes the industrial traditional procedure of transformation of cereals or potatoes in alcohol, as well as the preparation of the alcoholic drinks by diluting alcohol with water up to the consumption concentration: "if water is not pure, the brandy gets turbid and is like milk especially when water contain lime or plaster". About the still in Svoraste: "the income is considerable; that is why the construction of this distillery is monumental, having all the details taken to the utmost perfection." In county Putna (1869): "the quantity of brandy produced in Vrancea goes up to 72000 liters resulted from 600 thousand kilos of plums. This brandy is made in 600 stills"; "the dry nuts and prunes are a significant income especially for the free peasants who dealt with the making of brandy from plums and obtaining oil from nuts"; in commune Mărăşti "orchards occupy 69 hectares with plum trees from which they make like people from Câmpuri, Soveja and Vizanția, more dry prunes than brandy "; "Colacu is the property of 164 free peasants holding 1467 hectares ... 29 plum orchards whose fruit is partially turned into brandy and the other part is dried and smoked by wattle and then are put in barrels. At the Regional Exhibition of Focşani (1868), he mentions the samples of fruit, grapes and brandies exhibited, among which many got medals (fruits from Monastioara, Mărăşeşti, Focşani and Tecuci).

CONCLUSIONS

An interesting evidence is constituted by the presentation of the analytical data for 20 brandy samples belonging to diverse owners from the counties Dâmbovița, Mehedinți, Vâlcea, Olt, Muscel, Prahova, Argeş and Buzău, produced between 1870 and 1897, analyses effectuated by P. Rădulescu, doctor in chemistry and wine-growing inspector:

The maximum concentration was between 31,8 vol% (Valea Călugărească, Prahova, 1880), and the minimum one of only 15,3 vol% (Bujoreni, Vâlcea 1894 and Răsvad, Dâmbovița 189); the extract oscillated between 0,103 and 0,036; total mineral substances between 0,091 and 0,019; acidity expressed in H_2CO_3 between 0,126 and 0,061. For 6 samples they found out very low quantities of free prussic acid (between 0,000103 and 0,00042).

Pomohaci, N. (2002) mentions, according to studies published at the end of the 19th century, that the benefits achieved from the plum tree culture by turning it into brandy were 20 times bigger than for wheat, 15 times bigger than for corn, 10 times bigger than for linseed, 8 times bigger than for potatoes and more than 3 times bigger than for vine. At the beginning of the 20th century, the number of stills was 5709 in county Gorj, Vâlcea - 4890, Mehedinți - 3874, Argeş - 3706, Buzău -3715.

At present, distillation is a traditional manner of transforming fruits (or cereals) that do not have another use. The surplus from the years with rich crops or the areas with bad roads, far from the processing centers, or the non-harvested wild fruits may be turned into fermented marcs for distillation. Distillates concentrate a great value to a reduced volume being able to be manipulated, kept and used depending on the needs.

The economic interest manifests through the steady demand of distilled drinks, the excise paid, the work places occupied, the industries that sell equipment/materials and, last but not least, by limitation of importations. Alcoholism is a social danger but prohibition is not a solution. Like in other European countries or from other continents, there is a popular tradition to produce some assortments of high quality distillates in well known areas and also to consume alcohol at festive moments.

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Research to improve blueberry multiplication technology by hardwood cuttings

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Keywords: highbush blueberry, multiplication

ABSTRACT

Even if Romanian research in the field of blueberry culture are in progress since 1968, nowadays areas cultures with a commercially purpose are extremely reduced. In spite of the fact that in the last years the interest for blueberry culture registered a remarkable progress, large areas of the founded plantations have been used the imported biological material. In fact, during years 1980 at Bilcesti there was produced biologically material in a multiplication sector, with a capacity of 100.000 plants annually.

In such conditions, we have been proposed to improve the multiplication technology, with a view to obtain plants at an equally or superior quality, as against those imported. The main purpose of our experiences was to obtain rooted cuttings with a superior vigor, comparing with the standard technology. So, there were performed experiments in protected areas covered with resins (PAFS) characterized by different thickness and transparence. The protected areas have been build in two variants, as greenhouse type, with a roof in two "water" and with half round metallic skeleton - Finn type. The performed determinations emphasized that the best rooting results there were obtained in the protected areas with half round roof, with PAFS with a smaller thickness than 1 mm and a high transparence (over 90%). In such conditions, cuttings had a superior root system volume and growing during the first year has been in a sum of 28 cm at Coville and 23 cm at Blueray, respectively. In the second year, during rooted cuttings fortification the best results there were obtained at variants from the half circle protected areas, with PAFS under 1 mm thickness; for cuttings transplanted on pots with a diameter of 14 cm, growing being in a sum of 92 cm at Coville and 83 cm at Blueray, respectively. After observations performed during 2006-2007 period, it can be noticed that for planting, fortification of the biologically material during 2 years can decisive contribute to accelerate the field growing rate, with the condition to use in the second year pots or bags with a volume of minimum 2-3 L.

INTRODUCTION

Even if in Romania research regarding blueberry culture have been initiated 40 years ago, the culture have not been extended on significantly areas, in spite of the obvious favorable pedo-climatic conditions assured by many locations, especially in submontain areas.

Research carried out in North of the Arges District, at Bilcesti, at an altitude above 800 m have been emphasized that a blueberry culture success is mainly determined by the quality of the used planting material.

MATERIALS AND METHODS

Observations and determinations have been performed during 2003-2007 period, on Coville and Blueray cvs. cuttings - first year (rooting period) and during the second and the third years (fortification period). For the first year attention has been focused on rooting process in the case of hardwood cuttings. As a rooting substrata it was used a mixture of peat and perlite. In the second year, plants have been transplanted on pots having peat as substrata and there were performed determinations as regard as growing volume.

RESULTS AND DISCUSSION

The obtained results pointed out that assuring a favourable microclimate by rigorously temperature and humidity control at the substrata level, as well as on the greenhouse premises, the rooting process was over 90% at both cultivars. It was noticed that the high rooting percent is assured by maintaining a high humidity level on the greenhouses premises, using the artificially fog installations. The rigorously humidity and temperature control has been assured during all day length, between 8-20 hours, especially during May[15] - September[15], when exists the risk that the temperature into greenhouses can surpass 40-45^{0}C, in conditions when is not assured a corresponding air circulation. In conditions of the hot summers, as for instance that of 2007, in the PAFS high transparency protected areas, to avoid super heating, it can be used shadow net and results are positively, especially in the case of greenhouses longer than 18-20 m.

Determinations performed at the end of the vegetation period, corresponding to the end of October in the Bilcesti protected conditions areas showed that in spite of the high rooting percent and of satisfactory root system development, for the first year growing were reduced. The sum of the growing on a cutting had a mean value of 14 cm at Blueray and 22 cm at Coville, in the case of the greenhouses with PAFS roof of 2 mm, and 23 cm at Blueray, 28 cm at Coville, respectively, in the case of greenhouses with PAFS roof, with a thickness less than 1 mm (Fig. 1).

After transplanting to pots, in the second year, the sum of the growing lengths have been at a higher level, mean values ranging between 46 and 96 cm at Coville, and 41 - 83 cm at Blueray (Fig. 2.).

The highest values for the growing length have been determined for both cultivars transferred on pots with a diameter of 14 cm, in condition of greenhouse (PAFS roof with a thickness less than 1 mm) fortification. The superior growing number was determined by the higher level of the growing lengths (for these variants), but also by their mean length.

For the mentioned variants, the growing numbers was 6,4 buc. At Coville cv. and 5,8 at Blueary cv., in conditions when for the others variants this number was between 4,4 - 5,6 at Coville cv. and 4,2-4,7 at Blueray (Fig. 3).

There was registered the same situation as regard as mean growing length and the values were 14,4; 14,3 respectively at the mentioned variants, as against only 10,5-11,2 at Coville cv., and 9,8-11,5 at Blueray cv. for the others variants (Fig. 4).

Nevertheless, it can be appreciated that the growing level is sufficient taking into consideration that after the field planting, plants vulnerability degree as regard as water deficit is very high, due to the specifically superficially blueberry roots system. In such conditions, we appreciate that to found out blueberry plantation without an irrigation system, results are superior if the planting material is minimum 3 years old. Determination carried out during 2006-2007 period pointed out a significantly root system volume increase and of the growing length for 3 years old plants, fortified in protected areas, as comparing with those transferred in the field conditions. To prolong the fortification period, it is necessary to transfer plants in the third year in pots with minimum 2,5-3 L and minimum 5 L in the fourth year. If the fortification is prolonged more than one year, it must to apply severe pruning for the vigorously growing remaining 3-4 buds and to short the small growing with an entire elimination of the generative buds. Removing the generative buds is an obligatory operation, taking into consideration blueberry precocity, which develops generative buds just during the first year on the rooting substrata, but especially during fortification.

CONCLUSIONS

Hardwood cutting of cultured blueberry by respecting the multiplication technology and rigorously control of the water - temperature regime in the protected areas assures a rooting percent above 90%.

Growing/cutting during the first year is low, of about 25 cm/cutting, according with the mean values registered during 2003-2007.

During fortification process a higher growing level is favoured by the protected areas covered with higher transparency level and by a superior pots volume.

To found plantations without an irrigation system, superior results as regard as acceleration of the growing rate can be obtained when it is planted biological material which has been supposed to prolonged fortification period, with still minimum one year in the protected areas.

When the fortification period is prolonged, it is necessary to use pots with a minimum capacity of 2-3 L in the third year and 5 L in the fourth year.

During the fortification period, every spring, before the vegetation starting must apply pruning for the vigorously growing and shortening for the small growing.

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Figures

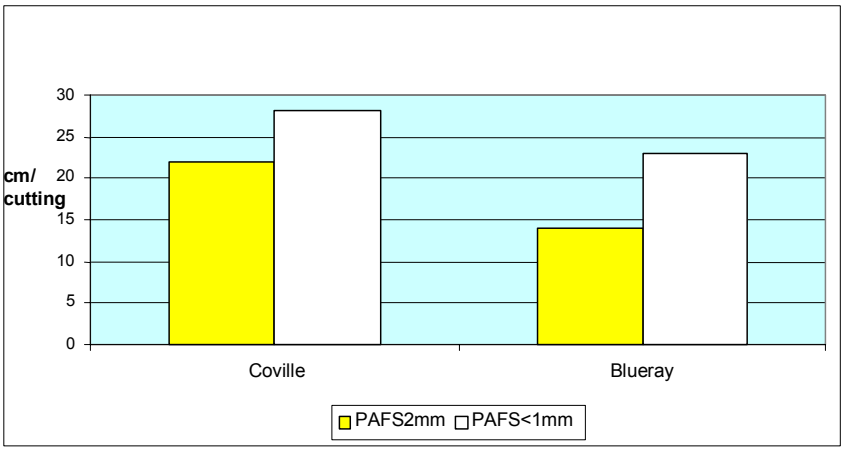

Fig.1. Sum of the growing lengths in the first year, after determination made at the end of the vegetation period (Rooting period)

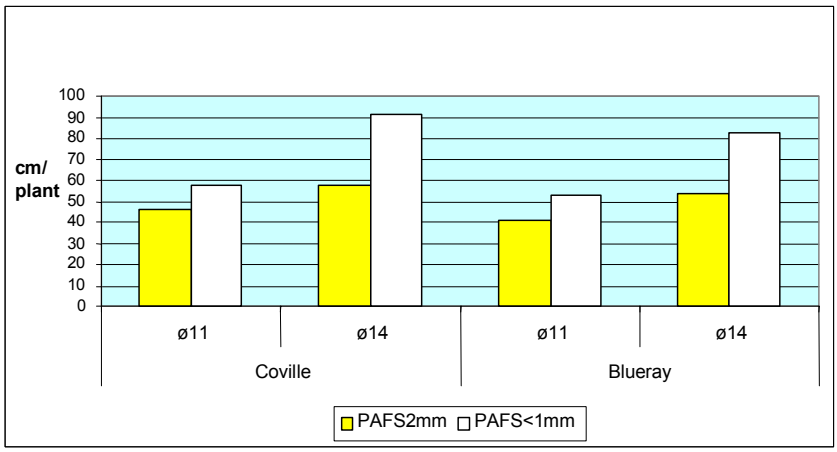

Fig. 2. Sum of the growing lengths for Covile and Blueray cvs. (Rooted cuttings in the second year - fortification)

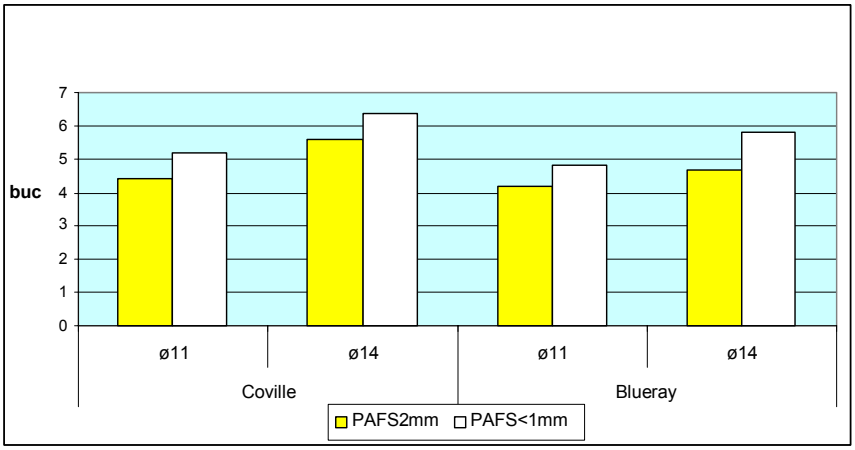

Fig. 3. Mean growing number on a rooted cutting in the second year (Coville and Blueray cvs.)

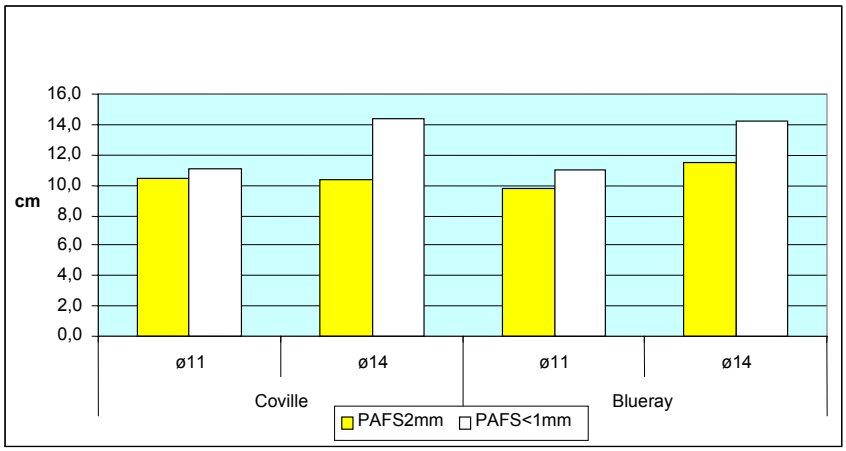

Fig. 4. Mean length of one growth at blueberry rooted cuttings in the second year

Evaluation of disease susceptibility of some native sour cherry genotypes, *ex situ* collected into Romanian National Germplasm

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Keywords: *Prunus cerasus*, evaluation, disease susceptibility, native germplasm

ABSTRACT

Romanian territory is located in the extended area limits of the geographic genetic center for cherries which grows wild or weedy, in a high genetic diversity, all over the country. In the past, cherry has been propagated to a large extent by seeds or suckers, resulting a wide range of variability. Subsequently, by selection and clonally propagation of valuable individuals within seedling population from different growing areas, many local cultivars were framed. Additionally, as a result of breeding programs started more than 50 years ago, 18 new varieties were released. Some of them are preserved in cherry collections which include 174 sour cherry (from which 43 are autochthonous biotypes, breeder's lines, old and new cultivars) accessions held in duplicate in two different locations. To give new opportunities for conservation of cherry biodiversity and sustainable use of genetic resources, 43 native genotypes have been evaluated regarding their disease susceptibility to *Blumeriella jaapii* and *Monilia laxa,* according to the numerical scale of IBPGR descriptors.

INTRODUCTION

Sour cherry, one of the most important fruit tree species, owing to his economical value given by the nutritive fruit quality and commercial opportunities, meets in Romania optimum natural environmental conditions to express his agro-biological potential. Moreover, as a matter of fact it is believed that some of the cherries are originated from the Black Sea surroundings (Kaska et al., 1998), including a significant part of the Romanian territory.

The wild or naturalized cherry types are abundant in most part of the country. So, wild or cultivated cherry is a traditional crop in Romania made extensively for commercial purpose or in home gardens for domestic consumption.

In the past, the richness in genetic diversity, gives the possibility to generations of fruit growers to select valuable individuals from seedling populations, on the basis of fruit quality. Propagation was made vegetative (by suckers) and clonally (by grafting) and most of these so called „local varieties" have names derived from their local origin (Crisana, Marculesti, de Botosani, de Targu Jiu, De Pitesti, de Cluj, Ilva, Topologu, Satmarean, Vrancean, etc.).

Collecting the new varieties was an activity made as a hobby by rich landowners, enthusiast fruit growers, monks in the abbey orchards, or disperse in different nurseries and research centers.

MATERIAL AND METHODS

Romanian cherry genetic resources have started to be methodically collected since 1967. At present, there is a total of 174 sour cherry accessions held in duplicate at the Research Institute for Fruit Growing, Pitesti – Maracineni and Fruit Research Station, Iasi.

Five sour cherry trees per genotype grafted onto Mahaleb seedlings, are planted in each location.

Collections contain foreign or indigenous cultivars, selections, clones, local varieties and landraces. All accessions are evaluated for morphological and biological characteristics according to the numerical scale of IBPGR descriptors. The main tasks are to estimate commercial value and/or to detect the possible useful sources of valuable genes for breeding program.

At this time, the target is to systematize collected data from the two institutions, like susceptibility to leaf spot (*Blumeriella jaapii*), blossom wilt (*Monilia laxa*) and brown rot (*Monilia fructigena*), check and re-examine in order to update or adjust.

In this circumstances, to give new opportunities for conservation of cherry biodiversity and sustainable use of local genetic resources, greater attention has been paid to characterize and preliminary evaluate 43 *ex situ* collected wild genotypes and indigenous varieties (Budan, 2005).

Descriptors used to evaluate *ex situ* collected native cherry genotypes

Susceptibility to *Monilia laxa* (scale steps and reference cultivars): 1. None; 2. Very low - Erdi Jubileum; 3. Low - Erdi Botermo, Ujfehertoi furtos; 5. Intermediate - Schattenmorelle; 7. High - Montmorency; 9. Extremely high.

Susceptibility to *Blumeriella jaapii* (scale steps and reference cultivars): 1. None; 2. Very low - Csengodi; 3. Low - Meteor Korai, Montmorency; 5. Intermediate; 7. High - Schattenmorelle, Erdi Botermo, Pandy, Crisana; 9. Extremely high.

RESULTS AND DISCUSSIONS

As a first benefit of characterization and evaluation of present time available germplasm fond, between 1950 – 2005, was facilitation of the knowledgeable use of different genitors in more than 150 cross combinations from which over 5,000 seedlings were obtained and 18 new varieties as Amada, Bucovina, Crisana 2, De Botosani, Dropia, Ilva, Mocanesti 16, Nana, Pitic, Rival, Satmarean, Scuturator, Timpurii de Cluj, Timpurii de Pitesti, Timpurii de Osoi, Timpurii de Targu Jiu, Tarina, Vrancean were released. (Budan et al., 2006 a).

Of course, in the breeding work in the overwhelming scale, foreign varieties but also, often, Crisana an indigenous old cultivar and some advanced new breed Romanian cultivars as Tarina, Bucovina, Nana, Dropia have been used.

Evaluation made over several years shows the great diversity of susceptibility to *Monilia laxa* (Aderh et. Ruhl) and *Blumeriella jaapii* (Rehm) Arx..(Table 1)

Low susceptibility to *Monilia laxa* (Aderh et. Ruhl) was showed by De Botoşani, HV 47/11, HV 45/90, HV 43/32, Mari timpurii cl. 1, Mari timpurii cl.2 and P1 Vie genotypes. Observations made on Băneasa 44/7, Bizigheşti, HV 45/40, HV 47/11, HV 43/32, Mari timpurii cl. 1, Mari timpurii cl. 2, P1 Vie, Suraia and Topoloveni 6 accessions lead to the same level of evaluation concerning the susceptibility to *Blumeriella jaapi* (Rehm) Arx..

The obtained data offer new possibilities to select valuable genotypes useful by their characteristics for breeding program and also to register other interesting local landraces quite important to domestic market.

Unfortunately, the lack of coordination and limited financial support means that only limited results have been achieved in recent years.

At present, for wild or weedy sour cherry trees there is no funding project for identification, evaluation, collection and *ex situ* or *in situ* conservation of these native genetic resources (Budan et al., 2006 b).

More over, as a result of privatizing government lands the massive clearing of cherry trees for timber is taking place endangering and dramatically decreasing the cultivated and natural biodiversity.

So, to avoid further losses, development of a national strategy and governmental and/or international financial support for cooperation projects regarding the enhancement of germplasm by exploration and selection of natural and semi-natural ecotypes and agro-types followed by *ex situ* preservation of the most valuable native genotypes is required.

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Table 1. Evaluation of the disease susceptibility of some native sour cherry genotypes from the Romanian national germplasm

No	Accession name	Origin	Location	Susceptibility to *Monilinia laxa*	Susceptibility to *Blumeriella japii*
1	Breznița	ROM	P, I	5	7
2	Băneasa 44/7	ROM	P, I	7	3
3	Bizigheşti	ROM	P, I	5	3
4	Crişana 2	ROM	P	5	5
5	Crişana 15/10	ROM	P	5	5
6	Crişana Cluj	ROM	P	5	5
7	Drobeta	ROM	P, I	5	5
8	Dropia	ROM	P	5	7
9	De Botoşani	ROM	I	3	5
10	Engleze timpurii cl 1	ROM	P, I	5	5
11	Engleze timpurii cl 2	ROM	P, I	5	5
12	Focşani 3	ROM	P, I	5	5
13	Ilva	ROM	P, I	7	5
14	HV 13/21	ROM	P	5	5
15	HV 47/11	ROM	P	3	3
16	HV 45/40	ROM	P	3	2
17	HV 43/32	ROM	P	3	2
18	Japonica	ROM	P	5	5
19	Locale de Bistrița	ROM	P, I	5	5
20	Mari timpurii cl 1	ROM	P	3	3
21	Mari timpurii cl 2	ROM	P	3	3
22	Mărculeşti 33/13	ROM	P, I	7	5
23	Mărculeşti 33/21	ROM	P, I	7	5
24	Mărculeşti 15/2	ROM	P	5	5
25	Mocăneşti 16	ROM	P, I	5	5
26	Mocăneşti 15/2	ROM	P	5	5
27	Nana	ROM	P, I	7	7
28	P1 Vie	ROM	P	3	3
29	P4 Vie	ROM	P	4	5
30	Pitic	ROM	P, I	5	7
31	Rival	ROM	P	8	5
32	Suraia	ROM	P, I	5	3
33	Scuturător	ROM	P, I	5	5
34	Timpurii de Piteşti	ROM	P, I	5	5
35	Timpurii de Mărculeşti	ROM	P, I	5	5
36	Turceşti	ROM	P, I	5	5
37	Topoloveni 6	ROM	P	5	3
38	Topologu Tulcea	ROM	P, I	5	5
39	Tg Jiu 200	ROM	P, I	5	5
40	Tg Jiu 404	ROM	P, I	5	5
41	Timpurii de Osoi	ROM	I	5	5
42	Țarina	ROM	P, I	5	5
43	Vrâncean	ROM	P, I	5	7

P = Research Institute for Fruit Growing Pitesti Maracineni
I = Fruit Research Station Iasi

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A study of qualitative properties of certain cherry cultivars

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Keywords: multiple correspondence methods, variance-covariance matrix.

ABSTRACT

A study of a set of qualitative properties of some varieties of cherries is made by means of the so-called method of multiple correspondences (founded and settled by J.-P. Benzécri and collaborators). More exactly, a study of the intercorrelations between some qualitative properties of certain cherry cultivars is performed. The main result consists in determining the coordinates of these cultivars as well as of the qualitative modalities of their analyzed qualitative properties along the principal directions of the set of characteristics used in analysis. These coordinates allow us to build a graph representing simultaneously both the cherry cultivars and the modalities of their qualitative properties, what suggests us to make some remarks about the possible similarities between cherry cultivars and about the compatibilities between the qualitative modalities of their qualitative properties.

INTRODUCTION

Unlike the study of quantitative properties of individuals in a population, where numerical functions are naturally used, where numerical functions are naturally used, the study of their qualitative properties is founded on logical functions and variables. That is why the studies of qualitative properties have to overcome great difficulties, starting with the quantification and registration of the data regarding such properties. An efficient method to study a set of qualitative properties of a population was worked out 40 years ago by J.-P. Benzécri and his collaborators (Benzécri, J.-P. et al., 1979).

In this paper, a study of the interdependence of several qualitative properties of some cherry cultivars, using the multiple correspondences method, is carried on. To this end it was firstly made a quantification of the qualitative modalities (fr. modalities or categories) of the qualitative properties of several cherry cultivars by using the membership logic. In order to save space, the registrations of data are made by means of the *reduced encoding table* (Table 3), only. Specifically, the corresponding disjunctive table can be built in a standard way so that we avoid its presentation. The used method allows an enlargement of the analysis frame for both the genotypes and phenotypes of cherry cultivars what means that, besides the specific genotypic and phenotypic properties, usually called *active variables*, other *derivate properties* such as *utility, production costs, possible benefits,* etc. can be introduced in study.

As active variables the *drought resistance*, *frost resistance*, *autofertility* & *interpollination*, *fruit-bearing type*, *strength*, and *resistance* of *diseases* are used; each active variable has either two or three qualitative modalities. A first appreciation of the similitude between the pairs of active variables is made by using Burt's table (Table 5) associated with available registrations, which actually is a synthetic definition of a similarity function (Burdujan, I., 2007). The *row* and *column profile tables* then allow determining the eigenvalues to be applied for both some interpretations of the collected data and the application of the multiple correspondences method. We avoid the presentation of the profile tables for saving space. It must be remarked that these results can be also used to find a partition of the analyzed population of cherry cultivars which could be used to give a hierarchic classification (whose utility is out of any discussion) of it.

MATERIALS AND METHODS

The whole information regarding the genotypes and phenotypes of analyzed cherry cultivars was taken over from the monograph by Budan et al. (2000) and the paper by Petre (2003). We are going to analyze only six characteristics for 27 cherry cultivars. These limitations are due, on one hand, by the huge volume of work to do for collecting, registering and processing the collected data and, on the other hand, by the limited capacity of the computer used in processing these data. To these difficulties we can add our lack of an efficient soft for statistic data processing as well as for computations with large sparse matrices.

As it was already noticed, the method used in our study is a well-known one in the descriptive statistics (Benzécri, 1979; Burdujan, 2008), namely the multiple correspondences method (shortly, MCM). In this frame it was preferred to use the matrix descriptions of MCM (Burdujan, 2007), because it allows for an appropriate use of the MAPLE 5 soft (available to us by the kindness of our colleagues from UAIC Iaşi).

RESULTS

List of the analyzed cherry cultivars, including their appropriate encodings, is given in Table 1. Table 2 contains the list of the analyzed active variables together with the corresponding encodings. By using the information from the book by Budan (2000) and the paper by Petre (2003), one gets the *reduced encoding table* for the properties of the analyzed cultivars (Table 3). Then, the 27×16-matrix X having as its entries the infor-mation regarding the active variables contained in the complete disjunctive table, cor-responding to the encoding table, is considered. The inertia of the set of the analyzed characteristics is 1,6667. The similitude between the pairs of active variables are resumed and analyzed by means of Burt' s table (Table 4) associated with all registered data, that can be identified with the 16×16-matrix $B = X^T \cdot X$.

Then, the profile tables (which allow us to get the contingence tables) are built, namely, the *row profiles* table P_L and the *column profiles* table P_C. Actually, table P_L can be identified with matrix $\frac{1}{6}X$, while table P_C can be identified with the 27x16-matrix XE^{-1}, where E is the diagonal matrix diag ($n_{.1}$, $n_{.2}$, ... , $n_{.16}$) (here $n_{.j}$ denotes the absolute frequency of jth modality). For our study one gets E = diag (7, 5, 15, 8, 14, 5,10, 8, 9, 8, 13, 6, 13, 14, 14, 13). Then, the covariance matrix V and the correlation matrix as well as the matrix $M = diag(\sigma_1^{-2}, \sigma_2^{-2}, ..., \sigma_n^{-2})$ of the normed metric have been computed. Among the 16 real eigenvalues of V, 5 are equal to zero, one is =1 and the other 10 are given in Table 6. In the column of *cumulated percents* we can see that the first two eigenvalues (in the decreasing order) contribute with 51.98% to the total inertia of population, while the first 5 eigenvalues (in decreasing order) contribute with 83.10% to the total inertia of population. The corresponding eigenvectors give the *principal directions* for the population.

According to these data one determine the coordinates of the qualitative moda-lities (Table 6) as well as for the cherry cultivars (Table 7). They are just the projections of each modality, respectively of each cultivar**,** on the first five principal directions identified by means of the eigenvectors.

DISCUSSIONS

The positioning of points in the graph (Fig. 1) suggests the existence of some similitude between the elements of some groups of cultivars or characteristics. The similitude of characteristics or of cultivars is stronger as they lie close to the 1-axis (which polarizes 29% from the total inertia – which is maxim among the five axes). Of course, it is also necessary to consider their positions relatively to the 2-axis. For example, the cultivars *Stella, Early Reavers, Hedelfinger* have similar properties but no too strongly, because they lie in a proximity of the 2-axis (which polarizes 23.08% from total inertia). On another hand, *medium type fruit-bearing, weak resistance to diseases and weak vitality* give a group of characteristics in a strong correlation. Certainly, the similitude's remarked on this graph must be confirmed by projections on every pair (i, j), with i, j = 1, 2, … , 5, of principal directions.

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Tables

Table 1. List of analyzed cultivars

Nr. crt.	Cultivar	Cod	Nr. crt.	Cultivar	Cod	Nr. Crt	Cultivar	Cod
1.	*Prunus fructicoza*	PRF	10.	*Golia*	GOL	19.	*Cerna*	CER
2.	*Biggareau Moreau*	BGM	11.	*Early Rivers*	ERV	20.	*Biggareau Drogan*	BGD
3.	*Maria*	MAR	12.	*Marina*	MRN	21.	*Biggareau Donissen*	BGDN
4.	*Hedelfinger*	HDF	13.	*Sam*	SAM	22.	*Kordia*	KOR
5.	*Cilegia di ottobre*	CGO	14.	*Stella*	STL	23.	*Van*	VAN
6.	*Cătălina*	CAT	15.	*Lambert*	LMB	24.	*Ulster*	ULS
7.	*Lapins*	LAP	16.	*Gold*	GLD	25.	*Boambe de Cotnari*	BCT
8.	*Biggareau Burlat*	BGB	17.	*Fromm*	FRM	26.	*New Star*	NSR
9.	*Summit*	SUM	18.	*Cetățuia*	CET	27.	*Hudson*	HUD

Table 2. List of active variables

Nr. crt.	Characteristic	Cod charact.	Modality*	Modality Cod		
			- + ++	-	+	++
1.	frost resistance	FR	- + ++	FR1	FR2	FR3
2.	drought resistance	DR	- + ++	DR1	DR2	DR3
3.	strength	S	- + ++	S1	S2	S3
4.	bearing type	BT	- + ++	BT1	BT2	BT3
5.	autofertility&interpollination	AI	- +	AI1	AI2	
6.	resistance of diseases	RD	- +	RD1	RD2	

*for 1-4, „-„ means *small*, „+" means *medium*, „++" means *large*, and 6, „-„ means *small*, „+" means *large*; for 5, AI1 means *autofertility* and AI2 means *interpollination*.

Table 3: Reduced encoding table

No. crt.	Modality→ / Cultivar↓	FR	DR	S	BT	AI	RD
1.	PRF	3	2	3	3	2	2
2.	BGM	1	1	1	1	2	1
3.	MAR	3	2	3	3	2	2
4.	HDF	2	2	2	2	2	2
5.	CGO	1	1	1	2	1	2
6.	CAT	3	3	1	3	2	1
7.	LAP	1	1	2	3	1	2
8.	BGB	1	1	1	1	1	2
9.	SUM	2	1	1	2	2	2
10.	GOL	3	2	3	2	1	2
11.	ERV	2	1	1	1	1	2
12.	MRN	3	2	3	3	2	1
13.	SAM	3	3	3	1	2	1
14.	STL	2	2	2	3	1	2
15.	LMB	3	2	2	2	1	1
16.	GLD	3	2	3	1	2	1
17.	FRM	1	1	2	2	2	2
18.	CET	3	2	2	1	2	1
19.	CER	2	2	2	2	1	2
20.	BGD	3	2	3	1	1	1
21.	BGDN	3	3	1	1	2	1
22.	KOR	1	1	1	1	1	2
23.	VAN	3	2	1	1	2	1
24.	ULS	3	3	1	2	2	1
25.	BCT	3	2	3	2	1	1
26.	NSR	1	1	1	2	1	2
27.	HUD	3	3	1	2	2	2

Table 4. Table Burt (Burt's Table)

	FR1	FR2	FR3	DR1	DR2	DR3	S1	S2	S3	BT1	BT2	BT3	AI1	AI2	RD1	RD2
FR1	7	0	0	7	0	0	5	2	0	3	3	1	1	6	5	2
FR2	0	5	0	1	4	0	1	4	0	0	5	0	0	5	3	2
FR3	0	0	15	0	10	5	4	2	9	5	5	5	12	3	6	9
DR1	7	1	0	8	0	0	6	2	0	3	4	1	1	7	5	3
DR2	0	4	10	0	14	0	0	6	8	3	7	4	7	7	8	6
DR3	0	0	5	0	0	5	4	0	1	2	2	1	5	0	1	4
S1	5	1	4	6	0	4	10	0	0	4	5	1	5	5	5	5
S2	2	4	2	2	6	0	0	8	0	1	6	1	2	6	5	3
S3	0	0	9	0	8	1	0	0	9	3	2	4	6	3	4	5
BT1	3	0	5	3	3	2	4	1	3	8	0	0	6	2	3	5
BT2	3	5	5	4	7	2	5	6	2	0	13	0	4	9	9	4
BT3	1	0	5	1	4	1	1	1	4	0	0	6	3	3	2	4
AI1	1	0	12	1	7	5	5	2	6	2	0	13	4	9	9	4
AI2	6	5	3	7	7	0	5	6	3	2	9	3	0	14	9	5
RD1	5	3	6	5	8	1	5	5	4	3	9	2	5	9	14	0
RD2	2	2	9	3	6	4	5	3	5	5	4	4	8	5	0	13

Table 5. The 10 eigenvalues of covariance matrix V and their contributions to the inertia

Nr. crt.	Eigenvalues	Percents%	Cumulated percents (%)
1.	0.4816	28.90	28.90
2.	0.3847	23.08	51.98
3.	0.2110	12.66	64.64
4.	0.1576	9.45	74.09
5.	0.1501	9.01	83.10
6.	0.1233	7.40	90.50
7.	0.0815	4.89	95.38
8.	0.0457	2.74	98.12
9.	0.0235	1.41	99.54
10.	0.0077	0.46	100.00
	Inertia=1.6667	Total = 100.00	

Table 6. Coordinates of categories by the axis 1-5

Nr. crt.	Modality	Coordinates				
		1	2	3	4	5
1.	FR1	1.178.	-0.923.	0.622	-0.212	0.022
2.	FR2	0.852.	0.232.	-1.024	-0.345	0.314
3.	FR3	-0.841.	0.021.	0.045	0.177	-0.113
4.	DR1	1.168.	-0.824.	0.363	-0.164	0.045
5.	DR2	-0.308.	0.819.	0.228	0.129	0.189
6.	DR3	-1.021.	-0.967.	-1.221	-0.067	-0.607
7.	S1	0.322.	-1.041.	-0.402	0.084	-0.306
8.	S2	0.603.	0.891.	-0.356	-0.379	0.374
9.	S3	-0.889.	0.374.	0.763	0.243	0.012
10.	BT1	-0.354.	-0.809.	0.345	-0.022	1.041
11.	BT2	0.368.	0.292.	-0.486	0.603	-0.150
12.	BT3	-0.340.	0.462.	0.602	-1.289	-1.056
13.	AI1	-0.844.	-0.289.	0.071	0.084	0.041
14.	AI2	0.778.	0.267.	0.058	-0.082	-0.037
15.	RD1	0.404.	0.193.	0.307	0.515	-0.351
16.	RD2	-0.429.	-0.210	-0.391	-0.545	0.367

Table 7. Coordinates of cultivars by the axis 1 - 5

Nr. crt.	Cultivar	Coordinates				
		1	2	3	4	5
1.	PRF	-0.322	0.421	0.103	0.211	0.124
2.	BGM	0.254	-1.100	0.192	-0.289	0.521
3.	MAR	-0.489	0.460	0.504	-0.583	-0.278
4.	HDF	0.451	0.878	-0.689	-0.256	0.456
5.	CGO	1.013	-0.552	0.162	0.354	-0.331
6.	CAT	-0.745	-0.545	-0.501	-0.662	-0.721
7.	LAP	0.911	0.019	0.578	-0.632	-0.434
8.	BGB	0.844	-0.842	0.474	0.094	0.178
9.	SUM	0.728	-0.081	-0.662	-0.189	1.101
10.	GOL	-0.117	0.533	0.333	0.656	-0.189
11.	ERV	0.645	0.989	-0.456	0.194	0.141
12.	MRN	-0.867	0.321	0.451	-0.512	-0.243
13.	SAM	-1.053	-0.509	-0.172	-0.061	0.324
14.	STL	0.484	1.038	-0.064	-0.604	-0.245
15.	LMB	-0.142	0.516	-0.121	0.467	0.003
16.	GLD	-0.883	-0.032	0.356	0.017	0.664
17.	FRM	0.878	-0.143	-0.054	-0.289	0.272
18.	CET	-0.516	0.112	-0.043	-0.241	0.819
19.	CER	0.645	0.994	-0.456	0.192	0.141
20.	BGD	-0.678	0.078	0.601	0.456	0.345
21.	GDN	-0.761	-0.889	-0.589	-0.133	0.183
22.	KOR	0.843	-0.844	0.474	0.092	0.181
23.	VAN	-0.667	0.423	0.689	-0.061	-0.545
24.	ULS	-0.578	-0.589	-0.891	0.134	-0.334
25.	BCT	-0.504	0.378	0.294	0.732	-0.162
26.	NSR	1.009	-0.554	0.156	0.352	-0.33]
27.	HUD	-0.378	0.492	-0.663	0.578	-0.644

Figure

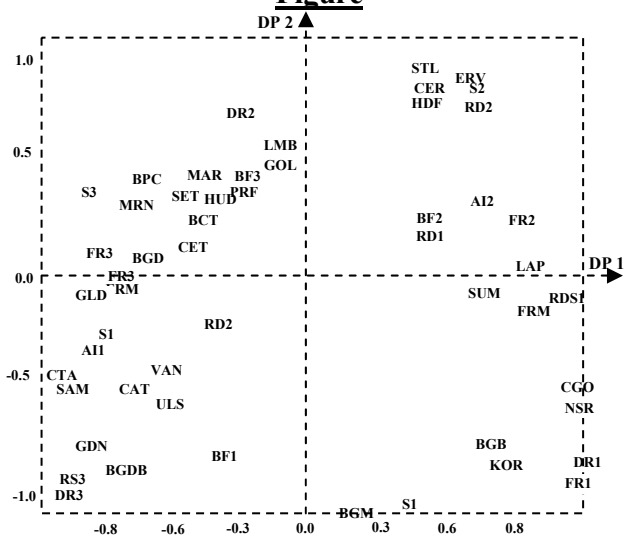

Fig. 1. Common graph (categories – cultivars) for first two principal directions (DP1-DP2)

The Structure and the Biotechnological Value of the Compact Columnar Apple Tree

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Keywords: hybrids, spur fructification, productivity potential, disease resistance

ABSTRACT

This paper treats the columnar apple type which is consider to have early productions and with maximum parameters. Nevertheless, the plant material proved to be much expensive and the great expected yields had realized every second (two) years. In these conditions, the members of the Pomiculture Desk from USAMV Bucharest have directed the researches for obtaining the compact-columnar apple type with a simplified technology and productivity efficiency at highest parameters.

INTRODUCTION

In the last decades, the spur and columnar apple varieties created have open wide perspectives for the intensification of the culture. It was always wished the yield maximization in the first years from planting, aiming the fastest recover of the orchard investment. This kind of apple develop a compact crown, with 4-7 upright branches, parallel to the ax of the tree which start close by the soil and are garnished with spur and short fruiting branches. Thus, it is assured a good illumination of the tree foliage, the raising of the photosynthetic output and the fruits color intensity. The trees perform freely, without pruning for 5-6 years. After that, there are made pruning cuts to limit the apple grow in highness. With low number of disease and insect treatments (5-6) and with easiness to harvest from the soil level, the compact columnar apple becomes a priority in the modern and economical pomiculture field.

MATERIALS AND METHODS

The biological material used in this research was represented by hybrids obtained from natural pollination between Wijcik and some Romanian apple varieties (Pionier, Voinea and Generos) and foreign varieties (Liberty, Novo Easigro, Sir Prize, Prima, Florina and Macfree). These selected hybrids 25 years long in the Baneasa didactic farm remark through the compact columnar habit and spur fructification type.

The studies and researches were made using a orchard planted in 1999 at SC Frasinu SA Buzau farm, which are composed by the next apple hybrids: H21-5/2, H35-5/7, H38-5/9, H61-6/1; H61-6/5; H73-6/4; H29/91; H46/93; H51/93 grafted on MM106 rootstock and planted at the 4 x 1,5 m distances (1666 trees/ha). The soil was maintained tilled, organic fertilized (30 t/ha manure – once every 3 years) and irrigated 4-5 times (depending on the climatic conditions). The phytosanitary treatments (4-5 treatments/year) were used only to prevent and control the insects attack, because all hybrids are very resistant to scab and tolerant to mildew. In the research period (2001-2006), it wasn't registered hard frosts and coming back spring frosts, which could affect the fruiting organs. In the vegetation time (March-September) it was realized an average temperature of 15,75 ^{0}C, a relative humidity of the air of 65,34% and 326,8 mm precipitations.

RESULTS AND DISCUSSIONS

The compact columnar habit research for the studied 9 hybrids, emphasize some specific particularities as a result of the naturally pollinations combinations. From these 9 apple hybrids, it was distinguished 5 with a strong compact crown, 3 with a medium-compact crown and only 1 with a poor compact habit (Table 1). The strong compact hybrids have a permanent structure composed from an axe, 4-7 first-order branches and 18-28 second-order branches. The medium-compact trees present the axe garnished with 7-8 first-order branches and 20-23 second-order branches. The poor compact apples permanent structure is constructed from an axe, 5 first-order branches inserted under large angles and 20 second-order branches.

The compact-columnar feature is defined by the thickness of the parametric values from base, middle and top of the crown, the number and the first-order and second-order ramifications position. From the recorded dates, it results that the 9 years old hybrids have a crown with the basal diameter of 0,64 m, at the middle 1,01 m and at the top 0,66 m.

The fructification structure of these hybrids is given by the short branches (spur branches) which dominate (H61-6/1 - 94% to H46/93 – 97,5%) disposed directly from axe (in the first years) and from first and second-branches (in the full fructification time). The standard branches (long bearing branches) are in percent of 2,6-5,9% and are formed from the indicatively shoots of the first and second-branches. The high values of the ratio between short and long fructification branches emphasize an over optimal fruiting potential of the H46/93 and H51/93 hybrids.

The rhythm and fructification intensity analyze of the compact-columnar apple hybrids during the 3-8 year period from planting, show a positive correlation between age and the productivity of each hybrid. A constant and high rhythm was observed at the H46/93 and H51/93 hybrids (Table 3). Same constant rhythm but at a lower level was noticed at H21-5/2 and H35-5/7 hybrids. These prove indicate that in during the 6 years of fructification (2001-2006), all the studied hybrids registered an increasing constant annual fructification. The average fruit production in this interval was between 17,25 kg and 24,85 kg/tree, respectively 28,75 tones and 41,4 t/ha. This capacity proved to be more superior to the columnar apple (which after supra optimal yields has the tendency to slip to fructification alternation) and to standard (classic) apple type (which request many interventions to maintain a good fruiting balance and normal harvests every year).

The productive efficiency of the compact-columnar apple trees (Table 4) expressed by the harvest linked to 1 liner meter of the crown (upright) and cm^2 trunk section, point out the fact that the hybrids H46/93, H35-5/7, H38-5/9, H61-6/1, H29/91 and H46/93 with a higher degree of crown compactness realized a more efficient production (13-14,5 kg/ml) than the hybrids H21-5/2; H61-6/5; H73-6/4 and H51/93 (10,1-11,5 kg/ml).

The different productivity, measured by ratio between the harvest and cm^2 trunk section show that these apple hybrids are self rate fruiting trees, the quota is maintained at the optimal parameters calculated by N. Cepoiu (1974). From the 9 hybrids, 6 had a productivity of 0,5-0,54 and 3 of them 0,60-0,71. The 3 hybrids demonstrate a bigger fruiting potential than the first 6 hybrids.

CONCLUSIONS

1. The compact-columnar apple conceived at the Faculty of Horticulture represents the perfect type for the high density ecological orchards;
2. H46/93, H51/93 and H29/91 associated with MM106 rootstock are recommended for economical and yearly production;
3. The decreased habit of these hybrids with the weight center near the soil eliminates any trellis.

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Tables

Table 1. The compactness degree of the columnar apple crown, grafted on MM106 rootstock at 8 years old

Hybrid	High (m)	Diameter (m)			Permanent structure of the crown (skeleton branches)			Compactness degree		
		base	middle	top	Total	First order	Second order	strong	medium	poor
H21-5/2	2,45	0,65	1,10	0,80	26	5	21	X	-	-
H35-5/7	2,36	0,60	1,05	0,80	27	7	20	-	X	-
H38-5/9	2,78	0,60	0,75	0,40	25	5	20	-	-	X
H61-6/1	3,10	0,70	0,80	0,35	24	6	18	X	-	-
H61-6/5	3,42	0,50	0,75	0,75	29	7	22	X	-	-
H73-6/4	3,26	0,80	1,15	0,85	30	7	23	-	X	-
H29/91	2,95	0,60	1,10	0,60	30	8	22	-	X	-
H46/93	2,85	0,50	1,10	0,75	32	4	28	X	-	-
H51/93	3,35	0,80	1,25	0,61	34	6	28	X	-	-
Average	2,95	0,64	1,01	0,66	28,5	6,1	22,4	-	-	-

Table 2. The predominant fructification type at the compact-columnar apples crown, grafted on MM106 rootstock at 8 years old

Hybrid	Fructification branches					
	Total	Spur type		Standard type		Ratio spur:standard
		No	%	No	%	
H21-5/2	568	543	96,6	25	4,4	23
H35-5/7	560	545	97,3	15	2,6	36
H38-5/9	511	481	94,1	30	5,8	16
H61-6/1	507	477	94,0	30	5,9	16
H61-6/5	463	438	94,6	25	5,4	17
H73-6/4	492	464	94,3	28	5,6	16
H29/91	657	626	85,2	31	4,7	20
H46/93	776	757	97,5	19	2,4	40
H51/93	611	591	96,7	20	3,3	30
Average	571,6	546,8	95,5	27,7	4,5	20
Variation limits	463-776	438-757	94,0-97,5	15-31	2,6-5,9	16-40

Table 3. The rhythm fructification of the compact-columnar apple hybrids during the 3-8 years from planting (2001-2006)

Hybrid	Year						
	III (2001)	IV (2002)	V (2003)	VI (2004)	VII (2005)	VIII (2006)	Average 2001-2006
H21-5/2	6,10	8,70	13,10	23,40	26,15	28,30	17,25
H35-5/7	7,10	9,46	16,30	18,21	25,10	31,40	19,92
H38-5/9	3,10	10,60	15,81	21,40	29,41	36,40	19,45
H61-6/1	4,75	8,30	15,20	21,30	30,10	41,20	20,15
H61-6/5	6,25	9,15	13,20	19,36	28,30	36,40	20,30
H73-6/4	8,10	11,60	18,10	25,15	30,50	33,10	21,09
H29/91	4,50	13,20	17,35	29,14	17,25	39,40	20,14
H46/93	10,41	15,70	21,40	32,10	28,17	41,30	24,85
H51/93	9,26	18,30	14,21	29,40	26,20	38,20	22,60
Average	6,62	11,67	16,07	24,38	26,80	36,19	20,42

Table 4. The yield and the production efficiency of the compact-columnar apples hybrids 8 years old

Hybrid	High (m	Trunck section area (cm^2)	Ratio high: section area	Fruit production		Productive efficiency	
				Kg/tree	t/ha	Kg fruits/high meter	Kg fruits/cm^2 trunk section
H21-5/2	2,45	56,3	4,35	28,3	47,1	0,50	11,5
H35-5/7	2,36	61,4	3,84	31,4	52,3	0,51	13,3
H38-5/9	2,78	71,3	3,89	36,4	60,6	0,51	13,0
H61-6/1	3,10	78,2	3,96	41,2	68,6	0,52	13,3
H61-6/5	3,42	70,1	4,87	36,4	60,6	0,52	10,6
H73-6/4	3,26	60,9	5,35	33,1	55,1	0,54	10,1
H29/91	2,95	65,5	4,50	39,4	65,6	0,60	13,1
H46/93	2,85	67,2	4,24	41,3	68,8	0,61	14,5
H51/93	3,35	53,5	6,26	38,2	63,6	0,71	11,4
Average	2,95	64,9	4,58	36,2	60,3	0,55	12,3

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Ecologically products consumer – demand analysis and stores potential

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Keywords: ecologically, consumers, distributions, questions, products, price

ABSTRACT

This study's objectives are to underline consumers preferences and their perception regarding the ecologically products. The image of a naturally and healthy product which doesn't contain chemically residue are between the most important features for the consumer. In fact, exist many consumers who request products with a quality and healthy guaranty, but they consider that these characteristics are intrinsic product features and are not disposed to pay a higher value.

INTRODUCTION

Ecologically products are in Romania at a crossing moment: become an important segment from the economically view point, efficient for the vegetables – fruits trade or remain with a marginally role, where they are appreciated by a diminished numbers of consumers, but fond of and qualified (Chira, 2005). Distribution criteria and sites, quality and moreover the price are factors which action to modify the actually tendencies.

In the last ten years, in Europe and SUA we assist to an increasing society interest as regard as the environment problematic. Due to this phenomena we assist to an increasing interest for an alternative agriculture, such as the ecologically one, which has a specially importance in the primary sector (Pasini et al., 1998).

European Union, sensible to the environment management and preserving its own established agriculturally politic, having as a major role that of using of naturally resources, as a part of its main objectives. In this direction subscribed the Reg, CEE 2092/1991 referring to the method to obtain the ecologically production. So, there was possible to define the ecologically production not only from the juridical- law view point but technically too, because there were established vegetal production methods, chemically products (not synthesis products) allowed to be used, cultures rotation, organic fertilization, against parasites fight and storage by a naturally technology.

MATERIALS AND METHODES

This study's objectives are to underline (show) consumers preferences and their perception regarding the ecologically products.

There was used a question set, for a pattern composed by 100 persons distributed in four selling points:

1. Usual food store;
2. Ecologically products specialized store;
3. Supermarket
4. Market

Every question is reported as a preference scale from 1 to 3.

The question set was structured in three parts. The first part is formed by a questions series as regard as the individuals social, demographically and culturally aspects. The second part comprises questions concerning to the ecologically products acquisition sites and the last part is referring to the utilized communications means to assure information referring to the ecologically products.

RESULTS AND DISCUSSIONS

Data analyzing offers consumers image that pay attention to their food.

In Table 1 there are presented results for a questions series used with the purpose to evaluate knowledge degree as regard as the ecologically products and customers impressions.

The questioned people affirm that these products are clear (87%) and important for health (95%). However 10% (a small percent) sustain that these products are less attractive comparing with the conventionally one and 25% say that is difficult to choose them. On the other hand, a lot of consumer (80%) sustain that this products are good quality guaranty. Data referring to the taste show that these products are very good (85%) and a small percent sustain that the taste is medium (15%).

It is interesting to underline that the majority affirm that "the ecologically" is important for the fresh products (75%) due to chemically residue absence (75%) and for their sanitary control (85%).

The ecologically products are very expensive for 60%. There is a group which affirms that these products are preferred by the diets fanatics (15%) and it can say that is only an image impression and a publicity producer to sell well (5%).

In Table 2 there are presented responses to the questions as referring to the ecologically products acquisition sites. The preferred sites are the specialized stores (75%) and the supermarkets (70%).

In Table 3 there are presented results referring to the utilized communications means to assure information referring to the ecologically products.

The best sources of information about ecologically products are generically reviewing (80%) and specialty review (25) compared with newspapers (20%), TV (20%), publicity (20%), retailers (15%) and producers (5%).

So, newspapers, televisions and marks publicity less orientate the consumer. Neither producers and/or retailers are not an information pathway.

CONCLUSIONS

The image of a naturally and healthy product which doesn't contain chemically residue are between the most important features for the consumer. In fact, exist many consumers who request products with a quality and healthy guaranty, but they consider that these characteristics are intrinsic product features and are not disposed to pay a higher value.

There is also another categories, less for that the preference for the ecologically products is accompanied by the disposition to pay a higher price as compared with those for traditionally products.

To successfully start the ecologically production first of all must to use techniques with a reduced environment impact, preserving an undamaged soil and in the same time to assure the consumer with safety and quality products (Orto, Frutetto Biologico, 1998).

PAC's objectives as according to the EU as regard as the organic production "specificity". The norms of Reg. CEE 2091/1992 have as a purpose an increased product attention, mentioning the origin area and the processing method (Ghid Legislativ pentru Agricultura ecologica).

So, there are norms which differentiate the quality class, even for the large consume products, with remarkable consequences and advantages for producers and consumers.

The question for this point is referring to the price.

The major causes that determine to remain at a higher price in the case of ecologically products can be summarized as followings:

a) Missing of an adequate commercialization structure (market economy);

b) The higher injury for more products, taking into account the total missing of the post-harvest treatments and of preservatives.

c) A reduced (unitary) production, especially during the conversion period:

d) A high workers request because in the ecologically agriculture there are performed more manually works:

e) Meticulously and slowly processing techniques;

f) A reduced consumer's number.

Between the ecologically producers there are present risks as regard as the own products selling. These can be significantly reduced when there was a production assuring and due to commercialization to a seriously dealer or to an industrially processing factory.

In the first case, if we discuss about a high agreement product amount it can determine (start) an efficient and penetrated marketing politic. In the second case, by some adequate processing process, it can be adequately used a product which respond to an important consumers part.

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Tables

Table 1. The mean responses to the question: „After your opinion, the ecologically products are:..” (The percent on preferences classes)

Question	A little %	Medium %	Much %
1. Nourishing	0	11	89
2. Adapted only for the diets fanatics	55	30	15
3. Adapted only for children	55	20	25
4. Important for healthy	0	0	95
5. Less attractive	40	50	10
6. Trustworthy	5	60	95
7. Difficult to choose them	15	45	25
8. Easily to distinguish from other products	5	35	50
9. Important for house processing products	0	5	65
10. Only the image to sell more	90	5	5
11. Important only for legumes-fruits	55	5	40
12. Free from chemically residue	20	20	75
13. Importante only for fresch products	5	20	75
14. Submit to an external control	5	10	85
15. Without preservatives products	0	10	90
16. Have a good taste (unique)	0	15	85
17. Don’t like it	55	25	20
18. Easily digested	0	25	75
19. Clear by the offered information	0	13	87
20. Good quality guaranty	0	20	80
21. Too expensive	15	20	60
22. Are similarly with the others but they have publicity	65	25	10

Table 2. Response to the question:” Where do you buy or must buy the ecologically products?” (Percent on the preferences classes)

Preference for the selling place			
Biologically stores	15	10	75
Supermarkets	10	20	70
Usually food stores	55	30	15
Market	40	25	35

Table 3. Responses to the question:”How are you informed about the ecologically products?”

The source of information about ecologically products	A little %	Medium %	Much %
Newspapers	62	19	19
Generically review	5	10	85
Specialty review	30	40	30
TV	45	35	20
Publicity	45	35	20
Producers	65	30	5
Retailers	70	15	15

Monitoring of some pathogen attack specifics to stone fruit trees species cultivated in Bucharest area

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Keywords: fungus, pathogen, attack

ABSTRACT

In pedo-climatically conditions from Bucharest area, stone fruit trees species preferred to agricultural cultivators are plum tree and apricot tree. These species are, usually, the most frequent in small or average orchard dimensions of the agricultural producers. Regard as plum tree cultivation, these species is perfect because present a high adaptability capacity at environment various conditions and can capitalize a large scale of soils, with different fertility degrees especially where other fruit trees species are not recommended or hasn't good efficiency. Concerning at apricot tree culture, this is good to conditions from South of the country. Been known like a species with low adaptability capacity, the apricot tree is value for that zone while winter conditions has no implicating consequences for summer period. Species with commune pathogens, plum and apricot trees have finding close in all orchards near Bucharest. Biology, ecology and treatment possibilities knowledge present a great importance for treatment schemes realizations an applying of some products with efficiency for commune disease of the two species.

MATERIAL AND METHODS

The researches were perormed in plum and apricot orchards area in frame of „Agral Prod SRL" society, on varieties Centenar and Rosu de Baneasa. *Stigmina carpophila* pathogen was monitoring, responsible for stone fruits leave fungal screening and *Monilinia laxa* that producing moniliose of the stone fruits species. Besides monitoring fungal diseases, it has followed and Plum pox virus attack incidence that determined plums viruses.

The observations were effected on leaves apparatus for *Stigmina carpophila* attack and on young shoots for *Monilinia laxa* attack. In case of the *Stigmina carpophila* attack it has determined attack frequency and intensity, used values in attack degree calculation. With a control variant out of the treatment scheme it has determined products used in treatment scheme efficiency.

The used products are presented in scheme that appear in this paper, and was applied in conformity with recommended using instructions.When it was necessary, the fungicides were applied blended with compatible insecticides for complex control of the specific pests.

Accounting of the applied treatments effectiveness, in function of attack degree, at variant treatment scheme and control variant, it has determined effectiveness bigger than 70% at treatment scheme applying.

It has followed and attack incidence of the moniliose (Table 3) and regard as attack frequency on plum young shoots it has noted a value by 6,5% at variant-treatment scheme and 5% at same variant in case of the apricot tree. Incidence diminished can be considered significant, because at control variant, attacked young shoots frequency was 18% at plum tree and 13,5% at apricot tree.

RESULTS AND DISCUSSIONS

From data presented in Table 2 result that as result of the applied treatments in conformity with scheme from Table 1, *Stigmina carpophila* attack on plum tree leaves was by GA=5,85% comparative with variant considered control, that attack degree were

by 19,75%. It has observed that applied treatments, was a high influence concerning attack frequency, that reduced from 79% at control variant to 45% in case of products applying in scheme. Also, attack intensity value was significant diminished, from 25% (control variant) to 13,0% at variant considered as result of treatments applying from scheme treatment. Regard as *Stigmina carpophila* fungus attack on apricot leave, data from same table shows that after treatments applying foresaw in scheme, attack degree arrived at 6,3%, value more diminished comparative with untreated variant, out of the scheme, that GA=24,65%. In this situation it has ascertained both a frequency reduction and especially a attack intensity reduction, that value was by 11%, comparative with control variant with an intensity by 24%.

CONCLUSIONS

It has assessing applying of the treatment scheme for controlling in complex of the commune pathogens for the two fruit trees species.

Stigmina carpophila attack it has diminished significant as result of the treatment scheme applying, at the two monitoring stone fruit trees species.

Applying treatments effectiveness was by 71% concerning *Stigmina carpophila* attack on plum trees leaves

Effectiveness of the products applied against leaves screening at apricot tree was by 76%.

Monilinia laxa attack incidence was considered low, as result of the treatments, comparative with control variant.

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Tables

Table 1. Treatment scheme used in vegetation period at plum and apricot tree for controlling of the *Stigmina carpophila* and *Monilinia laxa fungus*

Treatment and stage	Monitoring pathogen agent	Used product, concentration%, dose
1. Flowering beginning	**Stigmina carpophila** **Monilinia laxa** (*Phylostica rubrum*)	Turdacupral 0,4%
2. Beginning of the petals bolting	**Stigmina carpophila** **Monilinia laxa** (*Phylostica rubrum*)	Dithane M 45 0,2%
3-4. After 10-12 days	**Stigmina carpophila** **Monilinia laxa** (*Phylostica rubrum*) Transchelia pruni-spinosae	Folpan 0,3% (+insecticides)
5-6. After 8-10 days	**Stigmina carpophila** **Monilinia laxa** (*Phylostica rubrum*) Transchelia pruni-spinosae	Dithane M 45 0,2% Polyram 0,25% (+insecticides)
7-8. At 10-14 days from one treatment for pests at warning	**Stigmina carpophila** **Monilinia laxa** (*Phylostica rubrum*) Transchelia pruni-spinosae	Rovral 0,1%
9-10. After fruits falling down	**Stigmina carpophila** **Monilinia laxa**	Turdacupral 0,5%

Table 2. Effectiveness of the products applied treatments scheme in conformity with applied scheme of the treatments in vegetation period against *Stigmina carpophila fungus*

Variants	**Attack by *Stigmina carpophilla* on leaves**							
	Attack on plum tree			**Attack on apricot tree**			**Effectiveness**	
	F (%)	**I (%)**	**GA (%)**	**F (%)**	**I (%)**	**GA (%)**	**On plum %**	**On apricot %**
Treatment scheme in vegetation	45,0	13,0	5,85	57,0	11,0	6,3	71,0	76,0
Untreated control variant	79,0	25,0	19,75	85,0	29,0	24,65	—	—

Table 3. Attack incidence of the *Monilinia laxa* on young shoots

Variant	***Monilinia laxa* attack on plum tree young shots (%)**	***Monilinia laxa* attack on apricot tree young shots (%)**
Treatment scheme in vegetation	6,5	5,0
Untreated control variant	18,0	13,5

Nowadays research preoccupations as regard to *Venturia inaequalis* fungus apple interactions

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Mini-review of literature

Keywords: *Malus domestica*, apple scab

ABSTRACT

Plants are exposed to many biotic and abiotic stress factors and understanding the mechanism of their responses represents a precondition for a sustainable agriculture and for a high crop quality, in the context of the new concept of vital quality. Apples belong to the main fruit species and the main objectives of apple breeding in a number of breeding stations worldwide and also in Romania include: fruit quality, resistance to biotic stresses, environmental adaptability, changes in tree habitat, fruiting characteristics and yield efficiency and constancy of production. The European market for fresh apples is the largest in the world; therefore, the best strategy for the European fruit sector is to promote quality, safety and sustainable production system. Apple scab, caused by *Venturia inaequalis* (Cooke). Wint. is the most serious disease of apple (*Malus* domestica* Borkh.) and a limitation to apple production in the world, despite years of research and development. Innovative approaches for early detection of plants pathogens and characterisation of their impact on plant productivity and crop quality, taking as a case study apple scab produced by the fungus *V. inaequalis* must be develop. Next to the classical research methods, nowadays attention is focused on the new tools of spectroscopy, to detect early alteration induced by the pathogen, to discriminate between resistant and susceptible interaction or to distinguish and classify plants pathogens.

INTRODUCTION

Plants are exposed to many biotic and abiotic stress factors and understanding the mechanisms of their responses represents a precondition for a sustainable agriculture and for a high crop yield (Delian, 2006), in the context of the new concept of vital quality. Apples belong to the main fruit species, the most important fruit in the temperate zone, and the main objectives of apple breeding are being pursued in a number of breeding stations, worldwid and also in Romania. These objectives include, fruit quality, resistance to biotic stresses, environmental adaptability, changes in tree habit, fruiting characteristics and yield efficiency, and constancy of production (Braniste, 2000; Braniste, 2005; Gradinaru et al., 2002; Coman et. al, 2005; Sansavini et. al., 2005; Delalieux et. al., 2005; Lakso et al., 2006; Massonnet et al., 2006).

The European market for fresh apples is the largest in the world; therefore, the best strategy for the European fruit sector is to promote quality, safety and sustainable production system (Stich et al., 2005). During the first Food, Quality and Health (FQH) - project with apples, it was studied the possibility of introduction the new concept – vital quality-, based on the life processes: growth, differentiation and integration processes and the corresponding product aspects: vitality, structure and coherence (Bloksma et al., 2001).

Apple scab, caused by *Venturia inaequalis* (Cooke). Wint., is the most serious disease of apple (*Malus* domestica* Borkh.) and a limitation to apple production in the world, despite years of research and development.

So, innovative multidisciplinary research should be necessary to exhaustively study the host-pathogen interaction and the pathogen variability. A promising non-destructive method, that it is used successfully to identify, distinguish and classifies pathogens is FT-IR spectroscopy (Oberreuter et al., 2002; Wenning et al., 2002; Maquelin et al., 2003; Burgula et al., 2004; Rebuffo-Scheer et al., 2007). This technique has been used to detect early stress effects on the changes in chemical composition in *Arabidopsis* (Jang and Yen, 2002).

STATE-OF-THE-ART

In Europe, apple and pear represent major crops, grown on 490000 ha in 15 countries with a production of more than 11 million tons per year (FAO, 2003), but this production is under high economic pressure due to increasing imports from overseas countries. In this situation of market saturation, quality aspects are the most relevant and promising arguments to keep or even gain market share. The best strategy for the European and Romanian fruit sector is to promote quality, safety and sustainable production systems. Several large national and international breeding programs have been initiated and accomplished on apple and pear, the major objectives being those focused on combining high fruit quality and multiple disease resistance, according to the European preoccupation (as there was established at the new COST Action proposal - COST Action 864).

V. inaequalis is an ascomycete and the life cycle is divided into two phases: during winter, the fungus survives primarily in fallen leaves, as immature pseudothecia – winter phase. In the spring, ascospores develop in the pseudothecia and are actively discharged during rain. The fungus infects the aerial part of the apple tree, including leaves, petioles, flowers, sepals, fruit, pedicels, young shoots and bud scales.

Generally, researches have been focused on two main directions: control methods and breeding for resistance.

Control of apple scab is based on traditional methods using different kinds of chemical fungicides and modern strategies (alternative sprays, biological control etc.). To control apple scab in a sustainable fashion, there are three essential ways: maintaining a balanced soil with good fertility, preventing the development of ascospores, and preventing infection when some ascospores do survive. A fourth way, which may obviate the need for the latter two is to plant only scab resistant cultivars of apples.

Chemical control of apple scab is unsustainable. The use of large amounts of fungicides in orchards raises ecological problems, consumer's health concerns in addition to the economic cost (Lespinasse et. al., 2000). D.A.R.E. European Project (Durable Apple Resistance in Europe) contributes to developing knowledge, methodologies, plant and pathogen material, to achieve durable genetic resistance towards *V. inaequalis* and *Podosphaera leucotricha* in the future. In Italy recently, there was demonstrated that under high disease pressure, even the new products must be applied with flexible schedules adjusted to the climatic conditions (Brunelli et al., 2003). So, to overcome this problem, Hockenhull, Projectleader –of Project nr.VII.5-Control of scab in organic apple growing (as part of the research program- Research in organic farming 2000-2005 -DARCOF II), pointed out a range of potential control materials, that have been screened for the efficacy against the scab pathogen in growth chamber, greenhouse and orchard experiments. Also, promising materials have been investigated histopathologically to establish the mechanism of control and the other

studies are focused to characterise the effect of selected treatments on the secondary metabolites in apple.

A second strategy is to make use of ***apple's natural resistance to scab*** and the breeding and planting of scab resistant cultivars of apples should be encouraged, but cannot be relied on exclusively because the resistance will probably be overcome in time.

In Romania there are recent results concerning new columnar apple variety with genetic resistance to apple scab (Braniste et al., 2005). During 2000-2005, at Maracineni- Pitesti Research Institute, there were created new apple cultivars with the gene of apple scab resistance, such as Romus 5, Nicol and Rebra (Coman, 2005). Also, Sestras (2003) verified 75 varieties of apple in central Transylvania conditions and noticed that 41.3% were registered with no attack, both on leaves and fruits, including genetically resistant varieties Prima, Priscilla, Sir prize, Liberty, Florina, Priam, Pioneer, Voinea, Generos.

This attribute is under constant threat from changes in the virulence pattern of the pathogen and many of the varieties in current use have resistance originating from *Malus floribunda*. New races have recently been registered (Parisi et al., 2000; Bus et. al., 2005; Bilbao-Martinez and Murillo, 2005). Zeppa et al. (2002) presented status of breeding for apple scab resistance in Australia and Tartarini et al. (2002) presented a review on molecular studies in this field. Recent results on resistance to V. inaequalis has focused on the molecular aspects of R-gene resistances (Vinatzer et . al. 2004; Patocchi et al., 2004). Despite this emphasis on R-genes from different species, evidence has emerged that other resistance(s) also occur in apple cultivars (Durel et al., 2003; Liebhard et al., 2003).

Another group of defensive strategies are ***cultural practices*** and a number of such methods are currently under investigation as for instance the project: Development of sustainable production systems for apples (Bengtsson, 2001).

An integration of many control methods is necessary to reduce scab infection to an acceptable and sustainable levels in apple orchards (Holb et al., 2005; Holb, 2008) and from this viewpoint there are recent results in Romania too, results published by Mocanu (2004). Also, it is possible to take the level of cultivars susceptibility into consideration, within the framework of integrated fungicide protection (Brun et al. 2008).

As we referring to ***organic apple growing*** in Europe, in accordance with EU directive 2092/91, the plant protection products permitted for use, consists of material based on plant extracts (Trapman et al. 2004), micro-organisms (Fiss et al., 2000), utilising induced disease resistance (Jorgensen et. al., 2004; Lateur, 2002). In this context, Bengtsson et al. (2004) and Lindhard et al. (2003) presented interesting data. In Europe, southern states, especially Italy, Spain and France have the largest land area with organic fruit (Weibel et al. 2004) and it was pointed out during the International Congress (IHC) in Toronto, August 12-16, 2002 – The Toronto 2002 sustain ability declaration on research needs for a continuous development of sustainable horticultural systems for the 21st century-. The declaration shall provide some guidance to further activities to support a sustainable horticulture in applied research (Bertschinger, 2004; Bertschinger et al. 2004).

Fungus impact on apple physiology, morphology and biochemistry

Traditional apple physiology has majored on the effects of environmental factors on tree and fruit growth and development, but the challenge for the physiologists of the future is to make all systems of production more sustainable (Palmer and Wunche, 2004).

From the viewpoint of the *V. inaequalis* fungus impact on the main apple physiological processes, in Romania there are some recently results (Chira et al., 2000; Delian et. al, 2006). In the world there is a preoccupation for changes in photosynthesis and carbohydrates metabolism in response to whole plant source-sink manipulation (Tartachnyk and Blanke 2001; Zhou et al., 2003), or physiological and biochemical leaf and tree responses to crop load in apple (Wuusche et al., 2004).

As concerning the preformed and induced biochemical barriers, *phenols* are very important (Roemmelt et al.2002; Merzlyak et al. 2005; Delian et. al. 2007). In fact, ten years ago, Gutmann said that in the near future development on photochemical based on phenols lead with broad activity spectra against common pathogens is one of the most promising strategies. The resistance to *V. inaequalis* is related to the accumulation of phenolic compounds at the infection site and literature data mentioned that in apple leaves and fruit skin a positive correlation between phenol content and predisposition to apple scab has been established. Lux-Endrich et al. (2002) studied response of apple cell suspension cultures cv. Alkmene on elicitation with biotic elicitors and formation of novel flavonoids. Roemmelt et al. (2002) reported that in apple treated with prohexadione-Ca, substance which is known to reduce the incidence and severity of fire blight caused by *Erwinia amylovora* and other plant diseases flavonoid synthesis, also. Merzlyak et al. (2005) reported a spectral feature and technique for non-destructive assessment of apple flavonols.

Also, the nutrition status of apple leaves is very important. According to physiological and biochemical functions, nutrients are: C, H, O (basic building blocks for organic molecules); - N,S (building blocks of enzymes organic structure); K, Ca, Mg, Cl, Mn (balances osmotic pressures within the plant and activates enzymes); - Fe, Zn, Mo, Cu (takes part in photosynthesis by causing electron carriage through charge exchange).

So, innovative multidisciplinary research should be necessary to exhaustively study the host-pathogen interaction and the pathogen variability. A promising non-destructive method that it is used successfully to identify, distinguish and classifies pathogens is FT-IR spectroscopy (Oberreuter et al., 2002; Wenning et al., 2002; Maquelin et al., 2003; Burgula et al., 2004; Rebuffo-Scheer et al., 2007). This technique has been used to detect early stress effects on the changes in chemical composition in *Arabidopsis* (Jang and Yen, 2002) and Kizilksya et.al. (2007) reported first results by using the microspectroscopy beamline in the investigation of plant -pathogen interaction, apple -*V. inaequalis* causing scab.

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Studies concerning the quality of some walnut oils and grapeseed oils commercialized in Iaşi

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Keywords: edible oils, acidity, peroxide index, spectrophotometer, humidity

ABSTRACT

The Romanian edible oil market has enriched in new sorts, less known for consumption, such as grapeseed oil and walnut oil. These oils are very valuable, due to the essential fat acids, vitamins, antioxidants and other bioactive compounds they contain. The hypo cholesterol value of the essential fat acids is tightly related to the global quality of lipids from diet, the total caloric share as well as the size of the ratio between the essential polyunsaturated fat acids and the saturated fat acids (P/S) that must be supra-unitary with a value over 2. The production of grapeseed oil has developed consequently the economic and the ecological belief of capitalize the offals, so that the row materials be processed completely. The commercial units from Iasi from where come the samples studied are Carrefour, Gimma, Kaufland, Sellgros. In the vegetal oils, the contents of phosphatides vary between 1 and 2%, in the crude oils it varies depending on the nature of the raw material and technology (press oil or extraction oil). Among the substances always accompanying the glycerides (triacil glycerols) from the crude oils, we can also find the free fat acids that are extracted from oil by alkaline neutralization. In the presence of the atmospheric oxygen, the fat acids from the composition of fats may partially oxidize forming peroxides or hydro-peroxides. Their formation is noticed in the process of becoming rancid of fats and also during their dryness. This way, the peroxide index serves as an indicator of the oxidative changes suffered by fats. Through the refining process, they remove accompanying substances to make oil able to be used in alimentation or to insure the organoleptic features required by the standards within the validity terms.

INTRODUCTION

The present paper wants to demonstrate how grape seed oil and walnut oil, recently introduced in the Romanian market, deserve attention due to their very obvious dietary or medicinal qualities and also that they able to be used in alimentation and to insure the organoleptic features required by the standards within the validity terms Having between 73-84% polyunsaturated fat acids, the walnut oil situates on the first place among the unsaturated oils before the soy oil (50-60%) and the corn oil (40-50%), for their anti-cholesterol properties. The walnut oil obtained by cold pressing has a light yellow colour, a strong characteristic taste and smell. It is indicated for the protection of the cardiovascular system. It is also used in dermatosis, nephrolithiasis, milk legs, and burns. Grapes seeds oil is resistant to high temperatures (224ºC) and thermal degradation. It has a light yellow colour and a delicate taste. It is a rich source of antioxidants (vit. E) and essential fat acids. Among the essential fat acids absolutely necessary for the human body, the linolenic acid was identified in high quantities in the grapes seeds oil that has positive effects over the cardiovascular, circulatory and immunity system.

MATERIALS AND METHODS

We effectuated the analyses in the interval February-March 2008. The material used was taken from shops having different origins and manufacturing methods depending on the producer. The oils analyzed were the following: grape seed oil (produced by different companies) and walnut oil (refined and unrefined) (tab. 3). Through the analyses effectuated, according to the existing standards, we determined

acidity (% oleic acid), alkalinity (mg sodium oleate/kg), the phosphor contents (mg/kg, after our own method), the iodine colour (%), humidity (U%), the peroxide index (% I or meq O2/kg), the acidity index (mg KOH/g). The free acidity is the percentage of fat acids found in the oil analyzed and is expressed conventionally in the most representative fat acid. For the common oils from soy, sunflower, peanuts, pumpkin they express it in oleic acid; for the coconut and palmist oil they express it in lauric acid; for the palm oil they express it in palmitic acid; for the castor oil they express it in ricinoleic acid; for the rapeseed they express it in erucic acid. The work method consists in dissolving a quantity of oil in a mixture of alcohol-ether, afterwards it is titrated with a solution of sodium or potassium hydroxide in the presence of the indicator phenolphthalein. The appearance of the pink coloration indicates that all free fat acids were neutralized. By acidity index we understand the quantity in mg of potassium hydroxide necessary to neutralize the free fat acids from a gram of fat matter.

Oils may contain natural alkaline constituents (calcium soaps from bones) or accidental (sodium soaps from the improperly refined oils). The method principle to determine the contents of soaps from a sample consists in dissolving it in a warm mixture of acetone-water and its titration with chlorine hydride. Alkalinity may be expressed as a percentage of sodium hydroxide or in mg of sodium oleate per keg of sample.

The method principle to determine the contents of phosphor relies on the destruction of the organic matter by mineralization in the presence of magnesium oxide. The dissolving of ash and the formation among the phosphoric ions and molybdenum and vanadium ions of the complex ammonium phospho-vanado-molybdate gives a stable yellow coloration. The intensity of coloration is measured by spectrophotometer for a wave length of 390 nm as against a blank test obtained from distilled water and nitro-vanado-molybdenic reagent.

The iodine colour was determined by spectrophotometer measurement of light absorbance though oil for a wave length of 420 nm, on a UV/VIS spectrophotometer, as against an approval solution where the sample was replaced by water.

In the presence of the atmospheric oxygen, fat acids from the composition of fats may oxidize partially forming peroxides or hydro-peroxides. The determination of the peroxide index relies on the property of the fat peroxide to react in an acid environment with potassium iodide freeing iodine that is afterwards titrated with tiosulphate.

The determination of humidity and the contents of volatile matters was effectuated by the drying process in a drying chamber at the temperature of de 103°C, until we got a steady mass, then the samples were let to cool and were weighed in vile with a precision of 0,001g.

RESULTS AND DISCUSSIONS

The unrefined oils have a higher free acidity than the refined ones (tab. 1). Thus, the nut oil Gaianello has an acidity of 1,57% expressed in oleic acid.

Through the determination of the oil alkalinity (tab. 1) we identified the highest value of 7,6 mg/kg, for the nut oil Lesieur.

Higher values of the phosphor contents were registered for the unrefined nut oil Gaianello 13,61 mg/kg (tab.1). The lowest contents of phosphor, 0,93 mg/kg, was regieterd for the grapes oil Olitalia.

After determining the iodine colour (tab. 2) we could notice that the nut oil Gaianello had the most favourable index 47,7%, its hue insuring superior organoleptic characteristics.

The appreciation of the oxidative alteration level for the oils analyzed was effectuated depending on the values obtained: all the grape seed oils are fresh but they are not good for a long keeping (tab. 2). The nut oils, by the values obtained (0,09% I, 0,10% I respectively) appear like oils with a dubious freshness (tab. 2).

The humidity of the oils analyzed corresponds to the existing standards ranging between 0,01-0,05% (tab. 2).

CONCLUSIONS

The free acidity and the acidity index of the unrefined oils are higher than those of the refines oils. The nut oil Gaianello has an acidity of 1,78% expressed in oleic acid and the acidity index of 3,1 mg KOH/g. A high acidity index is also held by the nut oil Guenard, 1,7 mg KOH/g, since it also has in its composition unrefined oil (50%). These oils may be kept for a shorter period of time (up to 12 months), at low temperatures and in dark places.

Alkalinity registers high values for the nut oils Lesieur and Gaianello, but they correspond to the standard requirements.

The unrefined nut oil registers a high level of phosphor, 13, 61 mg/kg. All the refined oils registered a low phosphor level as we already expected.

The nut oil Gaianello had the most favourable index 47,7% and its hue insures superior organoleptic characteristics.

Through the appreciation of the oxidative alteration level, the grape seed oils proved to be the freshest, but they may not be kept for a long period of time.

The oils analyzed have a humidity ranging between 0,01-0,05% and correspond to the existing standards.

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Tables

Table 1. Determination of acidity, alkalinity and phosphor contents from the grapeseed and walnut oil

Oil	Acidity (% oleic acid)	Alkalinity (mg/kg)	Phosphor (mg/kg)
Grapeseed oil Olitalia	0,08	absent	0,93
Grapeseed oil Vinaciollo	0,12	absent	2,22
Grapeseed oil Monini	0,14	absent	1,04
Walnut oil Lesieur	0,23	7,6	8,57
Walnut oil Guenard	0,86	absent	7,50
Crude Walnut oil Gaianello	1,57	3,65	13,61

Table 2. Determination of moisture and oils index

Oil	Iodine colour (%)	Peroxide index (%)	Acidity index (mg KOH/g)	Humidity (%)
Grapeseed oil Olitalia	21,2	0,04	0,16	0,02
Grapeseed oil Vinaciollo	39,0	0,08	0,24	0,01
Grapeseed oil Monini	35,0	0,05	0,28	0,02
Walnut oil Lesieur	39,1	0,09	0,46	0,01
Walnut oil Guenard	22,0	0,09	1,7	0,05
Crude Walnut oil Gaianello	47,7	0,10	3,10	0,05

Table 3 Details about origins and manufacturing methods of the analyzed oils

Product	Weight (ml)	Producer	Utilization	Ingredients and temperature recommended for keeping
Grape seed oil Olitalia refined	500	Olitalia Ltd. Via Meucci, Italy	ideal for cooking, frying, salads	nutritional factors for 100 ml: fats 91 g, out of which monounsaturated 19g, polyunsaturated 62g, saturated 10 g an important source of vitamin E and antioxidants temperature recommended for keeping 10-25ºC
Grape seed oil Vinaciollo refined	1000	Costa d Oro S.p.A., Italy	ideal for cooking, frying, salads	nutritional factors for 100 ml: lipids 92 g, out of which monounsaturated 18g, polyunsaturated 64g, saturated 10 g an important source of vitamin E and antioxidants temperature recommended for keeping 10-25ºC
Grape seed oil Monini refined	1000	Italy	ideal for cooking, frying, salads	nutritional factors for 100 ml: fats 100 g, out of which monounsaturated 18g, polyunsaturated 71g, saturated 11 g. temperature recommended for keeping 10-25ºC burning point 180 ºC
Refined walnut oil Guenard	250	Levallois Cedex, France	ideal for salads, mayonnaise, raw vegetables	contains: 50% refined nut oil and 50% virgin walnut oil rich in omega 3 temperature recommended for keeping 10-25ºC.
Unrefined walnut oil Gaianello	250	Azienda Agraria Vesprini Elvasio and Nardoni Rita, Italy	ideal for salads	rich in omega 3 the turbid product with sediments on the bottom of the bottle prove that the oil is naturally obtained by cold pressing without any chemical products temperature recommended for keeping 10-25ºC
Refined walnut oil Lesieur	500	Lesieur, France	ideal for cooking, and salads	rich in omega 3 nutritional factors for 100 ml: fats 100 g, out of which monounsaturated 18g, polyunsaturated 72g, (out of which omega 3: 12g) saturated 10 g. temperature recommended for keeping 10-25ºC

Precocity and production potentialities of some apple varieties, grafted on M9, in a large density plantation

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Keywords: variety, parent plant, precocity, bearing branch, spur.

ABSTRACT

Studies on precocity in yielding of some apples varieties, grafted on vegetative parent plant M9 were tried to give an answer to the major objective from fruit growing, concerning yield increasing from the first years of orchard with the purpose of recovering in short time losses from the plantation.

MATERIALS AND METHODS

With that end in the years 2006 and 2007 there were made researches on a assortment of apple make up from: IONATAN (witness), DELBARD, ROYAL-GALLA, ELTON, ELSTAR, LIBERTY, SIR - PRIZE, MUTZU, IONAGOLD, GRANNY - SMITH and FLORINA, grafted on M9, planted at 3,5m/1,5m away (1904 trees/ha) and guided as a fruit hedge (PALMETA NEREGULATA) .

Starting with the third year of the plantation these varieties had started bearing (with the exception of the Mutzu variety) producing crops of 3,25-11,3 to/ha and a big quantity of bud fruit for the next year 2008.

The research of morpho-productive particularity of the new apple varieties from the competition cultures, pomological collections and short plantations have as a purpose to point out new performances concerning obtaining of some early crops, big, constant and quality, with a simpler technology.

In this context we have proposed to make a complex study in witch we analyzed how some varieties of apple act in South of Romania ecological conditions.

This necessity started from the fact that in the last 5 decades assortment of winter apple was dominate by the varieties: IONATHAN, GOLDEN DELICIOUS and STARKRIMSON (in the present on a loosing way), even if in the last time we tried to improve with the presence in the culture of some resistance varieties at *Venturia inaequalis.*

With some exceptions, this fruits have growing them fruits in summer, autumn or at the start of winter.

For giving an answer of the new tendencies, in the summer of 2004, there were founded at BELCIUGATELE DIDACTIC STATION, at MOARA DOMNEASCA farm, a superintensive plantation of apple on a surface of 12ha in witch is a collection of 16 varieties of apple, of witch 11 we study: IONATHAN (witness), DELBARD, ROYAL – GALLA, ELTON, ELSTAR, LIBERTY, SIR – PRIZE, MUTZU, IONAGOLD, GRANNY – SMITH and FLORINA.

The trees are grafted on parent plant M9 planted at 3,5m/1,5m away (1904 trees/ha) and guided as a fruit hedge (PALMETA NEREGULATA) limited at 2,5 m, from witch 0,5 m represent the trunk height of the trees and 2,00m the height of the tree crown.

The varieties tested are limited into a monofactorial experience put into line as variants. In each variant are 3 repetitions with 3 trees each.

During the researches the soil was maintaining as a black field fertilized with chemical complex fertilizer and irrigate trough drops.

To combat diseases and pests were apply 2 winter treatments and 12 treatments during the vegetation period.

RESULTS AND DISCUSSIONS

For estimate the productive quality of this varieties, have been following record of the number, have been measure the trunk width, the dimensions of the crown tree and have been calculate the surface of the transversal section of the trunk, the crown volume and the productive efficiency of each variety .

From the analyze of the growing potential and of the crown ramify (table 1) made in third year, autumn, after the leaves fall, have been result as medium surface of the trunk section passing the witness with 6,23 cm^2.

The biggest values of the investigated varieties were at MUTZU (20,41 cm^2), FLORINA (21,22 cm^2) and ELSTAR (24,61 cm^2).

The medium volume of the crown studied varieties was superior at the witness with 0,24 m^3.

The biggest volume of the crown was at GRANNY-SMITH (0,85 m^3) and SIR-PRIZE (0,82 m^3).

At the main varieties the permanent structure of the crown (frame) was made of 5-6 branches from the first year, and the nonpermanent one (semiframe) from 4-8 branches of second order.

On the third year of the orchard, the medium production of fruits on ha, at the studied varieties was of 1,96 biggest that witness variety .

The biggest crops were ensured by the GRANNY-SMITH (8130 kg/ha), SIR – PRIZE (9291 kg/ha) and FLORINA (11 305 kg/ha) varieties.

The productive efficacy of a cm^2 from a trunk section, was biggest at SIR – PRIZE (0,307), GRANNY-SMITH (0,294) and FLORINA (0,239) and lower at ELTON (0,151).

On a m^3 of crown have been made much more varieties FLORINA (7,61 kg) and SIR – PRIZE (5,95 kg).

The first harvest of fruits obtain in the third year was made of the 10-29 fruit (table 3). The only variety witch didn't fructify was MUTZU. All the fruits obtain weren't frame in the extra quality.

Because we show a medium diameter having dimensions between 71,85 cm and a medium weight from 165g (Elton) to 205g (Florina), the Ionatan variety is being on the last place in what concern the last 2 studied indicators.

In the conditions of obtaining this crops the studied varieties have taking part of a good treatment, amplify the crowns and differentiated a number of bud bear, and the crop waited being economical from a quantity and quality point of view.

From the dates show in table IV, result that Delbard, Royal - Galla, Liberty, Sir-prize, Ionagold, Granny – Smith, and Mutzu varieties (at the first fructification) buds bear belonging to the thorns, and at the Elton, Florina and Elstar, twig, sprout.

CONCLUSIONS

1. From the research made we could observe that 10 from the 11 graft varieties on M9 have started to fructify from the third year of orchard.
2. There were realized economical productions varieties: SIR-PRIZE, GRANNY-SMITH and FLORINA.

3. The biggest productivity was at: SIR-PRIZE (I 2 - 0,307 si I 3 – 7,07) and FLORINA (I 1 – 0,279 si I 2 – 5,13).

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Tables

Table 1. The variation of point numbers of yield at main varieties of apple

Nr. crt.	Variation	The surface of the transversal trunk section	Crown Volume	Branches of l order	Branches of all order	Points of bearing
	variety	cm	mc	nr.	nr.	stock
1	IONATAN (Mt)	11,33	0,56	5	8	25
2	DELBARD	13,84	0,64	4	4	16
3	ROYAL - GALLA	17,34	0,59	5	5	21
4	ELTON	19,62	0,73	5	5	24
5	LIBERTY	15,19	0,78	4	6	28
6	SIR - PRIZE	15,89	0,82	4	6	31
7	MUTZU	20,41	0,80	4	4	x
8	IONAGOLD	12,56	0,73	4	4	25
9	GRANNY - SMITH	14,51	0,85	5	8	36
10	FLORINA	21,22	0,78	6	7	33
11	ELSTAR	24,61	0,68	5	6	26
	MEDIUM	17,56	0,80	5,1	5,5	26,6
	LIMITS OF VARIATION	11,33 - 24,61	0,64 - 0,85	4. - 6.	4. - 8.	0. - 36.

Table 2. Capacity of production and efficacity of some apple varieties grafted on M9

VARIANTY	PRODUCTION (KG)		diference from Mt kg/ha	PRODUCTIVE EFFICACITY	
	on tree	on ha		I 1	I 2
V 1 Ionathan	1,71	3,255	X	0,150	3,05
V 2 Delbard	1,75	3,332	77	0,126	2,73
V 3 Royal - Galla	2,97	5,654	2399	0,171	5,03
V 4 Elton	2,97	5,654	2399	0,151	4,06
V 5 Liberty	2,64	5,026	1771	0,173	3,38
V 6 Sir - prize	4,88	9,291	6036	0,307	5,95
V 7 Mutzu	X	X	X	X	X
V 8 Ionagold	2,91	5,540	2285	0,231	3,98
V 9 Granny - Smith	4,27	8,130	4875	0,294	5,02
V 10 Florina	5,94	11,309	8054	0,279	7,61
V 11 Elstar	3,52	6,702	3447	0,143	5,17
MEDIUM	3,35	6389,3	3493,6	0,202	5,10
LIMITS OF VARIATIONS	1,71 - 5,94	3255 - 11309	77 - 7958	0,126 - 0,295	2,73 - 7,61

Table 3 Fruits quality expressed trough the size and weigh at some apple varieties, grafted on M9 in the third year from the plantation (2007)

VARIETY	Fruits number on a tree	FRUITS DIAMETER mm		Medium weight of fruits grams
		Medium values	Variations limits	
v 1 Ionathan	11	73	67 - 75	156
v 2 Delbard	10	78	71 - 83	175
v 3 Royal - Galla	14	81	80 - 85	193
v 4 Elton	18	75	73 - 78	165
v 5 Liberty	15	75	71- 79	176
v 6 Sir - prize	26	82	77 - 85	188
v 7 Mutzu	x	x	x	x
v 8 Ionagold	16	78	70 - 81	182
v 9 Granny - Smith	23	77	75 - 81	186
v 10 Florina	29	82	78 - 88	205
v 11 Elstar	21	78	71 - 85	168
Medium	**18,3**	**77,9**	**73 - 82**	**179,4**

Table 4. Potential of bearing of some graft apple varieties on M9 on the 4 year from plantation (2008)

VARIANT / VARIETY	FRUTIFY BRANCHES						RAPORT
	TOTAL	SHORT		LONGS			short branches/ long branches
		Thorns	Spur	Shoots	Twings	Sprout	
v 1 Ionathan	76	27	22	12	14	1	1,81
v 2 Delbard	86	24	31	13	18	X	1,77
v 3 Royal - Galla	79	55	5	4	4	11	3,15
v 4 Elton	61	13	15	11	21	1	0,84
v 5 Liberty	92	50	17	17	8	X	2,68
v 6 Sir - prize	104	26	29	9	39	1	1,14
v 7 Mutzu	103	38	31	7	27	X	2,12
v 8 Ionagold	86	42	21	11	12	X	2,73
v 9 Granny - Smith	76	27	22	12	14	1	1,81
v 10 Florina	84	25	16	10	25	8	0,95
v 11 Elstar	82	10	30	6	35	1	0,95
Medium	**84,43**	**30,63**	**21,72**	**10,18**	**19,72**	**2,18**	**1,81**

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The behaviour of some raspberry varieties cultivated in the Banat area *(Rubus idaeus L.)*

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Keywords: raspberry, behaviour, varieties.

ABSTRACT

From the data registered during the years of research, we can draw the conclusion that raspberry varieties cultivated in the sylvo-steppe conditions of the Banat area, react better if the right agro-technique is applied, starting with a good exposure, preparing and fertilizing the soil, ensuring the right amount of water. All these also ensure a constant production throughout the years. Protection against floods, dryness and late spring frosts is important as well. The Latham variety shows the highest annual growing in 2006 and 2007. The size of the berry has a variable characteristic within the same variety, according to the flowering time, the pedo-climatic conditions, the applied agro-technique etc. Still, each variety has a certain characteristic size. The research that has been undergone by this moment, are limited in Roumania and they do not refer to the area where we have done our research. It is desired that the cultivation of raspberry should be promoted, mainly because of the qualities mentioned above.

INTRODUCTION

The raspberry has great nutritional and diet-therapeutic qualities due to its rich content, namely 5-8% complete sugars, 0.9-1.9% organic acids, 0.2-0.5% peptin substances, 13-41% vitamin C and numerous mineral substances (Ghena N., Braniste N., 2003). Raspberry fruits are consumed fresh as well as in a processed state, like syrup, jam, candied raspberry juice, ice-cream and others. The fruits are also used in cosmetics and pharmaceutics (Radu Dana, Bobescu I., 2007). The research that has been undergone by this moment, are limited and they do not refer to the area where we have done our research. It is desired that the cultivation of raspberry should be promoted, mainly because of the qualities mentioned above, but also because of the economic advantages. It is a known fact that a raspberry plantation produces profit starting with the second year, that there are a lot of market possibilities. We also have to take into account the fact that most of the raspberry on the market comes from spontaneous fauna and does not cover the demand, the good price, the export demand.

MATERIALS AND METHODS

13 varieties of raspberry have been studied, the planting material was purchased from the Fruit Growing Research and Development Institute Pitesti-Maracineni, with the soil quality verified. The root suckers were planted at a o.5 m distance on the row and 2.5 m distance between rows, for each variety there were planted 15 pieces. The experimental plantation was started in 2005 in Icloda, Timis county, 28 km from Timisoara city, 45˚38′27″ N latitude, 21˚23′10″ E longitude and an average altitude of 90 m, on the bank of the Poganisul Sec river. The field is included in the Western Lowland of Romania, it has got a plan relief not characteristic for fruit shrub cultures, in this case for raspberry. The Banat area has a special climate, determined by its positioning on the continent, with a certain circulation of the western and eastern continental air masses, overlapping with southern air masses crossing the Mediterranean Sea. These air circulations determine a moderate continental climate with subtropical influences. The winters are shorter and calmer in Banat, the summers warm and the autumns long. In return, late spring hard frosts can occur due to north-east cold air invasions even in the second or third decade of April (Berbecel O., 1979).

RESULT AND DISCUSSION

Raspberry is sensitive to water, it needs 800-900 mm/year and in Banat we only get 550-700 mm/year. Studies show that in the plain area you can not get good results with raspberry cultures in Banat unless the water supply can be assured by irrigation (3-5 waterings of 300-400 m³/ha). In 2005, when the plantation was started, the annual precipitation sum was of 720.5 mm with a maximum of 154.4 mm in April, determining massive flooding and long water stagnation, which had a disastrous effect on the plantation.

The observations recorded refer to the vegetative growings and the root sucker number from the following years. The results are showed in tables 1 and 2.

Table 1. The sum and average of varieties studied in 2006

Variety	Growing sum	Shrub number	Growing average	Shrubs/ha	Growing sum/ha (m)
Cayuga	286,1	4	71,52	2133,3	1525,84
The Latham	1759,0	10	175,9	5333,3	9381,27
Malling Exploit	952,0	8	119,0	4266,6	5077,25
Citria	407,5	6	67,91	3200	2173,33
Heritage	358,0	4	89,5	2133,3	1909,33
Autumn Blis	374,0	3	124,66	1600	1994,66
Opal	132,0	3	44,0	1600	704,0
Camby	95,0	1	95,0	5333,3	506,63
Willarnette	844,5	9	93,83	4800	4503,99
Veten	390,0	6	65,0	3200	2080,0
Lyielin	273,0	4	68,25	2133,3	1455,97
Kilarney	487,0	5	97,4	2666,6	2597,26
Royalty	839,5	6	139,91	3200	4477,33

Table 2. The sum and average of varieties studied in 2007

Variety	Growing sum	Shrub number	Growing average	Shrubs/ha	Growing sum/ha (m)
Cayuga	514,5	4	128,6	2133,3	2743,9
The Latham	2854,0	10	285,4	5333,3	15221,2
Malling Exploit	1191,0	4	297,7	2133,3	6351,9
Citria	1106,0	5	221,2	2666,6	5898,5
Heritage	563,0	4	140,7	2133,3	3002,6
Autumn Blis	340,0	3	113,3	1600	1813,3
Opal	50,0	1	50,0	533,3	266,6
Camby	90,0	1	90,0	533,3	439,9
Willarnette	966,0	6	161,0	3200	5152
Veten	98,0	2	49,0	1066,6	522,6
Lyielin	301,0	4	75,2	2133,3	1605,3
Kilarney	1074,0	5	214,8	2666,6	5727,8
Royalty	401,0	2	200,5	1066,6	2138,5

Table 3. Root sucking degree of varieties studied in 2006

Variety	Shrubs number	Total root sucking number	Root sucking/shrub average	% root sucking
Cayuga	4	11	2,75	26,6
The Latham	10	21	2,10	66,6
Malling Exploit	8	16	2,0	53,3
Citria	6	6	1,0	40,0
Heritage	4	9	2,25	26,6
Autumn Blis	3	7	2,33	20,0
Opal	3	7	2,33	20,0
Camby	1	1	1,0	6,6
Willarnette	9	36	4,0	60,0
Veten	6	11	1,83	40,0
Lyielin	4	7	1,75	26,6
Kilarney	5	5	1,0	33,3
Royalty	6	11	1,83	40,0

Table 4. Root sucking degree of varieties studied in 2007

Variety	Shrubs number	Total root sucking number	Root sucking/shrub average	% root sucking
Cayuga	4	9	2,25	26,6
The Latham	10	38	3,8	66,6
Malling Exploit	4	18	4,5	26,6
Citria	5	14	2,8	33,3
Heritage	4	12	3,0	26,6
Autumn Blis	3	8	2,66	20,0
Opal	1	1	1,0	6,6
Camby	1	1	1,0	6,6
Willarnette	6	28	4,6	40,0
Veten	2	5	2,5	13,3
Lyielin	4	6	1,5	26,6
Kilarney	5	14	2,8	33,3
Royalty	2	10	5,0	13,3

CONCLUSIONS

The annual growings represent a significant and reliable biological criterion in evaluating the vigour of the variety and its adaptation to pedo-climatic conditions. With the raspberry the vigour is represented by the offshoots length and root sucking power. From the presented data we can observe that the Latham variety shows the highest annual growing in 2006 (with 1759 cm measured at surviving shrubs) as well as in 2007 (with 2854 cm), followed by the Heritage and Royalty varieties in 2006, and in 2007 by the varieties Malling Exploit (1191 cm), Citria (1106 cm) and Kilarney (1074 cm). The Latham variety behaved best regarding excessive soil humidity (in 2005) and also drought (in 2007). The Heritage and Royalty varieties are quite resistant to humidity, but not drought, whereas Malling Exploit, Citria and Kilarney varieties can do well with drought and make a quick recovery. The least vigorous varieties have proved to be Camby and Opal both in 2006 and 2007, regarding both humidity and drought.

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The quality of apple influenced by the area of culture

D. Hoza

Keywords: apple, varieties, eco-pedological condition,

ABSTRACT

The quality of fruits is very important from two points of view: to select genotypes and to zone correctly the varieties, in order to efficiently value the resources from a certain area.

The culture area had a direct influence on the varieties studied. None of the varieties had a well behavior in all areas, so the assortment was established depending on the behavior of varieties. For the three features studied: the average weight of fruits, the content of dry substance and the taste score, the varieties had different behaviors. Each of them had high values for two of features and smaller for the third one. The Starkrimson variety proved adjustable to Bistrita conditions, where all the parameters studied had big values.

The quality of fruits remains one of the major concerns of fruit growing farmers in order to efficiently value the production. Except the biological features, the quality is influenced also by the area and the technology applied on culture. The present paper presents the results obtained during the analysis of fruits exposed at the 2007 autumn fruits contest, original from different areas.

MATERIAL AND METHOD

The experiment was conducted in the 2007 Autumn, on a lot of fruits original from the main fruit growing centers: Voinesti, Bistrita, Falticeni, Pitesti and Iasi, at the fruits contest unfold at pomology research center Voinesti Dambovita. The analysis was made on varieties original from many areas: Ionathan, Idared, Florida, Generos, Starkrimson and Mutsu, original from Voinesti, but exposed by many producers. The features studied were: the average weight of fruits, the total content of dry substance (determined by refractometer method) and the score obtained after tasting the fruits, calculated as average of the sheets made by the commission members. Based on scores, the following medals were given for each test: gold, for up to 56,6 points, silver for up to 53,3 and bronze for up to 50,3 points.

RESULTS AND DISCUSSIONS

The analysis proved that the varieties have a limited ecological plasticity, the biological potential manifests only in certain areas. For the Golden delicious variety, the most common variety from our country, the average weight of fruits was bigger at the ones original from Bistrita and Voinesti and smaller at the ones original from Falticeni, showing a difference of 17% (fig.1).

The fruits original from Voinesti and Pitesti had more total dry substance than the ones from Iasi. The score obtained when tasting the fruits was less influenced by the other two features and more influenced by the general appearance of fruits, the type of fruit and the taste. This is the reason why some cases there is were no connections between the size of the fruit and the score obtained. So, the fruits original from Iasi had the highest score. The lowest score was obtained by the fruits from Pitesti area.

For the Florina variety, a relatively new one, resistant to diseases, the best area proved to be Bistrita. The fruits were big and contained much total dry substance.

Fig. 1. Golden delicious variety

Therefore, in what regards the average weight of fruits, the best results were obtained in Bistrita area, with over 11,6% over the average and over 33% over the fruits original from Iasi (fig.2).The content of total dry substance was bigger at the fruits original from Bistrita and Voinesti and smaller at the ones original from Iasi, with a difference of 20% between the areas. The scores obtained tasting the fruits showed higher marks for Bistrita fruits, with over 51 points and smaller marks for Iasi fruits, with only 40 points. The Florina variety proves adjustability to different climatic conditions and assures a good commercial quality.

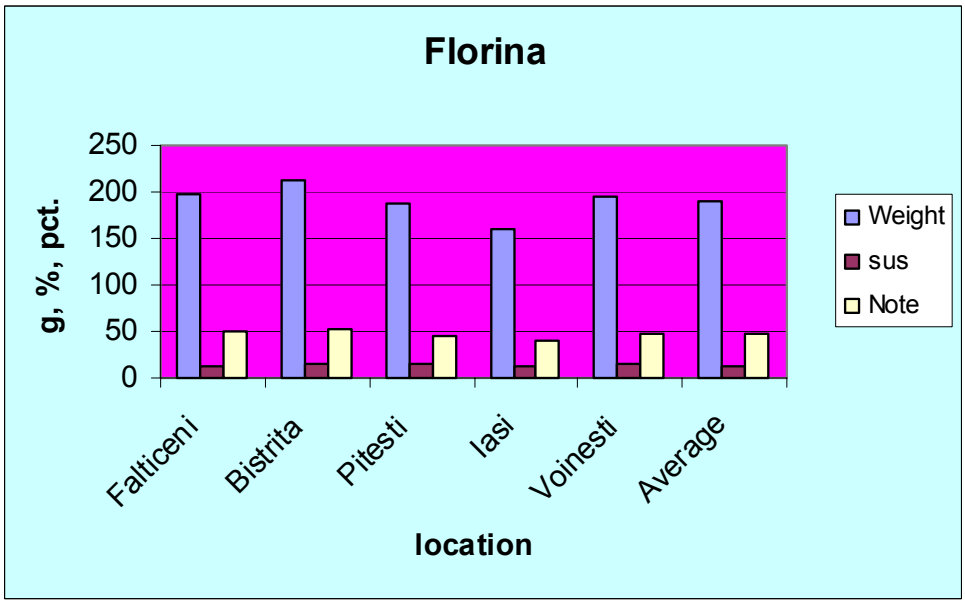

Fig. 2. Florina variety

The Generos variety, an also new variety, resistant to diseases, had a better behavior at Falticeni. The best results for the average weight of fruits were obtained at Voinesti, with over 216g, but the score obtained was only of 44,5 points, excluding the variety from a potential medal (fig.3). At Falticeni, although the average weight was small, of 183 g, the total content of dry soluble substance, of 15,3% and the score obtained, of 54 points, were the biggest.

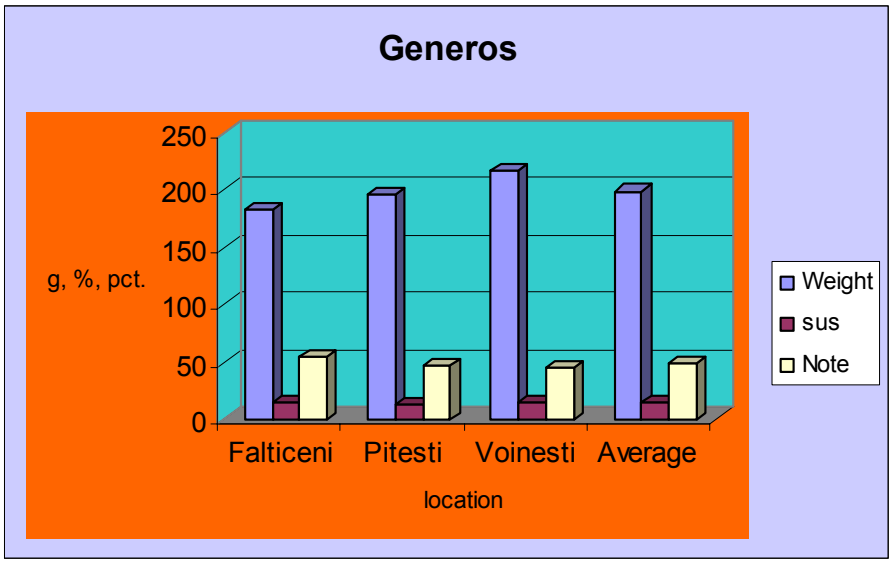

Fig. 3. Generos variety

The Idared variety had a good behavior in Pitesti area, were the average weight of fruits was of 225 g, the score obtained was high, 54,6 points and the total content od dry soluble substance was the biggest. The biggest fruits were obtained in Bistrita, but at tasting the score obtained was of 43 points and the fruits had only 3% of dry soluble substance. In Falticeni area, with this variety, the results obtained were unsatisfactory (fig.4).

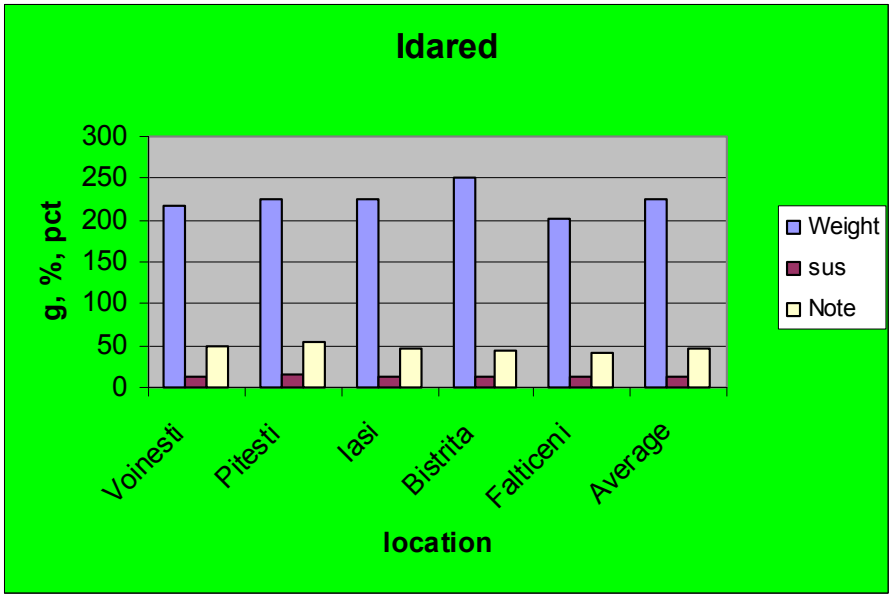

Fig. 4. Idared variety

The Ionathan variety, one of the most common from our country, had a very different behavior between areas. The average weight of fruits was between 225 g at the ones from Pitesti and 130 g at the ones from Iasi and Bilcesti (fig.5). The total content of dry substance was also different and it not depends on the average size of fruits. The smallest content of dry substance was obtained at the big fruits from Falticeni, 205 g and the small fruits from Bilcesti. The fruits from Candesti and from Voinesti had more dry substance, 16,6% and respectively 15.9%. The score obtained at tasting did not have any connections with the other parameters, especially with the dry substance content. Therefore, high scores were obtained in Falticeni and Iasi areas, 59 points, gold medal fruits and small scores at the fruits from Malu cu Flori, with only 45,3 points.

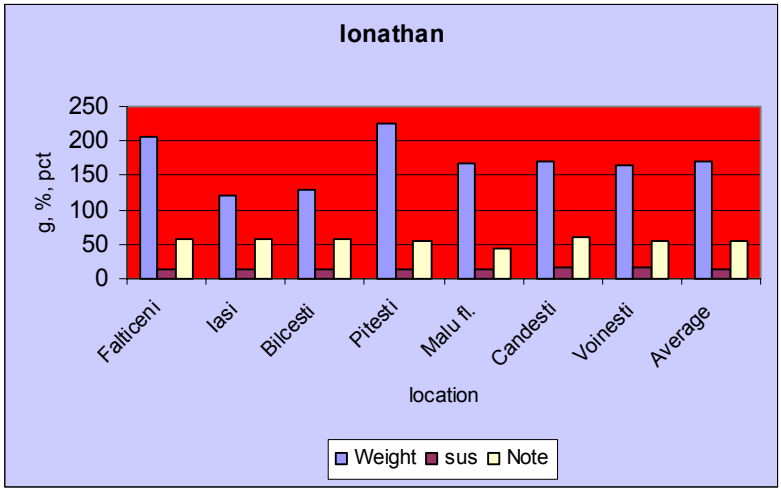

Fig. 5. Ionathan variety

The variety Starkrimson, represented by fruits from 3 different areas, Bistrita, Iasi and Voinesti. From this areas, Bistrita proved to be the area which provided the best conditions, because the fruits reached 257 g, with 16,8% dry soluble substance and 52 points for taste. Lower results were obtained with fruits from Iasi area (fig.6).

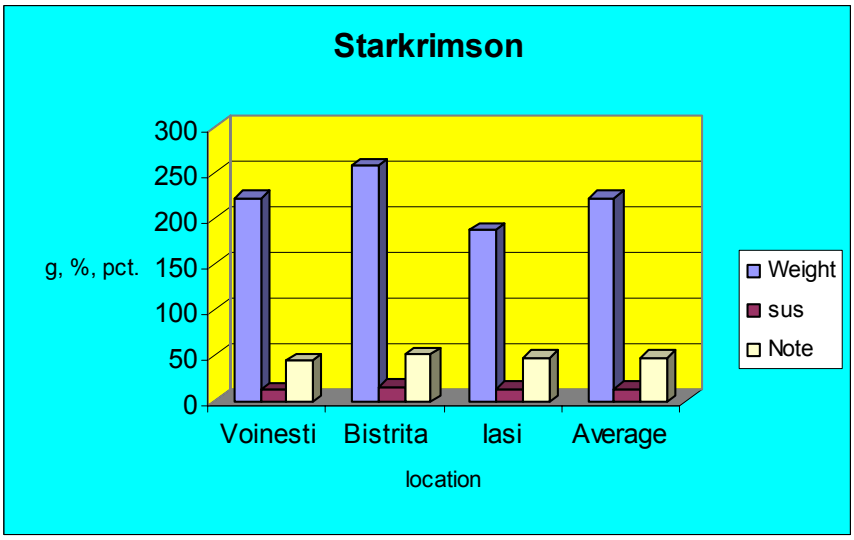

Fig. 6. Starkrimson variety

Comparing the fruits from Mutsu variety from the same area, but from different producers proved the importance of the technology used, not only of the climatic conditions. Therefore, the size of fruits was between 230 g and 330 g, the content of dry substance was between 15 and 19%, and the score for taste was between 61 and 50,3% points (fig.7).

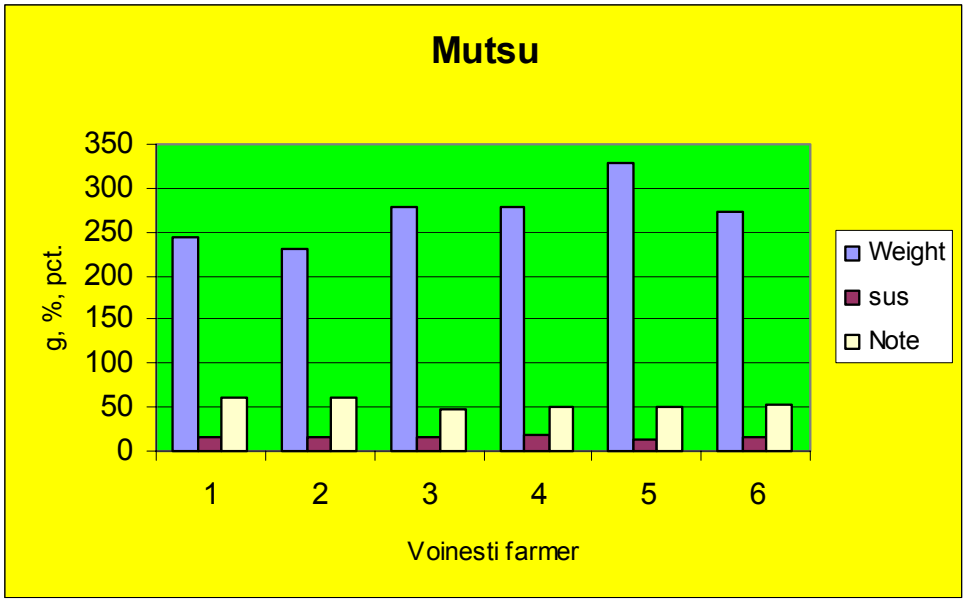

Fig. 7. Mutsu variety

CONCLUSIONS

From the present study the following conclusions can be drought:

1. The area of culture can assure good conditions for varieties to manifest their biological potential;
2. None of the varieties studied reached maximum values for all 3 features in the areas chosen;
3. The technology used in culture in very important in order to obtain quality fruits.

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"Florina" apple tree breed behaviour in different systems of crown pruning

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Keywords: pruning; designing; variants; fruit formations; skeleton branches.

ABSTRACT

The present study is meant to give the fruit farmers on dambovita valley a practical answer so that they could achieve the desired goals and to establish some strategies of managing and maintaining the apple tree orchards intended for biological yield, choosing the crown type being a key element for the capitalization of the fructification potential of these breeds. Florina trees, immune to the apple scab (*venturia inaequalis*) but partial resistant to the powdery mildew of apple (podosphaera leucotricha), planted in 2000, were studied. To improve the biologic potential of this vigurous breed 5 crown shapes were used: multilevel vase, slender spindle, bush-vase, fruit cylinder, discontinuous pyramid. Their results were compared to the typical crown that is dominant on dambovita valley. These crown shapes were taken into an experiment with 6 variants, each repeated 3 times. 3-4 trees were taken in each repetition, adding up to 70 trees. The results obtained after 3 years of crown shaping, which reflect the healthy growth of the trees,show that,compared to the testifier, the biometric values(trunk height, trunk diameter,tree height, crown volume) vary, the maximum values being reached in case of the fruit cylinder and the minimum ones for the bush-vase. Analysing the number of fruit formations, we can see that "florina" trees beared fruits mainly on short branches and less on long branches. This peculiarity will be the starting point for establishing the pruning method. Comparative to the average yield and the number of fruitful branches, the testifier distinguishes itself (the voinesti crown type) followed by the fruit cylinder the smallest yield is given by the discontinuous pyramid followed by the vase types of crown. These facts conduct us to the main conclusion that the voinesti type of crown is a suitfull one for"florina" trees, while the "bush-vase" is not recommended for the vigorous breeds of apple. The "fruit cylinder" crown is also recommended because, under the conditions of the present experiment this type of crown had values close to the maximum concerning the yield and the fruit load also having the advantage that it needed little interventions for shaping and maintaining.

INTRODUCTION

A multitude of crown shapes which enable rich, high quality and constant yields have been tested in apple tree growing worldwide lately.

Professor Nicolae Cepoiu has carried out such research since 1976. The result has been materialized in initiating new crown shapes, among which the belt-bush crown, conceived for the spur breeds of apple, based on the evolution and free fructification of certain branches that were retained on purpose (Cepoiu, 1987) and bush-vase, conceived at the University of Agricultural Sciences, for the "Nana" sour cherry tree (Cepoiu,1989).

Among the pruned crowns without support, the most frequent are: rarefied multi level pyramid, "Leader"pyramid, late vase, bush-vase, slender spindle, pin-bush crown, free flattened palmette, vertical band, Bouche Thomas, Lepage, Heckinger and conic crowns obtained by frame cuts. (N. Cepoiu – Applied fruit farming)

In order to give the fruit farmers on Dambovita Valley a practical answer so that they could achieve the desired goals, in 2004 I initiated a study meant to establish some strategies of managing and maintaining the apple tree orchards intended for biological yield, choosing the crown type being a key element for the capitalization of the fructification potential of these breeds.

The research and documentation activity for the present work was carried out at Voinesti Fruit Tree Research and Production Centre, situated within the Meridional Subcarpathian area, in the centre of the Dambovita fruit tree basin, having as the main goal the rehabilitation of the fruit tree patrimony and the fruit farming development in this area.

The study apple tree plantation is situated on a gentle slope hill side (maximum 5%), having an exposure favourable to a good crown lightning. The soil, with an argillaceous-clayish texture has medium-low fertility. The humus level is between 3.6 and 1.5%, pH is slightly acid 40%, neuter 4% and slightly alkaline 56%, within the Dambovita water meadow.

The climatic conditions were favourable for the fruit tree farming and for differentiating the fruit buds, the average temperature over many years being 8.8°C. The amount of the annual rainfalls was 782 mm, out of which 60% fell during the vegetation period (April – September).

MATERIALS AND METHODS

"Florina" trees, planted in 2000, were studied. The "Florina" breed resulted by interbreeding the "Jonathan" breed with a complex hybrid, the bearer of the resistance gene *Vf*, inherited from *Malus floribunda 821*. "Florina" is one of the breeds that are immune to the apple scab (*Venturia inaequalis*) but have partial resistance to the powdery mildew of apple (*Podosphaera leucotricha*).

The healthy tree bears fruit especially on long branches and blooms late.

The fruit is large, red-violaceous, with firm, juicy and sour pulp, with good gustative virtues.

The parent stock was MM 1o6. The trees were planted within 4/3 m, thus getting a density of 833 trees/ha.

To improve the biologic potential of this vigurous breed 5 crown shapes were used. Their results were compared to the typical crown that is dominant on Dambovita Valley.

These crown shapes were taken into an experiment with 6 variants, each repeated 3 times. 3-4 trees were taken in each repetition, adding up to 70 trees.

After designing (2001), the trees were pruning controlled according to the features of each crown shape.

The technology of controlling the studied crown shapes. Crown designing and controlling works:

For all variants the designing height of the first level was 60 cm. Afterwards shaping was differentiated according to the characteristics of each crown shape.

Variant 1- Testifier – Voinesti controlling type

This is a combination between the "slender spindle" and the "multilevel pyramid" crowns.

The insertion angle of the skeleton branch has an initial value of 70°.

Variant 2- Multilevel vase

Designing:

- 1st year – first level (average number of frames is 4);
- 2nd year – the second level (3 frames)

During the following years 3-4 skeleton branches are carried out for each level, at 50-60cm one from the other. A well balanced distribution is intended. The initial insertion angle of the skeleton branch is 47°.

Variant 3 - Slender spindle

The following procedures are applied for the shaping:

- Shortening the stem to 60 cm in the first year;
- Balancing the skeleton branches (4-5) by shortening them at the same level (60- cm), also in the first year;
- Driving the axis into a herringbone shape through transfer pruning.

The initial insertion angle of the skeleton branch is 47°. Afterwards, through pruning, a widening of the angles at the crown base is intended, so that the altered angle can reach 61° (after the 2nd year pruning) and 77° (after the 3rd year pruning).

Variant 4 – Bush-vase

Designing consists of:

- Shortening the axis to 80 cm
- Carrying out 5-6 permanent branches, first class
- Getting 3-4 second class offshoots on each first class branch.

The initial insertion angle of the skeleton branch is 56°. A widening to about 90°is meant through pruning.

Variant 5 – Fruit cilinder

- A crown enframed into a 1.75 – 2m diameter fruit cylinder is created through contour pruning. A crown volume development is intended.
- Limiting the tree height to 3 m.

The insertion angle of the skeleton branch is 45°. In the case of the fruit cylinder we do not intend to widen this angle, the extensions of the skeleton branches having a fairly vertical position. Rarefaction pruning of the inner crown is performed instead.

Variant 6 – Discontinuous pyramid

A typical pyramid is carried out. Its axis is discontinued after 3 levels.

Designing:

- 1st year – first level (4-5 skeleton branches);
- 2nd year – second level (3 skeleton branches);
- 3rd year – third level (3 skeleton branches).

The insertion angle of the skeleton branche is 48°. After pruning the angle changes, finally reaching the value of 75°.

RESULTS AND DISCUSSIONS

The results obtained after 3 years of crown shaping ,which reflect the healthy growth of the trees, the permanent and non-permanent structure of each crown shape are shown in tables number 1,2 and 3.

Table 1 shows that, while experimenting the 6 crown shapes, the trunk height maintained at the designing level, with unimportant alterations concerning the trunk diameter.

Compared to the testifier, the 5 crown shaped trees have a diameter between 7.4 cm (bush-vase and discontinuous pyramid) and 7.9 cm (fruit cilinder).

The designing height of the 2nd level was carried out at close distances from the testifier (Voinesti controlling type). For the levelled types (levelled pot and discontinuous pyramid), the designing was carried out at 117, respectively 120 cm. For the discontinuous pyramid, the designing of the last level was carried out at about 60 cm from the 2nd level.

The trees' height, for the experimental forms, was situated at close values to the testifier. The only crown shape that exceeds the testifier (10 cm) is the slender spindle.

The biggest difference compared to the testifier is the bush-vase, with a 59 cm smaller height.

With this permanent structure, the calculated crown volume, compared to the testifier, reached a maximum value in the case of the fruit cylinder (2.69 m^3 larger than the testifier's volume).

According to table 2,a variable number of fruit formations appeared on the permanent crown structure, the average value being 286 branches, out of which 183 short branches and 103 long branches. The ratio between these formations (1,78: 1) shows the fact that the trees bear fruit mainly on short branches and less on long branches.

But if we analyse the number of fruit formations, we will see that it is constantly balanced. Thus, out of 177 fruit buds (average value), 112 are situated on short branches – pricks and 65 fruit buds on long branches – twigs and scions. This peculiarity will be the starting point for establishing the pruning method.

Taking into account the average, the largest difference is given by the discontinuous pyramid (with 78 fruitful branches, that is 44% more), followed by the fruit cylinder (+23%) and the testifier (+19%). At the opposite end there is the bush-vase, whose number of fruitful branches is reduced to half compared to the average.

As for the number of fruit buds, the fruit cylinder distinguishes with an amount of 42 fruit buds (+23%) compared to the average. The least amount of fruit buds is met again in the bush-pot crown.

Instead, in the case of the bush-vase crown we meet the most balanced ratio between the number of fruit buds situated on long branches and number of fruit buds situated on short branches, respectively 1.2: 1 for the long branches.

The research carried out by professor Cepoiu (Doctoral Dissertation – 1974), proves that the Winter Banana breed, with a large number of fruit buds on a twig, enabled economic yields only on long branches.

Comparative to the average yield (added in 2 years), with a value of 12.14 t/ha, the testifier distinguishes itself (the Voinesti crown type) with an added amount of 2.89 t/ha, followed by the fruit cylinder, which outbalances the average amount with 1.68 t/ha (Table 3). The smallest yield is given by the discontinuous pyramid (-1.89 t/ha) followed by the vase types of crown (-1.21 t/ha for bush vase). Although the yield is relatively low in the case of the bush-vase crown, due to the small number of fruit, as well as to the small number of fruit buds, the quality of the fruit is superior to all other types of cown (a fruit weighs 178 g, 16 g over the average value).

CONCLUSIONS

The results show that, in the case of the "Florina" breed there is a positive correlation between the crown's volume and the number of levels, on one hand, and the load of fruit, respectively the yield's value, on the other. Thus, the types of levelled crowns had higher yields than the open shapes, such as the "vase" type.

For a deeper study the "Fruit Cylinder" crown is recommended. Under the conditions of the present experiment this type of crown had values close to the maximum concerning the yield and the fruit load. The "Fruit Cylinder" also had the advantage that it needed little interventions for shaping and maintaining.

The "bush-vase"shaped apple trees did not have a favourable reaction.They got the smallest yield compared to the other crown types.The type of crown is not recommended for the vigorous breeds of apple.

The Voinesti type of crown is a suitfull, balanced one, with a top yield of 15,03 t/ha and all the other values above the average ones.

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Tables

Table 1. Biometric measurements

Crown shape	Trunk height (cm)	Trunk diameter (cm)	1st level designing height (cm)	3rd level designing height	Tree height		Crown volume	
					Tree height (m)	Difference in contrast with the testifier	Crown volume (m3)	Difference in contrast with the testifier
V1- voinesti crown	60	6.3	117	-	3.52	-	1.81	-
V2-multilevel vase	60	7.5	117	-	3.17	-0.35	2.57	+0.76
V3-slender spindle	60	7.8	-	-	3.53	+0.01	3.24	+1.43
V4-bush vase	60	7.4	-	-	2.93	-0.59	3.47	+1.66
V5-fruit cilinder	60	7.9	-	-	3.28	-0.24	4.5	+2.69
V6-discontinuous pyramid	60	7.4	120	181	3.01	-0.51	2.4	+0.59

Table 2. The ability of trimming with fruitful branches

Crown shape	Total fruitful branches	Total fruitful buds	Total short branches	Total fruitful buds (short branches)	Spurs	Pricks	Total long branches	Total fruitful buds (long branches)	Twigs	Shoots	Scions
V1- voinesti type of crown	**342**	**190**	229	**149**	80	149	113	**41**	76	34	3
V2-multilevel vase	**280**	**190**	180	**105**	75	105	100	**85**	20	77	3
V3-slender spindle	**218**	**145**	133	**80**	53	80	85	**65**	24	60	1
V4-bush vase	**140**	**101**	72	**46**	26	46	68	**55**	17	50	1
V5-fruit cylinder	**352**	**219**	215	**130**	85	130	137	**89**	56	81	4
V6 Discontinuous pyramid	**364**	**215**	260	**158**	102	158	104	**57**	52	47	5
Average:	**286**	**177**	183	**112**	71	112	103	**65**	41	59	3

Table 3. Average yield – per crown type

Crown type	Average fruit weight (g)		Yield per tree (kg)		Yield per hectare (t/ha)		Added yield Anii VI+VII (t/ha)	Differences compared to the average value (+/-) (t/ha)	Yield per crown m3 (kg/m3)	
	Trees year VI	Trees year VII	Trees year VI	Trees year VII	Trees year VI	Trees year VII			Trees -year VI	Trees year VII
V1- voinesti type of crown	150	158	6.68	11.37	5.56	9.47	15.03	+2.89	1.69	4.42
V2 multilevel vase	142	164	4.97	7.87	4.14	6.55	10.69	- 1.45	1.93	2.42
V3-slender spindle	120	152	5.76	8.81	4.8	7.33	12.13	-0.01	1.77	2.53
V4-bush vase	132	178	4.06	9.07	3.38	7.55	10.93	-1.21	1.17	1.76
V5-fruit cylinder	134	165	5.89	10.72	4.9	8.92	13.82	+1.68	1.3	3.77
V6 Discontinuo us pyramid	127	158	4.58	7.74	3.81	6.44	10.25	- 1.89	1.9	4.27
Average:	134	162	5.32	9.26	4.43	7.71	12.14	-	1.62	3.2

The behavior of some nectarine varieties in conditions of Didactic Station Timişoara

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Keywords: nectarine, varieties, fruit binding degree, production, estimative production

ABSTRACT

The peach tree is the most important species by its fruit qualities and biological featuresof trees, being considered the IIIrd fruit culture as economical importance and culture perspectives in our country. In Romania, the peach tree occupies the 6th place after the apple tree, plum tree, sweet cherry tree, appricot tree and peat tree. If between this species was very cultivated, after 1990 the peach tree culture known a progressive decline. The nectarine culture amplified from 1970, thanks to the collaboration between dr. Vasile Cociu and prof. Leon Hough from the University Reurgers, New Jersey, U.S.A. researchers who made up the genetic bases of some nectarine varieties. The ample studies concerning the nectarine cultures were made by dr. Monica Murvai, dr. Antonia Ivascu, prof. Draganescu E. And others

INTRODUCTION

In Timisoara, the studies concerning peach and nectarine culture was begining in 1982-1990 period, was continous after taht and in present here is estabilished the national collection of peach and nectarine who content 259 varieties and hybrides on the globe.

In this paper we have observed the behavior of some nectarine varieties in conditions of the Didactic Station Timisoara, concerning their productivity, the anterior studies showing up the fact that in this conditions the productions obtained are smaller, on one side bacuse of the climatic accidents and on the other side because of the bad technology culture used in our country.

MATERIALS AND METHOD

The biological material content 8 nectarine varieties: Ark 90, Delta, Cora, Romamer (witness), Hardires, Durbin nectarin, Suntre nectarin and White Das.

The research goal was observing the fruit bending degree and the obtained productions, during the years 2006 and 2007.

The nectarine trees were planted in the spring of 2001 at a distance of 4 x 3 meters, obtaining a density of 833 trees/hectar. The nectarine trees were grafted on a mirobolam, the top tree system being a "free palmet" and the type of soil is cambic cernosiom. The culture tecnology was the common one.

The working method was of stationary type in two steps:

- first step: on field, base on observing the fruit bending degree, counting the fruits and weighting them;
- second step: in the laboratory, based on calculating and interpretation the collected data.

In the first step we did the following observations: marking 3 trees for each variety, counting the fruits that remained on the tree after the physiological and premature falling of these, collecting the fruit samples in order to weight them, determination of his mean weight and estimating the production.

The second step consisted in calculating the obtained data, the experiment being a monofactorial one and the interpretation of the data was made by the analysis variance method.

RESULTS AND DISCUSSIONS

The fruit binding degree was established after counting and calculating the fruits that were left on the tree after the physiological and premature falls, considering the fact that there were not done any chemical or mechanical procedures for the fruit rate-setting process and in that period there were not regeistered any climatic accidents that could have compromised the fruit production. The results obtained concerning this indicator are presented in tables 1 and 2.

Table 1. Fruit binding degree for the nectarine varieties in 2006

No.	Variety	No. of bind fruits after pollination - fecundation	No. of fruits after the physiological and premature falls	% of binding
1	Ark 90	220	178	80,9
2	Delta	415	250	60,24
3	Cora	238	210	88,23
4	Romamer	310	290	93,5
5	Hardired	324	285	87,9
6	Durbin nectarin	260	198	76,15
7	Suntre nectarin	230	180	78,26
8	White Das	356	215	60,39

In 2006, the greatest fruit binding degree was oobserved for Romamer variety (over 90%), followed by Cora and Ark 90 varieties, which over passed 80%. On the opposite side, there are Delta and White Das varieties which had a fruit binding degree of 60%.

Table 2. Fruit binding degree for the nectarine varieties in 2007

No.	Variety	No. of bind fruits after pollination - fecundation	No. of fruits after the physiological and premature falls	% of binding
1	Ark 90	196	140	71,42
2	Delta	311	216	69,45
3	Cora	191	128	67,01
4	Romamer	437	217	49,65
5	Hardired	234	198	84,61
6	Durbin nectarin	173	121	69,94
7	Suntre nectarin	193	165	85,49
8	White Das	487	184	37,78

In 2007, the greatest fruit binding degree was observed for Suntre nectarin variety, closely followed by Hardired variety, both of them having values around 85%. On the opposite side, there is White Das variety that had a fruit binding degree under 40%. The other varieties had values around 70% bind fruits.

Comparing the studied years, we can see that in 2006 the fruit binding degree was higher, when most of the varieties over passed 80%. Among the varieties, the most constant ones were: Hardired, Suntre nectarin and Durbin nectarin. The most different

fruit bending degree was obtained for Romamer and White Das varieties, to which this indicator was almost half in 2007 than in 2006.

The medium weight of nectarine fruits obtained in 2006 and in 2007 is presented in tables 3 and 4.

Table 3. The medium weight of nectarine fruits in 2006

No.	Variety	Medium weight of fruits (g)	Relative values %	Difference for the witness	Significance
1	Ark 90	65,67	98,01	-1,33	-
2	Delta	70	104,48	3,0	-
3	Cora	88,33	131,84	21,33	XXX
4	Romamer	67	100	0	mt
5	Hardired	61,67	92,04	-5,33	-
6	Durbin nectarin	67,33	100,5	0,33	-
7	Suntre nectarin	62,33	93,03	-4,67	-
8	White Das	49,66	74,13	-17,33	000

DL5%=7,88 DL1%=10,92 DL0,1%=15,17

Out of this table, we can see that in 2006 the biggest weight of fruits was obtained for Cora variety, the difference for the witness being very significant positive. The lowest value was registered for White Das variety, the difference for the witness being very significant negative. The other varieties had values close to the witness, which is why there was no significance obtained.

Table 4. The medium weight of nectarine fruits in 2007

No.	Variety	Medium weight of fruits (g)	Relative values %	Difference for the witness	Significance
1	Ark 90	76,33	112,04	8,20	X
2	Delta	63,33	92,95	-4,80	-
3	Cora	98,49	144,55	30,35	XXX
4	Romamer	68,13	100	0	mt
5	Hardired	61,0	89,53	-7,13	0
6	Durbin nectarin	60,0	88,06	-8,13	0
7	Suntre nectarin	58,0	85,13	-10,13	00
8	White Das	45,5	66,78	-22,63	000

DL5%=6,11 DL1%=8,47 DL0,1%=11,76

Out of this table, we can see that in 2007 the the biggest weight of fruits was also obtained for Cora variety, the difference for the witness being very significant positive. A relative high weight was registered for Ark 90 variety, the difference for the witness being significant positive. The lowest value was registered like in the passed year 2006 for White Das variety, the difference for the witness being very significant negative. We can see that in 2007 most of the fruits were small reason why there were distinct significant negative differences for Suntre nectarin variety and significant negative for Durbin nectarin and Hardired varieties.

Comparing the two studied years, we can say that in 2007 the fruits obtained were smaller than in 2006, and on the whole the obtained weights are under the mentioned value in spciality litarature, exceppting Cora variety, whch had bigger fruits in conditions of Timişoara.

The production per tree in 2006 is presented in tables 5.

Table 5. Nectarine production per tree in 2006

No.	Variety	Medium production value kg/tree	Relative value %	Difference for the witness	Significance
1	Ark 90	11,69	60,15	-7,74	000
2	Delta	17,50	90,07	-1,98	0
3	Cora	18,55	95,47	-0,88	-
4	Romamer	19,43	100	0	mt
5	Hardired	17,57	90,44	-1,86	0
6	Durbin nectarin	13,32	68,59	-6,10	000
7	Suntre nectarin	13,4	68,97	-6,03	000
8	White Das	8,94	46,01	-10,49	000

DL5%=1,68 DL1%=2,33 DL0,1%=3,24

In 2006, Romamer variety – witness of the experiment, registered the highest production per tree, reason why the other varieties registered negative significances. However, the lowest production was obtained for the White Das variety, followed by Ark 90 variety, in both cases the differences for the witness being very significant negative. A production close to the witness was obtained Delta and Hardired varieties, in both cases the significances being negative.

The estimative productions per hectare in 2006 varied between 16,18 t for Romamer variety and 7,44 t for White Das variety in both cases the obtained values being under those mentioned in the speciality literature.

The production per tree in 2007 is presented in table 6.

Table 6. Nectarine production per tree in 2007

No.	Variety	Medium production value kg/tree	Relative value %	Difference for the witness	Significance
1	Ark 90	10,67	75,06	-3,54	000
2	Delta	13,68	96,25	-0,53	-
3	Cora	12,60	88,69	-1,61	0
4	Romamer	14,21	100	0	mt
5	Hardired	12,05	84,78	-2,16	0
6	Durbin nectarin	7,76	54,63	-6,45	000
7	Suntre nectarin	10,64	74,90	-3,57	000
8	White Das	7,5	52,80	-6,71	000

DL5%=1,60 DL1%=2,23 DL0,1%=3,09

Even in 2007, Romamer variety– of the experiment, registered the highest production per tree, reason why the other varieties registered negative significances.. However, the lowest production was obtained for White Das variety, followed by Durbin nectarin and Ark 90 varieties, in all the cases the differences for the witness being very significant negative. A close production to the witness was registered for Delta variety, which had no significance. Cora and Hardired varieties obtained intermediary productions, the difference for the witness being significant negative.

The estimative productions in 2007 varied between 11,83 t for Romamer variety and 6,25 t for White Das variety, in both cases the value obtained being under those mentioned in the speciality literature and under the level of those registered in 2006.

CONCLUSIONS

Concerning the fruit binding degree, the higher values were obtained in 2006, when most of the varieties over passed 80%, the climatic conditions in spring 2007 determined the decrease of this parameter.

Concerning the fruit production we can say that it was higher in 2006 comparing them to those in 2007, but in both cases the registered values were smaller than tose mentioned in the speciality literature.

The explanation for these low productions might be: - the appearance of late frosts in April 2007;

1. not favorable climatic conditions in 2007, which were very hot and dry summer and the soil conditions of the orchard;
2. the soil of the Didactis Station Timisoara has a greater clay content and the phreatic water is very close to the surface, which represent more reasons for the decraese of the nectarine productions.

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Research on *ex vitro* rooting of raspberry microcuttings obtained from in vitro micropropagation

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Keywords: *Rubus idaeus* L., greenhouse, substrate, indolilacetic acid, number of roots, length of roots, cultivar

ABSTRACT

In the classical method of raspberry micropropagation, rooting phase is done *in vitro*. The trials were undertaken to replace *in vitro* rhizogenesis by a direct ex vitro rooting. The micropropagated shoots of raspberry, cultivars Bulgarski Rubin, Malling Exploit, Cayuga, Citria and Ruvi were trated as soft cuttings and rooted ex vitro (in non-sterile conditions). The evaluation of ex vitro rooting proved that the rhizogenesis happens normally in the perlite substrate versus that induced on *in vitro* culture. The highest percentage of the rooted plants directly on perlite substrate was obtained when 10.0 mg/l indolilacetic acid (IBA) application was done. Three varieties out of the five tried showed over 90.0% rooting frequency. Ex vitro rooting may provide a better quality of the rooted raspberry plants.

INTRODUCTION

Present trends throw a new light on the small fruits species. Several characteristics of thesespecies stand for their extended growing, the raspberry holding a major place thanks firstly to its rich content in nutrients with a therapeutic value. The studies on raspberry micropropagation (*Rubus idaeus* L.) enabled to develop an *in vitro* culture technology, efficient enough to be of help to the breeders and nurserymen for producing high amounts of uniform and disease free plants (Anderson, 1980; Donnelly and Daubeny, 1986; Hoepfner and Nestby,1991; Isac, 2000; Snir, 1981; Vertesy,1979; Welander,1985). Although the rooting techniques for raspberry are various, this stage remains difficult to attend and costs are also high. Some workers choose to root the raspberry directly into the soil (Sobczyewicz,1981), peat-perlite or peat-sand mixture, under mist or high air humididity with good results. To reduce costs of micropropagated plants, the aim of the present study was to evaluate posibility to replace *in vitro* rhizogenesis by a direct ex vitro rooting at some important and new raspberry cultivars.

MATERIALS AND METHODS

In order to establish ex vitro rooting, studies were done on five raspberry cultivars: Bulgarski Rubin, Malling Exploit, Cayuga, Citria and Ruvi. For this purpose microshoots micropropagated on Murashige-Skoog medium with 3 mg/l 6-benzylaminopurine (BAP), 0.1 mg/l indolilacetic acid (AIA) and 50 mg/l ascorbic acid were used (Isac, 2000).

After cutting, the microshoots were planted in the greenhouse on a sterile perlit substrate to which 5, 10 and 25 mg/l indolilbutiric acid (IBA) solutions were used. For each treatment, 100 microshoots of each variety were used. The air humidity was maintained by repeated moisturing. After two weeks, it was sprayed a nutritive solution, Knop.

In 30 days after planting, the rooting capacity and quality was evaluated by recording the number of rooted plants (%), the number and root length as well as the height of plants (as average).

RESULTS AND DISCUSSION

The experimental results regarding ex vitro rooting of the raspberry microshoots indicated that the percentage of plants rooted directly on the perlite substrate in the greenhouse was similar to that with *in vitro* rooting for all the five varieties tried (Isac, 2003). So, the average percentage of ex vitro rooted plants ranged from 70.8% with Bulgarski Rubin cultivar to 79.8% with Citria cultivar.

Under the glasshouse conditions, IBA applications (auxin largely used for *in vitro* rooting of the raspberry shoots) on the perlite substrate had favourable effects on the rhizogenesis capacity. For the four cultivars out of five (except to Bulgarski Rubin cultivar), IBA application (5.0 mg/l) increased the development of the *ex vitro* rooted microshoots (by 1.2% for Citria cultivar and by 12.7% for Ruvi cultivar). The highest percentage of the rooted plants directly on perlite substrate was obtained when 10.0 mg/l IBA application was done. The values were 10.3% with Malling Exploit and 15.9% with Cayuga cultivar (Fig 1), so three varieties out of the five tried showed over 90.0% rooting frequency.

Utilization of a much higher IBA concentration (25.0 mg/l) to induce *in vitro* rhizogenesis proved to be inadequate because under the same conditions, Malling Exploit and Cayuga varieties showed a lower average percentage of rooted microshoots than in case of absence of this phytohormone in the perlite substrate. The frequency of increase of the rooted plants of Bulgarski Rubin and Citria cultivars was nonsignificant (Fig. 1). The evaluation of ex vitro rooting proved that the rhizogenesis happens normally in the perlite substrate versus that induced on *in vitro* culture. In the former case, the average number of roots per shoot is slightly higher (3.8) than that of *in vitro* rooting (3.3 with Cayuga cultivar and 4.5 with Ruvi), (Fig. 2), with nonsignificant differences between all cultivars evaluated.

It was noticed that IBA application (5.0 mg/l) did not induce an important raise of the average number of roots developed, except to Cayuga cultivar. In return, when IBA concentration was 10.0 mg/l induced an increase in the average number of roots/shoot with four of the five varieties tried (ranging from 0.7 with Ruvi cultivar to 4.1 with Bulgarski Rubin cultivar). The favourable influence of IBA application is absolutely obvious by doubling the average number of roots developed per shoot with Bulgarski Rubin and Cayuga cultivars (Fig. 2), versus to the control where IBA was not added to the perlite substrate.

Application of a very high IBA concentration (25.0 mg/l) proved to be unfavourable because the average number of roots/shoot subjected to ex vitro rooting was similar to that of the control.

Another parameter for the quality evaluation of raspberry plants was the root length up to the end of ex vitro rooting stage which was similar to *in vitro* rhizogenesis. So, the average lenght of roots varied between 1.3 cm (Bulgarski Rubin cultivar) and 1.8 cm (Citria şi Cayuga cultivars), (Fig. 3). The application of 5.0 mg/l IBA solution did not show a significant influence on the root lenght developed from various varietal raspberry shoots except to Bulgarski Rubin cultivar where a strong interaction between auxin concentration and genotype was noticed. The application of 10.0 mg/l IBA solution had a favourable effect on both the average number of roots per shoot and also to a certain extent on the root lenght for all five varieties tried. Therefore, in case of ex vitro rooting, it was a difference of 1.1 cm root length (Bulgarski Rubin cultivar – Fig. 3) which represents over 25% increase versus the control. Our data showed that when

25.0 mg/l IBA was applied the ex vitro root length of raspberry shoots was close to that of the control.

The benefic influence of IBA application on the raspberry plants developed by ex vitro rooting became evident by the averge plant height at the end of rooting stage versus to the control (1.5 cm for Citria cultivar and 2.2 cm for Ruvi cultivar). In case of 10.0 mg/l IBA application, the differences in plant height varied between 0.6 cm (Ruvi cultivar) and 1.2 cm (Citria cultivar), (Fig. 4).

CONCLUSIONS

Ex vitro rooting of some raspberry varieties as an efficient and cheaper alternative to *in vitro* rooting, demonstrated that the rhizogenesis takes place normally in a perlite substrate with 10.0 mg/l IBA added under glashouse conditions. The percentage of plants rooted directly in the glasshouse was similar to that obtained *in vitro*, the maximum rooting frequency being 90% for all the cultivars tried.

The overall results of this experiment indicated that ex vitro rooting may provide a better quality of the rooted raspberry plants, having also positive consequences on the aclimatization in the glashouse and certainly on the percentage of viable plants for the field transfer.

Our data suggest also a sort of interaction between the auxin concentration applied in the rooting substrate and the genotype, that is a possible explanation for the increased percentage of the rooted microshoots of Ruvi cultivar even if a very high IBA concentration was applied.

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Figures

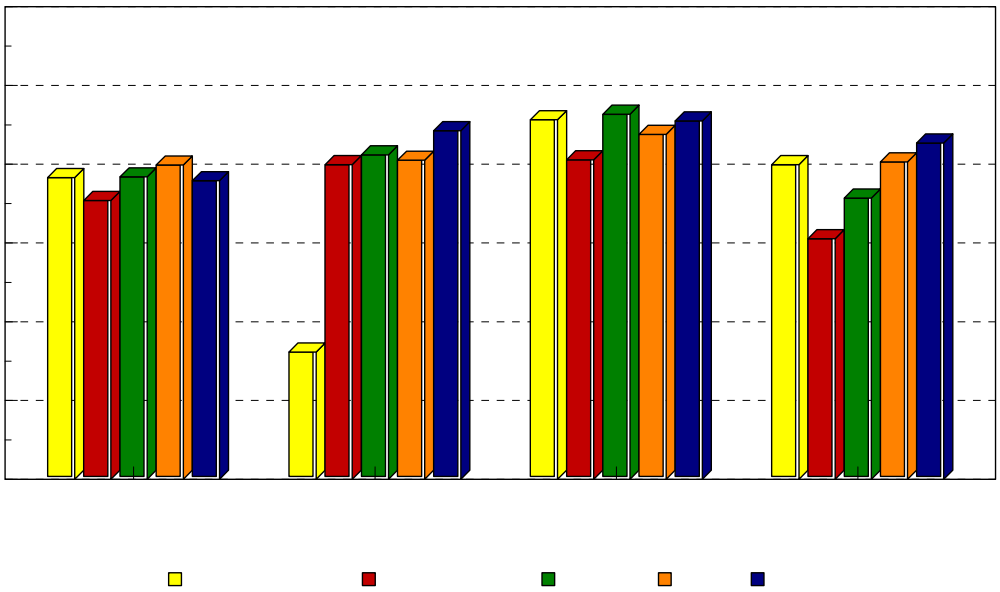

Fig. 1. *Ex vitro* rooting capacity of raspberry microshoots.

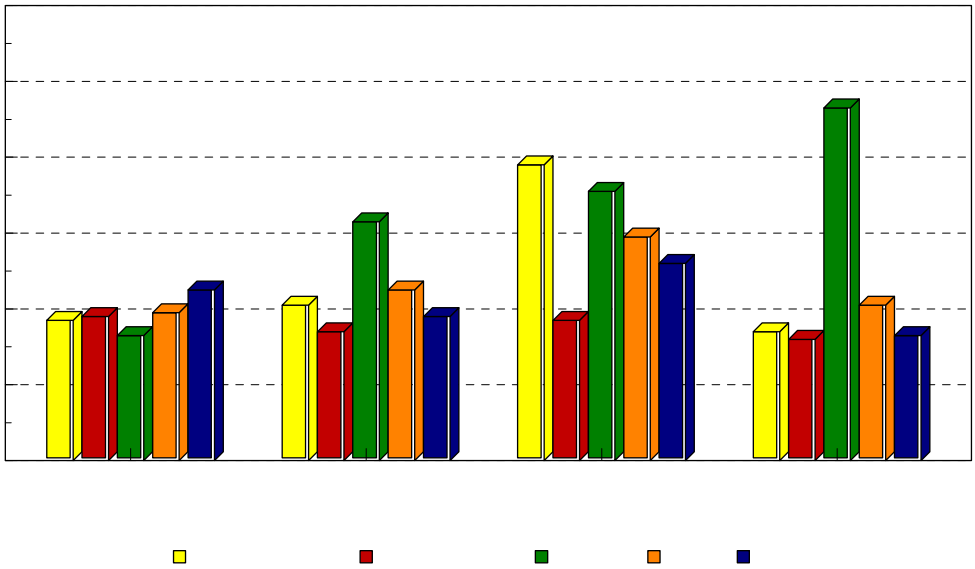

Fig. 2. Variation of the average number of roots developed ex vitro by the raspberry microshoots

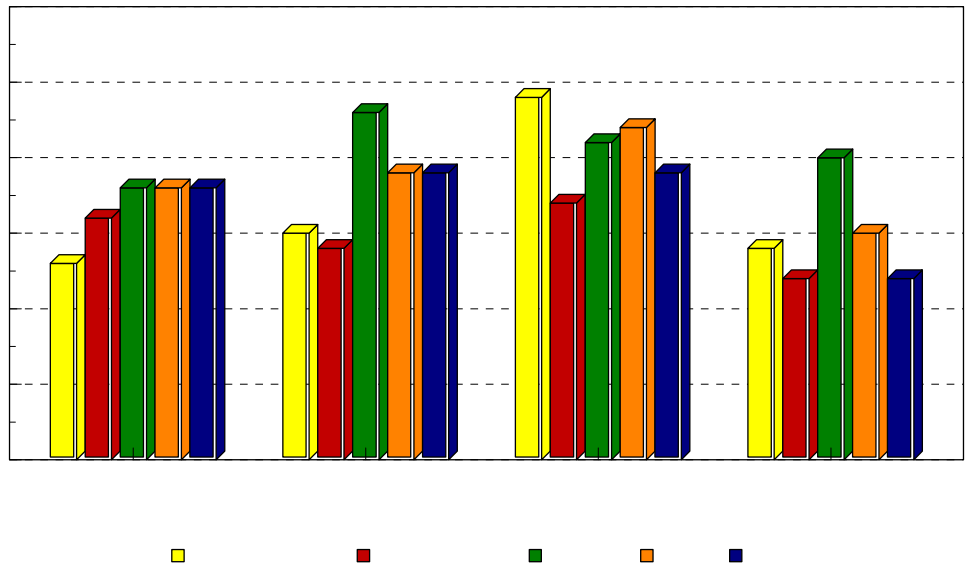

Fig. 3. Variation of the average length of roots developed ex vitro by the raspberry microshoots.

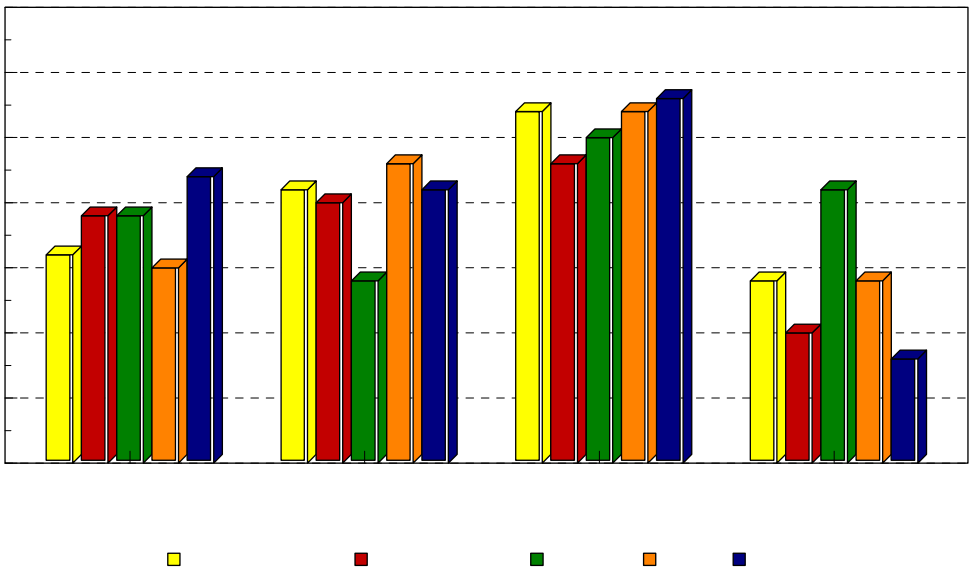

Fig. 4. Variation of the average height of raspberry plants developed by ex vitro rooting.

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Research concerning the establishment of the soil maintenance technology in apple orchards in the Voineşti-Dambovița area

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Keywords: herbicide, orchard, soil, variety, production.

ABSTRACT

In the new social-economic circumstances, the Romanian fruit growing has perspectives as a result of higher and higher demands for fruits, as well as because of the profitability of this cortical branch of production. This demands not just the usage of some associations of precious species with mother/father plant of low vim, adequate to the pedoclimatic, but also the usage of excellent technologies of soil maintenance and farming, weed killing, fertilization phyto protection, fighting against diseases and pest control, mechanization.

INTRODUCTION

In the tree farming an essential role is represented by soil maintenance work which is executed to insure the conditions in which trees will grow and yield fruit, through weed killing and maintaining an acceptable structure of the soil.

The soil work depends on the maintenance system and differs depending on the climatic conditions, the farming system, the cultivated specie.

MATERIALS AND METHODS

The purpose of the research:

Through the present thesis: “Research concerning the establishment of the soil maintenance technology in apple orchards in the Voinesti - Dambovita area” it is pursued: sorting the weeds in he apple orchards, establishing the efficacy of some herbicides in pest control and establishing methods of soil maintenance under economic efficiency circumstances.

Through the accomplished researches it has been established the degree of weeding, herbicides and the most efficient doses in pest control in the apple orchards, methods for soil maintenance with beneficial influences over the retention of soil fertility and humidity in the droughty periods, as well as the economic efficacy.

The objectives of the research

- The maintenance and improvement of the soil’s characteristics, as well as its improvement through the contribution brought by weeds formed between the lines.
- The establishment of founding and exploitation in the maintenance system technology with weeds in the apple plantation.
- Looking into knowing the weeding degree in the apple orchards and setting the optimal moments to apply the herbicide.
- The establishment of some technological sequences in soil maintenance in the apple orchards, referring especially to the utility of herbicides and pest control in a biological way, in order to obtain larger productions of apples at low as possible prices.

In order to reach the established objectives, the researches were organized in apple orchards belonging to SPDP Voinesti, Farm nr. 1, taking into account:

a) establishing the efficacy of some herbicides in pest control in apple orchards
b) establishing methods of soil maintenance in apple orchards.

RESULTS AND DISCUSSIONS

Establishing strategies concerning pest control in apple orchards:

Based on the results obtained previously showed, there were elaborated strategies in pest control in apple orchards, which are presented in diagram 1, 2 and 3.

Strategy nr. 1

When the orchards are infected with monocotyledonous weeds, especially *Agropyrum repens*, it is used for pest control one of the post-emergent products: Touvhdown CE, Ground-UP, Glifosat Borzesti, Roundup 360 CE, Gliphosat 360 SL NAF 595, FRY 24 LC, Glialka 36 EC, Folar 525 FW, Basta 14 SL, Glyphogan 480 SL, administrated when weeds are in full development and have a maximum height of 15-20 cm.

Strategy nr. 2

When dycotyledonous weeds predominate, a good efficacy in pest control is achieved through administrating post-emergent products Starane 250 EC , Salt DMA 21/ha or Icedin Forte. But, in order to combat monocotyledonous weeds as well, herbicide Starane 250 EC is administrated together with Glyphogan 480 SL. On the weed hearths it can be applied one of the exemplified products from Strategy nr. 1.

Strategy nr. 3

When annual mono and dicotyledonous weeds predominate, good results are obtained when applying pre-emergently in early spring one of the products: Simanex 50 SC in a dose of 3-3.5l/ha or Simadon50 PU 6-8 kg/ha, Stomp 33EC 5l/ha, Vegepron DC 5-8l/ha, which ensures protection for at least 3 months. In case weeds appear in the hearths, it can be applied one of the products mentioned at Strategy nr. 1.

For establishing the most convenient strategy, herbicides and optimal annual doses, it’s necessary to identify with care the weeds in the orchards.

After 3-4 years of total herbiciding, the weed reserve diminishes very much.

On trees, the recommended herbicides do not produce phyto toxictoxic effects.

Likewise, it is avoided touching the tree leafs with products like Starane and Icedin Forte.

Production and the medium weight of the fruit: from the figures presented in the table, we may notice that the medium production of Generos/MM 106 fruit was between 28,9 and 36,0 t/ha, being higher for the fourth variant, fallow on the interval, with vegetal material coming from the weed mowing on the in between tree rows. The same happens with Florina/MM106 whose production was between 30,3 and 35,3 t/ha, with a medium fruit weight between 156 -163 g for Generos and 148 -153 for Florina. The bigger fruit appear with the fourth variant.

CONCLUSIONS

For an efficient control of the weeds of the apple tree orchards, we should take into account the weeding level and the floristic composition.

The weeding level of the orchards should be established annually so that the most appropriate strategy is set up and also the herbicides and the optimal doses.

During 2000-2003, after testing herbicides period, three strategies for the total control of the apple tree orchards weeds were established as follows 1 strategy, 2 strategy, 3 strategy.

The maintenance system of the fallowing soil on the in between rows and worked in four year cycles can be extended for the rainy areas too ; having as a positive effect –moderation of the vegetative growing of the trees without a negative influence over the quantity and quality of the production ; by comparison with the ploughing system maintenance .

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Tables 1. Production and medium weight of the fruit
(Voinesti 2005-2006) - 833 trees/ha

Variety and variant	Fruit Production Sum up (t/ha)					The fruit medium
	2005	2006	t/ha	Dif.(+/-)	Semnificația	- g -
l GENEROS/MM 106 variety V_1 – (Mt) – worked field	28,7	37,5	33,1	-	-	156
V_2 – fallow on theinterval by manual work on the fruit tree row	24,4	36,4	30,4	- 2,7	-	157
V_3 – fallow on the interval and herbicide on the tree row	17,7	40,1	28,9	- 4,2	-	160
V_4 – fallow on the interval and “mulcit” the soil on the row with vegetal material resulted from mowing between tree rows.	28,4	43,7	36,0	+ 2,9	-	163
V_5 – fallow on the interval and cover the tree row with polyethylene sheet.	26,3	35,5	30,9	+2,2	-	156
FLORINA/MM 106 variety V_1 – (Mt) – worked field	26,2	38,2	32,2	-	-	149
V_2 – fallow on the interval by manual work on the tree row.	24,8	40,4	32,6	+0,4	-	149
V_3 – fallon on the interval and herbivided on the tree row	27,7	40,7	34,2	+2,0	-	148
V_4 – fallow on the interval and “mulcit” the soile on the tree row, with vegetal material coming from mowing the grass, on the interval between the tree rows.	25,4	45,3	35,3	+3,1	*	153
V_5 – fallow on the interval and cover the tree row with polyethylene sheet.	24,0	36,7	30,3	- 1,9	-	148

Variety Generos: DL 5% = 5,50; DL 1% = 8,14; DL 0,1% = 12,57
Variety Florina: DL 5%= 3,10; DL 1% = 4,63; DL 0,1% = 7,15

Specific technological measures leading to the increase of the apples quantity and quality

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Keywords: specific technological sequences, chemical and manual thinning out, foliar fertilization, quality

ABSTRACT

Obtaining quality fruits, according to the trading standards, so as their size uniformity in the tree crown is realized by applying supplementary of some specific technological measures: the chemical and manual thinning out of the fruits, the foliar fertilization, technological sequences that must be an integral part of the fruit production technology.

Through the chemical and manual thinning out of the fruits, based on applying 4 – 5 treatments with foliar fertilizers, the production increase was 36 - 43% greater at the Generos breed and with 27 - 40% greater at the Florina breed. The fruit weight was much superior, surpassing the witness with 8 – 34 kg at the Generos breed and with 18 – 24 g at the Florina breed. Over 990% of the obtained production was registered at the +I quality.

INTRODUCTION

The putting into account of the fruit production encounters big difficulties on the internal market, due to the low percentage of the extra quality fruits, which hardly cope with the massive fruit import of higher quality as commercial aspect, as compared with those obtained in our orchards. According to the trading standards and to the new rules of the law 348/2003, the producers, the owners, the traders and other categories of operators cannot expose for sale and cannot trade fruits in fresh estate unless the fruits fulfill the requirements imposed by the quality standards.

In order to become competitive on the internal and on the European market with fruits of quality, the integration and the generalization into the exploitation technology of the apple fruit bearing orchards of some technological sequences is needed, which lead t the increase of the fruits quantity and quality.

High and quality productions are obtained, when the whole range of technological measures is applied optimally, regardless of the surface owned by each tree grower.

The fruit quality has not been a major objective in the period before the year 1989, due to the lack of the internal competition system (Isac, 2002).

The additional technological sequences which make a significant contribution to the apples quantity and quality increase, are represented by the manual and chemical thinning out of the fruits, the foliar fertilization, which unfortunately are applied sporadically or even at all in the apple orchards. (Petre A.O. 2006).

Trough the researches performed at the Tree Growing Research – Development Station Voinesti, by integration and generalization of the technological sequences, specific for the fruits quality increase, into the exploitation technology of the fruit bearing orchards, through the increase of the competition level of putting into account, according to the trading standards imposed by the requirements of the market – and the search for obtaining supplementary income for the apple growers.

The purpose of the paper is to present some technological measures which shall lead to the quantity and quality increase of the apple production.

MATERIAL AND METHODS

The experimentation of foliar fertilizers, of the thinning out products and of the manual thinning out, in order to increase the apples quality, were performed at the Tree Growing Research – Development Station Voinesti in the years 2004 -2005, at the Generos and Florina apple breeds, grafted on the graft bearer MM 106. The tree had the age of 11 – 13 years, planted at a distance of 4x3m (833 trees/Ha), the crown form freely flattened on the tree row.

The following variants were organized:

V0 – untreated witness V1 – Chemical thinning out

V2 - manual thinning out V3 – Chemical thinning out + manual revision

V4 – Chemical thinning out, manual revision, foliar fertilization.

For the 3 foliar fertilization, at the first 2-3 treatment5s the Agrofeed product was used in a dose of 1%, having in its composition the 3 fertilizing elements (NPK) in the ratio of 19:19:19. At the 4th and 5th treatments, administered before the fruits harvesting, the DELTA E product (5:45:30+ME) was used in a dose of 0.5%.

For the chemical thinning out, the Rarex (Amid 80 Cluj) product was used in a dose of 0,1% ,when the fruits in the centre of the flourishing had a diameter of 10 – 14 mm – and the manual thinning out was performed about the end of the physiological falling, when the fruits had attained a diameter of 16 – 18 mm.

On the control trees of both apple breeds, Generos and Florina, the differentiation degree with fruit bearing buds after the performing of the cutting, the binding degree of the fruits was determined, the moment of applying the thinning out products and of the manual thinning out was established, the production and the dimensional repartition of the fruits (55 – 60 mm, 65 – 70 mm and 75 – 80 mm) was registered.

The production data were interpreted statistically with the methods of randomized blocks.

In the experimentation period (2004-2006), the climatic conditions were favorable for the growth and the fruit bearing of the trees, being characterized by a annual medium temperature greater with 0,3 Celsius degrees as the zonal norm (8,8 Celsius degrees), with an annual precipitations amount surpassing the norm of 782 mm (in the year 2005 1,113 mm were registered).

The soil on which the researches wee performed is brown eumezobasic weak pseudogleised, with clayish texture, with a weak ACID Ph (5.7 – 5.9). The humus content is medium at the surface (2.0 – 2.9%), medium supplied with nitrogen and weakly supplied with phosphorus and potassium.

In the orchard the soil w2as maintained fallow in the interval and weeded on the tree row. The other works were performed according to the technology specific to the intensive apple orchards.

RESULTS AND DISCUSSIONS

Besides the classical technological measures, respective the soil works, the soil fertilization, the fruit bearing cuttings, the phyto-sanitary treatments, an important role in the apple quantity and quality increase of the Generos and Florina breeds is played by some specific technological sequences consisting of the foliar fertilization (at least 5 foliar treatments with foliar fertilizers: 1 before the blossoming, 2 after the blossoming and 2 before the fruits harvesting).

The fruits thinning out (chemical with the Rarex product with 0,1% dose, when the fruits in the blossoming centre have between 10 and 14 mm, completed with manual thinning out, when the fruits of the flourishing centre have reached a diameter of 16 – 18 mm).

The mentioned sequences must be an integrated component of the fruit production technology at any of the cultivated fruit breeds. All these, followed by respecting of the other conditions regarding the harvesting, the storage, the conditioning and the putting into account lead to the increase of the competition level on the market, the preparation of the tree growing production for the integration into the E.U.

a) The fruits thinning out. The productive potential of the breeds and the production level, realized in the current year, is closely related to the differentiation degree with the fruit bearing buds, this being determining for the applying of the chemical thinning out of the only then formed blossoms or fruits.

The potential with fruit bearing buds was situated between 43.3 and 51.3% at the Generos breed and between 38.0 and 42.8% at the Florina breed, assuring a good blossoming degree in the years 2004 – 2006.

The Rarex product (0.1%) acted differently on the fruit thinning out, depending on the breed.

At the chemically, chemically +manually or only manually thinned out variants, the fruit binding percentage at harvesting was situated between 8.7 and 13.2% at the Generos breed and between 12.5 and 15.4% at the Florina breed. The bound fruit at harvesting was more diminished at the chemically and at the chemically + manually revised variants. At the witness variant the fruit binding percentage at harvesting was of 16.3% at the Generos breed and of 18.3% at the Florina breed.

As compared with witness variant without any special intervention, at the variants where chemical, manual, chemical +manual revision was applied, the production increase was situated between 3 and 40% at the Generos breed and between 27 – 39% at the Florina breed. The fruits quality increased also to the same degree, the increased, being registered increases of the fruit weight from 165 to 173 – 192 g at the Genros breed and from 142 g to 160 – 164 at the Florina breed (table 1).

b) The foliar fertilizing represents a complementary measure, having the advantage that the fertilizing products enter much faster into the metabolic circuit of the plant, as compared with the radicular fertilization, but which it cannot replace. The foliar fertilizers are applied simultaneously with the chemical thinning out or with the phyto-sanitary treatments, avoiding the mixing with products based on copper. The foliar fertilizers can be applied in 4 – 6 stages during the vegetation period, caring that those containing nitrogen shall be given during the first 2 – 3 treatments, immediately after the blossoming.

Based on the applying of 5 treatments with foliar fertilizers, the production increase was with 43% greater at the Generos breed and with 40% at the Florina breed. The fruits weight was much greater, surpassing the witness with 34 g at the Generos breed and with 24 at the Florina breed.

The statistically interpreted data point out at the Generos breed distinct significant positive differences, at the variants 1, 2, 3, only with fruits thinning out and very significant positive at the variant 4, with fruits thinning out and foliar fertilization (table 1). At the Florina breed the differences are very significant positive at the 4 studied variants, as compared with the witness variant.

At all variants and demonstrative lots organized for the Generos and Florina breeds, the entire fruit quantity registered per hectare is placed between the fruits with an diameter of over 65 mm, which are able to resist to the competition pressure on the Romanian market. A percentage of 6 – 8% was registered in the category of 55 – 60 mm at the witness variant, without special interventions.

Applying all the agro-technical measures of the fruit bearing orchard exploitation technologies, including the supplementary sequences, specific for the fruits quantity and quality increase, is realized with great financial efforts, but which can be retrieved – and important profits can be obtained, under the conditions of orchards I full economical potential, with high commercial value breeds.

The fruit harvesting output is greater, due to the greater dimensions of the fruits and their size uniformity, the apple quantity harvested by each harvester increases, so that at equal or greater productions, the apple harvesting costs can be equal or even less.

The supplementary financial efforts registered by applying the specific supplementary sequences, can be retrieved and great profits can be obtained due to the increase of the apple quality and their putting into account at much more advantageous prices. The value of the production increases with 13 – 22% at the Generos breed and with 13 – 44 at the Florina breed.

CONCLUSIONS

1. In order to increase the apple quantity and quality to the level of the Romanian

Trading standards, harmonized with the E.U. requirements, it is necessary to integrate into the exploitation technology of the orchards with resistant breeds – and not only these – a series of specific technological sequences, supplementary to the classical technology, respect5ively the chemical and manual thinning out of the fruits, the foliar fertilization.

2. Through the chemical and manual thinning out of the fruits, based on applying 4 – 5 treatments with foliar fertilizers, the production increase was 43% greater at the Generos breed and 40% greater at the Florina breed, statistically assured, with very significant positive differences, as compare to the witness variant.
3. The increasing of the apple quality of the breeds Generos and Florina contributes to increase of the apples and putting into account level and to overcome the competition pressure on the Romanian market, simultaneously obtaining important profits, concretized by the production's value increase with 13 – 44% at both apple breeds.

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Tables

Table 1. The influence of the specific technological sequences on the apple quantity and quality

Breed and variant	Fruit production			Medium fruit weight (g)	Fruit size categories (%)		
	T/Ha	Dif. to witness (t/Ha)	%		55 - 60 mm	65 - 70 mm	75 - 80 mm
GENEROS BREED							
V0 – untreated witness	33.8	-	100	165	16	35	49
V1 – chemical thinning out	47.0	+13.2**	139	191	5	15	80
V2 - manual thinning out	47.4	+13.6**	140	173	10	24	66
V3 – chemical thinning out + Manual revision	46.1	+12.3**	136	192	0	7	93
V4 - chemical thinning out, Manual revision, foliar fertilizing	48.3	+14.5**	143	199	0	5	95
FLORINA BREED							
V0 – untreated witness	30.7	-	100	142	18	48	34
V1 – chemical thinning out	39.8	+9,1***	129	162	8	27	65
V2 - manual thinning out	39.1	+8.4***	127	160	10	30	60
V3 – chemical thinning out + Manual revision	42.6	+11.9***	139	164	5	32	63
V4 - chemical thinning out, Manual revision, foliar fertilizing	43.0	+12.3***	140	166	3	28	69

Generos breed: DL 5% = 6.10; DL 1% = 9.03; DL 0.1% = 13.94.
Florina breed: DL 5% = 3.14; DL 1% = 4.64; DL 0.1% = 7.17.

The creation of new apple tree and pear tree breeds, genetic resistant against deseases, with quality fruits, suitable for ecological cultivation

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Keywords: apple breeds, genetic disease resistance, promising selections, yield potențial, fruit characteristics

ABSTRACT

The experiments organized for apple and pear trees had in view the creation of a selection base from the biological material, obtained at SCDP Voineşti and at ICDP Mărăcineni through inter- and intra-specific sexuate hibridation, material subjected to selection and evaluation in DUS and VAT testing cultures (competition micro cultures and cultures). From the biological material obtained beforehand, a series of genetic disease resistant elites have been selected, which have been enrolled at ISTIS for testing continuation in vue of homologation. From SCDP Voineşti were enrolled the elites: V.1/26-90; V.2/45-90; V.95/49; V.95/23; V.53/4; V.95/15, V.95/27 for aplle trees - 9/19-81; 2/8-86, 36/29-90, for pear trees. From ICDP Mărăcineni were enrolled at ISTIS the elites: 2/29P (Rustic) for apple tree and 5/24P; 7/33P for pear trees. In the years 2006-2007, at SCDP Voineşti the apple tree breeds Real şi Luca and the pear tree breed Tudor - genetic disease resistant productive breeds, with quality fruits - were homologated. At ICDP Mărăcineni, the genetic disease resistant columnar breeds Colmar and Colonade were homologated. By promoting the genetic disease resistant breeds in culture, the cost for performing the phyto-sanitary treatments are diminished with aprox. 50-60% and the negative impact on the environment is reduced.

INTRODUCTION

The realization of productions of ecological fruit productions, of superior quality and at as reduced as possible costs, represent major priorities, both on national and on world level.

An important chain loop in obtaining ecological fruits is represented by the creation and the promotion in culture of the genetic disease resistant breeds.

The orientation towards the desease resistant assortment was a permanent improvers' concern in the last decades, an activity liked by the cultivators, the consumers and the ecologists.

The apple and pear tree assortment was and continues to be dominated by very desease sensitive breeds, their control needing, depending on the meteorological conditions of the year or of the zone, 12 - 16 phyto-sanitary treataments applied anually, performed with a varios range of fungicides, at very high costs.

By promoting in the culture genetic disease resistant breeds, adapted to our country's ecological conditions, the quantity of apples and pears on the market increases, with fruits containing a low level of pesticide residues, with beneficial influences on the environment. The costs for performing phyto-sanitary treataments is reduced with 50-60%, a reduction positively reflected in the increase of the economical efficiency.

The researches performed at SCDP Voineşti and ICDP Mărăcineni in the period 2005-2007 point out the creation of a new selection base from the biological material obtained through inter- and intra-specific sexuate hybridization and the evaluation in testing cultures of the apple tree and pear tree elites which detached themselves by productivity, fruit quality and genetic disease resistance. The most valuable were homologated or further used in the improvement programs.

MATERIAL AND METHODS

a) The creation of a new selection base from the hibrid material obtained through the sexuate hibridation method.

In order to insure the continuity of obtaining the initial biological material in the selection process of new genotypes with perspective, in the years 2005 - 2006 sexuate hibridizations were performed, realizing new apple and pear tree hibrid series.

The selection of the genitors was made on genetic disease (scarf, mildew, bacterial burn) resistance criterias, production potential, superior quality and good fruit keeping capacity, adaptability to the local ecological conditions.

At SCDP Voineşti, 11 hibrid combinations were performed in the year 2005 and 16 hibrid combinations in the year 2006 at apple trees - and 4 hibrid combinations at pear trees.

The seedlings obtained from the hibrid combinations performed in the year 2005 in green house flower pots, were subjected to a negative selection after the infection with pathogenic strains, eliminating those with disease attack symtoms - the remaining were planted in the fortification field (the hybrid's nursery).

The artificial infection with patogenic scurf strains was performed when the seedlings had 4-5 leaves. Two weeks after the inoculation of the seedlings, the observation of the attack symtoms on the leaves was performed after the scale proposed by Shay and Hough.

At ICDP Mărăcineni, inter-specific hibridations were performed with genitors having the Vf gene and genitors with the columnar form feature, realizing a number of 12 hibrid combinations. The selection of the hibrid seedlings was made after the columnar habitat and the resistance against the scarf and mildew attack.

The negative selection in the fortification fields, both at SCDP Voineşti and at ICDP Mărăcineni, was made by elimination - in the juvenile phase - the apple tree genotypes presenting sensibility to scarf, the situation on hybrid combinations being established.

b) The experimentation of the apple and pear tree elites of the competition micro cultures.

The eliteles selected in the selection field were multiplied in the nursery and formed the base for the creation of competition micro cultures.

At SCDP Voineşti the study was performed in an apple tree micro culture, created in the year 2002, comprising a number of 9 elites with perspective, resistant against diseases. The used graft support is MM106. The planting distances are 4/3 m. Free crown form.

At the pear trees, the study was performed in 2 micro cultures, created in the years 2001 and 2003 on franc and quince tree graft supports. The planting distance is 4x2 m.

The experience organised at ICDP Mărăcineni comprises 29 apple tree elites with genetical resistence against scurf (Vf), 4 of which with standard habitus and 25 of the columnar type, grafted on M9 in th case of the standard elites and on own roots, those with columnar port. The trees are planted at a distance of 3,5x0,8 m (3500 trees/Ha) and those grafted on M9 gaft supports have an age of 6 years – and 11 years those on own roots.

At the pear trees, the 12 elites are grafted on quince tree BN 70 and comprise selections with autumn and winter fruit maturation. The trees are planted at the distance of 3,5x2 m (1400 trees/Ha).

On the apple and pear trees, 4-6 phyto-sanitary treataments were applied only with insecticides.

Observations and determinations were made regarding the tree growth strength, the resistance against diseases (*Venturia inaequalis*, *Podosphera leucotricha*) at the apple trees, *Venturia pirina*, *Erwinia amylovora* şi *Psyla sp*. at the pear trees, the production potential and the fruit quality expressed as weight (g), SU content (%), the pulp firmness (kg.f/cm^2), the aspect and the taste.

The elites, detaching themselves by genetic disease resitance, productivity and fruit quality, were enrolled for testing at ISTIS in vue of homologation.

RESULTS AND DISCUSSIONS

a) The creation of a new selection base from the hibrid material obtained through the sexuate hibridation method.

At SCDP Voineşti, at the apple trees, at the sexuate hybridization, the genotypes: Luca, Florina, Generos, Priscilla, Nova Easigro, Discovery (maternal genitors) and Royal Gala, Goldrush, Falstaf, Luca, Ciprian, Florina, a columnar breed (paternal genitors) were used.

In order to obtain biological disease resistant material, in the year 2005 - 11 hybrid combinations were performed, 3690 blossoms being pollinated, of which 445 fruits were harvested, 3340 hybrid seeds being obtained, which have been grouped in combinations, conditioned and kept till stratification (Table 1.).

From the 3340 hybride stratified apple seeds, 2,130 hybrid seeds were sown in the green house into floweer pots, from which a number of 1913 seedlings resulted, which were subjected to artificial infections with pathogenical scurf strains, when the seedlings had 4-5 leaves.

The seedlings planted in the hibrids' nusery were subject in August to a second selection regarding the resistance against scurf, remaining a number of 1451 apple tree hybrids with genetic disease resistance (75,8%).

The infection degree with scurf of the seedlings is different in the combinations, depending on the genitors used in the respective combinations.

The scurf resistance feature was transmitted to a high percentager (91,6% - 100%) in the case of the RXR type combinations (rezistant x rezistant), but there were also combinations, at which the lowest percentage of sensible seedlings was registered: Florina x Goldrush – 0; Priscilla x Luca – 2,4%; Generos x Goldrush – 8,4%.

In the case of the RxS (rezistant x sensible) type combinations, the infection of the seedlings with scurf breeds was stronger, so that the percentage of attacked and eliminatd seedlings oscillated between 37.2% (the combination Discovery x Florina) and 41.3% (the combination Priscilla x Falstaf).

In the year 2006 - 16 hibrid apple tree combinations were performed: V 34/41 x Liberty; V 34/41 x Idared; V 34/41 x Generos; V 34/41 x Ciprian; Florina x Luca; Goldrush x V 53/4; Falstaf x V 53/4; Royal Gala x V 31/1; Generos x V 53/4; Generos x Florina; Priscilla x Generos; Priscilla x Ciprian; Priscilla x Falstaf; Falstaf x Ciprian; Falstaf x Florina; Florina x Kolumnar (KB45).

6.620 blossoms were pollenated, between 150 and 780 blossoms per combination, obtainig 674 hybride fruits, of which 3960 seeds were extracted ,which were stratified and sown in flowerr pots in spring 2007.

At the pear trees, in spring 2006 four hybrid inter-breed combinations were realized: H7/78-81xWilliams Roşu; H8/106-81xWilliams Roşu; H8/106-81xPackham's Triumph; H9/19-81x Monica, 2.850 blossoms being pollinated. After pollination - and especially in the fruit forming and growth period – low temperatures were registered, which had a negative influence on the fruit binding percentage.

At ICDP Mărăcineni, the evaluation of the 2005 apple tree hybrid generation was performed in August in the green house and comprised 4,398 hybrid seedlings, choosen after the columnar habitus and the field resistance against scurf and mildew. A total of 357 elites were marked, representing 8,1% of the evaluaeted seedlings. Of the 19 combinations, the most resistant plants with columnar habitus were choosen at COL 108 (112 plants), respectively 9.7%. Greater percentages were registered at COL 2/22, with 22.1% resistant columnar plants – and the smallest at COL R12P87, with only 0.8% - or only 1 resistant columnar plant.

To be mentioned: the selected seedlings Co + Vf are obtained by free pollenation of the resistant (mother) selections with columnar habitus – and not from controlled hybridizations.

b) The experimentation of the apple and pear tree elites of the competition micro cultures.

The promotion in culture of a breed is based on the study in the comparative competition cultures and micro cultures, under the aspect of the growing strength, the production potential, the superior fruit quality and especially the disease resistance.

The medium data registered in the years 2005 and 2006 are presented in the Table 2.

Analyzing the data registered at SCDP Voineşti, it has been found that the growth strength expresed by the trunk section surface (TSS) at the 9 studied apple tree genotypes is situated between 24,6 and 47,8 cm^2.

Regarding the productivity, it is appreciated that the most precocious elites were V 95/45 (3,5t/ha), V 95/272 (4,3 t/ha), V 95/49 (8,2 t/ha) and V 95/230 (8,7 t/Ha).

The fruit caracteristics - expressed by the weigth, the size, the firmness and the content in dry substances (DS) – point out the fact that the majority of the elites frame into superior parameters:

- The fruit weight is comprised between 126–190 g, with an size of 60-75 mm;
- The good to very good firmness of the fruits, with 7.1-10.0 kg.f./cm^2 and a dry substances content of 10-14.6%.

Regarding the disease resistance, all studied apple tree selections present a very good resistance against scurf and a reduced mildew attack, with a frecvency of 0.8% at the V95/230 selection – to 8.8% at the 98/72 selection.

At the pear trees, none of the analyzed selections in the testing cultures doe's present bacterial burn attack symptoms, with the exception of the 5/104-84 elites, where in the rainy years 1-2 brownished offspring picks were observed. Also all elites are resistant against scurf. The reistance against the patogenical agents was tested in conditions of not sprinkling with fungicides.

Regarding the resiatance against Psylla sp., the selections: 1/63-81; 1/83-81; 5/104-84; 5/117-84; 24/2-86 and 2/8-86 showed an evident tolerance against this pest.

At the selections grafted on franc graft supports, the medium productions oscillated between 2,2 and 25 t/Ha, the greatest productions being atributed to the elites: 5/104-84; 7/78-81 and 1/63-81.

In the case of grafting of Quince tree A, the greatest production was registered at the selection 12/83-79 (15.75 t/Ha), 2/8-86 (14 t/Ha) şi 5/5 – 84 (12 t/Ha).

Based on the obtained results, the following elites are remarked: 5/104-84 (homologated under the name Tudor in the year 2007), 9/19-81, 24/2-86 and 2/8-86 ,with pleasant aspect and taste, suitable size and consumption period October - November, with longer keeping capability in cold conditions.

The data registered at ICDP Mărăcineni show that the tree growth strength, expressed as the trunk section surface in cm^2 , presents a very high variability at the columnar types on own roots, from 23 cm^2 (2-70 COL, 1-61 COL) to the double: 45.8 – 53.2 cm^2 (COL 114, COL 94, COL 92, COL 97, COL 108, 1-6 COL, 2-45COL).

Regarding the fruit caracteristics, the standard apple tree elites (4) presented fruits with a medium weight between 128 g and 170 g, a size between 60–65 mm and 75–80 mm, dry substance comprised between 11.0% and 15.3% - and the pulp firmness between 7.0 and 9.4 kgf/cm^2. At the columnar type elites, the registered values had very large limits regarding the fruit weigth, from 87 g (1–33 COL) to 216 g (COL 97); in the same manner, the size was variable, between 60–65 mm and 80–85 mm (3-47 COL, 29-13 COL, COL 94, 3-40 COL). In this respect, many elites frame into the extra size classes, being competitive with the market's requirements and the imposed standards. Regarding the firmness, the elites presented values between 3.8 (2-62 COL) and 10.0 (1-31 COL), meaning from soft, very lax pulp, to very firm. As known, a firmness of over 7,0 kgf/cm^2 at harvesting is corelated with a good keeping capacity, a fact pointed out at many of the studied elites.

Regarding the dry substance content, it shows that the most of the elites have attained an optimal treshold for the autumn harvesting maturation, respectively over 12.5 – 13%.

At the pear trees, the studied elites have fruits with a medium weigth between 116-236 g, a size between 55-60 mm and 70-75 mm – and the dry substance between 9.6 and 16.8%. More than half of the elites have lesser values than the normal ones, having an influence on the taste qualities.

Also at ICDP Mărăcineni in competition micro cultures 14 apple tree columnar type breeds and selections - and 7 pear tree selections were experimented .

At the aple trees, the production potential, expressed as the fruit quantity obtained per hectare, is presented in the graph 1. So, for the 14 columnar breeds and selections, different productions were registered, depending on the genotype.

The apple production, calculated per hectare for a planting density of 3500 trees/Ha (meaning 3.5/0.8m) shows the possibility to reallize levels 72,8 t/Ha (2-39 Col) and of 46,2 t/Ha (Col 109) or 44,4 t/Ha at the Colmar breed. Of course, the production of the next year must be also waited for, in order to be able to see the alternance degree in the framework of the studied genotypes.

At the pear trees, the production capacity was registered at 7 selections and the results are presented in the *graph 2*. Having in vue the weaker binding due to the less vavorable clinmatical condions of the blossoming and fecundation period, the selections detaching themselves by higher productions per tree were: sel. 5/24 P (7.3 kg) and 7,33 P (6.5 kg). Low productions had sel. 14/32 P (3.0 kg/tree) şi 15-54 P (3.7 tree/pom).

Calculated per hectare, at a density of 1,400 trees, the highest pear production was of 10.2 t at sel. 5/24 P – and thelowest of 4.2 t at sel. 15-54 P.

The growth strength of the columnar trees grafted on M9, in the 6th year after planting, is presented in the *graph 3*. After the thickness growth of the trunk (TSS cm^2), the selection differentiate on a scale from simple to double, so the strongest has 26.6 cm^2 (COL 92) – and the weakest has13,5 cm^2 (COL 109). The other columnar selections have intermediate values, but are grouped on genotypes with greater strength, e.g. Col 92, Col 45, Col 85, Col 94 – and selections with weaker strength, e.g. Col 97, Colmar (mt), Col 113 şi Col 109. This classification after the tree growth strength serves for choosing the planting distances and for establishing the thickness of the columnar selections in the orchard.

The biometrical determinations, performed at the apple tree selections on own roots in the year XI after planting, show that there is a very high variability of the selections after this parameter, from a very high strength (e.g. Col 114, 2/114 P, 29/13 Col, 1/33 Col, 29/95 Col), to a medium strength (most of the selections) and to a reduced strength (2-20 Col, 2-62 Col, 1/43 Col, 2-70 Col, 30/63 Col).

At the pear trees, the biggest trunk thickness growth was shown by sel. 15-55-87 P, followed by 21-53-86 P şi 15-54 P, the other selections framing into the group of those with a medium and a reduced strength (1-22 P).– *graph 4*

Following the testing in competition micro cultures, based on beforehand established criteria (production potential, fruit quality, disease resistance, etc), the selection which met the imposed conditions were promoted in the DUS and VAT testings.

In vue of testing and homologation, at ISTIS following elites were enrolled: V.1/26-90; V.2/45-90; V.95/49; V.95/23; V.53/4; V.95/15; V.95/27 for appple trees – 9/19-81; 2/8-86; 36/29-90 for pear trees from SCDP Voinesti. From ICDP Mărăcineni, at ISTIS were enrolled the elites: 2/29P (Rustic) for apple tree - and 5/24P; 7/33P for pear trees.

In the years 2006-2007, at SCDP Voineşti the apple tree breeds Real şi Luca and the pear tree breed Tudor, genetic disease resistant productive breeds, with quality fruits, were homologated. At ICDP Mărăcineni, the genetic disease resistant columnar breeds Colmar and Colonade were homologated.

CONCLUSIONS

1. The creation of new selection bases for apple and pear trees from the hybrid material obtained through the sexuate hybridation methods has a continuos character, annualy being performed 11-16 hibrid combinations with 2850 – 3600 pollenated blossoms, of which resulted 1451 – 1913 seedlings in flower pots, of which 75,8% with genetic resistance against deseases.
2. Out of 4398 hybrid apple tree seedlings, obtained by free pollenation, a percentage of 8.1% were evaluated after the columnar habitus and the field resistance against *Venturia inaequalis* and *Podosphaera leucotricha* – a higher percentage resulted at COL 108 (9,7%) and COL 2/22 (22,1%).
3. The pointing out of the apple tree growth strength shows that between the studied columnar selections exist significant differences, from simple to double- and the standard type selections frame into the medium strength group, with values oscillating at the apple trees from 24.6 – 47.8 cm^2 trunk section surfaces, at the age of 4-5 years, on the graft support MM 106 – to 38.5 – 45.8 cm^2 trunk section

surfaces, at the age of 9 years, on the graft support M 9. At the pear tree/Quince tree A, at the age of 3-4 years, 6.6 – 14.5 cm^2 trunk section surfaces are registred – and at the pear trere/GQuince tree BN 70, at the age of 9 years, 12.6 – 28.3 cm^2 trunk section surfaces.

4. The production potential, expressed by the fruit quantity, is variable - both at the columnar forms and at the standard ones, being selected those presenting a high production potential (over 25-30 t/Ha), with quality fruits (over 150g/fruit), firmness of 7 kgf./cm^2 , superior taste qualities and with an as long as possible keeping in natural conditions.
5. Based on the registered results, the following elites were selected: V.1/26-90; V.2/45-90; V.95/49; V.95/23; V.53/4; V.95/15, V.95/27 for apple trees - 9/19-81; 2/8-86, 36/29-90 for pear trees from SCDP Voineşti and the elites : 2/29P (Rustic) apple tree and 5/24P; 7/33P pear trees from ICDP Mărăcineni, being enrolled at ISTIS. In the years 2006-2007, at SCDP Voineşti the apple tree breeds Real and Luca and the pear tree breed Tudor - genetic disease resistant productive breeds with quality fruits - were homologated. At ICDP Mărăcineni, the genetic disease resistant columnar breeds Colmar şi Colonade were homologated.

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Tables

Table 1. Evaluation of the hybrid series 2005 at SCDP Voineşti

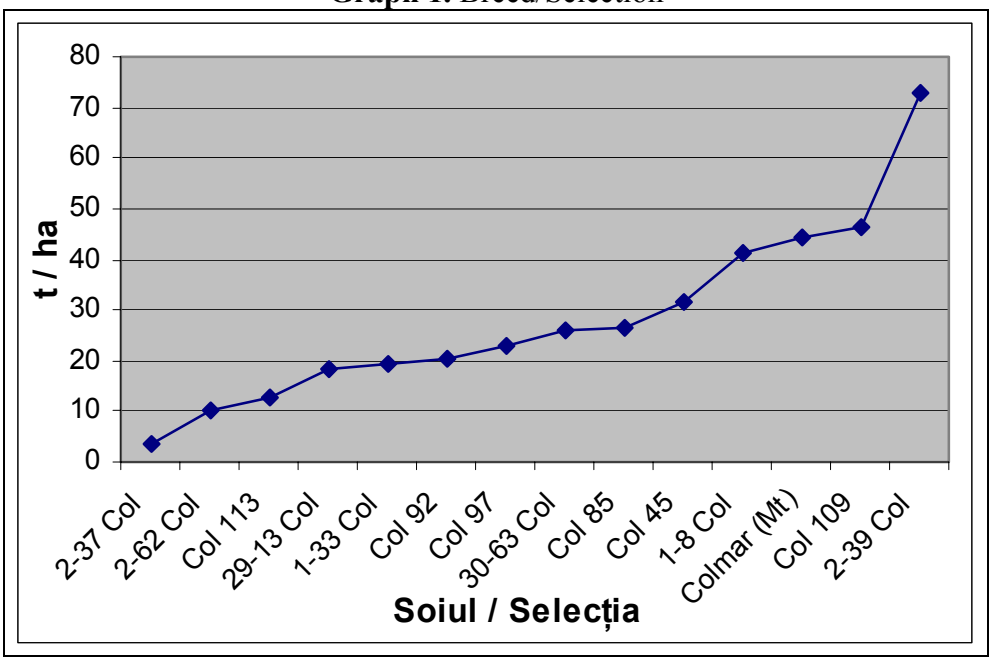
Nr.	Combination	Year 2005			Year 2006		
		Pollinated blossoms	Obtained seeds	Sown seeds	Seedlings obtained in flower pots	Resistant seedlings, passed into the fortification field	Resistant seedlings (%)
1	**Luca X Columnar**	190	390	140	139	92	66,2
2	Florina x Royal Gala	270	207	172	153	95	62,1
3	Florina x Goldrush	170	7	2	2	2	100,0
4	Florina x Falstaf	340	180	118	98	78	79,6
5	Generos x Goldrush	450	447	340	333	305	91,6
6	Priscilla x Luca	250	710	480	427	417	97,6
7	Priscilla x Falstaf	240	714	460	417	245	58,7
8	Nova Easigro x Idared	220	0	0	0	0	0
9	Discovery x Luca	510	98	80	74	48	64,8
10	Discovery x Ciprian	540	22	20	17	10	58,8
11	Discovery x Florina	510	267	318	253	159	62,8
	TOTAL	**3690**	**3340**	**2130**	**1913**	**1451**	**75,8**

Table 2. The strength, the production potential and the fruit caracteristics at the studied aplle and pear tree elites (2005 – 2006)

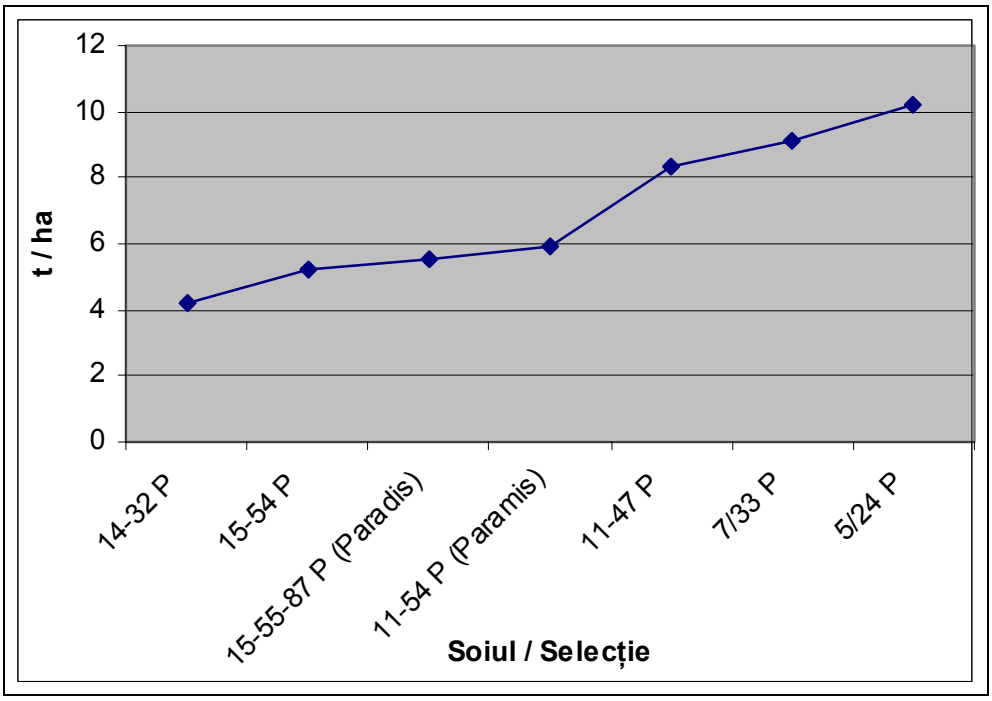
Breed/graft support	Nr. of studied selections	Tree strength TSS (cm^2.)	Productivity (t/Ha)	Fruit caracteristics			
				Weight (g)	Size (mm)	Firmness kg f/cm^2	Content in DS%
SCDP Voineşti							
Apple tree/MM 106 age 4-5 years (833 trees/a)	9	24,6-47,8	0,9-8,7	126-190	60-75	7,1-11,0	10,0-14,6
Pear tree/Franc age 4-5 years (1250 trees/Ha)	7	18,8-43,0	2,2-25,0	150-300	70-85	-	-
Peartre/Quince tree A age 3-4 years (1250 trees/Ha)	6	6,6 - 14,5	3,3-9,1	150-300	70-85	-	-
ICDP Mărăcineni							
Apple tree/M9 age 6 years (3500 trees/Ha) Standard elites	4	38,5-45,8	26,4-38,0	128-170	75-80	7,0-9,4	11,0-15,3
Apple trees/own roots age 11 years (1904 trees/Ha) Columnar type elites	25	23,0-53,8	16,4-38,8	87-216	60-85	3,8-10,0	12,5-13,0
Pear tree/Quince tree BN 70 age 9 years (1400 trees/Ha)	12	12,6-28,3	4,2-8,4	116-236	60-75	-	9,6-16,8

Figures

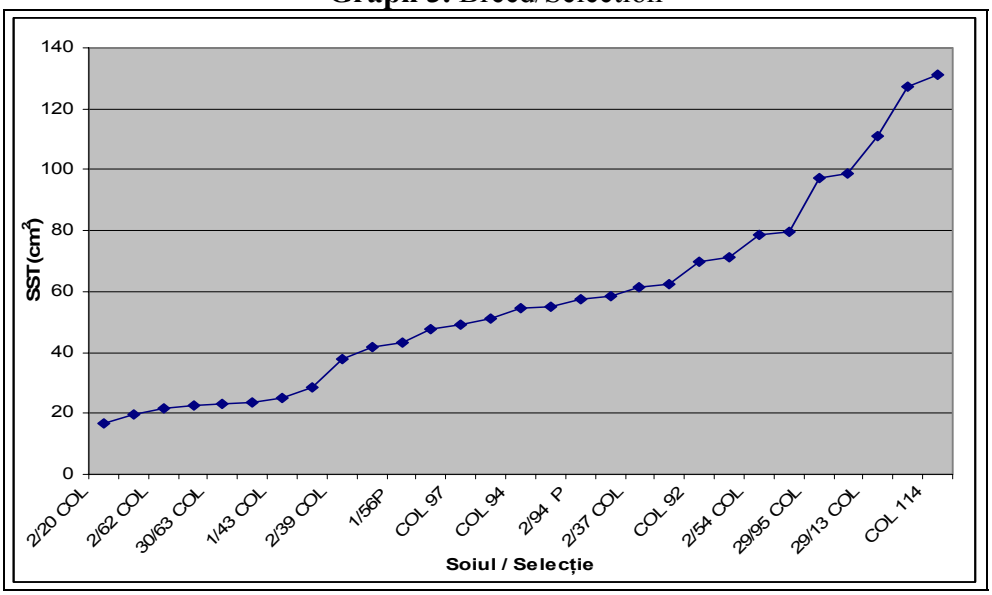
Graph 1. Breed/Selection



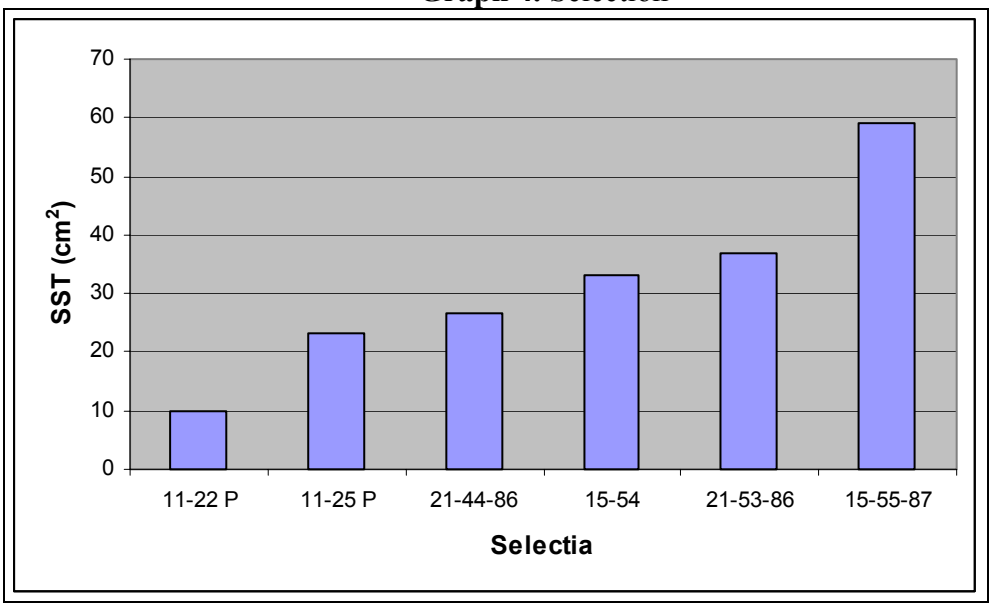
Grafic 2. Breed/Selection



Graph 3. Breed/Selection



Graph 4. Selection



Mycoplasma (Phytoplasma) detection in pear with pear decline, test plants and psyllids in Romania using dot blot immunoassay method

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Keywords: Pear decline, phytoplasma, *Cacopsylla pyri,* dot blot immunoassay.

ABSTRACT

Using two polyclonal antisera produced in rabbits, one with phytoplasma of apricot chlorotic leaf roll –ACLR (European stone fruit yellows group-ESFY) and another one with aster yellows phytoplasma isolated on axenic culture, an indirect dot blot immunoassay method was performed on nitrocellulose membrane stripes to detect PD, AP 15 and Aster yellows (AY) phytoplasmas in a comparative study. Goat anti-rabbit IgG conjugated with Alkaline Phosphatase or Goat anti-rabbit IgG conjugated with colloidal gold were used as secondary antibodies. Both antisera detected PD phytoplasmas from pear, *Cacopsylla pyri,* pepper experimentally infected by insect vector *Cacopsylla pyri,* apple proliferation AP 15 and AY, both multiplied in *Catharanthus roseus*. These results suggest that there are no serological difference between PD, apricot chlorotic leaf roll, AY and AP 15.

INTRODUCTION

Pear decline (PD) has been reported from Europe, North America and Australia and is considered one of the most dangerous diseases of pear trees. The disease was first described as "moria" in Italy (Refatti, 1948) and later on in North America as virus disease (Jensen *et al*., 1964) transmitted by pear psylla (*Psylla pyricola* Förster) denominated nowadays *Cacopsylla pyricola.* After 1970, mycoplasmalike bodies were detected in sieve tubes of pear trees affected with pear decline (Hibino and Schneider, 1970) and in the pear psylla vector of pear decline (Hibino et al., 1971). More recently transmission of the PD agent by *C. pyricola* was demonstrated in England and PD phytoplasma was detected in vectors collected in PD-affected orchards (Davies et al., 1998; Kucerová, 2007). Investigations developed in Europe have showed that PD is transmitted by a new vector, *Cacopsylla pyri*, in France (Lemoine, 1991) and Italy (Carraro et al., 1998; 2001).

Phytoplasma associated with PD disease was detected also in Germany (Lorenz et al., 1995), Spain (Garcia-Chapa, 2003), Hungary **(**Süle et al., 2007) and in Southern Australia (Schneider and Gibb, 1997). The first description of symptoms of PD with phloem necrosis in Romania was published by Bălăşcuţă et al. (1979) and molecular techniques for detection of PD and ESFY phytoplasmas were published by Ploaie (1981, 2006). Association of psyllids with pear orchards in Romania has been studied in the last ten years (Chireceanu, 1998, 2001).

The objective of this study was to detect PD phytoplasma in pear and vector *Cacopsylla pyri* using two polyclonal antisera in a comparative research with reference to two different phytoplasmas, apple proliferation and aster yellows.

MATERIALS AND METHODS

The following phytoplasmas (MLOs) were selected, purified by centrifugation at 20.000g for 2 hours, examined for purity by high resolution electron microscopy and stored at -20^{0}C: a) Pear decline MLO purified from petioles and midribs of pear leaves (figure 1C, E and from *Capsicum annum* L. (*cultivar*) artificially infected by the vector *Cacopsylla pyri* collected from pear orchards with symptoms of pear decline; b) Aster yellows MLO isolated from barley and purified from host plant *Catharanthus roseus*

(figure 1B) and used for cultivation on artificial media and for preparation of antiserum; c) Apple proliferation AP15 (kindly supplied by Erich Seemuller, Institut für Pflanzenschutz im Obstbau, Dossenheim, Germany in 1995) purified from *Catharanthus roseus* (figure 1D); d) MLO purified from the vector *Cacopsylla pyri*, overwintering generation (figure 1F); e) Apricot chlorotic leaf roll – ACLR (figure 1A), used for preparation of antiserum.

For detection of phytoplasmas from apple proliferation, apricot chlorotic leaf roll and aster yellows groups, an indirect dot blot ELISA method was performed on solid support (Ploaie, 2006; Ploaie et al., 2003). Using a polyclonal antiserum produced in rabbits with phytoplasma purified from petioles of apricot with symptoms of apricot chlorotic leaf roll (ACLR) or quick decline (Ploaie et al., 2003) and another polyclonal antiserum produced also in rabbits, using as antigen aster yellows phytoplasma isolated on axenic culture (Ploaie, 1983; Ploaie et al., 1988, 1994) a comparative study was undertaken to detect PD. The MLO was pipetted as 1-10µL/spot on SIGMA N-8017 nitrocellulose membrane stripes, 0,8/8 cm, having a porosity of 0.2 µm. The stripes were transferred in 10 ml volume plastic tubes filled with blocking buffer warmed at 45^0C (0,1M Tris-HCl pH 8, supplemented with 4% BSA, 0.1% gelatin and 0.2% sodium azide) for 30 min. The blocking solution was removed and the stripes were incubated in 1/10 dilution of primary antiserum for 5-8 hours or over night. Negative controls were carried out by omitting the antigen or the primary antibody.

The stripes were washed several times (10-15 min) with Tris-HCl buffer to remove unbound antibody. To detect antibodies bound to MLOs, the stripes were introduced in tubes filled with secondary antibodies (Goat anti-rabbit IgG-whole molecule) conjugated with Alkaline Phosphatase (SIGMA product No. 3779) or with Goat anti-rabit IgG (whole molecule) conjugated with colloidal gold, particle size 10 nm (SIGMA product No. 3779). The stripes were incubated over night in diluted secondary antibodies (1/100, 1/1000, 1/5000, 1/10.000). The membrane stripes were thoroughly washed with Tris-HCl buffer and ddwater and the positive reactions were identified for AP using SIGMA-*Fast*™ -BCIP/NTB substrate. With this substrate, color reaction of spots is blue-purple and end product is insoluble. The staining reaction was stopped by washing the stripes with ddwater. When Goat anti-rabbit IgG-coloidal gold was used the spots became red. If the spots were less visible, sliver enhancement procedure was used (BioCell Gold Conjugates, 1987), and the spots became black and strong visible.

Stripes were air-dried, mounted onto card board and stored at room temperature as document. For illustration the blots were photographed or scanned with a Logitech Page Scan Color and transferred in a PC.

RESULTS AND DISCUSSIONS

The antisera and dot blot technique for rapid diagnostic of MLOs were evaluated on numerous extracts of infected plants and insects. The following MLOs were selected and listed below from 1 to 6, spotted on nitrocellulose stripes and detected by indirect ELISA with secondary markers goat anti-rabbit IgG-PA or goat anti-rabbit colloidal gold.

1. Pear decline MLO purified from *Capsicum annum* L.(*cultivar*) artificially infected by the vector *Cacopsylla pyri* (2003);
2. Pear decline MLO purified from petioles and midribs of pear leaves (2004;

3. Pear decline MLO purified from *Capsicum annum* L.(*cultivar*) artificially infected by the vector *Cacopsylla pyri*, (2003);
4. Aster yellows MLO isolated from barley and purified from host plant *Catharanthus roseus*, (2003);
5. Apple proliferation AP15 (Germany) purified from *Catharanthus roseus*, (2004).
6. MLO purified from the vector *Cacopsylla pyri*, (2002-2004);

The results with the MLOs listed from 1 to 6 are illustrated in figure 2. The number on stripes corresponds with number in list. Antiserum anti AY MLO, produced in rabbits in 1988, 1992 and 1993, using as antigen the AY MLO, isolated in axenic culture, detected both AP15 (stripe 1) and AY (stripe 2) when goat anti-rabbit IgG-Gold as secondary antibodies were used. By symptoms in periwinkle AP15 from Germany and AY from barley were identical, inducing virescence and proliferation (figure 1, B, D). When AY (4) and AP15 (5) were tested with antiserum for apricot decline these MLOs gave positive reaction as seen on stripe 4 at position 4 and 5. Detection of PD MLO, extracted from pepper, infected by *Cacopsylla* (positions 1 and 3 in list) is illustrated as spot on stripes 3 and 4. Detection of PD MLO purified from pear (position 2 in list) was also positive with ACLR antiserum as shown on stripes 3 and 4. MLO purified from *Cacopsylla piry* (position 6 in list) was also detected and demonstrated on stripe 5. The diagnostic on stripes 3, 4 and 5 was of dot blot ELISA type performed with Goat anti-rabbit IgG-AP.

Our results demonstrate that AP15 and AY phytoplasmas are, serological, identical when purified from *Catharanthus roseus* and tested with primary antiserum produced in rabbits, using as antigen AY phytoplasma cultivated in artificial media, and goat antirabbit antiserum conjugated with gold. Phytoplasma of PD purified from pear, *Capsicum annum* and from *Cacopsylla piry* and AP15 and AY phytoplasmas were all identical when were tested with antiserum for apricot chlorotic leafroll phytoplasma.

Based on the analysis of 16S rDNA gene sequence Poggy *et al*. (2000) established that "the 16S rDNA sequence of AP and PD phytoplasma are similar and have a single base difference between the two probes, and a single base mismatch with the 16S rDNA sequence of the ESFY phytoplasma". Phylogenetic analyses of Apple proliferation (AP), pear decline (PD) and European stone fruit yellows (ESFY) revealed that the 16S rDNA sequences of strains of each of these pathogens were identical or nearly identical (Seemuller and Schneider, 2004).

CONCLUSIONS

The MLOs from Apple proliferation group (AP15, PD) are identical with Aster yellows when tested with antisera for AY and ACLR.

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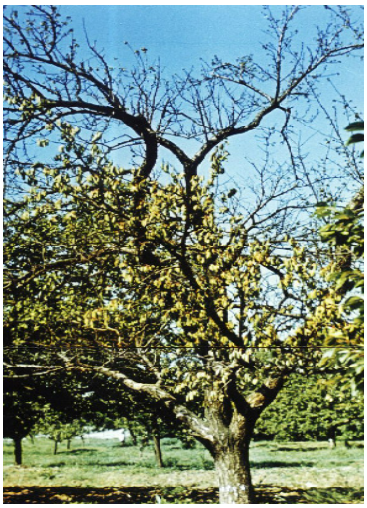
Figures



A Apricot with symptoms of apricot chlorotic leafroll (ACLR).

B Symptoms of aster yellows in periwinkle

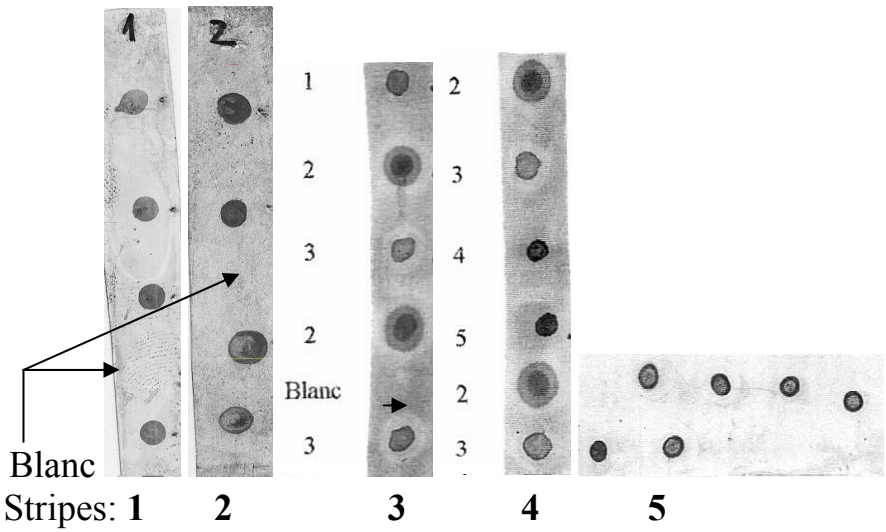
C Pear with pear decline (Williams)

D AP15 symptoms in periwinkle plant

E Leaves of pear with symptoms of reddening

F *Cacopsylla pyri*

Fig. 1. Symptoms caused by MLOs in different plant in natural and artificial infections



MLOs: 5 µL/spot on
1 and 2
Stripe 1: AP15 (5)
in 4 repetitions
Stripe 2: AYA (4)
in 4 repetition
Primary antiserum:
rabbit anti AYA MLO
Molecular marker:
Goat anti-rabbit IgG-Gold
Sliver enhancement
Blanc: antigen omitted

2µL/spot, on stripes 3, 4, 5
Stripes 3 and 4: spoted
with MLOs corresponding to the number from list
Stripe 5: MLO from 6 (*Cacopsylla*), in 6
repetitions
Primary antiserum: rabbit anti ACLR MLO
Molecular marker: Goat anti-rabbit IgG-AP
Positive detection: in 5 min

Fig. 2. Immunodetection of MLOs from AP and AY group

The behaviour of some plum cultivars and hybrids at Plum Pox Virus (PPV) in the south Romania conditions

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Keywords: virus, genotype, ELISA

ABSTRACT

The researches were made to show up the behaviour of some plum genotypes (*P. domestica* and *P. insititia*) at Plum Pox Virus. PPV is the most sever and spread of plum virus and influenced negatively the quantity and quality of the production. The next cultivars and local biotypes of plum were identified as tolerant or slow infected (10-12 years) with PPV: *Andreea, Flora, Mirabelle de Nancy, Oteşani 8, Gogoşele negre, Sâmbăta 3*. The diagnosis of PPV virus confirmed with biological analyses with woody indicators and serological analyses ELISA assured the phytovirotical negative selection of the genotypes of plum and their use in the breeding and propagation processes.

INTRODUCTION

Plum – pox (Sharka) became a big problem, with economical implication for stone plants crop, all over the world. The disease is extremely dangerous at fruit level because deteriorates the quality of the fruits. The quick proliferation of this disease in the entire plantation all over the world and the quick rate of falling ill of the plants, grow up a series of big problems all over the world and also in Romania.

In this paper work, there are presented dates regarding the evaluation and the behaviour of some plum genotypes at Plum pox, to show up plant genotypes which present high tolerance at this disease, meaning an important objective in genetic breeding and propagation of plum cultivars and rootstocks.

MATERIAL AND METHODS

The observations and the determinations were effectuated at S.C.D.P. Valcea, Romania during the period 1998-2004, using as biological material: Romanian and foreign plum cultivars, local biotypes and plum rootstocks.

It was analyzed the trees fruit production, the presence and the identification of the viral agent, the influence of these factors upon the fruits quality and quantity. It was found out and selected the plant forms with PPV tolerance or with an infection rate lower in time.

The diagnosis and the identification of the virus were realized through DAS ELISA analyses, through tests with biological herbal and woody indicators, recommended by International Committee for viruses study at fruit trees. For the test on woody indicators: *P. domestica cv. Tuleu dulce* and *P. persica cv. GF 305,* it was used double ocular chip budding grafts for healthy trees using inocul from the trees which were tested. The observations through indicators were effectuated periodically after 2 month, one and 2 years.

The herbal test plants, *Chenopodium foetidum* and *Nicotiana benthamiana* were inoculated with juice obtained from plum young leaves. At one gram of vegetal material were used 4 ml of phosphate tampon 0,05 M, with pH = 8. Through the technique (Double Antibody Sandwich Enzime Linked Immunosorbent ASSAY) ELISA, it were confirmed the viral infections and was determined the virus concentration in the tested

plants. The reagents for the analyzed plants were bought from BIO RAD and SANOFI companies.

RESULTS AND DICUSSIONS

The gender *Prunus* is well represented through many species, cultivars, rootstocks, local biotypes, hybrids etc. The characteristic of this biological material is the big diversity as geographical provenience, productivity, resistance at diseases, adaptability at different geographical conditions etc. As a fact, the available genetic base corresponds in a big part to the genetic breeding objectives to create new plum cultivars and rootstocks competitively on European markets, and for their propagation in the nursery. As far as it concerns the fruit production of the biological material that we evaluated, this is variable in function of genotype, from (13,4 t/ha) at (23,0 t/ha). Near by foreign cultivars very productive as well as *Stanley,* it were also remarked Romanian cultivars which registered high fruit productions: *Centenar* (23,0 t/ha)*; Flora* (20,0 t/ha*); Gras ameliorat* (17,0 t/ha)*; Andreea* (17,0 t/ha)*; Carpatin* (18,5 t/ha); *Silvia* (17,5 t/ha); *Tuleu gras* (15,5 t/ha); *Minerva (*17,0 t/ha) (Table 1).

At the base of created Romanian cultivars (*Centenar, Minerva, Pescăruş, Silvia,* etc.), there are the plum genotypes: *Tuleu gras, Rivers timpuriu, Renclod Althan* and other cultivars, which transmitted their productivity character and amplifying it through addition of new genes. These could serve in breeding for improving the productivity, near by the local biotypes (*Oteşani 8, Gogoşele negre, Buburuz* etc.). Although, the productivity and the quality of the fruits, the resistance at the diseases and especially at the virotics are ones of the most important problems in plum crop developing at national and world level. In the category of viruses with important economical implications, Plum – pox is considered the worst, and it is studied from the majority of the profile researchers all over the world.

Depending on genotype, the attack of Plum pox was different manifested. (Table no. 1). In the germoplasm fund, it were registered evidently symptoms of PPV at a number of plats from 0% (*Andreea; Gogoşele negre, Sâmbăta 3, Troianu 9) to 70% (Vânăt românesc).* The PPV attack manifests on some branches or all over the plant. The most important attack registers when the plant is completely affected and the specific symptoms of PPV are generalized (*Minerva, Centenar*) (Table 2). The symptoms could be present on the fruits and leaves with the exception of the genotypes at which the symptoms on the leaves are hardly to be recognized or are asymptomatically expressed (*Buburuz, Troianu 9, DNT 3, DNT 4 etc*.). The major importance is owned by the attack at the fruits level, because of the major economical damages that it produces. The infection of fruits production could be significant, being affected till 70-80% of the yield. Now there is no concept can consider that there is real tolerance in different degrees of manifestation. The statement is sustained by the fact that some cultivars tolerate the sever attack of the disease in the local area, depending on the manifestation on fruits and the influence on the production.

Over the sensibility report at the natural infections through vectors, over the quickness of the infection, it were considered sensitive and middle sensitive the cultivars: *Vânăt românesc, Centenar, Stanley, Silvia, Minerva*. There are infected slowly on natural way the cultivars: *Tuleu gras, Ialomiţa, Piteştean, Carpatin, Alina, Tita, Gras ameliorat etc.* The correlation between the system host/parasite and natural selection determine the promoting of the genotypes with high adapting value, tolerant at

PPV: *Flora, Mirabelle de Nancy, Andreea, Oteşani 8, Scolduş, Rival, Gogoşele negre, Troianu 9, Sâmbăta 3, DNT 3 etc.*

The infection with PPV virus at the cultivars: *Tuleu gras, Minerva, Carpatin, Alina, Silvia, Centenar, Anna Spath, Stanley* was confirmed through biological tests on herbal and woody plants. The virus was slowly transmitted through grafting, grafts at the bark at woody species and through juice inoculating at herbal species (Table 3).

The woody indicators plants: *P. domestica cv. Tuleu dulce* and *P. persica cv. GF 305* and herbal plants test: *Chenopodium foetidum and Nicotiana benthamiana* present on the leaves, bark, offshoots, specifics PPV virus symptoms, the intensity of these oscillates from slow evident (+) to evident (++) till the very evident symptoms (+++). These are characterised with chlorotic or sometimes necrotic spots and strips on the leaves, the massive yellowing of the leaves with necrotic spots on the edges, dwarf ness, wick epinasty or copper yellowing of the leaves.

The serological analyses through DAS - ELISA, indicate the viral concentrations variable in function by genotype. A big part of the tested biological material accumulates big quantities of virus appreciated by ELISA levels (Table 4). The PPV virus (Plum pox) showed up at the cultivars: *Centenar, Anna Späth, Carpatin, Stanley, Vânăt românesc, Minerva, Silvia, Tuleu gras,* with the values of the absorption between 1.203 – 1.432, comparatively with the negative witness of 0.032. The values of the extinction ELISA between 0.037 – 0.092 registered the genotypes: *Rival, DNT 4, DNT3, Sâmbăta 3, Troianu 9, Scolduş, Oteşani 8, Andreea* and *Tita* with values appropriated to the healthy control (0,035).

This demonstrates the fact that the analyzed biological material is free of PPV virus. The methods are used to assure the negative selection at the Plum Pox virus (PPV). The negative phytovirotical selection based on visual observations, serological tests, tests with woody and herbal indicators, made in the plum germoplasm fund assured healthy biological material, needed in the propagation and breeding processes.

CONCLUSIONS

1. The germoplasm fund of existent *Prunus* gender is well represented of geographical provenience forms with different resistance to diseases, very properly to the breeding objectives for creating new cultivars and rootstocks.
2. The productivity of the polygenic plum type with addition effect is very high at the following cultivars: *Centenar* (23 t/ha*), Flora* (20 t/ha), *Gras ameliorat* (17 t/ha), *Andreea* (17 t/ha), near by: *Stanley, Silvia, Tuleu gras* etc.
3. In the breeding process the genotypes: *Tuleu gras, Centenar, Stanley, Oteşani 8, Gogoşele negre, Buburuz, DNT 3, DNT4* etc., transmit the high productivity character and amplify it through the addition of new genes.
4. The *Plum Pox virus* (PPV) is the worst spread virus and influence negatively the fruit production. It manifests differently depending on genotype between 0% (*Andreea, Rival, Gogoşele negre, Sâmbăta 3)* and 70-85% infected trees at *Vânăt românesc.*
5. There are tolerant or slow infected (10–12 years) with PPV the following cultivars and biotypes: *Andreea, Flora, Mirabelle de Nancy, Oteşani 8, Gogoşele negre, Sâmbăta 3 etc.*
6. The biological tests with woody and herbal indicators and ELISA serological analyzes assured the PPV diagnoses. The negative phytovirotical selection offers the possibility to identify and select some plum genotypes (cultivars, clones, local

biotypes) free of PPV, with tolerance at this disease attack, with lent infection in time.

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Tables

Table 1. The fruit production and the influence of the plum pox attack over them.

No. crt.	Specification	The age of the plants at observation date	The fruit production	The fruit production attacked by PPV virus		The tolerance at Plum pox
				%	T/ha	
1	Tuleu gras	9-12	15,5	15	2,3	Gto.1
2	Minerva	7-12	17	18	3,1	Gto.2
3	Ialomiţa	9-12	15,3	10	1,5	Gto.1
4	Piteştean	9-12	16,2	12	2,0	Gto.1
5	Carpatin	9-12	18,5	10	1,8	Gto.1
6	Alina	5-10	15	15	2,3	Gto.1
7	Tita	5-10	12,5	10	1,3	Gto.1
8	Andreea	4-7	17,0	0	0	Gto.0
9	Vânăt romanesc	5-10	4,5	85	3,8	Gto.3
10	Silvia	5-10	17,5	20	3,4	Gto.2
11	Centenar	5-10	23,0	22	5,1	Gto.3
12	Gras ameliorat	7-12	17,0	12	2,0	Gto.1
13	Flora	7-12	20,0	5	1,0	Gto.1
14	Oteşani 8	6-10	16,5	11	2,0	Gto.1
15	Scolduş	6-10	12,5	10	1,3	Gto.1
16	Rival	3-6	15,7	0	0	Gto.0
17	Buburuz	7-10	16,3	5	1,0	Gto.1
18	Gogoşele negre	7-10	14,5	0	0	Gto.0
19	Troian 9	10-12	13,4	8	1,1	Gto.1
20	Sâmbăta 3	10-12	12,5	0	0	Gto.0
21	DNT 3	10-12	16,5	8	1,3	Gto.1
22	DNT 4	10-12	17,4	10	1,7	Gto.1
23	Mirabelle de Nancy	5-7	13,4	4	0,5	Gto.0
24	Anna Spath	7-10	16,5	11	2,0	Gto.1
25	Stanley	7-10	18,3	30	5,4	Gto.3

Gto.0 = cultivars with big tollerance Gto.1 = tolerant cultivars Gto.2 = medium tolerance cultivars
Gto.3 = low tolerance cultivars

Table 2. The phenotype manifestation mode of the plum pox attack at some plum genotypes

No. crt.	Specification	The localization of first visible symptoms of PPV at plants	The manifesting mode of PPV attack on fruits	When manifest the attack on fruits	The effect of the attack on fruits	The rate of the total infection of a tree (years)
1	Tuleu gras	The offshoots from lower 1/3 of the crown	Fruits with spots, without shape, red pulp stone	The foliar system presents over 20% symptoms	The fruits falls rarely	4-6
2	Minerva	Generalized symptoms all over the tree chlorotic strips and rings on the leaves	Symptoms evidently on skin, areas interrupted by fibres in the fruit pulp in the peduncule area	The foliar system presents over 40% symptoms	The fruits falls in percentage of 30-40%	2-4
3	Carpatin	The offshoots from lower 1/3 of the crown	Wick symptoms on the skin	The foliar system presents over 20% symptoms	The fruits falls rarely	4-5
4	Alina	The offshoots from lower 1/3 of the crown	No manifestations on fruits	-	-	6-7
5	Tita	The offshoots from lower 1/3 of the crown	No manifestations on fruits	-	-	6-5
6	Centenar	Real symptoms and generalized symptoms all over the tree	Fruits with spots, without shape, red pulp stone	The foliar system presents over 70 – 80% symptoms	The fruits falls in percentage of 30-40%	2-4
7	Andreea	Lower or without symptoms	No manifestations on fruits	-	-	6-7
8	Oteşani 8	The offshoots from lower 1/3 of the crown	No manifestations on fruits	-	-	8-10
9	Rival	Without symptoms	No manifestations on fruits	-	-	12-15
10	Gogoşele negre	Lower or without symptoms	No manifestations on fruits	-	-	6-8
11	DNT 3	Lower or without symptoms	No manifestations on fruits	-	-	6-8

Table 3. Some plum genotypes reaction at plum pox virus through biological tests.

No. crt.	Specification	Herbal test plants		Woody test plants		Observations
		Chenopodium foetidum	*Nicotiana benthamiana*	*Tuleu dulce*	*GF.305*	
1	Tuleu gras	(+)	(-)	(+)	(+)	Infected
2	Minerva	(+)	(-)	(-)	(+)	Infected
3	Ialomița	(-)	(-)	(-)	(-)	Healthy
4	Piteştean	(-)	(-)	(-)	(+)	Infected
5	Carpatin	(+)	(-)	(+)	(+)	Infected
6	Alina	(+)	(-)	(+)	(+)	Infected
7	Tita	(-)	(-)	(-)	(-)	Healthy
8	Andreea	(-)	(-)	(-)	(-)	Healthy
9	Vânăt romanesc	(-)	(-)	(+)	(+)	Infected
10	Silvia	(+)	(+)	(+)	(+)	Infected
11	Centenar	(+)	(+)	(+)	(+)	Infected
12	Gras ameliorat	(-)	(+)	(+)	(+)	Infected
13	Flora	(-)	(-)	(-)	(+)	Healthy
14	Mirabelle de Nancy	(-)	(-)	(-)	(-)	Healthy
15	Anna Spath	(+)	(+)	(+)	(+)	Infected
16	Stanley	(+)	(+)	(+)	(+)	Infected
17	Oteşani 8	(-)	(-)	(-)	(-)	Healthy
18	Scolduş	(-)	(-)	(-)	(-)	Healthy
19	Rival	(-)	(-)	(-)	(-)	Healthy
20	Buburuz	(-)	(-)	(-)	(-)	Healthy
21	Gogoşele negre	(-)	(-)	(-)	(-)	Healthy
22	Troian 9	(-)	(-)	(-)	(-)	Healthy
23	Sâmbăta 3	(-)	(-)	(-)	(-)	Healthy
24	DNT 3	(-)	(-)	(-)	(-)	Healthy
25	DNT 4	(-)	(-)	(-)	(-)	Healthy

Table 4. The plum pox diagnostic virus through das elisa test at some plum genotypes

No. crt.	Specification	DAS ELISA levels	Observations
1	Centenar	1,432	Infected
2	Anna Spath	1,072	Infected
3	Carpatin	1,240	Infected
4	Pescăruş	0,091	Infected
5	Stanley	1,301	Infected
6	Vânăt romanesc	1,402	Infected
7	Minerva	1,307	Infected
8	Silvia	1,203	Infected
9	Tuleu gras	0,794	Infected
10	Ialomița	0,072	Healthy
11	Tita	0,073	Healthy
12	Andreea	0,078	Healthy
13	Oteşani 8	0,092	Healthy
14	Scolduş	0,082	Healthy
15	Rival	0,037	Healthy
16	Gogoşele negre	0,059	Healthy
17	Troian 9	0,087	Healthy
18	Sâmbăta 3	0,056	Healthy
19	DNT 3	0,054	Healthy
20	DNT 4	0,042	Healthy
21	Healthy witness	0,035	Healthy

The valuation of some technological features of fruits at two new sweet cherry cultivars

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Keywords: cherry fruits, cultivars, chemical composition, deformation, antioxidant capacity.

ABSTRACT

The knowledge of the physical and chemical characteristics of fruits is a very important element to valuation of their quality. The cherry fruit yields in 2007 were smaller as against with the cherry fruit yields in the previous years. The principal negative factor was not the frost in February, but the excessive drought in April-June, which induced a pronounced physiological falling of the fruits and their low quality. We have valuated the technological features of the fruits at two new cherry cultivars (*Cetăţuia* and *Cătălina*) in the experimental field of Fruit Growing Development Station Iaşi (competition comparative culture), on the ground of fruit samples from the yield of the 2007 agricultural year. The following aspects have been studied: harvest period, stone size, percentage of the stone in the fruit, total and soluble dry matter, sugar content, total acidity, sugar/acidity ratio, resistance at deformation of the fruit and antioxidant capacity (rH) of fruits pulp samples.

INTRODUCTION

The knowledge of the physical and chemical characteristics of fruits is a very important element to valuation of their quality.

The alimentary value of fruits and the possibilities of integral showing to advantage by their keeping in fresh state and as a raw material for processing are reflected from these features.

We tried to establish some criteria for the characterization and appreciation of the fruits from the physical, chemical, technological and biochemical viewpoints, criteria that will be used to promote and maintain these breeds in the zonal assortment of influence of Fruit Growing Research Development Station Iaşi.

This paper comprises the description of the physical-chemical features for two new cherry breeds created at Fruit Growing Research Development Station Iaşi and the interpretation of the results obtained.

MATERIALS AND METHODS

The cherry fruit yields in 2007 were smaller as against with the cherry fruit yields in the previous years. The principal negative factor was not the frost in February, but the excessive drought in April-June, which induced a pronounced physiological falling of the fruits and their low quality. In our research, we used fruits harvested from two new sweet cherry cultivars, extant in the competition culture available at the Fruit Growing Research Development Station Iasi.

The following aspects have been studied: ripening date, stone size, percentage of the stone in the fruit, total and soluble dry matter, reducing glucids, total acidity, sugar/acidity ratio, resistance at deformation of the fruit and antioxidant capacity (rH).

The physical and chemical analyses on fruits were accomplished on base some parameters fixed in accordance with requirement processing sector.

For determined the fruit size, was have weight samples with 100 whole fruits (g) and 100 dry stones (g) using an high precision balance and than on the basis of this determinations were calculated flash/stone ratio.

Total acidity was determined through neutralization with sodium hydroxide 0.1 N till equivalence point as indicator used tymolphthalein.

Reducing glucids was determined through Schoorl method, while soluble dry substance refractometrically.

The total dry substance was determined through samples drying into drying stove and we calculated the difference between analyzed samples and dry produce, calculated the humidity%, too.

The deformation resistance was determined through reading on an original device of difference the fruit size (in mm), under 500 g or 1000 g weights.

The antioxidant capacity of those two cultivars taken to studied was determined through potentiometrical method with platinum electrode and reference electrode, expressed into rH parameter on fruits pulp samples.

RESULTS AND DISCUSSION

Ripening date. The *Cetăţuia* cultivar belongs to the very early ripening date needing only 31-39 days from the end of blossom until maturity.

The *Cătălina* cultivar belongs to the early ripening date needing a period of 48-51 days from the full blossom until maturity stage (*table 1*).

Fruits size. The sweet cherry cultivars we studied have fruits of small size to *Cetăţuia* (3.9 g) cultivar while medium size to *Cătălina* (5.7 g) cultivar (*table 2*).

The stone percent registered the value between 5.62% at *Cetăţuia* cultivar and 5.93% at *Cătălina* cultivar (*table 2*).

The flash/stone ratio registered the value between 16.79 at *Cetăţuia* cultivar and 15.88 at *Cătălina* cultivar (*table 2*).

The content of soluble dry substance. At sweet cherry cultivars which we studied, the content of soluble dry substance was registered the values between 17.2 ^{0}Bx at *Cetăţuia* cultivar and 16.2 ^{0}Bx at *Cătălina* cultivar (*table 3*).

The content of total dry substance registered the values between 18.3% at *Cetăţuia* cultivar and 17.77% at *Cătălina* cultivar (*table 3*). Basis that we calculated, the content of humidity registered values between 81.7% at *Cetăţuia* cultivar, and 82.23% at *Cătălina* cultivar.

The fraction glucids, the main constituent of dry substance registered value between 7.2% (*Cătălina)* and 10.4% (*Cetăţuia) to stand for* 40.51% and respectively 56.7% of the dry substance total content (*table 3*).

At sweet cherry cultivars studied the **total acidity** was registered between 0.92 g malic acid/100g at *Cetăţuia* cultivar and 0.79 g malic acid/100g at *Cătălina* cultivar (*table 3*).

The ratio sugar/acidity has a special role in establishing the direction for use especially for juice or fresh consumption. From this point of view, the breed *Cetăţuia* registered a sugar/acidity ration of 11.28, and the breed *Cătălina* a ratio of 9.13 (*table 3*).

Resistance of fruit against deformation. The two breeds were harvested at full maturity and we applied on the fruits weights of 500 g and 1000 g. When applying the weight of 500 g, the breed *Cetăţuia* registered an average deformation of 2.6 mm, and the breed *Cătălina* an average deformation of 2.2 mm. When applying the weight of

1000 g on fruit, the breed *Cetăţuia* registered a deformation of 7.9 mm, and the breed *Cătălina* a deformation of 7.8 mm (*table 4*).

The antioxidant capacity of cherries is superior to that of apples or pears but much more reduced than that of the species with small fruits such as wild strawberries, raspberries, bilberries or plumes, which have the highest antioxidant capacity.

For the cultivars studied, the rH value registered values of 26.29 for the *Cetăţuia* cultivar and 28.44 for the *Cătălina* cultivar.

Knowing that for a value of rH of 28.2 of a chemical system corresponds to neutrality from the reduction-oxidation viewpoint, we may say that the *Cetăţuia* cultivar has a reducing character and the *Cătălina* cultivar has an easily oxidizing character.

CONCLUSIONS

1. The *Cetăţuia* cultivar belongs to the very early ripening date needing only 31-39 days from the end of blossom until maturity, but it is smaller registering only 3.8 g the average weight of a fruit.
2. The *Cătălina* cultivar belongs to the early time ripening date needing a period of 48-51 days from the end of blossom until harvest, of average size, having an average weight of fruit of 5.7 g.
3. Although of a small weight, the *Cetăţuia* cultivar has a higher ratio pulp/stone (16.79) than the *Cătălina* cultivar (15.88), what makes it more advantageous for consumption.
4. The *Cetăţuia* cultivar is also superior to the *Cătălina* cultivar from the viewpoint of the contents of soluble dry substance, total glucids, level of acidity and sugar/acidity ratio, what makes it more important for consumption both in a fresh state and as a raw material for processing.
5. As for the resistance of fruit against deformation, the differences are very slight between the two cultivars they registering an almost identical deformation under a weight of 1000 g (7.8 mm and 7.9 mm, respectively), and under a weight of 500g, the deformation was 2.6 mm for the *Cetăţuia* cultivar and 2.2 mm for the *Cătălina* cultivar.
6. For the cultivars under study, the rH value registered values of 26.29 for the fruits pulp *Cetăţuia* cultivar and 28.44 for the fruits pulp *Cătălina* cultivar. That means the fruits pulp *Cetăţuia* cultivar has a more reducing character, whereas the fruits pulp of *Cătălina* cultivar has a slightly oxidizing character.

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Tables

Table 1. Ripening date at *Cetăţuia* and *Cătălina* cultivars

Cultivar	Full blossom date	Fruits maturity stage	Days between full blossom until fruits maturity stage
Cetăţuia	20.04	21- 29. 05	31-39
Cătălina	21.04	7- 10 .06	48-51

Table 2.The physical features of the fruits at two new sweet cherry cultivars created at the Fruit Growing Research Development Station Iasi

Features	**Cultivar**	
	Cetăţuia	**Cătălina**
Weight of 100 whole fruits (g)	389.60	569.71
Weight of 100 dry stones (g)	21.90	33.76
Stone percentage%	5.62	5.93
Flash/stone ratio	16.79	15.88

Table 3. The biochemical features of the fruits at two new sweet cherry cultivars

Cultivar	**SDS (^{0}Bx)**	**TDS (%)**	**Fraction glucids (%)**	**Acidity (g ac. malic/100g)**	**Sugar/acidity ratio**
Cetăţuia	17.2	18.3	10.38	0.92	11.28
Cătălina	16.2	17.77	7.21	0.79	9.13

Table 4. Deformation fruit resistence at two new sweet cherry cultivars created at the Fruit Growing Research Development Station Iasi

Cultivar	**Average deformation (in mm) of fruits, under weight of:**	
	500 g	**1000 g**
Cetăţuia	2.6	7.9
Cătălina	2.2	7.8

Perspective almond elites for fruit growing area of Oradea

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Keywords: selection, testing, homologate

ABSTRACT

The study of 55 elites, after 9 years after planting we recomand to extend 3 elites in the Oradea area: H4/1451/82, H1/2025/84 and H14/851/81, they have the biggest yield: 1443,8 kg/ha; 982,7 kg/ha and 930,5 kg/ha. Potato is one of the plants with the biggest requirement for continously water pro

INTRODUCTION

Is very known that essential problem for large spreading of the almond is the low level of the yield and rarelly, the accidents produced by late froisen in the spring. (V. Cociu 1967; V. Scheau et al. 2004, 2007).

The paper studies the almond selections very productive in the hybrids field on their roots in 4-5 years and after that the selections were planted in the competition microcrop.

MATERIALS AND METHODS

In 1997, after the selection in the hybrids field, 55 elites of almond were engrafted on almond in the own nurserry of the SCDP Oradea. In the spring of 1999 year, the elites were planted at 5/4 m in the competition microcrop with 5 tree on 1 variant.

Objectives studied:

- tree growth emphasized by trunck section surface;
- physics indexes of the fruits: size index (mm), weight index (g), broken report (% stone) double stone existence (%).

RESULTS AND DISCUSSIONS

In the table 1 is presented the trunk surface of the elites after 9 years from selection. We can observe that H16/1977/84, H16/1838/84, H31/1179/82, H16/1718/84, H24/719/82, H16/1610/84, H16/1939/84, H16/1979/84, H16/1992/84, H19/916/81, H1/2012/84, H4/1451/82 and H4/1465/82 are very vigorous, statistically assured, very significant; H16/1816/84, H9/1464/82, H24/811/81, H5/786/82, H15/2224/84 and H5/785/81, are distinque significant statistically and H23/2003/84, H16/1617/84 and H8/930/81 are significant statistically.

Very low vigorous had the next elites H46/1008/82, H23/2113/84, H6/2253/84 negative very significant statistically assured; H24/794/81, H8/1365/82 and H16/1986/84 distinque significant and H23/2104/84, H31/1426/81 and H31/1175/82 significant statistically.

In the table 2 the fruits and stones yield of the elites studied are presented. It was registered the yields between 2794,2 kg/ha (H9/1464/82) and 2090,7 kg/ha (H16/1939/84) in H1/2025/84, H14/851/81, H23/1501/82, H16/1974/84, H6/2253/84, H23/2003/84, H16/1608/84, H16/1986/84, H16/1838/84, H16/1992/84 and

H16/1828/84. Yield over 3000 kg/ha were registered in H5/786/81, 3014,8 kg/ha and H4/1451/82, 3662 kg/ha.

The record stones, very significant statistically was registered in H4/1451/82 with 1043,8 kg/ha, H1/2025/84 with 982,7 kg/ha, H14/851/81 with 930,5 kg/ha, H9/1464/82 with 899,1 kg/ha, H23/1501/82 with 770,2 kg/ha, H5/786/81 with 732,6 kg/ha, H16/1939/84 with 719,2 kg/ha and H16/1974/84 with 701,3 kg/ha.

Table 3 presents the physics properties of the fruits. Size index of the fruits was between 20,8 mm (H23/2076/84) and 31,3 mm (H31/1178/82) and the weight index was between 2,0 g (H8/1365/82, H16/1698/84 and H23/2076/84) and 6,1 g (H16/1992/84).

Big majority of the elites had a stones output between 30 and 40% with limits from 15% (H15/2224/84) to 63,9% (H5/785/81). Double stones over 10% were registered only in H5/786/81, H5/785/81, H8/1358/82 and H23/2113/84.

CONCLUSIONS

It is recomanded to spray in the crop of the following elites:

1. H4/1451/82, big vigorous, fruits yield 3662,5 kg/ha, stones yield 1043,8 kg/ha, median-big size of the fruit, weight of 3,7 g, brouken output of 28,5% and 5,9% double stones.
2. H1/2025/82, median-small vigorous, fruits yield of 2768,2 kg/ha, stones yield of 982,7 kg/ha, median-big size of fruits, weight of 3,5, brouken output of 35,5% and without double stones.
3. H14/851/81,median vigorous fruits yield of 2337,9 kg/ha, stones yield of 930,5 kg/ha, big size of the fruit, weight of 4,4 g, brouken output of 35,5% and 3,2% double stones.

All three elites recomanded are tested in ISTIS in the second year.

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Tables

Table 1. Elite growth emphasized by trunk section surface in the 9th year from planting

Nr. crt.	Elite	Trunk section surface		Difference (cm^2)	Signification
		Absolute(cm^2)	Relative (%)		
1	H16/1974/84	359,8	179,5	+159,3	***
2	H16/1838/84	290,8	145,0	+90,3	***
3	H31/1179/82	289,4	144,3	+88,9	***
4	H16/1718/84	288,4	143,8	+87,9	***
5	H24/719/82	282,3	140,8	+81,8	***
6	H16/1610/84	277,0	138,2	+76,5	***
7	H16/1939/84	274,0	136,7	+73,5	***
8	H16/1979/84	264,2	131,8	+63,7	***
9	H16/1992/84	261,9	130,6	+61,4	***
10	H19/916/81	252,7	126,0	+52,2	***
11	H1/2012/84	250,5	124,9	+50,0	***
12	H4/1451/82	250,4	124,9	+49,9	***
13	H4/1465/82	249,6	124,5	+49,1	***
14	H16/1816/84	245,6	122,5	+45,1	**
15	H9/1464/82	239,5	119,4	+39,0	**
16	H24/811/81	239,4	119,4	+38,9	**
17	H5/786/81	237,9	118,7	+37,4	**
18	H15/2224/84	236,3	117,9	+35,8	**
19	H5/785/81	236,0	117,7	+35,5	**
20	H23/950/81	234,4	116,9	+33,9	*
21	H23/2003/84	232,5	116,0	+32,0	*
22	H16/1617/84	228,7	114,1	+28,2	*
23	H8/930/81	227,9	113,7	+27,4	*
24	H23/1508/82	225,2	112,3	+24,7	
25	H16/1606/84	223,7	111,6	+23,2	
26	H46/985/82	223,4	111,4	+22,9	
27	H16/1828/84	218,6	109,0	+18,1	
28	H3/1421/81	216,4	107,9	+15,9	
29	H31/1178/82	216,1	107,9	+15,6	
30	H14/851/81	212,3	105,9	+11,8	
31	H16/1744/84	207,6	103,5	+7,1	
32	H19/912/81	201,7	100,6	+1,5	
33	PRIMORSKI (Mt)	200,5	100,0	-	
34	H24/818/81	199,0	99,3	-1,5	
35	H16/1919/84	198,0	98,8	-2,5	
36	H1/2006/84	197,7	98,6	-2,8	
37	H16/1730/84	194,0	96,8	-6,5	
38	H30/1125/82	193,7	96,6	-6,8	
39	H31/1223/82	191,1	95,3	-8,8	
40	H1/2025/84	190,2	94,9	-10,3	
41	H23/1501/82	189,7	94,6	-10,8	
42	H12/2148/84	187,2	93,4	-13,3	
43	H8/951/81	185,4	92,5	-15,1	
44	H8/1358/82	183,4	91,5	-17,1	
45	H46/953/82	181,9	90,7	-18,6	
46	H23/2076/84	176,2	87,9	-24,3	
47	H16/1698/84	174,8	87,2	-25,7	
48	H31/1175/82	173,4	86,5	-27,1	o
49	H31/1426/81	172,0	85,8	-28,5	o
50	H23/2104/84	171,7	85,6	-28,8	o
51	H16/1986/84	160,2	79,8	-40,3	oo
52	H8/1365/82	157,0	78,3	-43,5	oo
53	H24/794/81	155,8	77,7	-44,7	oo
54	H6/2253/84	147,3	73,5	-53,2	ooo
55	H23/2113/84	131,9	65,8	-68,6	ooo
56	H46/1008/82	121,5	60,6	-79,0	ooo

DL 5% = 26,1
DL 1% = 34,8
DL 0,1% = 45,3

Table 2. Fruits and stones yields of almond elites

Nr. crt.	Elite	Yield (kg/ha)		Relative (%)	Difference (cm^2)	Signification
		Fruits	Stone			
1	H4/1451/82	3662,5	1043,8	241,4	+611,4	***
2	H1/2025/84	2768,2	982,7	227,3	+550,3	***
3	H14/851/81	2337,9	930,5	215,2	+498,1	***
4	H9/1464/82	2794,1	899,7	208,1	+467,3	***
5	H23/1501/82	2533,6	770,2	178,1	+337,8	***
6	H5/786/81	3014,8	732,6	169,4	+300,2	***
7	H16/1939/84	2090,7	719,2	166,3	+286,8	***
8	H16/1974/84	2393,5	701,3	162,2	+268,9	***
9	H5/785/81	1094,4	699,3	161,7	+266,9	***
10	H6/2253/84	2583,9	692,5	160,2	+260,1	***
11	H23/2003/84	2433,8	691,2	159,9	+258,8	***
12	H1/2012/84	1061,8	664,7	153,7	+232,3	***
13	H24/719/82	1204,6	624,0	144,3	+191,6	***
14	H31/1426/81	1025,7	614,4	142,1	+182,0	***
15	H46/1008/82	1458,8	570,4	131,9	+138,0	***
16	H3/1421/81	1091,4	561,0	129,7	+128,6	***
17	H16/1606/84	2334,5	527,6	122,0	+95,2	**
18	H16/1816/84	1459,7	518,2	119,8	+85,8	**
19	H16/1730/84	1415,1	488,2	112,9	+55,8	
20	H16/1986/84	2408,4	486,5	112,5	+54,1	
21	H8/1365/82	1415,1	456,9	105,7	+24,5	
22	H16/1919/84	1923,5	440,5	101,9	+8,1	
23	H46/985/82	1012,5	436,4	100,9	+4,0	
24	PRIMORSKI (Mt.)	1225,0	432,4	100,0	-	
25	H16/1838/84	2183,3	419,2	96,9	-13,2	
26	H16/1992/84	2098,5	409,2	94,6	-23,2	
27	H8/1358/82	739,2	408,8	94,5	-23,6	
28	H1/2006/84	826,6	407,5	94,2	-24,9	
29	H16/1828/84	2338,4	402,2	93,0	-30,2	
30	H16/1744/84	1195,6	379,0	87,6	-53,4	
31	H23/2113/84	1481,8	349,7	80,9	-82,7	o
32	H31/1175/82	1398,4	342,6	79,2	-89,8	oo
33	H4/1465/82	1328,2	338,7	78,3	-93,7	oo
34	H24/811/81	1097,0	329,1	76,1	-103,3	oo
35	H12/2148/84	1318,8	315,2	72,9	-117,2	ooo
36	H16/1698/84	666,2	315,1	72,8	-117,3	ooo
37	H31/1179/82	1152,5	303,1	70,1	-129,3	ooo
38	H31/1223/82	1203,2	298,4	69,0	-134,0	ooo
39	H30/1125/82	766,1	295,7	66,9	-136,7	ooo
40	H23/950/81	1278,4	283,8	65,6	-148,6	ooo
41	H23/2104/84	947,7	282,2	65,3	-150,2	ooo
42	H16/1718/84	1084,7	262,5	60,7	-166,9	ooo
43	H16/1617/84	1243,4	254,9	59,0	-177,5	ooo
44	H8/951/81	641,7	223,3	51,6	-209,1	ooo
45	H24/818/81	821,4	218,5	50,5	-213,9	ooo
46	H19/912/81	750,5	215,4	49,8	-217,0	ooo
47	H23/2076/84	347,9	210,8	48,8	-221,6	ooo
48	H24/794/81	617,6	206,9	47,8	-225,5	ooo
49	H15/2224/84	1352,3	204,2	47,2	-228,2	ooo
50	H19/916/81	859,7	194,3	44,9	-238,1	ooo
51	H31/1178/82	519,2	178,1	41,2	-254,3	ooo
52	H16/1610/84	487,1	166,6	38,5	-265,8	ooo
53	H23/1508/82	654,9	165,7	38,3	-266,7	ooo
54	H16/1979/84	588,3	161,2	37,8	-271,2	ooo
55	H46/953/82	665,7	147,8	34,2	-284,6	ooo
56	H8/930/81	555,3	120,5	27,9	-311,9	ooo

DL 5% = 62,2
DL 1% = 82,7
DL 0,1%= 107,6

Table 3. Physics properties of the fruits in almond elites

Nr. crt.	Elite	Im (mm)	Ig (g)	Peeling (% stones)	Double stones (%)
1	H4/1451/82	25,3	3,7	28,5	5,9
2	H1/2025/84	25,1	3,5	35,5	-
3	H14/851/81	26,2	4,4	35,5	3,2
4	H9/1464/82	26,6	2,9	32,2	-
5	H23/1501/82	24,6	4,1	30,4	-
6	H5/786/81	26,2	2,4	24,3	18,9
7	H16/1939/84	26,7	4,1	34,4	-
8	H16/1974/84	25,5	4,4	29,3	3,6
9	H5/785/81	24,4	2,1	63,9	10,8
10	H6/2253/84	27,7	5,7	26,8	-
11	H23/2003/84	26,2	4,5	28,4	-
12	H1/2012/84	27,7	2,6	62,6	-
13	H24/719/82	27,2	3,4	51,8	3,4
14	H31/1426/81	25,5	2,7	59,9	-
15	H46/1008/82	26,8	3,2	39,1	-
16	H3/1421/81	28,8	2,7	51,4	7,1
17	H16/1606/84	27,1	5,5	22,6	-
18	H16/1816/84	27,3	5,2	35,5	-
19	H16/1730/84	25,9	3,4	34,5	-
20	H16/1986/84	25,6	4,4	20,2	-
21	H8/1365/82	23,0	2,0	32,3	2,9
22	H16/1919/84	26,2	4,8	22,9	-
23	H46/985/82	26,2	2,9	41,3	4,3
24	PRIMORSKI (Mt.)	22,4	4,0	32,1	2,2
25	H16/1838/84	26,6	5,2	19,2	5,6
26	H16/1992/84	27,0	6,1	19,5	-
27	H8/1358/82	28,2	2,7	55,3	10,0
28	H1/2006/84	25,8	2,5	49,3	-
29	H16/1828/84	27,6	5,2	17,2	-
30	H16/1744/84	26,6	4,1	31,7	-
31	H23/2113/84	26,8	4,2	23,6	13,0
32	H31/1175/82	24,9	4,7	24,5	2,8
33	H4/1465/82	26,9	3,8	25,5	-
34	H24/811/81	25,6	3,8	30,0	-
35	H12/2148/84	24,1	4,4	23,9	-
36	H16/1698/84	23,0	2,0	47,3	-
37	H31/1179/82	26,2	3,8	26,3	-
38	H31/1223/82	28,0	5,5	24,8	-
39	H30/1125/82	27,9	2,5	38,6	-
40	H23/950/81	27,2	4,8	22,2	-
41	H23/2104/84	25,4	3,7	29,8	-
42	H16/1718/84	28,3	6,1	24,2	-
43	H16/1617/84	25,6	4,8	20,5	-
44	H8/951/81	25,6	4,2	34,8	-
45	H24/818/81	27,1	3,6	26,6	-
46	H19/912/81	27,8	5,1	28,7	-
47	H23/2076/84	20,8	2,0	60,6	3,0
48	H24/794/81	25,6	3,1	33,5	4,2
49	H15/2224/84	26,6	5,4	15,1	8,3
50	H19/916/81	26,2	4,2	22,6	-
51	H31/1178/82	31,3	5,8	34,3	-
52	H16/1610/84	28,8	4,5	34,2	8,6
53	H23/1508/82	27,8	5,4	25,3	-
54	H16/1979/84	25,5	3,5	27,4	-
55	H46/953/82	26,6	4,9	22,2	-

Persimmon - a new specie for the southern Romanian area

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Keywords: grafting, rootstock, cultivars, quality, phenophases

ABSTRACT

As researches of Fruit Growing Tree Department results during 2003-2007 regarding persimmon's cultivars behaviour, result that in the Romanian Plain are the temperatures conditions, in most of the years. There are years with thermal shocks but flower buds loss are minimal and the trees have a very well regeneration capacity by pruning. The biggest productions are obtained at Sharon cultivar, which is the latest from the studied cultivars assortment.

INTRODUCTION

In 1998 were brought the first persimmon trees from Portugal and were planted in the Experimental-Didactical Field of USAMV Bucharest. One year late, were obtained by changing with Tras-Os Montes University, Alto Douro Vila Real, scions from the Hana Fuyu, Rojo Brillante, Coroa de Rei, Fuyu, O'Gosho and Sharon cultivars, which were grafted on *Diospyros lotus* rootstock. With these trees was established an orchard that made the object of a PhD thesis. By introducing some locale populations from China and U.S.A., the persimmon assortment in 2001 was formed by 5 cultivars and 2 populations remarkable by big productions.

The principal aim of these researches was to introduce progressively new species and cultivars from subtropical climate, that can adapts and have a normal fructification in the Southern Romanian areas, where the effect of the climatically changes requires changing of the pomological assortment.

MATERIALS AND METHODS

The trees used in researches were obtained at the Experimental-Didactical Field of Fruit Growing Trees Department from U.S.A.M.V. Bucharest; were used as grafting methods: grafting in the growing bud, grafting in the dormant budlanchier and bark grafting.

After one year of grows in the second field of the nursery, the trees were planted in a plot, as a dwarf peach intercrop, in decline phase. Planting distance was 4 x 3 m, with a density of 833,33 trees/ha.

For obtaining the first information about the possibility of cultivating this tree in Romania, were effectuated many phenophases observations finalized with establishing the vegetation period, the days number from blooming till harvest, to harvest registration and fruit quality appreciations after size, weight and biochemical content.

During the researches the soil was tillage, fertilized and irrigated at the optimal parameters for the growing and fructifying phenophases to develop in normal, optimal conditions.

RESULTS AND DISCUSSIONS

Developing of the phonological of vegetative and generative organs at persimmon during 2003-2007 in Bucharest aria, was dependent of the climatically factors level and of the biological particularities of every cultivar (tables 1 and 2).

During 2003-2007, budding took place from 24.03 till 25.04, earlier at CPL populations and at the cultivars O'Gosho, Rojo Brillante and Coroa de Rei and later at Sharon.

The growing start was during 1-30 April, in the same order established in the first phenophase.

Intense growing was registered in April, when the level of temperature rise and the amplitude was reduced very much. This phenophase was from 4.04 till 9.05.

The end of growing was develop in a bigger interval was during 23.06 - 27.07. The vegetation ends first at CPL, O'Gosho, Coroa de Rei, Hana Fuyu and later at Sharon and Rojo Brillante.

Generative organs phenophases evolution during 2003-2007 (table 2) was strongly influenced by the temperature fluctuations from the vegetation. In Bucharest, the first flower was bloomed at Rojo Brillante, fallowed by Coroa de Rei and O'Gosho, Hana Fuyu, Sharon and CPL. In the years 2003-2007, start of blossom and in blossom from 9 to 17 days. End of blossom, noticed when the first petals were drop, took place in a shorter time 12 -14 days.

Fruit set was registered from 16.05 till 27.06, earlier at Rojo Brillante, O'Gosho and CPL and later at Hana Fuyu and Sharon.

After the maturation season criteria, the fruits of the persimmon cultivars were grouped in: early cultivars (Hana Fuyu), midseason (Rojo Brillante, CPL, Coroa de Rei and O'Gosho) and late cultivars (Sharon).

For the vegetation from blooming till end of vegetation, persimmon's cultivars needs 191-217 days, with positive temperature. These conditions are assured completely in the South and South-West Romanian arias. Time from blossom till harvest was longer at Sharon and Rojo Brillante cultivars (147 days).

The trees started to fructifying in 2004 when the cultivar Hana Fuyu realizing 1,35 kg fruits/tree (table 3). In 2005 started to fructify Rojo Brillante, with a harvest of 2,63 kg/tree, then enter in an perpetual fruiting and fructifications again in 2007 with a harvest obtain of 3,26 kg/tree. In 2006 started to fruiting the cultivars Coroa de Rei (4,28 kg/tree), Sharon 1,52 (kg/tree) and the population CPL (3,13 kg/tree). In 2007 starting to fructification O'Gosho cultivar (4,51 kg/tree). The biggest harvest was realized by the cultivars Sharon (6,31 kg/tree), O'Gosho (4,51 kg/tree) and CPL (4,36 kg/tree).

Analyzing the harvest potential per hectare in 2007 situated on the first place the Sharon cultivar, fallowed by O'Gosho, CPL and Hana Fuyu (figure 1).

Regarding the harvest quality appreciated during this period through size and fruits quality, the biggest fruits were at Rojo Brillante (281,3 g – table 4) and the smallest at Coroa de Rei (120,5 g).

In this period, the biochemical content of the fruits (table 5) shows a different water content from 67,63 to 75,72%. The dry matter had a variation from 24,28% (CPL) to 32,37% (Hana Fuyu). A bigger sugar content was at Coroa de Rei, at the others the values were between 24,28 and 29,11%. NPK content registered variable values between 627-847 ppm for $N\text{-}NO_3^-$, 242,20-346,50 ppm for $P\text{-}PO_4^{3-}$ and 24,28-32,37 ppm for K^+.

CONCLUSIONS

Analyzing the growing and the fructification process in the ecological and technological conditions from Romania, resulted the following conclusions:

1. Development of phonological phases was dependent of the temperature and precipitations level during the vegetation.
2. Fruits maturation was determinate by genetically features of the cultivars and the conditions from the years of culture.
3. In 2007, the biggest fruits were obtained by Rojo Brillante and the biggest production by the Sharon cultivar.
4. An early maturation was noticed at Hana Fuyu cultivar (22 October) and a late one at Sharon, (7 November) and for the others cultivars was during 4-24 October.
5. The size and the biochemical content situated on the first place the Rojo Brillante cultivar.

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Tables

Table 1. Phenological phases development at the vegetative organs during 2003-2007

Cultivar/ population	Budding	The growing start	Intense growing	End of growing
Hana Fuyu	02.04-23.04	07.04-28.04	04.04-07.05	02.07-14.07
Rojo Brillante	28.03-23.04	03.04-30.04	12.04-09.05	05.07-27.07
CPL	24.03-18.04	01.04-23.04	11.04-02.05	23.06-02.07
Coroa de Rei	28.03-23.04	03.04-28.04	12.04-07.05	01.07-10.07
O'Gosho	27.03-18.04	02.04-23.04	10.04-02.05	03.07-12.07
Sharon	08.04-25.04	13.04-30.04	22.04-09.05	02.07-19.07

Table 2. Phenological phases development at the generative organs during 2003-2007

Cultivar/ population	Start of blossom	In blossom	End of blossom	Fruits set	Fruits maturation	Vegetation period (days)	Number of days from blooming to harvest (days)
Hana Fuyu	21.05-03.06	28.05-08.06	02.06-16.06	25.05-09.06	22.09-31.10	192-222	131-140
Rojo Brillante	11.05-28.05	18.05-02.06	28.05-10.06	16.05-31.05	04.10-24.10	197-224	135-147
CPL	21.05-01.06	23.05-05.06	28.05-12.06	21.05-07.06	04.10-30.10	188-199	136-137
Coroa de Rei	18.05-28.05	25.05-03.06	03.06-10.06	23.05-05.06	04.10-26.10	192-224	134-140
O'Gosho	18.05-29.05	23.05-04.06	25.05-09.06	21.05-24.05	04.10-23.10	198-225	140
Sharon	21.05-03.06	08.05-26.05	03.06-15.06	02.06-27.06	09.10-07.11	191-217	147

Table 3.The fruits production during 2004-2007

Cultivar/ population	2004		2005		2006		2007	
	kg/tree	kg/ha	kg/tree	kg/ha	kg/tree	kg/ha	kg/tree	kg/ha
Hana Fuyu	1,35	1125,00	5,21	4341,65	4,31	3591,65	3.49	2908,32
Rojo Brillante	-	-	2,63	2191,66	-	-	3,26	2716,66
Coroa de Rei	-	-	-	-	4,28	3566,65	3,06	2549,99
Sharon	-	-	-	-	1,52	1266,66	6,31	5258,31
O'Gosho	-	-	-	-	-	-	4,51	3758,32
CPL	-	-	-	-	3,13	2608,32	4,36	3633,32

Table 4. The harvest quality at persimmons during 2004-2007

Cultivar/ population	Diameter (mm)				Weight (g)			
	2004	2005	2006	2007	2004	2005	2006	2007
Hana Fuyu	52,3	73,2	67,1	68,3	86,5	185,2	120,6	141,3
Rojo Brillante	-	75,3	-	81,0	-	259,1	-	281,3
Coroa de Rei	-	-	68,2	66,3	-	-	163,1	156,3
Sharon	-	-	66,1	68,2	-	-	120,5	134,5
O'Gosho	-	-	-	65,4	-	-	-	126,3
CPL	-	72,3	65,1	66,3	-	155,1	120,6	121,3

Table 5. Mineral fruits content of some cultivars and persimmon population (2007)

Cultivar/ population	Water content (%)	Dry matter (%)	Total sugar (%)	Solubile sugar (%)	$N\text{-}NO_3^-$ (ppm)	$P\text{-}PO_4^{3-}$ (ppm)	K^+ (ppm)
Coroa de Rei	71,54	28,46	17,12	6,983	847	249,12	28,46
Sharon	70,89	29,11	16,56	6,984	627	276,80	29,11
Hana Fuyu	67,63	32,37	16,65	6,890	907	297,56	32,37
Rojo Brillante	68,47	31,53	16,75	6,877	627	290,64	31,53
O'Gosho	75,37	24,63	16,80	6,858	634	242,20	24,63
CPL	75,72	24,28	16,25	6,731	741	346,50	24,28

Figures

Fig. 1. Persimmon's fructification

Behaviour of Some New Resistant Romanian Apple Cultivars Under Different Planting Systems

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Keywords: *Malus domestica,* scab resistant cultivars, Spindle, Drilling, Mikado, vegetative growth, flower shoots,

ABSTRACT

Within the Romanian apple breeding program, Bistrița Fruit Research and Development Station, released in the last years an important number of scab resistant cultivars. At the Faculty of Horticulture in Bucureşti we started to test some of the most important ones: Aura**,** Auriu de Bistrița**,** Bistrițean**,** Jonaprim and Starkprim. Two of most diffused apple cultivars in the Romanian orchards: Florina and Idared were used as control. Trees were grafted on M 26 rootstocks and planted in the spring 2005 at 3.5 m between rows. The distance between the trees on the row varied from 1.5 m for Spindle, and 2.0 m for Drilling and Mikado canopies. Soil was maintained grass covert between rows and with polypropylene fabric mulch on the row. The canopy formation consisted mainly in summer pruning and shoots tiding. An integrated pests and diseases management was applied. In order to study the vegetative growth and the capacity of flower shoots formation, from the first growing season, the shoots type, shoots number and length was determined.

INTRODUCTION

Romania has an important apple breeding program. Starting with the 70', grace to the collaboration with Prof. Hough L.F., one of the promoters of PRI-COOP breeding program, a systematic program for scab and mildew resistance was initiated.

Bistrița Fruit Research and Development Station is one of the most important Romanian apple breeders and recently, it released few new cultivars: Aura, Auriu de Bistrița, Bistrițean, Jonaprim and Starkprim.

Apple planting systems had a constant and rapid evolution in the last century. Even the tendency in the late 80' was to develop very high density orchards (6000-10000 trees/ha) in Europe, most of the commercial apple orchards nowadays are planted under medium densities of 2000-3000 trees/ha.

The main raison for this approach was the necessity to reduce the initial investment required by high density planting systems as Super spindle. Alternative solutions were offered by multi axe canopies as Drilling and Mikado that proposed the reducing of the number of trees per hectare meanwhile maintaining the productive volume of the canopy (1, 2).

MATERIALS AND METHODS

In the Pomology Department experimental field within the Faculty of Horticulture in Bucureşti, five new released scab apple resistant varieties were compared with two of the most diffused apple cultivars in the Romanian orchards: Florina and Idared.

The studied Romanian apple cultivar are: Aura, Auriu de Bistrița, Bistrițean, Jonaprim and Starkprim.

Aura released in 1999 was obtained from the cross Prima x BN 33-39. The tree is vigorous and has a high an constant yield. Being resistant to scab and mildew, in

Bistriţa – Năsăud area is cultivated without pesticides. The fruit is big (161 – 186 g), conic-spherical, orange, with firm and juicy flesh.

Auriu de Bistriţa is a new scab resistant cultivar with big ovoid-spherical fruits, golden-yellowish colored and with good quality. Fruits rip in September and can be stored for1-3 months.

Starkprim was obtained from the cross between Starkrimson and Prima, and was released in 2000.

The tree has a medium vigor and produces on short flower shoots being spur type. The fruits are big (160-200 g), similar with the Calvil apple type, trunk-conical, colored in red-orange with a firm, juicy and flavored flesh. Ripening period is September with a fruit storage till December.

Trees were grafted on M 26 rootstocks and planted in the spring 2005 at 3.5 m between rows. The distance between the trees on the row varied from 1.5 m for Spindle, and 2.0 m for Drilling and Mikado canopies.

Soil was maintained grass covert between rows and with polypropylene fabric mulch on the row. A drip irrigation system was installed under the mulch.

The canopy formation consisted mainly in summer pruning and shoots tiding (3). An integrated pests and diseases management was applied.

In order to study the vegetative growth and the capacity of flower shoots formation, from the first growing season, the shoots type, shoots number and length was determined. Data were cummulated from 2005 to 2007.

RESULTS AND DISCUSSION

The formation of the vegetative shoots was influenced both by cultivar and by the planting system.

As one can see in the table 1, there is not a major difference between the cumulative number (2005-2007) of the long vegetative shoots and the formed spurs. However, in the first growing years, the long shoots number were dominant in Idared, Bistriţean and Jonaprim cultivars, meanwhile, Starkprim has a larger number of spurs.

Bistriţean was the most vigorous cultivar, having the strongest vegetative growth.

Between the three planting systems, Mikado showed also a higher cumulative number of spurs.

The cummulated number of fruiting spurs per tree varied from 71,7 in Spindle system to 126,6 in Mikado (table 2). Between the studied cultivars the highest number of fruiting spurs was registered in Starkprim, a typical spur cultivar, followed by Auriu de Bistriţa and Idared. The lowest number of fruiting spurs were produced by Aura.

Drilling and Mikado showed a similar capacity of supporting brindles formation with an average of 18,6 and respectively 19,9 brindles per tree, meanwhile Spindle system formed only 15,5 brindles per tree. The highest number of brindles per tree was counted on Auriu de Bistriţa (27) and the lowest on Florina (9,5).

The number of the crowned brindles was inferior to the number of brindles in all the studied cultivars. However, again Drilling and Mikado registered higher values than Spindle systems.

The studied cultivars had different behaviour related to the canopy and the rootstock used.

Drilling and Mikado systems showed a highest crown productive volume, to Spindle and between of them, Mikado is the most recommended to be extended in commercial orchards.

The most precocious and productive cultivar was Starkprim, followed by Auriu de Bistrița.

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Tables

Table 1. Influence of cultivar and planting system on vegetative shoots and spurs formation (2005-2007)

Canopy	Spindle		Drilling		Mikado		Average	
Cultivar	**Vegetative shoots**	**Spurs**	**Vegetative shoots**	**Spurs**	**Vegetative shoots**	**Spurs**	**Vegetative shoots**	**Spurs**
Florina	34.5	42.0	29.0	24.0	24.0	36.5	33.3	33.1
Idared	17.5	17.0	24.5	27.5	26.0	49.0	30.3	24.9
Aura	34.3	32.7	48.0	36.0	28.5	18.5	33.6	34.1
Auriu de Bistrița	36.8	16.8	33.5	35.0	32.5	67.0	45.8	32.5
Bistrițean	25.0	20.4	28.0	18.5	47.0	41.5	31.5	23.5
Jonaprim	25.5	19.0	23.5	17.5	31.0	43.5	30.8	22.4
Starkprim	15.0	32.5	28.5	45.0	27.5	52.5	32.0	36.5
Average	**26.9**	**25.8**	**30.7**	**29.1**	**30.9**	**44.1**		

Table 2. Influence of cultivar and planting system on flowering shoots formation (2005-2007)

Canopy	Spindle			Drilling			Mikado		
Cultivar	**Fruiting spurs**	**Brindles**	**Crowned brindles**	**Fruiting spurs**	**Brindles**	**Crowned brindles**	**Fruiting spurs**	**Brindles**	**Crowned brindles**
Florina	79.5	12.5	5.0	23.0	7.0	1.5	143.5	9.0	22.5
Idared	50.5	15.5	2.7	101.5	11.5	4.5	189.0	18.5	8.0
Aura	45.6	6.3	0.6	97.5	21.5	6.5	85.5	12.0	0.5
Auriu de Bistrița	86.0	19.4	4.8	130.5	29.0	10.5	97.0	32.5	2.0
Bistrițean	20.5	19.0	7.8	93.0	21.5	6.5	110.0	16.0	14.0
Jonaprim	104.5	17.0	6.5	82.5	17.0	8.5	116.5	28.5	6.5
Starkprim	115.5	18.5	7.5	107.0	23.0	16.5	145.0	23.0	7.0
Average	**71.7**	**15.5**	**5.0**	**90.7**	**18.6**	**7.8**	**126.6**	**19.9**	**8.6**

Figures

Fig. 1. Spindle system in the second year after planting

Fig. 2. Detailes of Drilling system in the second year after planting

Fig. 3. Drilling and Mikado system with antihail protection

Preliminary results concerning the evolution of main biochemical components of some excessively perishable fruits (berries) during the modified atmosphere storage

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Keywords: raspberry, currant, blackberry, biochemical component

ABSTRACT

Valorization of the excessively or excessively perishable fruits is important not only for the fruit producers, but also for the fruit sellers from the entire trade process (intermediate storage, storage between two delivering centers, specialized shops, supermarkets) and for the consumers. This category of fruit needs a special attention concerning the different steps of the valorization technology, starting with harvesting and finishing with selling. Moreover the technological factors related to the cropping technology, conditioning method (sorting, calibration, packaging), handling, transportation and storage have decisive influence in the success selling on the market of the product who correspond to the requirements of the quality standards operation and not lastly to respond to the increasing consumer exigencies. The purpose of the researches results presented in the paper is to prove the effect of storage under modified atmosphere conditions on the storage period and maintaining the quality of some berries fruits.

INTRODUCTION

The main factors which are involved in shelf life of the very perishable fruits as berries are the following: temperature, relative humidity of the air, light, atmospheric composition and mechanical damage. All these factors could action independently or in complex on the fruits growing in the field as well as in post harvest life in storage spaces.

There in no doubt that the most important factor affecting post harvest life is temperature. This is because temperature has a major affect on the rate of biological reactions as for example metabolism and respiration. The temperature for the majority of the physiological processes of most crops ranges between 0 and 30°C, increasing temperatures causing an exponential rise in respiration. Adequate O_2 levels are required to maintain aerobic respiration. The level of O_2 that reduces respiration still permitting aerobic respiration varies with commodity and variety. In most crops, O_2 level around 2 to 3% produces a beneficial reduction in the rate of respiration and other metabolic reactions. Increasing the CO_2 level in the case of some commodities reduces respiration, delays senescence and retards fungal growth.

Generally there is a reverse relationship between respiration rate and post harvest-life of fresh fruits. Respiration plays a major role in the post-harvest life of fresh commodities because it reflects the metabolic activity of the tissue that also includes the loss of water and food reserves in the tissue, loss of taste quality (especially sweetness) and food value for the consumer, the synthesis of new compounds, and the release of heat energy.

MATERIALS AND METHODS

In order to have relevant data concerning storage life for excessively perishable fruits there were harvested 3 varieties for each species from this category of fruits, respectively: red raspberry (Glen Moy, Willamette, Veten); black currant (Geo) and red currant (Roşu Timpuriu, Youthner); blackberry (Arapaho, Darrow, Lockness). These species and varieties are cultivated in the experimental collection of Research Institute for Fruit Production Piteşti and also are cultivated in small areas in Southeastern part of Romania.

The harvesting of fruits was made in the summer of 2007, in the stage of proper maturity for each variety. For fresh market, blackberry maturity can be determined by fruit color, gloss, and easy of detachment. Fully black berries should pull easily from the pedicel yet be firm, not mushy. Blackberries lose acidity with ripening and are quite astringent if harvested partially colored. For fresh market, raspberries were best harvested when get into bright-red (red raspberry) or fully-colored (purple raspberry). Berries should pull or shake easily from the receptacle, yet be firm, not mushy. Red and black fruits from currant were best harvested as soon as they are clear in color. It is necessary to pick whole cluster to avoid injuring the delicate fruit.

The fruits from all species were harvested manually, directly in little plastic boxes (250 g). The samples were considered 1 kg for each variety and species. The samples were put in refrigerating boxes immediately after harvesting and also in the storage cells.

According to Ryall, A.L. and col., 1978; Ryall, A.L. and col., 1979; Kader, A.A. and col., 1985; Salunkhe, D.K., 1992, for appreciation of fresh fruit quality were made sensorial analysis and biochemical analysis for each sample. The samples were stored under modified atmosphere (4% CO_2), in their original package, until 10% from samples quantity (each variety and each species) had lost the initial quality.

RESULTS AND DISCUSSIONS

At the harvesting moment, the fruits from all species and varieties were in conformity to quality standards concerning fruit maturity stage, firmness, taste, aspect and size. The optimal level of these quality components is very important in the process of maintaining the quality characteristics and increase shelf life in the process of storage.

The results of determinations concerning biochemical component from excessively perishable fruits were recording in the table 1.

The respiration process before the storage has the highest values at raspberry (Veten variety) and the lowest at blackberry (Arapaho variety) (Table 2).

The fruits of raspberry varieties can be preserved without losing firmness, taste, and aspect during 13 days. The content of total solids, after 13 days of storage, under modified atmosphere condition, indicate the highest loss on Willamette variety (from 10% to 7.6%) and the lowest loss on Veten variety (9.3% to 7.6%). Total acidity increase with 0.2% from initial value to final value on Glen Moy variety and also with 0.12% on Willamette variety. Total sugar decrease is the highest on Veten variety (with 2.12%) and the lowest on Glen Moy variety. According to the final values of the three components of the biochemical analysis, Glen Moy variety can be characterized as the most balanced after 13 days of storage (Table 2).

Black currant and red currant varieties can be stored under modified atmosphere conditions without losing firmness, taste, and aspect during 30 days. The content of

total solids, after 30 days of storage indicates the highest loss on Roşu Timpuriu variety (from 13.69% to 12.05%) and the lowest loss on Geo variety (17.79% to 17.41%). The highest decreasing of total acidity was noted on Roşii Timpurii (2.81%) and the lowest decreasing on Geo variety (1.65%). Total sugar decreasing was the highest on Roşu Timpuriu variety (1.77%) and the lowest on Jonkheer van Tets variety (0.36%). According to the final values of the three components of the biochemical analysis (total solids, total acidity and total sugar), Geo variety can be characterized as the most balanced after 30 days of storage (Table 2).

Blackberry varieties can be stored under modified atmosphere conditions without losing firmness, taste, and aspect during 20 days. The content of total solids, after 20 days of storage, indicates the highest loss on Arapaho variety (from 15.50% to 8.73%) and the lowest loss on Lochness variety (from 13.78% to 11.65%). The highest decreasing of total acidity was noted on Darrow variety (0.37%) and the lowest decreasing on Arapaho variety (0.17%). Total sugar decreasing was very high on Arapaho variety (6.28%) and the lowest on Darrow variety (0.63%). According to the final values of the three components of the biochemical analysis (total solids, total acidity and total sugar), Lochness variety can be characterized as the most balanced after 20 days of storage (Table 2).

CONCLUSIONS

1. On the harvesting stage, the berries fruits as excessively perishable commodities have proper biochemical composition in respect to be preserved a longer period under modified atmosphere.
2. Under modified atmosphere storage (4% CO_2) the raspberry fruits from each of three varieties were kept 13 days with preservation of taste quality and food value.
3. Under modified atmosphere storage (4% CO_2) the currant fruits from each of three varieties were kept 30 days with preservation of taste quality and food value.
4. Under modified atmosphere storage (4% CO_2) the blackberry fruits from each of three varieties were kept 20 days with preservation of taste quality and food value.
5. The content of total solids, total acidity and total sugar decreased after optimal storage period in different rates depending on variety with the exception that the content of total acidity increased on raspberry varieties.
6. The preliminary results give us the useful data regarding the evolution of the biochemical components and physiological processes in the post harvest life of excessively perishable fruits from some berry fruits (raspberry, currant and blackberries).

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Tables

Table 1. Biochemical components of some excessively perishable fruits studied (berries)

Raspberry	UM	Variety		
		Glen Moy	**Veten**	**Willamette**
Soluble solids	%	11.2	9,3	8.5
Total sugar	%	4,70	3,78	4,05
Total acidity	g acid malic/100g	0,36	0,64	0,66
Total solids	%	13.10	13.29	13.17
Water	%	82.9	86.71	86.63
Minerals	%	0.64	0.65	0.68
Antocyans	mg/100g	6.85	11.91	9.38
Ascorbic acid	%	41.3	40.71	43.48
Fructose	%	1.741	1.63	1.99
Glucose	%	1.03	1.47	1.92
Zaharose	%	0.98	1.03	1.47
Currant	**UM**	**Rosu timpuriu**	**Jonkheer**	**Geo**
Soluble solids	%	13,69	12,66	17,79
Total sugar	%	6,10	5,11	6,51
Total acidity	g acid malic/100g	4,84	4,60	5,40
Total solids	%	14.23	12.94	20.11
Water	%	85.77	87.06	79.89
Minerals	%	0.63	0.70	0.78
Antocyans	mg/100g	3.52	4.65	11.89
Ascorbic acid	%	42.61	46.28	119.56
Fructose	%	2.64	3.53	5.13
Glucose	%	2.40	3.68	3.42
Zaharose	%	0.34	1.17	1.76
Blackberry	**UM**	**Darrow**	**Lochness**	**Arapaho**
Soluble solids	%	10,45	13,78	15,50
Total sugar	%	4,75	11,21	11,08
Total acidity	g acid malic/100g	0,98	1,12	0,65
Total solids	%	10.88	15.71	14.73
Water	%	89.12	84.29	85.87
Minerals	%	0.30	0.53	0.50
Antocyans	mg/100g	22.21	31.86	19.23
Ascorbic acid	%	8.55	8.43	8.68
Fructose	%	0.20	0.12	0.20
Glucose	%	1.18	2.91	2.49
Zaharose	%	1.19	2.71	2.48

Table 2. Preliminary results concerning the main biochemical components evolution during the modified atmosphere storage of some excessively perishable fruits (berries)

Commodity/Variety		**Respiration (initial) (mg CO_2/kg/h at 20°C)**	**Total solids (%)**		**Total acididity (%)**		**Total sugar (%)**		**No. of storage days**
			initial	**final**	**initial**	**final**	**initial**	**final**	
Raspberry	Glen Moy	196.3	10.8	8.8	0.36	0.56	4.70	4.19	13
	Veten	200.5	9.3	7.6	0.64	0.81	4.70	2.58	13
	Willamette	189.4	10	7.6	0.66	0.78	4.05	3.58	13
Currant	Rosu timpuriu	145.0	13.69	12.05	4.84	2.03	6.10	4.33	30
	Geo	130.0	17.79	17.41	5.40	3.75	6.51	5.67	30
	Jonkheer van Tets	134.0	12.66	11.37	4.60	2.23	5.11	4.75	30
Blackberry	Darrow	81.83	10.45	7.75	0.98	0.61	4.75	4.12	20
	Lochness	80.47	13.78	11.65	1.12	0.80	11.21	7.28	20
	Arapaho	74.21	15.50	8.73	0.65	0.48	11.08	4.80	20

The methodology for analyzing the choice of various market channels by the Romanian fresh fruits and vegetables producers

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Keywords: survey, questionnaire, mathematical model, variables

ABSTRACT

The agricultural reform in Romania has broken in different lines the previously vertically integrated agri-food system. The agriculture structures was changed in general and horticulture retailing especially. These changes have also influenced the vertical structures in agri-food sector. The aim of this study is to identify the useful information and methodology for analyzing the alternatives the Romanian farmers have for various supply channels in fresh fruit sector, and also with respect to the transaction and economic costs. The analysis is based on a survey among actors involved in fresh fruit market from Southeastern part of Romania.

INTRODUCTION

The horticulture is a risky business and moreover the Romanian producers should face some additional difficulties. The actual state in fresh fruit market can be described by considerable uncertainties which were caused mainly by the incapacity of the farmers to have achievable commercial relations or achievable contracts, as well as practical and useful information concerning market channels for their specific activities. Furthermore, the public institutions are ineffective in ensuring enforceable contracts. In the absence of enforceable contracts the setting up of any kind of vertical coordination became extremely difficult.

Therefore, searching new long-term partners and getting specific investments have been associated with high transaction costs for farmers. In addition, this creates severe barriers in the situations of market exchanges. Under these conditions, it is expected that spot markets (the spot market or cash market is a commodities market in which goods are sold for cash and delivered immediately) dominate over other co-ordinate mechanisms. In those sub-sectors, where any type of production contracts does exist, agricultural producers face the hold-up problems (e.g. delayed payment for delivered products, or post price reduction by retailers), which are strongly stressed. But, these problems are very severe for those sub-sectors dominating fragmented and small-scale farms, like fruit and vegetable sector.

MATERIAL AND METHODS

Zaharieva et al. (2001) investigated the choice of supply channels by Bulgarian wine makers applying case study approach. They identified four types of channels which differ in the costs of using them and effectiveness of information transmission from processors to growers. The case studies revealed that despite the difficulties created by the underdeveloped market and barriers in finding investment financing, the expected long-run benefits of vertical integration offered sufficient incentives to firms to pursue alternative ways of accomplishing this initiative.

Boger (2001) has examined the marketing arrangements between Polish hog producers and buyers. She employs various multivariate techniques based on a sample of 200 Polish hog producers. The multinomial logit analysis suggests that producer's choice between large processors as opposed to traders and local slaughterhouses can be predicted by type of contract.

Romania can produce each year in average 2.000.000 tone of fruits (40% from total horticultural products). The most part of fruit production (98%) is obtained within the private sector (Table 1).

Starting with the information data and some conclusions from regional survey regarding farmers, producers and their fresh fruit market activity, we have formulated the hypothesis that the producer decision among various marketing channels is influenced by the transaction costs (Table 2, Table 3).

Associations or farmers groups by products or group of products are relatively weakly represented and generally the fresh fruit market has some general characteristics: lack of market information; generally high transport costs; no enforcement contracts; no commodity exchanges; extremely unbalanced competition. As a result, there is a tendency to the vertical integration in view to avoid the regional monopoly.

The coordinators/channels detected in Romanian for agricultural sector are the following: local market; wholesale markets; wholesalers; traders for domestic market; producer organizations; retailers; farmers.

As can be seen from the list, there is a variety of channels and markets for the horticultural producers. We have to underline however, that spot markets and different types of contracts (including in some cases contract production) are the most common forms of co-ordination. Different retail chains gain bigger and bigger share from fresh fruit and vegetable market. However, marketing co-operatives and producers' organizations also can solve the marketing problems of the fruit and vegetable producers.

RESULTS

A multinomial logit model is applicable to reveal on the determinants influencing the choice among various supply channels. The multinomial logit model assumes that data are case specific; each independent variable has a single value for each case. The multinomial logit model also assumes that the dependent variable cannot be perfectly predicted from the independent variables for any case. Collinearity is assumed to be relatively low, as it becomes difficult to differentiate between the impact of several variables if they are highly correlated. The independence of irrelevant alternatives is another assumption which the multinomial logit model makes. This assumption states that the odds do not depend on other alternatives that are available (i.e., that including additional alternatives or deleting alternatives will not affect the odds on the dependent variable among the alternatives that were included originally).When using multinomial logistic regression, one category of the dependent variable is chosen as the comparison category. Separate relative risk ratios are determined for all independent variables and for each category of the independent variable with the exception of the comparison category of the dependent variable, which is omitted from the analysis. Relative risk ratios and the exponential beta coefficient represent the change in the odds of being in the dependent variable category versus the comparison category associated with one unit change on the independent variable.

In view to use the matematical model there was built a relevant questionnaire (Table 4) with the transaction costs divided into three groups for empirical analysis: information costs, negotiation costs and monitoring costs. In addition, we attempted to measure the human and physical asset specificity.

CONCLUSIONS

1. This survey can offer the model and the way for next steps to build a data base for regional and national fresh fruit market information.
2. The questionnaire, analysis and mathematical model can offer the statistical data to demonstrate the choice of Romanian producers among various supply channel for fresh fruit market.
3. Producers selling to wholesale market can be strongly and negatively affected by the farmer's age, information costs, and negatively by the bargaining power and monitoring costs.

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Tables

Table 1. Dynamic of Romanian fruit production in total country and private sector
- thou tones-

Species	2001		2002		2003		2004		2005		2006	
	Total	**Private**	**Total**	**Private**	**Total**	**Private**	**Total**	**Private**	**Total**	**Private**	**Total**	**Private**
Plums	557.2	538.2	220.6	216.3	909.6	897.8	475.8	464.0	622.3	595.7	595.7	590.8
Apples	507.4	453.6	491.5	443.4	811.1	769.7	1097.9	1050.2	638.0	591.0	591.0	550.6
Pears	71.6	71.1	68.1	67.4	103.8	103.1	45.9	45.0	88.9	82.0	82.0	61.7
Peaches and nectarines	16.7	9.9	13	10.6	18.0	12.7	19.6	17.6	29.8	26.3	26.3	16.0
Cherries and sour cherries	91.2	83.1	66.3	63.7	98.5	93.6	51.0	48.0	117.9	109.7	109.7	100.9
Apricots	28.3	25.0	18.3	17.6	42.6	37.3	20.7	19.8	52.4	49.7	49.7	37.1
Nuts	33.9	33.7	37.5	37	50.8	50.4	15.6	14.9	47.8	45.1	45.1	38.3
Strawberries	18.4	18.2	16.9	16.8	14.9	14.8	14.5	14.2	18.2	17.6	17.6	18.0
Other fruit	28.1	27.8	19.6	19.2	39.2	38.5	3.4	3.3	31.7	30.3	30.3	13.5
Total	**1352.8**	**1260.6**	**952**	**892.3**	**2088.5**	**2017.9**	**1744.7**	**1677**	**1647**	**1547.4**	**1486.4**	**1426.9**

Table 2. Consumer price indices, for the main groups of vegetables and fruits

Commodities	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Vegetables and tinned vegetables	114.9	129.6	136.2	94.7	109.2	111.58
Potatoes	111.2	157.0	148.8	92.0	85.7	135.93
Other vegetables and tinned vegetables	107.1	121.4	136.7	92.7	123.0	104.17
Fruits and tinned fruits	112.4	126.4	112.2	102.7	108.2	103.29
Fresh fruit	**96.5**	**134.3**	**111.6**	**102.3**	**120.4**	**105.79**
Citrus and other southern fruit	140.7	118.5	112.4	104.2	94.7	99.69
Tinned fruit	116.9	114.1	115.3	107.5	104.9	103.35

Table 3. Volume indices of sales (buying up) of horticultural products

Previous year = 100

Commodities	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Crop horticultural products	99.8	94.9	92.5	94	119	83.2
Vegetables	83.3	137.0	98.7	100.1	94.8	100,3
Fruits	118.3	93.7	112,2	116.9	100.7	103,9

Table 4. Variable used for multinomial logit model and the content of questionnaire

Variable	**Questionnaire**
Dependent variable	
Chain	Which is the type of supply channels?
Independent variables	
Information costs	
Access to information	Have you problems in information access?
Time	How time you spend finding partners for transactions?
Phone	Do you have phone and/or fax?
Mobile phone	Dou you have mobile phone?
Price	Is it a problem not knowing the price before selling?
Negotiation costs	
Delivering products	Who delivers products to buyer?
Transport costs	Who carries the costs of transporting to the buyer?
Frequency of selling	How often did you sell products to the buyer?
Negotiation terms	Can you negotiate the transactional terms with the buyer?
Payment condition	Dou you satisfy with condition of payment?
Finished business	Would it be a problem if your buyer finished business relations with you?
Monitoring costs	
Graded aspects	Is it a problem if product may not graded as expected before selling to buyer?
Presence at grading	Is it a problem not being present when products are graded?
Physical asset specificity	
Investments	Have you invested in your business last year?
Future investments	Do you plan invest in the future years?
Human asset specificity	
Education	Which is your education?
Age	Which is the age of farmer/actor in fresh fruit market?

The behavior of some pear trees, grafted on quince trees in the conditions from the N-V part of the country

Aurora Venig
S.C.D.P. BIHOR

Keywords: pear

ABSTRACT

Pear is more and more preferred because of OTS aspect and taste. They are consumed rough or in industrialization. Through its high number of cultivated varieties, pear trees assure an important quantity of fruit all over the year (from july until autumn and by a good preserving until spring). The researches carried out at S.C.D.P. over pear varieties, permitted distinguish of the varieties that provide the best climatic conditions in the North-Western part of the qountry.

INTRODUCTION

Obtaining high fruit production with a good quality depends on the technology applied on plantations.

This implied some studies concerning the pear trees crop area and the applied technology.

Branişte, Parnia, Cociu and Drăgănescu described in their researches different types of technologies applied on the pear variety.

At S.C.D.P. Bihor was carried out an experience in order to appreciate the way behavior of different pear trees varieties in the N-V part of the country.

MATERIALS AND METHODS

The experience started in 2001, in a superintensiv system, at a distance of 3 m between the roots and 1,5 m on the row, on a „preluvisol" soil type with the horizon A0-B-C.

The used material is build from 6 pear varieties grafted on pear trees: Trivale, Aromată de Bistrița, Untoasă Precoce, Moretini, Napoca, Curé, Haydea. Each variety is geometrical arranged and is structured on three rows.

The aim of the experience was to the trees vigour, that was established after the following criterions: the trunk's diameter, the wreath's dimensions and its volume. The measurements were made in 2007.

RESULTS AND DISCUSSIONS

The results were presented in tables. Regarding the trunk's diameter (table nr.1), the values were situated between 51,7 mm at Napoca variety and 78,3 mm at Trivale variety, the varieties medium is 62,16 mm. The most vigorous varieties are Aromată de Bistrița and Trivale (78,3 mm), varieties that are considered the most valuable ones.

The smallest values regarding the trees' vigour were registered at Napoca variety (51,7 mm) and Curé (58 mm). The differences between the variants and Mt (the varieties medium) are from significant to very significant, positive or negative.

Table 1. Trees vigour (trunk's diameter mm) VII the vegetation year

Nr. crt.	Variety	x ± sx	s%	Difference to x	t	Signification
1.	Trivale	78,3±2,27	9,18	16,14	5,20	xxx
2.	Aromată de Bistrița	65,4±1,18	5,71	3,24	1,33	-
3.	Untoasă P. Morettini	58,2±2,33	12,66	-3,96	1,26	-
4.	Napoca	51,7±1,68	10,30	-10,46	2,70	0
5.	Curé	58,0±2,71	14,77	-4,16	1,20	-
6.	Passe Crassane	61,4±2,56	13,20	-0,76	3,32	00
	x Medium	62,16±2,12	10,97	-	-	-

DL 5% = 2,10; DL 1% = 2,90; DL 0,1% = 3,90

An important influence in establishing the planting densities has the trees' habitus. Regarding the wreath's diameter the obtained results are show in table nr.2.

Table 2. Wreath's diameter (cm) at the end of the VII vegetation year

Nr. crt.	Variety	x ± sx	s%	Difference to medium	t	Signification
1.	Trivale	167,5±7,46	14,08	40,3	4,62	xxx
2.	Aromată de Bistrița	145,5±2,83	6,15	18,3	3,43	xx
3.	Untoasă P. Morettini	134,0±5,15	12,14	6,8	0,99	-
4.	Napoca	99,5±4,04	12,85	-27,7	4,57	000
5.	Curé	99,5±3,45	10,96	-27,7	4,87	000
6.	Passe Crassane	117,5±4,23	11,38	-9,5	1,53	-
	x Medium	127,2±4,52	11,26	-	-	-

The wreath's diameter is situated between 99.5 cm at Napoca and Curé variety and 167.5 cm at Trivale variety. The varieties medium is 127.2 cm.

Concerning the wreath's volume, the results are shown in table nr.3.

Table 3. The peach trees behavior grafted on dwarf and standard parent stocks in the orchard

Nr. crt.	Variety	x ± sx	s%	Difference to medium	t	Signification
1.	Trivale	6,25±0,35	17,76	1,64	4,00	xxx
2.	Aromată de Bistrița	5,06±0,16	10,27	0,45	1,66	-
3.	Untoasă P. Morettini	4,90±0,21	14,08	0,29	0,96	-
4.	Napoca	3,38±0,12	11,53	-1,23	5,34	000
5.	Curé	4,15±0,31	24,33	-0,46	1,21	-
6.	Passe Crassane	3,92±0,19	15,56	-0,69	0,29	-
	x Medium	4,61±0,22	15,58	-	-	-

DL 5% = 2,10; DL 1% = 2,90; DL 0,1% = 3,90

CONCLUSIONS

The North-Western part of our country offers good conditions for pear trees' growing. The pear trees grafted on quince trees has good results in the superintensive plantations, but the success depends on the grafted variety.

The differences are registered in case of the trees' vigour, aspect that plays an important role in establishing the trees' density planted/ha.

From the six researched variants, there were distinguished the following varieties:

- vigorous varieties: Trivale, Passe Crasane;

- middle vigour varieties: Aromată de Bistriţa, Curé, Untoasă Precoce Moretini;
- small vigour varieties: Napoca.

After the carried out researches, there are recommended the pear trees plantations in a superintensiv system, with trees directed after the pillar system with densities of 2000-2500 trees/ha. The bests varieties seemed to be: Trivale, Passe Crasane, Aromată de Bistriţa, Curé, Untoasă Precoce Moretini, which are recommended to be extended in production.

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Economical efficency concerning dwarf and semidwarf on own roots

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Keywords: peach, dwarf forms,

ABSTRACT

The North-Western part of the country provides good growing and development conditions of the peach trees. Taking into consideration that peaches are the most appreciated fruits from Romania, there is a reason of enlarging the surfaces cultivated with peach trees. A problem in fruit-growing represent the cost and the work, so people try find out solutions in order to reduce or eliminate the toil. This might be done by using intensive and superintensive plantations, close related to obtain new low built peach forms(dwarf).This new forms should also have a high fruit production and trees' density/ha. The researches made at S.C.D.P. Bihor between 2001-2004 over 20 dwarf and semidwarf peach hybrid offspring (selected from two hybrid combinations Bonanza x Springcrest and Bonanza x Cardinal) demonstrated that the studied material is diverse and valuable and might represent the basis of creating new varieties.

INTRODUCTION

Obtaining dwarf forms with small vigour permit a significant enlargement of the trees' density/ha, so the fruit production/ha is high. During the time, the fruit growing activity went through a permanent process, so in the present is turning into an economical subsystem, with different roles and functions in the national economy. Reducing the cost/ha and enlarging the economical efficiency implies mechanization of the work in the fruit growing, which means using trees with structure and habitus suitable for mechanism(Cociu si Oprea, 1989).

This low built genotypes are more efficient from the economical point of view and that is the reason why it became a studying and economical subject at S.C.D.P Bihor in the period 2001-2004.

MATERIAL AND METHOD

The used material is compound from two hybrid combinations Bonanza (dwarf nectarin) x Springcrest (standard peach) and Bonanza x Cardinal (standard peach).In the experience there were included the F2 offspring of these hybrids and the year:

- from the Bonanza x Springcrest hybrid:
  - 13 dwarf and semidwarf peach offspring
- from the Bonanza x Cardinal hybrid:
  - 7 dwarf and semidwarf peach offspring

The experiences that were carried out in the period 2001-2004 were organized after the randomized blocks method with a number of three repetitions. Each repetition was built from three trees with a planting distance of 4/1,5m.As Mt there was used the experience medium at some types and at others the paternal genitor. During the vegetation, in all researching years there were carried out measurements and analysis on the field and in the laboratory concerning:

- variability of some morphological characters
- variability of the fruit production at the surface unit
- variability of some physiological property
- variability of the fruits' quality

OBTAINED RESULTS

Taking into consideration the comparison between the dwarf and Mt, the best offspring (concerning the economical efficiency at the dwarf and semidwarf hybrids on own roots from the Bonanza x Springcrest hybrid combination) were registered at the variants Oradea 13 and Oradea 14 with 5828 lei/ha each profit, Oradea 8 with 5820lei/ha and Oradea 6 with 5796 lei/ha profit.

Table 1. Economical efficiency concerning dwarf and semidwarf peach trees grown on own roots and resulting from Bonanza x Springcrest hybrid combination

Nr. crt.	Hybrid offspring	Obtained production (t/ha)	Entire costs (lei/ha)	Production cost (lei/kg)	Entire incomes (lei/kg)	Obtained profit (lei/ha)
1	Oradea 6	6,3	3654	0,58	9450	5796
2	Oradea13	6,2	3472	0,56	9300	5828
3	Oradea14	6,2	3472	0,56	9300	5828
4	Oradea8	6,0	3180	0,53	9000	5820
5	Oradea1	5,5	3575	0,65	8250	4675
6	Oradea5	5,0	3250	0,65	7500	4250
7	Oradea2	4,8	3264	0,68	7200	3963
8	Oradea4	4,2	2856	0,68	6300	3444
9	Oradea7	4,0	2800	0,70	6000	3200
10	Oradea3	3,8	2660	0,70	5700	3040
11	Oradea10	3,8	2660	0,70	5700	3040
12	Oradea12	3,5	2485	0,71	5250	2765
13	Oradea9	3,2	2272	0,71	4800	2528
14	**Medium (mt)**	**4,8**	**3076**	**0,64**	**7212**	**4136**

Medium selling price 1,5 lei/kg.

Table 1. shows that generally the production cost(lei/kg) is inverse proportional with the fruit quantity at the surface unity, being situated between 0,58 lei/kg at Oradea6 and 0,71lei/kg at Oradea 9.

Analysing the economical efficiency at the Bonanza x Cardinal, here is a inverse proportional relation between the production/ha and the production cost/kg. The production cost was situated between 0,53 and 0,60 lei/kg, meanwhile at the Bonanza x Springcrest combination offspring the production cost was situated between 0,58 and 0,71 lei/kg.

Table 2. Economical efficiency of dwarf and semidwarf peach trees grown on own roots and rising from Bonanza x Cardinal hybrid combination

Nr crt	Hybrid offspring	Entire production (t/ha)	Entire costs (lei/ha)	Production cost (lei/kg)	Entire incomes (lei/kg)	Profit (lei/ha)
1	Oradea4	6,0	3180	0,53	9000	5820
2	Oradea3	5,3	3074	0,58	7950	4876
3	Oradea5	5,3	3074	0,58	7950	4876
4	Oradea1	4,3	2537	0,59	6450	3913
5	Oradea2	4,2	2478	0,59	6300	3822
6	Oradea7	4,2	2520	0,60	6300	3780
7	Oradea6	3,3	1980	0,60	4950	2970
8	Medium(mt)	4,7	2692	0,58	6986	4294

Table 2. shows that there are registered high results at Oradea4 with 5828lei/ha profit, Oradea3 and Oradea5 each with 4876lei/ha profit.The highest profit obtained at the dwarf peach offspring was situated between 5820-5828 lei/ha.

CONCLUSIONS

The hybrid offspring Oradea6, Oradea13, Oradea14 and Oradea 8 which are obtained from the Bonanza x Cardinal cross-breeding on own roots might become a biological material for obtaining new peach varieties.

From the economical point of view, the best results are obtained at the Bonanza x Springcrest combination.The profit is obtained not only from the high productions but also from eliminating the upkeep costs,the good using of water, soil, light. All these advantages lead to a high profit at the surface unit in case of the dwarf peach plantations.

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Behavior of new apricot hybrids in the processing industry

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Keywords: control cultivar**,** quality**,** preserved product**,** technological parameters

ABSTRACT

There were studied 5 cultivars of apricots: B 7/56, B 21/3, B 21/39 (hybrids), Viorica and Carmela (control cultivars), during the period 2003-2005. Some technological parameters that contributed to the setting up of the processing directions were analyzed at these cultivars and hybrids. The new apricot hybrids were processed under similar technological conditions with control cultivars, according the technologies used in the production for the preservation of fruit by jam. After the stabilization period of the preserved products, of minimum 21 days from the processing, the following analyses were carried out: sensorial analyses, biochemical analyses with determining of the energetic value and microbiologic analyses.

INTRODUCTION

The research carried out has proved that for processing of the fruits two aspects are essential: the quality of the raw material and the applied technology that must keep or even improve the natural qualities of the fruit, in order to satisfy the consumer's taste (Beceanu and Chira 2003).

Within the practice of the new fruit varieties testing for homologation, besides the check up of the agricultural, biologic and technological qualities, the nutritional value and optimum qualities of processing into preservation product, which will use at maximum the characteristic qualities of these fruits, are also of consequence (Gherghi et al. 1999).

Within the process of fruits processing, their initial attributes change many times, intentionally, to obtain new products with high nutritive value and of better quality.

As a consequence, creation of new cultivars with a specific purpose of capitalization is desired and, at the same time, valuable fruits, from the nutritive point of view. (Williamson 1996)

The fruits intended for industrial processing are considered of quality if, besides the sensorial properties, they have also a high content of soluble dry substance, pectic substances and maintain their colour and the flavour during their processing into compotes, jellies, jams, juices etc. (Liu 2003).

Testing the new fruit cultivars the nutritive, sensorial and the technological qualities check is carried out.

Fruit jams are products obtained by boiling of fruit (whole, halves, etc.) with sugar syrup until the reaching a viscous consistency. Jams can be defined as fruits included in concentrated syrup (Enachescu 1995)

MATERIALS AND METHODS

The Baneasa Research-Development Station supplied the material intended for testing for the processing for Fruit Trees.

The experimentations were carried out at the Research-Development Institute for Processing and Marketing of Horticultural Products – HORTING Bucharest within the frame of the Laboratory of Research – Processing of Horticultural Products.

Two control cultivars of apricot: Viorica and Carmela and three new hybrids of apricots: B 7/56, B 21/3, B 21/39 were analyzed.

At these apricot cultivars and hybrids a series of technological parameters (sensorial, physical and biochemical) which have contributed to the setting up of the processing directions, were analyzed.

In experimentations were also used: auxiliary materials (sugar and citric acid); packaging (Twist-off jars 314ml, 220ml), lab equipment, reagents and lab glass.

The control and assessment of the technological quality of the apricot cultivars (hybrids) were carried out in three stages through:

- analysis of the raw material;
- analysis of behaviour on the processing flow sheet of jam;
- analysis of the preserved product.

In the experimentations carried out on the apricot cultivars and hybrids, the continuous concentration of fruits with previous diffusion in sugar procedure was used. This procedure consists of mixing the fruits with sugar or putting them in layers and after a period of time (8-24 hours) thermal concentration is used. The thermal concentration can be achieved also discontinuously by boiling the fruits in sugar syrup, applying 2-3 discontinuities of 5-10 minutes up to reaching the final concentration that must be of minimum 72% of soluble dry substance.

After the stabilization period of the preserved products obtained from processing of the apricot cultivars and hybrids, of minimum 21 days from processing, the following analyses were carried out:

- sensorial analyses (appearance, colour, taste, flavour, consistence, texture);
- biochemical analyses (soluble dry substance, sugars, lipids, proteins, vitamin C, acidity) determining the energetic value;
- microbiological analyses (aerobic and anaerobic mesofile bacteria, yeasts and moulds).

The biochemical and microbiologic analysis of raw material and preserved products was carried out using the methods presented in table 1.

RESULTS AND DISCUSSIONS

Following the analysis of the sensorial and physical characteristics of the cultivars and new hybrids of apricot, it was found that the size, shape and colour of the new apricot hybrids are similar to the control cultivars. The hybrids B 21/3 and B 7/56 have a sweet-sour taste similar to the control cultivar Carmela, and the stone is unadhesive to the pulp. The hybrid B 21/39 presents a sweeter taste, and the stone is semi-adhesive to the pulp.

The hybrids B 7/56 and B 21/39 have an intense flavour like the control cultivars Carmela, while the hybrid B 21/3 and the control cultivar Viorica have a finer flavour.

Also, the analyzed apricot hybrids have the pulp of firm consistency, the same as the control cultivar Carmela. The control cultivar Viorica has the pulp of more mealy consistency, slightly tasteless and the stone is adherent to the pulp.

In table 2 the average values of the biochemical characteristics analyzed at the apricot control cultivars and at the hybrids during 2003-2005 are presented.

Following the analyses, the best average values of the biochemical characteristics achieved during the three years study were registered with the control cultivar Carmela and with hybrid B 7/56, which presented an average value of the soluble dry substance ranging from 19 – 19.1%, and the average value of the total glucides content were 15.68% and, respectively, 16.02%.

The control cultivar Carmela has had the highest average value of vitamin C content 20.73 mg/100 g, followed by the hybrid B 21/3 that has had 17.05 mg/100g.

The highest average value of total acidity was registered with the control cultivar Viorica 1.8 g malic acid/100 g, while the average ratio glucides/acidity for this cultivar was of only 5.8 due to the low content of total glucides 10.44%.

The hybrids B 21/3 and B 7/56 have had the highest values of the average ratio glucides/acidity: 12.03 and respectively 12.32.

The new apricot hybrids B 21/3, B7/56 and B21/39 were processed as jam in similar conditions as the control cultivars Viorica and Carmela.

The qualitative assessment of the processed products from the apricot cultivars and hybrid was established by sensorial, biochemical and efficiency analyses. The results of these analyses for the product "Apricot Jam" are shown in table 3.

The biochemical analyses show that the product „Apricot Jam", achieved from the apricot control cultivars and new hybrids, meets the product standards by the fact that the soluble dry substance was comprised between 72-73%, and the energetic values ranged from 282.60 – 286.84 kcal/100 g .

The efficiency at the jam processing was very good both in case of the apricot new hybrids and the control cultivars. Thus, the processing refuse ranged from 4.5 to 7.4%.

After the stabilization period the product „Apricot Jam", achieved from the five cultivars (hybrids), was subject of the sensorial testing by granting qualificative for the following characteristics: appearance, color, taste, flavour (the STAS sensorial testing 12656-88). The general score obtained by each type has detached at the end the cultivar (hybrid) that behaved the best following the changes suffered at the jam processing.

Table 4 presents the results obtained as a consequence of sensorial analysis of the cultivars and new hybrids processed as „Apricot Jam".

The hybrids B 21/3, B 7/56, B 21/39 processed as jam have obtained the qualificative „very good", as well as the control cultivars Viorica and Carmela and were characterised by special senzorial qualities, as for example the sweet balanced taste and intense flavour.

CONCLUSIONS

The tested apricot new hybrids have globally shown special biochemical characteristics comparable to the control cultivars. The B 7/56 new hybrid distinguished itself with the highest content of soluble dry substance, total glucides, as well as the highest value of the ratio glucides/acidity.

All the analyzed new hybrids B 7/56, B 21/3, B 21/39 are suited for industrial processing as jam and have obtained the qualificative - "very good" - at the sensorial testing.

Based on these results, the premises of supply to consumers of quality products obtained from apricot hybrids with high sensorial and nutritional qualities are created.

The results of these experimentations are part of the project Agral N^{o}. 5023/2001 and are used at the registration of the new cultivars of fruits into the "Official Catalogue of Cultivated Plants Cultivars from Romania."

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Tables

Table 1. Methods of analyses

Analyses	Method of analyses
Sensorial analyses	STAS 12656-88
Soluble dry substance	STAS 5956 – 71
Titratable acidity	STAS 5952 – 79; SR EN 12147 - 99
Glucides	SCHOORL
Lipids	SOXHLET (STAS 5957 – 71)
Proteins	KJELDHAL
Vitamin C	STAS 5950 - 91
Aerobic, anaerobic mesophile bacteria	STAS 8924 – 96
Yeasts and molds	STAS 12964 – 91

Table 2. Average biochemical indicators of apricot cultivars and hybrids (2003-2005)

Cultivar/ hybrid	Soluble dry substance (%)	Total acidity (g malic acid/100g)	Total glucides (%)	Vitamin C (mg/100g)	Ratio glucides/ acidity
Viorica	14,4	1,8	10,44	13,56	5,8
Carmela	19	1,37	15,68	20,73	11,45
B 21/3	18	1,2	14,43	17,05	12,03
B 7/56	19,1	1,3	16,02	16,8	12,32
B 21/39	18,4	1,52	14,77	16,61	9,72

Table 3. Quality indicators of apricot cultivars and hybrids processed as „Apricot Jam"

Quality indicator	**Cultivar/hybrid**				
	Viorica	**Carmela**	**B 21/3**	**B 7/56**	**B 21/39**
Sensorial properties Fruits appearance	Fruit pieces are about the same size. They are uniform included in a concentrated syrup.				
Fruits texture	Soft				
Fruits colour	Yellow–reddish	Dark reddish	Dark reddish	Dark reddish	Yellow– reddish
Syrup appearance	Glassy liquid with fruit particles in suspension				
Syrup consistency	Viscous, non gelified and non sugared				
Syrup colour	yellow	Yellow–reddish	Dark reddish	Yellow–reddish	yellow
Taste and smell	Sweet, smell good				
Flavour	finer	intense	finer	intense	intense
Biochemical properties Soluble dry substance (%)	72,3	72,6	72	73	72,4
Glucides(%)	70,51	70,85	70,20	71,25	70,68
Lipids (%)	0,038	0,04	0,036	0,035	0,045
Proteins (%)	0,39	0,42	0,37	0,38	0,40
Acidity (g malic acid/100 g)	0,73	0,72	0,70	0,70	0,75
Energetic value (kcal/100g)	283,94	285,44	282,60	286,84	284,73
Refuse (%)	7,4	4,5	7,2	6,2	6,4

Table 4. Sensorial analyses of apricot cultivars and hybrids processed as „Apricot jam"

Cultivar/ hybrid	**Appearance**	**Colour**	**Taste**	**Flavour**	**Total medium score (P_{mt})**	**Qualificative**
Viorica	3.64	6	6	3,64	19,3	very good
Carmela	3,64	5,46	6	3,816	18,9	very good
B 21/3	3,64	5,328	5,724	3,64	18,3	very good
B 7/56	3,64	5,328	6	3,904	18,9	very good
B 21/39	4	6	6	4	20	very good

Stability of protection to sharka of C5 transgenic plums inoculated with *Plum Pox* Virus and heterologous viruses

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Keywords: Honey Sweet, engineered resistance, PPV-D, co-infection, PDV, PNRSV, ACLSV

ABSTRACT

Transgenic C5 'HoneySweet' is a clone of *Prunus domestica* L. transformed with the *Plum pox virus* coat protein gene (PPV-CP). This transgenic plum displays post-transcriptional gene silencing (PTGS) which makes it highly resistant to PPV infection. To test the effect of heterologous viruses on the efficacy and stability of PTGS against PPV, transgenic C5 trees were graft-inoculated with different combinations of *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and PPV-D strain. The potential for suppression of the silencing mechanism mediated by these viruses was evaluated. Challenge experiments were performed in Romanian experimental fields and under greenhouse conditions. Virus infections were evaluated by visual monitoring of symptom development and by serological and molecular diagnosis. Across all trials, the engineered resistance to PPV in C5 transgenic plums was stable and was not suppressed by the presence of the assayed heterologous viruses over a three-year experimental period.

INTRODUCTION

Transgenic plants expressing viral genes have been shown to exhibit varying degrees of resistance to the virus that provides the viral transgene, and to closely related viruses (Beachy *et al.,* 1990). The viral genes most commonly used in this pathogen derived resistance (PDR) strategy have been coat protein (CP) genes. Since the initial report of CP-mediated resistance against *Tobacco mosaic virus* infection in transgenic tobacco (Abel *et al*., 1986), this strategy has allowed the development of many virus-resistant crops. *Plum pox virus* is the causal agent of one of the most devastating diseases of *Prunus* species, producing important agronomic and economic losses (Cambra *et al*., 2006).

Since its first description in Bulgaria (Atanasoff, 1932), the virus has spread to a large part of the European continent, around the Mediterranean basin and Near and Middle East, South and North America (Chile, USA, Canada, and Argentina) and Asia (Kazakhstan, China and Pakistan) (Capote *et al.,* 2006). Nevertheless, there are control measures against PPV based on two strategies: the reduction or elimination of the viral inoculum by quarantine measures and eradication programs (Rodoni *et al.,* 2006; Lebas *et al.,* 2006; Thompson, 2006; Muñoz *et al.,* 2006; Speich, 2006; Myrta *et al.,* 2006; Ramel *et al.,* 2006), and the obtain of PPV resistant plants by conventional breeding (Karayianis, 2006; Badenes and Llácer, 2006; Bassi, 2006; Krska *et al.,* 2006; Hartmann and Neumüller, 2006) or through the use of genetically modified plants (Scorza and Ravelonandro, 2006). Following the last strategy, transgenic European plums (*Prunus domestica* L.) containing the CP gene of PPV were developed as an approach to obtain PPV resistant plums. One transgenic line, C5, subsequently named 'HoneySweet' (Scorza *et al.,* 2007) was found to be highly resistant to graft- and aphid-mediated inoculation by PPV in greenhouse and field tests (Ravelonandro *et al.,* 1997,

2000; Hily *et al.,* 2004; Malinowski *et al.,* 2006). C5 viral resistance is based on a RNA silencing (Scorza *et al.,* 2001).

RNA silencing is a sequence specific RNA degradation mechanism widely observed in animals, fungi and plants (where it is called PTGS) (Baulcombe, 2004; Hannon 2002). The roles of RNA silencing include the developmental regulation of gene expression and protection from transposable elements and viruses. Virus infection in plants can trigger the PTGS pathway in which siRNAs are produced (Hamilton and Baulcome, 1999; Hily *et al*., 2005). As a response to this defence mechanism, many viruses encode gene-silencing suppressor proteins acting at different points in the PTGS pathway (Anandalakshmi *et al.,* 1998; Voinnet, 2001). It has been shown that the helper component proteinase (HC-Pro) of potyviruses is a suppressor of the PTGS pathway (Brigneti *et al.,* 1998), which interferes with the maintenance of the silencing by inhibiting degradation of the target mRNA and, consequently, preventing siRNA accumulation (Anandalakshmi *et al.,* 1998). Suppressor protein 2b from cucumoviruses, is unable to reverse already established RNA silencing, but prevents its initiation at the growing points of the plant by inhibiting the long-range activity of the silencing signal produced during the silencing reaction (Béclin *et al.,* 1998; Guo and Ding, 2002). Viral PTGS suppressors have also been shown to suppress PTGS of non-viral transgenes (Beclin *et al.,* 1998). While viral suppression of gene silencing has been demonstrated in herbaceous species, it has not been reported in most crops including tree species or under field conditions.

The objective of this work was to determine the stability of RNA silencing in transgenic plums by assessing if suppression of PTGS occurs under mixed infection of PPV and heterologous viruses, such as *Prunus necrotic ring spot virus* (PNRSV) and *Prune dwarf virus* (PDV), viruses that commonly infect *P. domestica* and other *Prunus* species.

MATERIALS AND METHODS

Plant material and inoculated viruses. The studies were carried out at the Fruit Research and Development Station Bistrita, Romania. Two experiments were initiated in the field, in an experimental orchard and in a nursery, and another one in greenhouse conditions. The C5 transgenic clone was chip-bud graft inoculated with PPV (D strain) and with the combinations PPV + PDV or PPV + PNRSV. Buds infected with individual viruses were used for chip-bud inoculation. Conventional plums were similarly inoculated and used as controls.

Experimental orchard. Ten C5 transgenic clones grafted on Myrobolan rootstock were planted in the field in 1998 in an orchard with a high PPV infection pressure. Clone C5 exhibited a high resistance to natural infection of PPV. In September 2003 six C5 trees were subjected to chip-bud graft inoculation. Two C5 plum trees were used for each virus combination. On each plum tree, half of the branches were graft inoculated and the other half not. On each inoculated branch 10 buds individually infected with the test viruses were alternately grafted.

Experimental nursery. Myrobolan rootstocks were planted in an experimental nursery in April 2003 and grafted with buds from C5 or conventional plums on August 2003. The inoculation with the viruses was made as follow: buds of transgenic or conventional plums were inserted on the top of grafting area, buds with PPV were inserted in the middle and buds with heterologous viruses were inserted at the bottom. Ten plants were used for each virus combination.

Greenhouse assays. The graft inoculation method used in the greenhouse was the same as in the nursery. The budding and chip-bud graft inoculation were made on March 2004. Five plants were inoculated for each virus combination.

Virus monitoring. Virus infection (PPV and heterologous viruses) was evaluated by visual monitoring of symptom development and by serological and molecular methods. For testing, leaf samples were collected from different parts of the plants as follow: in the case of the field experimental orchard, leaves from graft inoculated branches, non-graft inoculated branches, and shoots developed from the grafted chip buds were collected; in the case of the greenhouse and nursery experiments, leaves from the basal half of the plants, leaves from the top half of the plants and shoots developed from the grafted chip buds were collected. Serological virus detection was achieved by DAS-ELISA using polyclonal antibodies (PPV, PDV and PNRSV) according to the manufacturer (Bioreba, Switzerland). Molecular detection was performed by Immunocapture - Reverse Transcription - Polymerase Chain Reaction (IC-RT-PCR) using the pair of primers P1/P2 for PPV (Wetzel *et al.,* 1991), PNRSV-10F/PNRSV-10R for PNRSV (Marbot *et al.,* 2003) and PDV-17F/PDV-12R for PDV (Kummert *et al*., 2001). Analyses were performed before grafting (to check the virus-free status of the rootstocks and C5 trees) and after inoculation in June 2004, July 2005 and June 2006. In June 2004 all plants were analyzed. In July 2005 and June 2006, in the case of experiments performed in the nursery and greenhouse, only two plants for each treatment showing the best symptoms and the highest absorbance values were subjected to analysis. In the case of C5 inoculated with PPV and PPV+PNRSV only one plant grown in the greenhouse was analyzed.

RESULTS

Experimental orchard *(Table 1).* PPV, PDV and PNRSV could not be detected in the inoculated C5 trees by symptom visualization and DAS-ELISA tests one year post-inoculation. Obvious PPV symptoms appeared on shoots that developed from the grafted chip buds. DAS-ELISA results confirmed the presence of all viruses on shoots derived from the inoculum. Low concentrations of PPV and PDV were detected in the inoculated conventional plums.

Two years post-inoculation DAS-ELISA tests revealed that the heterologous viruses (PDV and PNRSV) were translocated from the inoculum to the C5 trees. PPV could be detected with a very low titer in C5 trees only on a few discrete symptomatic leaves from the graft inoculated branches. Conversely, very severe PPV symptoms and high viral concentration appeared on graft inoculated branches from conventional plums. In addition, the PPV symptoms observed on the noninoculated branches indicated that the virus invaded a large part of the canopies of conventional plums.

The evaluation performed three years post-inoculation showed no spread of PPV infection in C5 trees. In all cases (both singular and mixed infections on C5), PPV could be observed (discrete diffuse spots and sporadic symptoms) and detected by DAS-ELISA and IC-RT-PCR only near the inoculum points. No PPV symptoms were observed in the non-inoculated part of the C5 canopy. The absence of the virus was confirmed by molecular testing. Although PDV and PNRSV showed no clear symptoms in the plum canopy, these viruses were detected both in the inoculated canopy and in the non inoculated part of the C5 trees.

No differences in symptom development or PPV spread and detection were observed in the C5 trees when PPV was inoculated alone or in combination with heterologous viruses.

Experimental nursery *(Table 2).* PDV readily invaded whole C5 plants inoculated with the PPV + PDV combination. PPV also translocated from the inoculum bud to C5 but the virus could only be detected at the basal half of the plants. Although PPV was detected one year post-inoculation, the infection did not prosper in the following two years (2005 and 2006).

In C5 plants inoculated with PPV + PNRSV, diffuse spots of PPV symptoms sporadically appeared on a few leaves at the basal part of the plants. DAS-ELISA (2004 and 2005) and IC-RT-PCR (2006) confirmed the presence of PPV in this part of the plants. However, PPV could not be detected by ELISA and IC-RT-PCR on the top half part of the inoculated plants. Although PNRSV produced no symptoms the virus was detected with high titer in the whole plant since the first year post-inoculation.

Similar PPV behaviour was observed in C5 plants inoculated with PPV alone. No difference in symptom development or PPV spread was observed on C5 grown in the nursery when PPV was inoculated alone or in combination with heterologous viruses

Greenhouse assays *(Table 3).* In C5 plants inoculated with the PPV + PDV combination, PPV was detected by DAS-ELISA at the basal part of the C5 plants, three months and one year (2005) after inoculation. In the next year (2006), IC-RT-PCR tests confirmed that the spread of PPV was blocked in C5. On the other hand, no defensive reaction was observed against PDV, the plants showing very severe PDV symptoms during all experimental vegetative period. In the case of C5 inoculated with PPV + PNRSV, one year post inoculation, very low concentration of PPV was detected by DAS-ELISA and only on a few symptomatic leaves from the basal part of the plants. Two years post-inoculation PPV could be detected by IC-RT-PCR but, also, only on a few very discrete symptomatic leaves from the basal half of the plant. PNRSV showed no symptoms but the presence of the virus was confirmed by DAS-ELISA and IC-RT-PCR, both at the basal and distal sampling points.

No differences in symptom development or PPV spread were observed on C5 grown in the greenhouse when PPV was inoculated alone or in combination with heterologous viruses.

DISCUSSION

Regardless of singular (PPV) or mixed (PPV+PDV, PPV+PNRSV) infection, C5 transgenic plums revealed a similar behaviour in regard to PPV infection: PPV could produce a mild and limited infection in C5 independently of the presence of the heterologous viruses. PPV symptoms were extremely mild, sporadic or absent, indicating an effective inhibition of the virus multiplication. Across all trials experiments (orchard, nursery, greenhouse), the infection remained close to the inoculation site and did not prosper. Malinowski *et al.* (2006) showed the same mild infection phenotype in C5 trees graft-inoculated with PPV in an experimental open-field trial in Poland.

Previous field and greenhouse results clearly demonstrated that C5 is highly resistant to PPV infection through aphid vectors and by graft inoculation and the stability of the resistance (Hily *et al*., 2004; Malinowski *et al.,* 2006). The resistance can be transferred to seedlings through cross-hybridization (breeding) (Ravelonandro *et al.,*

1998; Scorza *et al,* 1998). In addition, C5 fruit quality is excellent, and productivity appears to be very good. For all these characteristics, the transgenic C5 named 'HoneySweet' is considered for deregulation in the USA (Scorza *et al.,* 2007)

Based on results presented we have no evidence that heterologous viruses can affect the stability of the engineered protection in transgenic C5 plums.

CONCLUSIONS

Graft-inoculation of transgenic C5 plums containing the PPV CP gene with PPV and the ilarvirus *Prunus necrotic ringspot virus* (PNRSV), or the ilarvirus *Prune dwarf virus* (PDV) in the field and in the greenhouse did not affect the efficacy and stability of PTGS over a three-year period. As a consequence, resistance to PPV did not break down.

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Tables

Table 1. Evaluation of *Plum pox virus* (PPV) *Prunus dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) in different parts of the graft-inoculated transgenic C5 "HoneySweet"and conventional European plums in the Romanian experimental orchard over a three-year experimental period.

inoculation: September 2003

Tree type	Inoculum	Analysed tree part	2004			2005						2006					
			DAS-ELISA			DAS-ELISA			Symptoms intensity			IC-RT-PCR			Symptoms intensity		
			PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV
C5	PPV	graft (inoculum)	++++			+++++			+++++			+			+++++		
		grafted branch	-			+			±			+			+		
		non-grafted branch	-			-			-			-			-		
C5	PPV	graft (inoculum)	+++++			+++++			+++++			+			+++++		
		grafted branch	-			+			±			+			+		
		non-grafted branch	-			-			-			-			-		
C5	PPV PDV	graft (inoculum)	++++	++++		+++++	+++++		+++++	+		+	+		+++++	+	
		grafted branch	-	-		+	+++++		±	+		+	+		+	+	
		non-grafted branch	-	-		-	+		-	±		-	+		-	±	
C5	PPV PDV	graft (inoculum)	++++	+++		+++++	+++++		+++++	+		+	+		+++++	+	
		grafted branch	-	-		+	+++++		±	+		+	+		+	±	
		non-grafted branch	-	-		-	++		-	±		-	+		-	±	

C5	PPV PNRSV	graft (inoculum)	+++			+++	+++++			++++	+++++			+	+			+	+++++			±
		grafted branch	-			-	±			++	+			±	+			+	+			±
		non-grafted branch	-			-	-			±	-			-	-			+	-			-
C5	PPV PNRSV	graft (inoculum)	+++++			++	+++++			++++	+++++			+	+			+	+++++			±
		grafted branch	-			-	+			+++	±			±	+			+	±			±
		non-grafted branch	-			-	-			+	-			-	-			+	-			-
Conventional	PPV	graft (inoculum)	++++				+++++				+++++				+				+++++			
		grafted branch	++				++++				+++				+				+++++			
		non-grafted branch	-				++				+				+				++			
Conventional	PPV PDV	graft (inoculum)	+++++	+++++			+++++	+++++			+++++	+			+	+			+++++	+		
		grafted branch	+	±			++++	+++++			+++	+			+	+			+++++	+		
		non-grafted branch	-	-			++	++			+	±			+	+			++	+		
Conventional	PPV PNRSV	graft (inoculum)	++++			++++	+++++			+++++	+++++			+	+			+	+++++			+
		grafted branch	+			-	++++			+++	+++			±	+			+	+++++			++
		non-grafted branch	-			-	++			+	+			-	+			+	++			±

Table 2. Evaluation of *Plum pox virus* (PPV) *Prunus dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) in different parts of the graft-inoculated transgenic C5 "HoneySweet" and conventional European plums in the Romanian experimental nursery over a three-year experimental period.

inoculation: August 2003

Tree type	Inoculum	Analysed plant part	2004			2005						2006					
			DAS-ELISA			DAS-ELISA			Symptoms intensity			IC-RT-PCR			Symptoms intensity		
			PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV
C5	PPV	bottom half	++			+			+			+			+		
		top half	-			-			-			-			-		
C5	PPV	bottom half	+			+			±			+			+		
		top half	-			-			-			-			-		
C5	PPV PDV	bottom half	±	++++		+	+++++		+	+++		+	+		±	++++	
		top half	-	++++		-	+++++		-	+++		-	+		-	+++	
C5	PPV PDV	bottom half	++	++++		++	+++++		+	+++		+	+		±	++++	
		top half	-	++++		-	+++++		-	+++		-	+		-	++++	
C5	PPV PNRSV	bottom half	±		+++++	+		++++	+		-	+		+	±		-
		top half	-		+++	-		++++	-		-	-		+	-		-
C5	PPV PNRSV	bottom half	+++		+++++	++		++++	+		-	+		+	±		-
		top half	-		++++	-		+++	-		-	-		+	-		-
Conventional	PPV	bottom half	+++++			+++++			+++++			+			+++++		
		top half	+++			++++			++++			+			++++		
Conventional	PPV PDV	bottom half	+++++	++++		+++++	+++++		++	+++		+	+		+++	++++	
		top half	++	++++		++++	+++++		+	+++		+	+		++	++++	
Conventional	PPV PNRSV	bottom half	+++++		+++++	+++++		++++	+++++		-	+		+	+++++		-
		top half	+++		++	+++		++++	+++		-	+		+	++++		-

Table 3. Evaluation of *Plum pox virus* (PPV) *Prunus dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) in different parts of the graft-inoculated transgenic C5 "HoneySweet" and conventional European plums in the Romanian experimental greenhouse over a three-year experimental period.

inoculation: March 2004

Tree type	Inoculum	Analysed plant part	2004			2005						2006					
			DAS-ELISA			DAS-ELISA			Symptoms intensity			IC-RT-PCR			Symptoms intensity		
			PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV	PPV	PDV	PNRSV
C5	PPV	bottom half	+++			++			+			+			+		
		top half	-			-			-			-			-		
C5	PPV PDV	bottom half	+++	+++++		++	+++++		±	+++++		+	+		±	+++++	
		top half	-	+++++		-	+++++		-	+++++		-	+		-	+++++	
C5	PPV PDV	bottom half	++	+++++		++	+++++		+	+++++		+	+		+	+++++	
		top half	-	+++++		-	+++++		-	+++++		-	+		-	+++++	
C5	PPV PNRSV	bottom half	++		+++++	+		++++	+		-	+		+	±		-
		top half	-		+++++	-		++++	-		-	-		+	-		-
Conventional	PPV	bottom half	+++++			+++++			++++			+			++++		
		top half	++			+++			++			+			+++		
Conventional	PPV PDV	bottom half	++++	++++		+++++	++++		++++	+++		+	+		++++	++++	
		top half	+++	++++		++++	++++		++	+++		+	+		++	++++	
Conventional	PPV PNRSV	bottom half	+++++		+++++	+++++		++++	+++++		-	+		+	+++++		-
		top half	+++		+++	+++		++++	+++		-	+		+	++++		-

VITICULTURE & OENOLOGY

Grape sensory parameters to be monitored in order to obtain typical Merlot wines – assessment of grape maturation in 2007

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Keywords: Merlot, maturity, typicality, harvest, grape sensory analysis

ABSTRACT

The evolution of Merlot grapes during the pre-harvest period in the year 2007 was followed by means of a sensory analysis methodology. By applying this methodology the optimum time for harvest can be established, along with the parameters which determine the quality and typicality of this particular variety. It was found that the Merlot grapes should be harvested when the pulp and juice aroma and also skin aroma are at their peak. At that moment, the sugar accumulated in berries is at a good level for a quality red wine, while the acidity is not too low and the concentration of polyphenolic compounds seems to remain constant for several days. Delaying harvest with 10 days will not lead to a significant increase in sugar, but the acidity will drop substantially, while a part of the aroma which gives the variety its specificity and typicality would also be lost.

INTRODUCTION

Merlot is a French grape variety which found good cultivation conditions in Romania and is, consequently, the most widely spread variety for quality red wines. The variety is characterized by a specific fruity aroma, which reminds of red berries, especially raspberry. In order to obtain typical wines which exhibit this specific aroma, the grapes to be vinified should be harvested at a proper time, when the concentrations of several important classes of compounds are in a certain balance.

To establish the optimum period of harvest for the Merlot variety a sensory analysis methodology of grapes can be applied during the last weeks of ripening period. The evolution of the assessed parameters will lead to important conclusions regarding the appropriate time of harvesting.

MATERIAL AND METHODS

The sensory analysis methodology is consistent with the one presented in previous papers (Antoce, 2007), and is a variant of the methodologies described by Rousseau J. and Delteil D. in 2000, Delteil D. in 2002 and Guyot Ch. and Dupraz Ph. in 2004.

The methodology involves the evaluation by a panel of tasters of 21 sensory parameters regarding the various parts of the grape berries (skin, pulp, juice and seeds). The score sheet specially designed for this purpose requires the estimation of the magnitude of the perception for a parameter by using a continuous scale ranging from 1 to 5; some parameters are estimated on discontinuous scales, with values of 1, 2 or 3.

The averages of values given by the tasters for all the parameters are plotted on a radar-diagram, the result being a fingerprint of the Merlot variety on the day of the evaluation.

The analysis is continued during a period of 2-3 weeks, at intervals of 2-3 days. The evolution of fingerprints obtained for the grapes can be compared by overlapping them, but a better way of interpretation requires defining a standard, a most desirable sensory profile of the grapes, followed by a comparison of the fingerprints obtained each day with the control, standard fingerprint. Even though usually it is impossible to obtain a total overlap of the actual fingerprint with the most desirable one, the vintner can take an informed decision to harvest or not, in accordance to the values of the most important parameters for the type of wine intended.

In this paper Merlot grapes cultivated in the collection of the Department of Viticulture and Enology, Faculty of Horticulture, Bucharest, were assessed by sensory analysis during the period of August 24 and September 10, 2007 in order to estimate the best harvest period.

RESULTS AND DISCUSSIONS

The sensory parameters estimated as an average of the results given by the panelists are presented in Table 1, for all the 8 days in which they were measured, in chronological order. In this way, their evolution can easily be followed.

As it can be observed, the pulp and juice sweetness seems to be increasing from August 24 to September 9th. However, refractometric analysis performed in the same period, does not support this finding, the sugar content measured in °Brix during this period varying in the range of 21.20 to 22.60 (Table 2), which can be considered constant, considering the cumulated errors related to this type of measurement. Therefore, the perception reported by the panelists that the Merlot grapes are sweeter as the maturation process progresses are probably due, in this case, to a significant decrease in acidity (parameter P5). Taking this fact into consideration we can say that the physiological maturity is reached already on August 31st. The decision to harvest should, however, be based also on the results regarding polyphenols and aroma.

The parameters in Table 1 show that polyphenolic compounds tend to remain constant for several days during the period of the study, but the parameters regarding aromatic compounds tend to increase up to August 31st, and then decrease significantly. Figure 1 shows that pulp and juice aroma (parameter P9) is maximal on August 31st and skin aroma (parameter P13) is maximal on September 3rd.

For this reason, we can say that the aromatic maturity is attained between August 31st and September 3rd and, if we were to preserve the entire aromatic heritage of this variety and obtain typical wines, this is the period when we should have started harvesting in 2007. If we take a closer look at the Figure 2 we see that we can narrow even further the interval of "optimum harvesting", because in September 3rd we already have a decrease of skin thickness (Parameter P10) and seed astringency (parameter P17), which means a loss of some polyphenolic compounds that may be important for the color and structure of the future wine.

These observations lead to the conclusion that delaying harvest after August 31st is accompanied by a loss of acidity and aroma, while the sugar concentration is not increasing. Of course, in some cases when the polyphenolic and aromatic maturity is not reached, it is logical to wait until at least one of these types of maturity is accomplished; a drop in acidity is not of much concern, since it can be corrected according to the legislation. In this case, however, any delay in harvest after August 31st when the pulp and skin aroma are at their peak, means an irremediable decrease of aroma, which cannot be compensated in any way, having as consequence the production of less

typical Merlot wine. Otherwise, a 10 days delay will not affect the yield, as it is the case of Feteasca neagra variety, for example, for which the decayed berries reached 16.5% in the same conditions, Merlot variety is more resistant, recording almost no affected berry (Table 2).

As for the phenolic maturity, we can say that, as compared with other varieties less rich in polyphenols, for which the change in the colour of the seeds represents significant information for the establishment of the harvest time (as it is for Feteasca neagra variety – data not presented), for the Merlot variety this parameter remains quite constant during the last weeks of ripening (Table 1).

In order to facilitate the decision of harvesting for Merlot grapes, Fig. 4 presents the evolution of the sensory parameters related to the typicality of this variety, plotted against the ideal case constructed with the most desirable values of these parameters. Again it can be seen that the pattern obtained for our Merlot grapes on August 31st is closer to that derived from plotting the most desirable parameters.

CONCLUSIONS

The typicality of Merlot wines depends upon the wise selection of the harvest period. The sensory parameters of the grapes which should be followed in order to establish the appropriate moment are found to be: the parameters related to physiological maturity: P5- pulp and juice acidity and P8 - pulp and juice sweetness; the parameters related to varietals aroma: P9 - pulp and juice aroma, P13 - skin aroma and parameters related to polyphenolic maturity: P10 - skin thickness and P17 - seed astringency intensity.

As opposed to the usual behaviour, delaying harvest with 10 days for Merlot this year did not lead to significant increase in sugar, while the acidity dropped substantially, and a part of the aroma that gives the variety its specificity and typicality decreased too.

For the year 2007, the optimum period of harvest for Merlot cultivated in our collection was around August 31st, when the aroma profile was evaluated to be at its maximum and the acidity and some of the polyphenolic compounds did not drop too much to affect the structure of the future wine.

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Tables

Table 1. The evolution of sensory parameters of Merlot grape variety during the period of August 24th and September 10th, 2007

Code	Parameter	Desirable parameter (Control)	M 24.08.07	M 27.08.07	M 29.08.07	M 31.08.07	M 03.09.07	M 05.09.07	M 07.09.07	M 10.09.07	Maximum obtainable value
P1	*berry firmness*	**3.0**	3.8	4.3	4.0	4.2	4.0	4.2	4.5	4.2	**5**
P2	*berry witherness*	**1.5**	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	**5**
P3	*berry color hue*	**4.5**	3.9	4.5	4.5	4.0	4.3	4.5	4.7	4.5	**5**
P4	*berry color homogeneity*	**5.0**	3.8	4.0	4.0	4.5	4.7	4.5	4.0	4.5	**5**
P5	*pulp and juice acidity*	**2.5**	2.9	3.1	2.7	2.3	2.0	2.0	1.5	1.5	**5**
P6	*pulp consistency*	**2.0**	2.0	2.5	2.5	3.0	3.2	3.0	2.5	1.5	**5**
P7	*pulp and juice viscosity*	**2.0**	2.5	2.5	2.5	3.0	3.0	3.0	2.0	1.5	**5**
P8	*pulp and juice sweetness*	**4.0**	2.8	3.5	3.8	3.5	3.8	3.5	4.0	4.0	**5**
P9	*pulp and juice aroma*	**3.0**	1.9	2.2	2.5	3.7	3.5	3.3	3.2	3.0	**4**
P10	*skin thickness*	**4.0**	3.5	3.0	3.0	3.0	2.7	2.5	2.5	2.0	**5**
P11	*skin astringency intensity*	**2.5**	1.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	**5**
P12	*skin astringency quality*	**3.0**	2.3	3.0	3.0	3.0	3.0	3.0	3.0	3.0	**3**
P13	*skin aroma*	**2.0**	1.4	3.0	3.0	3.2	3.5	3.3	3.2	3.0	**4**
P14	*skin bitterness*	**2.0**	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	**5**
P15	*seed color hue*	**4.5**	2.9	4.0	4.0	4.0	4.0	4.0	3.5	3.8	**5**
P16	*seeds color homogeneity*	**3.5**	4.3	4.0	4.5	4.2	4.5	4.2	4.0	4.0	**5**
P17	*seed astringency intensity*	**2.5**	4.0	3.0	2.6	2.0	2.3	2.5	2.5	1.5	**5**
P18	*seed bitterness*	**2.0**	3.8	4.0	3.5	1.3	2.5	2.7	2.5	1.2	**5**
P19	*seed consistency*	**4.5**	3.6	4.0	4.2	4.5	3.5	3.8	4.3	4.0	**5**
P20	*seed astringency quality*	**2.0**	2.4	2.3	2.6	1.2	1.8	1.5	1.5	1.2	**3**
P21	*seed aroma*	**2.5**	2.4	2.0	2.1	3.0	2.0	2.2	2.5	2.5	**3**

Table 2. The evolution of some physico-chemical parameters of Merlot grape variety during the period of August 24th and September 10th, 2007

Parameter	M 24.08.07	M 27.08.07	M 29.08.07	M 31.08.07	M 03.09.07	M 05.09.07	M 07.09.07	M 10.09.07
% of normal berries	96.80	96.81	96.80	96.78	96.19	96.56	96.04	96.74
% of abnormal berries	0.00	0.38	0.00	0.00	0.60	0.00	0.20	0.00
% of stems	3.20	2.81	3.20	3.22	3.21	3.44	3.77	3.26
Sugar content (°Brix)	22.55	22.35	22.60	22.45	21.35	20.30	21.60	21.20

Figures

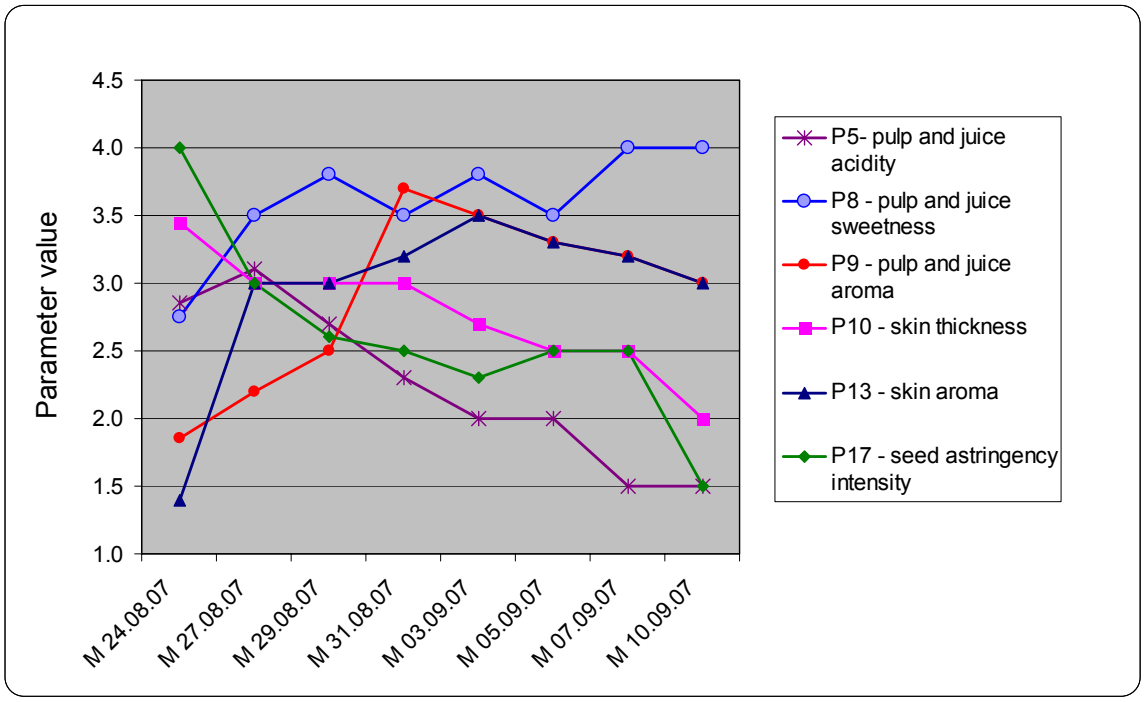

Fig. 1. The evolution of perceived pulp acidity and sweetness (P5 and P8) along with some sensory parameters related to aromatic tipicality of Merlot in 2007.

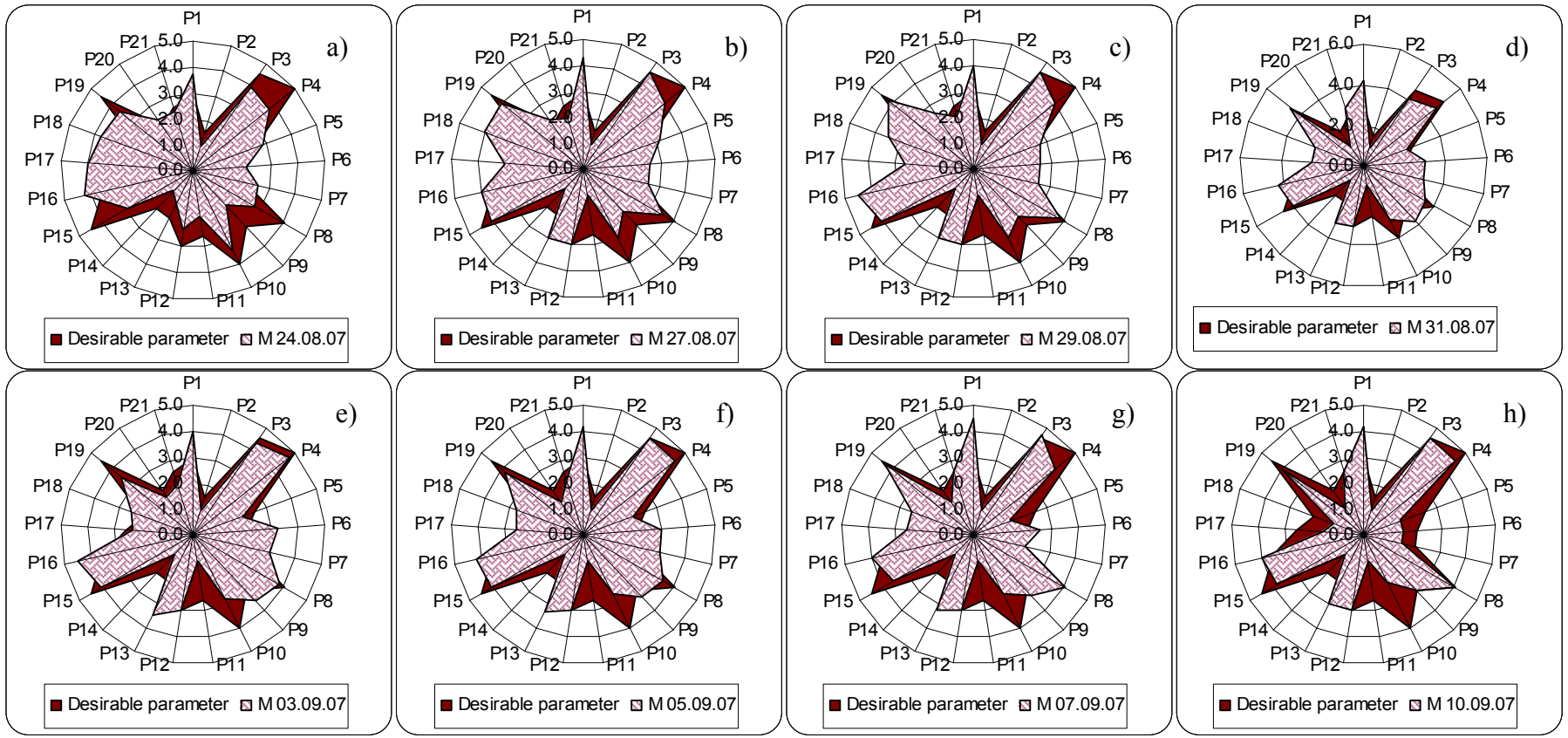

Fig. 2. The evolution of sensory analysis parameters of Merlot grapes during their ripening in the period of August 24 and September 10, 2007;
the solid pattern represents the control diagram (obtained with the desirable/ideal parameters);
the dotted patterns represent the actual parameters at a certain date.

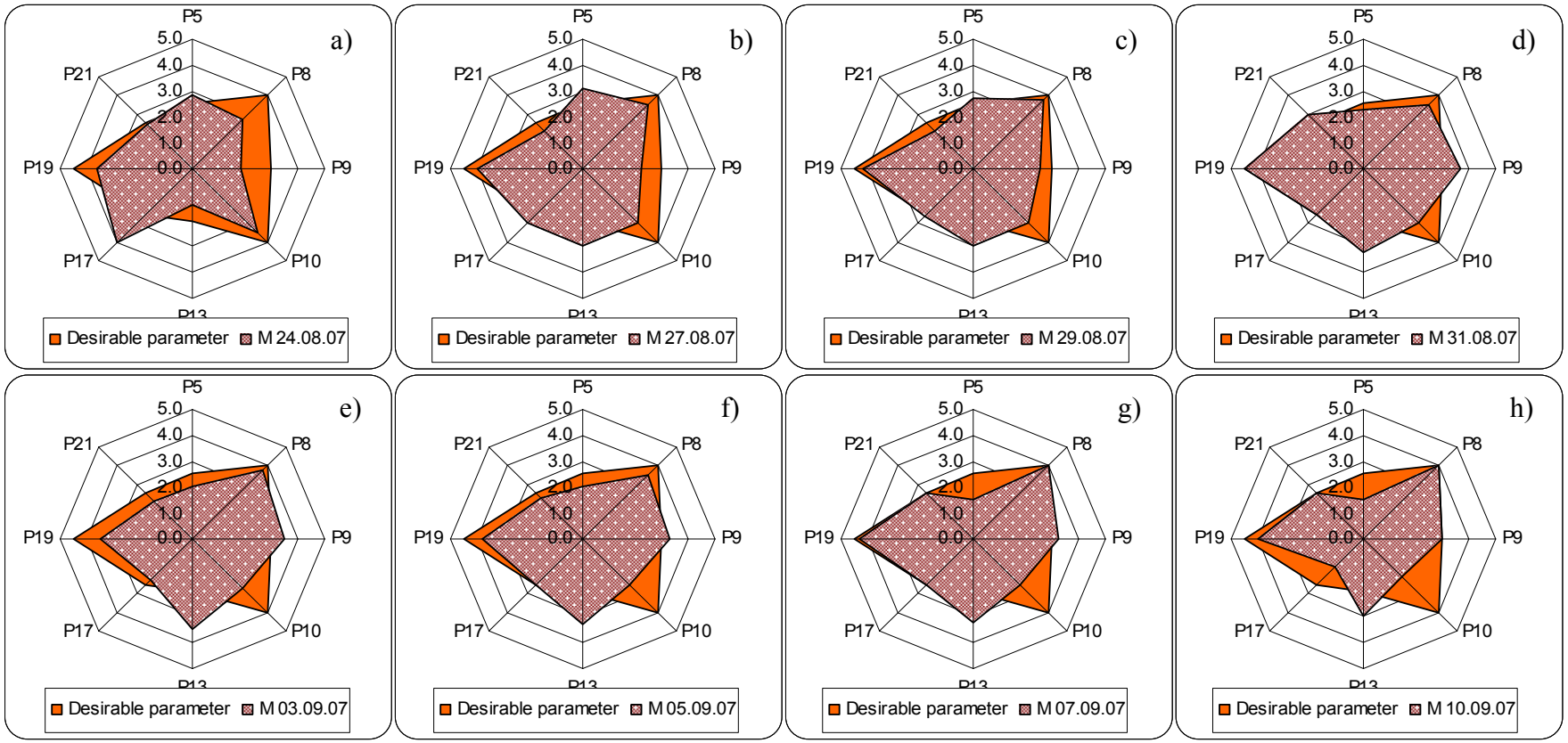

Fig. 3. The evolution of the sensory analysis parameters that define the typicality of Merlot grapes (P5, P6, P9, P10, P13, P17, P19, P21) during their ripening in the period of August 24 and September 10, 2007; the solid pattern represents the control diagram (obtained with the desirable/ideal parameters); the dotted pattern represents the actual parameters.

Particularities of the maturation of Pinot noir grapes in 2007 determined by sensory analysis

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Keywords: Pinot noir, maturity, harvest, grape sensory analysis

ABSTRACT

Pinot noir is a grape variety characterized by good tannin content but low anthocyan accumulation. The establishment of the phenolic maturity of this variety should take into account several other sensory parameters of grapes, which must be assessed periodically during the 2-3 weeks before the moment of harvest. By using a methodology of sensory analysis for grapes we found that for this variety we should monitored especially the evolution of the following parameters: pulp and juice acidity, pulp and juice sweetness, pulp consistency, pulp and juice viscosity, skin thickness and seed colour hue.

INTRODUCTION

Pinot noir is a variety characterized by relatively high accumulation of tannins, but lower anthocians as compared to other grape varieties for quality red wines. The optimum date for harvest should therefore take into consideration not only the phenolic maturity, but certain equilibrium between the levels of tannins and anthocians. Nonetheless, considering the high potential for the sugar accumulation of this variety and the rapid decay of the berries once they reach physiological maturity, the harvesting may be started before the polyphenolic maturity is reached.

This paper presents the maturation process of Pinot noir variety in the year 2007 cultivated in the collection of University of Agronomical Sciences and Veterinary Medicine of Bucharest, as assessed by sensory analysis of grapes.

MATERIAL AND METHODS

The grape analysis consisted of a sensory analysis performed in accordance with the methodology presented in a previous paper (Antoce, 2007), combined with physico-mechanical analysis. The sensory analysis involved the evaluation of 21 sensory parameters by a panel of trained tasters, during a period of three weeks, from August 24th to September 10th. The tasting was organized every 2 or 3 days and the results reported as averages. Also, at the same time with the tasting, the berries of a cluster were weighed and the percentages of healthy berries, decayed berries and stem weight were determined. The sugar content of the grape juice was determined by refractometry and reported in Brix units.

RESULTS AND DISCUSSIONS

Table 1 shows the data collected as a result of tasting the grapes of Pinot noir on 8 different days during the period of August 24th and September 10th, 2007. Among the 21 parameters evaluated we can see that in this period there are some parameters which remain almost constant or fluctuate within the range of the estimation error, since they reached already their maximum value according to the potential of this variety. This is the case, for example, of the parameters P11-P14, describing the aroma and astringence of the skins, or P16-P19 describing various aspects related to the polyphenolic compounds of seeds. This fact shows that the accumulation of the tannins in the skins

and seeds is not expected to increase anymore; the grapes being already ripen from this point of view. Harvesting anytime during this period would have led to wines of good tannic structure. As for the berry colour and colour homogeneity, the parameters which describe these characteristics, P3 and P4, are also constant and within the expected range for this variety.

Another parameter which remained almost constant during this entire time interval was the sugar content. This was proven by the sensory results regarding the pulp and juice sweetness (P8-Table 1), but also by the sugar content analysis reported in Table 2. The level of sugar determined refractometrically is between 25 to 27 °Brix, with an average of 25.76 °Brix; this sugar content shows that the variety is physiologically ripen and may be even overripe. For comparison, in the same interval the grapes of Merlot registered 24.8, Cabernet Sauvignon 23.9, Regent 24.0, Feteasca neagra 24.0, Dornfelder 21.43 and a white variety, Feteasca regala 21.4 Brix degrees. This aspect can also lead to the conclusion that the variety should have been harvested at the beginning of the period monitored. The fact is also supported by the high proportion of decayed berries recorded during this period (Table 2), which is up to 13.47%, by the berry firmness parameter (P1) which is under the ideal value (2.0 in comparison with an ideal 3.0 out of 5.0) and by the berry witherness (P2) with values of 4.0-4.5 out of 5.0.

However, there are some other parameters which should be taken into consideration. The perceived acidity of this variety is significantly decreasing during this period (P5 - Fig. 1), reaching its lowest level on September 7th. This fact indicates that the harvesting day should not be delayed too much. Moreover, in the same time with the loss of acidity some aroma and colour loss may also occur. The skin thickness (P10 - Fig. 1), perceived as thicker in the beginning of the ripeness evaluation period (August 24th-27th) and awarded 2.6-3.0 points, is perceived by the tasters as much thinner on September 10th, when it is given only 1.2. If this perception correlates to a reduction in the number of skin cells, we can expect a significant loss of colour in wines obtained from grapes harvested on September 10th compared to the wines produced of grapes harvested on August 24th. Due to the fact the Pinot noir is a variety that is naturally poorer in colour pigments, such a loss is in no way desirable, and this is the main sensory parameter that will decide the harvest day. Accordingly, the most appropriate day for the harvest of Pinot noir in 2007 was August 29th, a day in which most of the parameters, including P10, were close to the ideal parameters for this variety.

This is easier to observe if we plot the main parameters of the Pinot noir in a radar-diagram and superimpose it on the diagram obtained with the values considered ideal for the variety, as in Figures 2a-h. Also, to simplify the ripeness evaluation of Pinot noir, the sensory parameters of less importance can be excluded and the diagrams can include only those parameters which are essential for the decision. Figures 3 a-h show the simplified diagrams comprising only the parameters P5, P6, P7, P8, P10 and P15 and one can notice that the Pinot noir fingerprint that has the closest pattern to that of the desirable fingerprint is the one obtained on August 29th (Fig. 4c).

CONCLUSIONS

Pinot noir is a variety which accumulates a high quantity of sugar and this was especially the case in 2007. The maturity of the grapes occurs earlier than in the case of other grape varieties for quality red wine and, if not harvested in time, the grapes tend to lose colour. In the same time the yield may be affected by an increasing number of berries which lose consistency and firmness, becoming progressively softer until they decay.

The parameters which are essential to be monitored in order to establish the time of harvest were determined to be: P5 - pulp and juice acidity, P6 - pulp consistency, P7 - pulp and juice viscosity, P8 - pulp and juice sweetness, P10 - skin thickness, P15 - seed colour hue. These parameters allow the evaluation of the state of ripeness and help us select the appropriate time for harvest, when the polyphenolic compounds are at their optimum but the grapes are still not too advanced on the ripeness scale.

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Tables

Table 1. The evolution of sensory parameters of Pinot noir grape variety during the period of August 24th and September 10th, 2007

Code	Parameter	Desirable parameter	PN 24.08.07	PN 27.08.07	PN 29.08.07	PN 31.08.07	PN 03.09.07	PN 05.09.07	PN 07.09.07	PN 10.09.07	Maximum obtainable value
P1	*berry firmness*	3.0	1.5	2.0	1.5	2.0	2.5	2.0	2.0	2.3	**5**
P2	*berry witherness*	1.5	4.0	3.5	3.7	4.0	4.3	4.5	4.5	4.0	**5**
P3	*berry color hue*	4.5	4.0	3.8	4.2	4.0	4.2	4.5	4.0	4.0	**5**
P4	*berry color homogeneity*	5.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.5	**5**
P5	*pulp and juice acidity*	2.5	3.8	3.0	2.7	2.0	1.8	2.0	1.5	2.0	**5**
P6	*pulp consistency*	2.0	2.0	2.2	2.5	2.5	2.0	1.5	1.5	1.2	**5**
P7	*pulp and juice viscosity*	2.0	2.0	3.0	3.3	3.0	2.0	2.0	1.5	1.5	**5**
P8	*pulp and juice sweetness*	4.0	2.8	4.0	4.0	4.3	4.5	4.0	4.3	4.0	**5**
P9	*pulp and juice aroma*	3.0	3.2	3.2	3.5	3.5	3.5	3.7	3.5	3.3	**4**
P10	*skin thickness*	4.0	2.6	3.0	2.5	2.0	2.0	1.7	1.5	1.2	**5**
P11	*skin astringency intensity*	1.5	1.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	**5**
P12	*skin astringency quality*	3.0	1.3	2.2	3.0	3.0	3.0	3.0	3.0	2.8	**3**
P13	*skin aroma*	2.0	3.7	3.5	3.2	3.8	3.5	3.5	3.5	3.7	**4**
P14	*skin bitterness*	2.0	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	**5**
P15	*seed color hue*	4.5	2.4	3.0	3.5	3.0	3.7	4.0	3.5	3.8	**5**
P16	*seeds color homogeneity*	3.5	3.0	4.0	4.5	4.0	4.5	4.7	4.0	4.3	**5**
P17	*seed astringency intensity*	2.5	3.3	2.5	2.0	2.2	2.7	2.2	2.5	2.2	**5**
P18	*seed bitterness*	2.0	3.9	2.5	2.0	2.2	3.2	2.8	3.0	2.0	**5**
P19	*seed consistency*	4.5	4.0	3.8	4.0	3.8	3.5	4.0	4.0	3.3	**5**
P20	*seed astringency quality*	2.0	1.5	1.5	1.5	1.3	1.5	1.0	1.0	1.5	**3**
P21	*seed aroma*	2.5	2.4	2.7	2.8	2.5	1.8	2.2	2.5	3.0	**3**

Table 2. The evolution of some physico-chemical parameters of Pinot noir grape variety during the period of August 24th and September 10th, 2007

Parameter	PN 24.08.07	PN 27.08.07	PN 29.08.07	PN 31.08.07	PN 03.09.07	PN 05.09.07	PN 07.09.07	PN 10.09.07
% of healthy berries		**82.95**	**93.81**	**94.25**	**86.33**	**82.99**	**92.47**	**90.64**
% of decayed berries		**13.23**	**3.39**	**2.94**	**10.31**	**13.47**	**4.81**	**6.60**
% of stems		**3.81**	**2.79**	**2.81**	**3.35**	**3.55**	**2.73**	**2.76**
Sugar content (°Brix)	25.45	25.45	26.25	26.90	24.90	24.95	25.50	26.65

Figures

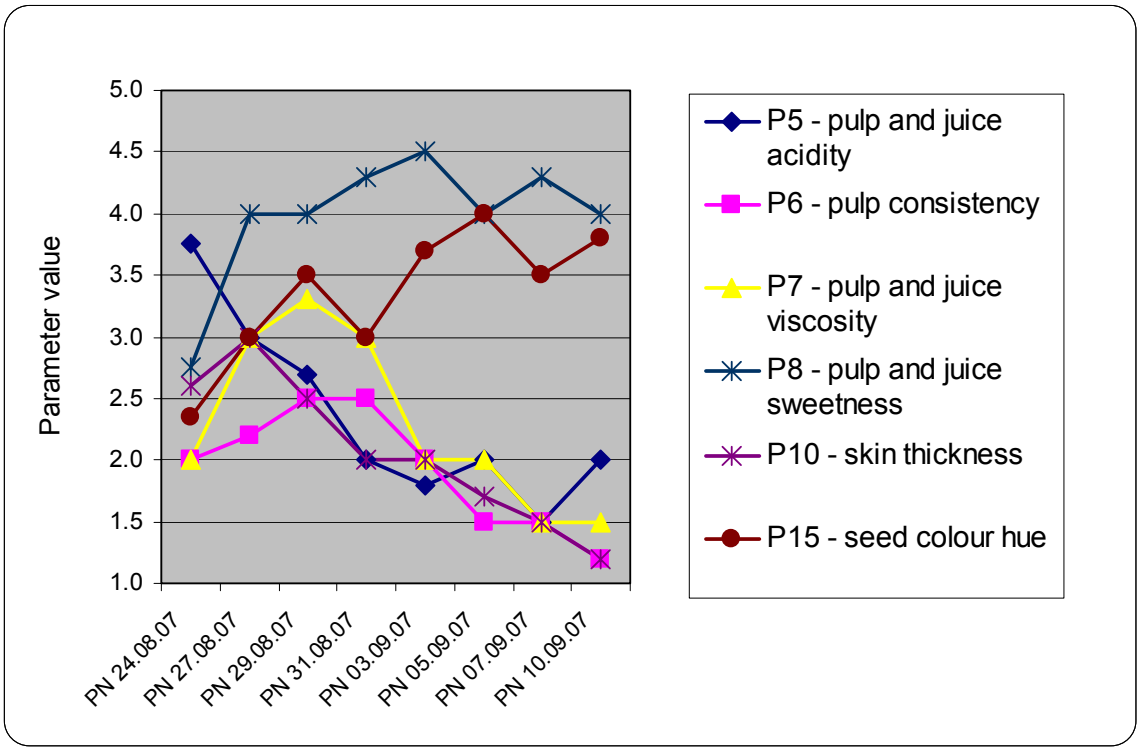

Fig. 1. The evolution of perceived pulp acidity and sweetness (P5 and P8) along with some sensory parameters related to aromatic typicality of Pinot noir in 2007.

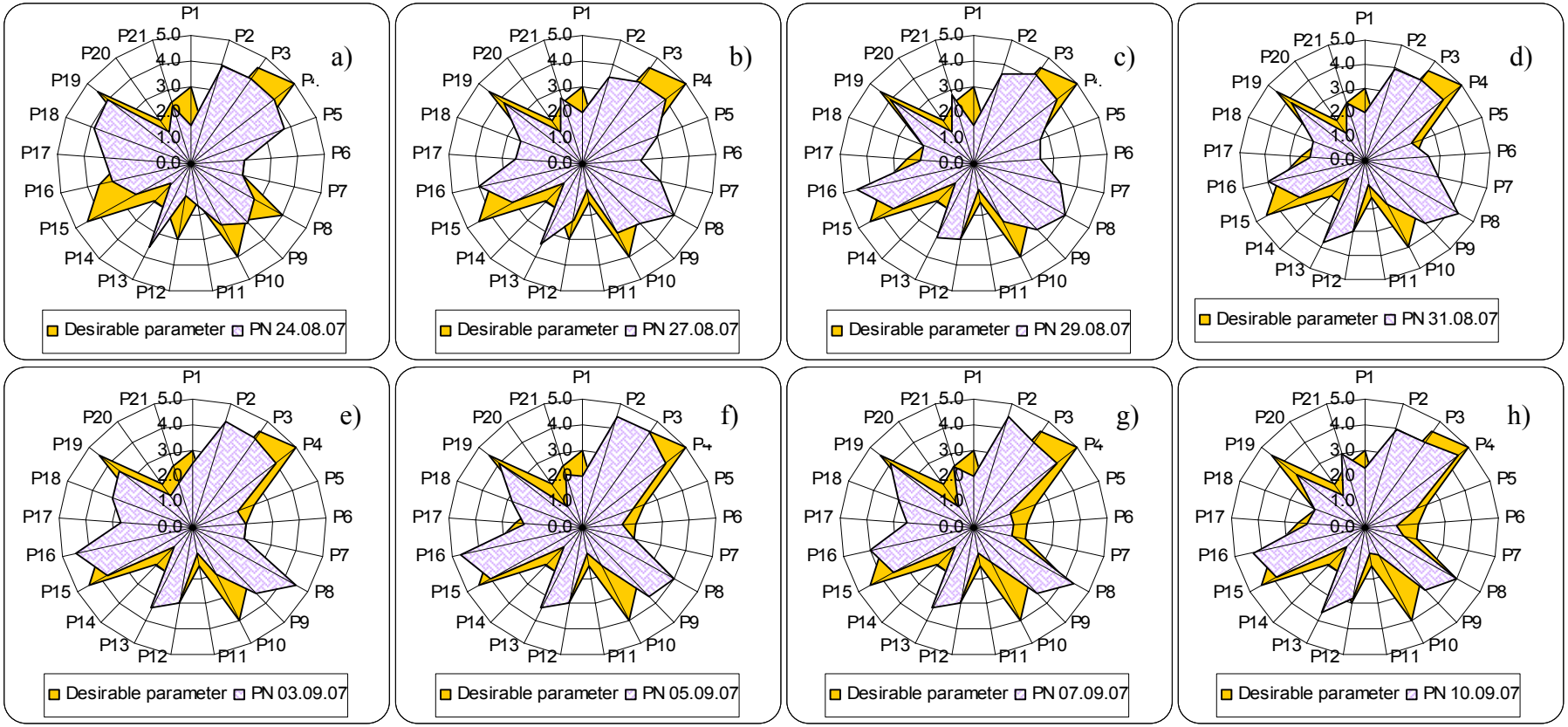

Fig. 2. The evolution of sensory analysis parameters of Pinot noir grapes during their ripening in the period of August 24 and September 10, 2007; the solid pattern represents the control diagram (obtained with the desirable/ideal parameters); the dotted patterns represent the actual parameters at a certain date.

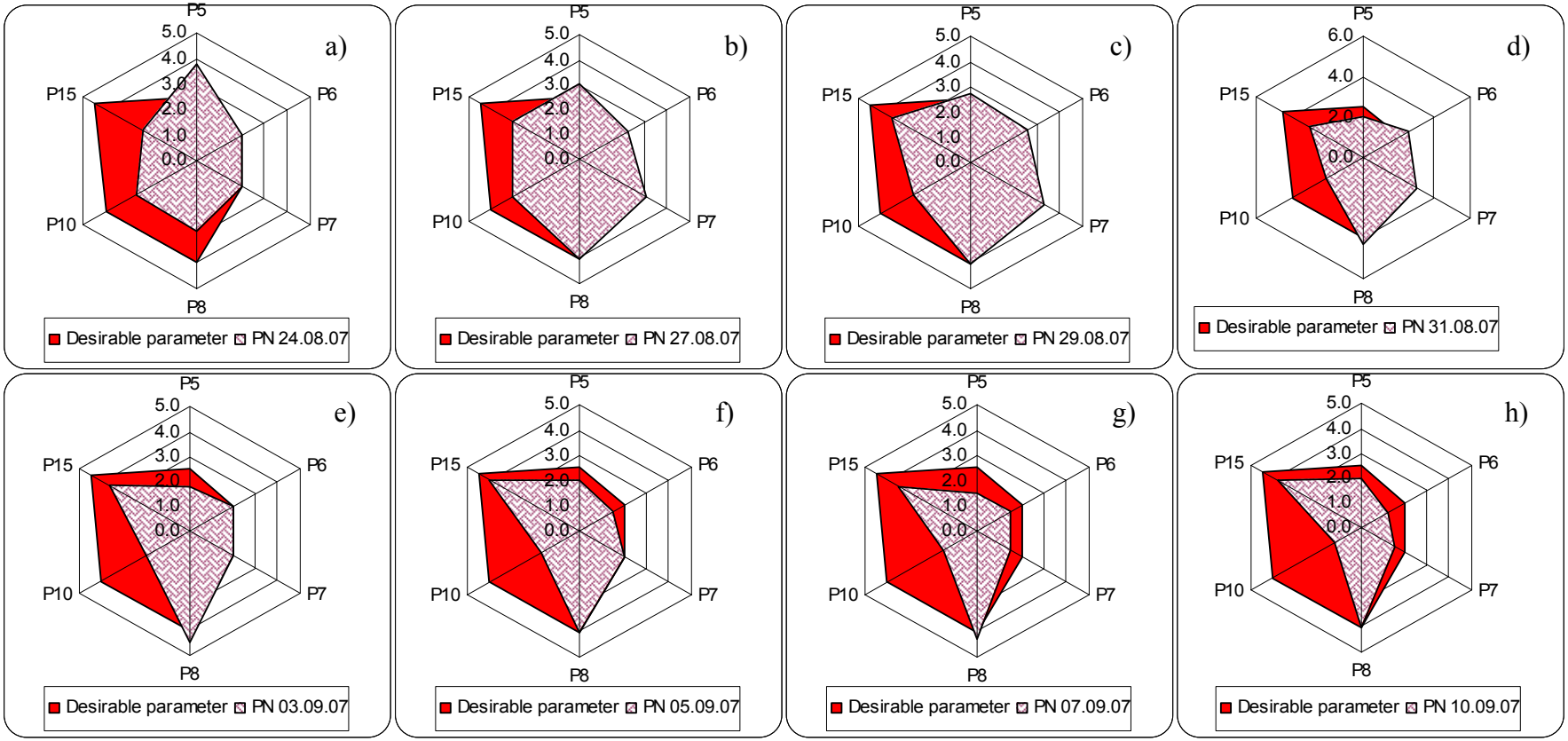

Fig. 3. The evolution of the sensory analysis parameters that define the tipicality of Pinot noir grapes during their ripening in the period of August 24 and September 10, 2007; the solid pattern represents the control diagram (obtained with the desirable/ideal parameters); the dotted pattern represents the actual parameters. (P5, P6, P7, P8, P10, P15).

Researches concerning the influence of weed control measures on grape yields from vine plantation of Timişoara Didactic Station

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Keywords: grape vine, control measures, post-emergent herbicides, manual weed control, grape yields.

ABSTRACT

Grapes represent an important and valuable food product due to their chemical composition. They contain a series of nutritive substances useful for human body with increased energetic, food and medicinal values.

The researches were performed in the period (2002 and 2004) and aimed the influence of weed control measures on grape yields in case of wine variety "Burgund mare" from Didactic Station Timisoara.

The main purpose of this study was to study the influence of weed control on grape yields as a result of applying post-emergent herbicides and manual weed controls for "Burgund mare" variety.

INTRODUCTION

Integrated weed control from grape vine plantations represent an important issue taking into consideration the ongoing reduction of labourers as well as the increases of grape yields.

The fast spreading of vegetative propagated weed species by rhizomes and root buds imperiously require the reduction of mechanical measures and applying herbicide-based weed controls.

The elimination of weeds from grape vine plantations can be diversely attained, the results being confirmed by studies performed during several research years (Lăzureanu, 1993, 1994; Cârciu, 2004).

MATERIAL AND METHODS

The researches were organized in the grape vine plantation of Didactic Station Timisoara considering a grape variety of 26 years old for white wine representing "Burgund mare".

The soil considered to set up the experiment was a cambic chernozem, slightly gleyed, with high content of humus matter 3,16%, moderately supplied with phosphorus, with underground moisture, decarbonated with fine medium leossoide deposits, medium clay-like loam/medium clay-like loam.

The plantation was represented by classical cultivation system with 2,0 m between rows and 1,2 m between vines per row insuring a normal total density of 4166 vines/ha (Dobrei, 2003).

The performed experience of single factor type was organized according to randomized block designs (Săulescu et Săulescu, 1967) in 4 replications and 8 experimental variants: 1- without herbicides and manual weed control; 2- Roundup 3 l/ha; 3- Basta 4 l/ha; 4- Touchdown 4 l/ha; 5- Gallant super 1,5 l/ha; 6- 2 manual weed controls; 7- 3 manual weed controls; 8- 4 manual weed controls. The experience was developed on a study period of three years (2002 and 2004).

Herbicides were applied on vine rows considering lanes of 60 cm wide. Herbicides were applied using Vermorel type spraying machine.

Grape harvesting was made considering mean grape samples on replications and variants. The weighing of mean grape samples was made using hand scales while statistical processing of experimental data was attained using variance analysis (Săulescu et Săulescu, 1967).

RESULTS AND DISCUSSION

The temperature regime of the experimental years (2002 and 2004) was regarded as normal for the area taken into study. Concerning the pluviometrical regime, the experimental years showed differences.

The rainiest year proved to be 2004, when precipitation level had reached 706,7 mm.

In tables 1.,2. and 3, there are presented data regarding the influence of weed control measures on grape yields in case of „Burgund mare" variety for the experimental years 2002 and 2004.

The highest grape yields per hectare in case of Burgund mare variety considering the experimental year 2002 was obtained for the variants using Roundup (3 l/ha), Touchdown (4 l/ha) and Basta (4 l/ha) with registered values of 12.51 t/ha, 12.35 t/ha and 12.24 t/ha, the differences comparing the control being positively very significant.

Considering the data of table 2, it has been observed that absolute yield showed values comprised between 6.51 t/ha (without herbicides and manual controls) and 8.86 t/ha (Roundup 3 l/ha) while relative yields were between 87,03% and 118,44%.

The highest yield was obtained for the variant with Roundup 3 l/ha (8.86 t/ha), the differences comparing the control being positively distinct significant.

The smallest grape yield was obtained for the non-treated variant (6.51 t/ha) with differences comparing the control negatively significant. In case of the remaining variants, no significance was registered, the values being close to control a variant.

Analyzing the mean of experimental years, it may be concluded that, the highest grape yields/ha were obtained for the variants treated with the following herbicides: Roundup (3 l/ha) and Touchdown (4 l/ha), with means of 10.68 t/ha and 10.48 t/ha, differences comparing the control variant being positively distinct significant (fig. 1.).

CONCLUSIONS

1. Considering the conditions of the year 2002, the highest grape yield per hectare in case of Burgund mare variety for the variants treated with the following herbicides: Roundup (3 l/ha), Touchdown (4 l/ha) and Basta (4 l/ha) showed values of 12.51 t/ha, 12.35 t/ha and 12.24 t/ha.
2. It could be observed that, in the year 2004, absolute yields were comprised between 6.51 t/ha (without herbicides and manual weed control) and 8.86 t/ha (Roundup 3 l/ha) while relative yield ranged between 87.03% and 118.44%. The highest grape yield was obtained for the variant treated with Roundup 3 l/ha (8.86 t/ha), the difference comparing the control variant being positively distinct significant. The smallest grape yield has been obtained for the non-treated variant (6.51 t/ha), the difference comparing the control variant being negative significant.
3. Analyzing the mean of the experimental years, it can be concluded that, the highest grape yields/hectare were obtained for herbicide treated variants using Roundup (3 l/ha) and Touchdown (4 l/ha), with yield means of 10.68 t/ha and 10.48 t/ha.
4. Taking into consideration the presented data regarding the influence of weed control measures on grape yields for the studied variety, it resulted that the best weed control

during the experimental period was performed using Roundup (3 l/ha) and Touchdown (4 l/ha) herbicides and thus being recommended for the West part of Romania.

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Tables

Table1. The influence of the weed control measure application on grape yield in Burgund mare, in the year 2002

Variant	Absolute yield (t/ha)	Relative yield (%)	Yield difference comparing control variant (t/ha)	Significance of difference
V_2-Roundup 3 l/ha	12,51	122,28	2,28	XXX
V_4-Touchdown 4 l/ha	12,35	120,72	2,12	XXX
V_3-Basta 4 l/ha	12,24	119,64	2,01	XXX
V_8-4 manual weed controls	11,43	111,73	1,20	X
V_5-Gallant super 1,5 l/ha	11,16	109,09	0,93	_
V_7-3 manual weed controls	10,74	104,98	0,15	_
V_6-2 manual weed controls	10,23	100,00	Control vt.	_
V_1-without herbicides and manual controls	9,52	93,05	-0,71	_

$DL_{5\%}$ = 1,08 t/ha; $DL_{1\%}$ = 1,47 t/ha; $DL_{0,1\%}$ = 1,98 t/ha;

Table 2. The influence of weed control measures on grape yield in Burgund mare grape variety, in the year 2004

Variant	Absolute yield (t/ha)	Relative yield (%)	Yield difference comparing the control variant (t/ha)	Significance of differences
V_2-Roundup 3 l/ha	8,86	118,44	1,38	XX
V_4-Touchdown 4 l/ha	8,62	115,24	1,14	X
V_3-Basta 4 l/ha	8,35	111,63	0,87	X
V_8-4 manual weed controls	7,96	106,41	0,48	_
V_5-Gallant super 1,5 l/ha	7,81	104,41	0,33	_
V_7-3 manual weed controls	7,64	102,13	0,16	_
V_6-2 manual weed controls	7,48	100,00	Control vt.	_
V_1- without herbicides and manual controls	6,51	87,03	-0,97	0

$DL_{5\%}$ = 0,85 t/ha; $DL_{1\%}$ = 1,16 t/ha; $DL_{0,1\%}$ = 1,56 t/ha;

Table 3. The influence of the weed control measures on grape yield in Burgund mare grape variety (mean values 2002 and 2004)

Variant	Absolute yield (t/ha)	Relative yield (%)	Yield difference comparing the control variant (t/ha)	Significance of differences
V_2-Roundup 3 l/ha	10,68	120,36	1,83	XX
V_4-Touchdown 4 l/ha	10,48	117,98	1,63	XX
V_3-Basta 4 l/ha	10,29	115,63	1,44	X
V_8-4 manual weed controls	9,69	109,07	0,84	-
V_5-Gallant super 1,5 l/ha	9,48	106,75	0,63	-
V_7-3 manual weed controls	9,19	103,55	0,15	-
V_6-2 manual weed controls	8,85	100,00	Mt.	-
V_1- without herbicides and manual weed control	8,01	90,04	-0,84	-

$DL_{5\%}$ = 1,10 t/ha; $DL_{1\%}$ = 1,49 t/ha; $DL_{0,1\%}$ = 2,02 t/ha;

Figure

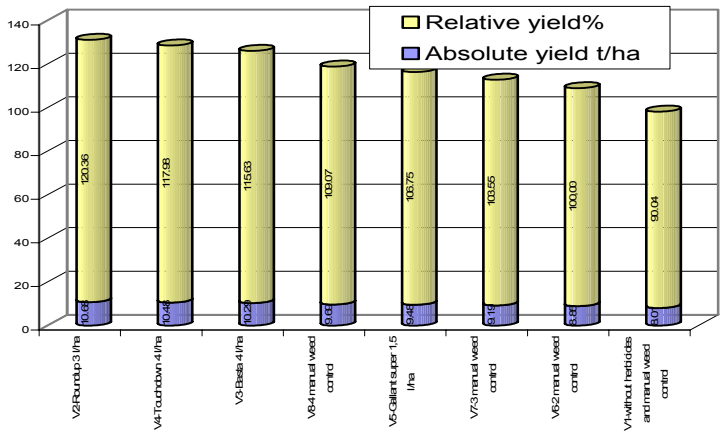

Fig. 1. The obtained grape yield in Burgund mare variety, in terms of weed control method (mean values 2002 and 2004)

The *Phytodietus* species (Hym: Ichneumonidae) – biology and contributions to the reducing of the grape leaf-roller, *Sparganothis Pilleriana* (Den. et Schiff.) (Lep: Tortricidae) populations in Southern vineyards of Romania

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Keywords: pest, vine, host larvae, ectoparasitoid, percentage of parasitizing.

ABSTRACT

As a result of the rearing of grape leaf-roller *Sparganothis pilleriana* (Den. et Schiff.) larvae collected in 1998, 2000-2003 from two vineyards, Ştefăneşti (Ag) and Dăbuleni (Dj), 3 species of *Phytodietus* have been obtained as primary larval ectoparasitoid: *Phytodietus* sp., *Phytodietus ornatus* Desv. and *Phytodietus polyzonias* (Först.). The host parasitizing by the *Phytodietus* species occurs during the month of May, depending on the local and annual climatic conditions. *P. polyzonias* (Först.) was present in both vineyards; at Dăbuleni, its activity was more obvious. Three host-parasitoid relationships have been recorded, all of them new to science. The role played by these parasitoids in the limitation of grape leaf-roller populations is generally minor, the parasitism ratio being 1.2%.

INTRODUCTION

The populations of many pests are controlled by parasitoid Hymenoptera, which have a contribution to reducing the damage they cause. As part of the Icneumonidae family, the Tryphoninae subfamily, the genus *Phytodietus* stands out, with approximately 21 species, known all over Europe. The species belonging to that genus are parasites of several lepidopterous larvae. In accordance with the literature in the field, they parasitize the caterpillars that have already completed their development. The egg is laid on the surface of the host organism, after the latter has been temporarily paralysed (Mills and Carl, 1991).

In the present paper the species of *Phytodietus* are presented, which were obtained for the first time through rearing larvae of *Sparganothis pilleriana* (Den. et Schiff.).

MATERIALS AND METHODS

The researches were carried out in two vineyards of South of Romania, Ştefăneşti-Argeş and Dăbuleni-Dolj. The larvae host collected in May-July period, 1998, 2000-2003 years. The caterpillars were reared in isolation up to the apparition of tortricid or parasitoid adults, their food being the vine leaves. 14 individuals of *Phytodietus* species were produced under laboratory conditions.

RESULTS AND DISCUSSIONS

3 species of *Phytodietus* have been obtained as larval ectoparasitoid: *Phytodietus sp., Phytodietus ornatus* Desv. and *Phytodietus polyzonias.* (Först.). All *Phytodietus* species was present in Ştefăneşti, only *Phytodietus polyzonias* (Först.) in Dăbuleni. In Fig. 1 is presented the percentage of larvae parasitized by the *Phytodietus* species, in Ştefăneşti.

The data about the recorded parasitoids have been arranged in the following order: locality/stage of host/collecting date/date of emergence/individuals (♀ and ♂) obtained.

1. *Phytodietus* sp. was obtained as a larval solitary, primary ectoparasitoid in: Ştefăneşti/mature larva/08.06.1998/27.06.1998/1♂.

At the moment of the sampling, the larva of *S. pilleriana* Den. et Schiff. was outstandingly mobile, though the ectoparasitoid larva was 4 mm in length. As of the 17.06, the larva of the parasitoid had already made a white, transparent 8-mm cocoon, with a very thin wall, and, after another 10 days, hatched a male individual.

The role of this parasitoid in limiting the host populations in Ştefăneşti was minor: 0.2% (Table 1).

The host-parasitoid relationship is new to science.

2. *Phytodietus ornatus* Desvignes, 1856 was obtained as a larval solitary, primary ectoparasitoid only in Ştefăneşti: immature larva/19.05.2002/14.06.2002/1♂; immature larva/4.06.2002/20.06.2002/1♂; mature larva/1.06.2003/16.06.2003/1♂; mature larva/1.06.2003/18.06.2002/1♀; mature larva/1.06.2002/21.06.2003/1♀; mature larva/1.06.2003/24.06.2003/1♂;

In Europe, it is known in Germany, Latvia, Moldova, Romania, and the United Kingdom.

Host: *Parasyndemis historiana* Frol., *Choristoneura murinana* Hb., *Archips rosanus* L. (Tortricidae), *Anacampsis populella* Cl. (Gelechiidae) (Pisică, 2001).

In Romania, it was obtained from *Archips rosanus* L. (Diaconu, 1999).

During the researches conducted, two cases out of six were represented by the parasitizing of the immature larvae of *Sparganothis pilleriana*, corresponding to 3rd and 4th instars of host larvae.

At the moment of the sampling of the parasitized larvae of *S. pilleriana* (Den. et Schiff.), the parasitoid was in the stage of an egg or larva.

The eggs under observation were white, elongated, and 1 to 1.5 mm in length; they were located dorsally, on the caterpillar's meso- and metathorax.

Thus, on the 19.05.2002 a 3rd instars caterpillar of *S. pilleriana* was collected, which had an elongated egg on the mesothorax (Photo 1a). The caterpillar continued its growth, passing on to the following age period, without that process affecting the egg of the parasitoid. Subsequently, out of the egg was hatched a larva of *P. ornatus* that began feeding, and the parasitized caterpillar kept its mobility. The posterior end of the larva of *P. ornatus* fixed itself near the prothoracic shield of the *S. pilleriana* larva, as the mandibles showed distinctly at the anterior end. The caterpillar kept its mobility until the parasitoid's larva reached the last larval age, when the larva of *P. ornatus* became aggressive and killed its host. Eventually, the *P. ornatus* larva completely consumed its host, with only the chitinous remains left out of the latter (Photo 1b). Then, close to the remains, the mature larva of *P. ornatus* built its cocoon with a view to the nymph stage. From the eclosion of the parasitoid larva out of its egg, up to the moment of the building of the cocoon, under laboratory conditions, at an average temperature of cca. 22^{o}C, ten days passed. The duration of the cocoon building was of about 24 hours, and from the date hen the cocoon was finished up to the emergence of the adult (a female) eleven days passed. Fed with sugared water, the adult survived for 17 days.

For the eclosion, the adults of *P. ornatus* make a narrow, long subterminal opening into the cocoon.

In all the cases observed, he parasitized caterpillars kept their alertness until the parasitic larva reached complete development. The parasitoid can be noticed to occur only in the years 2002 and 2003, and the percentage of parasitation of the host larvae

was greater in 2003 (Fig. 1).

The role of this parasitoid in limiting the host populations in Ştefăneşti was minor: 1.20% (Table 1).

According to the literature consulted, the host-parasitoid relationship is new to science.

3. *Phytodietus polyzonias* (Förster, 1771) was obtained as a larval solitary, primary ectoparasitoid in:

Ştefăneşti/mature larva/16.06.2001/27.06.2001/1♂;
Dăbuleni/mature larva/28.05.2001/15.06.2001/1♀;
Dăbuleni/mature larva/28.05.2001/18.06.2001/1♂;
Dăbuleni/mature larva/28.05.2001/21.06.2001/1♂;
Dăbuleni/mature larva/28.05.2001/2.07.2001/1♂;
Dăbuleni/mature larva/19.06.2001/1.07.2001/1♀.

In Europe, it is known in Denmark, France, Germany, Italy, Latvia, Moldova, Romania, and United Kingdom.

Host: a known polyphagous species, belonging to the Tortricidae, Yponomeutidae, Pterophoridae, Pyraustidae species.

In Romania, it was obtained from *Tortrix viridana* L., *Archips rosanus* L., *Pandemis heparana* Den. et Schiff., *Hedya dimidioalba* Retz., *Adoxophyes orana* Fisch. v Rösl. (Tortricidae) (Pisică, 2001).

P. polyzonias (Först.) has behaviour similar to the related species *P. ornatus* Desv. All the parasitized caterpillars of *Sparganothis pilleriana* were mature at the moment of the sampling. Although the parasitoid larva comes to be 4-5 mm long and 1.5 mm broad, the *S. pilleriana* larvae are still mobile.

The males obtained were measured to be between 7.1–8 mm and 1.5–1.9 mm long, and the females, between 10.1 and 10.75 mm long, and the breadth of their abdomen is 1.5–2 mm, as they are larger.

The colour of the cocoon varied from white-transparent to white-yellowish, yellowish-brownish, and even brownish, with a median, nacreous ring. The wall of the cocoon is very think and seems to be transparent.

On eclosion, the adults tear, with their mandibles, a jagged-lipped opening, located terminally or subterminally, at the opposite end of the excretion one.

P. polyzonias was present in both locations, in 2001 alone; at Dăbuleni, its activity was more noticeable (Table 1). Its absence in the other years was probably due to the small number of samples, or its preference for other hosts.

On the whole, its role in reducing the populations of *S. pilleriana* was greater than that of the other species of *Phytodietus*: 0.6%, against 0.09% - *Phytodietus* sp. and 0.51% - *P. ornatus* Desv. (Table 1).

The host-parasitoid relationship is new to science.

CONCLUSIONS

In the Ştefăneşti and Dăbuleni vineyards, the grape leaf-roller *Sparganothis pilleriana* (Den. et Schiff.) larvae was parasitizing through 3 *Phytodietus* species: *Phytodietus ornatus* Desv*, P. polyzonias* (Först.), and *Phytodietus* sp.

The host parasitizing by the *Phytodietus* species occurs in the course of the month of May, depending on the local and annual climatic conditions. Cases when the immature caterpillars of the host were parasitized were noticed, as well. In all the cases

under observation, the parasitized caterpillars kept their alertness until the parasitized larva reached its complete development. All *Phytodietus* species was present in Ştefăneşti.

The role played by the *Phytodietus* species in the reducing of the grape leaf-roller populations is minor: 1.2%.

3 host-parasitoid relationships have been recorded, all of them new to science.

ACKNOWLEDGEMENTS

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Table

Table 1. Efficiency of the *Phytodietus* species in limiting the host populations

Place	Year	No. of ind.	No. par.	%	***Phytodietus* sp.**							
					No	%	***Phytodietus* sp.**		***Phytodietus ornatus***		***Phytodietus polyzonias***	
							No	%	No	%	No	%
Dăb. (Dj.)	2000	215	61	28.37	-	-	-	-	-	-	-	-
	2001	308	67	21.75	6	1.95	-	-	-	-	6	1.95
	2002	146	16	10.96	-	-	-	-	-	-	-	-
Subtotal		669	144	21.52	6	0.9	-	-	-	-	6	0.9
Ştef. (Ag.)	1998	27	5	18.51	1	3.7	1	3.7	-	-	-	-
	2000	64	12	18.75	-	-	-	-	-	-	-	-
	2001	124	18	14.52	1	0.8	-	-	-	-	1	0.8
	2002	164	30	18.29	2	1.22	-	-	2	1.22	-	-
	2003	122	34	27.87	4	3.28	-	-	4	3.28	-	-
Subtotal		501	99	19.76	8	1.6	1	0.2	6	1.2	1	0.2
Total		1170	243	20.77	14	1.2	1	0.09	6	0.51	7	0.6

Dăb. = Dăbuleni vineyard
Ştef. = Ştefăneşti vineyard
No. of ind. = number of individuals collected (larvae and pupae)
No. par. = number of individuals parasitized by parasitoids complex

Figures

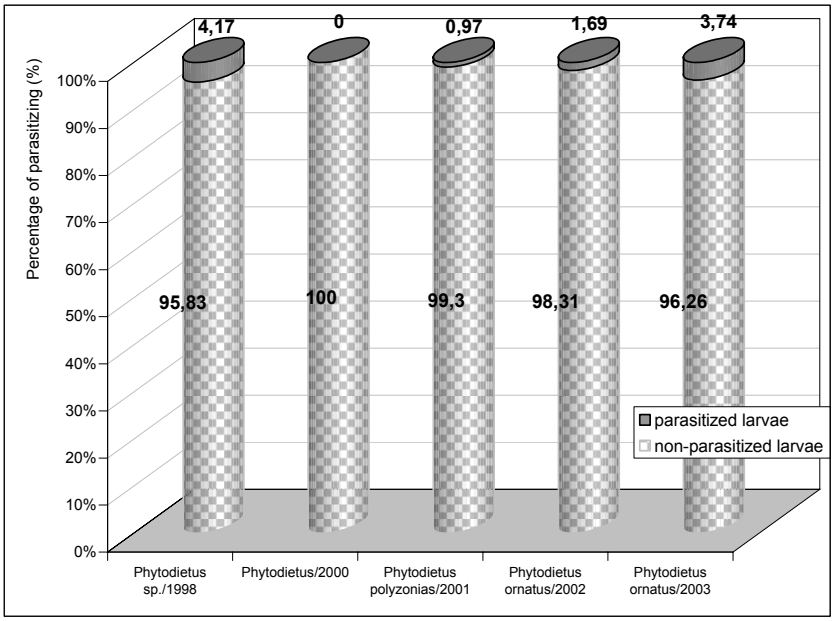

Fig. 1. The percentage of parasitizing of *Sparganothis pilleriana* larvae by *Phytodietus* species, in Ştefăneşti.

Photo 1a. The *Phytodietus ornatus* Desv. egg on mesothorax of *Sparganothis pilleriana* caterpillar

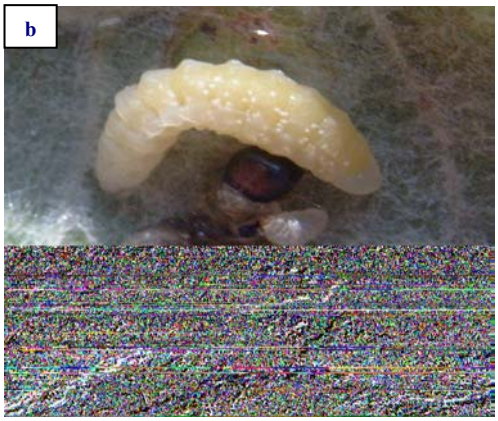

Photo 1b. The *Phytodietus ornatus* Desv. larva near the remains of caterpillar

Photo 1. The *Phytodietus ornatus* Desv. egg on mesothorax of *Sparganothis pilleriana* caterpillar

The multicriterial climatic groups from the Romanian viticultural level

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Keywords: geographical delimitation, climatic index, macroclimate, vineyard

ABSTRACT

Within the last decade a worldwide *multi-criteria climatic classification* methodology was adopted. The multi-criteria method is based on three climatic index applications (multi-criteria): drought index (IS), heliothermal index (IH) and night cooling index (IF). Thereby, applying the multi-criteria method for each wine-growing region of Romania and according to the values expressed by the three indexes analyzed, we have created thirteen climatic groups in all the country, each of them characterizing the win-growing climate; the groups are presented in the present paper.

INTRODUCTION

The desire to join the efforts related to the creation of a data base concerning the viticultural ecoclimate, integrated in an international system, determined us to proceed to the determination of the values concerning *the drought index, the heliothermic index and the index of the night cooling* for all the viticultural centres afferent to the eight regions of Romania, then we have established the *viticultural climates* for each centre, as well as different *climatic groups*. The establishment of these two concepts allows the comparative study of the viticultural climate and the appreciation of the favourability degree for the vine culture of the areas of reference.

MATERIALS AND METHODS

From the multitude of tested synthetic indicators with ecoclimatic character, those who proved their validity were the indexes which integrate the hydric resources of the soil, the heliothermic ones and the low thermal resources from the nights of September, decisive for the quality of the grapes maturation, and namely:

The drought index (IS) – allows the characterisation of the hydric component of the ecoclimate from a viticultural region, rendering the availability degree of the water from the soil. This index takes over 5 classes of variation: $\mathbf{IS_{00}}$ – moist climate, $\mathbf{IS_0}$ – under-moist climate, $\mathbf{IS_1}$ – climate of moderate drought, $\mathbf{IS_2}$ – climate with pronounced drought and $\mathbf{IS_3}$ – climate with very pronounced drought.

The heliothermic index (IH) – delivers the necessary information with respect to the level of the thermal potential, and it is calculated for the period of conventional vegetation of 6 months. This index takes over 6 classes of variation: $\mathbf{IH_1}$ – very cool climate, $\mathbf{IH_2}$ – cool climate, $\mathbf{IH_3}$ – temperate climate, $\mathbf{IH_4}$ – warm temperate climate, $\mathbf{IH_5}$ – warm climate and $\mathbf{IH_6}$ – very warm climate.

The cooling index of nights (IF) – represents the average of the minimum temperatures, (J. Tonietto, 1999), from the cooling nights of the month of September. The cooling index of nights is framed in 4 classes of variation and namely: $\mathbf{IF_1}$ – climate with warm nights, $\mathbf{IF_2}$ – climate with temperate nights, $\mathbf{IF_3}$ – climate with cool nights and $\mathbf{IF_4}$ – climate with very cold nights.

The calculation of these three synthetic indicators with climatic character was performed for all the viticultural centres from the eight regions of Romania, in terms of the climatic data taken over, in a primary form, from the specialised literature (Climate R.P.R., 1961, VOL. II; The Climate of Romania, 1990, vol. II); and in case of the

viticultural centres where there weren't any meteorological stations, it was resorted to the *method of translation* (M. Buiuc, 1999), used currently in the specialised literature.

RESULTS AND DISCUSSIONS

The representative climatic groups for the viticultural region of the Transylvania plateau

In this region, the 19 viticultural centres are framed in ***three climatic groups***, of which two are obviously in majority and namely: $IS_{00}\ IH_2\ IF_4$ (moist climate, cool, with very cold nights – 47% of the total of viticultural centres), and $IS_{00}\ IH_3\ IF_4$ (moist climate, temperate, with very cold nights – 47%); the group with the lowest share is $IS_0\ IH_3\ IF_4$ (under-moist climate, temperate, with very cold nights), having a percentage of 6%. The two dominant groups are accredited with the greatest hydric resources and moderate heliothermic resources, on the ground of some very cold nights.

The group $IS_{00}\ IH_2\ IF_4$ holds, in this area, the highest percentage from the territory of our country and is located in the most northern viticultural area of Romania. We find ourselves therefore at a latitude from which, towards north, the vines cannot live in this part of Europe.

The representative climatic groups for the viticultural region of Moldavia Hills

In the viticultural region of the Moldavia Hills, the 53 viticultural centres of this region are a part of a number of ***six climatic groups***, unequally constituted, with variations from those with a single viticultural centre such as $IS_{00}\ IH_2\ IF_4$ (moist climate, cool, with very cold nights – c.v. Bozieni) or $IS_2\ IH_4\ IF_3$ (climate with pronounced drought, cool, with cool nights – c.v. Smardan), up to the climatic group $IS_1\ IH_3\ IF_4$ (climate with moderate drought, cool, with very cold nights, which includes 38 viticultural centres, the most famous in the area), which holds a percentage of 71%. All the other groups present lower shares, contained between 2% and at most 13% but cover an enough varied spectre of climatic conditions.

The representative climatic groups for the viticultural region of Muntenia and Oltenia Hills

For the viticultural region of the Hills of Muntenia and Oltenia, the 37 viticultural centres belong to a number of ***seven climatic groups***.

In this viticultural region we find two climatic groups obviously dominant and namely $IS_1\ IH_4\ IF_4$ (climate with moderate drought, temperate warm, with very cold nights) and $IS_1\ IH_4\ IF_3$ (climate with moderate drought, temperate warm, cool nights, which represents 61% of the total). This two dominant climatic groups are accredited with the lowest hydric resources and the highest heliothermic resources.

The representative climatic groups for the viticultural region of Banat Hills

We find here ***four climatic groups*** within which the number of the viticultural centres is different, but constant, beginning with a single centre in the groups $IS_0\ IH_3\ IF_4$ (Silagiu), $IS_0\ IH_4\ IF_4$ (Recas) and $IS_1\ IH_4\ IF_4$ (Teremia), up to three viticultural centres included in the group $IS_0\ IH_3\ IF_3$ (New Moldavia, Tirol and Jamu Mare).

Here is predominant the under-moist, temperate climate, with cool nights ($IS_0\ IH_3\ IF_3$) which holds a share of 49% of the total. We also find here a climatic group characterized by a moderate drought, temperate warm, with very cold nights ($IS_1\ IH_4\ IF_4$), specific to the viticultural centre Teremia.

The representative climatic groups for the viticultural region of Crisana and Maramures Hills

The viticultural centres of this region are found in ***three climatic groups*** (towards the north-west part of our country), which contain a different number of centres, starting from one, in the group *IS_0 IH_4 IF_4* – 7% (Minis, the vineyard Minis-Maderat), continuing with the group *IS_{00} IH_3 IF_4* – 40% which gathers six viticultural centres, and the majority of the number of centres (equal with eight) is held by the group *IS_0 IH_3 IF_4 – 53%*.

The representative climatic groups for the viticultural region of Dobrogea Hillocks

The 14 viticultural centres of this region are divided in ***two climatic groups***, respectively *IS_2 IH_3 IF_3* and *IS_2 IH_4 IF_3*. The number of consecutive centres is different, group IS_2 IH_3 IF_3 contains five centres – 36%, whereas the group IS_2 IH_4 IF_3 is predominant (contains nine viticultural centres, respectively 64%).

These climatic groups are accredited with the highest heliothermic resources and the lowest hydric resources, predominant being the group IS_2 IH_4 IF_3 characterized by a climate with pronounced drought, temperate warm, with cold nights.

The representative climatic groups for the viticultural region of the Danube terraces

For this viticultural region, we have ***four climatic groups***, unequally constituted, with variations from a single centre (Zimnicea - *IS_1 IH_4 IF_3*, Aliman - *IS_2 IH_4 IF_3*), up to maximum four centres for the climatic group *IS_2 IH_4 IF_4* (clomate with pronounced drought, temperate warm, with very cold nights – Ostriv, Baneasa, Oltina and Fetesti).

The representative climatic groups for the viticultural region of sands and other favorable lands from the south of the country

The climatic groups specific to this region ***are in number of four*** and have in their structure a different number of viticultural centres, the greatest group being *IS_1 IH_4 IF_3* (climate with moderate drought, temperate warm, with cold nights, which contains 13 centres), followed by *IS_1 IH_4 IF_4* (with four viticultural centres), and the other two groups IS_1 IH_5 IF_3 and IS_2 IH_4 IF_4 contain each of them a viticultural centre, respectively Poiana Mare and Suditi.

► In the conditions of our country, among the components of the viticultural climate the drought index is the most movable (figure 1), namely it was framed in four classes of variations (IS_{00}, IS_0, IS_1, IS_2), followed by the heliothermic index (figure 2), also with four classes (IH_2, IH_3, IH_4, IH_5), on the last place being placed the cooling index of the night (figure 3), which was included in two classes of variations (IF_3, IF_4).

It must be mentioned that the drought index was the most movable; the heliothermic index, although he was framed in four classes of variations, the last class of the warm climate (IH_5) contains a single viticultural centre, having a very small share, even insignificant.

It may be noticed from the graphics the share that each type of climate may reach on the territory of our country, according to the number of the viticultural centres that it contains.

CONCLUSIONS

1. From the combination of the classes of variation, calculated for the criterial indicators, it was performed the arrangement of the *viticultural climates* in *homogenous groups*. On the assembly of the eight viticultural regions of Romania, results a number of 13 climatic groups, which represents 11% from the total of the 120 groups theoretically possible. Given the fact that in reality, at the level of Geoviticulture have been identified 38 existent climatic groups (of the 120 possible groups), results that in those eight Romanian viticultural regions we find 34% of the climatic groups actually possible on Terra and approximately 60% of the climatic groups encountered at the European level.
2. From the calculation of the new criteria climatic indicators (the drought index, the heliothermic index and the index of night cooling), results on the territory of Romania a number of 13 climatic groups that are encountered in the eight existent regions, as it follows:
   - $IS_{00}\ IH_2\ IF_4$ – Transylvania Plateau, Moldavia Hills;
   - $IS_{00}\ IH_3\ IF_4$ - Transylvania Plateau, Muntenia and Oltenia Hills, Crisana and Maramures Hills;
   - $IS_0\ IH_3\ IF_4$ - Transylvania Plateau, Moldavia Hills, Muntenia and Oltenia Hills, Banat Hills, Crisana and Maramures Hills;
   - $IS_0\ IH_3\ IF_3$ - Banat Hills;
   - $IS_0\ IH_4\ IF_4$ - Muntenia and Oltenia Hills, Banat Hills, Crisana and Maramures Hills;
   - $IS_0\ IH_4\ IF_3$ - Muntenia and Oltenia Hills;
   - $IS_1\ IH_4\ IF_4$ - Moldavia Hills, Muntenia and Oltenia Hills;
   - $IS_1\ IH_3\ IF_4$ - Moldavia Hills, Muntenia and Oltenia Hills, Banat Hills, Danube Terraces, the sands and other favourable lands in the south of the country;
   - $IS_1\ IH_4\ IF_3$ - Muntenia and Oltenia Hills, Danube Terraces, the sands and other favourable lands in the south of the country;
   - $IS_2\ IH_3\ IF_3$ – Dobrogea Hillocks;
   - $IS_2\ IH_4\ IF_4$ - Moldavia Hills, Danube Terraces;
   - $IS_2\ IH_4\ IF_3$ - Moldavia Hills, Dobrogea Hillocks, Danube Terraces.

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Figures

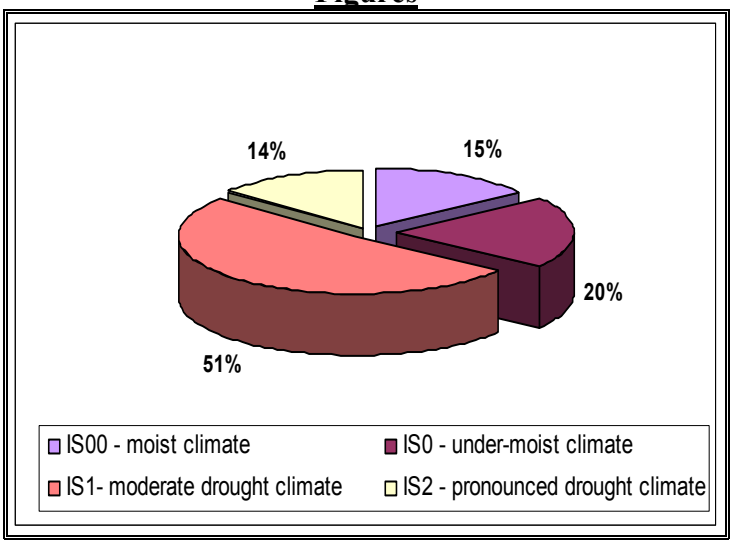

Fig. 1 – The viticultural climate according to IS

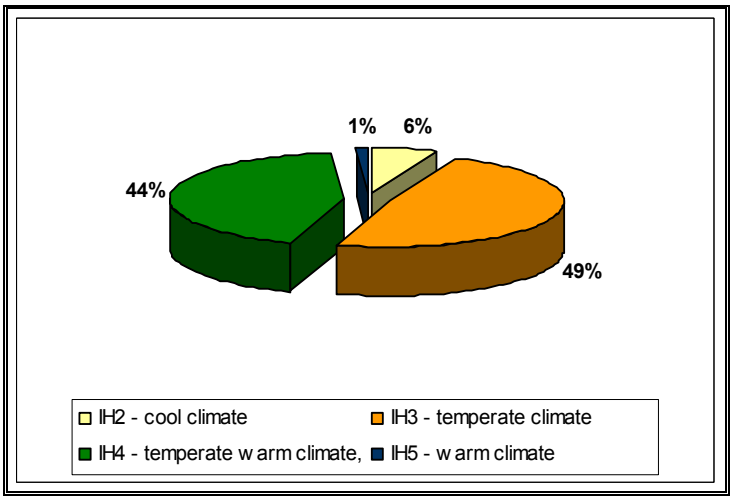

Fig. 2 – The viticultural climates according to IH

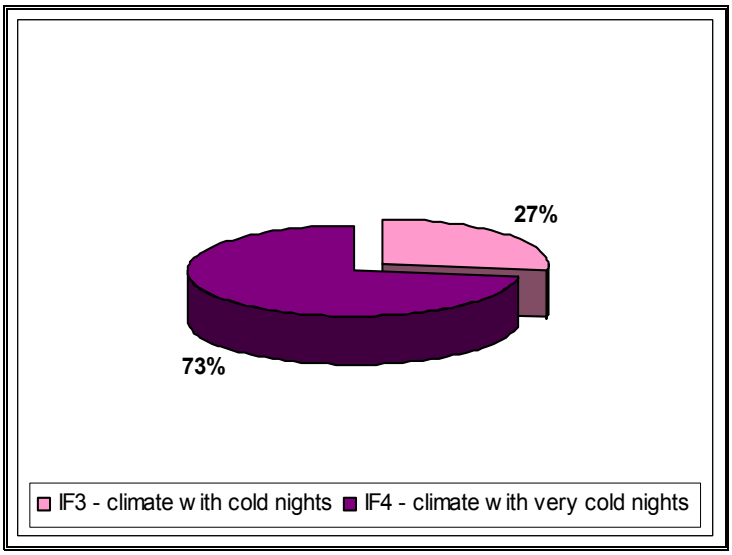

Fig. 3 – The viticultural climates according to IF

Studies regarding the elaboration of some instruments for the evaluation of the suitability of viticultural areas for ecological viticulture

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Keywords: ecological viticulture, Valea Călugărească

ABSTRACT

The paper proposes some instruments for the evaluation of the plots of land destined to the cultivation of vine in ecological system. The application of the proposed instruments is exemplified and discussed for some representative plots located in the viticultural centre of Valea Călugărească.

INTRODUCTION

The principles of ecological viticulture are based on a sound mastering of production systems which maximize the efficient use of local economical and ecological resources, integrating traditional knowledge and scientific progress. Each plantation is a complex system with specific and unique traits, which requires adequate solutions. From a technical viewpoint this involves the elaboration of certain flexible instruments for the evaluation of the ecological, climatic, soil-related, technological and social-economic conditions of each viticultural centre.

This paper aims to propose such an instrument, named environmental diagnosis, applicable to the evaluation of the suitability of viticultural areas for the implementation of ecological viticulture.

MATERIALS AND METHODS

The diagnosis requires three steps:

- selection of the evaluation criteria;
- acquiring the information and structuring the diagnosis;
- evaluation and interpretation of results, formulating the decisions.

The selection of the evaluation criteria takes into consideration the following:

1) the evaluation of the ecology-soil-climatic conditions in view of the requirements of the ecological viticulture system;
2) the evaluation of the effects of various technological parameters on the plants (planting density, training systems, health state, soil parameters etc.);
3) the evaluation of the social-economic potential.

From the viewpoint of the nature of information the diagnosis is structured in 4 chapters:

Chapter I) information regarding the initial state of the land and of the plantation which is going to be converted; these aids in establishing the decisions regarding the technological solutions to be chosen;

Chapter II) information regarding the ecological parameters of the site, many of them of essential importance for the suitability of the site for ecological viticulture;

Chapter III) environment-related aspects which must be considered in order to minimize any potential environmental risks which might arise as a result of the intended project;

Chapter IV) social-economic aspects which give us information on the human and material resources.

The evaluation of the performance of the area is made by comparing the results obtained using the evaluation criteria with the values considered optimal from an ecological point of view. The indicators which cannot be measured physically were granted marks on a scale from 1 to 10, where 1 means the criterium is failed and 10 mean the criteria is fully met (optimal). The larger the differences observed in this comparison of the evaluation results and the optimal situation, the more difficult and costly is presumed to be the implementation of ecological viticulture. If 80-100% of the indicators received maximum or close to maximum marks we can say that there is a significant potential for establishing an efficient ecological production.

RESULTS AND DISCUSSIONS

For demonstration purposes the diagnosis procedure was tested using the information gathered for certain representative lots from the Valea Călugărească viticultural centre.

Tables 1-4 contain information regarding the state of the plantations, the ecology-soil-climate conditions, the environment, social-economic data – presented in comparison with the requirements of viticulture. The tables also include the marks obtained after evaluation, observations and recommendations.

CONCLUSIONS

1. Prior to the elaboration of projects for setting up an ecological plantation or converting towards ecological production it is necessary to evaluate the site from the viewpoint of the ecology-soil-climate, technological and social-economic potential.
2. The evaluation indicators can be adapted according to specific traits of each area, bearing in mind the principle “as ecological as possible”.
3. All indicators have the same maximum value (no indicator is considered less important).
4. The evaluators must be informed and objective.
5. The ecologic potential of the viticultural centre Valea Călugărească is very good; there is a need, however, of technological intervention and substantial economic input (investments).

Tables

Table 1. Indicators regarding the initial state of the plantation

No.	Indicator	Specifications		Mark	Observations and recommendations
		Optimal	Actual		
Chapter I – Initial state of the land and of the plantation which is to be converted (state indicators)					
1	Nature of the Vinifera varieties cultivated	Certified biologic material*, resistant varieties or varieties with increased resistance	Variety with increased resistance (Cabernet Sauvignon)	8	Varieties with weak resistance are difficult to protect from diseases; use of uncertified material to create new plantations infringes on the legislation in force.
2	Rootstock	Adapted to the area	K 5 BB, vigurous, cosmopolite variety	5	It does have the biological capability of fully exploiting the ecological conditions.
3	Planting density	Max 5000 vines/ha	5000	10	Higher densities create shading and increase the danger of infection.
4	No. of lost vines	Less than 5%	> 15 %	0	Significant decrease of production.
5	Training system	Semi-high	Semi-high training (not well-mainteined)	5	Contributes to the creation of a favourable microclimate
6	Soil management	Winter cover crops, permanent cover crops, bare soil	Bare soil	10	Will be differentiated based on soil structure and fertility and water availability. The cover corps protect from erosion and may improve the soil features.
7	Vegetation state of plants	Very good, good	Good	8	Allows the optimization of vegetation structure.
8	Plantation health state	Very good, good	Good (bacterian cancer, Eutipa present)	8	Good health state allows the reduction of phytosanitary treatments
9	Plantation yield	According to the ecological potential of the variety	Good	10	Allows the optimization of the production level
10	Surfaces with cover crops (alleys, grassy lands, bushes, etc.)	5% of the active area	Very good	10	Ensures an optimum ratio of useful/damaging phyto-fauna
TOTAL mark: **74**					
Maximum mark: **100**					
% of the maximum mark: **74 %**					

Table 2. Indicators regarding the ecological-soil-climate conditions

No.	Indicator	Specifications		Mark	Observations
		Optimal	**Actual**		
Chapter II. information regarding the ecological-soil-climate parameters					
1	Frequency of harmful low temperatures (-20 … -22°C)	1/10 for unprotected cultures; 2/10 for semi-protected cultures; 3/10 for protected cultures)	One in ten years presents temperatures below -20°C	10	Increase of the sensitivity of rootstocks to diseases; requires improvements in training systems etc.
2	Frequency of low temperatures in the flowering periode <15-17°C	1/10	In one year in ten there are late frosts	10	Significant production loss; resistant varieties must be selected and the varieties with a tendency for millerandge and colour avoided.
3	Frequency of hail	NO	Annual frequency requires crop insurance	10	Biological and financial adverse effects.
4	Landslides	NO	No landslides	10	Surface and deep antierosion works are required.
5	Land slope	< 15 %	< 15 %	10	Eliminates need of massive earth moving works at plantation time and decreases costs
6	Land exposure	S, E and in between	S-E	10	Positive effects on energy input and quality.
7	Length of bioactive period	Minimum 160 days	172 days	10	Avoids late frosts in spring and early frosts in autumn.
8	Helio-thermic index	>1.3	2.53	10	Allows grape maturation.
9	Bio-climatic index	> 4		10	Values under 4-6 mean low sunlight and thermal resources
10	Oenoclimatic index	>3600°C	4740	10	It is a quality index.
11	Hydro-thermal coefficient	>0.7-0,8; <1,8	1.0	10	Values lower than 0.7-0.8 require irrigation; values over 1.8 indicate excessive humidity.
12	Humidity in the upper layer of soil profile	Nt present	Not present	10	Such humidity would lead to root asphyxiation; draining works required.
13	Content of soluble salts	Within the tolerance of grapevine; rootstocks: 0.2-0.5 ‰; Vinifera 1.5-40 ‰	Within limits	10	Limits are set depending on the soil sensitivity and checked by analysis.
14	Amount of nitrogen in soil	<50 kg/ha/year	Within limits	10	Determined by analysis. Larger amounts lead to longer conversion periods; adapted fertilization programs required, to help diminish the nitrogen content by washing it away.
15	Amount of phosphorus in soil	For correction, 0-100/kg/ha based on analysis; For maintenance 0-30/kg/ha (probably rarely used)	Within limits	10	Determined by analysis. Larger amounts lead to longer conversion periods; adapted fertilization programs required, to help diminish the phosphorus.
16	Amount of potassium in soil	For correction 250-500 kg/ha based on analysis. For maintenance 0-120 kg/ha (probably rarely used)	Within limits	10	Determined by analysis. Larger amounts lead to longer conversion periods; adapted fertilization programs required, to help diminish the phosphorus.
TOTAL Mark: **160**					
Maximum mark: **160**					
% of the maximum mark: **100**					

Table 3. Indicators regarding environmental aspects

No.	Indicator	Specifications		Mark	Observations
		Optimal	Actual		
Chapter III. Environmental aspects					
1	Presence of pesticide residues in soil	Under allowed limits	Not present	10	Determinations and comparison to allowed limits. If limits are exceeded and there are pesticides which decompose very slowly, the site may need to be abandoned.
2	Presence of polution risks generated by adjacent activities in the farm (incorrect storage and management of pesticides and herbicides etc.)	No risk of polution (according to legislation)	No risk	10	The plantation must avoid the proximity of urban areas, motor roads or industrial areas. The plot must be away from any source of polution.
3	The management of package materials, waste materials and waste waters	According to the legislation in force	There is no waste manage-ment contract	0	These can represent a main polution source.
TOTAL mark: 20					
Maximum mark: **30**					
% of the maximum mark: **67 %**					

Table 4. Indicators regarding social-economic aspects

No.	Indicator	Specifications		Mark	Observations
		Optimal	Actual		
1	Level of technical endowment	Good, professional facilities with low energy consumption	Old, energy consuming	0	Energy consumption must be reduced.
2	Possibility of obtaining loans	Yes	No	0	Guaranty that the production process can be sustained.
3	Possibility of insuring the plantation	Yes	Yes	10	
4	Possibility of providing specialized labour force (% persons working in agriculture)	Specialized personnel, trained continuosly	Seasonal personnel	5	Guaranty that the work is done correctly
5	Access la to information sources	Yes	Yes	10	Efficiency, progress
6	Delivery market	Yes	Yes	10	A feasibility study is required.
TOTAL Mark: 25					
Maximum mark: **60**					
% of the maximum mark: **42**					

Figure

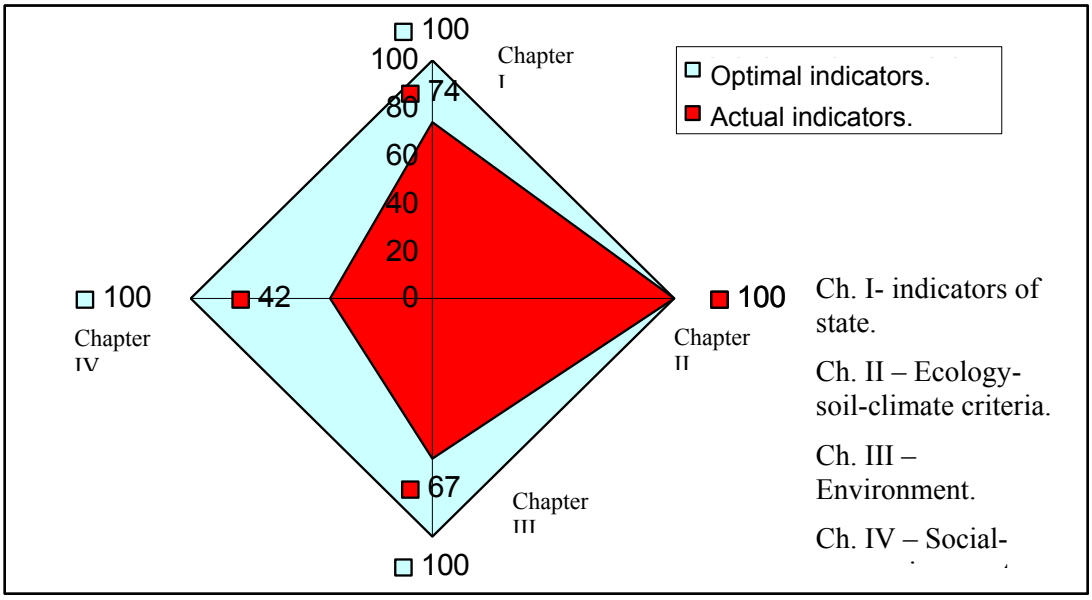

Fig 1. Graphic representation of the results of the evaluation criteria for ecological viticulture in the viticultural center Valea Călugărească.

The implications of globalisation on Romanian viticulture

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Keywords: globalisation, wine market, quality, world trade, tendencies, wine offer

ABSTRACT

The acceptance of Romania in the European Union, in 2007, the alignment at the communitarian legislation and standards, within this domain, imply a continuous preoccupation for maintaining the viticultural heritage, improvement of the quality, diversification of the production, satisfaction of the consumers' requests, ensuring the durability of the sector. If 30 years ago the international trade affected 15% of the global wine production, during the past years this percent has been exceeding 33%. The changes which have occurred lately on the wine market, which is very complex, in the framework of the international competition, which is stronger and stronger, are mainly influencing the viticultural sector in Romania. Romania has a privileged position in the viticultural countries; taking into consideration the surface cultivated with vines (192 600 ha), Romania occupies the 5th position in Europe and the 9th in the global hierarchy; taking into consideration the yearly wine production (between 4,9 and 6,1 millions hectolitres), Romania occupies the 12th position in the world and the 6th position in Europe. As main objectives, Romania has to improve its range structures of the plantations, increasing of the high quality production (the wines with denomination of controlled origin - DOC is representing only 10-12% from the total, compared to over 44% in the European Union), the development of the exports (which are very little our days, with a total value of only 4-8% from the total production).

INTRODUCTION

The evolution of world viticulture depends very much not only on the current organization, historical, cultural, scientific, social, religious influences but also on the globalization phenomenon.

The globalization, that lately affects the vine sector, is defined by the growing circulation of the capital, merchandise, services, persons, as well as the information and technological knowledge.

The intense exchanges of technological information in the entire viticulture world are knows, as well as the launching of great companies specialized in the production and commercialization of wines (Bisson et al., 2002; Silverman et al., 2003; Coelho and Rastoin, 2004; Rebelo et al., 2007).

The globalization phenomenon has been perceptible for about two decades in the vine and wine sector. Step by step, market structures in which the request and offer are concentrated on the traditional producing countries (France, Spain, Italy, etc.) have been replaced by an increased extension of the production and consume areas, with the apparition of new offertant (especially in the south) and solicitors (especially in the northern regions).

MATERIALS AND METHODS

This paper intends to analyze the request and offer on the very complex market of wine, the mutations that took place lately and Romania's place as a country of the European Union in the context of a more and more intense international competition.

RESULTS AND DISCUSSIONS

Tendencies on the international market of wine. The world wine request in the latest 40 years knew four stages: a significant increase between 1965 and 1975 (with 20%), due, mainly, to the forming of European common market, followed by a

stagnation until 1985, then a decrease until 1995 determined by the reduction of consumption per inhabitant in the European countries (Rastoin et al., 2006). In the last 10 years there has been a slow increase until 240 million hl in 2006.

The consumption decreases in the traditional producing countries and increases in the new consumer countries (North America, Asia).

About 90% of the world consumption of wine is insured by 25% of the world population, a number of 15 countries insuring 80% of the market. This proves that there is a significant increasing potential.

The wine offer depends on the cultivated surfaces and the obtained productions, with particularities related to the perennial character of the grapevine, on the multitude of kinds existing in the culture, on the applied technologies, on the regulations in the field, on the investors and companies' behaviour.

The world vineyard surface decreased with 25% in the last 30 years, to stabilize to 7.9 million ha, as a result of the decrease of the European Union's surface (with 34%), while, beginning with the 90', the countries in the New World adopted strategic programs for the vine production development.

The world wine production reached 280 million hl in 2006, under the conditions of a medium grapes production of 7 t/ha in the European Union, while in the New World it is close to 10 t/ha.

The maintenance of grape productions on a smaller level through strict regulations in the European Union determined not only the expansion of the New World on the markets in Great Britain, USA, northern countries, but also the extension of international trade, the volume of the exports increasing to more than 70% in the last 20 years, fact that constitutes one of the essential factors of the sector's globalization (O.I.V., 2007).

As a result of the geographic distribution of vine production in different regions of the world favourable to this culture, **the world trade** (wine import and export) intensified in the last decades, reaching 34.5% of the production of 2006, comparing to 18% at the beginning of 80'.

On international level **two tendencies** are noticed: the European one that grants more attention to quality, the capitalization of "terroir", with an adequate enactment and a limitation of the new plantings (Failla and Scienza, 2006) and the one of the "New World" (Australia, USA, South Africa, Chile, Argentina, New Zeeland), where the rhythm of plantings is high, the quality of the wines is corresponding, and the wine export from these countries affects, year by year, the European supremacy.

This tart competition on the world market led lately to harsh disputes regarding the profound reform of the common organization of the wine market.

The countries in the New World are the basis of a wine homogenization on international level, which manifests through the precedence choice, the vineyard establishment on plane fields, without vine vocation, the extension of irrigation through dripping, fertilization, the use of new training systems, the extension of mechanization, etc, leading to the increase of production.

To this, the extension on large scale of international varieties: Merlot (that today is placed in the world on a surface of more than 200 000 ha); Cabernet Sauvignon (160 000 ha); Syrah (65 000 ha); Pinot noir (60 000 ha); Chardonnay (130 000 ha) and Sauvignon (45 000 ha), which contributed to the unification of the wine quality, is added (Fregoni, 2005).

The wine preparation techniques also contributed to the unification of chemical and organoleptic characteristics of wines with weak aging aptitudes, with uniform flavours (especially in the case of using barique and then, the "chips"). This way, the prices are more accessible, as a result of the smaller production costs, especially in the New World.

Romanian's Viticulture and Vinification in the world and European contexts. The Romanian viticulture benefits from some important aces up the sleeve: the existence of a long tradition; the diversity of favourable pedo-climatic conditions; a valuable array of varieties, adapted to our culture conditions, allowing the attainment of diverse wine products, of high quality; the wine is associated to the art of life, using the local gastronomy; the existence of a great number of specialists in this domain, etc.

In the same time, we must consider the handicaps that negatively influence the wine preparation sector; the old plantations; the slow rhythm of new plantations; the great surface occupied by hybrids directly producing; financial and organizational capacity that is too weak; accentuated breakage of properties; the predominance of small exploitations; too long process of decision making; the loss of traditional export markets etc.

Considering the surface cultivated with grapevine, Romania stands on a privileged position among the wine producing countries, being on the 5th place in Europe and 10th in the world, holding 5% of the vine surface of the European Union (table 1).

Considering the annual wine production (between 5.5 and 6.1 million hl), our country stands on the 12th place in the world hierarchy and on the 6th place in the European Union (table 2), insuring 3% of its total wine production.

Concerning the vineyard on production area with table grapes it was reduced to half in the last 17 years; from a surface of 25 900 ha, in 1990, in 2006 there were only 12 578 ha (table 3).

A concerning situation is the surface of vineyard new established, that do not insure the annual rhythm of replanting of approximately 3.5% necessary for keeping the current national vine patrimony. With new planted surfaces, of up to 859 ha (in 2006), the annual rhythm for replanting is of approximately 7 times less (0.5%).

In the last years, Romania produced between 5 and 6 million wine hl; of this quantity, the share of the ones with controlled origin denomination (DOC) is of 9-12%, the ones with geographical indication (IG) 12-15%, the rest (over 75%) being represented by table wines (VM).

Romania exports annually a quantity of wine between 376 000 hl (2004) and 503 000 hl (2002), that represents between 4 and 10% of the annual production, compared to 33-34% on world level. Regarding the wine exports, our country is on the 20th place in the world hierarchy and on the 10th place within the European Union.

The main destination countries for Romanian wine exports are Germany, Russia, USA, The Republic of Moldova and Great Britain.

Appreciating the share of the wine export of the total production of the states of the European Union (table 4), Romania doesn't have quite a favourable situation, being overcome by almost all the wine productive countries except Czech Republic.

In figure 1 the annual exports of Romanian wines are presented in the interval 1991 – 2006.

Furthermore, in the first half of 2007, our country exported 86 200 hl at a medium price of 1.04 euro/litre and imported 210 000 hl at a medium price of 0.65 euro/litre.

Figure 2 presents the evolution of wine imports performed by our country, with intensification in the last years.

The main states from which we imported wine in the last years are Italy, Spain, France, Moldavia and Germany.

The Romanian wine market is assessed at about 450 million euro. The main producers are: Murfatlar (with a market share of 27.8%), Jidvei (14.5%), Cotnari (13.1%), Vincon (7.8%), Recas (3.1%), holding together 66.3% of the wine market.

It is interesting that many Romanian producing companies reconvert on imports of wine to increase profits. The massive penetration of imports may affect the small producers and the exports will be troubled by the imports getting cheaper and cheaper.

CONCLUSIONS

Considering the irrefutable importance of the international market in the wine products consumption, the complexity of the adaptation to globalization process, as well as the avoidance of registering some disastrous effects of this phenomenon over the Romanian wine production sector we need to urgent the following measures:

1. improving the quality of products, increasing the share of wines with controlled origin denomination (DOC) and the ones with geographic indication, to the detriment of the table wines;
2. replanting of table grapes varieties;
3. restructure of directly producing hybrid plantations and the plantations destined to obtaining table wines, as well as their conversion by promoting the valuable Romanian varieties and the ones requested by the market;
4. the increase of competitiveness of the sector, reduction of production costs;
5. maximizing the efficiency of anthropic interventions;
6. promoting the large dimensions exploitations or developing a cooperative system;
7. a commercial aggressiveness more powerful than the companies';
8. promotion and information campaigns, integrated in a communication based on the benefits for health through moderate and regulate consumption of wine;
9. transmitting to the market a prestige image corresponding to our tradition and culture, history and geography.

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Tables

Table 1 – The main wine producing countries of the world (after International Organization for Vine and Wine)

No.	Country	Surface (thousands ha):					
		1986 – 1990	**1991 – 1995**	**1996 – 2000**	**2002**	**2003**	**2004**
1.	Spain	1 506	1 290	1 184	1 202	1 207	1 200
2.	France	996	940	915	898	888	889
3.	Italy	1 063	985	909	872	862	849
4.	Turkey	636	615	584	570	570	570
5.	China	148	153	218	421	455	471
6.	U.S.A.	329	331	376	415	415	398
7.	Iran	228	244	274	302	300	296
8.	Portugal	282	269	257	251	250	247
9.	**Romania**	244	251	253	243	239	222

Table 2 – Wine production in the main wine producing countries (after International Organisation for Vine and Wine)

No.	Country	Wine production (milion hl):					
		1986 – 1990	**1991 – 1995**	**1996 – 2000**	**2002**	**2003**	**2004**
1.	France	64 641	52 886	56 271	50 353	46 360	57 386
2.	Italy	65 715	60 768	54 386	44 604	44 086	53 000
3.	Spain	33 519	26 438	34 162	33 478	41 843	42 988
4.	U.S.A.	18 167	17 619	20 386	20 300	19 500	20 109
5.	Argentina	19 919	15 588	13 456	12 695	13 225	15 464
6.	Australia	4 285	4 810	7 380	11 509	10 194	13 811
7.	China	2 734	5 140	9 581	11 200	11 600	11 700
8.	Germany	10 012	10 391	9 989	9 885	8 191	10 047
9.	South Africa	7 742	8 228	7 837	7 189	8 853	9 279
10.	Portugal	8 455	7 276	6 828	6 677	7 340	7 481
11.	Chile	4 135	3 326	5 066	5 623	6 682	6 301
12.	**Romania**	7 133	5 529	6 173	5 461	5 555	6 166

Table 3 – The situation of Romanian viticulture
(after the Statistic Yearbook of Romania, MADR)

Specification	2003	2004	2005	2006
Total viticulture surface (ha)	239351	193150	199566	192611
Vines in production - total (ha)	238300	191821	190556	189663
from which: - for wine	223870	178458	177743	177085
- for table grapes	14430	13363	12813	12578
Vines not in production – total (ha)	1051	1329	1000	2948
from which: - for wine	1023	1270	952	2857
- for table grapes	28	59	48	91
from which: - new plantings	245	144	223	859
Wine production (milion hl)	5.555	6.166	2.602	5.014
from which: - DOC	0.443 (8.0%)	0.560 (9.1%)	0.404 (15.5%)	0.615 (12.3%)
- IG (VS)	0.847 (15.2%)	0.871 (14.1%)	0.326 (12.5%)	0.601 (12.0%)
- VM	4.265 (76.8%)	4.734 (76.8%)	1.872 (72.0%)	3.797 (75.7%)
from which: - white wines	3.037 (54.7%)	3.216 (52.2%)	1.518 (58.3%)	2.861 (57.1%)
- red wines	2.518 (45.3%)	2.950 (47.8%)	1.084 (41.7%)	2.153 (42.9%)

Table 4 – The share of wine export in total production of wine producing countries in the European Union (after International Organisation for Vine and Wine)

No.	Country	Share (%)							
		1986 – 1990	1991 – 1995	1996 – 2000	2002	2003	2004	2005	2006
1.	France	20	22	27	31	33	25	27	28
2.	Italy	19	25	26	34	29	27	30	33
3.	Spain	14	28	26	29	30	33	41	35
4.	Portugal	18	27	31	32	43	43	39	41
5.	Germany	27	26	23	24	34	27	32	36
6.	Bulgary	41	36	48	40	33	46		
7.	Austria	4	8	9	15	27	27		
8.	Hungary	16	26	23	23	18	10		
9.	**Romania**	6	4	8	9	7	6	10	4
10.	Cyprus	42	75	16	26	28	15		
11.	Slovakia		18	19	35	24	14		
12.	Slovenia		22	17	22	9	10		
13.	Cehia		3	3	6	4	6		

Figures

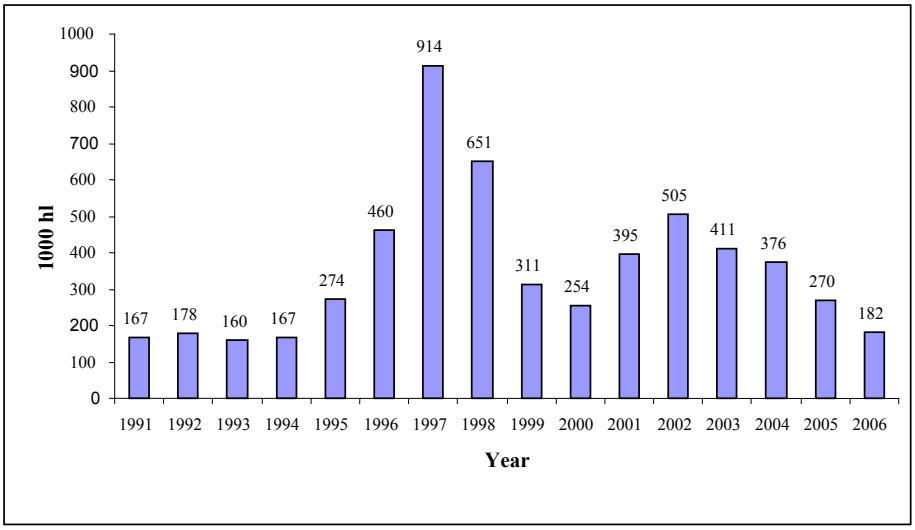

Fig. 1 – Romanian wines exports, 1991 – 2006

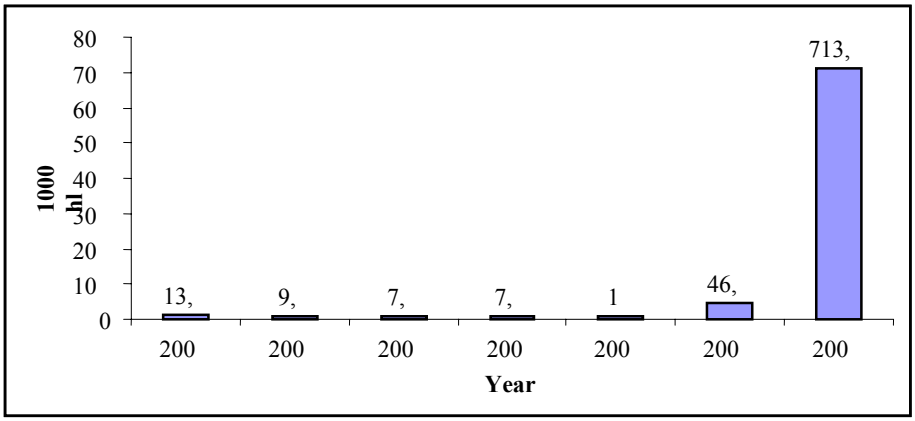

Fig. 2 – Romania's wine import, 2000 – 2006

The effectiveness of weed control measures applied in a plantation with table grape varieties

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Keywords: *chemical weed control, manual chemical control, control extent, Muscat de Hamburg, Chasselas doré*

ABSTRACT

The researches were performed during the experimental years 2002 and 2004 considering some table grape varieties: Chaselas doré and Muscat de Hamburg in order to select the best methods to control weed species from vineyards. The applied methods were represented by chemical methods (weed control) and agrotechnical methods (manual practices). The experiences have demonstrated that best results were obtained by combining herbicides with manual practices.

INTRODUCTION

Weeds create a microclimate favourable for development on grape vine varieties of numerous diseases like powdery mildew, grey mould and downy mildew. In this regard, in a weed covered vineyard, downy mildew on grapes registers rates of 34% comparing with vineyards where soil is maintained by manual and mechanical weed control. In addition, weeds synthesize toxic compounds with negative influences on development of root system of grape vines (Bernaz et. al. 1970).

Integrated weed control from grape vine plantations aims the reduction of labour volume in agricultural sector. The integrated weed control from grape vine plantations aims the reduction of labour volume in agriculture. Weed elimination from grape vine plantations may be diversely achieved using different methods (Carciu, 2004; Lăzureanu, 2002)

The performed researches have demonstrated that the extent of weed cover decreased below 10 % in case of combined use of a systemic product (Gesatop 50, Caragard) with amythrol-based therapeutic product or contact product (Gramoxone). (Bernaz Gh. et. al., 1976).

The above mentioned authors have concluded that applied herbicides have neither negative influences on grape vine growth and fructification nor on grape yields and their quality.

The knowledge regarding the weed extent in grape vine plantations is attained by weed mapping in vineyards performed the period May-July. Weeds are divided in two large categories: monocotyledonous and dicotyledonous species (Chirilă et al., 1998).

Weed control on grape vine plantations represent a permanent concern for viticulturists.

MATERIAL AND METHODS

Our experience has been focused on weed control using agro-technical measures (manual control measures) and chemical control (herbicides) separately for Muscat de Hamburg and Chasselas doré varieties considering the experimental period 2002-2004.

Table grape varieties were placed at Didactic Station of USAMVB Timisoara. The crop system is of unprotected type, on semi-trunk with planting distances 2 m between rows and 1,2 m between plants per row resulting a total density of 4166 vines/ha.

Manual control measures used to discard monocotyledonous and dicotyledonous weeds were differently performed for each row of studied grape varieties. These measures had also the role of soil aeration and superficial tillage.

The doses of herbicide were established in terms of weed extent and their application was done using a portable spraying device of "Vermorel" type. The selected herbicides were post-emergently applied, considering lanes of 30 cm wide on each side of vine rows while determination of weed extent was performed using numeric quantitative method. The measurements were made for each experimental variant one day prior to herbicide's treatment and 30 days after their application.

The experience was of single factor type arranged according to randomized block design with 10 variants in 4 replications. The experimental variants in case of this study were: V_1 – without herbicides and manual control; V_2 - Touchdown (4 l/ha); V_3 – Roundup CS (3 l/ha); V_4 – Basta 14 SL (4 l/ha); V_5 – Gallant super (1,5 l/ha) + 2 manual weed controls; V_6 – Fusilade super EC (4 l/ha) + 2 manual weed controls; V_7 – Goal 2 E-RV (5 l/ha) + 2 manual weed controls; V_8 – 4 manual weed controls; V_9 – 3 manual weed controls; V_{10} - 2 manual weed controls.

RESULTS AND DISCUSSION

During the experimental year 2002, the extent of weed control for Muscat de Hamburg variety was comprised between 61.52% (2 manual controls) and 98.25% (Gallant super - 1,5 l/ha + 2 weed controls). Efficient weed controls are attained also by herbicides like Fusilade super EC (4 l/ha) and Goal 2 E-RV (5 l/ha)associated with two manual controls, the extent of weed control registering rates of 94.36% and 93.16%, respectively.

Precipitation deficit registered for the experimental year 2003 favoured the effectiveness of manual weed control. The extent of weed control ranged between 60, 84% and 97.12%. The variant including 4 manual controls insured levels of weed control of 97.12%. Control rates exceeding 90% were registered by the variants where Fusilade super EC (4 l/Ha) and Gallant super (1,5 l/ha) were applied and associated with 2 manual controls. The variant with 3 manual weed controls proved its efficiency insuring control rates for weeds of the 84, 56%.

High weed level registered for the experimental year 2004 generated lower control rates comparatively with previous experimental year, ranging between 59, 36% and 92.93%. Also for this year, the best effectiveness regarding weed reduction is obtained for variants where herbicides are associated with two manual controls, the control proportion ranging between 89.39% and 92.93%. The variants with manual weed control attained lower extents of weed control (below 85%) due to the large amount of precipitation that favoured fast weed spreading.

Analyzing the mean of all three research years, it has been confirmed once again that best results concerning weed control are obtained by combining herbicides with manual controls, the control rate being comprised between 91.53% (Goal 2 E-RV – 5 l/ha + 2 manual weed controls) and 92.36% (Fusilade super EC 4 l/ha + 2 manual weed controls) (table 1 and figure 1.).

Weed extent was higher in case of Chasselas doré variety that indicated a reduced rate concerning weed control.

In the year 2002, as a result of applying weed control measures, it has been reached control levels comprised between 60, 63% and 97.12%. The variant Gallant super (1, 5 l/ha) + 2 manual weed controls prove to be most efficient with control rate of 97.12%. The number of three or four manual controls attained control rates of 87.36% and 83, 65%, respectively. Among all there herbicides with total action, Roundup CS (3 l/ha) proved to be most effective with control rates of 80.27%.

In 2003, the variant with 4 manual controls attained best weed controls with values reaching 95.78%. The variant with 3 manual controls insured weed control rates of 83.52%, while the variant with 2 manual controls provided rates of only 60.03%. Systemic herbicides (Goal 2E-RV, Fusilade super and Gallant super) associated with 2 manual weed controls insured control rates between 87.47% and 92.36%.

In the year 2004, it is registered the most reduced weed control as a consequence of a high weed spreading. The extent of weed control registered in this case values between 58.07% and 90.35%. The association of systemic herbicides with 2 manual weed controls provided the best control comprised between 58.07% and 90, 35%. The variant with 3 and 4 manual controls insured weed control extents of 78.02% and 84.47%, respectively.

Analyzing the mean of all three research years (2002-2004), we have concluded that best results were obtained when weed control is associated with chemical or agro-technical methods, these being materialized on weed extents ranging between 88.96% and 92.11%.

Manual weed controls attained close values, the differences being due to an additional manual control in case of variant with 4 manual weed controls 88.91% comparing with 81,42% in case of three manual practices. Contact herbicides with total action (Basta 14 SL – 4 l/ha, Touchdown – 4 l/ha and Roundup CS – 3 l/ha) generated weed control rates between 69.30% and 78.67%, respectively. The variant with 2 manual controls proved to be the least efficient with registered values of 59, 4% (table 2. and figure 2).

CONCLUSIONS

1. Taking into account the high extent of weeds in the studied region, it is recommended to perform weed control using combined methods with herbicides and manual controls;
2. In case of Chasselas doré variety the best results during the research years (2002-2004) concerning weed control was materialized on control rates of 88.96% and 92.11%.
3. The most efficient weed control for Muscat de Hamburg variety was comprised between 91.53% (Goal 2 E-RV – 5 l/ha + 2 manual weed controls) and 92.36% (Fusilade super EC 4 l/ha + 2 manual weed controls).
4. The highest percentage of uncontrolled weeds for both table grape varieties has been registered for the variant including only 2 manual controls.
5. Among herbicides used to control weeds from grape vine plantations, the mostly recommended are: Fusilade super – 4 l/ha, Gallant super EC – 1,5 l/ha and Goal 2 E-RV - 5 l/ha;

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Tables

Table 1. Influence of control measures on the extent of weed spreading, in case of Muscat de Hamburg variety, mean of years 2002-2004

Variant	Number of weeds/m^2	Controlled number of weeds//m^2 comparing the control variant	Extent of weed control (%)	Significance of differences
V_6- Fusilade super EC (4 l/ha) + 2 manual weed controls	11,14	134,67	92,36	***
V_5-Gallant super (1,5 l/ha) + 2 manual weed controls	11,30	134,51	92,25	***
V_7- Goal 2 E-RV (5 l/ha) + 2 manual weed controls	12,35	133,46	91,53	***
V_8- 4 manual weed controls	16,39	129,42	88,76	***
V_3- Roundup CS (3 l/ha)	29,16	116,65	80,00	***
V_9 - 3 manual weed controls	30,89	114,92	78,81	***
V_2- Touchdown (4 l/ha)	32,30	113,51	77,85	***
V_4- Basta 14 SL (4 l/ha)	43,15	102,66	70,41	***
V_{10} – 2 manual weed controls	57,66	88,15	60,46	***
V_1 – without herbicides and manual weed control	145,81	Control variant	0,00	-

DL5% = 4,32 weeds/m2
DL1% = 5,84 weeds/m2
DL0,1% = 7,78 weeds/m2

Table 2. Influence of control measures on extent of weed spreading in case of Chasselas doré variety, mean of years 2002 – 2004

Variant	Number of weeds/m^2	Controlled number of weeds//m^2 comparing the control variant	Extent of weed control (%)	Significance of differences
V_5- Gallant super (1,5 l/ha) + 2 manual weed controls	12,19	142,23	92,11	***
V_6- Fusilade super EC (4 l/ha)+2 manual weed controls	13,83	140,59	91,04	***
V_7- Goal 2 E-RV (5 l/ha) + 2 manual weed controls	17,05	137,37	88,96	***
V_8- 4 manual weed controls	17,12	137,30	88,91	***
V_9 - 3 manual weed controls	28,69	125,73	81,42	***
V_3- Roundup CS (3 l/ha)	32,94	121,48	78,67	***
V_2- Touchdown (4 l/ha)	38,25	116,17	75,23	***
V_4- Basta 14 SL (4 l/ha)	47,41	107,01	69,30	***
V_{10} – 2 manual weed controls	32,63	91,79	59,44	***
V_1 – without herbicides and manual weed control	154,42	Control variant	0,00	-

DL5% = 5,08 weeds/m2
DL1% = 6,86 weeds/m2
DL0,1% = 9,15 weeds/m2

Figures

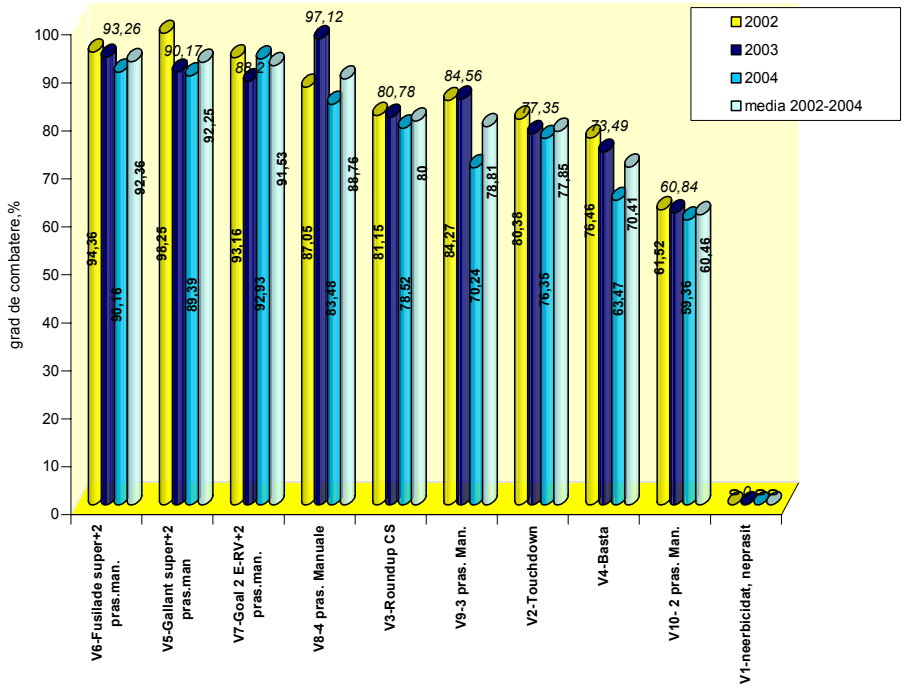

Fig. 1. The extent of weed control in case of Muscat de Hamburg variety

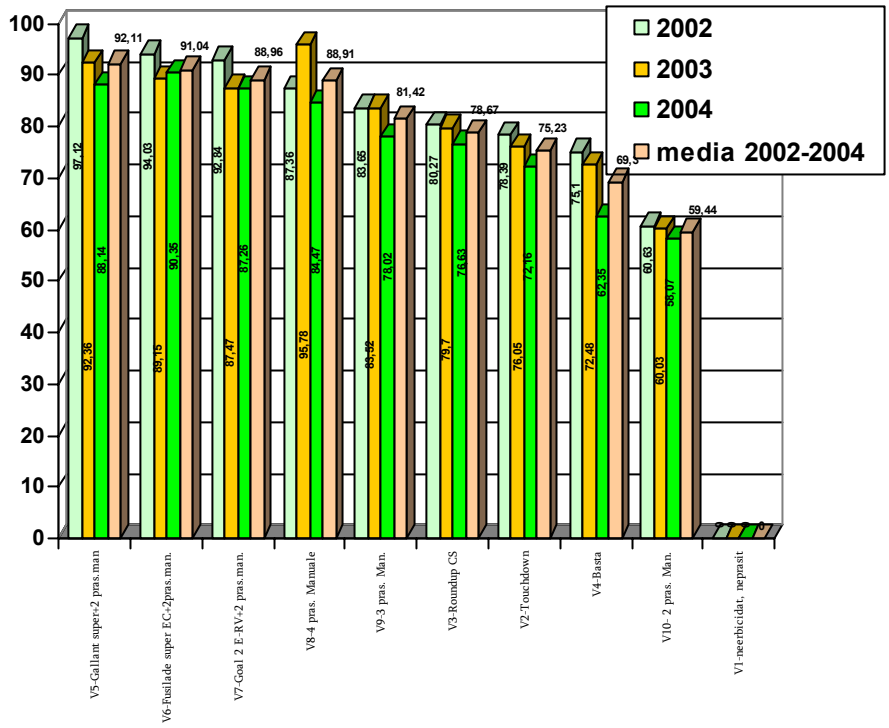

Fig.2. The extent of weed control in case of Chasselas doré variety

Exploitation of ecological resources by managing the processes which influence the quantity and quality of grape production

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Keywords: indicators of the vegetal/production balance, ecological viticulture

ABSTRACT

The optimization of fruit loading of the Cabernet Sauvignon variety was attempted in the viticultural center Pietroasa, in order to ensure efficient functional relationships in the given microclimate and to reduce the incidence of disease outbreaks under the conditions of ecological viticulture.Measurements were made regarding the productivity of exposed foliar surfaces, grape production and its quality, the state of health of the plantation and crop.The characteristics of the foliar surfaces allow for the exploitation of ecological resources, also influencing the quantity and quality of production. From the viewpoint of ecological viticulture often a reduction of the vegetative mass is achieved in order to provide a favourable microclimate, to maintain the health state of grapes, while ensuring a balance between the foliar surface and the grape production. From the results of the research it appears that the ecological system for fighting diseases ensures a healthy crop, very little diminished in quantity due to the lower bud loading on each vine, but superior in quality.

INTRODUCTION

The principles of ecologic agriculture are based on the detailed knowledge of the production systems that capitalize ecological resources to the maximum, integrating traditional know-how with scientific progress. Each agricultural holding constitutes a complex system, a self-supporting organism which requires adequate solutions.

The orientations of Romanian viticulture regarding the obtaining of ecologic products have led to research in viticultural centers such as Valea Călugărească, Pietroasa and Odobeşti, which are known for their special eco-pedo-climatic offer and for the high class wines obtained. The purpose of the present research is to verify and put into practice the principles of ecologic viticulture within a CEEX project.

Since a good quality production is obtained by achieving a balance between the foliar surface and the production of grapes, within the Pietroasa viticultural center an attempt was made to optimize the fruit vintage, by assuring useful functional relationships in the given microclimate which can contribute to reducing the incidence of diseases in the conditions of ecologic viticulture.

In the year 2006, within the Pietroasa center an experimental plot was set up in a plantation of Cabernet Sauvignon variety, where two culture systems were monitored: ecologic and conventional.

The factors considered were: the conditions of the vintage year, the fruit load with three graduations (16, 24, 40 buds/vine stock) and the phyto-sanitary system with two graduations (ecologic and conventional protection system). The determinations performed took into consideration the productivity of the exposed foliar surface, the grape production and its quality, the health condition of the plantation and the vintage.

MATERIALS AND METHODS

The research was carried out in the period 2006-2007 within the experimental plot set up in a plantation of Cabernet Sauvignon fifteen years old, with planting distances of 2.0/1.2 m, on a proluvial coluvial soil, medium humificated, carbonated and layered. Leading form – semi-trunk, clipping type - Guyot on semi-trunk.

The experimental plot comprised the following variable factors:

Factor A. – year of culture: a1– 2006; a2 – 2007

Factor B – grapevine protection system: b1– ecological; b2 – conventional.

Factor C – the number of buds per vine stock: c1 – 16 buds/vine stock; c2 – 24 buds/vine stock; c3 – 40 buds/vine stock.

The climatic conditions in the experimental period, which influence the development of plants and pest agents, may be characterized as follows:

In September 2005, after a cold and rainy viticultural season, the weather improved in terms of temperatures and rain. The month of October was especially warm. Then there was an alternance of very high temperatures, with waves of cold and extremely low temperatures, characteristic to the months of January, February, March. After March 20th, positive temperatures settled in and the Cabernet Sauvignon variety started in vegetation. In April, the average temperature for the second decade decreased again to only 7.5°C, meaning below biological zero, and the rains were in large quantities. All throughout spring, the days with cold and rain alternated with the warm ones, with temperatures of 20°C, having as consequence the non-uniformity of budbreak. There followed repeated waves of cold, even in the months of May and June, when the blooming pheno-phase is disturbed.

The summer of 2006 was normal, from the point of view of temperatures and rains. The autumn was long and draughty, vegetation prolonging for a very long time. The grapes' health condition was, generally, good.

The viticultural year 2006-2007 may be characterized, as a whole, as excessively draughty and very warm. The annual average temperature recorded was of 12.2°C, compared to the average 11.4°C. The absolute maximum temperature in the air was 41.1°C. The climatic elements of the months of January, February, March are significant, with very large amplitudes of temperature variation: from minus 2°C to plus 20°C, from 15.6°C to plus 17°C.

In January the sum of the temperature degrees reaches 137.4°C, compared to -33.3°C, the normal value for this month. In February there were summed up 66.2°C, compares to 24.3°C, the multi-annual average, and in March, 220.8°C, compared to 153.4°C and with exceeding regime of rains. These temperatures favoured the rapid vegetation growth, and then, after these three warm months, April followed, with a significant deficit of temperatures and rains, disturbing the normal passing of the pheno-phases. These determined the installing of a non-uniform vegetation start and an attack of parasites that exceeded by much the pest threshold; the April - July interval with an accentuated water deficit determined a decrease in the air hygroscopic character below the optimum level for performing the physiological processes of the plant and for the development of the pathogenic fungi; the rains of August and September determined a ripening of the grapes within the normal limits, but favoured the installing of grey rot.

Within the ecologic culture system, the blocks' health condition was ensured with treatments allowed by the legislation in effect (products based on copper and sulphur, in legally allowed doses), and in the conventional one with products specific to this culture system.

The determinations performed were: statistics of the fruit elements; foliar surface; grape crop, its quality and the attack degree determined by the pest agents.

The foliar surface was determined by means of the round parts method at the end of the twig growing period.

The frequency(F%) and intensity(I%) of the attack were determined using the grading scale with six classes (Method C. Rafailă). The expression for calculating the attack degree(Ga%) is Ga = (F x I)/100.

The dry substance of the grapes was determined by multiplying the production (kg/vine stock) x 0.23 (this value representing the ratio between the dry weight of the grapes/their fresh weight).

The quantity of sugars/block was determined through the relation: g. sugars/block = kg. grapes/vine stock x 0.70 (efficiency rate of transforming grapes into must) x g. sugars/l (rephractometrically determined).

In order to determine the optimum ratio between the foliar surface and the grape production, the productivity of the total foliar surface was computed (cm^2 of foliar surface/grapes weight), and in order to determine the foliar surface necessary for obtaining an optimum content of sugars, the ratio between the foliar surface and the quantity of sugars accumulated on the vine stock was computed.

The relation quantity-quality was determined by comparing the grapes' dry substance (kg. grapes x 0.23), to the accumulations of sugars on the vine stock (kg. grapes x 0.70 x g. sugars/l must).

In order to establish the connections between the variables studied the following correlations were determined:

- Productivity of the foliar surface (cm^2/g. grape) with the accumulations of sugars (g sugars/vine stock);
- Productivity of the foliar surface (cm^2/g. grape) with the attack degree produced by the grey rot (Ga %);
- Ratio between the foliar surface and accumulated sugars with the attack degree produced by grey rotten stuff (Ga %);
- Grapes' dry substance (g/vine stock) with the accumulations of sugars (g/vine stock);

For these correlations the average values of the indicators studied in the period 2006-2007 were used. The optimum solution was established by statistical calculation.

RESULTS AND DISCUSSIONS

From the graphical analysis of the fertility elements there may be noticed (Fig. 1) that the two systems of culture did not influence, throughout the two years of study, the fertility of the winter buds.

The characteristics of the foliar surface allow the capitalization of the ecologic resources, influencing the quality and quantity of production. The productivity of the foliar surface ("cm^2 foliar surface/g grape" or "m^2 foliar surface/kilogram of grapes) explains the differences of sugars accumulation in different eye loads (*Dejeu and collab., 2006*). The values comprised between 7 and 17 cm^2 foliar surface/g grape are considered optimum for the proper ripening of the beans (*P. May and collab., 1969; Kaps M. L., Cahoon G. A., 1992*).

By examining the values of the ratio between the productivity of the foliar surface(cm^2/g. grapes) and the sugars on the vine stock (Fig. 2), a significant correlation was obtained. The highest accumulations of sugars in grapes were recorded in the conditions in which the foliar surface (cm^2) compared to the production of grapes took

values comprised between 10.86 and 10.90, values which correspond to a load of 24 eyes/vine stock in the ecologic system. It is obvious that, for the accumulation of each gram of glucides as a result of the photo-synthesis activity, a certain foliar surface is necessary, which is influenced by the technological factors (eye load, in the case at hand).

By examining the connection between the productivity of the foliar surface and the attack degree produced by the grey rot (Ga %) it was established that the minimum attack values are recorded for a foliar productivity comprised between 7.21 and 10.90 cm^2/g grape (Fig. 3).

The attack degree records minimum values when the ratio foliar surface/g sugars is comprised between 59.15 and 75.96 (Fig. 4), which corresponds to a load of 16 – 24 eyes on the vine stock.

By statistically analyzing the grape production in the two years of study (Tab. 1, Tab.2), the two systems of culture and the three fruit loads attributed on the vine stock, the following aspects were established:

- The existence of a distinct significant difference of production between the two systems (1.68 instead of 1.95);
- Between the minimum fruit load attributed/vine stock for each system and the one recommended for the type studied there is a very significant negative difference of production; this conclusion was also verified in other situations as well (1.40 compared to 1.90 and 1.75 instead of 2.10);
- By Analyzing the combined influence of the fighting system and of the fruit load, for a load of 16 eyes/vine stock a significant negative difference of production was observed, between the ecologic and the conventional system (1.60 compared to 2.10). For the other graduations no significant differences were recorded.

CONCLUSIONS

In order to verify and put into practice the principles of ecologic viticulture two production systems, ecologic and conventional, were monitored. The factors studied were: the conditions of the vintage year, the fruit load with three graduations (16, 24, 40 bud/vine stock) and the fighting system with two graduations (ecologic and conventional fighting). The determinations performed took into consideration the productivity of the exposed foliar surface, the grapes production and its quality, the health condition of the plantation and the vintage. From the results obtained, the following conclusions can be drawn:

- The highest accumulations of sugars in grapes were recorded in the conditions when the foliar surface (cm^2) compared to the production of grapes took values comprised between 10.86 and 10.90, values obtained at the load of 24 buds/vine stock in the ecologic system.
- In the two culture systems analyzed, the minimum values of the attack are recorded for a foil productivity comprised between 7.21-10.90 cm^2/g grape.
- From the statistical results it is derived that the ecologic fighting system provides for a healthy crop, insignificantly diminished but qualitatively superior at a load of 24 buds/vine stock.

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Tables

Table 1. The influence of the number of buds per vine stock upon grape production depending on the culture sistems and the crop year.

Year	Fruit loading viticulture system	The number of buds per vine stock			Average
		c1(16)	c2(24)	c3(40)	
a1	b1	1.60 ^{o}	2.00	2.20	1,93^{oo}
	b2 (Mt)	2.10	2.30	2.40	2,27
Average		**1,85**	**2.15**	**2.30**	**2.10**
a2	b1	1.20 ^{o}	1.50	1.60	1,43^{o}
	b2 (Mt)	1.40	1.70	1.80	1,63
Average		**1,30**	**1.60**	**1.70**	**1.53**

Table 2. The influence of the number of buds per vine stock upon grape production depending on the viticulture sistem analized.

Fruit loading viticulture system	c1(16)	c2(24)	c3(40)	Average
b1	1,40^{ooo}	1,75	1,90(mt)	**1,68^{oo}**
b2	1,75^{ooo}	2,00	2,10(mt)	**1,95(mt)**
Average	**1,57^{ooo}**	**1,87**	**2,0**	**1,81**

Figures

Fig. 1. The influence of the viticulture system upon the fertility of winter buds.

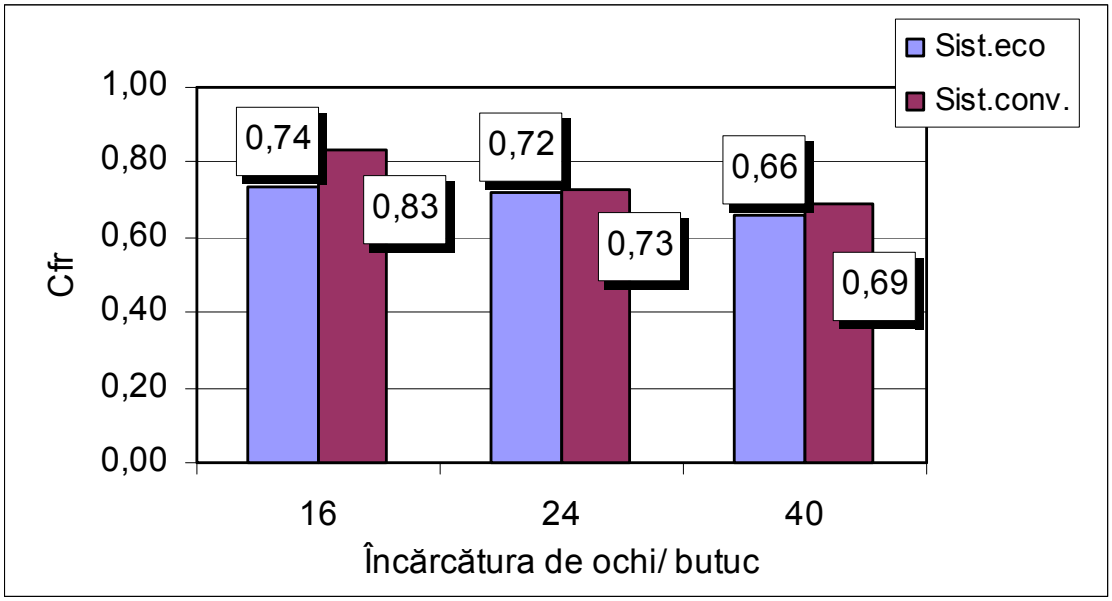


Fig. 2. Corellation between the productivity of the foliar surface and the sugars on the vine stock(cm2/g) .

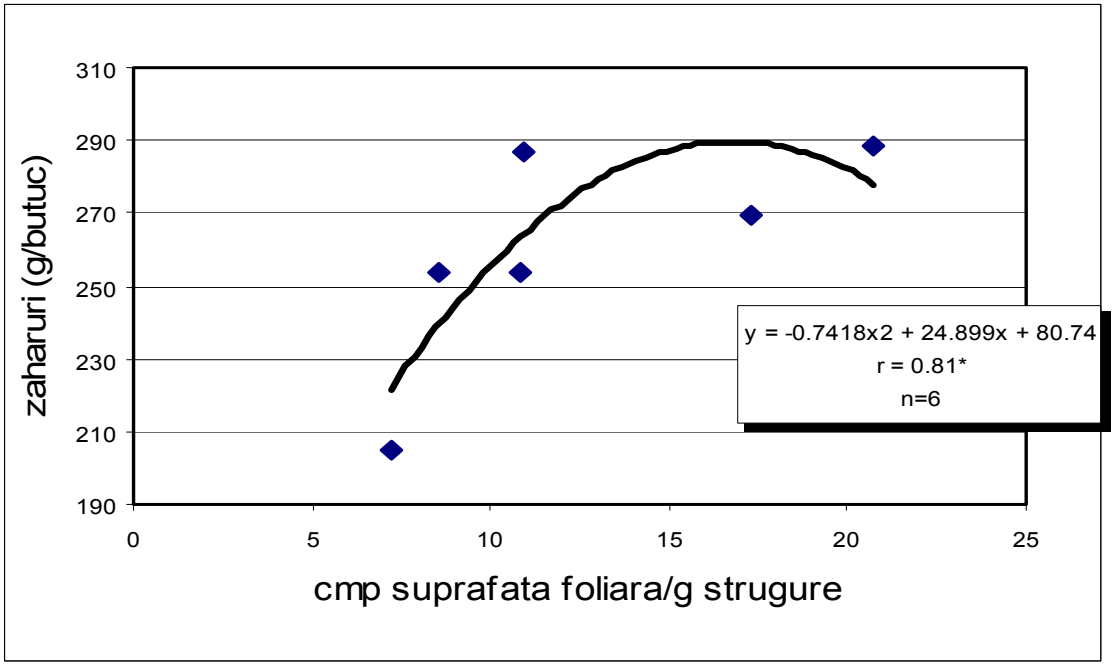


Fig. 3. Corellation between FS/g grape and the attack degree produced by the grey rot.

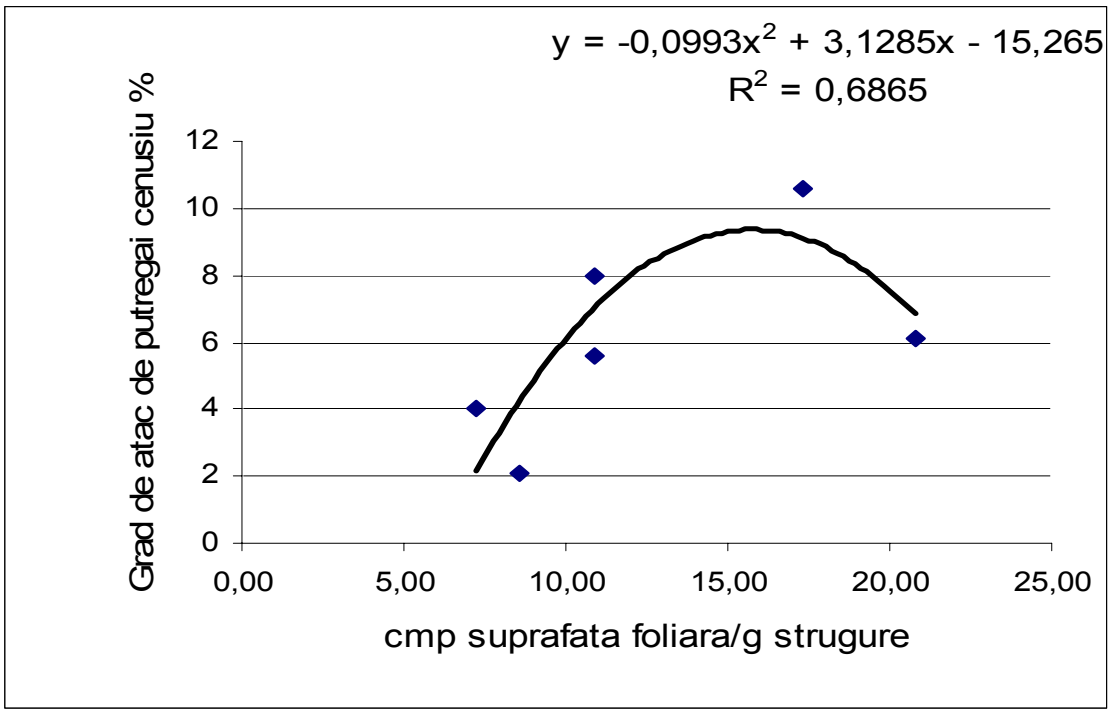
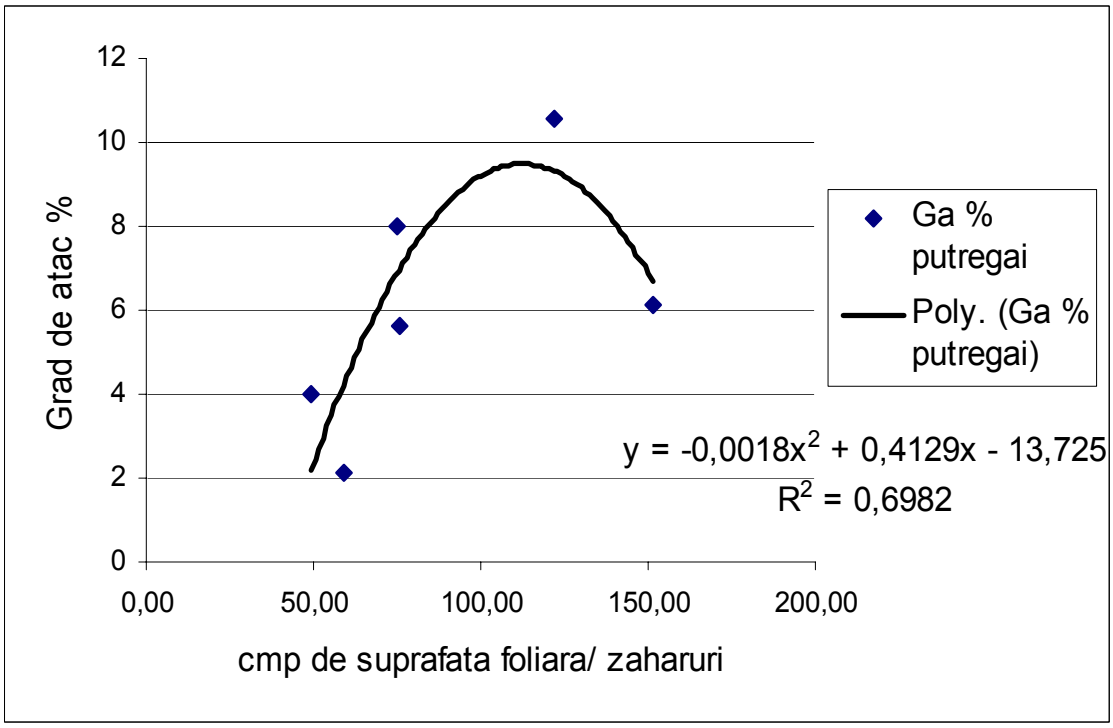


Fig. 4. Corellation between cmp FS/g sugar acumulation and the attack degree produced by the grey rotten stuff.



Lucrări ştiinţifice U.Ş.A.M.V.B., Seria B, vol. LI, 2008

The influence of the winemaking technology on the aging capacity of the Grasă variety wines from the Dealu Mare Vineyard – Pietroasa Wine Center

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Keywords: winemaking, aroma, typicity, tasting notes

ABSTRACT

The purpose of this study is to evaluate the influence of the various technologies used in winemaking on aging capacity of the wines obtained from the Grasă grapes from Pietroasa wine region, using the sensorial analysis. It was analised wines from Grasă obtained in 2 diferent winemaking trials. The samples were collected from homogeneous lots of must from Grasă grapes from Pietroasa region (breed purity 100%). Basic chemical analysis were done on the fresh must, the fermented must and on the young wine. Wines were sensorially analysed 1 year, 2 years and 3 years after winemaking by a panel of five autorised winetasters. The results have proven that some winemaking techniques significantly influence the intensity and complexity of wine flavour and the aging capacity of the wines.

INTRODUCTION

The production of aromatic medium dry and sweet DOC wines with sugar content is traditionally made in Romania in strictly delimitated areas, in which the favourable pedoclimatic conditions are capitalized through the means of grape varieties with a high capability of flavour and sugars accumulation and concentration in the berries. In this context, it is very well known the capability of sugars accumulation of the Grasa variety, in Pietroasa, Dealu Mare region.

In this work we will try to evaluate, using the sensorial analysis, the influence of the different winemaking technologies on the capacity of these wines of aging in bottles, being known, at one side, the very good results of this variety, traditionally winemade in aging conditions, but also the present tendency of changing the winemaking technologies in order to produce lighter, more aromatic and less extractive wines.

MATERIALS AND METHODS

To make this study there were used as raw materials grapes from the Grasă variety from the Pietroasele region, manually harvested on end of september 2004.

Two winemaking technologies were used:

- tradional industrial used technology in present that include 24 hours clarification process and natural fermentation (GM coded)
- the new technology that include enzyme treatment, 24 hours clarification, fermentation with selected yeasts (GN coded)

The grapes were destemmed-crushed with an electric destemmer-crusher, and the juice was obtained by outflow – pressing in a 20 litres capacity hydraulic press (max. 2 atm.). The resulting juices (free-run and press juice) was assembled, homogenized and sent to processing where it was distributed in 30 litres glass recipients (3 recipients for each winemaking method). The mash was added a 5 g/hl SO2 dose using potassium metabisulphite and the clarification of the must was made by gravity in glass beakers. The enzyme treatment was performed by adding a 2g/hL dose, directly in the juice, after the juice was distributed in the clarification recipient. The product used is a commercial one, an enzymatic pectolytic concentrate with secondary glycosidic activities (Lallzyme Cuvee Blanc). For inoculation we used 20g/hl of Lalvin QA23, a Saccharomyces

bayanus strain recognized for its abilities of producing fermentation flavours and revealing the aromatic character of wines. The fermentation was stopped for all wines by adding an 8 g/hl dose of SO2 and granulated sodic bentonite BentoClar (100 g/hl). After filtration the wines were bottled in 0,75 lt glass bottles and stored in a wine cellar at a temperature of approx. 12°C. The musts and wines were subject to basic chemical analysis, according to the standard analysis methods: alcohol content, sulphur dioxide (free and total), reducing sugars, total dry extract, volatile acidity, total acidity. The wines were tasted by a group of 5 authorized expert winetasters on a regular basis, as following: 3 months after production, one year, 2 years, 3 years. The global value of each wine was appreciated using a scale from 1 to 100 points and the wines were also analysed according to visual, olfactive, taste and touch descriptors. Each descriptor was evaluated on a scale from 1 to 9 points.

RESULTS AND DISCUSSIONS

1. The sugar content of Grasă grapes was 228 g/l (harvest time);
2. The alcoholic fermentation was conducted at a temperature of about 20ºC, in 3 recipients of 30 lt for each technological variant. At the end of the fermentation all the samples were analyzed. The samples coded GM needed 8 days to get to approx. 25 g/l residual sugar and to end the fermentation. The samples clarified using the enzymes and inoculated with selected yeasts – coded GN – reached within 5 days the necessary parameters to stop the fermentation.
3. Chemical parameters of wines: The fermentation was stopped in order to obtain semi-sweet wines. The chemical analysis of the samples are presented in table 3 (after stopping the fermentation and the chemical analysis of each recipient, these were racked and assembled, because there were not noticed differences between the different recipients of the same technological variant).
4. Sensorial analysis:The wine tasting was carried out in march 2004, march 2005, march 2006. There were used two types of tasting sheets: One sheet used for the general description of wines, with an evaluation scale from 0 to 100 points that evaluated the following characteristics: aspect, colour, aroma, taste, harmony and one sheet in which there were noted a series of visual, olphactive, gustative descriptors, each on an evaluation scale from 1 to 9. The results of the global sensorial analysis of the variants (aspect, taste, colour, aroma, harmony – total up to 100 points) are presented in Fig. 1 and Fig. 2. The results obtained at sensorial analysis for the olphactive descriptors are presented in Fig 3 and fig.4.

CONCLUSIONS

During the years, after the sensorial analysis of the different technological variants of the Grasa variety the following were observed:

1. The technological variant that included enzymatic clarification of the juice and the use of selected yeasts obtained better grades for the aroma and taste after the first year after winemaking; aromatic intensity, floral and fruit aroma were more pronounced at this technological variant;
2. In the aroma panel used one could observe that the honey aroma considered typical for the Grasa variety was not significantly influenced by the winemaking technology during the first year after winemaking but it has enhanced in time at the wine produced with the classic technology (GM);

3. The selected yeast significantly influenced the aroma of the Grasa variety wines, the variant that used it got very good grades for the aromatic intensity, floral aroma and especiallt elder flower aroma, during the first year after winemaking;
4. New and unusual aroma for Grasa variety (e.g. elder flower aroma) can influence the aromatic typicity of the variety, if the wine is put on sale in the first year after winemaking. This atyipical aroma disappears after few yeas of aging;
5. Typical honey aroma is much more intense at the Grasa variety wines that were produced by the traditional method after three years from harvesting comparing to the wines that were produced using a modern winemaking technology;
6. The use of pectolytic enzymes for clarification, as well as of the selected yeasts for juice fermentation leads to faster fermentations and to fresher wines, more pleasant and more aromatic, but less appropriate for aging.

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Table

Table 1. Chemical parameters of Grasă wines at bottling time

Variant Code	Alcohol, %vol.	Sugar, g/l	Dry extract, g/l	Total acidity, g/l	Volatile acidity, g/l	Total SO2, mg/l	Free SO2, mg/l
G M	12,2	21,4	24,84	5,57	0,68	184	59
G N	12,3	22,2	22,54	5,78	0,51	156	54

Figures

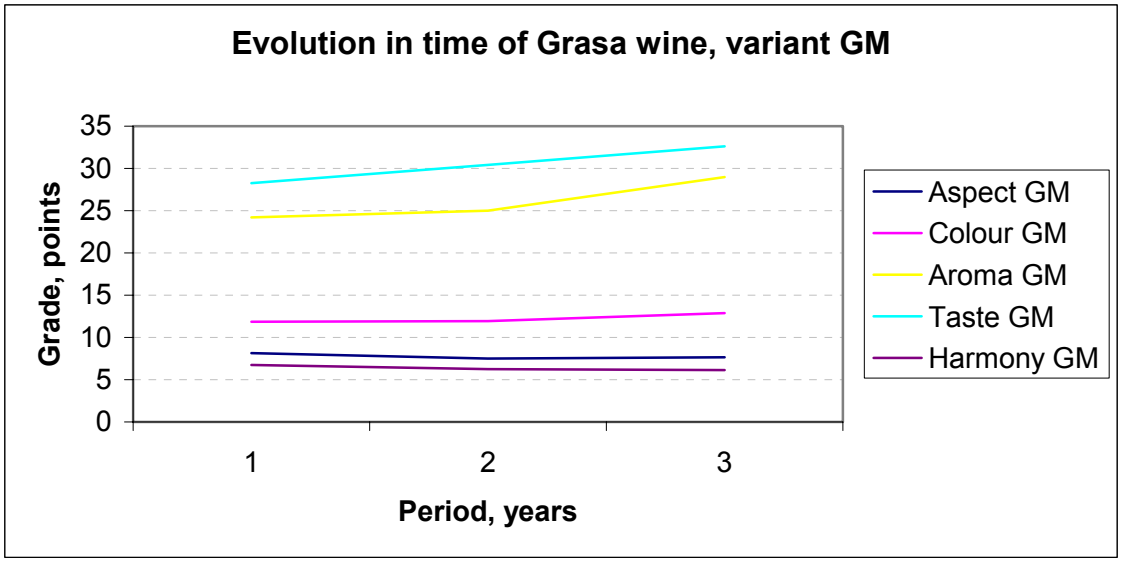

Fig. 1. Wine tasting grades for the global sensorial analysis obtained by a group of 5 winetasters for the GM variant

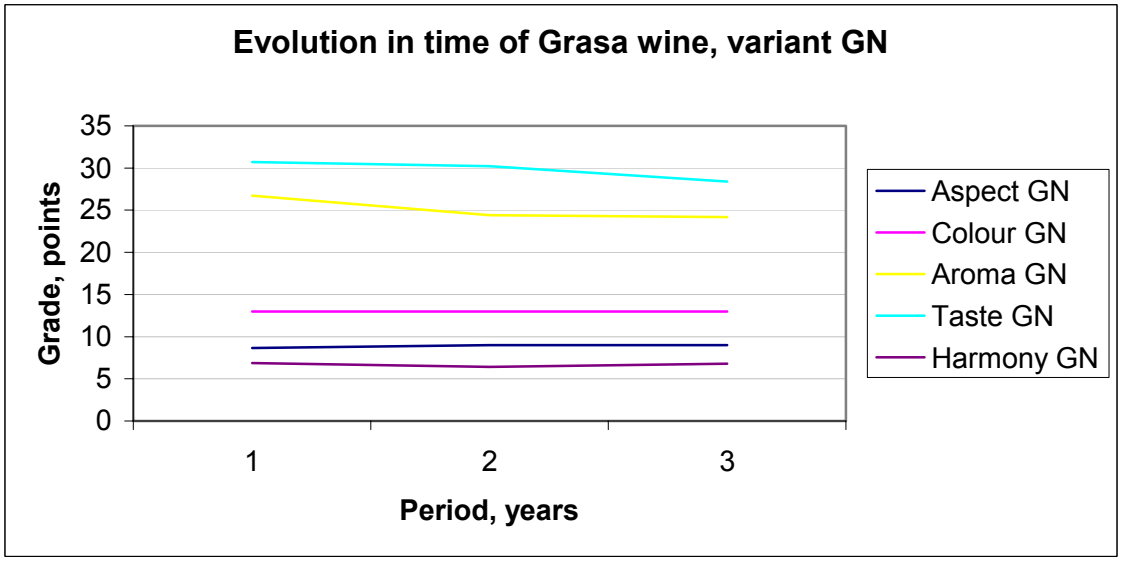

Fig. 2. Wine tasting grades for the global sensorial analysis obtained by a group of 5 winetasters for the GN variant

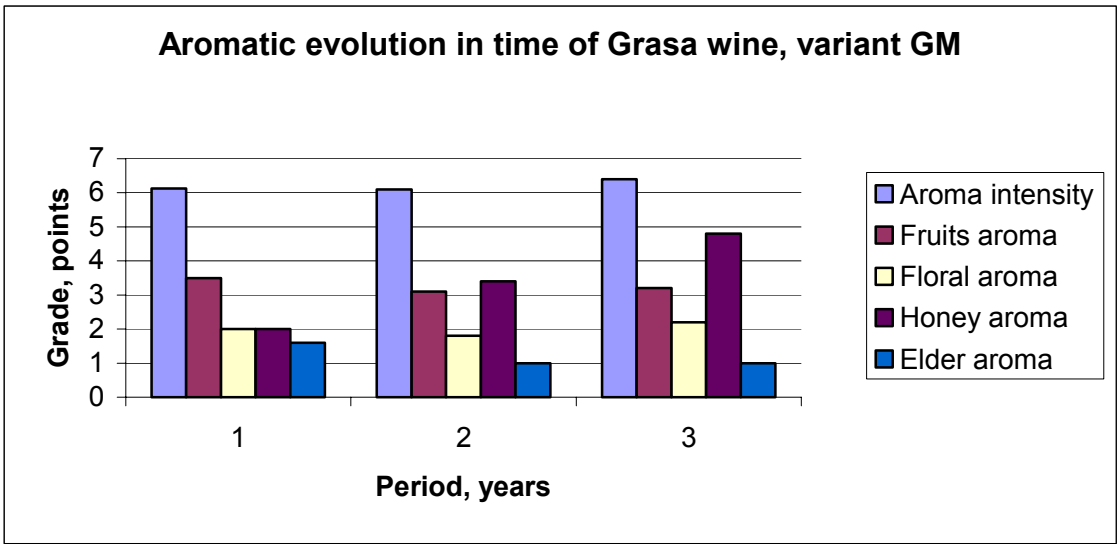

Fig. 3. Wine tasting average grades for the olphactive descriptors for the GM

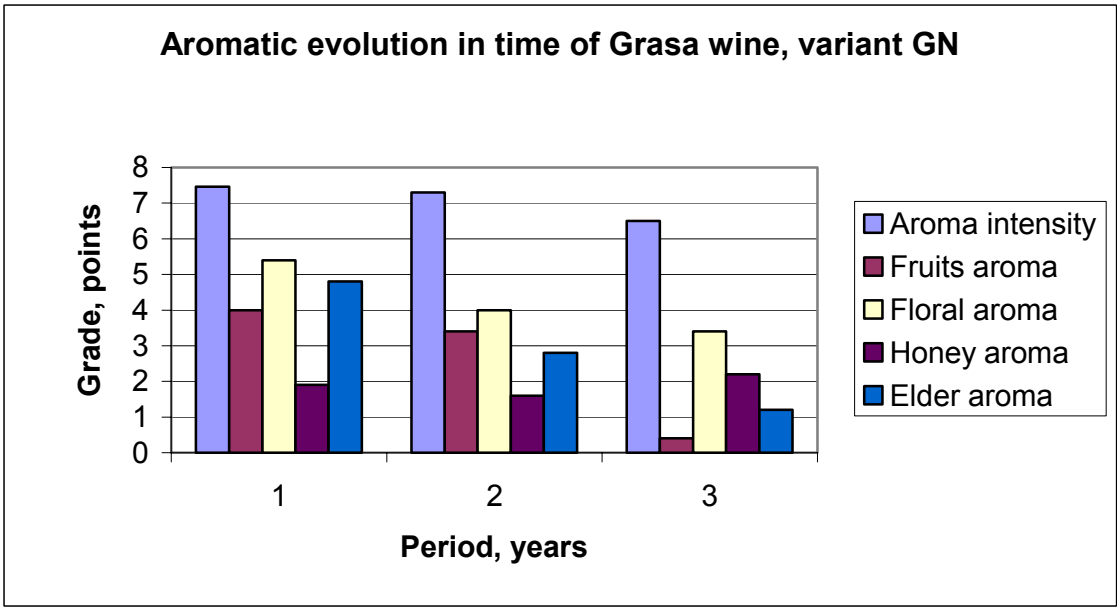

Fig. 4. Wine tasting average grades for the olphactive descriptors for the GN variant

The effect of electric field on in v*itro* regenerative processes and grapevine virus elimination

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Key words: *Vitis vinifera*, micropropagation, GLRaV 1+3, continuous electric current, sanitation, ELISA

ABSTRACT

In order to study the effect of electric field on *in vitro* regenerative processes and virus free plant achieving in *V. vinifera* L., Cabernet Sauvignon variety plants infected with grapevine leafroll associated virus serotype 1+3 (GLRaV 1+3) were used. The virus infected plants obtained from one bud woody cuttings were subjected to electric field to 10, 20 and 40V/cm applied for 5, 10 and 20 minutes in each variant.. Also, a prolonged treatment of four days, 8 hours/day to 40 V/cm was realised. The shoot apices collected both from treated and control plants for *in vitro* culture on regenerative media were used. ELISA testing for GLRaV 1+3 detection was performed on acclimatized plants. A percent of virus free plants were obtained in each variant as follows: 83 % to 10V/cm - 5 min; 60% to 20V/cm - 5 min; 72,7% to 40V/cm - 5 min; 57,1% to 40V/cm - 20 min and 100 % to the other short variants. Any virus free plant in the prolonged application of electric field was identified. No correlations between period and intensity of electric field and percent of GLRaV 1+3 free plants achieving have been found. The *in vitro* multiplication and rooting rates were not uniform influenced by the electric field comparatively to the control.

INTRODUCTION

The thermotherapy and/or *in vitro* culture are individually or in combination the most frequent methods of obtaining virus free grapevine plants (Buciumeanu at al., 2001; Vişoiu and Buciumeanu, 2004). The alternative sanitation methods as chemotherapy, electrotherapy, cryopreservation, less used with grapevine, present satisfactory results for different crop plants (Hansen and Lane, 1985; Bittner et al., 1989; Burger, 1989; Helliot et al., 2002; Igarza Castro et al., 2007; Panattoni et al., 2007).

The paper purposes the study the *in vitro* regenerative processes and the possibility of grapevine sanitation in the presence of electric field. The work has in view grapevine leafroll associated virus serotypes 1+3 (GLRaV 1+3) which presence in the grapevine planting material affects genetic potential of infected cultivars (Martelli and Boudon-Padieu, 2006).

The setting up of new plantation with healthy planting material would lead to a rise in the technical-economic competitiveness by the efficaciousness of all obtaining and exploitation steps from grafting to preserving the production characteristics.

MATERIAL AND METHODS

The effect of electric field (continuous electric current) on virus elimination and regenerative processes in *V. vinifera* L. were investigated. Cabernet Sauvignon variety grapevine plants infected with GLRaV 1+3 were used. The infected plants obtained from one bud woody cuttings were subjected to electric field at 10, 20 and 40V/cm for 5,

10 and 20 minutes in each variant. In addition, a prolonged treatment of four days, 8 hours/day, to 40 V/cm was led. After each day of treatment leaves from the potted plants were collected for ELISA testing. The apparatus used during the experiment was developed in the Faculty Electronic and Telecommunication of the University of Piteşti. The shoot apices collected both from treated and control plants for *in vitro* culture on regenerative media were used (Vişoiu and Teodorescu, 2001). The cultures were established starting with two explants/variant. ELISA testing for GLRaV 1+3 detection was performed on leaves collected from acclimatized plants, using SEDIAG reagents.

RESULTS AND DISCUSSION

Electric field was applied on 15-20 cm rooted plants in vegetative pots. The plants were not damaged under the presence of electric field. The shoot apices were excised after each period of electric field exposure. *In vitro* cultures on regenerative media were established corresponding to each variant. The virus infected plants used as controls were not subjected to electric field action. Electric field exposure induced discordant behaviour of *in vitro* culture material from the point of view of micropropagation processes (multiplication and rooting rates). The multiplication (Fig. 1) and rooting (Fig. 2) rates were influenced by the electric field comparatively to the control (C). The multiplication rate increased to 10V/cm - 10 min, 20 V/cm – 20 min and 40 V/cm – 5 min variants. The rooting rate increased to 10V/cm - 20 min, 20 V/cm – 10 and 20 min and 40 V/cm – 5 and 10 min variants. Both the multiplication and rooting rates were increased only to 20V/cm – 20 min and 40V/cm – 5 min variants comparatively to the control. Equal or lower rates comparatively to the control were obtained in the other short periods of electric field application variants. Prolonged electric field produced insignificant modifications of multiplication and rooting rates. No necrosis or abnormal microshoots were observed in the multiplication (one sub-culture of 30 days) and rooting (30 days) periods.

A number of 89 of acclimatized grapevine obtained in short variants of electric filed action were ELISA investigated (6–16 plants/variant) for the presence of GLRaV1+3 infection. Continuous electric current displayed antiviral activity on virus infection of grapevine. In all short variants of electric field application were obtained GLRaV 1+3 free grapevine plants in different percent, from 57,1 to 100% (83 % to 10V/cm - 5 min; 60% to 20V/cm - 5 min; 72,7% to 40V/cm - 5 min; 57,1% to 40V/cm - 20 min and 100 % to the other short variants)**.** The best treatment was 20V/cm – 20 min achieving sanitation of 100% of the acclimatized plants and also significantly increased multiplication and rooting rates (1:2,75 and 1:5,2 respectively) were obtained. No correlations between period and electric field and percent of GLRaV1+3 free plants achieving have been found (Table 1).

In the case of prolonged application of electric field any virus free plant was identified after *in vitro* regeneration. The ELISA readings in leaves from the treated plants were fluctuant comparatively before the electric field action but the GLRAV 1+3 presence was detected every time. ELISA values (A_{420nm} readings) registered in the leaves in the period of prolonged treatment of plants were the following: 0,680 (control); 0,553 (after 1st day); 0,352 (after the 2nd day); 0,889 (after the 3d day); 0,745 (after the 4th day). The readings did not decrease with increased time of treatment.

CONCLUSSIONS

1. Continuous electric current displayed antiviral activity on virus infection of grapevine. In all short variants of electric field application were obtained GLRaV 1+3 free grapevine plants in different percent. The best treatment was 20V/cm – 20 min achieving sanitation of 100% of the acclimatized plants and also significantly increased multiplication and rooting rates were obtained. Any virus free plant was regenerated in the variant of prolonged application of electric field.
2. No correlations between period and electric field applied and percent of GLRaV1+3 free grapevine achieving have been found.
3. Both the multiplication and rooting rates were increased in grapevine after electric field application to 20V/cm - 20 min and 40V/cm – 5 min variants comparatively to the control.
4. Further investigations will allow the optimization of the factors for a method of virus free plants regeneration under the influence of electric field.

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Figures

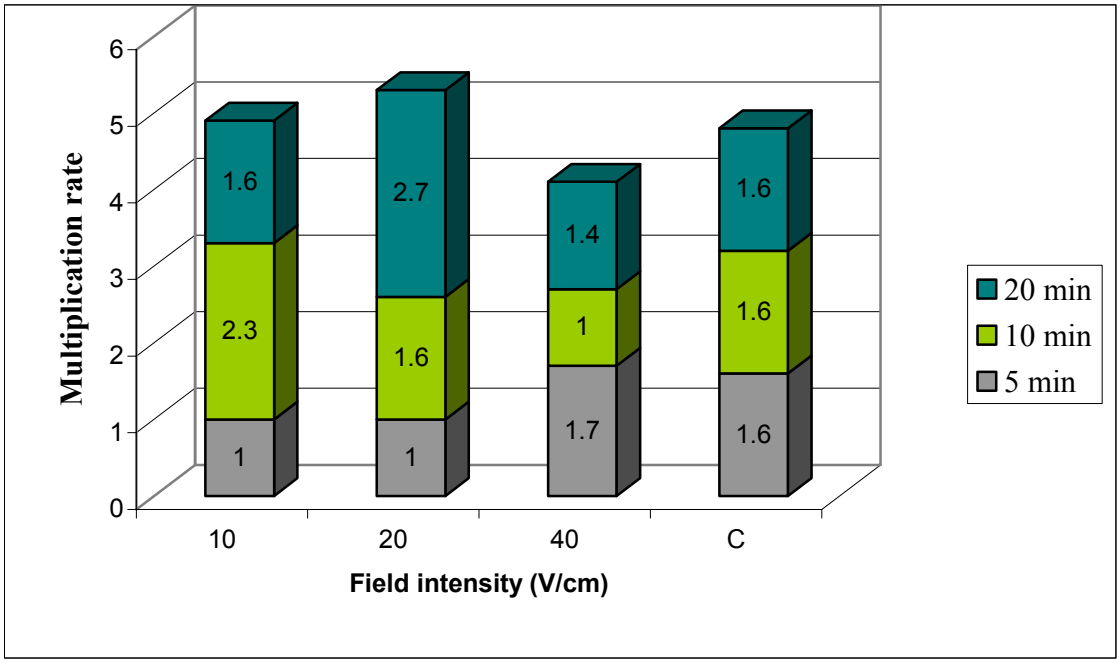

Fig. 1 Multiplication rate of grapevine explants excised from GLRaV1+3 infected Cabernet Sauvignon exposed to electric field, comparatively to the control (C)

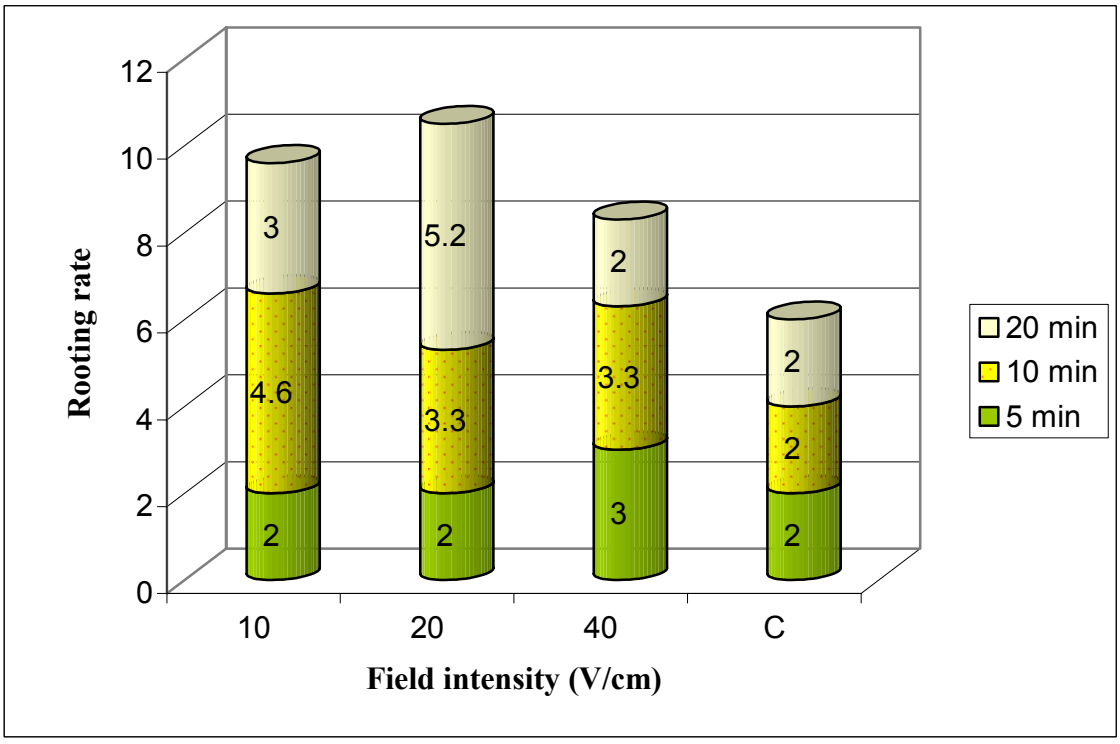

Fig. 2 Rooting rate of grapevine multiplied explants from GLRaV1+3 infected Cabernet Sauvignon exposed to electric field, comparatively to the control (C) .

Table 1 Evaluation of *in vitro* regenerated virus free grapevines from GLRaV1+3 infected Cabernet Sauvignon exposed to electric field

Field intensity (V/cm)	Exposure period (min)	ELISA tested plants (No.)	Virus free plants (No.)	Sanitation (%)
10	5	6	5	83,3
	10	14	14	100
	20	8	8	100
20	5	7	7	100
	10	10	6	60
	20	16	16	100
40	5	11	8	72,7
	10	10	10	100
	20	7	4	57,1

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Agro-biological and phenolic potential for area extension of Romanian grapevine varieties for high-quality red wines

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Keywords: red wines, autochthonous grape varieties, Novac, Negru de Dragasani, colour intensity

ABSTRACT

The national strategy for the Romanian wines market penetration has started a decade before and is still running. The results presented in this paper have been obtained mainly a few years ago but only now they have been completed.

As a result of a hard selection work, they have been homologated, more than 10 years ago, two new red grapevine varieties at S.C.D.V.V. Drăgăsăni: Novac and Negru of Drăgăşani. From 2004 we can find on the market the wine named "Negru de Dragasani", but a comparative characterization of these varieties haven't been published.

This paper presents some results of the grapevine behavior in the USAMV Bucharest vineyard compared with the native area Dragasani and the consecrated winemaking area Valea Calugareasca. A special attention was given to the color characteristics of the obtained wines. Both new varieties proved an upper productivity comparing with Merlot variety as control, and one of them, Negru de Dragasani has a good color intensity compared to the control. The obtained data come to sustain the extension of the cultivation area for Negru de Dragasani in order to obtain higher quantity of good quality autochthonous red wines.

INTRODUCTION

During the last decade researchers and business people have given a special attention to the market penetration of autochthonous wines. To obtain such a wine there is a need in new selected grape varieties and also to be used local isolated yeast as starter culture.

One important goal in this strategy is to improve or to select new grapevine varieties proving superior elements in terms of productivity and quality. A strong accent was focused to improve the local red grape varieties in order to obtain new one and to extend their cultivation area.

More than ten years ago at SCDVV Stefanesti Arges, two new varieties Novac and Negru of Drăgăşani have been obtained starting from the same the same genitors (Negru Vârtos and Saperavi) and they have been homologated in 1997, respectively in 1993.

Their behaviour has been tested in their local vineyard (Dragasani), but in order to prove their potential for an extended area cultivation they have been cultivated in the consecrated area for winemaking Valea Calugareasca and in the experimental vineyard of USAMV Bucharest - Faculty of Horticulture and from the grapes was obtained wine by micro-vinification (laboratory conditions).

MATERIALS AND METHODS

Two Romanian red grapevines, Novac and Negru de Dragasani, having as genitors Negru Vârtos and Saperavi varieties have been cultivated and studied in Valea

Calugareasca area and in the experimental vineyard of USAMV Bucharest during five years. The data have been collected after 3 years of cultivation (from 2000 to 2002).

The cultural behavior was described by using the following indicators and compared with a control grapevine (Merlot): fertility (expressed by the percent of fertile offshoots and by the relative and absolute fertility coefficient); productivity (average variation of grape weight; variation of 100 g grains); for the sugar content (by refractometry) and the total acidity in g/l H_2SO_4 (by titration).

Samples of 20 kg grapes have been pressed, kept 3 days for the maceration-fermentation, than the extracted juice completed the fermentation under laboratory conditions. The wines have been characterized mainly for their phenolic elements: total polyphenols, tannins and anthocyan (Puisant-Leon method). Also spectrophotometric measurements have been performed in order to obtain the ration of the main colors (yellow at 420 nm, red at 520 nm and blue at 620 nm).

As a control for the experiments was considered the consecrated red grapevine variety Merlot cultivated in the experimental vineyard of USAMV Bucharest.

RESULTS AND DISCUSSIONS

The fertility expressed by the proportion of fertile offshoots and by the values of the two fertility coefficients was studied at U.S.A.M.V. Bucharest (Fig.1). From this point of view the new varieties are inferior to the control. However, compared with the control, productivity of the new varieties are bigger as a result of their grain weight, and the grape (Fig.2).

At the same level productivity for the control Merlot of 9.1 t/ha and 9.6 t/ha for Negru of Dragasani variety and a productivity of 13.9 t/ha for Novac variety, the sugars content it is superior for the new varieties (Fig.3). In the same time the acidity (g/l H_2SO_4) shows almost the same level for the control and the new varieties (Fig. 4).

The obtained wines from the two new Romanian varieties, Novac and Negru de Drăgăşani, show a high similarity regarding the polyphenols content, with a maximum content in galic acid of 1600 mg/l, while the control (Merlot) reached only 700 mg/l average (Table 1). Instead, the tannins, which are part of he total polyphenols rises the same level for the control and the new variety Novac.

A very important compound of the red wine grape varieties is the anthocianins content. From the two new varieties, only the Negru de Dragasani variety accumulates constantly the same level as the control Merlot (115 mg/l, respectively 93 mg/l). The Novac variety is proved to be in a deficit regarding these compounds, their values arriving at half level from the control value (35 mg/l).

Because of its anthocyanins content the Negru de Dragasni variety shows a pronounced color intensity, very close to the control level (5.5 - 7.0), while the Novac variety it is close to the value of 4.5.

The three colors ratio contribution in the final color of the wine it is almost the same for the two new varieties and the control and varies between the following limits as it is shown in the Table 2:

- blue 9.9 - 15.5 %
- red 48.46 - 63.46 %
- yellow 26.6 - 37,.66 %

CONCLUSIONS

The two studied red wine grape varieties Novac and Negru of Dragasani proved to have in all three cultivation area superior productivity indicators comparing with the control (Merlot) and a higher sugar content during the three years.

For the obtained wines a correct color intensity, closer to the control, was founded for the Negru of Dragasani variety because of its content in anthocianins.

The data are proving that Negru de Dragasani has a high potential for an extended area cultivation and it can be recommended to the vine and wine makers to obtain good quality autochthonous red wines. Novac can be kept in culture for its high productivity.

ACKNOWLEDGEMENTS

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Many thanks to our collaborators from S.C.D.V.V. Valea Calugareasca for providing local data regarding the two studied varieties.

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Tables

Table 1. The average level of the phenolic compunds in the obtained wines

Variety	Total polyphenols (mg/l galic acid)	Tannins (mg/l galic acid)	Anthocian (mg/l)
Novac	1450	130	35
Negru Dragasani	1600	400	93
Merlot	700	120	115

Table 2. The average values of the coloring compounds and the color intensity of the obtained wines

Variety	% blue (620 nm)	% red (520nm)	%yellow (420nm)	Colour Intensity
Novac	9.9	63.46	26.64	4.52
Negru Dragasani	15.2	61.39	23.41	6.85
Merlot	10.6	63.46	25.94	7.56

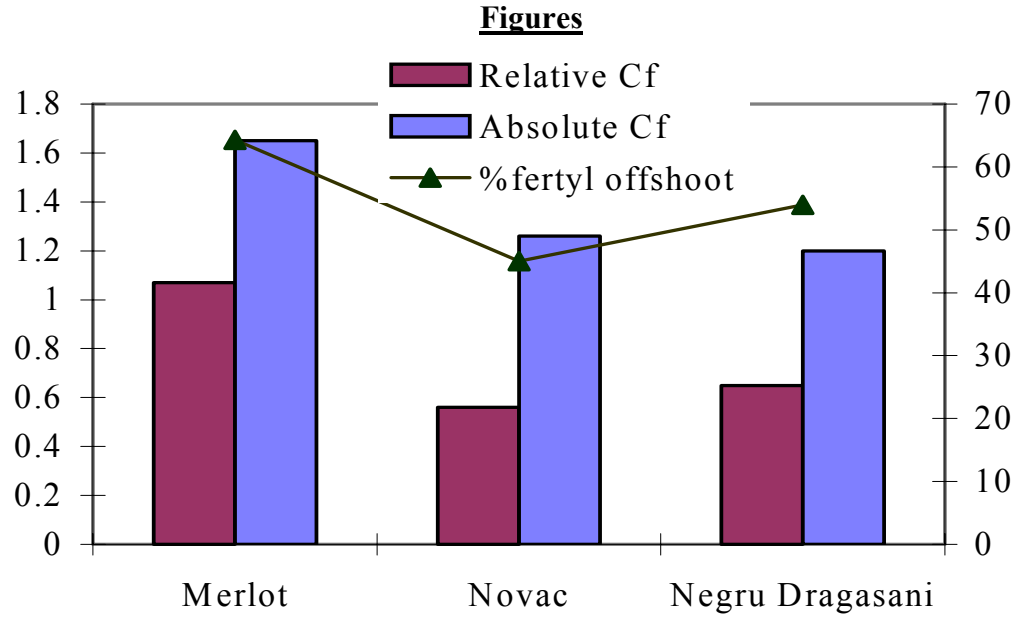

Fig. 1. The fertility indicators of the red wine grape varieties (data from U.S.A.M.V.B).

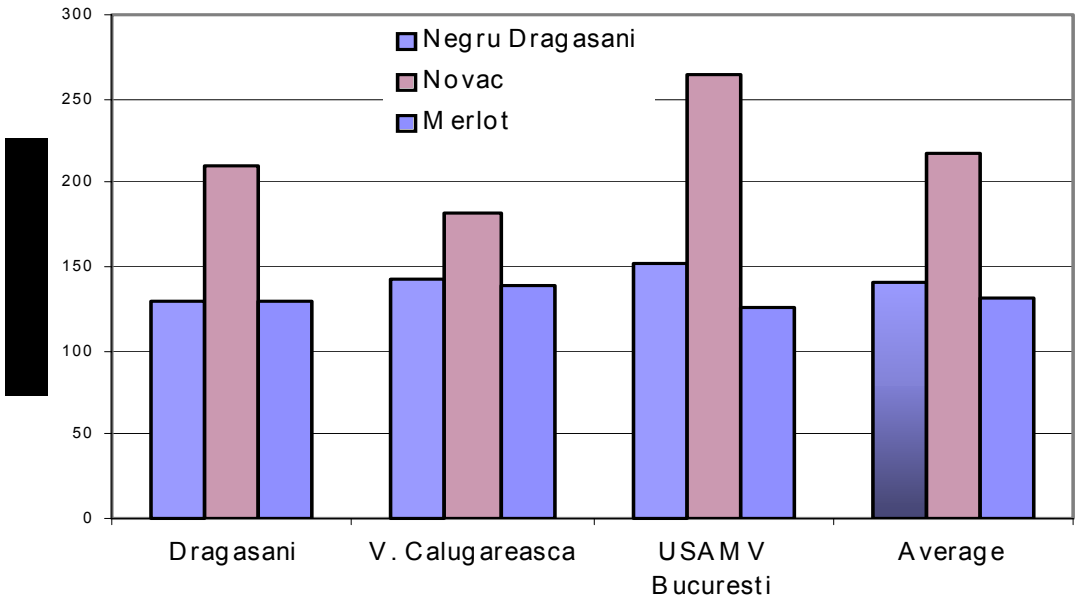

Fig.2. Variation of the 100 grains weight for the new red wine varieties.

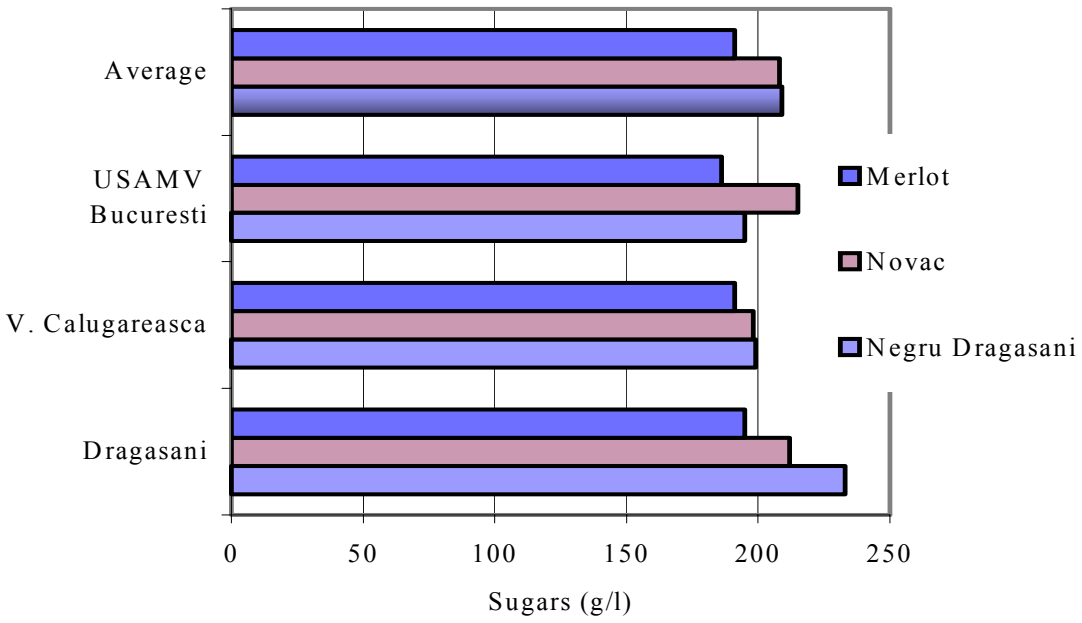

Fig.3. Average sugars content during three years of cultivation in different area

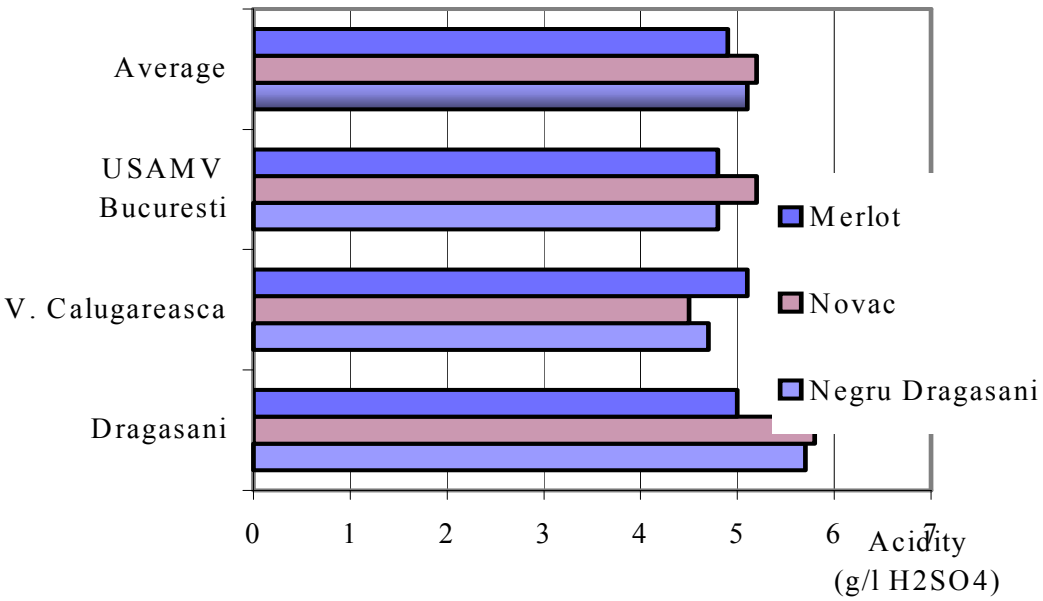

Fig.4. Average total acidity during three years of cultivation in different area

Procedure of reverse osmosis used in conditioning and stabilization of wines – its effect on preservation wines quality

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Keywords: membranes, filtrate, concentrate, biotechnology, ecological.

ABSTRACT

The work paper „Procedure of reverse osmosis used in conditioning and stabilization of wines – it's effect on preservation wines quality" has as the aim the substitution of traditional technology (the deferrization with ferrocyanide of potassium), with a negative impact on environment with a new biotehnology based on the extraction of the excess of ferric and copper ion from wine using membranes of reverse osmosis. The studied wines are passed through the reverse osmosis module, abaft this process diminishes the iron and copper ions concentration from the wine submitted to the osmosis, in the end obtaining a wine which maintains the parameters and the naturalness of the initial wine. Through new biotehnology is obtained an ecological product from viewpoint of conditioning and stabilization.

INTRODUCTION

The method of reverse osmosis used in the technology of conditioning and stabilization of white the red wines replaces an old tehnology with negative impact on environment due to the ferric ferrocyanide deposit constituted after ferrous iron complexation with ferrocyanide of potassium with a new biotehnology which has as the aim the biological stabilization of the product, quality preservation of the product.

MATERIALS AND METHODS

During the scrool of the project 2005-2008, at SCDVV Murfatar reserchers are made regarding conditioning and stabilization technology of white and red wines, with special reference to the ferric and copper ions, their elimination through membranes of reverse osmosis, to a content of 3-4 mg/l iron and 0,1-0,3 mg/l copper, without unbalances in the wine composition.

The experiments were performed on white red wines from types: Pinot gris, Chardonnay, Riesling Italian, Columna, Muscat Ottonel and Cabernet Sauvignon, Merlot, Pinot Noir, Mamaia, Feteasca Neagra. After obtaining wines, they were conditioned through treatment gelatine and bentonite, filtrate with semisterile plates KK7, physico-chemical analized and appreciate from organoleptic viewpoint. After these operations are made, wines are passed through the reverse osmosis module equiped with polyamidic membranes.

RESULTS AND DISCUSSIONS

Wines obtained from the harvest of the year 2007, were conditionized and physico-chemical analized before and after passing through reverse osmosis module and results are presented in table 1 and 3 for white wines and table 2 and 4 for red wines.

The degustation was made close without knowing the samples or the assortment, they were presented after their sugar content.

Organoleptical apreciation was made by a jury composed of 7 members, authorized tasters being in ADAR evidence. The order of presentation begain with dry, demi-dry, demi-sweet and sweet wines, white and red, the notation being on a scale from 1 to 10.

The notes obtained at the organoleptical appreciation of white and red wines, before and after passing through the reverse osmosis module are presented in table 5 and 6.

CONCLUSIONS:

1. From the physico-chemical analyses of white and red wines, corelate with organoleptical appreciations, from harvest of the year 2007, wines are well-balanced, extractive, typical for the cultivated kinds in the Murfatlar vineyard.
2. The evolution of white and red wines from harvest 2005, emphasizes their superior quality after application of a new technology to stabilize the ferric and copper ions using membranes of reverse osmosis.
3. New technology preserves the primary aromas of each kind, further emphasizes the kind and the vineyard character.
4. Biotehnology application to stabilize the ferric copper ions through the use of the membranes of reverse osmosis, leads to the preservation of the wine components, don't modifies physico-chemical structure of the wine on the contrary kept it's naturalness, asuring him a positive evolution in time.
5. New biotehnology feels like solve a series of problems related to conditioning and stabilization of wine, raising thus lath quality, placing the wine in a row of foods with big degrees of complexity and protector effect on consumers.

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Tables

Table 1. The physico-chemical analyses of white wines from the harvest of the year 2007, before passing through the reverse osmosis module

Nr crt	Assortment / Determinate compounds	Pinot gris	Chardonnay	Riesling italian	Columna	Muscat Ottonel
1	Alcohol contens (% vol)	11,8	12,1	11,6	11,8	12,0
2	Reduced sugar (g/l)	42,0	58,0	2,6	4,0	28,0
3	Total acidity (g/l H_2SO_4)	4,8	5,1	4,6	5,3	4,2
4	Volatile acidity (g/l CH_3COOH)	0,32	0,34	0,30	0,36	0,38
5	Reduced extract contents (g/l)	26,4	27,2	22,6	21,7	25,8
6	Ash contents (g/l)	2,3	1,94	1,86	1,84	1,98
7	Ash alkalinity (g/l	1,90	1,84	1,72	1,76	1,83
8	Glycerine (g/l)	8,9	8,4	7,9	7,85	8,4
9	Acetaldehyde mg/l	38	36	32	24	38
10	D_{420} nm (1 cm)	0,030	0,034	0,029	0,028	0,040
11	Total polyfenols (mg/l)	280	285	264	270	290
12	Taninic polyfenols (mg/l)	105	100	95	100	115
13	Total azote (mg/l)	380	350	340	345	385
14	pH wine	3,20	3,25	3,30	3,25	3,15
15	rH wine	15,0	15,40	14,08	15,0	15,10
16	Total polysaccharides (mg/l)	370,0	375,0	320,0	325,0	318,0
17	Total gums (mg/l)	192,8	187,0	185,0	190,4	194,7
18	Acid tartric (g/l)	4,24	4,5	4,17	4,25	3,42
19	SO_2 free (mg/l)	65	60	50	52	58
20	SO_2 total (mg/l)	170	189	120	95	165
21	Iron (mg/l)	9	8	7	8	7
22	Copper (mg/l)	0,45	0,70	0,90	0,85	0,70

Table 2. The physico-chemical analyses of red wines from the harvest of the year 2007, before passing through the reverse osmosis module

Nr crt	Asortment / Determinate compounds	Cabernet Sauvignon	Merlot	Pinot noir	Mamaia	Fetească neagră
1	Alcohol contens (% vol)	12,5	12,7	11,8	11,7	12,4
2	Reduced sugar (g/l)	2,4	2,6	38,0	42,0	3,7
3	Total acidity (g/l H_2SO_4)	4,2	4,5	4,3	4,0	4,2
4	Volatile acidity g/l CH_3COOH	0,42	0,38	0,36	0,41	0,78
5	Reduced extract contents (g/l)	28,4	27,6	25,9	27,6	26,6
6	Ash contents g/l	1,84	1,82	1,79	1,84	1,80
7	Ash alkalinity g/l	1,38	1,34	1,42	1,50	1,59
8	Glycerine g/l	8,82	8,74	8,90	8,74	8,84
9	Acetaldehyde mg/l	40	38	39	42	41
10	Total polyfenols mg/l	2038	1545	2040	1190	1195
11	Tanin polyfenols mg/l	650	505	675	320	405
12	Simple polifenols mg/l	280	235	305	240	245
13	Colorant intensity (Is)	3,669	2,525	4,137	2,210	4,156
14	Tent colour (Ts)	0,610	0,822	0,540	0,590	0,744
15	Total azote mg/l	590	600	582	495	570
16	D_{280} nm(1 cm)	63	61	60	58	62
17	Antocians mg/l	640	320	466	364	369
18	pH wine	3,50	3,49	3,52	3,40	3,46
19	rH wine	16,0	15,72	15,42	15,24	16,0
20	Total polysaccharides mg/l	428	389	326	289	416
21	Total gums mg/l	298	282	224	220	300
22	Acid tartric g/l	3,7	3,67	3,60	3,58	3,62
23	SO_2 free mg/l	45	40	58	50	42
24	SO_2 total mg/l	90	86	140	156	82
25	Iron mg/l	8,6	9,8	7,2	8,0	8,6
26	Copper mg/l	0,56	0,64	0,46	0,72	0,84

Table 3. The physico-chemical analyses of Murfatlar white wines, harvest of the year 2007, after passing through the reverse osmosis module (Phase I and II)

Nr crt	Asortment / Determinate compounds	Pinot gris	Chardonnay	Riesling italian	Columna	Muscat Ottonel
1	Alcohol contens (% vol)	11,9	12,1	11,7	11,8	12,1
2	Reduced sugar (g/l	43,0	60,0	2,8	4,2	30,0
3	Total acidity (g/l H_2SO_4)	4,6	4,9	4,5	5,1	4,0
4	Volatile acidity (g/l CH_3COOH)	0,35	0,36	0,32	0,36	0,39
5	Reduced extract contents (g/l)	27,4	27,6	21,8	22,9	26,5
6	Ash contents (g/l)	2,8	2,3	2,1	2,1	2,4
7	Ash alkalinity (g/l	1,9	1,8	1,7	1,7	1,8
8	Glycerine (g/l)	9,1	8,6	8,2	8,1	8,6
9	Acetaldehyde mg/l	40	38	35	28	39
10	D_{420} nm (1 cm)	0,028	0,032	0,027	0,026	0,036
11	Total polyfenols (mg/l)	282	288	270	274	301
12	Taninic polyfenols (mg/l)	108	102	98	104	117
13	Total azote (mg/l)	380	350	340	345	385
14	pH wine	3,15	3,20	3,35	3,30	3,25
15	rH wine	16,0	15,6	14,8	15,2	15,4
16	Total polysaccharides (mg/l)	375	380	325	336	324
17	Total gums (mg/l)	194	190	187	192	195
18	Acid tartric (g/l)	4,20	4,30	4,10	4,15	3,48
19	SO_2 free (mg/l)	54	56	48	47	50
20	SO_2 total (mg/l)	165	180	115	90	156
21	Iron (mg/l)	4,5	3,6	3,9	4,01	3,9
22	Copper (mg/l)	0,22	0,16	0,24	0,28	0,21

Table 4. The physico-chemical analyses of Murfatar red wines, harvest of the year 2007, after passing throug the reverse osmosis module (Phase I and II).

Nr crt	Asortment / Determinate compounds	Cabernet Sauvignon	Merlot	Pinot noir	Mamaia	Fetească neagră
1	Alcohol contens (% vol)	12,6	12,7	11,9	11,8	12,5
2	Reduced sugar (g/l)	2,6	2,8	40	44	4
3	Total acidity (g/l H_2SO_4)	4,1	4,2	4,0	3,8	4,0
4	Volatile acidity g/l CH_3COOH	0,38	0,36	0,34	0,39	0,35
5	Reduced extract contents (g/l)	29,5	28,7	29,0	26,5	27,0
6	Ash contents g/l	2,05	2,0	1,85	1,94	1,90
7	Ash alkalinity g/l	1,40	1,38	1,42	1,48	1,53
8	Glycerine g/l	8,90	8,85	9,0	8,94	8,95
9	Acetaldehyde mg/l	38	36	37	40	39
10	Total polyfenols mg/l	2075	1640	2055	1200	1250
11	Tanin polyfenols mg/l	750	560	680	320	395
12	Simple polifenols mg/l	278	245	310	260	255
13	Colorant intensity (Is)	3,740	2,620	1,250	1,300	2,350
14	Tent colour (Ts)	0,620	0,830	0,560	0,600	0,750
15	Total azote mg/l	570	610	590	490	575
16	D_{280} nm(1 cm)	65	63	61	59	64
17	Antocians mg/l	645	340	470	380	375
18	pH wine	3,40	3,35	3,4	3,25	3,30
19	rH wine	16,20	15,80	15,35	15,30	15,90
20	Total polysaccharides mg/l	410	360	315	290	385
21	Total gums mg/l	290	275	210	210	295
22	Acid tartric g/l	3,6	3,5	3,55	3,45	3,54
23	SO_2 free mg/l	40	38	52	46	38
24	SO_2 total mg/l	88	82	135	150	80
25	Iron mg/l	3,2	4,0	3,86	3,50	3,67
26	Copper mg/l	0,14	0,23	0,18	0,24	0,20

Table 5. The notes obtained at the organoleptical appreciation of white wines

Nr. crt.	Members of jury / Asortment	M1	M2	M3	M4	M5	M6	M7	Media
1	Riesling italian ***	16,4	17,1	16,6	16,7	15,9	16,0	16,2	16,41
2	Riesling italian**	15,6	15,9	14,8	15,2	15,6	16,4	15,8	15,61
3	Riesling italian *	14,8	15,2	14,6	14,9	15,4	16,1	15,5	15,21
4	Columna ***	16,8	16,5	16,9	17,6	17,0	16,7	16,3	16,83
5	Columna **	16,2	15,8	14,9	15,1	15,9	16,2	15,6	15,67
6	Columna *	14,2	14,8	14,5	14,9	14,7	15,2	15,0	14,76
7	Pinot gris ***	18,2	18,0	18,3	18,4	17,9	17,8	18,6	18,17
8	Pinot gris **	16,4	16,5	17,0	16,6	16,7	16,9	16,5	16,66
9	Pinot gris *	15,9	16,0	16,1	15,9	15,8	16,2	16,0	15,98
10	Chardonnay ***	18,6	17,9	18,2	19,0	19,8	18,4	17,8	18,53
11	Chardonnay **	17,0	17,4	16,9	17,2	17,8	17,9	17,5	17,38
12	Chardonnay *	16,7	16,9	16,5	17,2	16,2	16,4	16,3	16,60
13	Muscat Ottonel ***	18,2	17,8	19,0	18,6	18,3	19,1	18,9	18,56
14	Muscat Ottonel **	16,0	16,7	15,9	16,8	17,0	17,2	17,4	16,71
15	Muscat Ottonel *	15,8	15,9	15,6	16,0	15,8	16,3	15,4	15,83

Table 6. The notes obtained at the organoleptical appreciation of red wines

Nr. crt.	Members of jury / Asortment	M1	M2	M3	M4	M5	M6	M7	Media
1	Cabernet Sauvignon ***	17,6	18,4	16,9	18,9	17,8	17,4	18,0	17,85
2	Cabernet Sauvignon **	16,2	16,7	16,8	16,5	17,1	16,6	16,3	16,6
3	Cabernet Sauvignon *	15,6	16,1	16,4	15,9	16,2	16,5	15,9	16,08
4	Merlot ***	17,2	17,6	16,7	16,9	17,0	17,3	17,6	17,18
5	Merlot **	17,3	17,5	16,9	16,8	17,0	17,6	17,9	17,28
6	Merlot *	16,4	16,8	15,9	16,4	16,9	17,1	16,8	16,61
7	Fetească neagră ***	18,6	19,4	18,9	19,0	18,2	17,9	19,0	18,71
8	Fetească neagră **	17,2	16,9	17,0	16,6	16,7	17,2	17,0	16,94
9	Fetească neagră *	16,2	16,4	15,8	15,7	16,0	15,9	16,4	16,05
10	Pinot noir ***	18,1	17,8	18,4	17,9	17,6	18,8	18,9	18,21
11	Pinot noir **	16,0	15,9	14,8	14,9	15,3	15,6	16,0	15,5
12	Pinot noir X*	15,4	14,8	14,6	15,1	15,6	14,7	14,9	15,01
13	Mamaia **	16,8	17,1	17,6	18,0	16,9	15,9	17,0	17,04
14	Mamaia *	15,2	14,8	15,0	14,7	15,4	16,0	16,2	15,32

Note:
*= white (red) wine, harvest of the year 2007, before the extraction of the excess of ferric and copper ions
**= white (red) wine, harvest of the year 2007, after the extraction of the excess of ferric and copper ions
***= white (red) wine harvest of the year 2005.

Soil water reserve dynamics in grapevine plantations and its influence on the production of grapes under the environmental conditions characteristic for the year 2007 in the Vineyard of Odobeşti

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Keywords: environmental conditions, water deficit, grape vine varieties reaction

ABSTRACT

Although grapevine is considered to be resistant to hidric stress, persistent pedological drought may significantly affect vines and their productive ability. The precipitation deficit is characteristic for the years 2006-2007, in the Vineyard of Odobesti, starting with the autumn of the year 2006 and all through the cold season, excepting January and March, when values of above average were recorded and increased considerably in the months of April, May, June, and July. The precipitation deficit superposed on a period of high temperatures (between the 16^{th} -30^{th} of July) when maximum temperatures on ground level of 50 and 60˚C were signaled. Under these conditions the active water reserves decreased dramatically, descending under 50% of the AIU in the months of June (41,3%), July (16,2%) and August (41,6%), which has determined disturbances in the progress of growth and development stages of the plants, respectively: the yielding of grapes, the intensive growth of offshoots and grapes, with direct implications on production and its quality.

INTRODUCTION

This paper aims to present, in progress, the evolution of the water reserve in the active soil layer, in grapevine plantations in the Vineyard of Odobesti under the specific conditions of 2007 year, and also its influence on the quality and quantity of production.

MATERIAL AND METHODS

Research has been conducted in the experimental field, respectively the experimental viticultural agrotechnics area of R-D Wg. Wm. S. Odobesti, described from the pedological point of view as aric-cambic black soil with carbonates at a depth of at least 100 cm, formed on loessoid clay of weak settlement and erosion. The research perimeter contains terrains on a slope of 3 to 10% with a mainly eastern exposure.

The quantity of soil-water has been determined through the gravimetric method. For determining the active water reserve in the soil, samples were collected with a tubular probe, on successive layers of 20 cm each, down to a depth of 100 cm, and corresponding to each month from the beginning of the year. The active reserve or the available soil-moisture at a given moment (Uacc) and the deficit were calculated with the aid of hydrophysic indices. Also, in order to establish the degree to which available humidity is provided for the plants, the active water reserve or the available soil moisture (Uacc), with reference to the available soil water capacity (AWC) or the active moisture interval(AIU), calculated previously for the Vineyard of Odobesti.

RESULTS AND DISCUSSIONS

Although statistically, 2007 year may be described as having a surplus of precipitation – rainy, the most part of the vegetation period (April, May, June, July and the first ten days of August) received little precipitation.The continuous decrease in the soil-water reserve up to the last decade of April 2007 has been caused primarily by the aggravation of the precipitation deficit recorded in the autumn of 2006 (comparative to

the multiannual average), starting with the month of October but also by the high temperatures during the vegetation period. The climatic elements contributing to the water-reserve decrease are shown in Table1.

By comparing the total of precipitation recorded in the interval October 2006-August 2007 with the normal values for this period in the Vineyard of Odobesti, a constant precipitation deficit is observed, with the exception of the months of January and March, when a slight surplus was recorded.

Thus, as is shown in Table 1 and Figure 1, in October 2006 only 13.1 mm were recorded, compared to the multiannual value (41.3 mm), in November 1.8 mm compared to 33.7mm, in December 14.0 mm compared to 33.7mm. From the pluviometric point of view, the year 2007 started with a slight surplus of precipitation, recorded a surplus in March, after which, simultaneously with plants entering the period of vegetation (starting with April and up until the third decade of August), the precipitation deficit increased on a monthly basis (38.8mm compared to 85.4 in April; 28,8mm compared to 68.5 in May; 50.0 mm compared to 56.6mm in July; in August, the entire amount of precipitation, namely 127.0mm, was recorded in the last decade of the month).

By analyzing the data in Table 2 it may be observed that, although the sum-total of precipitation in 2007 year (917.1mm) is considerably higher than the multiannual average (598.0mm), there was an uneven distribution throughout the year, with a deficit in the first half of the year and an excess from September to December.

By analyzing the sum of precipitation in the vegetation period (653.4mm), despite being superior to the multiannual average value (450.65mm), it may be observed from Figure 2 that most of the vegetation period (the month of April-half of the month of August), which is the proper period for the development of the main physiological processes in the plant, benefitted from only 23.7% of the total amount, while the rest of over 75% of the precipitation occurred towards the end of the vegetation period (the third decade of August and the months of September and October).

This precipitation deficit combined with the average monthly temperatures recorded in the vegetation period, which were mostly above the normal multiannual values, presented in Table 3 and Figure 3, (respectively : 11.4 ˚C compared to a normal of 11.0˚C in the month of April; 19.0˚C compared to a normal of 16.3 ˚C in the month of May; 23.1˚C compared to a normal of 20.1˚C in June; 25.3˚C compared to a normal of 21.9˚ C in July;23.9˚C compared to a normal of 21.3˚C in August, as well as the maximum temperatures recorded at ground level of up to 60˚C in the month of July), led to a constant decrease in the available water reserve in the soil.

In the mentioned agroclimatic context we specify that from the specialized literature (M. Oşlobeanu and Contributors, 1980:Tardea and Dejeu, 1995) and the actual experimental data, the optimal soil moisture for grapevine culture has a value of 50 to 70% of the active water reserve or the available water capacity of the soil (AWC). The higher registered values are favorable to de-budding and the intensive growth stage of the off shoots and grapes while the lower values favor the blooming phenological phases, the ripening of the grapes and the maturing of the wood.

Thus, if up to the month of May the active water reserve of the soil registered values of over 50% of the AIU throughout the entire observed profile (0-100 cm), starting with the month of June, the active water supply in the soil decreased

considerably as it may be observed from the data in Tables 3 and 4 and graphical representation in Figure 3

The most drastic reduction of the active water reserve in the soil has been observed during July and the first decade of August, when very low values were recorded (under 6% of the AIU in the 0-20 cm layer and 16.2% of the average AIU throughout the entire profile). Thus, the water deficit correlated with a high average temperature (25.3˚C compared to the multiannual of 21.9˚C in July and 23.89˚C compared to the multiannual of 21.3˚C in August) produced metabolic disturbances in the growth and development processes and respectively, the normal development of the phenological phase of growth in the offshoots and the grapes, quickening the maturation of the grapes.

We may observe through the graphical representation of data (fig. 4) that the time interval with the maximum deficit of soil-water overlaps the plant's maximum consumption period which amplifies the negative systemic effects, respectively: metabolic disturbance, decrease in assimilation activity , excessive physiological fall of the grapes, abnormal premature physiological maturation etc.

Starting with the third decade of August and continuing through September and October, the hydric regimen of the soil improved, as a consequence of the precipitation (127mm compared to a normal of 37.6 in the month of August; 148 mm compared to a normal of 49.6mm in the month of September and 221.6 mm in October, compared to a normal of 41.3mm).

The aggravation of soil-water deficit in the vegetation period, combined with extremely high temperatures, recorded in air as well as at ground level (close to 60˚C), accelerated the ripening of the grapes with an average of 10 to 12 days and it reflected upon the quantitative and qualitative characteristics of the grape harvest.

Thus, in the varieties studied in the comparative analysis of data registered in 2007 compared to the multiannual data, centralized in Table 4, it may be observed that: the average weight of a cluster of grapes was lower in 2007 relative to the multiannual values, respectively 106g compared to 120g in the Royale Feteasc ă variety and 78g compared to 85 g in the Riesling italian variety. This resulted in a lower average output compared to the multiannual average obtained on a surface unit, respectively 11656kg/ha compared to 12861 kg/ha in the Royale Feteasc ă variety and 7815kg/ha compared to 9148kg/ha in the Riesling italian variety.

From the point of view of sugar content, a higher content is observed compared to the average data registered over a period of 5 years, respectively: 198g/l compared to 189h/l in the Royale Feteasca variety and 203g/l compared to 191 g/l in the Riesling italian variety.

CONCLUSIONS

The water deficit and the high air temperature (38.6˚C) and at ground level (about 60˚C) in the months of June-the first half of August determined metabolic disturbances in plants which reflects in the slow growth and development of the offshoots and the grapes, the forced untimely maturation of the grapes, premature ripening, small grapes with an insufficiently developed mesocarp.

The combination of drought and high temperatures also led to an acceleration in the ripening of grapes with approximately 10 to 12 days compared to the multiannual average.

Under such conditions the negative effects may be observed at the production level: the average obtained output is lower by about 1250kg/ha(12%).

Positive influences may also be observed in the sugar content which exceeded the average by about 11.5g/l , respectively 5% (average values).

According to submitted data and the results obtained, the continuation of research is recommended, concerning the control of the negative effects of the drought on the grapevine by developing the adequate technological measures.

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Tables

Table 1. Temperatures and precipitation registered in the Vineyard of Odobesti during the interval October 2006 – October 2007

Climatic element		Oct.06	Nov.06	Dec.06	Ian.	Feb.	Mar.
Temperature average	Monthly	13.1	7.3	2.0	5,3	2,4	7.4
	Multiannual	11.0	4.3	0.2	-0.9	0.2	4.5
Precipitation average	Monthly	13.1	1.8	14.0	44.1	24.3	100.9
	Multiannual	41,3	33.7	33.9	31.7	49	75.6

During the vegetation period (April-October)

Climatic element		Apr.	May	June	July	Aug.	Sept.	Oct.
Temperature average	Monthly	11.4	19.0	23.1	25.3	23.89	15.9	11.5
	Multiannual	11.0	16.3	20.1	21.9	21.3	16.7	11.0
Precipitation average	Monthly	38.8	28.8	50.0	37.6	127.0	148.0	221.6
	Multiannual	85.4	68.5	56.6	42.9	37.6	49.6	41.3

Table 2. Temperature and precipitation progress in the Vineyard of Odobesti in 2007

Climatic element/Month		Jan.	Feb.	Mar	Apr.	Mai.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Temperature°C average	Monthly	5.3	2.4	7.4	11.4	19.0	23.1	25.3	23.89	15.9	11.5	3.1	1.05
	Multiannual	-0.9	0.2	4.5	11.0	16.3	20.1	21.9	21.3	16.7	11.0	4.3	0.2
Precipitation (mm) average	Monthly	44.1	24.3	100.9	30.0	28.8	50.0	37.6	127.0	48.0	221.6	32.0	62.4
	Multiannual	31.7	49.0	75.6	85.4	68.5	56.6	42.9	37.6	49.6	41.3	33.7	33.9

Table 3. The active water reserve dynamics during January-March 2007 in the Vineyard of Odobesti, expressed in mc/ha and percentages of AIU

Depth (cm)	Month					
	I		II		III	
	mc/ha	% of AIU	mc/ha	% of AIU	mc/ha	% of AIU
0 - 20	226.66	76.66	180.55	67.49	223.37	74.93
20 – 40	230.26	75.17	219.16	75.6	238.47	77.56
40 – 60	187.84	58.02	163.95	54.11	166.58	50.81
60 - 100	214.05	36.89	122.04	36.87	185.24	39.13
Average (0–100)	214.7	61.69	171.43	58.52	203.42	60.61

Table 4. The active water reserve dynamics during April-October 2007 in the Vineyard of Odobesti, expressed in mc/ha and percentages of AIU

Parameters/Indicators			Depth (cm)				
			0 – 20	**20 – 40**	**40 – 60**	**60 – 100**	**Media**
Month	IV	mc/ha	233.69	250.82	230.31	267.54	247.84
		%of AIU	78.89	82.05	71.14	62.82	73.73
	V	mc/ha	188.88	209.82	243.93	299.78	235.60
		%of AIU	64.01	68.74	75.79	68.41	69.24
	VI	mc/ha	106.52	145.49	142.49	178.21	143.18
		%of AIU	34.96	46.64	44.07	39.7	41.34
	VII	mc/ha	17.42	78.04	58.5	17.98	54.9
		%of AIU	5.96	25.63	65.64	14.9	16.2
	VIII	mc/ha	134.07	124.64	122.47	159.7	134.47
		%of AIU	51.91	40.62	37.58	36.56	41.66
	IX	mc/ha	177.43	213.06	189.89	177.02	189.10
		%of AIU	58.66	69.38	58.87	38.46	56.34
	X	mc/ha	289.98	293.27	297.47	269.23	287.49
		%of AIU	99.2	96	92.54	59.81	86.89

Table 4. Qualitative and quantitative characteristics of the grape production in Royal Feteasca and Italian Riesling varieties registered at R-D Wg. Wm. S. Odobesti in 2007

Variety		**Weight of a cluster (g)**	**Sugar (g/l)**	**Production**	
				Kg/but.	**Kg/ha**
Royale Feteasca	Values 2007	106	198	3.49	11.656
	Multiannual average	120	189	3.86	12.861
Riesling italian	Values 2007	78	203	2.34	7.815
	Multiannual average	85	191	2.67	9.148

Figures

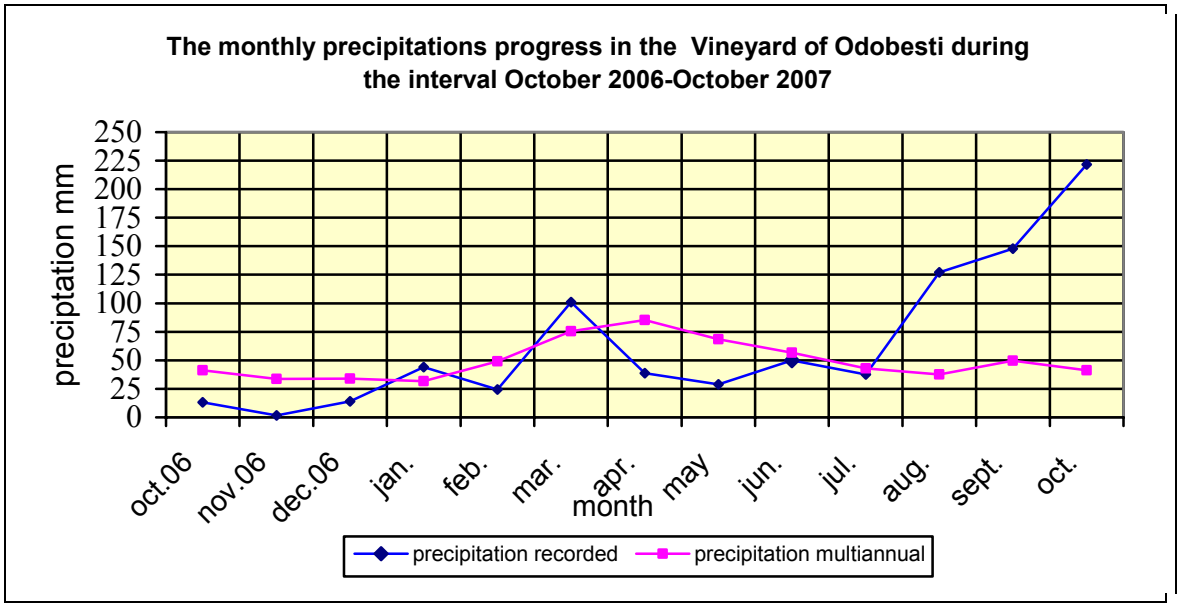

Fig. 1. The monthly precipitation progress in the Vineyard of Odobesti during the interval October 2006-October 2007

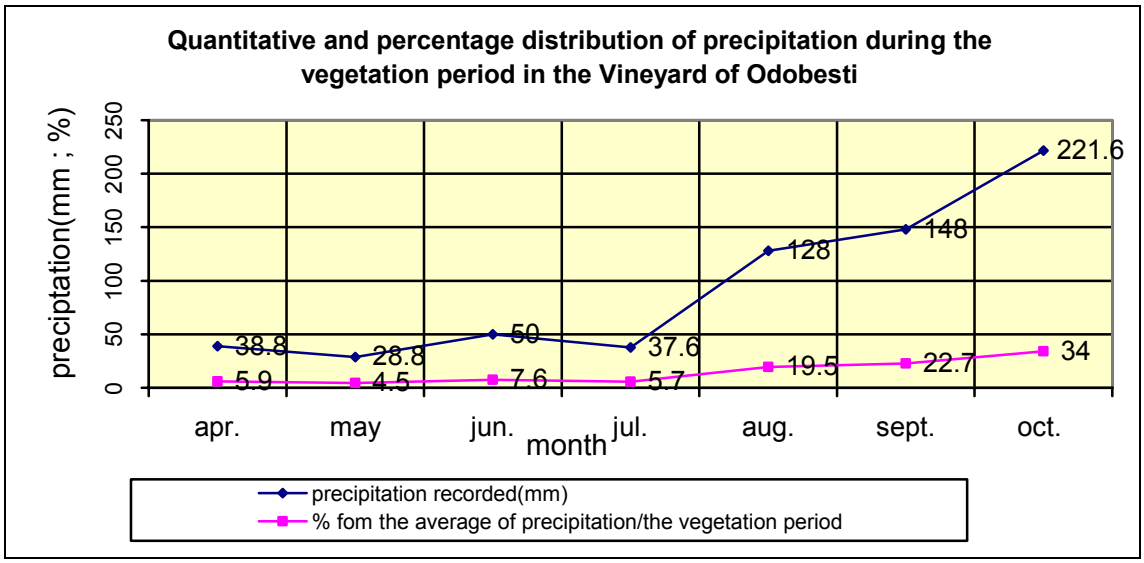

Fig. 2. Quantitative and percentage distribution of precipitation during the vegetation period in the Vineyard of Odobesti

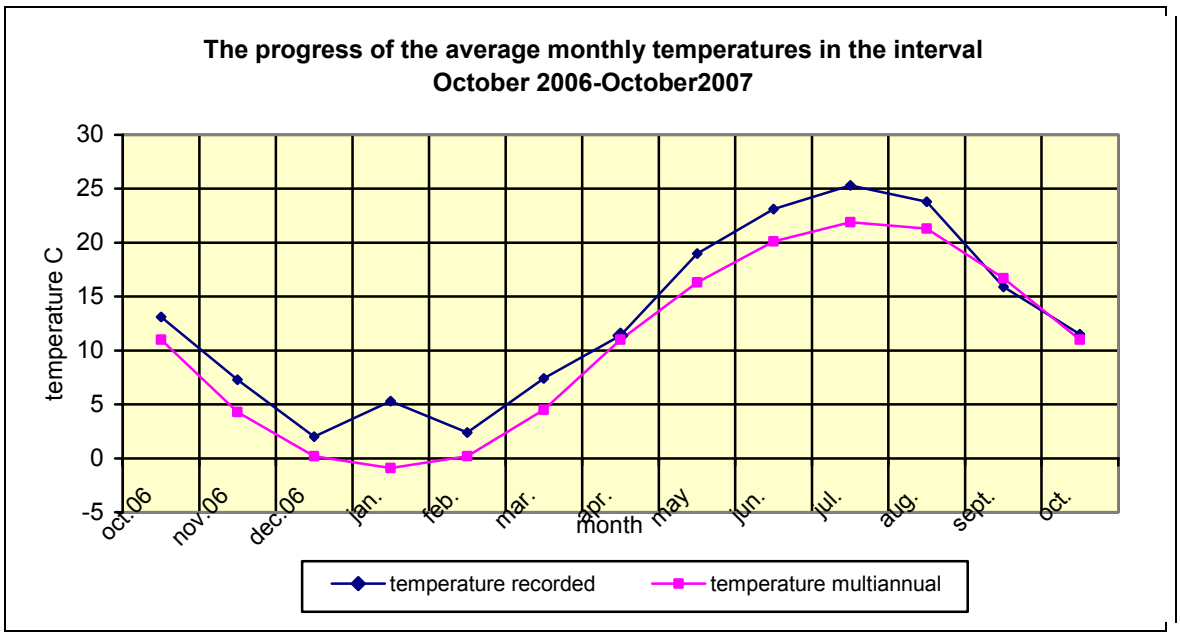

Fig. 3. The progress of the average monthly temperatures in the interval October 2006-October 2007

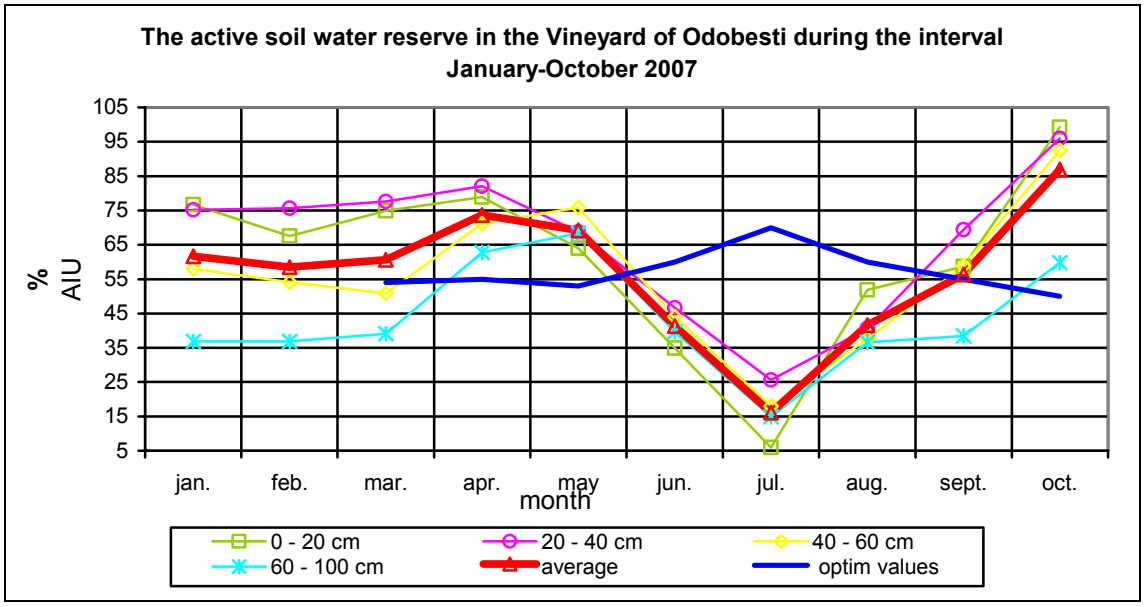

Fig. 4. The active soil water reserve in the Vineyard of Odobesti during the interval January-October 2007

Results regarding Feteasca Alba wines analysis using an „electronic nose" instrument

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Keywords: sensory analysis, Feteasca alba, electronic nose, volatile components, gas chromatography

ABSTRACT

In our country the methodology for food products discrimination is not very clear definite, priority being sensory analysis. In many cases this method is very good, but the vacuity of good defined methodologies makes sensory analysis specialists activity more difficult. Preoccupations regarding the correlation of tasters responses with data obtained from electronic nose instrumentation demonstrated the necessity of a good repeatability and accuracy of both sets of information. The present study regard the evaluation of wine discrimination methods using the „electronic nose" equipment. Using two different polarity separation columns, which provide a specific response for each wine volatile component, similar to human nose, the Heracles analyzer (Alpha MOS) provide an unique impress for each product. The instrument is based on ultra-fast gas chromatography and the translation and interpretation process, specific to human olfactory sense is substitute by a powerful software (Alpha Soft ver. 11).

INTRODUCTION

The present paper provides some results regarding the Feteasca Alba wine analysis using the „electronic nose" analyzer.

Analytical methods such as gas chromatography-mass spectrometry (GC-MS), or near infrared (NIR) provide the mainstay for measurement of volatile components in food, agricultural, chemical or environmental industries. Although data obtained give very precise measurements of individual components in a mixture, they give very poor indications of the sensory quality perceived by the human nose or tongue. The control of odour quality within these industries is associated with problems that are unique, because they also rely on human perception and preference for particular types of odours or tastes.

On international plan were performed many tests to obtain the olfactory impress of a product with a view to compare it after one hour, one day, one week with a new sample or to classify different lots reporting them to a quality standard for origin identification, possible contamination or peculation. The use of high performance sensors lead to better results in a shorter time but also increase low volatility components discrimination power. Key features include versatility and speed of analysis, giving the product cost and productivity advantages over the more traditional gas chromatographs.

Less requirements for sampling, simplicity and speed of analysis gives the electronic nose a big advantage over current methods used for peculation tracking or verify the presence and the concentration of certain substances in food products.

MATERIALS AND METHODS

To realize the proposed study it was used the Heracles Alpha MOS analyzer form Sensofood Laboratory – Department of Viticulture and Enology, University of Agronomical Sciences and Veterinary Medicine, Bucharest. The HERACLES instrument is a programmed temperature gas chromatograph using syringe or valve inlets to a flash evaporator. The sample is delivered to an adsorbent trap to concentrate the sample for delivery to twin capillary columns (GC#1 DB-5, 2m, Apolar/GC#2 DB-

1701, 2m, Medium polarity) and flame ionization detectors (FID) simultaneously. The hydrogen used as carrier gas must be FID Grade (Ultra High Purity 99.999%). To start the analysis you must set and verify the method parameters (Table nr. 1): injector parameters (sample volume, syringe type, injection time and speed, columns, trap, injection port and detectors temperature and pressure, gas pressure = 2.8 bar) and autosampler HS 100 parameters (injection coordinates for trays, injector and oven, incubation temperature, time and pressure). A set of 8 "Blank Run" (no sample) should be performed before the first analysis run of the day. This will flush any contaminants such as septum bleed or other high boiler buildup from the system. After that we verify the system components communication (injector and autosampler) for the automatic method. The sample is injected into the hot Injection Port, vaporizes (if necessary) and passes into the ambient temperature trap, hydrogen gas carrier passing through the glass injection port liner. After samples are focused and concentrated onto the trap, internal pressures within the injector/trap assembly equilibrate for a few seconds and then the trap is heated to its appropriate desorbtion temperature. During the analysis time, the instrument injects the sample into the separation columns and the chromatographic separation takes place. At the end of an analysis, all internal instrument components return to their initial temperatures and flow states, and the instrument becomes ready for the next analytical cycle.

RESULTS AND DICUSSIONS

Primary results are obtained as chromatograms (Fig.1). Each peak on those chromatograms correspond to a sample's volatile chemical substance, detected by the instrument's chromatographic columns after a period from sample's injection (retention time). The peak's area and height are correlated with the detected substance concentration. The construction principle of this electronic nose stipulates that every peak may be considered as a response provided by a virtual sensor of the instrument. A big number of peaks, correlated with the existence of two chromatographic columns with complementary properties, lead to a big number of sensors assuring the instrument a very good sensitivity.

Processing the chromatograms data we achieve integration tables which redound to database = "library" constitution. Finally the obtained data are processed with the instrument's software (Alpha Soft ver. 11.0) through multivariate statistic methods (PCA – principal component analysis, DFA – discriminant function analysis) leading to graphic representation which allows samples discrimination and identification visualization.

A first attempt regarded the possibility to discriminate the tested wine samples in accordance with production year. Table nr.2 present 5 samples of Feteasca alba wine from 2006 and 8 samples of Feteasca alba wine from 2007. After we obtain the database from all these wines chromatogram's processing the instrument's software allows the selection of sensors (peaks) with the highest discrimination power. Five sensors (peaks) were established by the software as adequate to discriminate the two wine groups (Fig. 2). The parameter named "discrimination index" establish how good is the obtained discrimination; its value must be positive and high; a 39 value can be considered acceptable, especially when the analyzed wines are from different vineyards, with different production methods (Fig. 3).

PCA (Principal component analysis) analysis pursue the identification of a smaller number of new variables constituted as linear combinations of initial variables,

analytic determined which may explain better the initial experimental data variability. Fig. 2 show that the software has identified a principal component (PC 1) which explain 99,328 % from initial data variability and represent a linear combination of the five sensors used. The second principal component, perpendicular on the first one, explains 0,622% from initial data variability.

Another multivariate statistic method, similar to PCA and permitted by the AlphaSoft program is DFA (discriminant function analysis). In this case the program identifies those initial data linear combination, named discriminant functions which assure a better separation (discrimination) of analyzed sample groups. Fig. 3 presents the DFA diagram concordant with the same data and sensors which provided fig. 2. Although in every group the wines are very different on the graphic we can see a very good group separation. A used program peculiarity shows that if we analyze only two groups of samples, the DF1 discriminant function has a 100% weight.

CONCLUSION

The obtained results indicate that the HERACLES instrument, due to its sensitivity and accuracy, has a great wine discrimination power, even if the discrimination criterion is the production year. Although the studied wine are different regarding vineyard, producer, technology, etc. the instrument was able to do an accurate distinguish between the two production years. Based on these results we can imagine many applications of the instrument and the presented method, applications which will be broached into posterior researches.

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Tables

Table 1. Method parameters

Parameter	Value
Sample volume	4 ml wine
Incubation temperature	60°C
Incubation time	600s
Injection volume	2500µl
Sampling time	20s
Trap temperature	40°C
Trap prepurge time	5s
Other trap parameters	Trap desorbtion temperature 250°C; trap preheat time 20s; incubation time 60s
GC program	Initial temperature 40°C (initial hold time 2s), final temperature 200°C (final hold time 5s), column heating rate 5°/s
Data acquisition time	40s
Injector temperature	200°C
Detector temperature	220°C

Table 2. Feteasca alba tested samples

Nr.	Production year	Vineyard	Producer
FA01	2007		Unicom Production
FA10	2006	Lechinţa	Prescon Mureş SA
FA11	2006	Terasele Dunării	Ostrovit SA
FA16	2007	Cotnari	Cotnari SA
FA17	2007	Cotnari	Cotnari SA
FA22	2007	Dealu Mare	Domeniile Viticole SRL
FA35	2007	Cotnari	Cotnari SA
FA41	2007	Dealu Mare Tohani	Oenotera SRL
FA68	2007		Vinia Iaşi SA
FA74	2006	Dealu Mare Tohani	Oenotera SRL
FA98	2006	Pietroasele	SCDVV Pietroasa
FA103	2006	Murfatlar-Medgidia	Fruvimed Medgidia SA
FA105	2007	Huşi	Duda Epureni

Figures

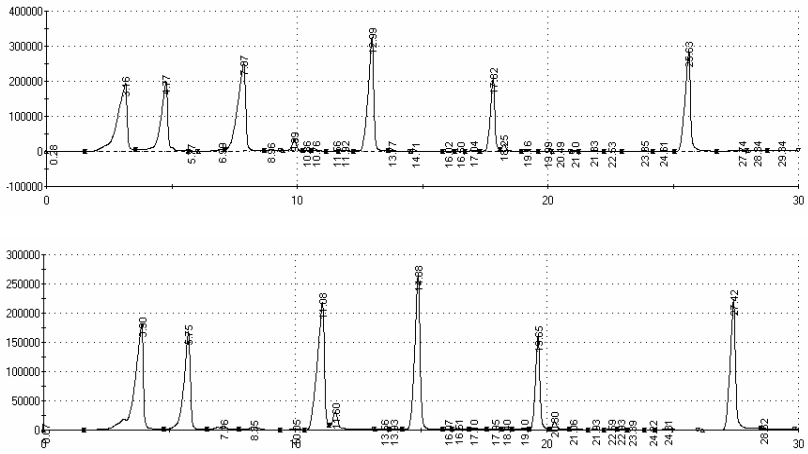

Fig. 1. The Feteasca alba (FA01) chromatograms obtained from the two chromatographic columns of Heracles analyzer.

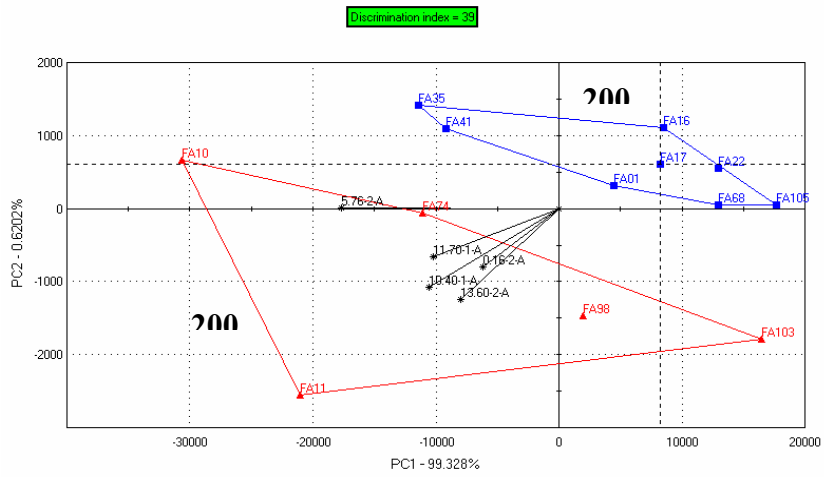

Fig. 2. PCA diagram for Feteasca alba wine discrimination in accordance with production year.

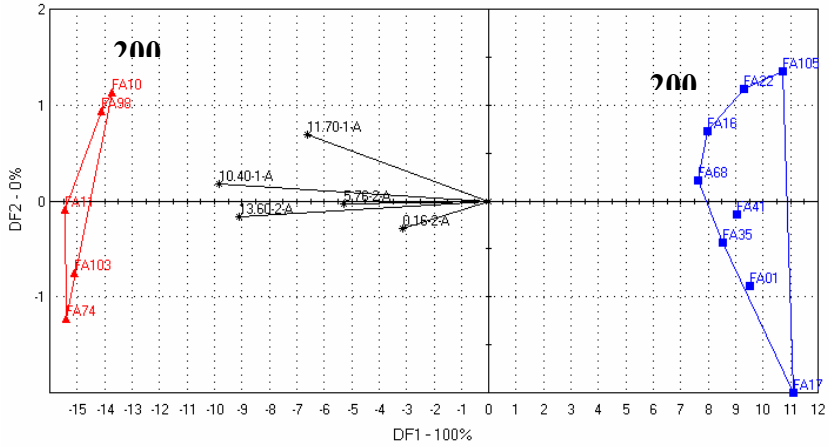

Fig. 3. DFA diagram for Feteasca alba wine discrimination in accordance with production year.

The behaviour of the Gros Sauvignon Variety in the ecological culture system in the Vineyard of Cotesti

Aurelia Podosu, Ghica Mihu, Lacramioara Miron

Keywords: indicators, technology, ecopedoclimatic conditions, pollutant, physiological processes

ABSTRACT

Ecological viticulture aims to develop a viable and durable agricultural system through the improvement of soil fertility and eliminating any type of polluting technology. Information is obtained by ascertaining and substantiating, through the experiment conducted in the Cotesti vineyard, the innovative demonstration of the ecopedoclimatic and socio-economical technological risk factors, which measure the level of observation of the ecological viticulture principles. For this purpose, at the Vine and Wine Research-Development Station Odobesti, an experimental lot with the Gros Sauvignon variety was studied, located in the Cotesti vineyard, respecting all phytosanitary safety precautions established by the control plan and aiming to obtain a less sensitive grapevine and a less aggressive parasite.

INTRODUCTION

Researches for the BIOTECH no 67 projects aimed to create a guiding methodology which would allow the ecological viticulture principles to be put to practice in accordance with the ecopedoclimatic and socio-economical conditions of the Romanian viticultural areas, with the purpose of attaining the levels of conformity requested by the national legislation aligned to the E.U. norms.

Ecological viticulture aims to create a durable and viable agricultural system through improving soil fertility and the elimination of all polluting inputs.

For this purpose, an experimental lot with the Gros Sauvignon variety was studied, in the Cotesti vineyard, by evaluating all the inductive factors which allow implementing culture technology in conformity with the principles of ecological viticulture.

The information is obtained, in the experiment conducted in the Cotesti vineyard, by ascertaining and substantiating the innovative demonstration of the eopedoclimatic and socio-economical technological risk factors which measure the observation of the ecological viticulture principles.

MATERIAL AND METHODS

The location of the experiment - The experiment is located in the Cotesti vineyard, the Carligele viticultural centre, T 45/1 Campeneanca, from the S.C.D.V.V. Odobesti Farm no. 5 and covers a surface of 1ha. Topographically, the experimental lot no.24 V is positioned on the no.45/1 field, on the farm no.5 territory Campeneanca, in the part called “Highroad”. The variety experimented on is the Gros Sauvignon, grafted on the SO4 rootstock. The plantation was set up in 2003, with a 2.2 planting scheme between rows and 1.1m between the vines on the rows, which resulted in a number of 4132 vines/ha.

Experimental alternatives - The experiment comprises two experimental technological soil maintenance variants, 0.5 ha with the standard soil maintenance variant – fallow land-conventional culture technology and 0.5 ha with ecological technology- fallow land. The form of guiding vines is semi-high; the pruning system is mixed, with the Guyot pruning type on half stem.

Other data on the experimental lot:
- slope ≤3%
- terrain exposure N-E
- weak alkaline reaction with pH values ranging between 8,28 - 8,25
- base saturation degree (V%) is of over 96%
- the humus content is fairly small 0.7%

The observations and ascertaining follow the influence of climatic factors in the experimentation period on the frequency and intensity of pathogenic agents' attack during growing season.

OBTAINED REZULTS

The year 2007 may be described as an atypical year from the climatic point of view fort the viticultural ecotope in the Cotesti vineyard, due to the lack of precipitation, in the form of snow during the out growing season and rain during the first part of the growing season, but with very high temperatures (compared to the multiannual average) recorded during July and the first decade of August, in the air and on ground level.

From analyzing the data about the main climatic indicators, showed in table 1, it may be observed that although the sum total of precipitation in 2007 (917.1mm) was above the multiannual average value for 10 years (647.2mm), the distribution was uneven, causing a deficit in the first half of the year and excess in the interval August-December.

The analysis of the precipitation sum in the growing season in 2007, shows a value superior to the multiannual average (653.4 in 2007 and 450.65mm the multiannual value). In the April-August interval, the proper period for the main physiological processes, the plant only received 23.7% of the total value while the rest of 75% of precipitation occurred toward the end of the growing season (late August, September and October) proving useless for the plants' metabolism.

The restrictive climatic indices of the development of pathogenic agents hold great importance in the ecological culture system, more than in the conventional culture system. The precipitation deficit and high temperatures are factors which have a negative influence on the pathogenic agents' development dynamics and also the power of the attack, with direct reference to the downy mildew of the grapevine - *Plasmopara viticola Berk et Curt* and the powdery mildew - *Uncinula necator Schw.* The analysis of the data in table 1 indicated favourable values for ecological viticulture being put into practice. Thus, for preventing and fighting against disease and pests in grapevine, two treatment schemes have been drawn up: for the ecological alternative and for the conventional alternative (table 2 and table 3).

Five preventive treatments were administered in the ecological alternative:
- the first treatment for the powdery mildew (*Uncinula necator Schw.*) on the 7th of May 2007 with lime sulphur wash, 15l/ha when offshoots had reached a lenght of approx. 5-7cm;
- the second treatment was applied on the 25th of May, before the blooming phenological phase, in order to prevent the downy mildew infections (*Plasmopara viticola Berk et Curt.*) and powdery mildew, using Funguran OH-50 WP -2 kg/ha and Kumulus S*-3 kg/ha;
- the third treatment was applied after petal fall (when 80% of the caps have fallen), for preventing downy and powdery mildew infections with Bordeaux mixture 5 kg/ha and Thiovit JET 80 WG -3kg/ha;

- the fourth treatment has been applied preventively during the growing of grapes, on warning.

In the conventional alternative - five treatments for preventing and fighting disease and pests, after the scheme in table 3, after EDT was reached.

The last treatment against downy mildew, powdery and grey mildew was done with a fermented nettle mixture (1kg fresh plant – 200 gr. vegetative material, dry substance, sun-steeped for 12-14 days, filtered and dilluted in 100l of water).

Pheromone traps have been placed for fighting against moth attack.

In the pathogenic agents monitoring program the evolution of the grey mildew of grapes (*Sclerotinia fuckeliana De Barry*) was observed, recording a frequency of 4.90% on 10.09.2007 (table 4), considering the atmospheric conditions during the ripening season and that the Gros Sauvignon variety is sensitive to the grey mildew (V1 –ecological variant, V2 – ecological variant).

From the analysis of the quantitative characteristics of the grape yield in the two presented variants, we may observe the following:

- concerning the average wight of a cluster of grapes, its value, both in the ecological and standard variants (58.2gr in the ecological variant and 48.1 in the standard variant) is noticeably lower than the average weight in the Gros Sauvignon variety (120 gr);
- the yield per vine (1.22 in the ecological variant and 1.24 in the standard variant) and the yield per ha (5,050 kg in the ecological variant and 5,150 kg in the standard variant) is a relatively low yield compared to the productive potential of the Gros Sauvignon variety;
- the sugar content varies between 211g/l in the ecological variant and 204g/l in the conventional variant;
- the acidity of the grapes varies between 5,7 g/l H_2 SO_4 in the ecological variant and 5,9 g/l H_2 SO_4 in the conventional variant.

CONCLUSIONS

From the presented data we may conclude the following:

1. From the agroclimatic point of view, the year 2007 may be described as atypical for the Cotesti vineyard, due to lack of precipitation under the form of snow, with very high temperatures recorded in July-August, both in the air and on ground level.
2. Cryptogamic diseases (downy and powdery mildew) manifested themselves atypically according to the source of infection, represented by the biological reserve of the bordering area, the primary and secondary infections, oily spots and fungus fructification, biology and evolution of pathogenic agents.
3. The disease and pest management treatments were aplied preventively, taking into consideration the economic damage threshold (EDT).
4. The average yield per vine (1.22 in the ecological variant and 1.24 in the standard variant) and the yield per ha (5,050 kg in the ecological variant and 5,150 kg in the standard variant) is low compared to the productive potential of the Gros Sauvignon variety.
5. As for the qualitative features, the values are normal, with the sugar content between 200 – 220 g/l, and the acidity between 5,7 g/l H_2 SO_4 in the ecological variant and 5,9 g/l H_2 SO_4 in the conventional.

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Tables

Table 1. Climatic indicators comparing the year 2007 with the average of the last 10 years

Climatic indicator	2007	Average 1996-2006	Differences
Duration of the air bioactive phase (no. of days)	203,0	192,9	10,1
The multiannual aver age temperature(°C)	12,3	10,9	1,4
Average temperature in June			
1st decade	21,8	19,4	2,4
2nd decade	23,9	20,8	3,1
Average temperature during the warmest month (July)	25,3	22,5	2,8
Maximum temperature average in August	28,8	26,6	2,2
The number of days with temperatures of over 30°C	49,0	23,8	25,2
The absolute maximum temperature in August	37,2	34,1	3,1
The absolute minimum temperature (°C)	-13,4	-15,5	-2,1
The global thermic assessment during growing season	3817,2	3622,9	225,3
The active thermic assessment	3678,0	3452,7	225,3
Thermal utility assessment (°C)	1838,0	1599,11	238,89
The sum of sunshine hours of day during growing season	1747,5	1558,69	188,81
The sum total of precipitation(mm)	917,1	647,2	269,9
The sum of precipitation during growing season	653,4	450,65	202,75
Air higroscopicity during growing season (%)	79,9	68,6	11,3
Heliothermic index real (IHr), Branas	3,21	2,75	0,46
Hydrothermic quotient (HQ), Seleaninov	1,77	1,43	0,34
Bioclimatic viticultural index (Ibcv),	4,87	7,30	-2,43
Oenoclimatic aptitude index (OAI), St.Teodorescu	5025,7	5294	-268,3

Table 2. Damaging agents control program for grapevine 2007– ecological variant

No.	The moment of treatment application	The treated disease or pest	Reccomended product *Active substance*	MU	Dosis Ha
0	1	2	3		4
1	50% of offshoots are 5-7 cm in lenght	Powdery mildew+ Grape bud mites (*Eriophyes vitis Nal.*)+ Grapevine thrips	Lime sulphur wash	l	15.00
2	Before blooming, when the offshoot has around 10-15 leaves. *Safety treatment*	Downy mildew+ Powdery mildew+	Funguran OH	Kg	2,0
			Thiovit JET 80WC	Kg	4,0
		Grape moth	Pheromone traps	Buc	2
3	After blooming, when 80% of the caps have fallen *Safety treatment*	Downy mildew+ Anthracnose+ Read leaf spot	Funguran OH	Kg	2,0
		Powdery mildew+ Grey grape mold	Thiovit JET 80WC	Kg	4,0
4	Grape growth, 8-10 days after treat.3	Downy mildew+	Champion 50 WP	Kg	3.00
		Powdery mildew	Thiovit JET 80WC	Kg	4,00
5	Grapes and clusters reached the characteristic size for the variety before ripening	Downey mildew+ Powdery mildew+ Grey grape mold	Plant extract treatment (live nettle + common yarrow*) 2/5 dilution	l	solution diluted*

*fermented mixture from 1 kg of fresh plant or 200g of dry vegetative material and 100 l of water

Table 3. Damaging agents control program for grapevine 2007 - traditional variant

No.	The moment of treatment application	The treated disease or pest	Reccomended product *Active substance*	MU	Dosis Ha
0	1	2	3		4
1	50 % of offshoots measure 5-7 cm in lenght	Powdery mildew+ Grape bud mites (*Eriophyes vitis Nal.*)+ Grapevine thrips	Lime sulphur wash	l	15.0
2	Before blooming, when the offshoot has around10-15 leaves. *Safety treatment*	Downy mildew+	Dithane M 45	kg	2,0
			Wettable sulphur	kg	4,0
		Powdery mildew+ Grape moth	Pheromone traps	pieces	2,0
3	After blooming, when 80% of the caps have fallen *Safety treatment*	Downy mildew+ Anthracnose+ Read leaf spot Powdery mildew	Antracol 70 WP	kg	2,0
			Falcon 460 EC	l	0,3
4	Grape growth, 8-10 days after treat.3	Downy mildew+ Powdery mildew	Dithane M 45	kg	2.0
			Falcon 460 EC	l	0,3
5	Grapes and clusters reached the characteristic size for the variety before ripening	Downy mildew + Powdery mildew+	Bordeaux mixture	kg	10.0
			Wettable sulphur	kg	4.0
		Grey grape rot	Topsin	kg	1.0

Table 4. Disease attack response to the two control schemes a for the Gros Sauvignon variety in the Cotesti vineyard 2007

Variants of treatment	Disease								
	DOWNY MILDEW			POWDERY MILDEW			MOULD (10.09.2007)		
	F%	I%	G.A.%	F%	I%	G.A.%	F%	I%	G.A.%
V1	0.00	0.00	0.00	20.00	5.00	1.00	4.90	3.70	0.18
V2	2.90	2.00	0.06	28.09	10.50	2.95	21.09	4.97	1.05
V3	5.10	3.90	0.20	49.15	14.89	7.32	12.95	15.91	2.06

Table 5. Quantitative and qualitative characteristics of the grape yield under the conditions of the year 2007

No.	Variant	No. of clusters/ vine	Average weight/ cluster (g)	Yield			
				Quality		Quantity	
				Sugar (g/l)	Acidity g H_2SO_4	Kg/vine	Kg/ha
1	Ecological	21	58.20	211.0	5.7	1.22	5.050
2	Conventional	26	48.10	204.0	5.9	1.24	5.150

Wine-growing habitats from Oltenia-Romania, with vocation for obtaining red quality wines, with controlled origin denomination (C.O.D.)

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Keywords: viticultural areal, aptitude, soil, controlled origin denomination, Oltenia.

ABSTRACT

For each viticultural wine growing habitat from Oltenia-Romania, we established the profile of the predominant soil, and we determined the physical-chemical characteristics of the obtained wines. Using the metodology of multicriterial delimitation in ecological concept of the viticultural areals of obtaining high quality COD products we traced in Oltenia-Romania, the habitats of five names of controlled origin: Banu Mărăcine, Segarcea, Mehedinți, Drăgăşani, Sâmbureşti.

INTRODUCTION

There are few the countries, like Romania, that can produce wine of very good quality, but there is a smaller number of capable countries, like Romania, to produce, to the highest level of quality, the entire range of wines that can be made out of grapes – white and red, dry – semidry or sweet – licorice, exceptional muscatel or frothy wines.

The multiple microclimates and types of soil found in the wine-growing regions of Oltenia, but also the complete range of the types of wine (white, red, aromatic), distillate of wine and table grapes and raisins that can be obtained, constitute arguments that Oltenia to be named a true minimalist wine-growing Romania. The wine-growing habitats in Oltenia have represented and still represent between 15-20% of the national wine-growing patronage, the quality red aromatic wines assuring over 40% of the national balance of wines that belong to this category.

In this paperwork, we wanted to define the wine-growing habitats of Oltenia-Romania that has vocation for obtaining red quality wines with controlled origin denomination.

MATERIALS AND METHODS

In order to express the oenological vocation of a wine-growing habitat, we established the value of the (A) oenoclimatic aptitude index, which is given by the sum of the temperature degree (T) and the hours of effective sun exposure (I), in the vegetative period (01.04-30.09), correlated by the subtraction of the precipitation excess (P-250) at the same period:

$$A=T+I-(p-250)$$

Catching the most favorable premises for the development of grape maturation, in conditions safe from hygrometric hardness, it was possible by monitoring the climatic characteristics in the months of grape harvesting and wine-making (September and October).

For each wine-growing habitat, there were identified the dominant types of soil and, there were determined the characteristics of composition of the obtained wines, using methods agreed by O.I.V. adopting the multi criteria methodology of quality wines with controlled origin denomination (C.O.D), we defined for the area of Oltenia,

five names of controlled origin like: Drăgăşani, Banu Mărăcine, Sâmbureşti, Segarcea, Mehedinți).

RESULTS AND DISCUSSIONS

Based on the ecopedoclimatic studies of the Romanian vineyards, these wine-growing areas were included in the wine-growing zones of the European Union (Law 244/2002). The wine-growing habitats of Oltenia-Romania (table 1) belong to the wine-growing regions of CI(a)-Banu Mărăcine, Drăgăşani, Corcova, Sâmbureşti and CII-Mehedinți Severin, Mehedinți-Vânju Mare, Mehedinți-Plaiurile Drâncei, Segarcea. Wine-growing habitats that belong to the wine-growing region CI(a) are used mainly to obtain quality wine, especially red and aromatic white ones. The ones that belong to the CII, are used to obtain red wines of high quality.

Based on the oenoclimatic aptitude index (A), there were made hierarchies of all wine-growing spaces of Oltenia-Romania (table 2).

One can notice that the wine-growing habitats of Oltenia belong to different oenoclimatic areas, depending on their geographical placement, but more importantly by the sum of the temperature degrees (T), the hours of sun brightness and the precipitations that were in the period of vegetation (01.04-30.09). To the oenoclimatic area A0, belongs the habitat of Polovragi-Dobrița, the oenoclimatic aptitude index is of de 3978 and assuring favorable conditions to obtain quality dry white wines. The wine-growing centers of Tg. Jiu and Râmnicu Vâlcea, belong to the A2 oenoclimatic area, with a value of the oenoclimatic aptitude index between 4437 and 4500, giving the chance to obtain mainly white wines, and secondly quality red wines.

The great majority of the wine-growing habitats of Oltenia-Romania, belong to the oenoclimatic A3 hill area and A3 meridian hill, that beneficiate of lots of warmth and light, lack of some excess precipitation, long sunny autumns and with no thermal stress. The most elevated values of the temperature degrees and effective sun exposure sums are registered in the wine-growing habitats of Mehedinți. The oenoclimatic aptitude index value reaches quotes between 4627 and 4939, offering the possibility to obtain red wines, white semidry wines, and sweet ones, liquory, aromatic wines, of the highest quality.

There can be observed that in the wine-growing habitats of Oltenia-Romania, in the important step of maturing process definition of the grapes (table 3), dominates a favorable time, with moderate temperatures, that do not create difficulties, either by excess, or insufficiency, both to the process of maturation definition and over maturation of the grapes and, to the harvesting and fermentation development during the wine-making process. In their turn, the small precipitation and the quite sunny time, represent also favorable premises for the realization of less depreciated harvests. The hottest month of the year is July, after which, the temperatures begin to decrease, more slowly in august and, faster in September and October, this way creating some of the most favorable premises for the unfolding of grapes maturation, in the conditions of bigger and bigger safety from the hygrometric hardness, the more the skin of the grape gets thinner the more the grape itself gets more vulnerable. Generally, the precipitations also know a decreasing influence, starting with the month of august and decreasing more in September and October, fact that positively contributes to keeping good sanitary state of the harvest, this way preventing mostly the grey grape rot to appear.

The types of soil predominant in the wine-growing habitats of Oltenia (table 4) are among the most preferred by the vine, deep soils, with sufficient porosity, big tampon capacity, sufficiently well supplied. The soft and hot soils, rich in lime, made out of river gravel are often encountered in the wine growing habitats of Vânju Mare-Orevița, Oprişor and Corcova. The forest red brown ones are dominant in Banu Mărăcine-Craiova, and the brown soils and red brown forest ones, some averagely podzolite, rich in lime, situated on the river gravel, there are found in the great wine-growing region of Drăgăşani, where the massive plantation occupies a length of over 60 km, the slopes of 3 rounds of hills parallel to the Oltul.

To Sâmbureşti, where there are obtained the most known red wines like Cabernet Sauvignon of Romania, there are predominant the forest red brown soils, while to Segarcea, on rendzinic-lime soils are produced exceptionally red wines from Pinot Noir.

As a natural consequence of the geographical position, of the climatic characteristics and the very physical-chemical features of the types of soil present in the wine-growing habitats of Oltenia-Romania, there were obtained along the years, veritable red wines (table 5), which distinguish themselves by a subtle delicacy, full of temperament, and the tonality and intensity of the color are two attributes that impose to them a powerful personality.

By aging, because of an ideal equilibrium among alcohol quantity, fix acidity and no reducing extract, it gets a cache typical for the great red wines obtained by sorts of grapes with a rich genetic package for quality, placed in the habitats with big pedoclimatic availability for the forming of the valuable chemical constituents of the grape.

Starting from the oenoclimaic aptitude index there were made hierarchies of all the wine-growing habitats of Oltenia-Romania, superior quality wine producers. In order to determinate, to the parcel level, the favorable habitats for obtaining quality wines with controlled origin denomination, there was used the method of their delimitation. Based on these methodologies we traced in Oltenia-Romania, the habitats of five names of controlled origin (Banu Mărăcine, Segarcea, Mehedinți, Drăgăşani, Sâmbureşti) of quality wines, that at present, have also juridical protection (Law no. 244/2002; HG 1134/2004 and Ord. MAPDR 690/2006).

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Tables

Table 1. Inclusion of the wine-growing habitats of Oltenia-Romania in the wine-growing area of the European Union

Oltenia's wine-growing habitat	European Union wine-growing zone	
	CI(a)*	CII**
Banu Mărăcine-Craiova	*	
Drăgăşani	*	
Sâmbureşti	*	
Corcova	*	
Mehedinți-Severin		*
Mehedinți-Vânju Mare		*
Mehedinți-Plaiurile Drâncei		*
Segarcea		*

*Corresponds to the Cahors, Charante, Bordeaux, Bourgogne vineyards in France.
** Corresponds to the Narbone, Frontignan, and Montpellier, Auribes vineyards in the South of France and the vineyards of the Italian Piemont (Milan, Torino, Asti, San Michele, and Toscana).

Table 3. Climate characteristics of the harvest months of grapes and winery, in the main habitats of wine growing in Oltenia-Romania (average data of 50 years)

Wine growing habitat	September			October		
	Average temperature(°C)	Average precipitation (mm)	Sun exposure (hours)	Average temperature (°C)	Average precipitation (mm)	Sun exposure (hours)
Banu Mărăcine	17,7	37	236	11,9	16	186
Segarcea	17,9	30	214	11,8	20	163
Vânju Mare-Orevița	17,9	31	225	12,1	29	174
Corcova	17,4	39	222	11,8	32	173
Drăgăşani	17,3	39	239	12,4	29	191
Sâmbureşti	16,6	40	232	11,0	31	188

Table 2. Climatic and geographic characteristics of the main wine-growing habitats of Oltenia-Romania and their oenoclimatic capacity

Centers and habitats with wine-growing spaces	Lat. N	Alt. (m)	Average annual temp.	Yearly amount of precipitations (mm)	Sum 01.04-30.09			Index of oenoclimatic aptitude A=T+I-(P-250)	Oeno-climatic area	Quality wines that can be obtained
					Grades of temperature (°C) T	Effective sun exposure in hours I	Precipitation (mm) P			
Polovragi-Dobrița	45°11”	530	9.3	893	2938	1302	513	3978	A0	White dry quality wines
Tg. Jiu Rm. Vâlcea	45°02”	210	10.4	816	3233	1450	433	4500	A2	Mainly white wines, secondly red quality wines
	45°06”	242	10.2	710	3173	1452	411	4437		
Corcova Drăgăşani Sâmbureşti	44°35”	150	10.7	741	3313	1546	374	4682	A3 on hills	Mainly red wines, white wines, semidry, sweet, liquor, aromatic and of superior quality.
	44°30”	182	10.8	684	3316	1576	385	4754		
	-	260	10.5	682	3226	1536	395	4627		
Drobeta Tr. Severin	44°38”	116	11.6	762	3487	1546	354	4929	A3 meridian hills	
Vânju Mare-Orevița	44°25”	86	11.0	634	3388	1549	309	4878		
Oprişor	-	130	10.7	587	3339	1550	287	4892		
Plenița-Orodel	44°13”	150	10.6	637	3340	1487	338	4762		
Segarcea	45°05”	145	11.2	565	3448	1439	288	4843		
Tâmbureşti	44°02”	73	10.9	575	3353	1540	312	4805		
Brabova	44°28”	-	10.4	612	3301	1525	312	4764		
Brădeşti	44°29”	200	10.7	634	3278	1516	336	4702		
Craiova-Banu Mărăcine	44°19”	195	10.9	543	3403	1574	288	4939		

Table 4. Types of soil, depending on their predominance, in the main wine-growing habitats of Oltenia-Romania

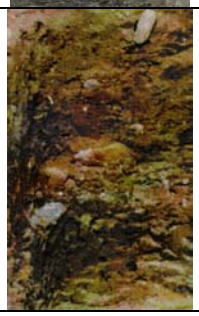
The habitat	Type of soil	Profile of the predominant soil
Banu Mărăcine	Red eroded preluvo-soil	
Segarcea	Cenozoic limestone Faeoziom gleic Limestone rendzine	
Vânju Mare-Oreviţa	Luvosoil typical for gravel Skeletal eutricambosoil Skeletal type luvosoil Litho-soils on skeletal limestone	
Drăgăşani	Preluvosol eroded limestone Preluvosol skeletally eroded Preluvosol lime Typical Faeoziom Faeoziom pelic Lime Regosoil	
Sâmbureşti	Lime Regosoil Luvosoil typical for gravel Luvosol typically skeletal Lime Preluvosol	

Table 5. Physical-chemical characteristics of red wines (obtained in the main wine-growing habitats in Oltenia-Romania)

Habitat / Type of wine	Physical-chemical characteristics (limits of oscillation)						
	Alcohol volume %	Total acidity g/l (H_2SO_4)	Glycerol g/l	Non reducing extract g/l	Ash g/l	Color intensity $DO_{420}+DO_{520}$	Tonality DO_{420}/DO_{520}
1.Banu Mărăcine							
Cabernet Sauvignon (1970-1990)	12,6-13,5	4,90-5,00	9-9,9	25,1-28,4	2,0-3,20	1,26-1,38	0,48-0,54
Pinot Noir (1970-1990)	13,4-14,1	3,64-3,95	9,2-10,8	25,0-27,2	2,90-3,10	0,72-0,86	0,78-0,80
2. Segarcea							
Cabernet Sauvignon (1921-1985)	11,62-14,94	3,44-5,05	9,0-17,99	23,74-43,73	1,73-2,91	1,30-1,42	0,94-1,62
Pinot Noir (1920-1985)	14,52-15,45	3,70-5,06	6,9-20,73	24,50-37,41	1,80-2,84	0,34-1,00	1,07-1,86
3.Vânju Mare-Oreviţa							
Cabernet Sauvignon (1970-1985)	12,8-15,8	4,3-4,5	7,3-11,0	25,2-30,6	2,90-3,80	1,92-2,01	0,58-0,66
Merlot (1970-1985)	12,7-13,0	4,4-4,5	7,8-10,0	27,8-28,9	2,40-3,10	1,47-1,51	0,43-0,56
4. Drăgăşani							
Cabernet Sauvignon (1990-2005)	11,76-13,64	4,7-4,90	9,0-9,84	22,0-28,90	2,30-2,96	0,86-1,52	0,40-0,64
5. Sâmbureşti							
Cabernet Sauvignon (1990-2005)	12,58-13,76	4,36-5,00	9,0-10,50	27,84-29,40	2,84-3,10	0,89-1,59	0,45-0,69

Figure

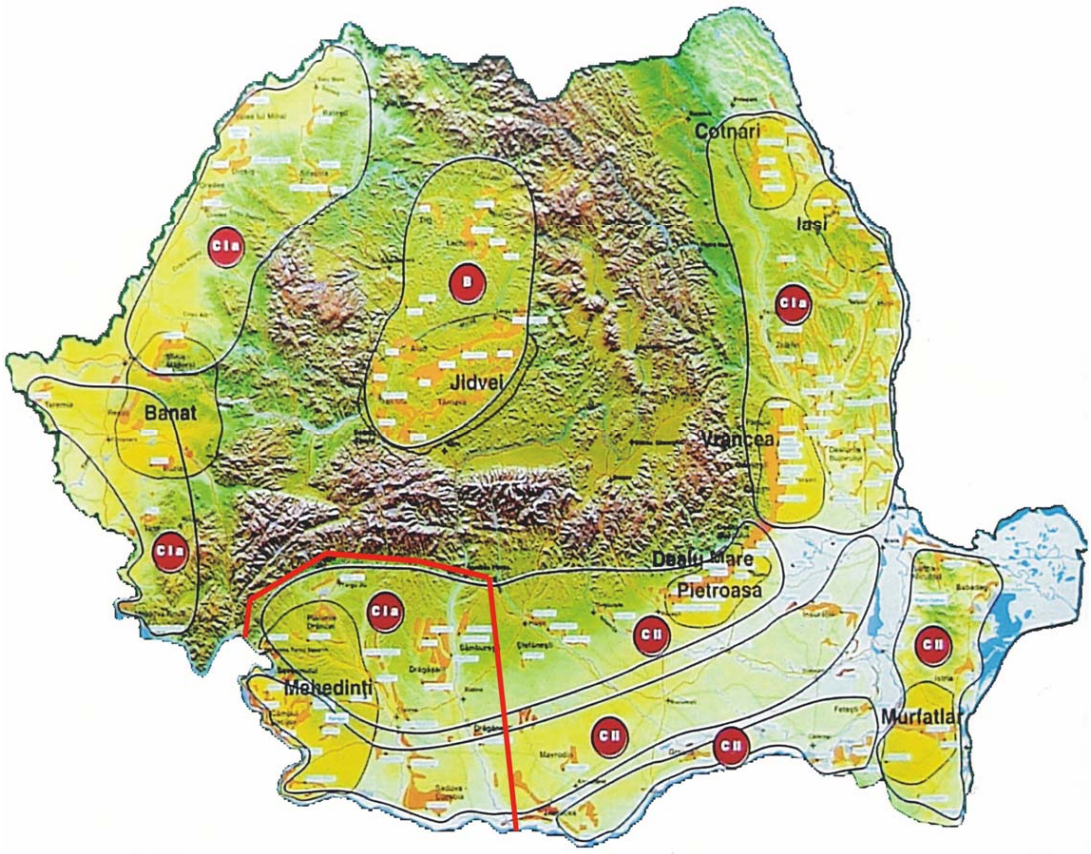


Fig. 1 – Framing vineyards from Oltenia Romania in the wine-grower regions of European Union

Comparative study regarding the degree of adaptability of two German varieties – Regent and Dornferlder on the experimental field of USAMV Bucharest

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Keywords: varieties, grape vine, favorability, quality, adaptability.

ABSTRACT

The obtainment of quality wines is an important goal for the present Romanian viticulture. The wine quality mostly depends on the production technologies, the applied treatments and last, but not least on the quality of grapes. The present work refers to a preliminary study that informs on the behavior of German varieties Regent and Dornferlder in ecopedoclimatic conditions registered in Ampelographic Collection from U.S.A.M.V. Bucharest. The experiment was realized to establish these varieties adaptability degree in a habitat with middle favorability for obtaining red quality wines, and to determine the capabilities and the possibilities to use these varieties to increase the number of suited varieties for obtaining red quality wines. The two varieties were studied mostly because of their popularity they have in their origin country (Germany), but also because of their resistance in *Mildery* and *Grey rot.*

INTRODUCTION

In wine growing, an important and actual problem is represented by extending areas cultivated with varieties for quality red and white wines, which are very apreciated in global market, though it is known that his areas have decreased in the last years. He study aims improving and completing the existing variety both in quality and in quantity of the production.

In this context, this work represents a preliminary – informative study for the bearing of Regent and Dornferlder German varieties in ecopedoclimatic conditions provided by the Ampelographic Collection of USAMV Bucharest. The experiment was conducted during 2005-2007 and its main purpose was establishing the degree of adaptability of these two varieties in a habitat with medium favorability for obtaining red superior wines by establishing the main abilities and ways of using of these sorts, so that we can extend the range of red wines varieties in Romania

MATERIALS AND METHODS

For accomplishing the established objective, two German varieties were studied; Regent and Dornferlder, and Merlot variety was studied as a control. Regent was obtained by G. Alleweldt, at the end of the 80's in Viticulture Institute Geilweilerhof, Germany, by crossing varieties (Silvaner x Müller Thurgau) x Chamburien, and Dornferlder was obtained by August Herold, in 1995 in Weinsberg, by crossing varieties for red wines Helfensteiner x Heroldrebe.

The studied varieties can be found in Ampelographic Collection of The University of Agronomic Sciences and Veterinary Medicine-Bucharest, and they are guided Goyot semi high, with 16 buds on a vine.

For establishing the agrobiological and technological potential of the experimented variants studied, the following indicators were analyzed: the percentage of viable buds/vine, the absolute and relative fertility coefficients, productivity index (absolute and relative), the medium weight of a grape, the weight of 100 grams of grape, production both by quantity (kg/vine) and by quality-sugar concentration (g/l), the amount of acid (g/l of tartaric acid), the amount of anthocyans (mg/l). The enumerated

aspects were established for all experimental variants which were completely mature, for each wine-growing year, but for the preliminary study an average value of the three years was analyzed.

RESULTS AND DISCUSSIONS

Following the unfolding of phonologic spectrum mostly aimed at the pheno-phases: The budding, the blooming, at colour appearance, the full maturity. The beginning of each pheno-phase was appreciated on the basis of specific literature as it follows: The budding it was ascertain that these pheno-phases started at the beginning of the third decade of April for Regent and Merlot varieties and in the second decade of April for Dornferlder variety.

The blooming*:* Generally starts in the last decade of May through the first days of June, but an early debut was observed for the control variety and a delay in Regent in contrast with Dornferlder variety. The registered differences in the two pheno-phases analyzed disappeared in ripening stage, when Regent had only two days in advance. In the matter of complete maturing, the pheno-phases analyzed after determinations in the lab (the evolution of the amount of sugar, acid, anthocyans, weight oh 100 grams of grape), it was observed that it appeared in the second decade of september for the years 2005 and 2006, and in the first decade of september for the year 2007, so the varieties are distinguished by an early complete maturing (the fifth stage of maturing).

Fertility and productivity elements, which are mostly influenced by the hereditary basis, by the used parent stock, by the agrotechnics applied to the plant and also by the ecopedoclimatic conditions of each harvest year, reveal differences between the three varieties: the highest percentage of fertile offshoots is registered in Dornferlder (94%), followed by Merlot (90%) and Regent, which only has a percentage of (87%).

The analysis of fertility coefficients reveals that registered values of Merlot are lesser than the other two varieties (1,6; 1,1,), and between the last two Regent (2,2; 1,37) is more fertile than Dornferlder (1,81;1,17) variety, so these varieties mostly depend on weather conditions registered during the analysis, but also on the agrotechnics applied.

The analysis of agrobiological and technological aptitudes of the studied varieties, in ecopedoclimatic conditions of USAMV Bucharest, realized through the productivity parameters reveals that: the genotype is directly connected with this parameter and the average values of this parameters is influenced by the average values of absolute and relative fertility coefficients and by the average weight of the grape. Though the registered differences are not significant, it can be observed a higher average value of these differences in Dornferlder (300,5 g/sprout) as against 275 g/sprout obtained for Regent variety and 151 g/sprout for Merlot.

The higher or the lesser weight of a grape has a practical importance, as it is a biological feature of each variety, wich can be dramatically modified in certain limits of ecopedoclimatic conditions, crop technology, used parent stocks etc. It can be noticed that grapes of Regent variety weight lesser than Dornferlder (125 g versus 166 g) but they weight more than Merlot (124 g). So we can consider that the analyzed varieties join the group of average-great size grapes varieties for obtaining red wine.

Besides productivity, the quality of the product constitutes an essential element which finally determines the quality of the wine. Table number presents the results of analysis done for establishing the qualitative value of the production, values that allowed establishing the optimum timing for harvest.

It can be noticed the studied varieties accumulated planty of sugars, resembling from the point of view of quantity, values which oscillate between 215-238 g/l and which indicates the obtaining of certain wines with an alcoholic degree between 12,5v%- 14,0v%. Acidity's level was correlated with the sugars volume accumulated in the grapes, remarking that the two varieties of German grapes have almost equal values (3,89g/l for the Regent and 3,90 g/l for the Dornferlder) and a little bit raides for the Merlot which was a sort of witness in this experience.

During the same period the volume of 100 grapes was analyzed and it can be noticed a resemblance between the two German varieties , the grapes weight depending on the sugars volume and on the total acidity's decrease at full growth, being an hereditary feature. It can be remarked a positive correlation between the volume of the 100 grapes and the volume of anthocyans accumulated in the membrane of the grapes. These accumulations represent a basic technological condition for obtaining high quality red wines and facilitate the quality forecast, the technological shaping and accommodation of wine-making.

The analyzed varieties registered large quantities of anthocyans of 638-785 mg/1000 grapes which can guarantee the chromatic feature according to the range of high quality wine-making, in agreement with the new aspirations. The production of grapes varied from 2,7 kg/vine for the Regent till 3,3 kg/vine for the Dornferlder, the genotype trough the hereditary dowry has a very important role expressing the level of the production, having a meaningful action in this case.

Regarding the quality of the production according to the health aspect it can be noticed that the production was 100% healthy, the degree attack with Grey rot - (++ a good resistance, + means medium-low resistance.

CONCLUSIONS

The ecopedoclimatic conditions registered in the Ampelographic Collection of the U.S.A.M.V. Bucharest, the Regent and the Dornferlder, puts good use in their quantitative and qualitative production potential proving a good adaptability and a high rate of productivity, succeeding to answer to all the actual requests of producing high quality wines.

The set of results obtained under the aspect of agrobiological an technical behavior lead to the idea that the two varieties can complete the scale of varieties for the making of red wines from Romania.

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Table 1. The synthesis of main fertility and productivity elements in Regent and Dornferlder varieties

Experimental varieties	Parameters				% Fertile offshoots	Coefficient of fertility		Productivity index		Average weight of a grape	Weight of 100 grapes
	Budding	Blooming	At colour appearance	Harvest		cfa	cfr	ipa	ipr	g	g
Regent	20.04	1.06	15.07	19.09	87	2,2	1,37	275	171	125	212
Dornferlder	15.04	28.05	17.07		94	1,81	1,17	300,5	262	166	226
Merlot	23.04	24.05	17.07		90	1.6	1.1	151	101	124	116

Table 2. The syntesis of grapes production by quality and quantity in Regent and Dornferlder varieties

Experimental varieties		Parameters					
		Sugars(g/l)	Total acidity (g/l tartaric acid)	Average weight of a grape (g)	Anthocyans (mg/l)	Production (kg/vine)	Resistance Grey rot* ++ a good resistence + means medium-low resistance
Average years (2005-2007)	Regent	238	3,89	212	737,5	2,7	++
	Dornferlder	215	3,90	226	785	3,3	++
	Merlot	226	4,3	125	638	3,0	+

Researches concerning fertility and productivity of grape varieties cultivated in Teremia Viticulture Centre

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Keywords: Majarcă albă, Creaţă, Steinschiller, Fetească regală, Italian Riesling, Burgundy, relative fertility coefficient, absolute fertility coefficient, relative productivity index, absolute productivity index

ABSTRACT

The traditional growing of grapevines in Teremia viticulture centre has witnessed numerous changes in time by modernizing crop technologies and changing assortment of grape varieties and moreover during transitional period many vineyards have been cleared or abandoned.

Re-establishment of viticulture centre by re-conversion of grapevine plantations requires knowledge relating to yield potential of cultivated varieties. The results obtained during the research period 2002-2004 approaching fertility and productivity of Majarcă albă, Creaţă, Steinschiller, Feteasca regală, Italian Riesling and Burgund mare varieties have demonstrated that Majarcă albă variety registered the best values for fertility coefficient and productivity indices proving to have the highest adaptability in the region and thus, it is recommended to be further maintained for cultivation.

INTRODUCTION

Traditional viticulture using sandy soils from Banat region and considering local grape varieties (Majarcă albă, Creaţă and Steinschiller roz) 10 –12 vines/ha using stakes with "rabbit-head" cut protected during winter by burying was modernized and changed by: introduction of novel grape varieties, enlarging planting distances between rows, changing trellis attaching and supporting system with 2 or 3 wires and fructification pruning using double Guyot system.

All these changes induced also microclimate changes for grape vines and implicitly for grape vine responses. The behaviour of local and foreign grape varieties in the climate conditions of Teremia Centre was widely studied by Constantinescu Gh.(1957, 1971), Elena Negreanu (1957), Gh. Calistru (1974) and Mihalca I.(1968,1987).

The study of yield potential for the cultivated grape varieties is focused on supporting and preserving for cultivation the most efficient and valuable varieties and thus to enable restoring old vineyards from Banat's field areas.

MATERIALS AND METHODS

The grape varieties taken into study were: Majarcă albă, Creaţă, Steinschiller, Feteasca regală, Italian Riesling and Burgund mare, grown in Teremia Viticulture Centre, during the experimental period 2002-2004.

The plantation comprising local grape varieties was set up in 1981 using grafted vines and planting distances of 2,0 x 0,6(m). Pruning was represented by "Teremia" type, with 3-4 spurs bearing 3-4 fruiting buds while trellis system consisted of two rows of wire.

The grapevine plantation consisting of Fetească regală, Italian Riesling and Burgund mare varieties was established in 1987 using grafted vines and using planting distances of 2,0 x 0,8(m). Pruning system is represented by double Guyot with two canes with 8-12 buds each, and renewal spurs with 2-3 buds, the supporting grapevine system being three-rowed trellis.

We have determined fertility coefficients by counting fertile and non-fertile shoots and also emerged inflorescences using known calculating relations.

The experience was set up using randomized block design considering 25 vines for each variety with 5 experimental variants and three replications

The processing of experimental data was performed using bi-factor variance analysis, experimental year with three graduations and variety with 6 graduations.

The control variant was considered Majarcă albă, being also the main grape variety in the studied local assortment.

RESULTS AND DISCUSSIONS

The mean values of fertility coefficient and productivity indices registered by the studied grape varieties considering the climate conditions for experimental year 2002 are summarized in table 1 and fig. 1.

Relative and absolute fertility coefficients of Majarcă albă variety have registered mean values of 1,12 and 1,62, respectively, the remaining varieties showing negative insignificant differences, except for Burgund mare that registered negative distinct significant differences.

The mean weight of grapes in case of control variant was of 123g, while the rest of the considered varieties registered negative insignificant differences for Steinschiller and Fetească regală varieties.

Relative and absolute productivity indices in case of Majarcă albă variety was of 138 g and 199g and comparatively all studied varieties registered negative distinct significant differences, distinct significant for Creață variety and very significant for the rest of considered varieties.

Mean values concerning fertility coefficients and productivity indices were attained for the experimental year 2003 and are presented in table 2 and fig. 2.

Relative fertility coefficient of the studied varieties registered the highest mean value for Majarcă albă (0,65) and comparatively all varieties registered statistically negative insignificant differences, except for Burgund mare variety with negative significant difference.

Absolute fertility coefficient registered minimal values of 1,04 for Burgund mare variety and maximum values of 1,25 for Majarcă albă and Fetească regală varieties. Compared with control variety, except for Fetească regală variety, all studied varieties registered negative insignificant differences.

The mean weight of grapes registered for Majarcă albă variety values of 107g and comparatively the mean weight of studied varieties: Steinschiller (66g), Fetească regală (71g) and Italian Riesling (62g) have registered negative very significant differences while Creață (110g) and Burgund mare (117g) positive insignificant differences

Relative and absolute productivity indices of Majarcă albă variety have registered mean values of 70g and 134g, respectively and comparatively the rest of the studied varieties registered negative insignificant differences for Creață variety (60g and 129g) and very significant Steinschiller (37g and 82g) and Italian Riesling (32g and 70g).

Fetească regală (40g and 88,50g) variety has registered negative distinct significant difference for relative productivity index and very significant for absolute productivity index while for Burgund mare (51 g and 119g) variety it has been observed negative significant difference for relative productivity index and insignificant for absolute productivity index.

The variability of mean values regarding relative and absolute productivity indices of grape varieties studied in the year 2004 is presented in table 3 and fig. 3.

Relative and absolute productivity indices in case of the studied varieties have registered values between 0,68 for Creață and 0,89 for Italian Riesling, respectively and between 1,17 for Fetească regală and 1,46 for Burgund mare variety, respectively.

Comparatively with the value of relative fertility coefficient of Majarcă albă variety representing 0,83, Fetească regală (0,86), Italian Riesling (0,89) and Burgund mare (0,84) varieties showed positive insignificant differences, Creață (0,68) and Steinschiller (0,82) varieties negative insignificant differences while comparing the mean value of absolute fertility coefficient (1,30) of control variant, insignificant positive differences were observed for Steinschiller (1,36), Italian Riesling (1,43) and Burgund mare (1,46) varieties and negative insignificant differences for Creață(1,19) and Fetească regală(1,17).

The mean weight value of grape clusters that have been attained for the experimental year 2004 was minimal in case of Italian Riesling (56g) and maximum for Burgund mare variety (115g). The control variety has registered mean weight of grape cluster of 110g and comparatively the rest of considered varieties have presented negative insignificant difference for Creață (103g) variety, negative very significant for Steinschiller(67g), Fetească regală (69g) and Italian Riesling (56g) while Burgund mare (115g) positive insignificant differences.

Relative and absolute productivity indices of the studied varieties have registered the smallest average values for Italian Riesling (50 g and 80g) and the largest mean values for Burgund mare (97g and 168g). Comparing the control variety, that has registered mean values of 92g and 144g, Steinschiller roz (60g and 91g), Fetească regală (59 and 81g) and Italian Riesling (50g and 80g) have attained negative very significant differences, Creață (70g and 123g) variety registered negative distinct significant difference for relative productivity index and insignificant for absolute productivity index. Burgund mare (116g and 169g) variety performed positive differences, distinct significant regarding relative productivity index and significant for absolute productivity index.

CONCLUSIONS

Grape varieties taken into our study are regarded as productive varieties. Fertility and productivity indices have registered the largest values for Majarcă albă variety followed by Fetească regală and Burgund mare while smallest values were observed for Steinschiller roz and Rieling Italian varieties.

Fertility and productivity of studied grape varieties registered variable values in terms of experimental years, therefore, the largest grape yields were obtained in 2002, the experimental year with normal conditions and the smallest values in 2003, when grapevines suffered losses due to minimal absolute temperatures of –25,5^{o}C.

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Tables

Table 1. Variability of values concerning fertility and productivity indices for grape varieties studied in 2002

Variety	Relative fertility coefficient		Absolute fertility coefficient		Relative productivity index (g)		Absolute productivity index (g)	
	Value	Dif. and signifi-cance comparing the control	Value	Dif. and signifi-cance comparing the control	Value	Dif. and signifi-cance comparing the control	Value	Dif. and signify -cance comparing the control
Majarcă albă	1,12	-	1,62	-	138	-	199	-
Creață	1,02	-0,10	1,47	-0,15	110	-28^{00}	159	-40^{00}
Steinschiller	1,04	-0,08	1,48	-0,14	76	-62^{000}	108	-91^{000}
Fetească regală	1,10	-0,02	1,45	-0,17	83	-55^{000}	109	-90^{000}
Italian riesling	1,07	-0,05	1,39	-0,23	77	-61^{000}	100	-99^{000}
Burgund	0,85	-0,27^{oo}	1,23	-0,39^{oo}	99	-38^{000}	144	-55^{000}
	DL 5% 0,17 DL 1% 0,23 DL 0,1% 0,30		DL 5% 0,27 DL 1% 0,36 DL 0,1% 0,47		DL 5% 19,09 DL 1% 25,39 DL 0,1% 33,02		DL 5% 23,50 DL 1% 31,25 DL 0,1% 40,65	

Table 2. Variability of values concerning fertility and productivity indices in 2003

Variety	Relative fertility coefficient		Absolute fertility coefficient		Relative productivity index (g)		Absolute productivity index (g)	
	Value	Dif. and significance comparing the control	Value	Dif. and significance comparing the control	Value	Dif. and significance comparing the control	Value	Dif. and signifycance comparing the control
Majarcă albă	1,12	-	1,62	-	138	-	199	-
Creață	1,02	-0,10	1,47	-0,15	110	-28^{00}	159	-40^{00}
Steinschiller	1,04	-0,08	1,48	-0,14	76	-62^{000}	108	-91^{000}
Fetească regală	1,10	-0,02	1,45	-0,17	83	-55^{000}	109	-90^{000}
Italian riesling	1,07	-0,05	1,39	-0,23	77	-61^{000}	100	-99^{000}
Burgund	0,85	-0,27^{oo}	1,23	-0,39^{oo}	99	-38^{000}	144	-55^{000}
	DL 5% 0,17 DL 1% 0,23 DL 0,1% 0,30		DL 5% 0,27 DL 1% 0,36 DL 0,1% 0,47		DL 5% 19,09 DL 1% 25,39 DL 0,1% 33,02		DL 5% 23,50 DL 1% 31,25 DL 0,1% 40,65	

Table 3. Variability of values concerning fertility and productivity indices in 2004

Variety	Relative fertility coefficient		Absolute fertility coefficient		Relative productivity index (g)		Absolute productivity index (g)	
	Value	Dif. and significance comparing the control	Value	Dif. and significance comparing the control	Value	Dif. and significance comparing the control	Value	Dif. and signifycance comparing the control
Majarcă albă	0,83	-	1,30	-	92	-	144	-
Creață	0,68	-0,15	1,19	-0,11	70	-22^{0}	123	-21
Steinschiller	0,82	-0,01	1,36	0,06	60	-32^{00}	91	-53^{000}
Fetească regală	0,86	0,03	1,17	-0,13	59	-33^{00}	81	-63^{000}
Italian riesling	0,89	0,06	1,43	0,13	50	-42^{000}	80	-63^{000}
Burgund	0,84	0,01	1,46	0,16	97	5	168	26*
	DL 5% 0,17 DL 1% 0,23 DL 0,1% 0,30		DL 5% 0,27 DL 1% 0,36 DL 0,1% 0,47		DL 5% 19,09 DL 1% 25,39 DL 0,1% 33,02		DL 5% 23,50 DL 1% 31,25 DL 0,1% 40,65	

Figures

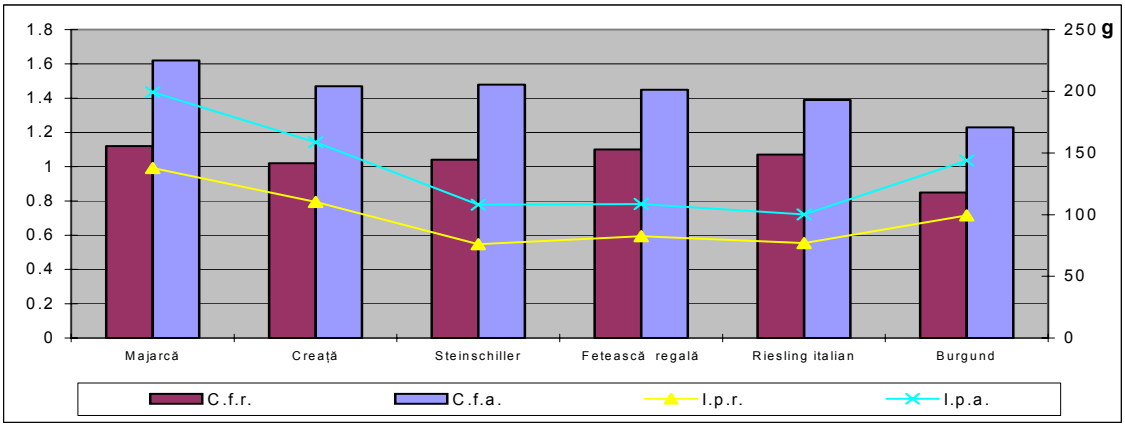

Fig. 1. Variability of values concerning fertility and productivity indices for grape varieties studied in 2002

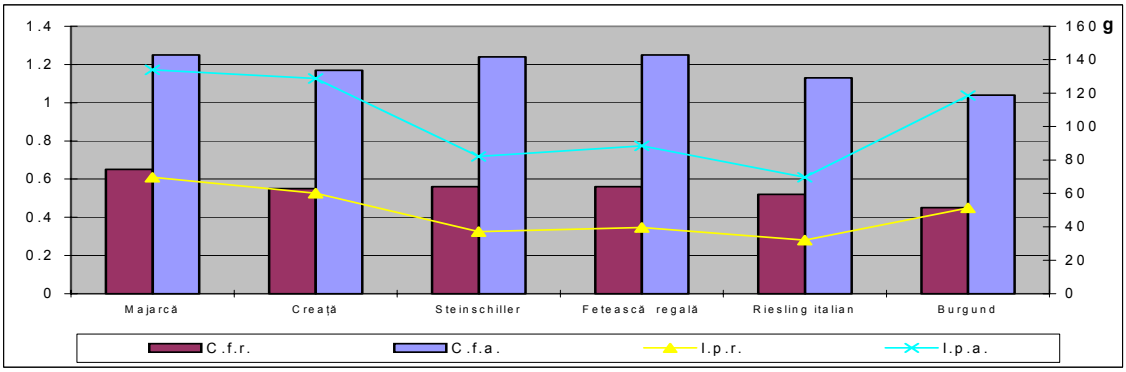

Fig. 2. Variability of values concerning fertility and productivity indices for grape varieties studied in 2003

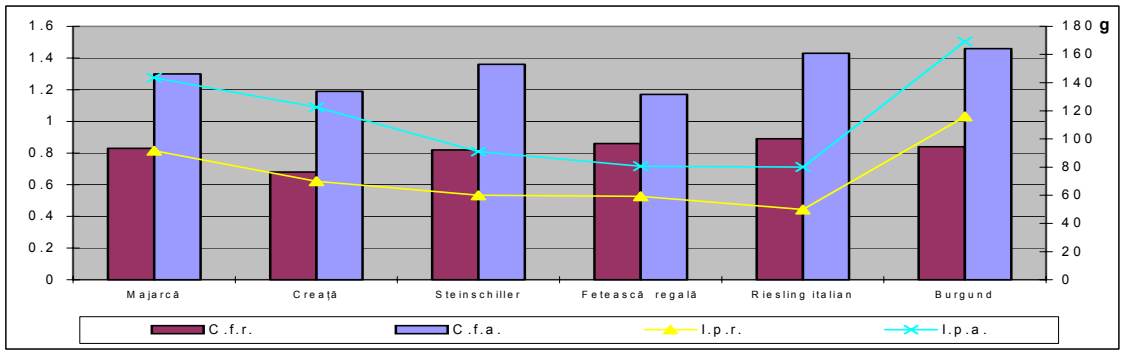

Fig. 3. Variability of values concerning fertility and productivity indices for grape varieties studied in 2004

Some aspects regarding the chemical behavior of some nutritive substrates used for grapevine growing in a closed system

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Keywords: drainage recuperation, macronutrients, biodegradation, grape cutting production

ABSTRACT

Use of virus free propagation material is an important factor to improve quality and quantity of grape production. Obtaining and storage of mother plants are important steps in this protocol. The present work evaluates the qualities of some organic materials used as active medium components for growing grapevine plants in containers, in a closed system. For the establishment of an optimal moisture content, some mixtures, with different rate of manure (M), peat (P), celery soil (CS), forest soil (FS) and a 1:1 (V:V) mixture of perlite and sand (PS) were investigated. A clone of Cabernet Sauvignon grafted to Kober 5BB was used as biological material. It were examined the influence of substrate and nutrient level on the plant growth parameters and productivity. The depend variables measured were pH, electrical conductivity (EC), organic matter (OM) and some macro-nutrients as $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, P, K, Ca and Mg. Some biometric measurements were performed: the plant highness, the shoots lengthiness and diameter, the number of leafs, the foliar surface, the dry and fresh matter. The results show that the evolution of the substrates was different. The analysis regarding nutrient concentrations in substrates show that the variants based on two active components (M and FS, P and FS or M and CS) have a better response to plant request by ensuring an optimum content of macronutrients. Correlated to the substrate nutrient content, the plants in this variants exhibit highest growth parameters and productivity. The best efficiencies of cuttings production was reported when a substrate formed by M and P was replaced by one having equal rates of M and FS (B_{III}) or P and FS (B_{IV}).

INTRODUCTION

The culture of grapevine in containers is used in the research departments as technology of cloner and vegetative multiplication of genetic material, especially new improved variety, cloner selections and biological material which is passed by virus elimination technology. The obtaining of mother plants, also play an important role in the protocol for the regeneration of virus-free plants through *in vitro* cultures of *vitis vinifera*. To increase productivity and quality of biological material an intensive culture system were developed by using nutritive substrates under greenhouse conditions. An optimal equilibration of physical and chemical properties of substrate with growth parameters of grapevine were followed by testing some different active substrates.

MATHERIALS AND METHODS

Some aboriginal materials were used to obtain the nutritional mixtures: oligotrphic peat (P), cattle manure (M), forest soil (FS), celery soil (CS) and a 1:1 (V:V) mixture of perlite and sand (PS). Thirteen variants of substrate were obtained by using different ratios of these materials (table 1). A closed system culture with drainage recuperation was investigated. For this purpose, the mixtures were located in inox pots, with double walls and drain pipe (figure 1). A clone of Cabernet Sauvignon grafted to Kober 5BB was used as biological material. Twelve repetitions for each nutritional mixture were installed under greenhouse conditions. The evolution of chemical composition of substrates correlated with the developed and growth parameters of the plants were followed for 4 years of culture. Biometric measurements were performed to

plants by investigate the grapevine highness, the shoots lengthiness and diameter, the number of leafs, the foliar surface, the dry and fresh matter.

The substrate samples were collected, air-dried and ground to pass through 2mm sieve for the analysis of pH, organic matter (OM), hydro soluble N, P, K, Ca and Mg. Substrate pH was determined with a pH electrode at a substrate to water ratio 1:5 (g:g) for the celery soil, the forest soil and the sand, and 1:10 (g:g) ratio for the others components. The same extract ratios were used for the macronutrients. The OM was analyzed by calcinations. The macronutrients in substrates were dosage by using classical methods of specialty literature.

The grapevine plants and substrate samples were analyzed in two phases: the beginning and the end of vegetation period.

RESULTS AND DISCUSSIONS

Table 2 shows the initial chemical composition of the substrates. All pH values were situated between 5.5 and 8.0 accounted as limits of pH domain for grapevine plants. Starting from the premise that optimal levels of macronutrients in substrate for this kind of culture are: 50-60ppm N, 30-40ppm P_2O_5 (13-17.5ppm P) and 180-300ppm K_2O (50-250ppm K) we can observe that the nutrient levels in all substrates were higher than the inferior limit.

As shown in figures 2-5, the evolution of macronutrients in the substrates in the years of cultures was different. The organic nature of some components, as M and P, induced, by the contact with water and root system, a serial of transformations defined as chemical reactivity of the substrates. These consist of a matter transfer between substrate and substrate solution. We can suppose that some biodegradation process were developed. The intensity of these processes varied with the substrate composition and the offered conditions. As we can see in figure 2, the decreasing in $N\text{-}NO_3$ level was faster in the substrates A_I, A_{II} and A_{IV}. After 4 years of culture in these substrates the $N\text{-}NO_3$ was lower than the critical value of 40ppm. Better results have been obtained with the variants B_{III}, C_I, B_I and B_{IV}. In this case, even in the 4^{th} year of culture, the level of $N\text{-}NO_3$ was situated in optimum limits (36-41 ppm). The level of phosphor (figure 3) has continuous decreased from one year to another, but without significant difference between variants. In the last year of culture, the concentration of hydro soluble P was lower than critical value of 13 ppm for the variants A_{III} (10.5 ppm), A_{IV} (11.3 ppm) and A_I (11.2 ppm). Similar to P, the evolution of K concentration (figure 4) can be described as decreasing continuous, especially in the last two years of experiment. K concentration for the variants A_{IV}, B_{III} and A_I were with 21%, 27% and 40% respectively, lower than the limit of 180 ppm. The other substrates provided higher levels of K due to the manure content. Regarding Ca and Mg content (figures 5-6) we can affirm that organic matter in substrates, as chelating agent, played an important role in this elements supplying. The mixtures containing different rates of peat or manure conferred better levels of 270-380 ppm Ca and 113-176 ppm Mg.

Still from the first year of researching program significant differences regarding growth and developing parameters were observed among the variants. Better conditions offered by substrates in variants B_I, B_{III}, B_{IV} and C_I induced higher values of the shoots lengthiness (figure 7) and of the foliar surface (figure 8). In October the average shoots lengthiness and foliar surface values are with 25-36%, respectively 33-42%, higher than the references' variant (V_r).

Fertility and structure components of nutritive mixtures affected the distribution of fresh and dry matter in the vegetative organs of the grapevine. As we can remark (Figure 9-10), in B_{III}, B_I, B_{IV} variants, well balanced with macronutrients, a higher rate of dry and fresh matter was stoked in annual growth elements (leafs and shoots). At the same time, in A_I, A_{II}, A_{IV} and B_{II} variants with lower levels of macronutrients, the higher rates of dry and fresh matter were accumulated in multiannual organs (root and stem).

Production of one node cuttings was strongly affected by substrate composition (table 3). The number and the quality of cuttings significantly and differently increase on the years of vegetation. The biggest efficiency was recorded in the second and the third year of vegetation. The highest number of cuttings per plant was reported for B_{III} (106 cuttings/plant) and B_I (104 cuttings/plant), that is a production enhance of 24.7% and 22.4% comparative with V_r. The better quality of cuttings was reported for B_{IV} variant with 92% woody cuttings and 8% green cuttings. Similar results were obtained for B_{III}, C_I and C_{II} variants.

CONCLUSIONS

By using some available materials, it was obtained substrates with optimum nutrients content, well structured and with a good drainage capacity reported to grapevine demands.

The analysis regarding nutrient concentrations in substrates show that variants B_I, B_{III}, B_{IV} and C_I have a better response to plant request by ensuring an optimum content of macronutrients. Correlated to the substrate nutrient content, the plants in this variants exhibit highest growth parameters and productivity.

We can appreciate that the best efficiencies of cuttings production is obtain when a culture substrate formed by manure and peat is replace by one having equal rates of manure and forest soil (B_{III}) or peat and forest soil (B_{IV}).

Comparatively to classical methods, this technology offer better conditions for a faster multiplication of valuable biological material and a better monitoring of quality and authenticity of primary biological materials destined to multiplication.

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Tables and Figures

Table 1 Nutritional substrate variants

Variant	Substrate component (%)				
	Acid peat (P)	Manure (M)	Forest soil (Fs)	Celery soil (Cs)	Perlite + Sand 1:1 (PS)
A_{I}	70				30
A_{II}		70			30
A_{III}			70		30
A_{IV}				70	30
B_{I}	35		35		30
B_{II}	35			35	30
B_{III}		35	35		30
B_{IV}		35		35	30
C_{I}	20	30	20		30
C_{II}	20	30		20	30
C_{III}		30	20	20	30
C_{IV}	25	25	25	25	-
Vr	35	35			30

Table 2 Initial chemical composition of nutitional substares A.E. 1/10, g/g

Variant	pH	$N\text{-}NO_3$ (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S.C. (%)	O.M. (%
A_{I}	5.61	46.2	21.6	118.2	314.5	187.3	0.278	48
A_{II}	7.26	53.6	32.1	785.4	420.4	239.5	0.462	55
A_{III}[1]	6.35	28.7	14.3	146.2	225.8	121.2	0.094	12
A_{IV}[1]	6.44	26.6	12.6	127.4	148.3	165.1	0.112	15
B_{I}	5.86	42.5	23.4	168.3	268.4	147.8	0.214	29
B_{II}	6.08	38.4	19.5	132.6	237.2	163.7	0.186	23
B_{III}	6.74	47.9	27.4	483.9	340.9	183.9	0.332	35
B_{IV}	6.93	42.2	22.8	436.4	317.1	176.5	0.315	32
C_{I}	6.55	51.4	29.7	394.8	371.5	223.3	0.389	44
C_{II}	6.73	47.3	26.5	375.8	353.3	194.1	0.367	40
C_{III}	6.84	42.8	24.2	348.0	307.0	186.4	0.320	34
C_{IV}	6.5	41.2	23.6	335.9	329	177	0.295	30
Vr	6.49	53.5	30.2	432.5	395.7	206.3	0.428	53

[1] A.E. 1/5, g/g

Fig. 1 Experimental variants

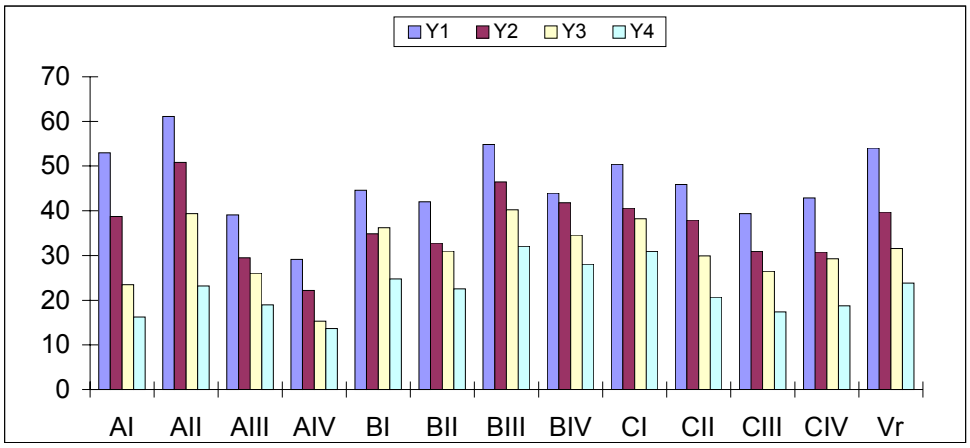

Fig. 2 The evolution of N-NO_3 concentration in substrates

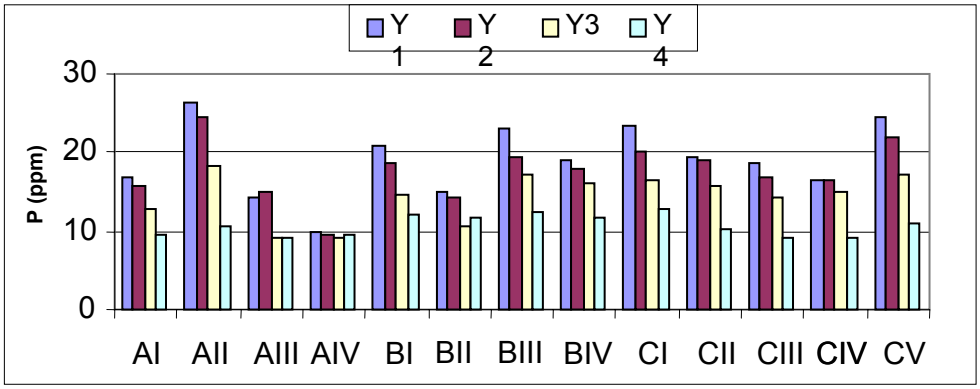

Fig. 3 The evolution of P concentration in substrates

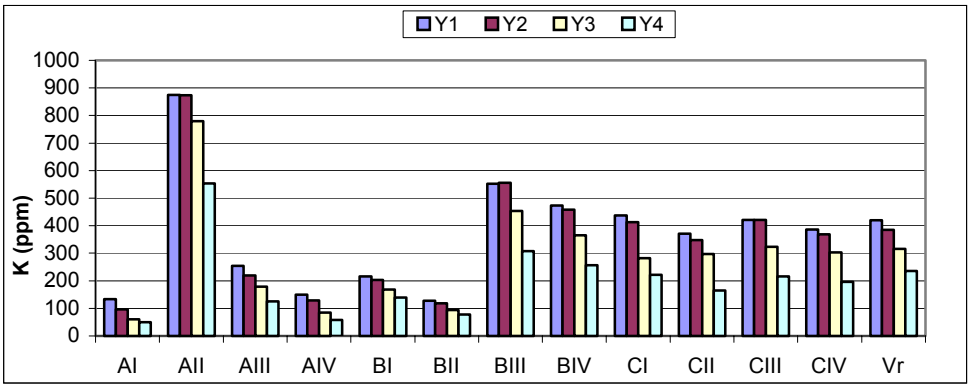

Fig. 4 The evolution of K concentration in substrates

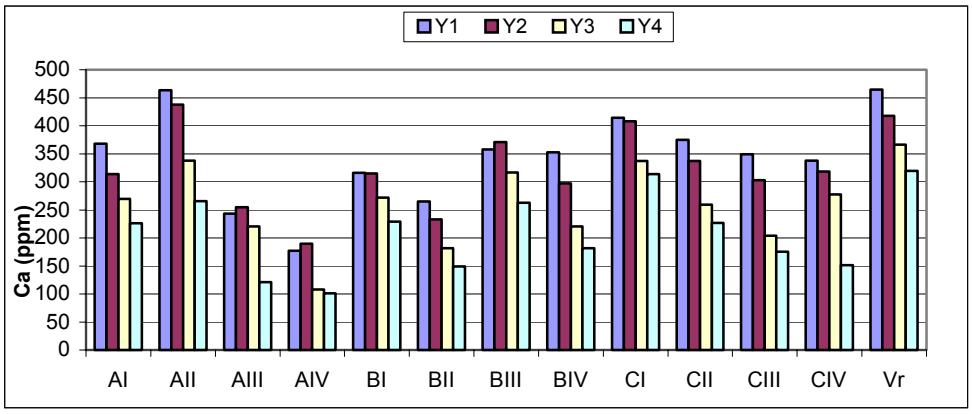

Fig. 5 The evolution of Ca concentration in substrate

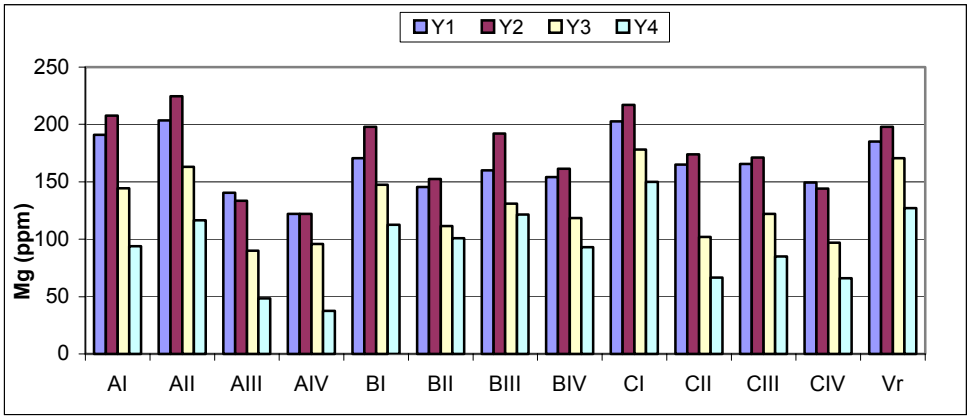

Fig. 6 The evolution of Mg concentration in substrates

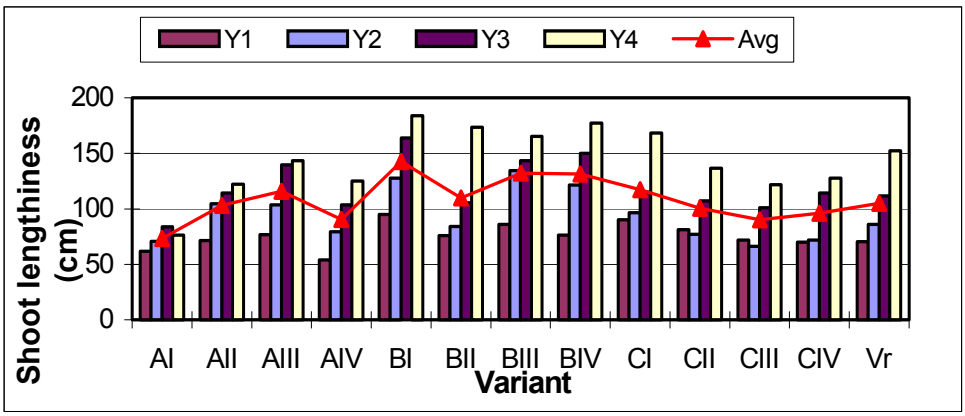

Fig. 7 The influence of substrate on shoots developing

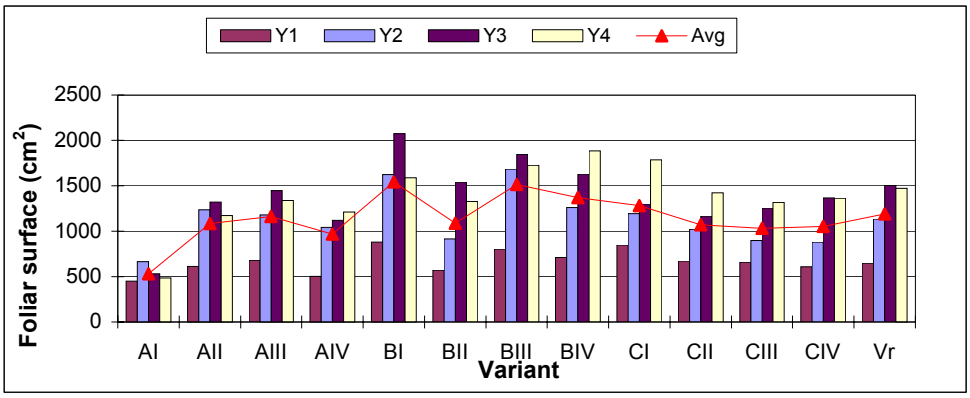

Fig. 8 The influence of substrate on foliar system developing

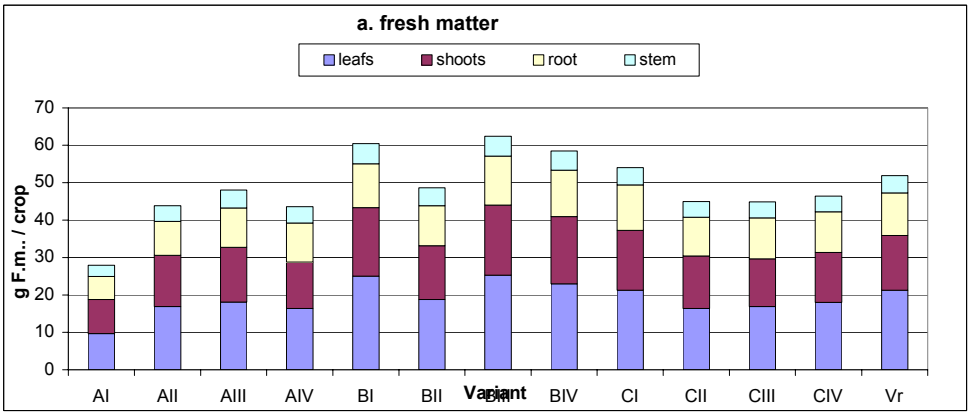

Fig. 9 The influence of substrate on fresh matter repartition

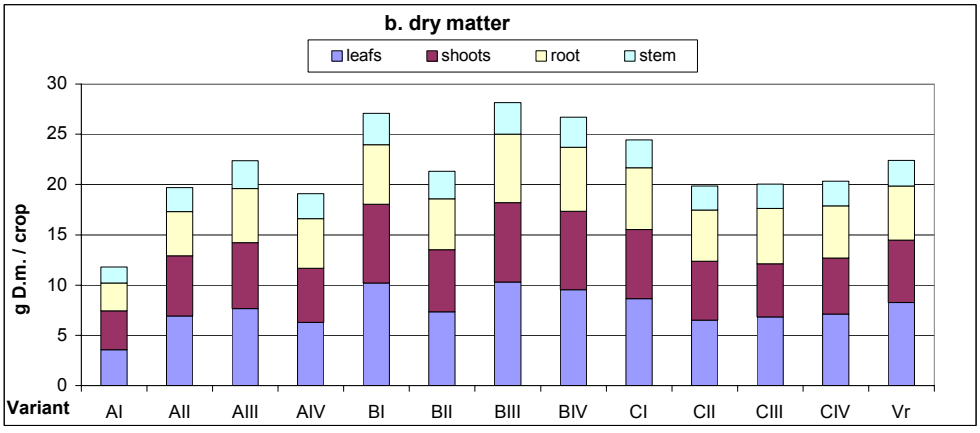

Fig. 10 The influence of substrate on fresh matter repartition

Table 3 The influence of nutritional substrate on the average rates of one eye grape cutting by crop

Variant	A I	A II	A III	A IV	B I	B II	B III	B IV	C I	C II	C III	C IV	Vr
Total grape cutting by crop	67.7	84.4	81.3	74.5	104.8	83.9	106.0	96.5	93.4	85.2	84.2	79.8	85.0
Green (%)	38.1	30.8	20.1	20.3	17.4	14.4	11.4	8.0	12.4	15.2	12.8	16.3	16.8
Woody (%)	61.9	69.2	79.9	79.7	82.6	85.6	88.6	92.0	87.6	84.8	87.2	83.7	83.2

BOTANY & PHYSIOLOGY

Obtaining and characterizing flavonoids and polyphenolic acids from *Cynara scolymus L.* (Artichoke) leaves and *Arctium lappa L.* (Burdock) roots

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Keywords: polyphenolic acids, flavonoids, artichoke, *Cynara scolymus L*., burdock, A*rctium lappa L.*

ABSTRACT

This paper presents a biochemically safe process of obtaining active substances from medicinal plants with economical potential: *Cynara scolymus L.* (artichoke) leaves and *Arctium lappa L.* (burdock) roots. A fast and reliable method based upon classic extraction was used to obtain flavonoids and polyphenolic compounds such as cynarin and chlorogenic acid from *Cynara scolymus* L. *and Arctium lappa* L. There are a few comparative studies regarding the total content of polyphenols and flavonoids of watery and ethanol extracts of medicinal plants from Romanian wild flora. These results are in agreement with those indicated by Romanian Pharmacopoeia. It was observed that the highest total flavonoids content was found in the *Cynara scolymus L.* leaves 55^0 ethanol extract (0.61%) and the highest total polyphenol acids content was found in the *Arctium lappa L.* roots. roots 70^0 ethanol extract (2.76 %), w/w (%).

INTRODUCTION

Cynara scolymus L. (artichoke) leaves and *Arctium lappa L.* (burdock) roots contain a variety of chemical compounds, such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids and amino acids. The medicinal plants composition depends upon the harvesting area vegetation. For example, the polyphenols amount is related to the harvest time, seasonal variations, geographic localization, and the part of the plant harvested as well as the diversity of the species.

Over the past 10 years, researchers and food manufacturers have become increasingly interested in polyphenols. The chief reason for this interest is the recognition of the antioxidant properties of polyphenols and their probable role in the prevention of various diseases associated with oxidative stress, cancer, cardiovascular, neurodegenerative or metabolic diseases (Sharaf-Eldin et al., 2007). In addition, polyphenols have several other specific biological actions that which are still poorly understood (Wang. et al., 2006).

The phenolic acids can be discriminated in two major classes: benzoic acid or cinnamic acid derivatives, as can be seen from Figure 1 (Neamtu et al., 1989).

The flavonoids, which share a common structure consisting of 2 aromatic rings (A and B) that are bound together by 3 carbon atoms that form an oxygenated heterocycle (ring C), may themselves be divided into 6 subclasses as function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins) (Neamtu et al., 1989).

MATERIALS AND METHODS

Regents and plant material

Gallic acid and rutoside were used as standard chemicals.

Distilled water, ethanol solution, Folin-Ciocalteu reactive, sodium carbonate 10%, sodium acetate 100 g/L and aluminum trichloride 25g/L were used.

Cynara scolymus L. (artichoke) leaves and *Arctium lappa L*. (burdock) roots were collected from Romanian wild flora, from different areas of Banat.

Plant materials were dried. They were stored in dark bags to protect them from humidity and light and, before each bunch of experiments, they were cut into pieces of different sizes having the appropriate equivalent diameter.

Extraction

10 g of dry powdered material with 0.315 mm particle size was macerated with distilled water and a mixture of water and ethanol (55^0 and 70^0) using a sample weight to solvent volume ratio of 1/10. The mixture was left at room temperature for 24 h in closed Erlenmeyer flasks. After the completion of the extraction process, the liquid phase (the filtrate) was separated from the residual plant material by vacuum pump filtration. The filtrate was analyzed by spectrophotometry.

Apparatus

The analysis was performed with a spectrophotometer CECIL 1011 model, with VIS detector.

All the calculations concerning the quantitative analysis were performed using external standardization by measurement of absorbance of a specific wavelength of light.

Standard Solutions

Stock solution of gallic acid (0.1g/L) and rutoside (0.1 g/L) was prepared in distilled water (v/v). Standard series in the concentration range of 25- 200 µg/mL for flavonoids and 4-16 µg/mL for polyphenols acid were obtained from the stock solution.

Spectrophotometry conditions

Spectrophotometry analysis was performed by total flavonoids and polyphenols analysis methods presented in Romanian Pharmacopeia.

Volumes of 2 mL solution prepared from each sample and placed into quartz cuvettes were analyzed into the spectrophotometer.

The total content of polyphenols was quantified with Folin-Ciocalteu reactive and Na_2CO_3 10%; the maximum of absorption of the colored solution was evaluated with respect to the calibration curve. The polyphenols are expressed as mg/g gallic acid corresponding to a final set concentration of 16 µg/mL.

The total content in flavonoids was quantified using an ethanol solution of aluminum trichloride 25g/L and sodium acetate 100 g/L; again, the maximum of absorption of the colored solution was evaluated with respect to the calibration curve. The total content of flavonoids is expressed as mg/g rutoside corresponding to a final set concentration of 200 µg/mL.

The blank solution was prepared in the same conditions as samples and was used for all experimental spectrophotometry studies.

Quantification was realized measuring the absorbance at the wavelength of 430 nm for flavonoids and 765 nm for polyphenolic acids to determine the concentration of a known solute in a given solution by the application of the Beer-Lambert law. Optical density (OD) is the absorbance per unit length, although it is sometimes used as a synonym for the absorbance with a base-10 logarithm.

The results were obtained as a mean value of three separate samples.

RESULTS AND DISCUSSIONS

The determination of flavonoids and polyphenolic acids from autochthonous medicinal plants was performed with the aforementioned spectrophotometry method.

The assay results of *Cynara scolymus* L. (artichoke) leaves and *Arctium lappa L.* (burdock) roots are presented in Table 1. The proposed spectrophotometry approach gives accurate results, as reflected by the good agreement observed with the literature data.

Our results show that content of total flavonoids and polyphenolic acids of *Cynara scolymus* L. (artichoke) leaves and *Arctium lappa L.* roots ethanolic extracts is significantly higher than content of aqueous extracts.

Therefore, the highest total flavonoids content was found in the *Cynara scolymus* L. leaves 55^0 ethanol extract (1/10 w/v) obtained through maceration at room temperature in 24 h (0.61 %, Table. 1), while the highest total polyphenol acids content was found in the *Arctium lappa L.* roots 70^0 ethanol extract (1/10 w/v) obtained through maceration at room temperature in 24 h (2.76 %, Table 2). These results are fairly comparable with those presented in Romanian Pharmacopeia.

The results coincide for the flavonoids content found in the *Cynara scolymus L.* leaves and the polyphenol acids content from the *Arctium lappa L.* roots.

CONCLUSIONS

A spectrophotometry method developed and applied for the determination of flavonoids and polyphenolic acids content of two medicinal plants: *Cynara scolymus* L. (artichoke) leaves and *Arctium lappa L.* (burdock) roots is presented in this study. The proposed spectrophotometry approach gave reliable results for the analysis of flavonoids and polyphenolic acids in all samples, their content being in good agreement with the data presented in the Romanian Pharmacopoeia.

The results of the present work form a basis for the future studies in this area, when intensification of the extraction will be sought using ultrasonic or microwave fields.

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Table 1. The dry soluble substances and total flavonoids content of *Cynara scolymus* L. (artichoke) leaves and *Arctium lappa L.* (burdock) roots.

	***Cynara scolymus* L. (artichoke) leaves**		***Arctium lappa L.* (burdock) roots**	
Samples	**Dry soluble substances g/100 g plant material**	**Total flavonoids content g/100 g plant material**	**Dry soluble substances g/100 g plant material**	**Total flavonoids content g/100 g plant material**
ethanol 55 ^{0}, sample weight to solvent volume ratio (w/v): 1/10, 0.315 mm particle size	17.50	**0,61**	29.69	***0,35***
ethanol 70 ^{0}, sample weight to solvent volume ratio (w/v): 1/10, 0.315 mm particle size	17.19	0,19	26.23	0,35
distilled water, sample weight to solvent volume ratio (w/v): 1/10, 0.315 mm particle size	19.23	0,04	35.87	0,04

Table 2. The dry soluble substances and total polyphenolic acids content of *Cynara scolymus* L. (artichoke) leaves and *Arctium lappa L.* (burdock) roots.

	***Cynara scolymus* L. (artichoke) leaves**		***Arctium lappa L.* (burdock) roots**	
Samples	**Dry soluble substances g/100 g plant material**	**Total polyphenolic acids content g/100 g plant material**	**Dry soluble substances g/100 g plant material**	**Total polyphenolic acids content g/100 g plant material**
ethanol 55 ^{0}, sample weight to solvent volume ratio (w/v): 1/10, 0.315 mm particle size	17.50	0,39	29.69	2,44
ethanol 70 ^{0}, sample weight to solvent volume ratio (w/v): 1/10, 0.315 mm particle size	17.19	***0,47***	26.23	**2,76**
distilled water, sample weight to solvent volume ratio (w/v): 1/10, 0.315 mm particle size	19.23	0,37	35.87	1,69

Research regarding the composition of *Agastache* Genus (*Lamiaceae*) cultivated in Romania

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Keywords: *Agastache*, dry matter, minerals, pigments, volatiles, essential oil

ABSTRACT

The researches were performed on six species of *Agastache* genus, originally from America, cultivated by SCDL Bacau. The plants were analyzed from viewpoint of water content, dry matter, minerals, pigments and volatiles contents. The essential oils were obtained by hydrodistillation, and analyzed by GC-MS. The main compounds were identified by mass spectra and retention-time correlations. The essential oils of *Agastache mexicana* held as main compounds: pulegone (36.78%), menthone (26.03%) and limonene (23.66%), and that of *Agastache rupestris*: estragol (methyl chavicol) (53.91%), pulegone (21.58%) and menthone (16.25%). The main oil constituents of *Agastache foenicullum* are menthone (36.63%), pulegone (28.31%), estragol (11.97%) and isomenthone (6.47%). Estragol is the main constituents of *A. anisata* (63.23%) and *A. hibrida* (89.19%). The essential oil extracted from *Agastache cana* held as main compounds: β-phelandren (23.9%), β-cubebene (15.16%), limonene (12.57%), γ-terpinene (11.52%), β-pinene (7.49%) and caryophyllene (6.75%). Pulegone and menthone are not present in the essential oils of this species. All investigated species have been studied for the first time in Romania from phytochemical point of view.

INTRODUCTION

The *Agastache* genus plants belong to *Lamiaceae f*amily and spread in the USA and north of Mexico. They are considered ornamental plants, but they are used as spicy herbs (Facciola, 1990) or medicinal plants – infusion for the flu treatment (Foster and Duke, 1990). Thanks to their volatiles content, the most *Lamiaceae* cultivated species hold a great economical importance (Burzo et. al., 2005).

The mature leaves of six *Agastache* species, originally from America and cultivated by SCDL Bacau, were analyzed from the viewpoint of water content, dry matter, minerals, pigments and volatiles contents. Moreover it was studied the variation of dry matter and minerals contents in each plant part of A. *foeniculum.* All investigated species have been studied for the first time in Romania from phytochemical point of view**.**

MATERIALS AND METHODS

The researches were performed on six species of *Agastache* genus, cultivated by SCDL Bacău: *A. cana* (Hook) Wot.& Standl, *A. foenicullum* (Pursh) Kuntze, *A. mexicana* (Kunth) Link & Epling, *A. rupestris* (Greene) Standl, *A. anisata* and *A. hybrida*.

Dry matter content and water content were determined by tissues drying at 105 $^{\circ}$C. The leaf pigments were determined using a spectrophotometer after extraction with acetone 80%. The minerals were determined by tissues calcinations at 560 $^{\circ}$C, followed the solubilization in HNO_3 conc. and the solution 1% were analyzed by a spectrometer inductively coupled with plasma (ICP-AES) IRIS INTREPRID.

The volatiles were obtained by hydrodistillation using a Singer-Nickerson apparatus, and analyzed by GC-MS. The components of essential oils were separated by

GC-MS Agilent using the follow system: a capillary column DB 5 (25m x 0.25 mm), gas carrier He, the gradient temperature 60 °C - 280 °C and a rate of 4 °C. The components were identified using the NIST databank and Kovats index.

RESULTS AND DISCUSSIONS

The leaves of the *Agastache* species and hybrids studied hold a water content between 20.86 % (*A. hybrida*) and 23.63 % (*A. cana*), dry matter between 76.37 % (*A. cana*) and 78.14 % (*A. hybrida*) and minerals between 2.51 % (*A. hybrida*) and 3.59 % (*A. mexicana*) (Table 1). This variation was a consequence of the plant adaptation in this new cultivation area with environmental conditions different from originally area.

The analyses performed of plants organs of *A. foeniculum* (Table 2) revealed that the lowest water content was determined in roots (17.59 %) and the highest in leaves (23.24 %). The highest mineral content was measured in roots (5.01%), and the lowest in stem (1.65%). The leaves of *A. foeniculum* contained 76.76% dry matter and 3.02 % minerals.

The mineral content (Table 3) analyzed revealed that the K^+ (an osmotic active element) had a high level in leaves of *A. mexicana* (1323.85 mg/100 g f.w.), *A. cana* (1314.59 mg/100 g f.w.) and *A. rupestris* (1130.23 mg/100 g f.w.), species adapted to hydric stress. The leaves of *A. mexicana* contained the highest level of Al (29.90 mg/100 g f.w.), Cu (0.38 mg/100 g f.w.) and B (0.60 mg/100 g f.w.), and the leaves of *A. hybrida* had the highest level of Ca (574.21 mg/100 g f.w.), Mg (129.34 mg/100 g f.w.), Mn (0.98 mg/100 g f.w.) and Zn (1.33 mg/100 g f.w.).

The investigated minerals of studied *Agastache* species revealed that the K had the great variations (345.60 – 1323.85 mg/100 g f.w.) between the cultivars, while the Cu had the lowest variation (0.22 – 0.38 mg/100 g f.w.). The leaves of *A. foeniculum* hold the lowest mineral content of all 11 minerals investigated.

The evaluation of minerals content in plant parts of *A. foeniculum* (Table 4) emphasized that the roots hold the highest level of K (537.91 mg/100 g f.w.), the stem holds the highest level of Ca (252.25 mg/100 g f.w.), and the leaves the highest level of Mg (69.29 mg/100 g f.w.) and P (9.37 mg/100 g f.w.). In flowers was determined the highest content of microelements like: Al (84.98 mg/100 g f.w.), Cu (45.11 mg/100 g f.w.), Fe (55.11 mg/100 g f.w.), Na (42.53 mg/100 g f.w.), Mn (1.86 mg/100 g f.w.), Zn (1.01 mg/100 g f.w.) and B (0.47 mg/100 g f.w.).

The leaf pigments content of the studied *Agastache sp*. emphasized a low variation of total chlorophyll (180.07 – 228.63 mg/100 g f.w.) and carotenes (35.10 – 44.85 mg/100 g f.w.) (Table 5). The chlorophyll a/b ratio varied between 1.53 and 2.21, and the chlorophyll/carotenes ratio varied between 4.48 and 5.36. The highest chlorophyll level was measured in *A. cana* leaves (228.63 mg/100 g f.w.) (Burzo et.al., 2004).

The main compounds of volatiles were identified by mass spectra and retention-time correlations (Figure 1). ***The essential oil*** of *Agastache mexicana* cultivated in Romania (Table 6) held as main compounds: pulegone (36.78%), menthone (26.03%) and limonene (23.66%). These results are consistent with the informations reported by Estrada-Reyes et al. (2004) for other subspecies of *Agastache mexicana*. Their GS-MS analyses showed that methyl chavicol, limonene and linalool were the main constituents of the essential oils of *A. mexicana* subsp. *mexicana*, while pulegone, menthone and isopulegone were the major constituents found in *A. mexicana* subsp. *xolocotziana.*

The essential oil (Table 6) from *Agastache rupestris* contains mainly estragol (methyl chavicol) (53.91%), pulegone (21.58%) and menthone (16.25%), while the main oil constituents of *Agastache foenicullum* are menthone (36.63%), pulegone (28.31%), estragol (11.97%) and isomenthone (6.47%). Estragol is also the main constituent of *A. anisata* (63.23%) and *A. hibrida* (89.19%). The essential oil extracted from *Agastache cana* held as main compounds: β-phellandrene (23.9%), β-cubebene (15.16%), limonene (12.57%), γ-terpinene (11.52%), β-pinene (7.49%) and caryophyllene (6.75%). Pulegone and menthone are not present in the essential oils of this variety.

CONCLUSIONS

The water content varied between 20.86 % (*A. hybrida*) and 23.63 % (*A. cana*), dry matter between 76.37 % (*A. cana*) and 78.14 % (*A. hybrida*) and minerals between 2.51 % (*A. hybrida*) and 3.59 % (*A. mexicana*).

The leaves of *A. mexicana, A. cana* and *A. rupestris* hold a high level of K^+. *A. mexicana* leaves hold a high level of Al, Cu and B, and *A. hybrida* leaves hold a high level of Ca, Mg, Mn and Zn.

The leaf pigments content varied between 180.07 – 228.63 mg/100 g f.w. (total chlorophyll) and 35.10 and 44.85 mg/100 g f.w. (carotenes).

The essential oils of *Agastache mexicana*, *Agastache rupestris* and *Agastache foenicullum* hold as main compounds: pulegone and menthone. Estragol is the main constituent of *Agastache anisata* and *Agastache hibrida*, while *Agastache cana* contain any traces of pulegone and menthone. The main compounds of *Agastache cana* were β-phelandrene, limonene, γ-terpinene.

ACKNOWLEDGEMENTS

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Tables

Table 1. The content of water, dry matter and minerals from leaves of *Agastache* species

Cultivar	Water %	Dry matter %	Minerals %
Agastache cana	23,63	76,37	2,64
Agastache foenicullum	23,24	76,76	3,02
Agastache mexicana	21,66	78,34	3,59
Agastache rupestris	21,20	78,80	3,20
Agastache hybrida	20,86	79,14	2,51
Agastache anisata	22,32	77,68	2,84

Table 2. The content of water, dry matter and minerals in *Agastache foeniculum* organs

Plant organ	Water %	Dry matter %	Minerals %
Roots	17,59	82,41	5,01
Stems	20,00	80,00	1,65
Leaves	23,24	76,76	3,02
Flowers	20,77	79,23	2,02

Table 3. Mineral content of *Agastache* sp. leaves (mg/100 g f.w.)

Cultivar	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
A. cana	30,19	0,46	295,38	0,35	**17,13**	1.314,59	74,34	0,73	35,62	12,97	0,82
A. foeniculum	17,23	0,34	190,00	0,22	8,86	345,60	55,70	0,38	17,19	9,37	0,60
A. mexicana	**29,90**	**0,60**	425,97	**0,38**	9,06	**1.323,85**	55,75	0,33	27,40	**22,11**	1,03
A. rupestris	25,12	0,51	450,34	0,36	10,02	1.130,23	56,12	0,37	22,11	21,30	1,00
A. hybrida	14,92	0,57	**574,21**	0,36	11,46	424,06	**129,34**	**0,98**	13,41	20,24	**1,33**
A. anisata	23,33	0,38	310,22	0,32	9,23	823,00	61,23	0,53	21,13	19,21	0,96

Table 4. Mineral content of plant parts of *Agastache foenicullum* (mg/100 g f.w.)

Organ	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
Roots	9,68	0,27	156,73	0,18	4,04	**537,91**	31,66	0,18	23,44	5,96	0,40
Stem	28,29	0,29	**252,25**	0,12	18,76	186,55	53,19	0,68	12,61	5,62	0,52
Leaves	17,23	0,43	190,00	8,86	18,86	345,60	**69,29**	0,38	17,19	**9,37**	0,60
Flowers	**84,98**	**0,46**	124,08	**45,11**	**55,11**	186,23	55,70	**1,86**	**42,53**	1,49	**1,01**

Table 5. The chlorophyll and carotene content of *Agastache* sp. leaves (mg/100 g f.w.)

Cultivar	Chlorophyll a	Chlorophyll b	Total chlorophyll	Chlorophyll a/b ratio	Carotenes	Carotenes/Chlorophy
A. cana	138,12	90,52	**228,63**	1,53	44,85	5,10
A. foeniculum	112,12	58,78	180,90	2,08	40,38	4,48
A. mexicana	120,19	68,13	188,32	1,76	35,10	5,36
A. rupestris	125,11	59,23	184,34	2,11	37,20	4,95
A. hybrida	123,93	56,14	180,07	2,21	37,91	4,75
A. anisata	128,13	58,33	186,46	2,19	39,88	4,67

Table 6. The main components identified in the essential oils of *Agastache sp.*

Compounds	*A. mexicana*	*A. rupestris*	*A. cana*	*A. foenicullum*	*A. hybrida*	*A. anisata*
Octene-3-ol	0,19	-		-	-	-
Thujene	-	-	1,41	-	-	-
α-Pinene	0,17	0,50	2,19	0,05	-	0,04
Sabinene	0,36	0,24	2,53	0,13	-	0,08
1-Octene-3-ol	-	-	-	1,16	-	0,76
3-Octenone	-	-	-	0,32	-	0,33
β-Pinene	-	0,58	7,49	-	0,45	-
β-Felandren	-	0,16	23,97	0,82	-	0,26
α-Terpinene	-	-	7,47	-	-	-
Limonene	23,66	3,61	12,57	7,83	4,81	28,45
trans-Ocimene	-	-	3,41	-	-	-
Linalool	11,47	-	-	-	0,12	0,08
γ-Terpinene	-	-	11,52	-	-	-
1- Octenyl acetat	0,27	-	-	0,53	-	2,16
3-Octyl acetate		-	-	-	-	0,03
Verbenol	0,23	-	-	-	-	-
Menthadienol	5,93	-	-	0,42	-	-
Isomenthone	5,94	1,44	-	6,47	2,31	0,02
Menthone	26,03	16,25	-	36,63	3,53	0,10
Isopulegone	0,61	1,01	-	1,09	1,31	0,08
Estragol	-	53,91	-	11,97	89,19	63,23
Menthol	-	2,09	-	-	1,97	-
Isomenthol	-	0,15	-	-	-	-
Pulegone	36,78	21,58	-	28,31	0,98	0,09
Anethole	-	-	-	-	-	0,04
Piperitone	0,59	4,09	-	-	0,35	-
Isopiperitone			-	0,50		0,03
Bicyclo elemene	-	-	0,74	-	-	0,15
Dihydrocarveol	-	0,16	-	-	-	-
Eugenol	-	-	-	-	-	1,34
Hydroxymenthone	-	0,24	-	-	-	-
Menthyl acetate	-	0,19	-	-	-	-
Isopulegyl acetate	-	0,50	-	-	-	-
Piperitone	-	2,00	-	0,16	-	-
Piperitone oxide	-	0,69	-	-	-	-
Methyl eugenol			-	0,26		
β-Caryophyllene	0,38	-	6,75	0,93	-	0,31
D-Germacrene	-	-	5,13	0,16	-	0,31
Bicyclogermacrene	-	-	1,72	0,36	-	0,16
Amorphene	-	-	0,18	0,08	-	0,12
Caryophyllene oxid	-	-	-	0,07	-	0,06

Figure

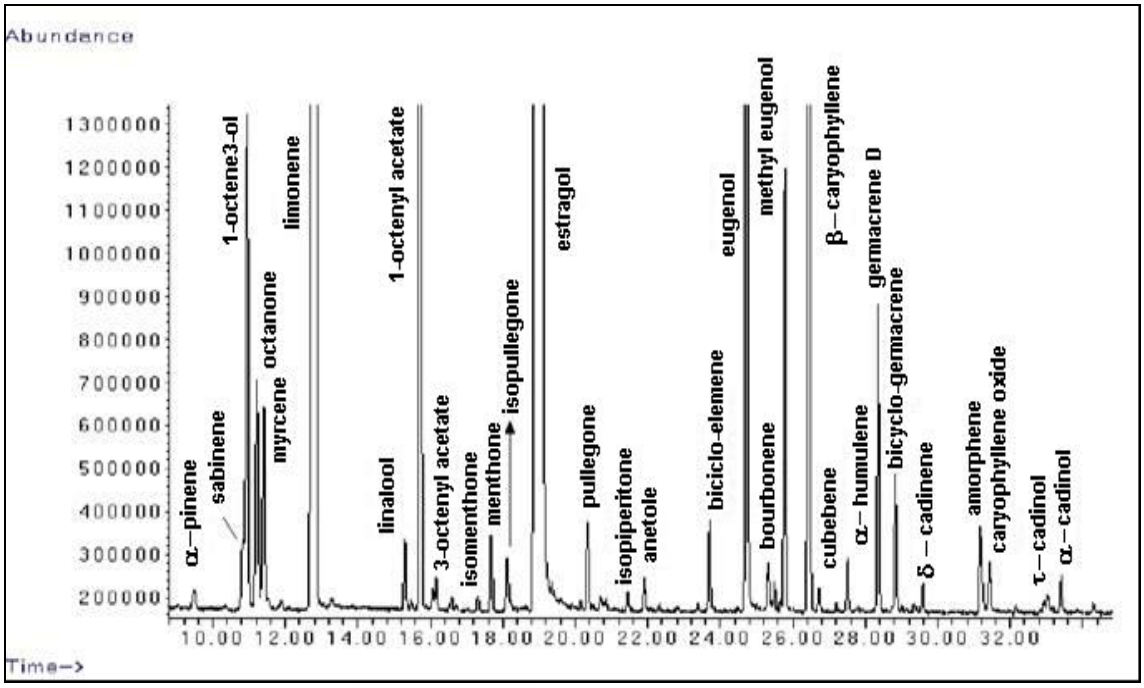

Fig.1. The chromatogram of essential oils of *Agastache anisata*

Physiological responses of Banat's common bean landraces (*Phaseolus vulgaris* L.) seedlings to osmotic stress

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Keywords: Proline, dry matter, germination, tolerance, hydric deficiency

ABSTRACT

Phaseolus vulgaris has a great variability regarding the tolerance to osmotic stress. In our experiment we tested osmotic stress tolerance of starting with values 1 MPa and up to 4 MPa induced by polyethylene glycol-6000 (PEG-6000). The experimental results achieved made evident the existence of some bean genotypes with a good tolerance to osmotic stress during germination (Berini, Bocsa Romana, Dudestii Noi, Sudrias urcatoare, Sacu, Sudrias pitica, Ciresu, Santana, Tincova, Comoraste). These genotypes have recorded during germination normal intensities of radicle growth and cotyledon development, and they have synthesized important amounts of free proline with osmoprotector role. We also measured the dry matter content of stressed genotype.

INTRODUCTION

Osmotic stress tolerance in plants is a complex phenomenon that involve morphological and developmental changes as well as physiological and biochemical processes. Two components have been identified as the probable cause of salinity toxicity, osmotic stress and ion toxicity.

Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semi-arid region of the world, reducing the value and productivity of the affected land (Gama et al., 2007).

The identification of tolerant genotypes that may sustain a reasonable yield on salt affected soils has been a strategy adopted by scientists to overcome salinity (Lee Dilly et al.,1994).

Bean protein is very valuable from qualitative point of view, including the majority of essential amino acids, at a lower cost comparative with animal protein. From this consideration, our research followed the tolerance showed by 10 bean genotypes at osmotic stress.

MATERIALS AND METHODS

The biological material used in our study consists of 10 *Phaseolus vulgaris* local landraces: Berini, Bocsa Romana, Dudestii Noi, Sudrias urcatoare, Sacu, Sudrias pitica, Ciresu, Santana, Tincova, Comoraste. The experimental variants were: V_0 – control (distillated water), V_1 – PEG -1 MPa, V_2 – PEG – 2 MPa, V_3 – PEG- 4 MPa). During seed germination we have determined the seed germinating rate, and free proline content (mg/g f.w.). The germination seed rate was determined by counting germinated seeds and it was repeatedly done during the experimental period (Bewley and Black, 1994). The proline accumulation is a common metabolic response of superior plants affected by water deficit and osmotic stress condition. This subject was intensely debated in the scientific world in the last 20 years (Sumalan and Dobrei Carmen, 2002). The dry matter was obtained by the diference between fresh weight of biological

material and his humidity and was determined by thermobalance Kern MLS 50-3ha160. For this determination we used embrions axes and cotyledons.

RESULTS AND DISCUSSIONS

From the results obtained regarding the germination potential measured at various time intervals, it has been noticed that Dudestii Noi local land race showed the higher germination rate on V_3, but this result can be inconclusive because of the fact that this land race have origin unknown. From the data analysis presented in Table 1, after 96 hours it has been noted that the best result regarding growing rate of plants was obtained in Dudestii Noi genotype and the lower growing rate was observed in Berini genotype on V_3. After 192 hours the best results regarding growing rate was noted at genotype Dudestii Noi (V_3)

Regarding the free proline accumulation the results are not very different in the V0 and V1 but the differences are evident in V_2 and V_3 confirming the theory that the proline is an osmoprotectant component. The best tolerant genotype was Dudestii Noi with 3,247 mg/g f.w. (Table 2).

The dry matter percent showed that in V_3 the most tolerant genotypes was Dudestii Noi with 15,22 % d.m. (Figure 1).

CONCLUSIONS

The osmotic stress induced using PEG (-1,-2,-3,-4 MPa) generated reduction of the germinating rate during the entire experimental period.

The best results regarding germinative capacity was noted in Dudestii Noi genotype.

Regarding the dry matter amount osmotic stress produce an increase of dry matter percent and some genotypes showed some tolerance (Dudestii Noi).

The results confirm the correlation between the synthesis of free proline and tolerance to osmotic stress, the best genotypes was Dudestii noi with 3,247 mg/g f.w.

ACKNOWLEDGEMENTS

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Table 1. Experimental results regarding germination potential of studies genotypes after 96 and 192 hours (%)

	96 hours				192 hours			
	V_0	V_1	V_2	V_3	V_0	V_1	V_2	V_3
Comoraste	95	70	65	50	100	75	70	55
Santana	100	85	65	60	100	90	75	65
Tincova	100	100	85	65	100	100	90	70
Sudrias pitica	95	90	80	40	100	90	85	40
Ciresu	90	85	65	50	100	80	70	50
Sacu	100	65	55	35	100	85	60	50
Sudrias urcatoare	85	70	45	35	100	85	80	50
Bocsa Romana	80	90	60	40	85	95	70	50
Dudestii Noi	90	100	85	75	90	100	95	80
Berini	100	100	95	30	100	100	100	55

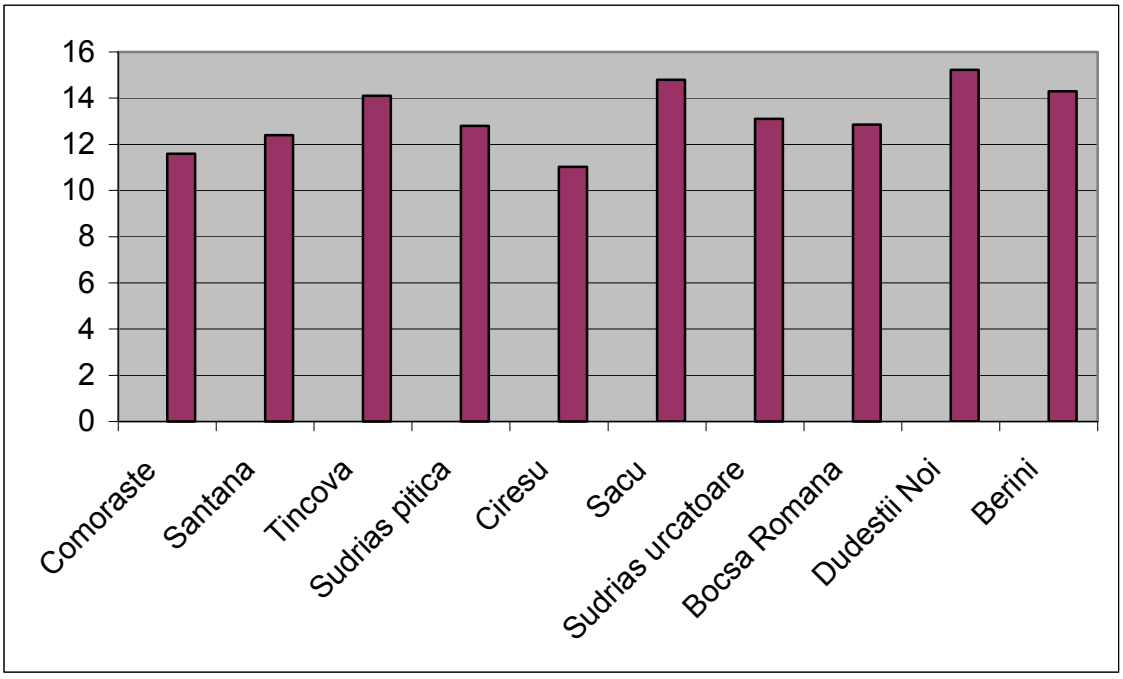

Fig. 1. Dry matter percent at studied genotype on V_3 (- 4 Mpa PEG)

Table 2. Free proline accumulation in stressed genotypes (mg/g f.w.)

Genotype	Variant	Mean	%	Dif. Mt.
COMORASTE	V_0	0,857	100	0
	V_1	0,975	113,833	0,118
	V_2	1,134	132,345	0,277
	V_3	1,268	148,041	0,411
DUDESTII NOI	V_0	1,111	100	0
	V_1	1,321	118,952	0,210
	V_2	1,340	120,693	0,229
	V_3	3,247	292,334	2,136
CIRESU	V_0	1,179	100	0
	V_1	1,235	104,727	0,055
	V_2	1,627	137,988	0,448
	V_3	1,733	146,971	0,554
SACU	V_0	1,223	100	0
	V_1	1,110	90,779	-0,1128
	V_2	1,378	112,703	0,155
	V_3	3,001	245,347	1,778
BOCSA ROMANA	V_0	1,107	100	0
	V_1	1,193	107,767	0,086
	V_2	1,399	126,385	0,292
	V_3	1,417	127,926	0,309
SANTANA	V_0	1,133	100	0
	V_1	1,236	109,070	0,102
	V_2	1,368	120,784	0,235
	V_3	2,979	262,843	1,845
SUDRIAS URCATOARE	V_0	1,078	100	0
	V_1	1,337	124,002	0,258
	V_2	1,396	129,466	0,317
	V_3	1,805	167,344	0,726
TINCOVA	V_0	0,969	100	0
	V_1	0,921	95,085	-0,047
	V_2	1,155	119,227	0,186
	V_3	1,891	195,151	0,922
SUDRIAS PITICA	V_0	1,394	100	0
	V_1	1,107	79,383	-0,287
	V_2	0,998	71,564	-0,396
	V_3	1,272	91,231	-0,122
BERINI	V_0	1,696	100	0
	V_1	1,219	71,885	-0,476
	V_2	2,453	144,647	0,757
	V_3	1,661	97,942	-0,034
SACU	V_0	1,172	100	0
	V_1	1,401	119,604	0,229
	V_2	1,559	133,048	0,387
	V_3	2,241	191,248	1,069

Preliminary study regarding the qualitative characteristics of a genotype from *Pyrethrum Cinerariifolium* (trevir.) specie, as a premise in the control of pests, through the specific methods of ecologic agriculture

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ABSTRACT

At VRDS Bacău the cultivation of *Pyrethrum cinerariifolium* (Trevir.) specie, started in the year 2005. The establishment of the crop was done using an autochthon biological material, a biotype with a large genetic variability. This perennial specie is known as a plant with multiple uses (can be decorative, in China is utilized also as medicinal, especially for vermifuge proprieties) but is recognized as a plant that plays an important role in biologic agriculture practice as a prime material for the extraction of pyrethrin, especially from flowers. In gardens, only the presence of this plant keeps away the insects from the plant from near-by thus being repellent. The plant can be dried and utilised latter because the dried plant conserve the insecticide and repellent proprieties of fresh plant. The study focuses on the possibility to adapt to the agro-pedologic conditions from the East of Moldavia as well as for cultivation in ecologic conditions. Due to the fact that one of the most important technological links in the cultivation of this specie is harvesting and drying of plants, the experimental variants are concentrated on the optimal moment for flower's harvest, the position of flower on plants (central or marginal). The aim of the present study is to establish if this biotype cultivated at VRDS Bacau, in the "bio" experimental polygon, has insecticide or repellent properties and can be utilized in the pest control, as a method specific for biologic agriculture.

INTRODUCTION

Pyrethrum cinerariifolium (Trevir.), with the common name "piretrul", heaven's flower, "romoniță", "tămâioară", dalmatian chrysanthemum, has as the main active principle extracted from the plant pyrethrum or piretrine (in fact it represents six natural compounds), with insecticide proprieties, term that is utilized in production and marketing when referring to the powder made from dried flowers.

Name in: english – dalmation pellitory; french – pyrèthre de Dalmatie; German – Asehenkrautblättrige Wucherblume; Family: *Compositae;* Synonym: *Ttanacetum cinerariifolium* (Trev.) Schultz-Bip, *Chrysanthemum cinerariifolium* (Trevir) Vis), *Chrysanthemum cinerariaefolium.*

The native area is in the east of Europe and in Caucasian area, near the ex-Iugoslavia but is well spread also in China, Iraq, Turkey, Spain, and Italy. In the present the dalmatian chrysanthemum is cultivated mainly in commercial purposes, in the mountain area of Kenya, Tanzania and in Ecuador. Although the commercial production of pyrethrum is in the Ecuadorian mountain area, the plant can grow also in our region, although the concentration of pyrethrum and the number of harvests/year is much lower than in native regions.

The dalmation pellitory is a perennial plant, of 0,45 – 0,65 m high. It blossom from July till September, the flowers are hermaphrodite and are pollinated by bees and butterflies. The utility rate of plant is 2 : 5. The flowers are middle typical, white colour on the edge and in the central area the tubular, fertile flowers are yellow. The floral canes are powerful and rigid. The leaves from the entire plant are green-blue.

The plant prefers relatively dry, heavy and calcareous soils. The area that is in shadow should be avoided, because only in full sun the quantity of pyrethrum is at maximum level. It grows well also in soils that are rich in humus, humid climate but the concentration of pyrethrine will be lower.

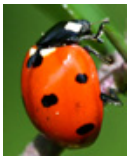
The plants doesn't require strong fertilizations, doesn't respond to nitrogen, but the presence of phosphor in quantities sufficient for the plants leads to an increase in the flower's production. During cultivation there are no special problems with the pests and pathogen attack, the only exception is sometimes with the trips that can appear in the flowers.

Utilization

Culinary. Is not recognized as edible plants.

Ornamental. The plant is not decorative, but due to its multiple utility can be utilized in the garden near flower and utile species, conferring a pleasant aspect.

Medicinal. The specie is not considered as medicinal, but the flowers have an antibiotic activity, being utilized in China against helminthes.



In biologic agriculture. The most important utilization is as prime material for the extraction of pyrethrine, from flowers (especially from the tubular flowers from inflorescence); the biggest concentration is in the floral buds (1,22 %). In the garden, only the presence of this plant acts as a repellent for insects from nearby. The plant can be dried and utilized much latter because it maintain its insecticide and repellent proprieties.

Dr. James A. Duke, in 1992, mention the main compounds from the plants of *Pyrethrum cinerariifolium* (Trev) Schultz-Bip: in flowers – Sesamin, Beta-Amyrin, Beta-Cyclopyrethrosin, Chrysanin, Chrysanolide, Chrysanthemic-Acid-Ester, Cinerins, Jasmolins, Pyrethric –Acid, Pyrethryn (7,000 – 20,000 ppm), Pyrethrol, Pyrethrotoxic-Acid; in plant – Choline, Chrysanthemine, Pyrethrosin, Stachydrine; in stem and shoots – ASH (71,000 ppm), Calcium (5,3000 ppm), Carbohydrates (794,000 ppm), FAT (5,000 ppm), Fiber (236 ppm), Phosphorus (2,400 ppm), Protein (130,000 ppm).

MATERIALS AND METHODS

At VRDS Bacău the cultivation of *Pyrethrum cinerariifolium* (Trevir.) specie started in 2005 year. The establishment of the crops was made with seeds original from our country and the biological material has its origin in a population with a large genetic variability. The crop was established generatively, from seedlings and during May the collection o plants were planted in the experimental polygon of biologic agriculture.

The applied technology was the one that is specific for "bio" cultivation: without chemical fertilization or application of pesticides, the maintaining of clean soil through hoes with specific equipments between the rows and manually on the rows

In 2007 year the culture passed well over the winter, the protection of the plants was not necessary.

The study focuses on the possibility to adapt to the agro-pedologic conditions from the East of Moldavia as well as for cultivation in ecologic conditions. At USAMV Bucharest the main compounds from the volatile oil were determined. The volatile oils

were extracted from Pyrethrum *cinerariifolium* (Trevir.) flowers, on experimental variants different from the point of view of the moment of flower's harvest, number of harvests and the position of flowers/plant (central or marginal).

Experimental variants: V1 - three harvests/plant (V1 - 30.05.2007; 07.06.2007; 15.06.2007); V2 - two harvests/plant (07.06.2007; 15.06.2007); V31 - two harvests/plant and central flowers (07.06.2007; 15.06.2007); V32 - two harvests/plant and marginal flowers (07.06.2007; 15.06.2007); V4 – two harvests, flowers harvested with cane, kept for 24 hours in water (13.06.2007; 20.06.2007); V5 - two harvests, flowers harvested with cane, kept for 48 hours in water (18.06.2007; 25.06.2007);; V6 - two harvests, media for 41 plants (07.06.2007; 15.06.2007); V7 - one harvest, media for 28 plants (18.06.2007).

Due to the fact that one of the most important technological link in the cultivation of this specie is the flower's harvest and drying, the experimental variants (Table 1 and 2), the study is referring to the date of flower's harvest, number of harvests, the position of flowers on plant (central or marginal), the way in which the plant is prepared for drying. Quantitative, different measurements were made for the establishment of the weight of fresh and dried flowers per plant, determining also the ratio of dried weight/fresh weight.

The harvesting of flowers was accomplished at full opening, after 5 days from the beginning of the blossom, according with the experimental variants.

At dried flowers, the main compounds from the volatile oils were determined, utilizing a hydro – distillation equipment type Clevenger. The separation of the compounds was accomplished with a gaseous chromatograph.

For the identifications of compounds from the volatile oils the gaseous chromatograph was coupled with a spectrometer in infrared with Fourier (FT-IR) NICOLET, and the quantitative analyses was accomplished with a ionization detector in flame coupled in parallel, the detector FT-IR being not destructive.

Also, different retention indices Kovats were utilized for the confirmation of the exact position of drops in chromatogram through the utilization of a series of n-alcans as reference.

RESULTS AND DISCUSSIONS

The analyses realized allowed the identification and dosing of the volatile oil extracted from the *Pyrethrum cinerariifolium* (Trevir.) specie of a number of 22 compounds.

From the dates presented in table 3, on remark the fact that, the main compounds identified in the volatile oil extracted from dried flowers of *Pyrethrum cinerariifolium* (Trevir.) from VRDS Bacău in condition of ecologic cultivation on the experimental variants, are different both as a number and as a percent (%) from the total of volatile oil extracted.

The results obtained on each experimental variant show that:

- the best results regarding the three compounds of pyrethrine were obtained on variant V1 with three harvests/plant: Cinerin I - 2,32 %, Piretrin I - 1,35 % şi Jasmolin I - 0,84 5; a high percent was identified also for the compounds Germacren D (38,76 %), β – Farnesen (14,91 5), Trans – Neroledol (8,46 %);
- at variant V2, the percent of pyrethrine is lower, of Cinerin I - 0,58 %, Piretrin I - 0,46 % and Jasmolin I - 0,22 %; higher percents were obtained at the compounds β – Farnesen (28,15), Germacren D (18,02) and Trans – Neroledol (15,92 %);
- variant V3.1 presents a good percent at the three pyrethrine when comparing with the variant's average Cinerin I - 2,20 %, Piretrin I - 1,05 % and Jasmolin I - 0,70 %; a higher percent was identified at the compounds Germacren D (35,74 %), β – Farnesen (14,91 %), Trans – Neroledol (8,46 %);
- at variant V3.2, were identified all three pyrethrine, the higher ratio being registered at cinerin I – 1,45 %, followed by Piretrin I – 0,75 % and Jasmolin I – 0,45 %; the most important percentages were identified Germacren D cu 34,84 %, β – Farnesen with 14,91 %, Trans – Neroledol with 7,05 % and Spatulenol with 5,85 %;
- variant V4, the three pyrethrine have values between 0,35 % (Jasmolin I) and 1,52 % (Cinerin I), while Piretrin I has 0,59 %; in what concern the other products identified, the highest percents were registered at Germacren D cu 30,82 %, β – Farnesen cu 29,32 % and Spatulenol with 7,50 %;
- at variant V5 the values of the three pyrethrine were of: Cinerin I – 1,16 %, Piretrin I – 0,80 % and Jasmolin I - 0,41 %, a little under the average on variants; a higher percent was identified at the compounds β – Farnesen (39,88 %), Germacren D (9,27 %) and Spatulenol with 9,27 %;
- at variant V6 the three pyrethrine have values similar with the average of variants, Cinerin I – 1,27 %, Piretrin I – 0,78 % and Jasmolin I - 0,55 %; a higher percent was identified at the compounds Germacren D (28,76 %), β – Farnesen (19,37 %) and Trans – Neroledol (10,38 %);
- at variant V7, the three pyrethrine have values under the average values of variants, Cinerin I – 1,12 %, Piretrin I – 0,64 % and Jasmolin I - 0,16 %; a higher percentage was identified at the compounds β – Farnesen (25,32 %), Germacren D (15,82 %) and Spatulenol with 9,68 %;

From the dates presented previous we can conclude that good results (with values over the average) were registered at variants V1 (three harvests/plant) and V3.1 (two harvests/plant at marginal flowers from plant), and at V3.2 (two harvests/plant at central flowers of plant), values similar with the average of studied variants.

The graphical representation of the main compounds identified in the volatile oils extracted from the dried flowers, on experimental variants is presented in graphic 1.

The analyses of the main compounds that determine the plant's qualities as insecticide and repellent, respectively Cinerin, Piretrin and Jasmolin, identified on the experimental variants studied in our experiment are presented in graphic 2 (Cinerin I), graphic 3 (Piretrin I) and graphic 4 (Jasmolin).

On observe that at Cinerin I were registered the highest percents in volatile oil extracted at variant V1 (17 %) and V31 (17 %), Piretrin I at variants V1 (19 %) and V31 (15 %), while Jasmolin I at variants V1 (21 %) and V31 (17 %)

From the preliminary studies accomplished at VRDS Bacau, the variants V1 (three harvests/plant - 30.05.2007, 7.06.2005, 15.06.2002) = 2,32 % (Cinerin I), 1,35 % (Piretrin I), 0,84 % (Jasmin I) and V31 (two harvest/plant - 7.06.2005, 15.06.2002) =

2,20 % (Cinerin I), 1,05 % (Piretrin I), 0,70 % (Jasmin I) are the highest, the average of experimental variants being of 1,45 % (Cinerin I), 0,80 % (Piretrin I), 0,46 % (Jasmin I).

CONCLUSIONS

1. The analyses accomplished allowed the determination, identification and dosing in volatile oil extracted, from the biotype *Pyrethrum cinerariifolium* (Trevir.) of a number of 22 compounds.
2. The main compounds identified in the volatile oil extracted from dried flowers of *Pyrethrum cinerariifolium* (Trevir.) from VRDS Bacău, in condition of ecologic cultivation of plants, on the experimental variants differ both as a number and as a percent (%) from the total of volatile oil extracted.
3. From the preliminary studies accomplished at VRDS Bacau, variants V1 (three harvest/plant - 30.05.2007, 7.06.2005, 15.06.2002) = 2,32 % (Cinerin I), 1,35 % (Piretrin I), 0,84 % (Jasmin I) and V31 (two harvests/plant - 7.06.2005, 15.06.2002) = 2,20 % (Cinerin I), 1,05 % (Piretrin I), 0,70 % (Jasmin I) are the most valuable, the average of experimental variants being of 1,45 % (Cinerin I), 0,80 % (Piretrin I), 0,46 % (Jasmin I).

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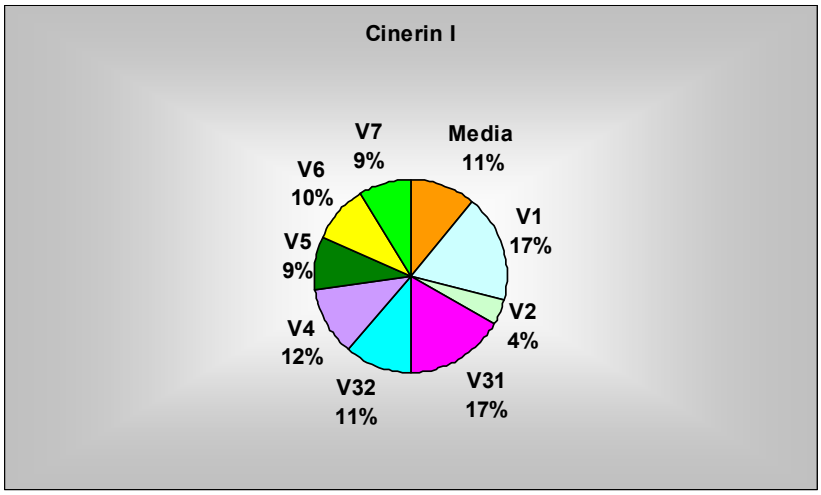
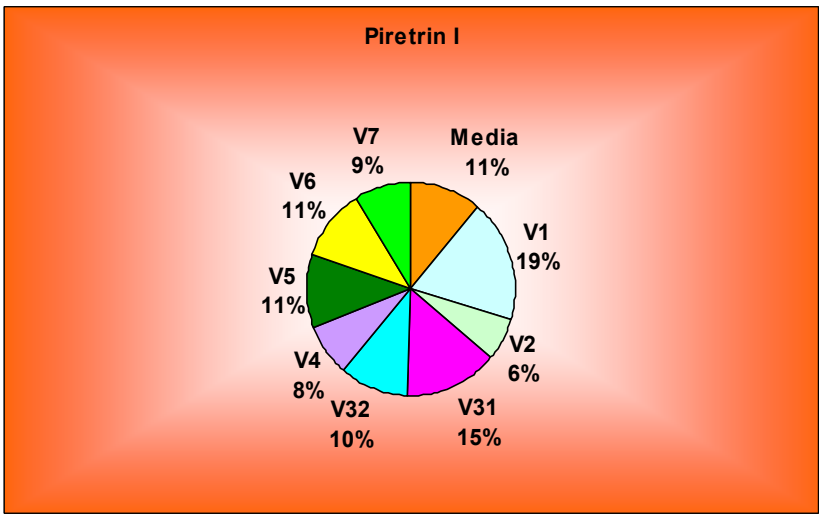
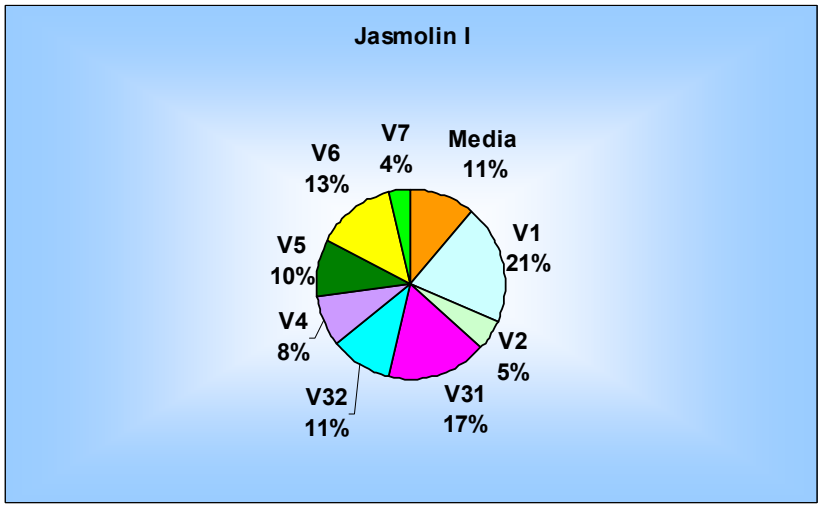


Graphic 2. Cinerin I (%) in extracted volatile oil



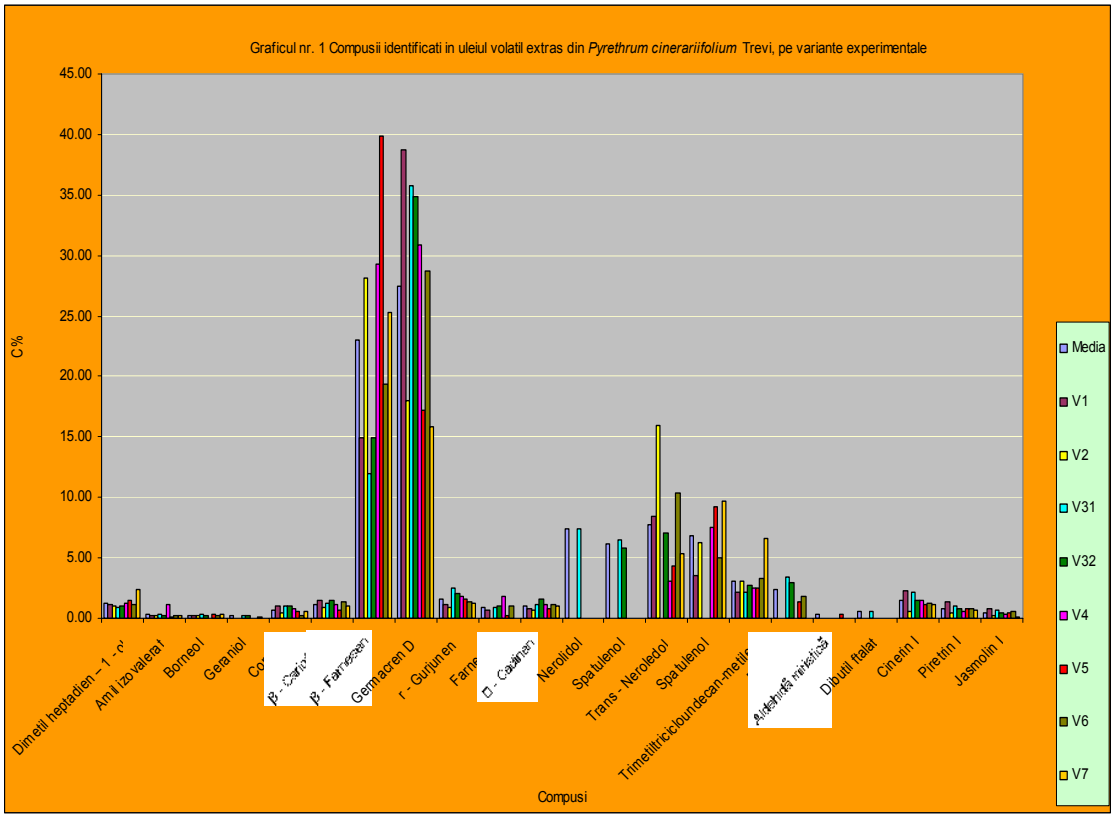
Graphic 3. Piretrin I (%) in extracted volatile oil



Graphic 4. Jasmolin I (%) in extracted volatile oil

Table 1. The main compounds identified in the volatile oil extracted from dried flowers of *Pyrethrum cinerariifolium* (Trevir.) at VRDS Bacău, on studied experimental variants

Compounds from extracted volatile oils	Experimental variants %								
	Media	V1	V2	V3.1	V3.2	V4	V5	V6	V7
Dimetil heptadien – 1 - ol	1.31	1,10	1,06	0,94	0,97	1,29	1,52	1,19	2,39
Amil izovalerat	0,35	0,21	0,26	0,29	0,23	1,15	0,16	0,23	0,27
Borneol	0,25	0,20	0,21	0,29	0,19	-	0,30	0,28	0,29
Geraniol	0,20	-	-	0,24	0,20	-	-	0,16	-
Copaen	0,71	1,08	0,47	0,99	1,08	0,75	0,53	0,19	0,60
β - Cariofilen	1,18	1,51	0,94	1,24	1,51	1,11	0,71	1,34	1,07
β - Farnesen	22.98	14.91	28,15	11,94	14,91	29,32	39,88	19.37	25,32
Germacren D	27.5	38.76	18,02	35,74	34,84	30,82	17,24	28,76	15,82
r - Gurjunen	1.59	1,17	0,94	2,48	2,09	1,80	1,63	1,32	1,27
Farnesen	0,94	0,68	-	0,86	1,07	1,84	0,22	0,97	-
□ - Cadinen	1,04	0,85	0,70	1,15	1,60	1,13	0,80	1,12	0,97
Nerolidol	7,40	-	-	7,40	-	-	-	-	-
Spatulenol	6,15	-	-	6,45	5,85	-	-	-	-
Trans - Neroledol	7,79	8,46	15,92	-	7,05	3,04	4,30	10,38	5,38
Spatulenol	6,87	3,58	6,21	-	-	7,50	9,27	4,98	9,68
Trimetiltricicloundecan-metilen	3,12	2,13	3,08	2,12	2,75	2,47	2,55	3,26	6,61
Farnesol	2,39	-	-	3,41	2,92	-	1,37	1,86	-
Aldehidă miristică	0,36	-	-	-	-	-	0,36	-	-
Dibutil ftalat	0,59	-	-	0,59	-	-	-	-	-
Cinerin I	1,45	2,32	0,58	2,20	1,45	1,52	1,16	1,27	1,12
Piretrin I	0,80	1,35	0,46	1,05	0,75	0,59	0,80	0,78	0,64
Jasmolin I	0,46	0,84	0,22	0,70	0,45	0,35	0,41	0,55	0,16



The geotropically modifications of mustard plantlets due to the phytocrom reversibility

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Keywords: mustard plantlets, phytocrom, geotropically motion

ABSTRACT

In this paper we observed the geotropically modifications of mustard plantlets due to the phytocrom reversibility at the variation of wavelength (λ_1 = 660nm - red, λ_2 = 730nm – far-red). The vegetal material consists of mustard seeds, which has been illuminated for one minute with special filters and then maintained in darkness. After five days in growth chamber, the deviation angle to vertical axe was estimated. In the plants maintained in darkness the phytochrom is only in an inactive form (P_i), which may be convert in an active form (P_a) by illumination with red light (λ = 600nm). These two forms P_i and P_a are photoconvertible. So, the mustard plantlets have a higher randomize by illumination for one minute with red light (λ_1 = 660nm) due to the phytochrom activation. For the other experimental lots the plantlets have a normal negative geotropism.

INTRODUCTION

The sensitivity and precision of the phototropic response towards unilateral light sources in laboratory studies fascinated physiologists and they began a century of work that focused on the mechanisms of light perception and the mechanisms for causing the differential elongation that brought about curvature. Such work has culminated with the discovery of photoreceptors responsible for phototropic perception but there is still disagreement as to how differential elongation is controlled.

The visible spectrum of light is from 400 nm to 760 nm. Blue light has a wavelength between 430 nm and 510 nm, while that of red light is about 660 nm; yellow 600 nm and far-red 700 to 760 nm. These wavelengths play an important role in the germination and plant motion. Plants remove the blue and the red lights and transmit the far-red light.

In the present paper were studied the geotropically modifications of mustard plantlets due to the phytocrom reversibility at the variation of wavelength (λ_1 = 660nm - red, λ_2 = 730nm – far-red).

MATERIAL AND METHOD

The mustard seeds were sown in 50 mm plastic petri dish, in agar and illuminated for 1 minute with monochromatic light (660 nm and 730 nm). Then, each box was cover with aluminum folia and maintained in dark. After 5 days in a plant growth room (25^{o}C 16h day/20^{o}C 8h night), in vertical position, the deviation angle to vertical axe was estimated.

The experimental variants were:

- V1 - Seeds in dark
- V2 - Seeds illuminated 1 minute with red light (660 nm) and then maintained in dark
- V3 - Seeds illuminated 1 minute with red light (660 nm),1 minute with far red (730 nm)and then maintained in dark
- V4 - Seeds illuminated 1 minute with far red light (730 nm) and then maintained in dark

The experiments were repeated for three times.

RESULTS AND DISCUSSIONS

For the first experimental variant, it was noticed that 49 mustard plantlets have a vertical orientation (see figure 1).

From figure 2, it remarks that the number of vertical plantlets was lower than the first variant; the randomize grad for values are higher due to the conversion of inactive phytocrom P_{660} in active formP_{730}. 29,1% plantlets illuminated with red light (660 nm) have an angle between(+/- $5^{0)}$

In variants 3, the hypocotyls of mustard were illuminated for 1 minute with red light and then another minute with far red light. It was noticed that 35,7% plantlets are vertical and 15,6% have the angle between vertical and hypocotyl around (+/- 5^0). The last flux, far red light, annulated the red light effect, so the phytocrom is in an inactive form $P_{660.}$ This proves that the phytocrom is essential in geotropic motions in plants.

From figure 4 we can see that 43,5% plantlets are vertical and 63,4% have an angle around (+/- 5^0), after illuminate for 1 minute with far red light.

For V_1, V_3 and V_4 experimental variants the phytochrom is in an inactive form P_{660}, so the geotropism are not affected. From statistic analyze it remarks that the most frequent deviation angle noted for V1, V3 and V4 variants is 0^0.

CONCLUSIONS

In plantlets maintained in dark the phytocrom is only in an inactive form (Pi), witch can be transformed in the active form (Pa) by illumination with red light (600 nm). The maxim absorption is at λ=730 nm, for the active form and at λ=660 nm for inactive form. For the rest visible domain, the spectrum for the two forms is superposing. This two forms are photoconvertible: in red light the inactive form (Pi) is changed in the active form (Pa), which in far red became inactive.

The *Sinapis alba plantlets* in V2 variants have a high randomize due to the phytochrom activation.

The plantlets from V_1, V3 and V_4 variants have a negative normal geotropism of hypocotyls.

The phytocrom has an essential role in geotropism motion at *Sinapis alba* plantlets due to the modifications of sucrose photoassimilation

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Figures

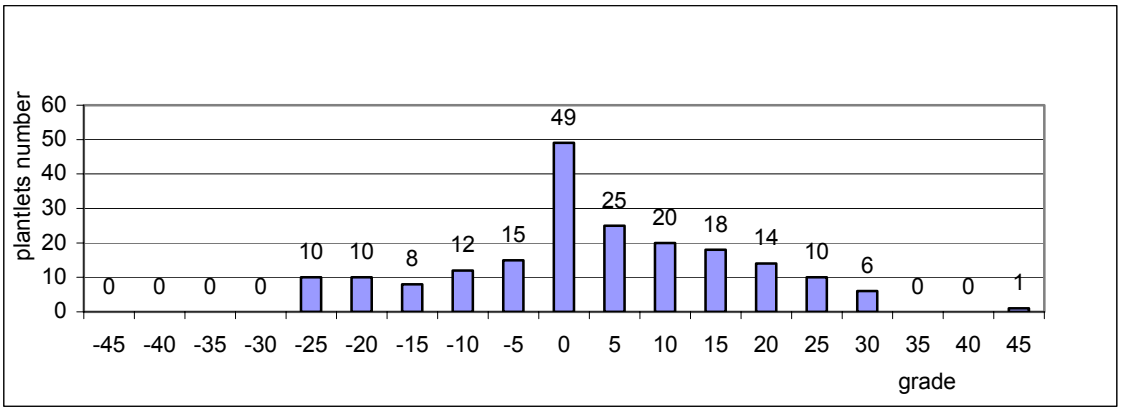

Fig. 1. The hypocotyls orientation in darkness

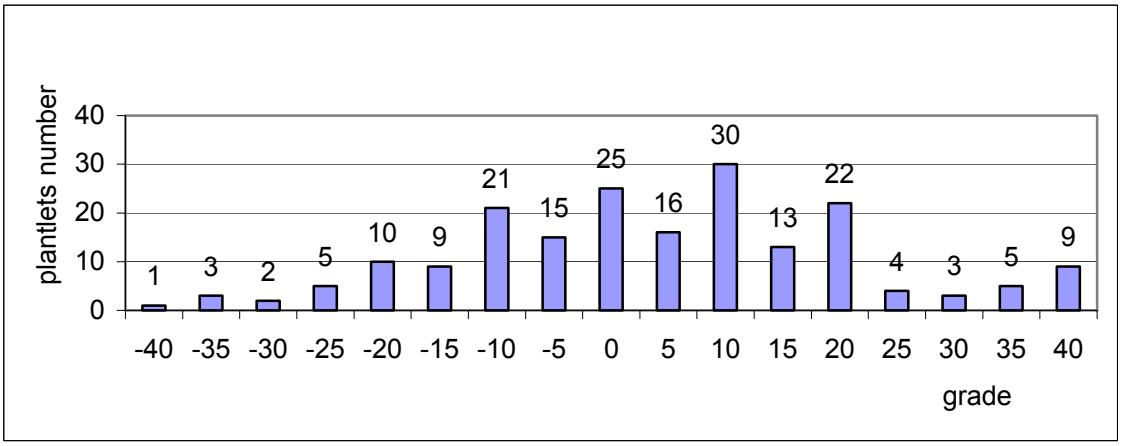

Fig. 2. The hypocotyls orientation in red light

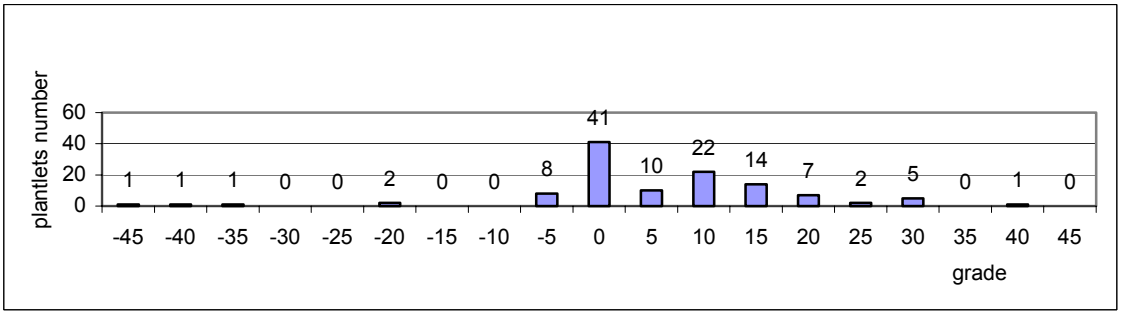

Fig. 3. The hypocotil orientation for plantlets illuminated 1 min in red light and 1 min far red light

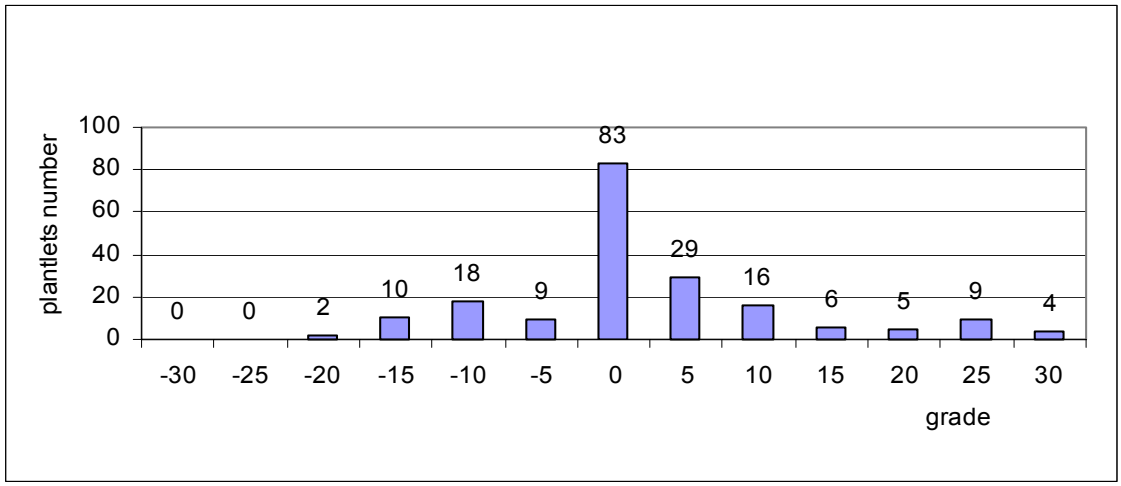

Fig. 4 The hypocotyl orientation in far red light

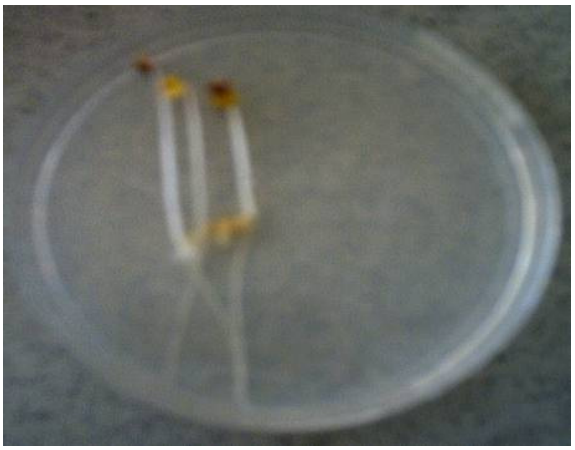

Photo 1 Plantlets in darkness

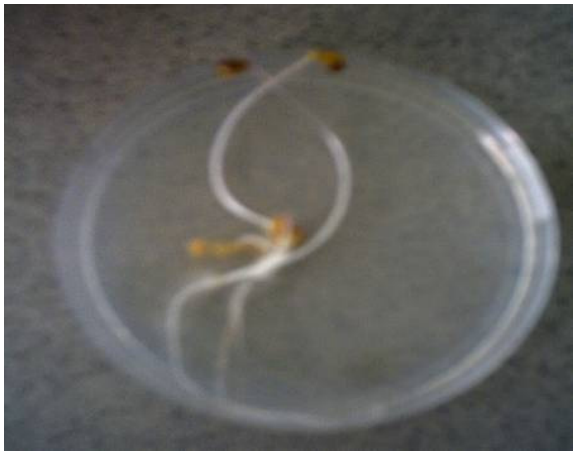

Photo 2 Plantlets in red light

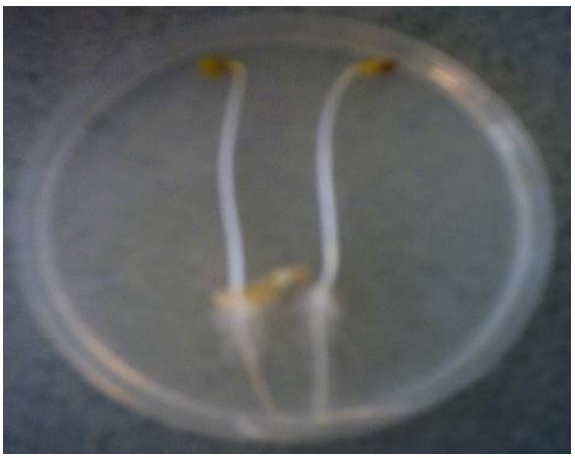

Photo 3 Plantlets 1 minute in red light and 1 minute in far red light

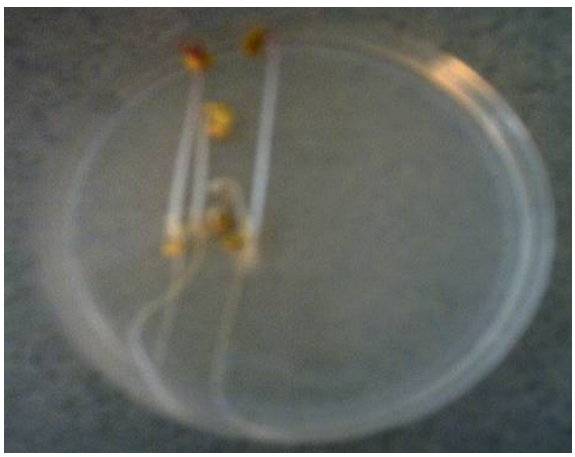

Photo 4 Plantlets in far red light

Contributions to the knowledge of the physiology and biochemistry from *Tilia platyphillos*

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Keywords: photosinthesis, transpirations, respirations, pigments, minerals

ABSTRACT

The physiology and biochemical analysis effectuated on the species *Tilia platyphillos* resulted that the intensity of the photosynthesis vary depending on the age of the leaves between 1,48 μmoli $CO_2/m^2/s$ and 7.27 μmoli $CO_2/m^2/s$, the intensity of the transpiration process varied between de 1.36 mmoli $H_2O/m^2/s$ and 3,28 mmoli $H_2O/m^2/s$, and the respiration process between 424.20 mg CO_2/kg/h and 87.50 mg CO_2/kg/h. The maximum mineral elements contents was determined in the leaves, flowers and bract and the predominant elements were Ca, K, Mg, P.

INTRODUCTION

Tilia platyphillos flowers are a popular domestic remedy for a number of ailments, especially in the treatment of colds and other ailments where sweating is desirable (Neval, 1996). A tea made from the fresh or dry flowers is antispasmodic, diaphoretic, expectorant, hypertensive, laxative and sedative (Grieve, 1984). Lime flower tea is also used internally in the treatment of indigestion, hypertension, hardening of the arteries, hysteria, nervous vomiting or palpitation (Brickell, 1990, Bown, 1987). The flowers are harvested commercially and often sold in health shops etc . Lime flowers are said to develop narcotic properties as they age and so they should only be harvested when freshly opened (Bradley,1992). In references data is few data regarding the intensity of physiological processes, and this paper intends to characterize some aspects concerning the *Tilia platyphillos* physiology.

MATERIAL AND METHODS

Research were made on species *Tilia platyphillos* , from the Botanical Garden of the USAMV Bucharest.

The intensity of the photosynthesis process and of transpiration were determined with the electronic analyser LCA – 4. The intensity of the respiration process was measured with the analyser of Ricken carbon dioxide. The analyses consisted of the measurement of the carbon dioxide given off in the time unit by the analysed material, with a known mass and volume. The dynamics of the content in chlorophyll and carotenoid pigments in leaves (Jasko spectrophotometer), in the acetone extract with a 80% concentration. The contain in mineral elements from the leaves, the flowers, bracteea and the fruit of the species *Tilia platyphyllos,* was determined by multi-element analysis techniques - Inductively coupled plasma optical emission spectrometry (ICP OES).

RESULTS AND DISCUSSIONS

The determinations performed with the electronic analyser LCA-4 at the leaves of the plants of *Tilia* kind in the study of the twig Fig1. at a relatively reduced interval of variation of the active photosynthetic radiations 1725-1803 μmol $CO_2/m^2/s$ and a temperature: 27,5-28,4 ^{0}C, showed that under similar normal conditions of environment,

environment, the maturity degree of the leaves influenced the intensity of the photosynthesis process, at the mature leaves being registered the lowest value(1,48 μmol $CO_2/m^2/s$), whereas the younger ones a maximum value(7,27μmol $CO_2/m^2/s$).

Similar to the intensity of photosynthesis, the respiration process varied according to the maturity of the analysed organ (Burzo, 2005), with minimal values of the meture leaves (1,36 mmol $H_2O/m^2/s$) and maximum (3,28 mmol $H_2O/m^2/s$) for the younger leave (Fig.2). Analysis made shows a negative correlation between the transpiration process and age of the leaves

The intensity of the respiration process was determined beginning with April and culminating with October, when the leaves reached he senescence

The intensity of this process has varied within the wide limits, according to the phase of development and of the analysed organ. The intensity of the respiration process registered a maximum value in case of the flowers of 466,3mg CO_2/hour and of the leaves.

From the analytic data we conclude that the highet water content was found in the young leaves, in April (74,18%), but the lowest content was found in the mature leaves, in October, 60,45 % (Table 1). In the case of the flower, the highest water content was found in April (75,61%) and decreased to (75,38 %) in June when we obtain a content of 74,86% (Table1)

In parallel was studied the influence of the chlorophyll pigment quantity and the carotenoide on the intensity of the photosynthesis process, which, in the same time with the increasing of the synthesized chlorophyll quantity.

The pigment content in the leaves was fixed in the whole period of vegetation, beginning in the senescence period. The total chlorophyll content have had the lowest values in April (68,85 mg/100g). The maximum value was establish in august (346,36 mg/100g) when the quantity of the chlorophyll content increases but decreases in September (285,24 mg/100g) and October (248,63 mg/100mg)

After that we obtain the mineral element content in leaves, flowers and bract (fig.3), 12 elements, in large quantity were found Ca, K, Mg, P. leaves was the organ which had Ca (1224,73 mg/100g), Mg (632,21 mg/100g) and P (734.73 mg/100mg) \. K has had a maximum value in the fruit (1078,7 mg/100mg).

CONCLUSIONS

1. Determination made for the leaves of *Tilia platyphillos* shows that the intensity of the photosynthesis process varied between 1,48 μmoli and 7.27 μmoli $CO_2/m^2/s$, but the transpiration intensity varied between 1,36 and 3,28 mmoli $H_2O/m^2/s$.
2. The intensity of the respiration process have had a maximum value at the beginning of the development period (466,3 mg CO_2/kg/h), but the minimum value, at the senescence period (203,7 mg CO_2/kg/h).
3. The minimum content of chlorophyll content was fixed in the young phase of the leaves in April (68,85 mg/100g).
4. From the mineral element of the leaves, flowers fruit and bract the highest content was in the case of Ca, K, Mg şi P
5. Leave was the organ in which Ca (1224,73 mg/100g) , Mg (632,21 mg/100g) and P(734,73mg/100g) had have the highest values.

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Tables

Table 1. The water and dry matter contents of *Tilia platyphillos* organs

Data	Water (%)			Dry matter (%)		
	Leaves	Flowers	Bract	Leaves	Flowers	Bract
April	74,18	-	-	25,92	-	-
May	69,28	75,61	79,92	30,72	24,39	20,08
June	63,60	75,38	78,30	36,40	24,62	21,60
July	62,36	74,86	77,86	37,64	25,14	22,14
August	61,67	-	-	38,33	-	-
September	61,25	-	-	38,75	-	-
October	60,45	-	-	39,55	-	-

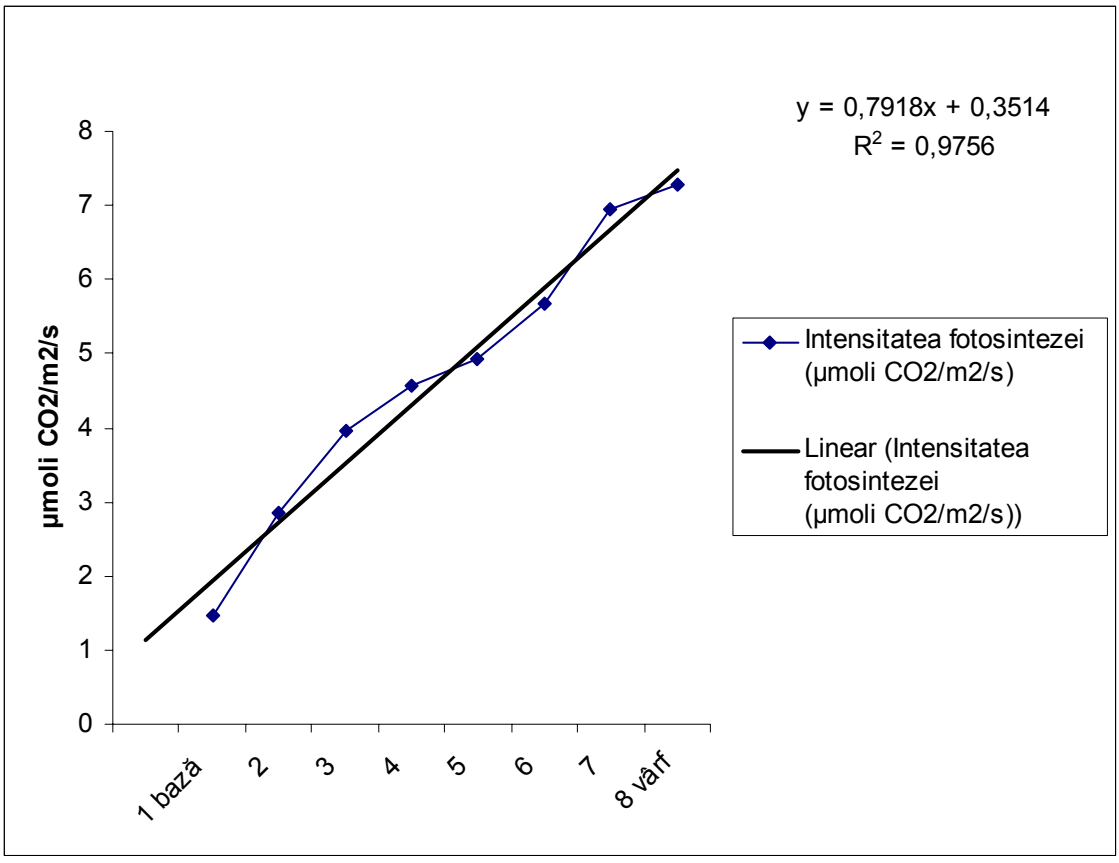

Fig. 1. Variations of the photosynthesis process

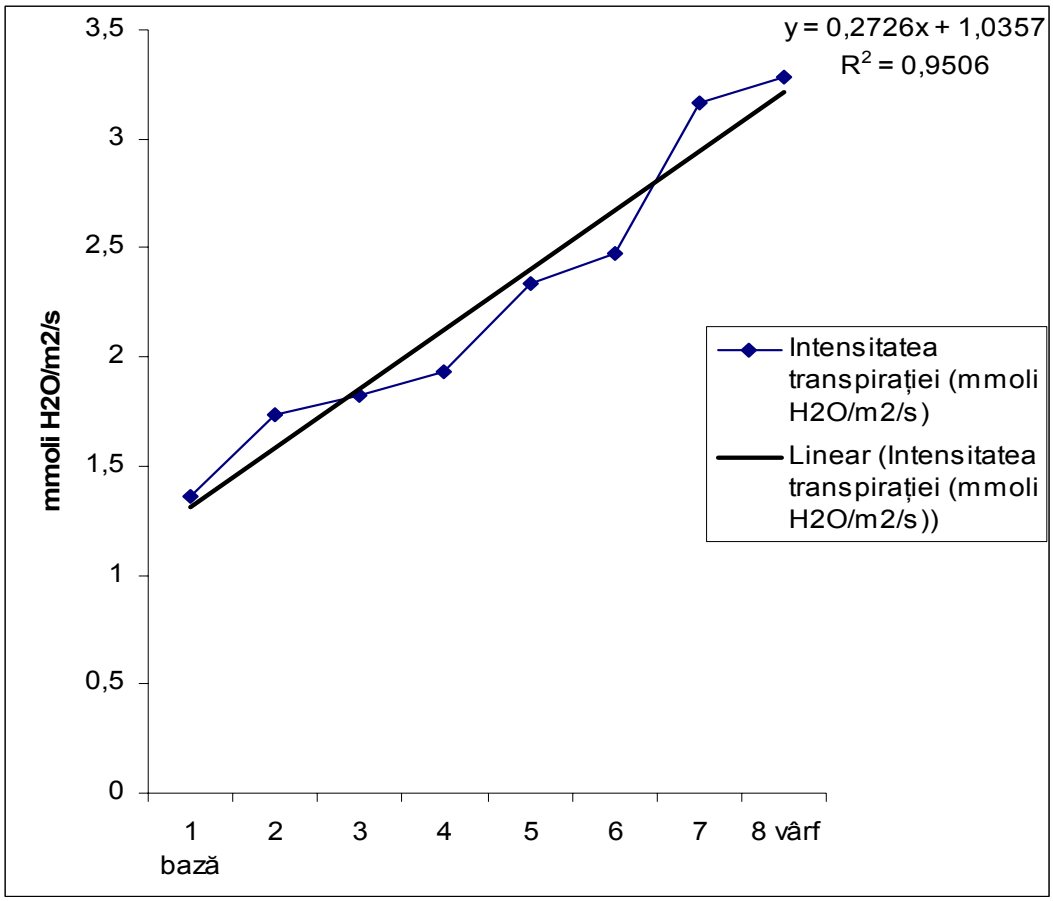

Fig. 2. Variations of the transpiration process

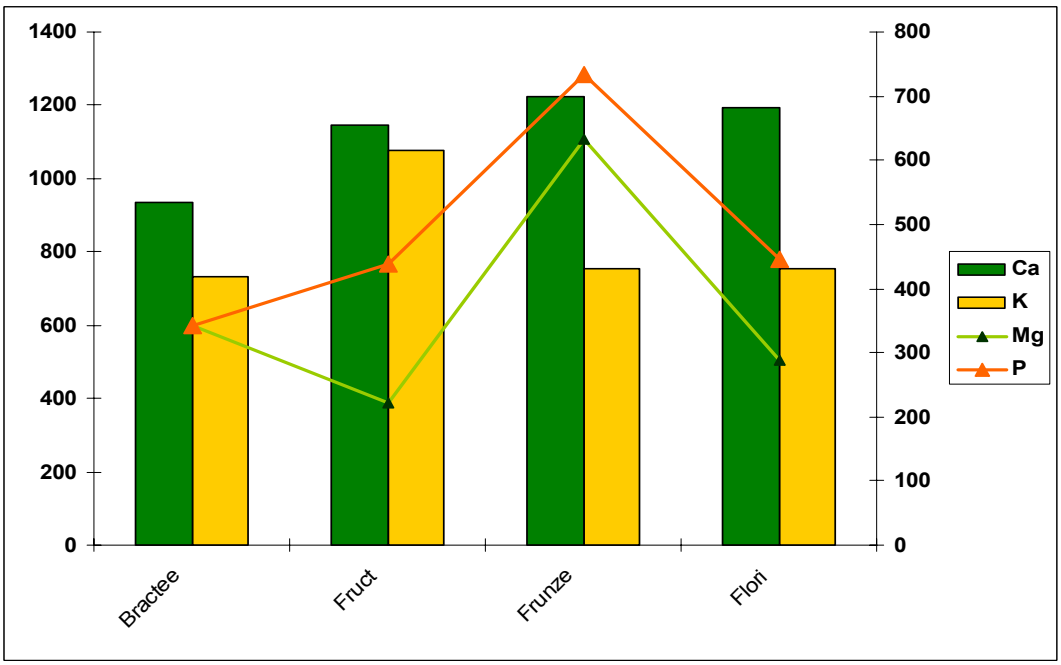

Fig. 3. The Ca, Mg, P , K contents from *Tilia platyphillos*

Contributions to the knowledge of the composition of essential oils from *Tilia tomentosa, Tilia americana and Tilia platyphillos*

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Keywords: *lime, volatil oil, flowers*

ABSTRACT

Essential oils which are extracted from flowers of 3 species genus *Tilia* by hydrodistilation, but the separation and the identification of their components were released in the chromatograph in gaz phase with masspectrometric detector. Data obtained had marke out a variation in the essential oils composition. Thus, the essential oils extracted from those 3 species contained in principal tricozan, identificate in all of 3 species, benzil benzoat (*T. americana*), phytol (*T. tomentosa*) and kauren (*T. platyphillos*).

INTRODUCTION

The first research upon the composition of volatil oils were done (Brickell, C., 1990, Burgess, 1991) in 1929. After 1960, when there were used gas chromatograph , were intensificate researches in this field. Were identificate 31 components in the extract obtain from *Tilia* flower (Neval, 1996, Wichtl, 1994). From which were identificate 80 components, including acids, hydrocarbure and esther (Magherini, 1993). In 1983, more than 300 components were identification.

The paper contains a comparative study for the essential oils compounds extracted from 3 *Tilia* species flowers.

MATERIAL AND METHODS

Research were made with 3 species of genus *Tilia* (*Tilia tomentosa, Tilia americana si Tilia platyphillos*) taken from Bucharest (Botanical Garden of the USAMV) for each of them at the flowering moment (June or July).

Essential oils from flower were extracted by hydroditillation, using an apparatus Clevenger type.

For the component separation from the volatile oil was used a gas chromatograph Agilent, provided with a capillary column DB5, with a length of 25 m and an interior diameter of 0.25 mm. The oven temperature was grown isotherm, with a gradient of 4 ^{o}C/minuts, from 40 ^{o}C, to 280 ^{o}C, but for a carrying- gas was used helium, with a debit of 1,2 ml/minut. Identification of the components was made cu mass spectrometric detector Agilent, and a bank specter NIST. For the positive confirmation the drop position in the chromatograph were used a retention Kovats indices .

RESULTS AND DISCUSSIONS

Researches permit the identification and quantification volatile oil components from 3 species of genus *Tilia* 31-32 of its components (Table 1).

For the flowers of *Tilia americana* specie there were identify 31 of its components, benzyl benzoate (13,16%) and tricosane (15,66%) had the highest value from all of the identify component. Other component with significantly percentage were hexahydrofarnesyl acetone (8,55%), limonene (5,97%), α terpinolen (4,65%), pentacosane (4,57%), ocimene (3,94%).

The oil extract from flowers of species *Tilia tomentosa* was characterised by the presence in high quantities for 2 components : tricosane (16,29%), commun for the *Tilia americana* species and phytol (12,33%), followed by the benzyl benzoate(7,40%), terpinolene (7,36%), limonene (6,92%) and pentacosane (6,82%). A characteristic composition of the extracted volatile oil from the flower of *Tilia platyphillos* in high quantities was the triconazol (11,06%) and kaurene (19,37%). Other components with significantly percentage were β Cariophilen (4,48%), γ terpinene (2,35%), hexahidropharnesol acetona (1,88%).

From the data presented in table 1, we observe a variation in the composition of volatile oil extracted from flowers of those 3 species of genus *Tilia* depending on the specie. From approximate 30 of the components identified for each specie, the common components for the 3 species were: 4 caren, α terpinene, benzyl benzoate, heneicosane, limonene, nonanal, tetrametil phenol, tricosane, hexenil acetate, ocimene.

CONCLUSIONS

1. Species from the genus *Tilia* were characterized by the specific composition of the volatile oil, from which 2 components were chosen for each specie, tricosane cause it is found in all the 3 species, benzyl benzoate (13,16%) for *Tilia americana*, phytol (12,33%) for *Tilia tomentosa* and kaurene (19,37%) for *Tilia platyphillos.*
2. From approximate 30 of the volatile components identify for each specie, common for the 3 species were only 8 components **:** 4 caren, α terpinen, benzyl benzoate, heneicosane, limonene, nonanal, tetrametil phenol, tricosane, ocimene, hexenil acetate .

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Table 1 The chemical composition of the essential oil extracted from *Tilia* species

SUBSTANCES	*Tilia americana*	*Tilia tomentosa*	*Tilia platyphillos*
4 - caren	2,197	2,034	0,91
α farnesene	0,484	3,649	-
α terpinene	0,292	2,38	0,569
α terpinolene	4,657	-	-
Palmitic acid	0,569	0,786	-
metil-ester salicilic acid	-	-	0,361
Anethol	-	-	0,682
β Cariophilen	-	3,97	4,483
Benzil benzoat	13,164	7,401	0,238
Cimen	-	1,951	-
cis hexenil benzoat	-	-	1,749
Ocimene	3,945	2,682	4,112
Cis-3-hexen-1-ol	0,92	1,322	-
Decanal	0,881	-	-
Dimethyl heptan	-	1,342	0,636
Dimethyl tetradecan	3,585	-	-
Docosane	-	0,938	-
Farnesyl acetone	-	-	0,71
Phenyl etil benzoate	0,682	-	-
Phytol	-	12,33	-
γ terpinene	2,128	-	2,358
Geranyl acetone	0,449	-	-
Heneicosane	1,325	1,162	1,978
Heptanal	-	-	0,552
Hexadecane	0,979	-	-
Hexahidropharnesol	0,5	-	-
Hexahidropharnesol acetona	8,551	3,881	1,881
Hexenile acetate	0,286	0,776	0,424
Hexenile benzoate	0,904	-	-
Izoamil benzoate	-	-	0,987
Izoeugenol methal eter	-	-	1,405
Kaurene	-	1,052	19,374
Limonene	5,97	6,925	1,102
Linalol	-	1,845	-
Menthatriene	-	1,191	-
Metyl eugenol	1,44	-	-
Metyl pentadecan	-	2,542	-
Mircene	0,6	0,526	-
Neroledol	-	-	0,689
Netoxioctilbenzen	-	-	0,447
Nonan	0,318	-	-
Nonanal	3,119	2,312	1,623
Octen-3-ol	0,358	0,419	-
p cimene	1,874	-	-
Pentacosane	4,575	6,823	-
Pseudolimonene	-	-	0,501
Terpinen -4- ol	-	0,74	0,923
Terpinolene	-	-	0,307
Tetracosane	0,883	0,998	-
Tetrametyl phenol	0,395	0,637	0,786
Tetrametil hexadecatetraen-1-ol	-	0,717	-
Trans neroledol	0,364	-	-
trans -Nerolydol	-	0,679	-
Tricosane	15,667	16,296	11,06

Structural peculiarities of *Polygonatum verticillatum*'s (L.) all. and *Streptopus amplexifolius*'s (L.) dc. aerial vegetative organs

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Keywords: anatomy, flowering stem, leaf

ABSTRACT

The structure of *Polygonatum verticillatum* 's and *Streptopus amplexifolius* 's aerial vegetative organs shows, on one hand, the morphological differences between the two species observed in flowering stems and leaves, and, on the other hand, the adaption to the enviroment's specific conditions - high soil's humidity and lower light's intensity.

INTRODUCTION

In this paper, a comparison between the flowering stem and the leaf structure of the two species is made.

Both of them, *Polygonatum vericillatum* and *Streptopus amplexifolius*, are herbaceus perennial species, with the same ecological requirements, which are growing in the spruce (*Picea abies*) forests. They are included in *Asparagoideae*, *Liliaceae, Liliales*, after the Ilustrated Flora of Romania (Ciocârlan, 2000), or *Convallariaceae*, *Asparagales*, after other plant classifications (Judd et al., 1999).

Polygonatum verticillatum (Solomon's seal) requires a medium to high soil humidity (meso-mesohigrophytes) and shady situation (sciadophytes). In the soil it forms a withish and branched rhizome which bears circular scars from the last flowering stems. The rhizome of a related specie (*Polygonatum odoratum* - Butură, 1979) was used in different zones of our country in obtaining red colour, or in folk medicine, in back pains or rheumatic tratament. On the stiffly, angular, fistlous or pith filled up flowering stem appears sessile, whorled leaves with a multiveins, narrow-lanceolate blade, hairy on their abaxial side (especially on the veins) (Flora RSR, 1966). Plants are used in the gardens for theirs bell-shaped, greenish-white flowers, produced in early summer (The Royal Horticultural Society, 1999).

Streptopus amplexifolius (Wild cucumber) is a sporadical specie. It forms a short rhizome with nest appearance, bearings many adventitious roots. The flowering stem is circular in transsection, with ovate to ovat-oblong-lanceolate, sessile, heart-shaped and stem-clasping (amplexicaul) leaves. On the abaxial side, the blade is pruinous and it shows many arcuate and oblic-anastomosing veins (Flora RSR, 1966). The plant's ecological requirements are the same as those of the preceeding specie - medium to high soil humidity (mesohigrophytes), shady situation (sciadophytes) (Ciocârlan, 2000). On the Northen American continent they are used as Edible and Medicinal plants: tender young shoots, raw in salads or cooked like asparagus, have a cucumber-like flavor; a tea from the whole plant has been used to treat stomach complaints and loss of apetite; the plant is considerate with tonic proprieties (Plant-life.org)

There are also anatomical studies on other species from the same family (*Asparagus officinale* L., *Convallaria majalis* L., *Colchicum autumnale* L., *Veratrum album* L.) presented in our specific literature (C.Toma, Rodica Rugină, 1998).

MATERIALS AND METHODS

The vegetal material, flowering stems and leaves, were obtained from plants bering fruits which are growing on the calcarous and rocky soil in the Zănoaga Mare Pass (Ialomița District). Observations were made on transverse sections of the stem's middle internod and leaves blade, processed by the usual proceedings used in the USAMV's Botany Laboratory. The photos were obtained with the microscope Biolux NG.

RESULTS AND DISCUSSION

Flowering stem - At *Polygonatum veriticillatum* is circular-costate, with 10 ribs, in transsection (Fig.1). The one-layered epidermis has isodiametric cells, with thicked external and internal walls; the external walls bear a thin cuticular layer; on the epidermis, there are also rare bicellular hairs, with a basal cell, bigger than an epidermal one, and a terminal conical cell (Fig.2). The cortex has 3-4 layers of homogenous parenchimatic cells between the ribs and an angular collenchyma below the ribs; there is no differentiated endodermis inside (Fig.1,3). The cental - cylinder is externally delimitated by a 3-4 layered sclerenchymatous pericycle, with their cell walls lignificated; this structure is present also in other species of *Asparagoideae* (C.Toma, Rodica Ruginã, 1998). Many closed collateral-bundle (18-25), situated on 3 rings, are present on the inner side of the pericycle; the external ones are smaller and are connected to the sclerenchymatous pericycle; rare sieve vessles are included in the perycicle; the second ring's collateral bundles are bigger and are connected to the pericycle by sclerenchymatous bridges (Fig.4); the inner ring collateral bundles are included in the stem ground parenchyma; the latter one is presented as a desorganized structure in the middle of the stem and, sometimes, even round about the bundles (Fig.5); the collateral bundles are made from sieves vassels with auxiliary cells, protoxilem and metaxilem distributed on the one and the other side of the sieves' elements.

The Streptopus amplexifolius's flowering stem is circular in transsection; the epiderma consists of one cell layer, slightly tangenial elongated, with thicked external and internal walls and a cuticular layer on the external wall. Below the epiderma there is a one-layered hypoderm consisting of isodiametric cells with cellulosic walls and no intercellular spaces (Fig.6). The cortex has 4-5 layers of parenchymatic cells with intercellular spaces. The cortex's cells are increasing in diameter from the external layers to the internal ones. Between the inner cells' layer of the cortex protude sclerenchymatous cells from the pericycle. Attached to the sclerenchymatous pericycle there are the first ring of the closed collateral- bundles; the bundels of the second ring are like those of the preceding specie, are bigger than the first ring's bundles and are connected by sclerenchymatous bridges with the pericycle; towards the inner side of the section there is the third ring of bundles, including in the ground, meatic, desorganized parenchyma (Fig.7). The bundles have sieves vassels with auxiliary cells, protoxilem and metaxilem distributed on semicircle around the phloem.

Although the structure of the flowering stem's main tissues appears similar in both species there are some differences on the cortex level: the presence of the angular collenchyma below the ribs in *Polygonatum verticillatum* makes the stem costate. The bicellular hairs of the same *Polygonatum verticillatum* are another differentiating element between the two structures. Specific to *Streptopus amplexifolius* is the presences of the hypoderma layer and the circular form of the section. The number and

the distribution of the metaxilem vessels are also specific to each structure.

The leaf - The *Polygonatum verticillatum*'s leaf is an amphistomatic type with a homogenous mesophyll. Between the upper epidermal cells there are some bigger, cone-shaped, cells which are wather depositaries, named hydromorphic cells (Andrei, 1978). The mesophyll, consisting of 4-5 layers of similar cells, whith intercellular spaces, is penetrated by several closed collateral- bundles. The bigger ones are connected with the two epidermises by sclerenchymatous strings. The lower epiderma shows bicellular trichomes in front of the collateral- bundles (Fig.8). In the mesophyll, there is a collateral bundle, representing the primary nerve, promiently on the adaxial side of the blade, which is separated from upper epiderma by two sclerenchymatous cells layers and from lower epiderma by a multilayered cellulosic parenchyma (Fig.9).

Streptopus amplexifloius's blade shows a homogenous mesophyll limited by two unilayerd epidermis with hydromorphic cells and stomata. The collateral bundles are different in size: some are bigger and connected to the epidermis by sclerenchymatous strings, some are smaller and are situated between the bigger ones (Fig.10).

The blade's inner structure of the two species is proving their ecology: the epidermal hydromorphic cells are characterizing mesohygrophytic species; the slim and homogenous mesophyll - adapting to a feeble light intensity.

CONCLUSIONS

The differences between the two species at the flowering stem's inner structure level are made by the epidermal trichomis and the cortex's collenchima presence to *Polygonatum verticillatum* and the *Streptopus amplexifolius*'s cortex hypodermis.

The sclerenchymatous pericycle, the collateral bundles situated on 3 rings, and the sieve vessles which are included in the perycicle, are characteristic to *Asparagoideae* subfamily.

The hydromorphic cells of leaves' epidermis, equivalent to the bulliform cells of the grass species, are characterizing the two mesohygrophytic species.

The homogenous mesophyll consists of few cell layers which permit the photosynthesis processes on feeble light intensity.

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*** Streptopus amplexifolius: http://montana.plant-life.org/species/strepto_ample.htm

Figures

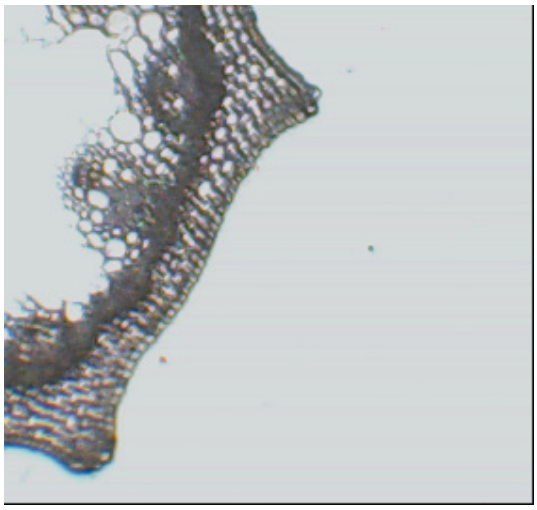

Fig. 1. Flowering stem - At *Polygonatum veriticillatum* is circular-costate

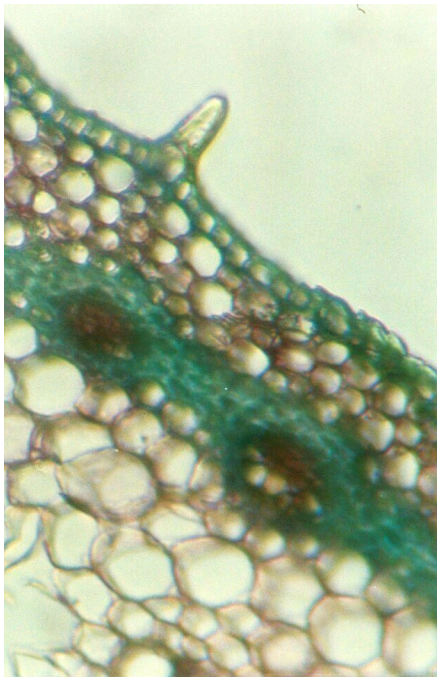

Fig. 2. Bicellular hairs on the stem epiderma of the *P. verticillatum*

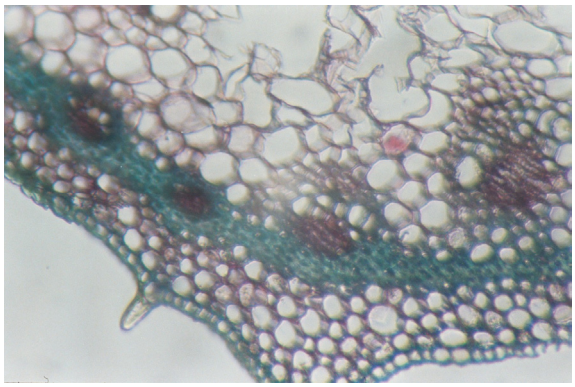

Fig. 3. Angular collenchyma below the ribs in *P.verticillatum*'s stem

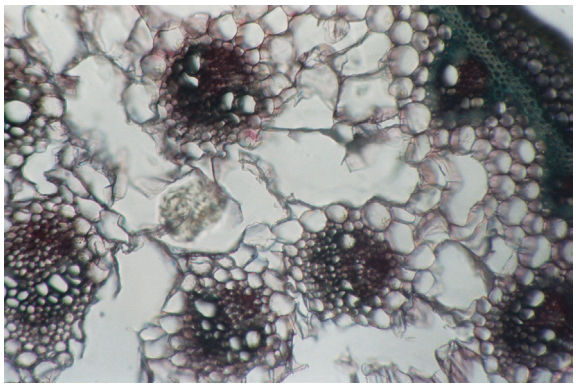

Fig. 4. In *P.verticillatum*'s stem the second ring's collateral bundles are connected to the pericycle by sclerenchymatous bridges

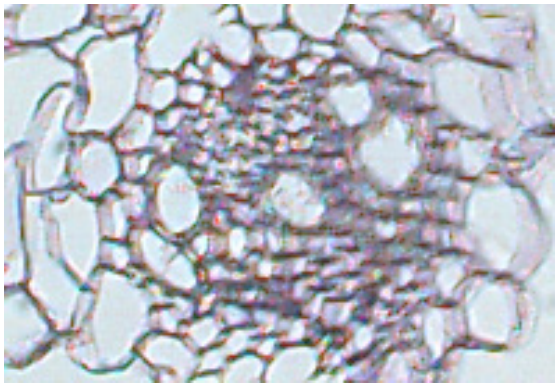

Fig. 5. The ground parenchyma is presented as a desorganized structure round about the bundles in *P.verticillatum*'s stem

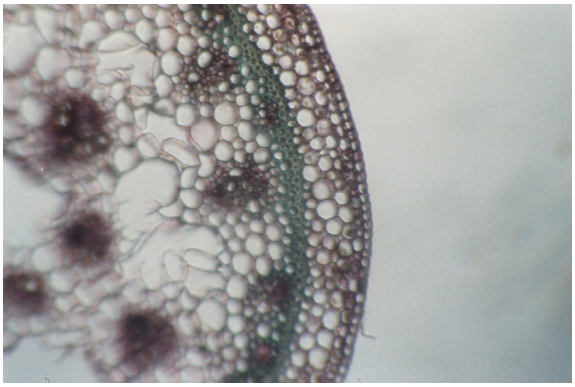

Fig. 6. The hypoderm of the *Streptopus amplexifolius*'s flowering stem

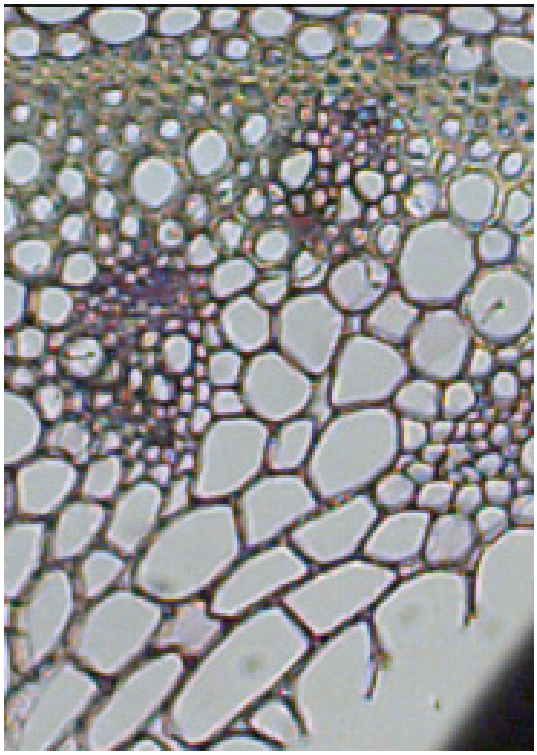

Fig. 7. In the *Streptopus amplexifolius*'s flowering stem second ring of collateral bundles are connected by sclerenchymatous bridges with the pericycle; towards the inner side of the section the third ring of bundles is included in the ground, meatic, desorganized parenchyma

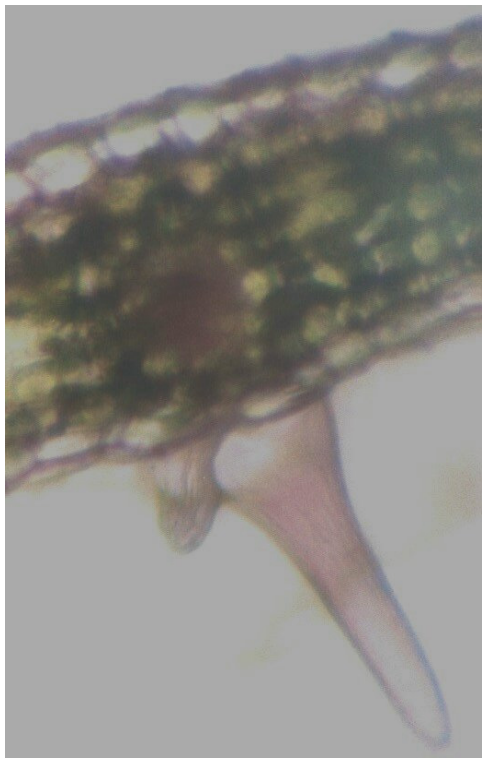

Fig. 8. Leaf of the *P.verticillatum* with the lower epiderma showed bicellular trichomes in front of the collateral- bundles

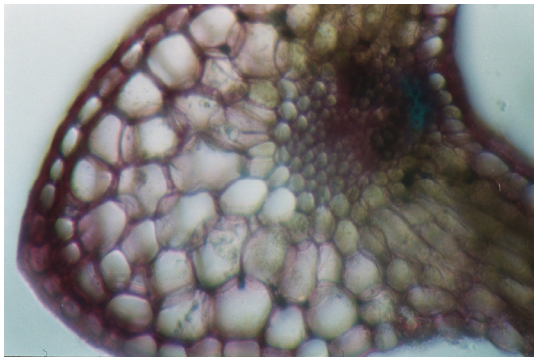

Fig. 9. In the leaf of the *P.verticillatum* the primary nerve, promiently on the adaxial side of the blade, is separated from upper epiderma by two sclerenchymatous cells layers and from lower epiderma by a multilayered cellulosic parenchyma

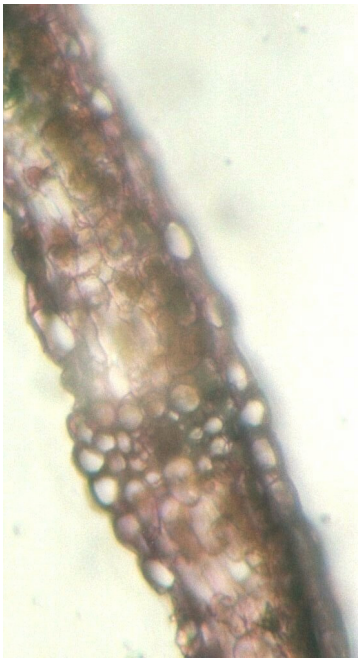

Fig. 10. Streptopus amplexifloius's blade has a homogenous mesophyll limited by two unilayerd epidermis with hydromorphic cells and stomata

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Contributions for knowledge of the content in mineral elements from the leaves of three species of Thuja

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Keywords: mineral elements, Thuja

ABSTRACT

The researches were made with the leaves from the species of Thuja orientalis, T. occidentalis and T. plicata, from the plants of Botanical Garden from USAMV Bucharest.

The results obtained presented the fact that the mineral substances from the leaves of Thuja varied between 1,96% for Thuja plicata and 2.36% for Thuja orientalis. The leaves of Thuja orientalis had the highest content of mineral elements which are important from the physiological point of view: calcium (997,10 mg/10g fresh substance), phosphor (107,20 mg/100 mg) and magnesium (39,27 mg/100g), and the ones of Thuja plicata had the highest content of potassium (194,60 mg/100 g), which is the most important element with an active osmotic role.

INTRODUCTION

The most detailed researches referring to the physiology and the biochemistry of wood plants and especially the conifer plants were made in USA and Canada. Kozlowki and Pallardy (1996) found out that the conifer plants have a smaller content of mineral substances, comparing to the deciduous plants. Van den Driessche (1984) mentioned that the leaves of the conifer plants have a higher content of mineral elements, comparing to the other organs, and Marschner (2000) mentioned the physiological role of the mineral elements.

Burzo and Dobrescu (2005) mentioned that the accumulation of the mineral substances into the conifer plants' leaves vary depending on the pedo-climatic conditions, but also depending on their age and their position on the plant.

The researches made on the global plan were focused more on Pinus, Picea and Pseudotsuga species, lacking the data referring to Thuja species and especially to the ones derived from the climatic conditions from Romania.

MATERIAL AND METHODS

The researches were made with plants from the species Thuja orientalis L. (Arbor Vitae), Thuja occidentalis L. (Canada Thuja) and Thuja plicata D. Don. (Giant Thuja).

The leaves gathered into the month of April were weighted and maintained 24 hours in stove, at the temperature of 105^0 C. After reweighing it was calculated the content in water and the total dried substance, which was expressed in percents. After that, the tests were calcined at the temperature of 550^0 C, and the mineral substances were established gravimetrically and they were expressed in percents.

The mineral substances were dissolved in 1 ml of nitric acid concentrated; they were brought to 50 ml with bidistilated water, and the mineral elements were established to a spectrometer with a plasma connected inductively IRIS INTREPRIED. The results were expressed in mg/100 g fresh substance.

RESULTS AND DISCUSSIONS

From the data presented into the table 1 it is noticed the fact that the leaves of Thuja plicata had the higher content of total dried substance (51,06%), while the leaves derived from Thuja orientalis had the smaller content: 46,99 %.

The content of mineral substances had an inversed variation. Thus, the highest content of mineral substances was established into the leaves derived from the species Thuja orientalis (2,36%) and the smaller content, into the ones of Thuja plicata (1,96%).

The analytical results referring to the content in mineral elements from the leaves of the three species of Thuja are presented into table 2. Among the 14 established mineral elements, the highest content was found into the case of the calcium, potassium and phosphor.

Thus, the content in **calcium** from the leaves was of 997,10 mg/100 g fresh substance for the species of Thuja orientalis, comparing to the species of Thuja plicata where it was smaller with 14 % and Thuja occidentalis, where it was smaller with 22%.

The content in **potassium** of the leaves varied between 162,90 mg/100 g of fresh substance for Thuja orientalis and 194,60 mg/100 g for Thuja plicata.

The **phosphor** hold the third place from the point of view of the quantity established into the Thuja leaves. The content of this mineral element varied between 59,43 mg/100 g for Thuja plicata and 107,20 mg/100 g for Thuja orientalis.

The leaves of the species Thuja orientalis presented a higher content in **magnesium:** 39,27 mg/100 g, comparing to the species Thuja plicata for which the content in magnesium was of 38,67 mg/100 g, smaller with 16% than Thuja plicata. The leaves of Thuja occidentalis species had the smaller content of magnesium: 33,24 mg/100 g.

It is mentioned the fact that the highest content of mineral substances important from the physiological point of view (calcium, phosphor and magnesium) was established into the leaves of Thuja orientalis species, and the higher content of potassium, important from the osmotic active point of view, was established into the leaves of Thuja plicata species.

The content of **iron** of the leaves was of 28,48 mg/100 g fresh substance to the species Thuja occidentalis, comparing to the species of Thuja orientalis where this was smaller with 14% and Thuja occidentalis, where it was smaller with 47%.

The leaves of the species Thuja occidendalis had a higher content of **sodium:** 12,55 mg/100 g, comparing to the species Thuja orientalis which has a smaller content of sodium: 11,97 mg/100. The smaller content of sodium was established to the species Thuja plicata 9,14 mg/100 g, smaller with 27 % comparing to the one established into the leaves of the species Thuja occidentalis.

The leaves of the species Thuja occidentalis had a higher content in **aluminum:** 22,25 mg/100 g plant comparing to the species Thuja orientalis which had a content of aluminum of 18,74 mg/100 g and Thuja plicata which had a very low content of aluminum: 12,36 mg/100 g.

The content of **manganese** varied between 3,16 mg/100 g into the leaves of the species Thuja plicata and 1,17 mg/100 g for the ones of the species Thuja orientalis.

The leaves of the species Thuja occidentalis had a high content in **copper** (1,027 mg/100 g, than the ones of the species Thuja orientalis (0,23 mg/100 g). The leaves of the species Thuja plicata had the smaller content in copper: 0,225 mg/100 g fresh substance.

The **zinc** is an element which stimulates the evolution of the plants. The findings made revealed the fact that this mineral element varied from the quantitative point of view in the following limits: 1,09 mg/100 g for the species Thuja occidentalis and 0,49 mg/100 g for the species Thuja plicata.

The smaller quantities were established for the case of **plumb**, which varied between 0.06 and 0,32 mg/100 g, **boron** which varied between 0.544 and 0,763 mg/100 g, **barium** (0,80 – 1,06 mg/100 g) and **chrome**, which had a similar concentration into the leaves of the three species of Thuja: 0,23 mg/100 g fresh substance.

CONCLUSIONS

1. The content of mineral substances from the leaves varied between 1,96 % for the species Thuja plicata and 2,36 % for Thuja orientalis.
2. The leaves of the species Thuja orientalis had the higher content of mineral elements important from the physiological point of view: calcium (997,10 mg/100 g fresh substance), phosphor (107,20 mg/100 g) and magnesium (39,27 mg/100 g).
3. The leaves of the species Thuja plicata had the higher content of potassium (194,60 mg/100 g), the most important element with an active osmotic role.
4. A medium content of mineral elements was established for the case of magnesium (33,24 – 39,27 mg/100 g), iron (28,48 – mg/100 g), sodium (9,14 – 12,55 mg/100 g), aluminum (12,36 – 22,25 mg/100 g), manganese (1,17 – 3,16 mg/100 g) and zinc (0,49 – 1,09 mg/100 g).
5. The smaller quantities were established for the case of plumb (0,06 – 0,32 mg/100 g), boron (0,544 – 0,763 mg/100 g), barium (0,80 – 1,06 mg/100 g) and chrome (0,23 mg/100 g fresh substance).

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Table 1. Content in water, total dried substance and mineral substance from the leaves of the three species of Thuja.

Species	Dry water	Water	Mineral substances
Thuja orientalis	46.99	53.01	2.36
Thuja occidentalis	49.02	50.98	2.36
Thuja plicata	51.06	48.94	1.96

Table 2. The content of the mineral elements from the leaves of the three species of Thuja (Mg/100 g fresh substance)

Mineral elements	Al	B	Ba	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	P	Pb	Zn
Thuja orientalis	18.7	0.54	1.06	997.10	0.23	0.27	24.41	162.90	38.27	1.17	11.97	107.20	0.16	0.86
Thuja occidentalis	22.25	076	0.82	902.60	0.23	1.03	28.48	190.05	33.24	1.25	12.55	83.81	0.32	1.09
Thuja plicata	12.36	0.58	0.80	783.90	0.23	0.23	15.23	194.60	38.67	3.17	9.14	59.43	0.06	0.49

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Variance of the mineral content from different organs of two Virginia tobacco cultivars

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Keywords: *Nicotiana tabacum*, chemical composition, calcium, potassium, magnesium

ABSTRACT

This paper presents the content in mineral elements from the organs of two *Nicotiana tabacum,* cultivars namely Virginia 180 and Virginia 196. Determinations were made during flowery time, the analysed organs being the root, the stem, leaves from the superior half of the plant, basal leaves and flowers. The content of mineral elements was determined in a larger amount in the Virginia 180 cultivar. Analysis of mineral substances from the plant organs marked out a larger content in the basal leaves (25,39%), and the minimal quantity was found in the flowers (9,66%). Analysis of mineral elements was made with a inductively coupled plasma spectrometer. Among the mineral elements, there has been determined the calcium percentage, which content varied between 11995.75 mgr./100gr.of dry substance in the leaves from the base of the plant and 943.3 mgr./100gr. in the flowers, potassium percentage that varied between 5776.45mgr/100gr. in the stem and 1629mgr. in the root, and magnesium percentage that varied between 2409.68 mgr. in the basal leaves and 509.55 mgr. in the root.

INTRODUCTION

Mineral substances are absorbed by tobacco plants in a selective way, beyond their needs and their accumulation is different, depending on species and their organs. Accumulation of mineral elements depends in principal on the concentration of the soil solution and the interaction between these elements (Marschner 1995). Coming from the results cited by Anitia and Marinescu (1993) and recently by Crispino and Kelly (2007), tobacco plants accumulate a larger quantity of potassium, calcium, magnesium, natrium and aluminium. Analysis of world published data showed that it was a smaller concern in this way. The purpose of this study is to complete existing data.

MATERIALS AND METHODS

There have been studied two cultivars of Virginia tobacco type: Virginia 196 and Virginia 180. The vegetal material was obtained by seedling and transplanting in the field. During flowering, the biologic material was gathered from the two tobacco cultivars and it consisted in: the root, the stem, basal leaves, leaves from the top of the plant and flowers.

The fresh vegetable material was dried for 24 hours at 105 °C, cooled in a exicator and after that it was weighed for the determination of the entire dried substance, then was calcinated at 550°C. After cooling in exicators, mineral substances were quantitative determined. They were dissolved in 1 ml of nitric acid and were put in a 50 ml quoted recipient with bidistillated water.

The aqueous solution obtained was used to determine the mineral elements with the ICP spectrometer IRIS Intreprid.

The results were expressed in mgr/100gr. dried substance.

RESULTS AND DISCUSSION

Study of analytic data presented in the table. 1, showed that from the point of view of the mineral elements quantity, the most significant one was determined in the organs of the Virginia 180 tobacco, excepting the root, that has had a smaller content (8,30%), comparing to Virginia 196 (11,26%).

The largest quantity of mineral substances was determined, for both species, in the leaves from the base of the plant, as it follows: 25.39% for the Virginia 180 cultivar, respectively 16.89% for the Virginia 196. Small quantities of mineral elements were determined in the stem of Virginia 180: 12,33% , respectively Virginia 196: 11,96%. Leaves from the top of the tobacco plants contained smaller quantities of mineral elements: 8,56% at Virginia 196 and 10,66% at Virginia 180.

The lowest quantities of mineral substances were determinate in the flowers: 9.66% for Virginia 196 type and 9.51% for Virginia 180.

Qualitative determinations of mineral elements, using ICP spectrometer are presented in the table 2. There have been identified 13 mineral elements, with different content, depending on species and organ type.

Comparing the content in mineral elements from the **two cultivars**, it has been noticed that Virginia 180 had the largest content in calcium (11995.75mgr./100grs dried substance), potassium (4439.34mgr./100grs. dried substance) magnesium (2409,68 mg/100g), manganese (16,88 mg/100g), copper (7,42 mg/100g) in basal leaves.

Virginia 196 cultivar had a larger content of potassium (5776,45 mg/100g) and calcium (4,76mgr./100gr.) in the stem and aluminium (268,66 mgr./100gr.), iron (215,38 mgr./100gr.) and cobalt (0,15 mgr./100gr.) in the root.

Variation analysis of principal mineral elements from different organs of the two cultivars showed the following:

The root of the tobacco plants had the larger content in calcium (2912,36 mgr./100gr.) and aluminium (268,66 mgr./100gr.) at Virginia 180 cultivar and iron (215,38 mgr./100gr.) and cobalt (0,15 mgr./100gr) at Virginia 196 cultivar.

The stem of tobacco plants had a maximum content in potassium (5776,75 mgr./100gr.) and in crom (4,76 mgr./100gr. dried substance) at Virginia 196 cultivar.

The basal leaves were characterized by the highest content in mineral elements. The highest weight was obtained for calcium (11995,75 mgr./100gr. dried substance), magnesium (2409,68 mgr./100gr.), natrium (117,10 mgr./100gr.), barium (24,44 mgr./100gr.) manganese (16,88 mgr./100gr.), copper (7,42mgr./100gr.) and boron (5,18 mgr./100gr.) for Virginia 180 cultivar.

Young leaves from the top of the plant and **flowers** from both tobacco cultivars had the smallest content in mineral elements.

CONCLUSIONS

1. The largest content in mineral substances was noticed for the Virginia 180 tobacco (25.39% in basal leaves and 9.66% in the flowers)
2. Comparing the content in mineral elements from the two cultivars, it resulted that Virginia 180 had the largest content in calcium (11995,75 mgr./100gr.), potassium (4439,34 mg/100g), magnesium (2409,68 mgr/100gr), manganese (16,88 mgr/100gr), cupru (7,42 mgr/100gr).
3. The basal leaves had the highest content in: calcium (11995,75-8814.59 mgr./100gr.), magnesium (2409,68 - 1837 mgr/100gr) and natrium (117,10 mgr/100gr - 87,33 mgr/100gr)

4. Young leaves from the top of the plant and flowers from both tobacco cultivars had the smallest content in mineral elements.

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Tables

Table 1. Content of mineral substances in the organs of the two tobacco cultivars (gr/100gr dried substance)

Cultivar	Root (%)	Stalk (%)	Top leaves (%)	Basal leaves (%)	Flowers (%)
196	11,27	11,96	8,56	16,89	9,51
180	8,30	12,33	10,66	25,39	9,66

Table 2. Variance of the mineral elements content in the organs of tobacco cultivars: Virginia 180 and Virginia 196

Element	Virginia 196 (mgr./100 gr dried substance)					Virginia 180 (mgr./100gr dried substance)				
	Root	Stem	Top leaves	Basal leaves	Flowers	Root	Stem	Top leaves	Basal leaves	Flowers
Al	268,66	44,71	32,39	168,78	51,85	171,28	96,03	49,51	171,14	52,37
B	2,30	2,72	1,96	3,09	2,80	1,53	2,66	3,38	5,18	3,69
Ba	5,11	5,85	4,02	17,78	1,84	6,60	5,89	7,59	24,44	0,88
Ca	2912,36	1939,48	2541,86	8814,59	1267,70	2464,90	2035,50	4901,54	11995,75	943,31
Co	0,15	0,01	0,03	0,10	0,04	0,07	0,09	0,03	0,09	0,04
Cr	3,57	4,76	2,23	3,75	4,65	2,32	4,51	2,53	4,28	4,03
Cu	3,47	4,43	3,27	4,68	4,74	2,62	3,34	5,12	7,42	4,88
Fe	215,38	2,50	18,05	116,77	20,69	121,75	50,80	32,51	121,84	19,58
K	2519,76	5776,45	2767,10	2804,75	3843,6	1629,00	4614,00	2595,20	4439,34	3922,29
Mg	509,55	770,44	1018,10	1837,00	1042,00	477,23	563,82	1370,93	2409,68	1113,46
Mn	7,15	1,57	5,05	9,43	3,68	3,87	3,91	6,27	16,88	3,60
Na	114,94	79,22	45,87	87,33	90,50	99,99	78,89	42,60	117,10	73,67
Zn	3,08	0,00	14,06	16,38	5,26	4,12	0,54	20,50	25,41	6,00

Contributions to the knowledge of physiological and biochemical processes of the "Mangetout" Pea cultivar Sugar Snap

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Keywords: *Pisum sativum,* chemical composition, respiration, transpiration, photosynthesis

ABSTRACT

This paper presents data concerning evolution of physiological and biochemical processes of the "mangetout" pea cultivar Sugar Snap. Determinations were made during vegetable growth and generative period, the analysed organs being the root, stem and leave from the superior half of the plant, basal leaves, flowers and pods. It has been determined the content of assimilating pigments from leaves, the intensity of the photosynthesis and transpiration, variance of respiration process in different organs, water content, total dried substance and mineral substances, also content of mineral elements of the pea plant organs. The chlorophyll quantity from leaves varied between 113,56 mg/100gr and 209,19 mg/100g and the carotenes from basal leaves varied between 51,46 mg/100g and 46,91 mg/100g. Intensity of the photosynthesis process and transpiration varied between 12,55 μmoles CO2/m²/s, respectively 14,78 mmoles H2O/m²/s in the basal leaves and 10,5 μmoles CO2/m²/s, respectively 12,81 mmoles H2O/m²/s in the leaves from the top of the plant. Respiration intensity varied between 760,87 mgCO2/kg/h for the flowers and 114,02 mgCO2/kg/h for roots. The highest content in water was found in pods (89,14%), and in total dried substance and mineral substance was found in roots. Among the mineral elements the most resulted was calcium. The calcium content varied between 450,48 mg/100g fresh substance (in the leaves from the base of the plant) and 65,86 mg/100 g (in pods), potassium, which varied between 240,16 mg/100g (in the leaves from the top) and 123,85 mg/100g (in pods) and phosphorus with a maximum of 112.18 mgr in flowers and a minimum of 36.15 mg in stem.

INTRODUCTION

The „mangetout" pea is little known in Romania. Although mangetout peas had been in existence for over 100 years, their popularity was limited to de amateur market (Green 1995). It differs from cultivated cultivars in our country because edible organs are represented by pods in which peas are 4-5 mm diameter (Atanasiu, 2000). Plants can achieve 120-180 cm height, being necessary to palisade them.

The development of shorter cultivars is a slow process. As due to the gene interaction only a small reduction in stem length could be achieved with each crossing.(Biddle, 1988)

Less productive than other species, it is cultivated discontinuous, due to its technological particularities. The studied cultivar comes from Grand Britain, where it has the finest growing conditions. At world level, there has been a low concern for the study of physiological and biochemical processes of this species

MATERIALS AND METHODS

It has been studied the "mangetout" pea cultivar Sugar Snap native from Grand Britain. The vegetable material needed for physiological and biochemical determinations were obtained from seedling that was planted in the field. The analytic samples were taken during vegetative growth and when flowery began, and consisted in leaves from the top and the bottom of the pea plant, stem, root, flowers and edible organs for determination of mineral elements.

The chlorophyll pigments from leaves were determined in 80% acetonic extract, colorimeter at wave length of 663 nm, 646 nm and 470 nm. Results were calculated using Mackiney formulas and values were reported to 100 mg vegetable material.

Intensity of photosynthesis and transpiration were determined in the field, using automatic analyzer LCA-4. Results were expressed in μmoles $CO_2/m^2/s$ respectively mmoles $H_2O/m^2/s$.

Determination of respiration intensity was made using the CO_2 RIKEN analyzer for the following organs: root, stem, basal leaves, leaves from the top half of the plant and flowers. These results were expressed in mg $CO_2/kg/h$.

The total dried substance, water and mineral elements were determined for leaves, stem, root, flowers and pods. The fresh vegetable material was dried for 24 hours at 105°C, was cooled in the dryer, that was weighed after that, to determine the total dried substance and water, and then was calcinated at 550 ºC. After cooling in exicators, mineral substances were determined gravimetrically. They were dissolved in 1ml nitric acid, and put with bidistilated water in a 50 ml quoted recipient.

The aqueous solution obtained was used to determine mineral elements with the ICP spectrometer.

RESULTS AND DISCUSSIONS

Interpretation of analysed data from tables, showed that intensity of physiological and biochemical processes are conditioned by the analysed organ and by the period when determinations were done.

The quantity of assimilated pigments, represented by the total chlorophyll, varied between 209,19 mg/100g in the basal leaves at the beginning of the flowery, and 113,56 mg/100g in the leaves from the top during the vegetative growing period. The maximum of chlorophyll **a** was achieved in the basal leaves, and in chlorophyll **b** in the leaves from the top, at the beginning of flowery. The quantity of carotenes was higher during the vegetative growing (46,91 mg/100g) in the basal leaves, followed by a decreasing evolution.

Intensity of photosynthesis and transpiration were determined in the field, using the LCA-4 analyzer, and results are presented in the table 2. It can be noticed that the intensity of the processes varied related to the age of the analysed leaves, the mature ones having a photosynthesis intensity (12,55 μmoles $CO_2/m^2/s$) and transpiration (14,78 mmoles $H_2O/m^2/s$) higher than the young leaves (10,50μmoles $CO_2/m^2/s$ respectively 12,81 mmoles $H_2O/m^2/s$).

Intensity variation of the respiration process was determined to different organs of the pea cultivar Sugar Snap, and results are presented in the table no.3. Intensity of the respiration process varied related to the organ type and to its age. It was established that the organs with the highest intensity of respiration are the flowers (760,87 mgr CO_2/kg hour) and the lowest respiration was found at roots (114,01 mgr. CO_2/kg/hour). Related to the organs age it was noticed that, at the same time with the increase of age, intensity of respiration decreases. During the vegetative period, there were determined higher values of intensity of respiration (540,69 mgr.CO_2/kg/hour) at top leaves, 371.6mgr at basal leaves and 250,25mgr. in the stems. At the beginning of the flowery the intensity of respiration was 524,08 mgr.CO_2/kg/hour at top leaves, 184.33mgr. at basal leaves and 220,59 mgr in the stems. The roots were excepted, having the respiration increased during flowery (286,03 mgr. CO_2/kg hour).

From data presented in the table 4 it resulted that the organs with the highest content of water were the pods (89,14%), and the top pea leaves (88,11%) and the lowest water content was found in the root (84,76%). The quantity of total dried substance varied between 15,24 % in the root and 10,86% in the pods.

The highest quantity of mineral substances was found in the plants roots (1,55%). Smaller quantities of mineral elements were found in the stem (1,38%), flowers (1,10%), basal leaves (1,05%) and in top leaves (0,93%).

The lowest quantities were determined in the pods (0,55%).

Qualitative determinations of mineral elements using ICP spectrometry are presented in the table 5. A number of 13 mineral elements from pea cultivar Sugar Snap were identified, whom content variation was related to the organ type.

Comparison of content in mineral elements, the most resulted was calcium (450,48mgr./100gr. fresh substance), potassium (240,16mgr./100gr.), phosphorus (112,18 mgr./100gr.) and natrium (64,58mgr./100gr.).

Annalysis of variation of principal mineral elements from the "mangetout" Sugar Snap pea cultivar showed the followings:

The root of pea plants has the highest content in mineral elements. The most found was sodium (64,58 mgr./100gr.), magnesium (62,49 mg./100gr.), aluminium **(25,7**1 mgr./100gr.), iron (20,98 mgr./100gr.), manganese (2,25 mgr./100gr.) and copper (0,78 mgr./100gr.).

The basal leaves had a high content of calcium (450,48 mg/100gr.fresh substance), zync (2,9 mgr./100gr.), boron (2,13 mgr./100gr.), chrom (1,27 mgr./100gr.) and barium (0,42 mgr./100gr.).

In the **top leaves,** the most found mineral was potassium (240,16 mgr./100gr.) and in **flowers** the most was phosphorus (112,18 mg/100g fresh substance).

The **stem** and **pods** had the lowest content in mineral elements

CONCLUSIONS

1. The leaves of the "mangetout" Sugar Snap pea cultivar were characterized by a content in total chlorophyll that varied between 113,56 mgr./100gr. and 209,19 mgr./100gr., intensity of photosynthesis that varied between 10,5 $CO_2/m^2/s$ and 12,55 µmoles $CO_2/m^2/s$ and a transpiration intensity that varied between 12,81 and 14,78 mmoles $H_2O/m^2/s$.
2. The organs with the highest respiration intensity are the flowers (760,87 mgr. CO_2/kg/hour). From the point of vue of the organs age it was noticed that once increasing the age, respiration intensity decreases.
3. The total dried substances and mineral substance were found mostly in the roots (15,24% respectively 1,51%), the lowest quantity of the total dried substance and mineral substances being found in the pods (10,86% respective 0,55 %).
4. The highest quantity of mineral substances was determined in the root (1,51%), mostly, being found: calcium (450,48 mgr./100gr. fresh substance), potassium (240,16 mgr/100gr.), phosphorus (112,8 mgr/100gr), sodium (64,58 mgr./100gr.), magnesium (62,49 mgr./100gr).

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Tables

Table 1 Content in assimilating pigments from leaves of the "mangetout" pea cultivar Sugar Snap (mgr/100gr)

Date	Leaf type	Cl A (mgr/ 100 gr)	Cl B (mgr/ 100 gr)	Cl T (mgr/ 100gr)	A/B	Carotenes (mgr/ 100gr)	ClT/ carotenes
22.04	Top leaves	81,49	32,08	113,56	2,54	32,89	3,45
	Basal leaves	130,96	51,46	182,42	2,54	46,91	3,89
10.05	Top leaves	107,10	61,03	141,55	3,11	25,68	5,51
	Basal leaves	148,16	42,88	209,19	2,43	41,75	5,01

Table 2 Intensity of photosynthesis and transpiration process on 3.05.2007

Light intensity (µmoles m²/s)	Temperature (ºC)	Photosynthesis (µmoli CO_2/m²/s)	Transpiration (mmoles H_2O/m²/s)
1781	23,6	12,55	14,78
1856	25,0	10,50	12,81

Table 3 Variation of the respiration process of the Sugar Snap cultivar organs.

Date	Organ type	Respiration (mgr./CO_2/kg/hour)
22.04	Root	114,01
	Stem	250,25
	Basal leaves	371,60
	Top leaves	540,69
10.05	Root	286,03
	Stem	220,59
	Basal leaves	184,33
	Top leaves	524,08
	Flowers	760,87

Table 4 The content of water, total dried substance and mineral substances in the organs of Sugar Snap cultivar

Organ type	Water (%)	Dried substance (%)	Mineral substances (%)
Root	84,76	15,24	1,51
Stem	89,09	10,91	1,38
Basal leaves	88,11	11,89	0,93
Top leaves	86,85	13,15	1,05
Flowers	86,87	13,13	1,10
Pods	89,14	10,86	0,55

Table 5. Variance of the mineral elements content in the organs of "mangetout" pea cultivar Sugar Snap

Element	Root (mgr./ 100gr.)	Stem (mgr./ 100gr.)	Basal leaves (mgr/ 100gr)	Top leaves (mgr/ 100gr)	Flowers (mgr/ 100gr)	Pods (mgr/ 100gr)
Al	25,71	9,88	3,90	1,52	0,74	0,33
B	1,99	1,42	2,13	1,48	1,28	0,48
Ba	0,38	0,33	0,42	0,15	0,05	0,08
Ca	121,69	219,02	450,48	136,84	66,97	65,86
Cr	1,25	0,82	1,27	0,79	0,47	0,15
Cu	0,78	0,52	0,66	0,45	0,43	0,17
Fe	20,98	4,63	2,74	1,63	1,33	0,80
K	217,84	176,94	132,33	240,16	176,40	123,85
Mg	62,49	26,16	53,20	35,05	32,05	28,00
Mn	2,25	0,36	1,29	0,46	0,34	0,16973
Na	64,58	41,262	36,86	20,78	10,81	6,57
P	69,49	36,15	61,63	102,16	112,18	71,21
Zn	1,21	0,44	2,90	0,46	0	0

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The influence of isoproturon on the dynamic of the population density and the assimilatory pigments content in *Chlorella vulgaris* and *Botryococcus braunii*

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Keywords: *Chlorella vulgaris* Beij., *Botryococcus braunii* Kuetz., isoproturon herbicide, growth, assimilatory pigments.

ABSTRACT

The *Chlorella vulgaris* and *Botryococcus braunii* cultures were used for the testing of the effect of the different concentrations of isoproturon on the growth and the chlorophyll a, b and carotenoid pigments content after 7 and 14 days from the adding of the herbicide in the medium culture.

Concomitantly, there were recorded significantly variations in the assimilatory pigments content from the variants treated with herbicide. It has been shown through the analyse of the regressions between the cells number and the pigments content that the variation of the assimilatory pigments content may be explained through an adaptation reaction to self shadowing. The 0.04 μM isoproturon concentration produced a seemingly stimulating effect on assimilatory pigments content of *Botryococcus* after 7 days exposure, but, even this concentration was clearly placed on the regression line between the number of cells and assimilatory pigments content.

INTRODUCTION

The unicellular green algae represent an useful model for the study of the herbicide impact on plant physiology (Böger et al., 1990) because those algae can multiply very quick and the effects of the individual variations are eliminated due to the great number of cells from the volume unit of the algal suspension.

Very often the specific herbicidal effects are still unknown and only become evident generally as growth inhibition (Pfister et al., 1983). The herbicidal action of chemical compounds depends on their interaction with a multitude of major biochemical reactions in the plant. Some of the commercial herbicides interfere with the pigments synthesis (Böger et al., 1995). Usually an herbicide affects more than one metabolic reaction in plants (Devine et al., 1993; Fedtke, 1982).

The purpose of the present investigation was to study the influence of an ureic compound, isoproturon (N-4-isopropylphenyl)N', N'-dimethylurea) (Şarpe, 1987) on growth and assimilatory pigments content in *Chlorella vulgaris* Beij. and *Botryococcus braunii* Kuetz.

The question is if there is a direct effect of the studied herbicide on the pigments content or the found variations may be explained through an adaptive reaction to self shadowing induced by the variation of the cells number.

MATERIALS AND METHODS

Chlorella vulgaris alga was cultivated on Arnon nutritive medium (Boldor et al., 1983) and *Botryococcus braunii* - on Zehnder-Gorham nutritive medium (Vlădeanu et al., 1988). The experiments were carried out in a chamber with artificial illumination of 8000 lx. The ambient temperature varied between 24-25°C. In order to reduce the mutual shadowing, the algal suspensions, in cylindrical glass recipients of 1000 ml, were bubbled with a steady stream of air produced by aquarium aeration pumps. The

culture medium was inoculated with an amount of algal biomass adjusted to 100000 cells/ml suspension in all experiment variants. The results represent average values of three repetitions for each variant.

The herbicide was introduced in the culture medium before inoculation. The concentrations of the tested herbicide solutions were 0.03, 0.08 and 0.13 µM for *Chlorella vulgaris*. In case of the *Botryococcus braunii* the tested concentrations were 0.04, 0.2, 0.4, 0.6 and 0.9 µM. The chosen concentrations were based on a preliminary study on each of the two species.

The commercial product Izoguard was used as a source of isoproturon. The Izoguard herbicide has 50% active substance (Şarpe, 1987).

At least each two days within the culture cycle, the growth of algae was estimated by the cells number from the volume unit of the algal suspensions, counted with a Thoma haemocytometric mount.

After 7 and 14 days the absorbance of the solutions extracted with 100% acetone were measured at 661.6, 644.8 and 470 nm and the calculation of the content in a, b chlorophylls and carotenoids was performed according to Lichtenthaler (1987); the values were expressed in mg/g dry matter (DM).

The affiliation to the regression line was the criterion to decide if the variation of the assimilatory pigments content is due to the self shadowing effect.

RESULTS AND DISCUSSIONS

The tested herbicide influenced the growth of *Chlorella vulgaris* and *Botryococcus braunii*, which demonstrates a certain degree of toxicity, variable in accordance with the used dose. In general, the number of cells grows in the first days from the adding of the herbicide; after this it maintains relatively constant and then it diminished itself. The recorded values from the herbicide treated cultures were generally below the control values. Two previous studies described the influence of isoproturon on growth of *Chlorella vulgaris* and *Botryococcus braunii* under similar experimental conditions. For the first 7 days, the herbicide dose which decrease with 50% the growth of the cells number of *Chlorella* was 0.023µM (Lazăr, 1998). For *Botryococcus*, the 50% growth inhibition dose was 0.950µM (Lazăr and Lazăr, 2001).

The growth and the assimilatory pigments content were below control levels in all used concentrations of isoproturon at 7 and 14 days after the herbicide adding in the culture of *Chlorella vulgaris*.

In case of *Botryococcus braunii* alga, for the concentration of 0.04µM isoproturon, after 7 days, transitory stimulating effects were noticed both for cells number per ml of algal suspension and seemingly for the assimilatory pigments content. This stimulating effect was not visible after 14 days when the values of both indicators were at the control level. The values obtained for the concentrations higher than 0.04µM were below the values observed for the control.

There were calculated the linear regression equations ($Y = a + bX$) and the correlation ratios (R^2) for the variation of the assimilatory pigments content in accordance with the cells number per ml of medium.

The results (fig. 1 and fig. 2) suggested the necessity of the separated calculation of the dates obtained from the first and the second week, respectively; in all cases the regression lines were distinct. These relationships have different parameters for each of the two sampling days, the adaptation reaction to self shadowing evolving in time in accordance with availability of the nutrient resources and the feed back mechanisms of

the characteristic assimilatory pigments synthesis for each species. The statistical approach assessing differences in regressions slopes is described below.

To appreciate the significant level of the differences between the regression ratios 'b" (the slopes of the regression line) from the 7^{th} and 14^{th} days there were calculated the standard errors S_b associated to these coefficients. These values were used to calculate the transgression probabilities α [%] of the distribution of the function t for the differences between the mentioned regression coefficients (Ceapoiu, 1968).

The slopes of the regressions lines of chlorophyll a content versus the number cells per ml (Fig. 1) was significantly different for 7 and 14 days, respectively, after the exposition of the *Chlorella* cells to isoproturon (the parallelism hypothesis was rejected).

In general, the correlation ratios (R^2) were statistically significant (at p=0.05). This means that the variations of the pigments content may be explained by the density of the algal population (the cells number per ml of culture medium), which is determined by the herbicide concentration from the culture medium. At higher herbicide concentrations, the light energy available is distributed to a reduced number of cells which will require a lower content of assimilatory pigments.

In all the analysed cases, excepting the chlorophyll a in *Chlorella*, the relationships between the number of cells and the assimilatory pigments content were those expected for the cultures with different cell populations but without any herbicide treatment. Even the 0.04 μM isoproturon concentration producing a seemingly stimulating effect on assimilatory pigments content of *Botryococcus* after 7 days exposure was clearly placed on the regression line between the number of cells and assimilatory pigments content (Fig. 2).

The strong correlation found between the cells number and the pigments content from the algal cultures treated with isoproturon suggests that it's not a direct effect of this herbicide on the assimilatory pigments content. In the studies performed with metribuzin on the same species direct effects (which can not be explained by self shadowing) were pointed out, but these results will be published in another paper.

CONCLUSIONS

The influence of the isoproturon herbicide on the assimilatory pigments content of *Botryococcus* and *Chlorella* is indirect; the recorded variations may be explained by a self shadowing effect. (At higher herbicide concentrations, the light energy available is distributed to a reduced number of cells which will require a lower content of assimilatory pigments). A possible exception may be the case of chlorophyll a in *Chlorella*.

The linear relationships between the number of cells and assimilatory pigments content presented different slopes (chlorophyll a from *Chlorella*) or different intercepts (especially for *Botryococcus*) at 7 and 14 days after the adding of the herbicide.

In case of *Botryococcus braunii* alga, for the concentration of 0.04μM isoproturon, after 7 days, transitory stimulating effects were noticed both for cells number per ml of algal suspension and seemingly for the assimilatory pigments content.

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Figures

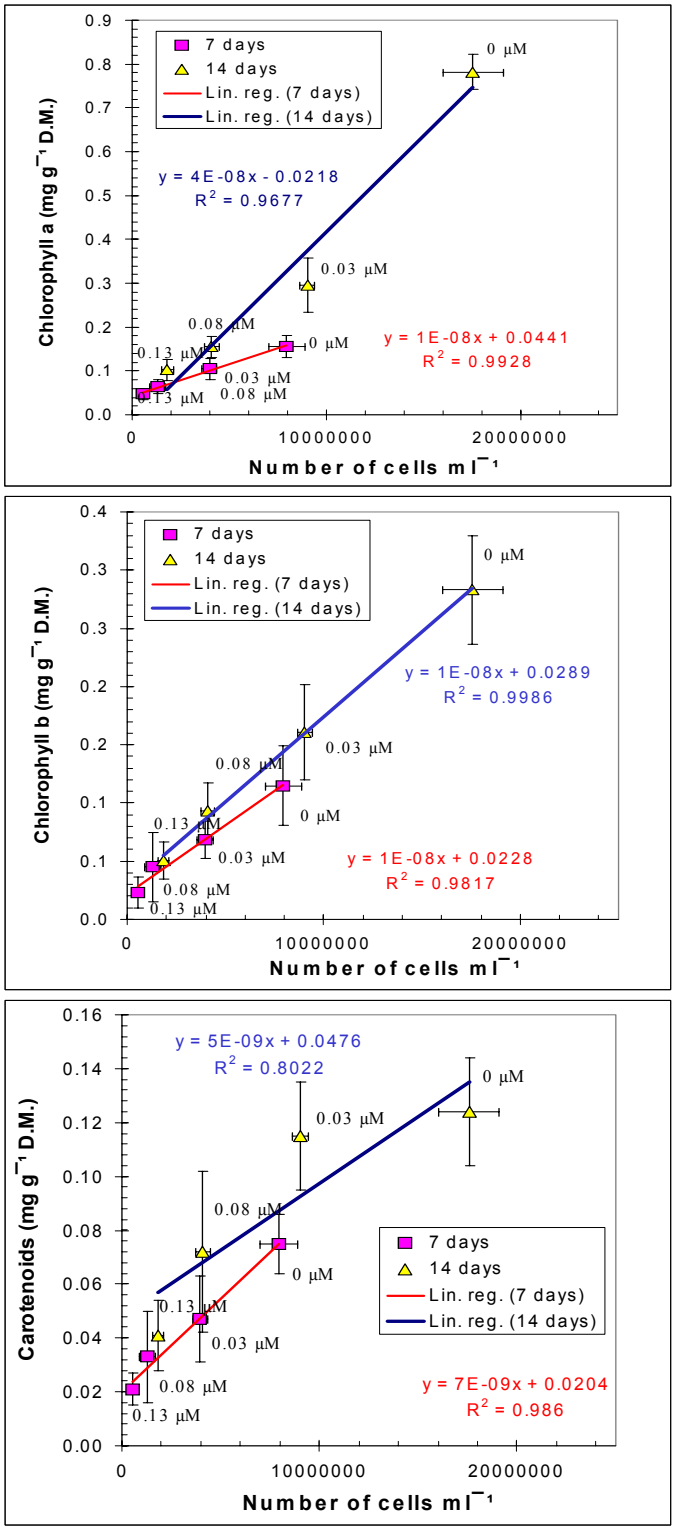

Fig. 1 Relationships between the cells number and the assimilatory pigments content in the *Chlorella* culture after 7 and 14 days from the adding of the isoproturon.

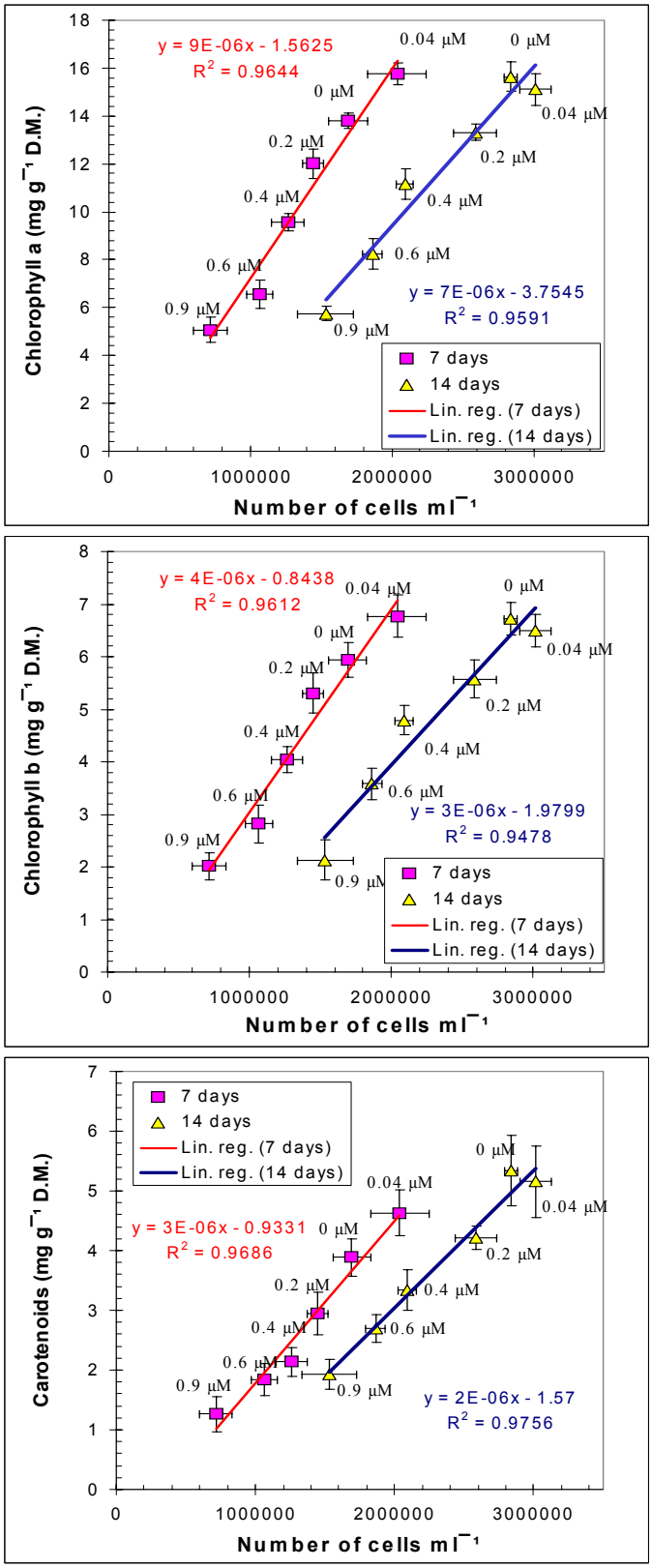

Fig. 2 Relationships between the cells number and the assimilatory pigments content in the *Botryococcus* culture after 7 and 14 days from the adding of the isoproturon.

The balance of mobile phosphorous in some substrates

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Keywords: mobile phosphorous, marc compost, phosphatase activity, nutritive solution

ABSTRACT

Culture substrates, formed by mixing different proportions of organic compounds are generally poor in nutrients (N, P, K), because the main role for the substrates used for containerized ornamental plants is to assure the optimum physical and chemical proprieties (porosity, aeration, water retention, pH, total salts content). For this reason, the nutrients are usually completed by applying nutritive solutions during plants vegetation. In the last decades, the use of this kind of substrates imposed the study of their properties in order to recommend them for a certain culture according to required agrochemical indicators and plants needs.

The aim of the present research was to study four variants of substrates based on marc compost as the recyclable component and forestry compost, leaves compost and peat.

Considering that phosphorous is one of the most important element for plants' nutrition but its solubility and mobility in the substrates create problems, we concentrate the studies on the balance of its mobile forms (the principal source for plants' nutrition) and the intensity of the phosphatase activity (a podoenzyme involved into phosphorous cycle) that supply the accessible phosphate for plants (Ștefanic G. et al., 2001).

INTRODUCTION

In the containerized culture of ornamental plants, which acquired in the last decade a great expansion, the soil was replaced with the culture substrate, resulted from a mixture of organic compounds that provide physical and chemical proprieties for an optimum growth of rooting system. The aeration and water regime, porosity, water retention capacity and nutrients of the substrate coming from either organic components decomposing or nutritive solution imply that each substrate variant to be study for further recommendation to a certain species.

The research tried to establish the phosphorous balance in four substrate variants with marc compost (waste resulted from grape processing) mixed with forestry compost, leaves compost and peat in equal volumetric quantities.

MATERIALS AND METHODS

The mixture ratio of the organic compounds used in substrate variants is presenting in experimental scheme (table 1).

The pH and mobile phosphorous of the four substrate variants were determined.

The studies were conducted in lab modules using glass columns of 3cm in diameter and 25cm height. The columns were filled with 20 cm substrate variants (V1, V2, V3, V4) sustained by glass wool on the base of those.

After the saturation of the substrates with distillate water, they were percolated with water or acidophil nutritive Coïc solution in order to establish the exchange of nutritive ions. The percolate solution was collected in conic glass, while its velocity was considered and then the volume was measured. The mobile phosphorous content was analyzed in the collected percolate after the percolation of water and Coïc solution. Also, the variants substrate were analyzed after percolation for mobile phosphorous, extracted in water (1:10) and exchangeable phosphorous in ammonium acetate 0.5 M, pH 4.65 (1:3), after Gäbriels and Verdonck method.

RESULTS AND DISCUSSIONS

The content of mobile P (extractible with $AcNH_4$, 0.5 M, pH = 4.65, ratio 1:3, Gäbriels and Verdonck method) in the substrate variants varied between 249.75 ppm (V2) and 346.00 ppm (V3). After water percolation the quantities of dislocated mobile P for variants V3 and V4 were 76.43%, respectively 81.35%. Almost 23.56%, respectively 18.65% from the total of displaced phosphorous was undislocated. In substrate remained 71.82% (V3) and 75.77% (V4) phosphorus, while in percolate, 4.61% (V3), respectively 5.58 % (V4) phosphorus, from total mobile phosphorous (table 4).

For substrates V1 and V2, with 107.62, respectively 110.42% mobile phosphorus, we remarked the absence of undislocated P_{mobile} and a quantity of 27.75, respectively 27.85 ppm phosphorus (in percents 2.55%, respectively 3.91%) that was leached with the water (table 3).

After the $AcNH_4$ extraction and percolation with Coïc nutritive solution, the exchangeable phosphorous forms, quantities between 64.36% (V3) and 87.80% (V2) of mobile phosphorous were dislocated and 53.46% (V3), 56.22% (V4), 62.48% (V1) and 76.55% (V3) P_{mobile} remained in the substrate (table 5). The P_{mobile} founded in percolate after Coïc solution washing varied between 9.06% (V2) and 12.24% (V4) (table 4).

The studies concerning the substrate P_{mobile} extracted with $AcNH_4$ (a method tested on numerous soil analyses which established the regularities of exchange and the factors like pH, the concentration of extractive solution) represents an innovation in the case of substrates and presents difficulties concerning the physico-chemical proprieties of their compounds (Davidescu et al., 2005).

The substrate variant V3 with highest content (346ppm) of P_{mobile} (table 2) provide 71.82% P_{mobile} in substrate water percolated and 53.46% P_{mobile} in substrate Coïc solution percolated (table 5). P_{mobile} from water percolate, respectively Coïc solution percolate was found 4.61%, respectively 10.89% in this substrate (V3).

The phosphatase activity of the nutritive substrate (table 6). Phosphorous organic compounds decomposition is active due to phosphatase enzymes and have as result the release of the orthophosphate ion, accessible for substrate micro-flora, micro- and mezzo-fauna and also for vegetal pad (Aendekerk Theo G.L.,1997; Ştefanic G. et al., 2006).

The quantity of the phosphorous enzymatic release in substrate varied between 7.21 mg P/100 g substrate at V3 (with 2 parts marc compost) and 10.16 mg P/100 g substrate at V1(with 0.5 parts marc compost). The highest quantity of phosphorous was determinate in the lowest pH (6.55) substrate (Madjar R. et al., 2007).

CONCLUSIONS

1. The mobile phosphorous quantity was higher in the substrates with low acid pH.
2. Coïc nutritive solution, with high content in nutritive elements, determined higher quantities of phosphorous in percolate.
3. The culture substrates variants are characterized by a high vital and enzymatic level and for this, a rich biological activity.
4. The higher ratio of marc compost in the substrate, the higher biological activity.
5. The substrate life level directly expresses the chemical and physical conditions of substrate that it offers to the microorganisms.
6. The water irrigation simulated in lab confirmed that in the containerized culture, the nutrients leaching phenomenon due to the frequently water supply was present.

7. The fertilization with nutritive solution simulated in lab determined different reaction of ions into the substrate, phenomena which have to be considered in containerized plant technology and the nutrients supply must be correlated with species requirements.

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Tables

Table 1. The variants substrate scheme

Variant	Raport componente			
	Forestry compost	**Leaves compost**	**Peat**	**Marc compost**
V1	1	1	1	0,5
V2	1	1	1	1
V3	1	1	1	2
V4	1	1	1	3

Table 2. Agrochemical characteristics of the substrate variants (pH and P_{mobile})

No. crt.	Substrate variants	pH	P_{mobile} ppm
1	V_1-1(forestry compost):1(leaves compost): 1(peat):0.5(marc compost)	6.55	306.00
2	V_2 -1(forestry compost):1(leaves compost): 1(peat):1(marc compost)	6.74	249.75
3	V_3 -1(forestry compost):1(leaves compost): 1(peat):2(marc compost)	7.35	346.00
4	V_4 -1(forestry compost):1(leaves compost): 1(peat):3(marc compost)	7.89	333.50

Table 3. The phosphorous mobile balance in substrate variants

Variant	P, ppm				
	Mobile in initial substrate	**Mobile in substrate after percolation with distillate H_2O**	**In percolate (H_2O)**	**Mobile in substrate after percolation with nutritive Coïc solution**	**In percolate (Coïc)**
V1	306.00	321.50	7.82	191.20	27.75
V2	249.75	266.00	9.78	191.20	27.85
V3	346.00	248.50	15.96	185.00	37.70
V4	333.50	252.70	18.62	187.50	40.85

Table 4. P mobile (%) dislocated, undislocated forms and P in percolate with distillate water leaching on recyclable organic waste columns substrate

Variant	P (%)			
	Mobile total dislocated	**Mobile undislocated**	**Mobile in substrate after percolation**	**In percolate (H_2O distillate)**
V1	107.62	-	105.06	2.55
V2	110.42	-	106.50	3.91
V3	76.43	23.56	71.82	4.61
V4	81.35	18.65	75.77	5.58

Table 5. P_{mobile} (%) dislocated, undislocated forms and P in percolate with Coïc nutritive solution leaching on recyclable organic waste columns substrate

Variant	P (%)			
	Mobile total dislocated	**Mobile undislocated**	**Mobile in substrate after percolation**	**In percolate (Coïc solution)**
V1	71.55	28.45	62.48	9.06
V2	87.70	12.30	76.55	11.15
V3	64.36	35.64	53.46	10.89
V4	68.47	31.53	56.22	12.24

Table 6. The level of phosphatase activity in nutritive substrate variants

Nutritive substrate	mg P/100 g substrate
V_1-1(forestry compost):1(leaves compost): 1(peat):0.5(marc compost)	a 10.16
V_2 -1(forestry compost):1(leaves compost): 1(peat):1(marc compost)	b 7.28
V_3 -1(forestry compost):1(leaves compost): 1(peat):2(marc compost)	b 7.21
V_4 -1(forestry compost):1(leaves compost): 1(peat):3(marc compost)	a 9.80
Dl 5% = 1.68* mg P/100 g substrate Dl 1% = 2.44 mg P/100 g substrate Dl 0.1% = 3.67 mg P/100 g substrate	

Preliminary results regarding the influence of Cytokinin on micropropogation of *Magnolia soulangiana* Soul. Bot

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Keywords: *in vitro* culture, micro propagation, explant, microshoot, cytokinin, multiplication rate

ABSTRACT

Achieved results show the different influence of benzilaminopurina cytokinin, 6-dimethylallylamino purina, thiadiaduron, kinetin and their concentration on the rate of propagation *in vitro* of explants and micro shoots elongation.

INTRODUCTION

Magnolia species are known as ornamental plants, planting for their beautiful leaves, flowers and fruits. There are used even in medicinal goal, due poliphenol compounds.

For quickly propagation of magnolia was made a lot of studies. In this sense in the literature specialty the researchers made study with the following interest: factors which influenced the development of magnolia obtained *in vitro* culture (Biederman, 1987), micro propagation by explants taken from mature plant (Kamenicka, 1994), factors that influenced the growing explants in the initiation phase (Isac, 1996; Radomir, 2004), the influence on nutritive medium on the forming of *in vitro* bud (Kamenicka et al., 1996).

Initiation of our experiments, regarding the influence of different types and concentration of cytokinin on the micro propagation of magnolia is justify by the bigger request of multiplied material, inefficiency of classic multiplication and the success registered in the last years in the field of tissue culture for ornamental plants.

MATERIALS AND METHODS

Biological materials used for the experiment was represented by micro shoots achieved in the initiation phase of culture. Nutritive media had the composition based on the Murashige–Skoog medium (1962), Miller vitamins (1982) A, 32 mg/l NaFeEDTA, 40 g/l dextrose and agar 7 g/l. Before these factors we add the following:

- A - tip citokinine: A1 – benzilaminopurina (BAP);
  A2 – 6-(dimethylallylamino) purina (2iP);
  A3 – thiadiazuron (TDZ);
  A4 – kinetin (K)
- B - concentration citokinine: B1 - 0,25 mg/l;
  B2 si 0,5 mg/l

Nutritive media was sterilized by autoclavation at one atmosphere for 20 minutes.

The experience has 8 variants with 3 repetitions (table 1).

The transfer of explants on the nutritive medium was made in aseptic conditions.

The explants pasted at nutritive substrates were at 22-24^{0}C temperature, 16 hours photoperiod and 3500 lux light intensity, in the growing room.

The results were registered as rata of multiplication expressing in micro shoots/explant and elongation of micro shoots expressing in cm and was interpretation by Duncan test.

RESULTS AND DISCUSSIONS

From interaction between cytokinins and concentration we can observe the influence of kind of cytokinins on propagation rate.

Taken in consideration the medium effect we can see the manifestation of kind of citokinine in the rate of propagation, which had values between 0 and 8.5 micro shoots/explants. An significant influenced had BAP, which have the bigger rate of multiplication (8.5 micro shoots/explants) in contrast with the low effect of K (5.5 micro shoots/explants) and zero in the case of using 2IP and TDZ.

From point of view of cytokinins influenced for each graduation concentration factor we see that BAP and K express better the multiplication capacity for 0.5 mg/l concentration, the values of rate of propagation being 10 and 8 micro shoots/explants (fig 1). At the constant level of concentration for 0.25 mg/l BAP and K express the capacity of multiplication by small rate of propagation.

Regarding the 2iP and TDZ influence we can see the lake of multiplication express by 0 rate of multiplication for the both level of concentration (fig. 2).

Analyzing the action of concentration factor for different citokinine, starting from medium effect, we can observe that 0.5 mg/l concentration ensure o bigger rate of propagation in contrast with 0.25 mg/l concentration. Following the effect of concentration for constant level of citokinine observed the increasing of propagation rate from 7 to 10 in BAP case and from 3 to 8 micro shoots/explant in the K case. For constant level of 2iP and TDZ using the both variants of concentration, the rate of propagation was zero (fig. 3; 4).

The elongation of micro shoots influence the efficiency of micro shoot transfers to the rooting medium, and it was influenced by both variants of factors.

Medium effect of different concentration on the constant level of citokinine lead to bigger elongation of micro shoots in the using 0.25 mg/l than 0.5 mg/l.

Following the action of both concentration for each citokinine observed that in the case of 0.25 mg/l BAP concentration the micro shoots have 2.4 cm in comparison with 0.5 mg/l BAP concentration where the length of micro shoot was 1.8 cm. Both variants of concentration have the same effect for 2iP constant level when the length of micro shoots was 2 cm. Length of 2 and 1.8 cm was realized under concentration influence for TDZ. The smaller length of micro length was registered when using the both concentration of nutritive medium with K (fig. 5).

The interpretation of medium effect regarding the influence of citokinine for constant level of concentration lead to the conclusion that the bigger micro shoots (2.1 cm) are realized on the nutritive medium supplemented with BAP. The decreasing values of micro shoots length have the following order: BAP, 2iP, TDZ, and K.

For constant level of 0.25 mg/l concentration the values of micro shoots elongation are 2.4 cm under BAP influence and 1.6 cm for K influence. TDZ and 2iP have influenced in the same measure the elongation of micro shoots, the values being 2 cm.

At 0.5 mg/l concentration the bigger values of micro shoots elongation was realized under 2iP influence, and the smaller values was determinate by the K. Intermediary values was achieved on the nutritive medium with BAP and TDZ (fig. 6).

Achieved results confirmed the conclusion of other specialists which consider that the nutritive medium composition is one of the very important factors for the success of magnolia *in vitro* culture.

CONCLUSIONS

For micro propagation of magnolia the better efficiency citokinine was BAP and K in 0.5 mg/l concentration.

Using BAP and K in 0.25 mg/l concentration we have smaller rate of propagation but influence in a good way the elongation of micro shoots and will be easier to individualize the micro shoot for transferring on the rooting medium.

The achieved results will be used to continue the ulterior research regarding the in vitro technology of magnolia.

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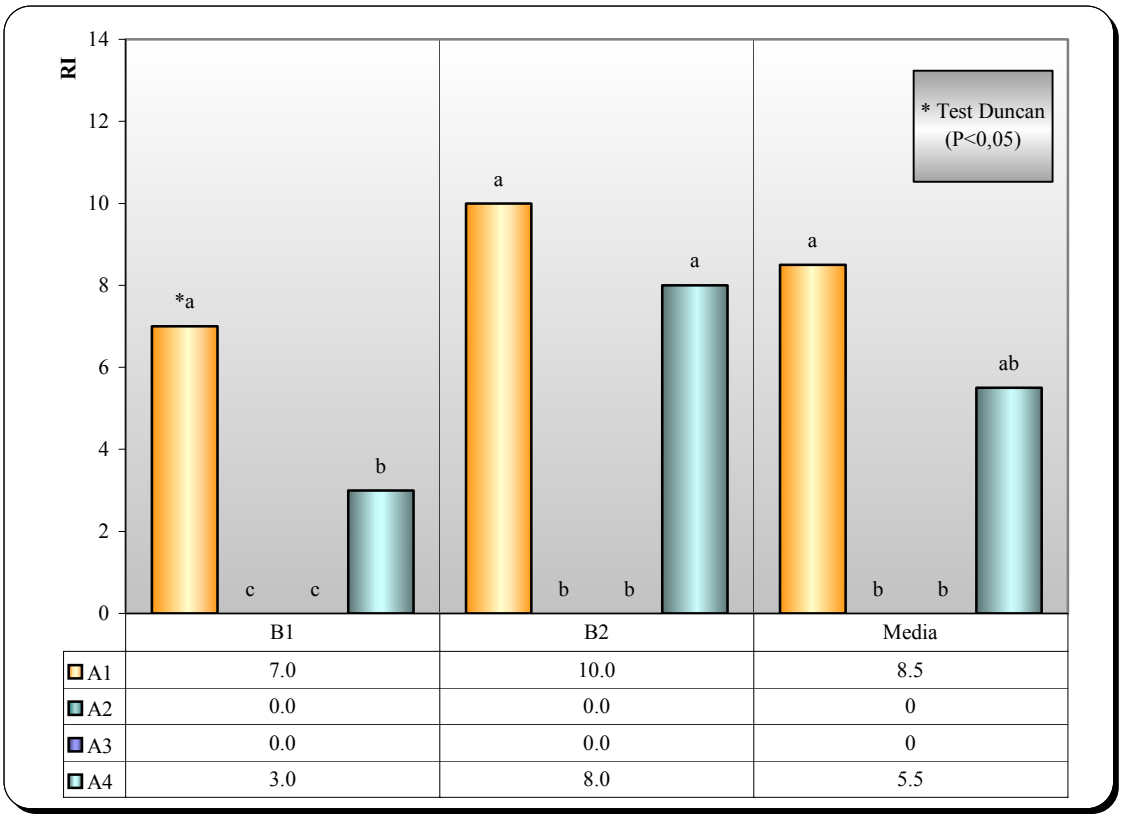
Tables

Table 1. Experimental variants for *in vitro* multiplication stage of Magnolia

Variants	Variable factors	
	Kind of citokinine	**Concentration of citokinine**
V1	A1	B1
V2	A1	B2
V3	A2	B1
V4	A2	B2
V5	A3	B1
V6	A3	B2
V7	A4	B1
V8	A4	B2

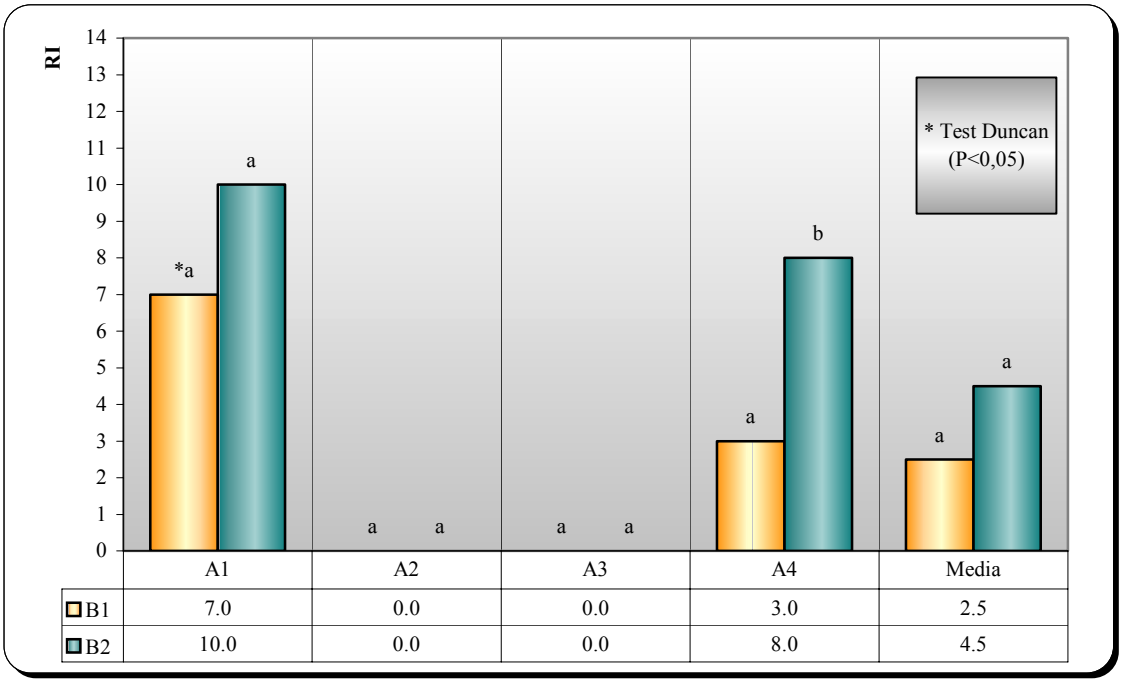
Figures

Fig. 1. BAP influence for 0.5 mg/l concentration on the Magnolia micro propagation



	B1	B2	Media
A1	7.0	10.0	8.5
A2	0.0	0.0	0
A3	0.0	0.0	0
A4	3.0	8.0	5.5

Fig. 2. Rate of *in vitro* propagation in function of citokinine for different concentration



	A1	A2	A3	A4	Media
B1	7.0	0.0	0.0	3.0	2.5
B2	10.0	0.0	0.0	8.0	4.5

Fig. 3. Rate of *in vitro* propagation in function of tested citokinine concentration

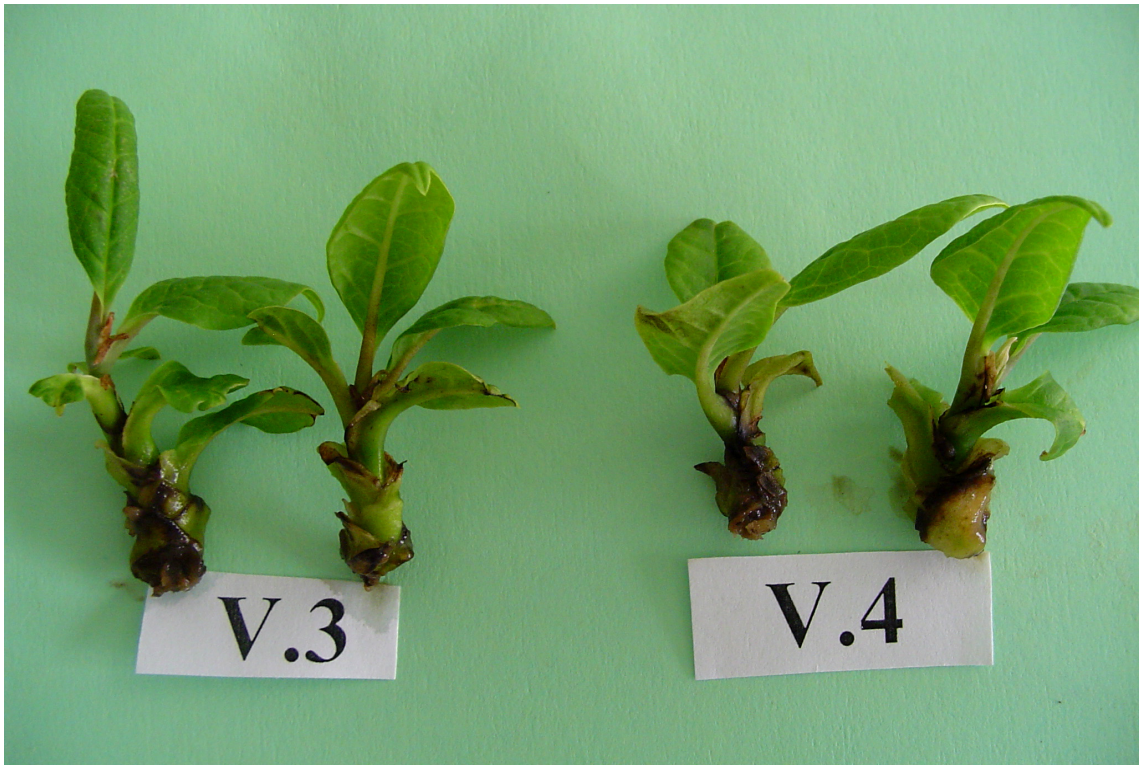


Fig 4. The influence of different concentration of 2iP on the *in vitro* propagation of Magnolia

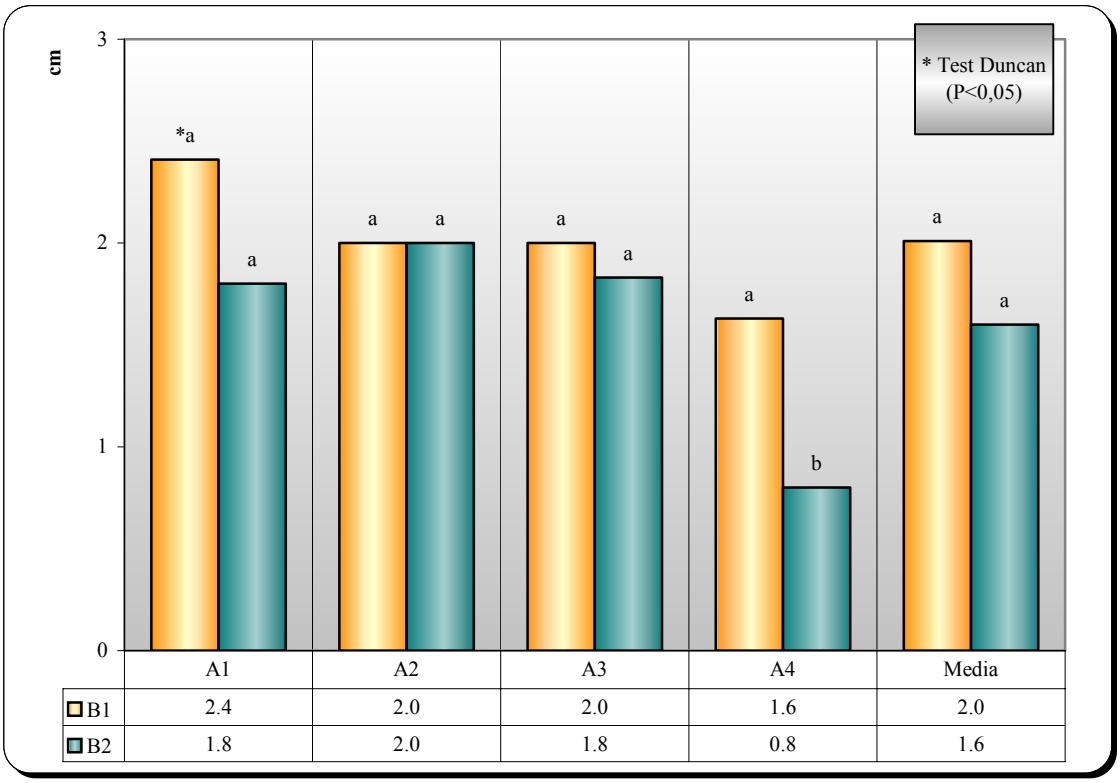
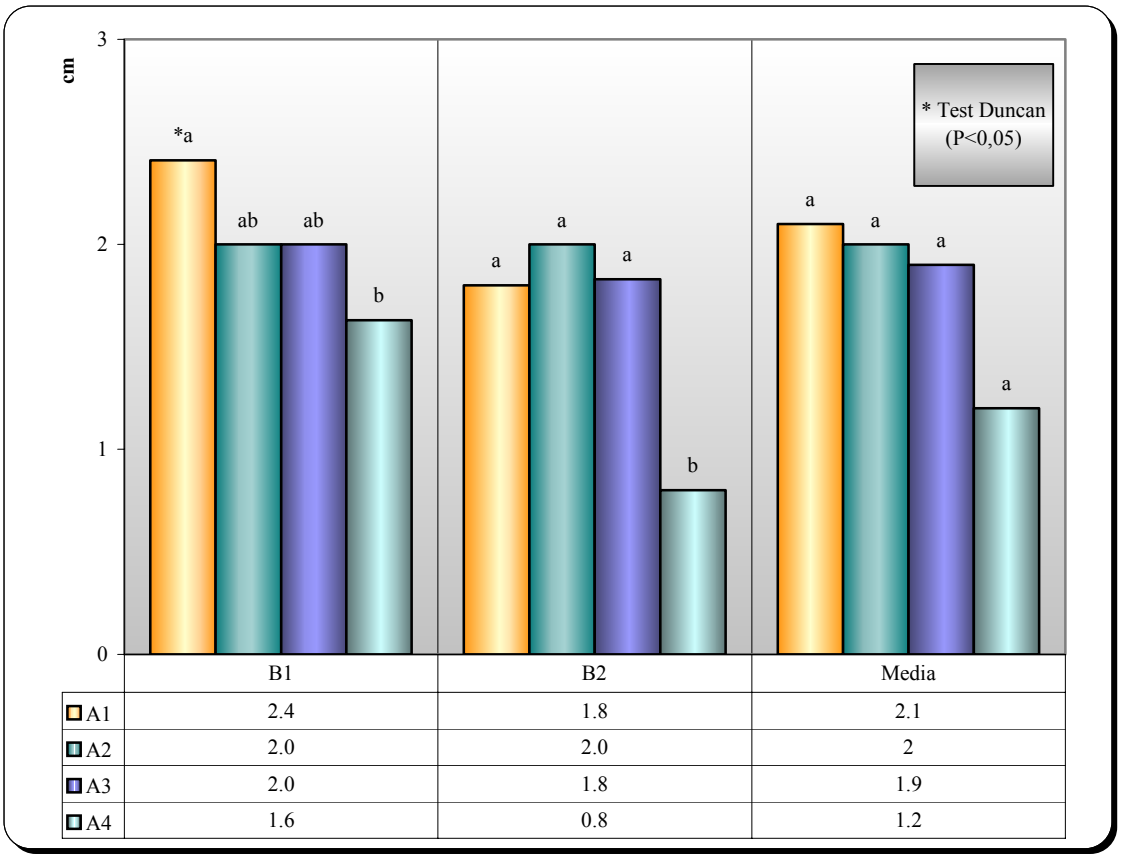


Fig. 5. Variation of micro shoots elongation in function of citokinine concentration



	B1	B2	Media
A1	2.4	1.8	2.1
A2	2.0	2.0	2
A3	2.0	1.8	1.9
A4	1.6	0.8	1.2

Fig. 6. Elongation of micro shoots in function of kind of citokinine for different concentration

The influence of photoperiod on *in vitro* culture in the multiplication phase at *Eustoma grandiflorum*

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Keywords: explants, Eustoma, tannins, assimilatory pigments, reducing sugars

ABSTRACT

The purpose of this paper was to study the main physiological indicators at *Eustoma grandiflorum* under photoperiod influence. We used two variant of photoperiod with 12 hours and 16 hours. The biological material was achieved used the *in vitro* technology. The observations of explants made in the multiplication stage of *in vitro* culture. In principal, these aspects show that the explants obtained in *in vitro* culture have small content in reducing sugars, tannins and mineral elements know the fact that the mass of material are very small.

INTRODUCTION

Lisianthus, a relatively new floral crop to the international market, quickly ranked in the top ten cut flowers world wide due to its rose-like flowers. Lisianthus is a perennial herbaceous ornamental species, original in the South of United States, which is used as cut flower due to its big and attractive flowers, long stalks and long duration in vases (Havely and Kofranek, 1984, Gill et al, 2000, Uddin et al 2004). In the first year of production, the number of cut flowers is small, due the fact as the transplant has a reduced potential to make shoot flowers (Farina and Ruforti, 1993). Lisianthus are available in various colours, such as blue, purple, plum, white, pink, and bicolour (Kunitake et al., 1995). Lisianthus grows to 50-70 cm in height with 20-40 flowers, flowering mainly in summer. The longevity of cut flowers and pot flowers is 2-5 weeks (Roh and Lawson, 1984). The cultures of explants with Lisianthus were kept at an air temperature of 25 +/- 2^0C with 16 hours photoperiod (Kee et al., 1999).

Micropropagation has been extensively used for the rapid production of many plant species and cultivars (Hartmann et al., 2002). Leaves produced in vitro also have low chlorophyll content (Grout and Aston, 1977), restricted leaf blade expansion (Kozai, 1992), low stomatal density (Ziv, 1995), poorly differentiated spongy and palisade tissues, low percent dry matter, and/or hyperhydrated shoots.

MATERIALS AND METHODS

The researches were carried out at Biotechnology and Physiology Laboratory.

The objectives of the study were to monitor *Eustoma* physiological aspects *in vitro* culture. We study the influence of photoperiod on the main physiological indicators. We used two variant of photoperiod with 12 hours and 16 hours.

We used explants obtained *in vitro* culture. The cultivars used are in the multiplication phase of biotechnology.

The determination of dry weight and total water was achieved by using the thermo-balance. The content of mineral elements was determinate by using the

spectrophotometer – Spectro MIDEX M. The quantity of tannins was made by Lowenthal method. By Schorl method we analyze the content of reducing sugars

The content in assimilatory pigments was determinates spectrophotometricaly.

The statistic interpretation of the results was performed by means of the SPSS 13,0 program for Windows.

RESULTS AND DISCUSSIONS

Figure 1 show the influence of photoperiod on the content of tannins and reducing sugars. The content of tannins analyzed in the micro shoots in the multiplication stage is the same for both variant of photoperiod (0,017%). The content of reducing sugars is 0, 44% for 16 hours photoperiod respectively 0,376% for 12 hours photoperiod. The statistic interpretation shows the facts that between both variants of photoperiod don't are significant differences for $p < 0,005$.

The quantity of chlorophyll a and b are bigger for 16 hours photoperiod (fig. 2). Regarding the carotenoids between both variants of photoperiod don't are significant differences.

The content in some mineral elements from leaves, for 12 hours photoperiod was registered bigger values for chlorine and manganese and smaller values for iron and zinc (fig. 3). The value of calcium don't are significant for used variants of photoperiod.

Influence of photoperiod on dry weight and total water don't registered significant differences for $p < 0,005$ (fig. 4).

CONCLUSIONS

In principal, the main physiological indicators registered lower values for *in vitro* culture.

The content of tannins registered the same values for both variants of photoperiod.

Dry weight registered for 12 hours photoperiod was 9,54% and for 16 hours photoperiod was 11,3.

In general, we obtained the bigger values in the variant with 16 hours photoperiod.

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Figures

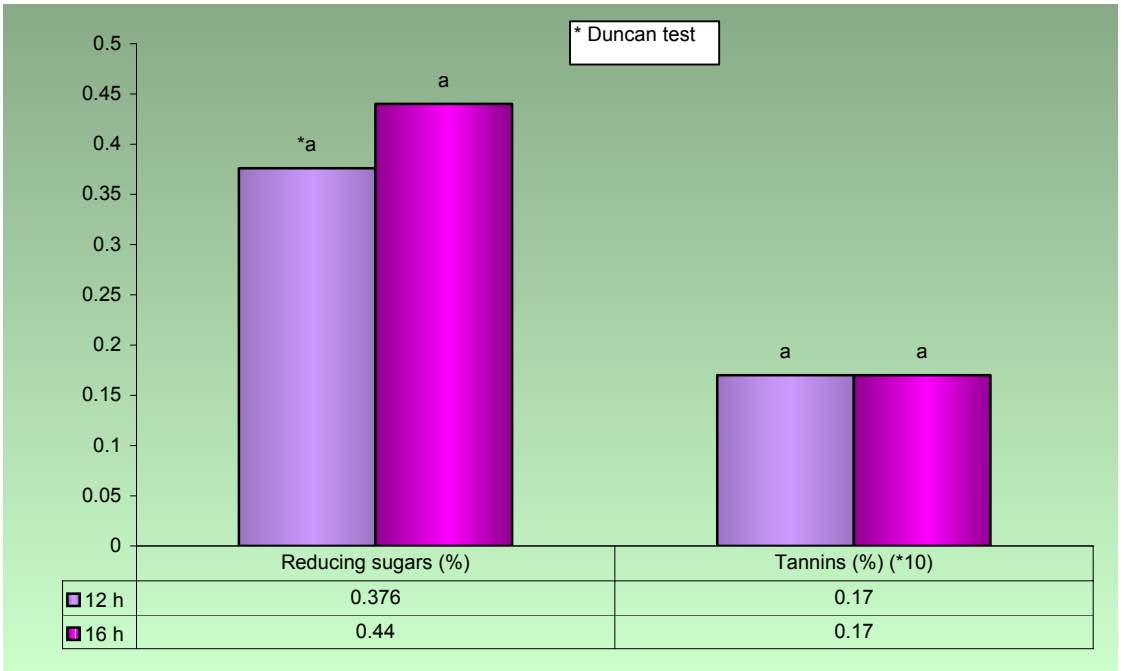

Fig. 1. The influence of photoperiod on tannins and reducing sugars content at *Eustoma grandiflorum*

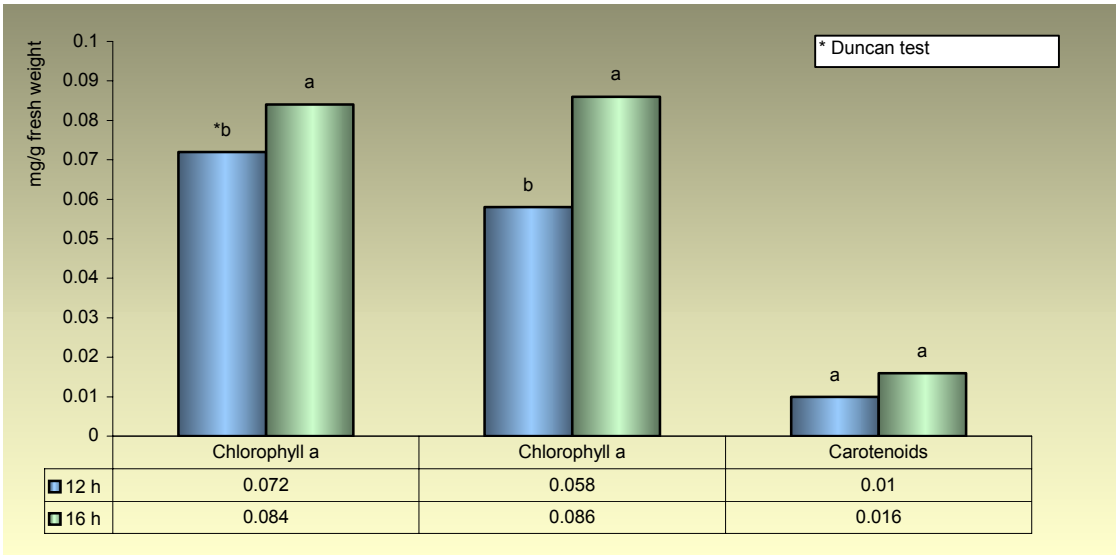

Fig. 2. The influence of photoperiod on assimilatory pigments quantity at *Eustoma grandiflorum*

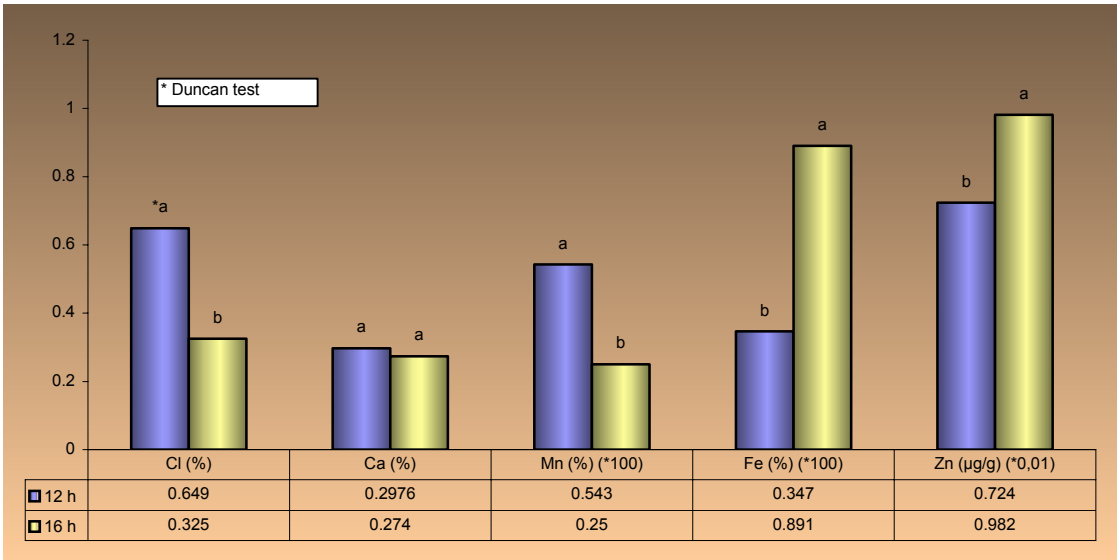

Fig. 3. The content of mineral elements for 12 and 16 hours photoperiod at Eustoma grandiflorum

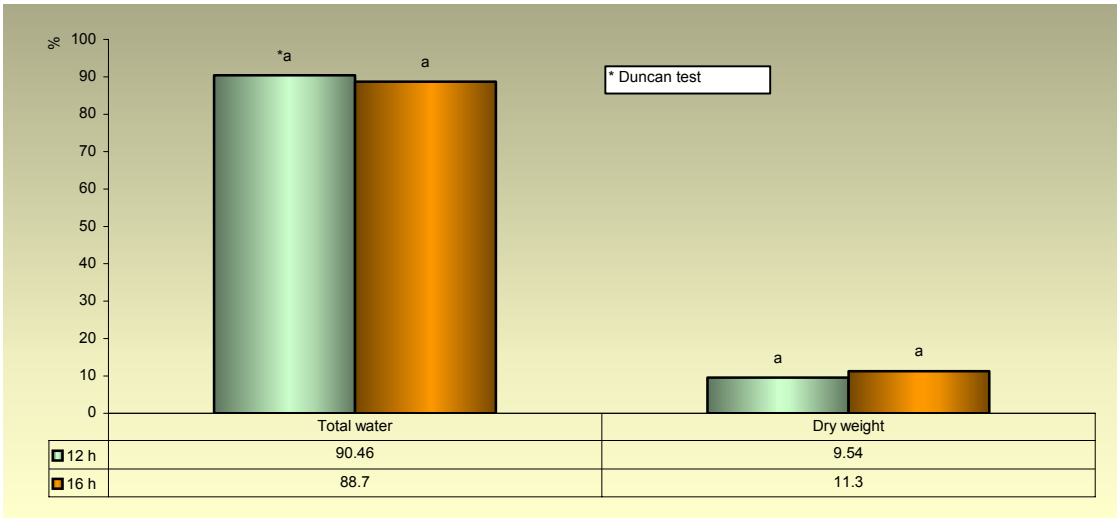

Fig 4. The influence of photoperiod on dry weight and total water

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Physiological behaviour of strawberry *in vitro* culture in the multiplication phase

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Keywords: Vitroplants, Elsanta, Premial, photosynthesis, assimilatory pigments, respiration

ABSTRACT

Regarding this study, we use two cultivars: Elsanta and Premial. The porpose of this paper was to study the main physiological indicators. In principal, these aspects regarding both cultivars show that the explants obtained in *in vitro* culture have small photosynthetic capacity. Between both cultivars exist significant differences regarding the physiological parameters.

INTRODUCTION

Hdider & Desjardins (1994) demonstrated that strawberry plants grown at 0.1% sucrose had a higher photosynthesis rate, hence were more autotrophic. Leaves produced in vitro also have low chlorophyll content (Grout and Aston, 1977), restricted leaf blade expansion (Kozai et al., 1992), low stomatal density (Ziv, 1995), poorly differentiated spongy and palisade tissues (Donnelly et al., 1985), low percent dry matter, and/or hyperhydrated shoots (Ziv, 1991). All of these characteristics negatively impact the potential for ex vitro acclimatization.

Micropropagation has been extensively used for the rapid production of many plant species and cultivars (Debergh and Zimmerman, 1991; Jeong et al., 1995; Hartmann et al., 2002). However, despite its extraordinary potential, this technology is still confronted with many problems. Among these, one of the most important is the poor survival of plantlets following ex vitro transfer, during acclimatization to greenhouse or field conditions (Pospíšilová et al., 1999). This problem originates from poor development of photosynthetic capacity in vitro, which has been attributed to the presence of sugar in the medium (Pospíšilová et al., 1992), low light and inadequate CO_2 supply (Kozai and Iwanami, 1988), and poor control of water loss caused by high relative humidity within the vessel (Desjardins, 1995; Estrada-Luna et al., 2001). These conditions can ultimately influence plant development and photosynthetic performance (Preece and Sutter, 1991). Kozai et al. (1997) demonstrated that most chlorophyllous plantlets/microcuttings in vitro have photosynthetic ability provided that the environmental conditions are favourable for photosynthesis. However, low ventilation rates, characteristic of conventional culture vessels, limit CO_2 availability during almost the entire photoperiod (Kadleček et al., 2001). Consequently, plantlets commonly exhibit low net photosynthetic rates caused by low CO_2 concentrations in the vessels during the photoperiod and low light intensities typical of culture rooms (Heo and Kozai, 1999).

In vitro culture conditions frequently result in alterations in mesophyll development as well as chloroplast structure, namely grana development (Wetzstein and Sommer, 1982). At the biochemical level, low ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) activity (Grout, 1988) and high phosphoenolpyruvate carboxylase (PEPC) activity (Triques et al, 1997) is often encountered in C3 species. These conditions also contribute to low photosynthetic activity.

MATERIALS AND METHODS

The researches were carried out at Biotechnology and Physiology Laboratory with the following types of strawberry: Elsanta and Premial.

Elsanta is a very sturdy strawberry with a good flavour and a strong aroma. This variety is ideally suited to both the grower and the market through its form, high productivity, sturdiness, and long shelf life.

We used explants obtained *in vitro* culture. The cultivars used are in the multiplication phase of biotechnology. The nutritive substrate used the Lee Fossard (1977) medium improved with BAP 0.4 mg/l, AIA 0.2 mg/l, NaFeEDTA 32 mg/l, sucrose 40 g/l, agar 7 g/l. The pH was 5.6-5.8.

The determination of the rate of photosynthesis and respiration were performed by measuring the gas exchange by means of Warburg device. The results were in cm^2 O_2/g/h. The determinations were performed at a lighting rate of 8000 lux (for photosynthesis) and a temperature of 20^0C. The content in assimilatory pigments was determinates spectrophotometricaly. The statistic interpretation of the results was performed by means of the SPSS 13,0 programme for Windows.

RESULTS AND DISCUSSIONS

The *in vitro* explant of Elsanta and Premial for dry weight determination registered the following values: 9,234 %, respectively 8,804 % (figure 1). The standard deviations of the values are presented in table 1. The value of dry weight for Elsanta cultivar is significant bigger than Premial, for 0.05 level (table 1).

The rate of respiration for Elsanta cultivar is 0.136 cm^3 O_2/g/h, and for Premial is lower (0.096 cm^3 O_2/g/h) (figure 2, table 2. The value of t (t test of significance) is 13.819, which mean significant differences for 0.05 levels.

Determination of photosynthetic rate showed in the figure 3 that at Elsanta was registered the higher value. Table 3 shows the value of photosynthesis, average and standard deviation for both cultivars. By statistical interpretation between these cultivars was a significant difference.

Figure 4 show the content of assimilatory pigments, chlorophyll a, chlorophyll b and carotenoids. At Elsanta cultivar we can observe that the content in chlorophyll a registered the value 4.266 mg/g fresh weight.

We realized five repetitions to determinate the content of pigments.

We registered at Elsanta cultivar the following values of pigments content: chlorophyll a – 4.266 mg/g fresh weight, chlorophyll b – 0,783 mg/g fresh weight, carotenoids – 0.238 mg/g fresh weight (figure 4, table 4). The content in assimilatory pigments for Premial are: chlorophyll a – 1.961 mg/g fresh weight, chlorophyll b – 0,309 mg/g fresh weight, carotenoids – 0,160 mg/g fresh weight (figure 5, table 5).

For all three pigments studied, exist significant difference between the value registered for Elsanta and Premial, for 0.05 level.

CONCLUSIONS

In principal, the main physiological indicators registered lower values for in *vitro* culture strawberry.

The value registered for both cultivars studied show significant differences regarding the main physiological indicators.

In comparison with Premial cultivar, at Elsanta we can see a higher content of chlorophyll a, chlorophyll b and carotenoids.

Determination of photosynthetic rate showed that at Elsanta was registered the higher value.

Regarding the respiration intensity at Elsanta observed the bigger value.

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Tables

Table 1. Dry weight: the value of repetition, average and standard deviation

	Elsanta	**Premial**
Repetition 1	9,26	8,74
Repetition 2	9,14	8,68
Repetition 3	8,87	9,15
Repetition 4	9,67	8,81
Repetition 5	9,23	8,64
Average	9,234	8,804
Standard deviation	0,288149267	0,203789107

Table 2. Rate of respiration: the value of repetition, average and standard deviation

	Elsanta	**Premial**
Repetition 1	0,138	0,123
Repetition 2	0,145	0,110
Repetition 3	0,124	0,089
Repetition 4	0,130	0,067
Repetition 5	0,145	0,089
Average	**0,136**	**0,096**
Standard deviation	0,009	0,022

Table 3. Rate of photosynthesis: the value of repetition, average and standard deviation

	Elsanta	**Premial**
Repetition 1	0,00340	0,00288
Repetition 2	0,00321	0,00319
Repetition 3	0,00470	0,00289
Repetition 4	0,00876	0,00198
Repetition 5	0,00981	0,00279
Average	**0,00598**	**0,00275**
Standard deviation	0,00310	0,00045

Table 4. Determination of assimilatory pigments: the value of repetition, average and standard deviation – Elsanta

	Chlorophyll a	**Chlorophyll b**	**Carotenoids**
Repetition 1	4,870	0,698	0,288
Repetition 2	4,342	0,776	0,189
Repetition 3	3,897	0,557	0,266
Repetition 4	4,234	0,998	0,255
Repetition 5	3,987	0,885	0,190
Average	**4,266**	**0,783**	**0,238**
Standard deviation	0,383	0,170	0,045

Table 5. Determination of assimilatory pigments: the value of repetition, average and standard deviation – Premial

	Chlorophyll a	Chlorophyll b	Carotenoids
Repetition 1	1,897	0,243	0,155
Repetition 2	1,675	0,432	0,187
Repetition 3	1,987	0,324	0,144
Repetition 4	2,576	0,255	0,135
Repetition 5	1,670	0,290	0,177
Average	**1,961**	**0,309**	**0,160**
Standard deviation	0,371	0,076	0,022

Figures

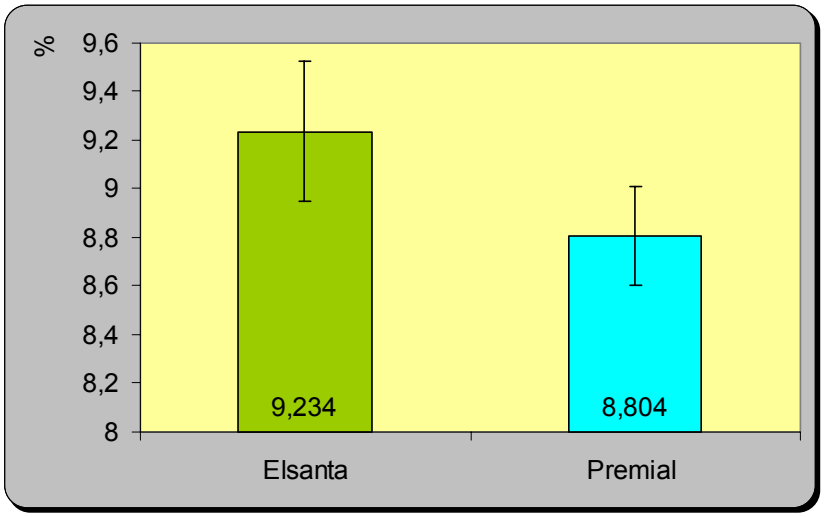

Fig 1. Dry weight to the strawberry explants

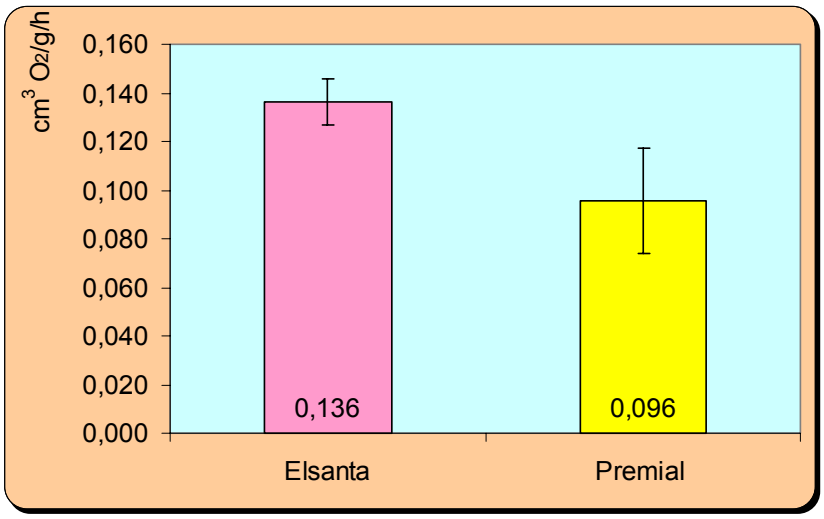

Fig 2. Rate of respiration to the strawberry explants

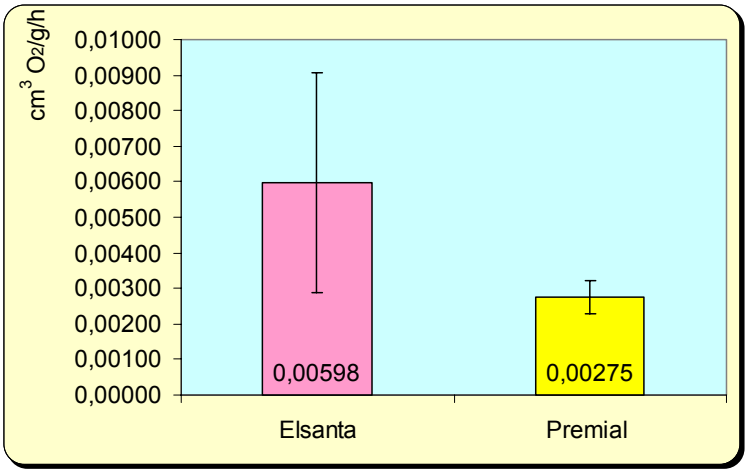

Fig. 3. Rate of photosynthesis to the strawberry explants

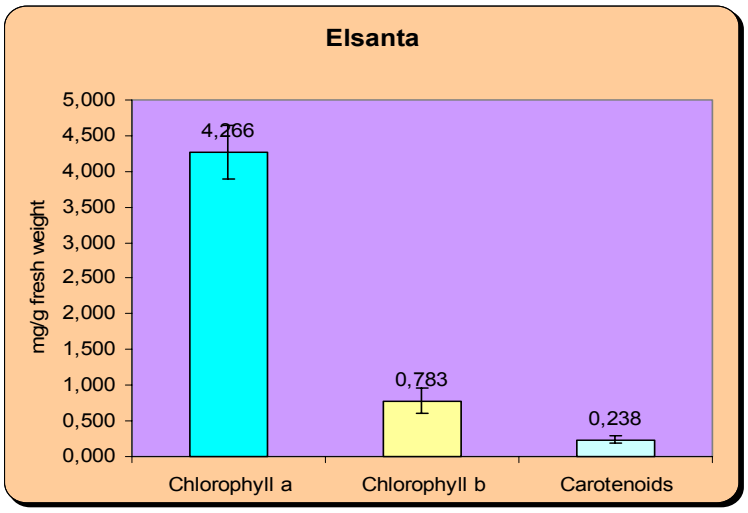

Fig 4. Content of assimilatory pigments - Elsanta

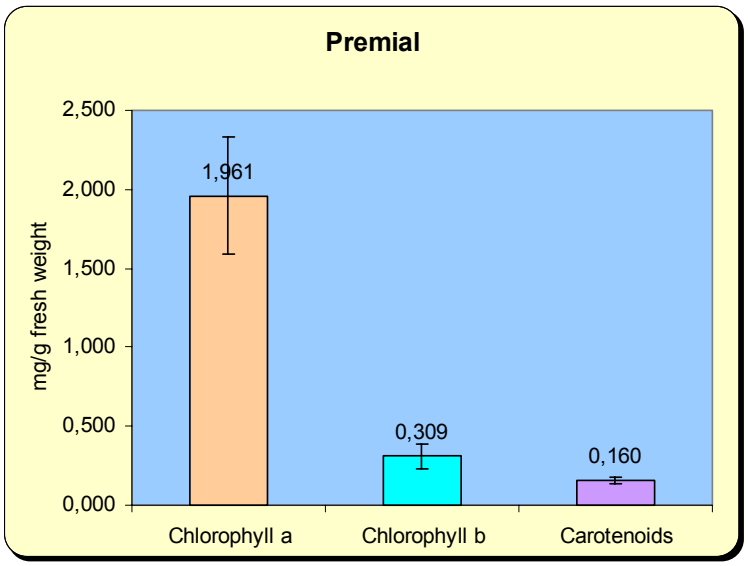

Fig 5. Content of assimilatory pigments - Premial

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Variability of the main anatomical characteristics for leaves and fruits of some apple trees varieties and hybrids (*Malus domestica* L.)

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Key words: epidermis, stomata, mesophyll, cuticle

ABSTRACT

This study shows the comparative anatomical study for leaves and fruits of some apple tree varieties and hybrids to making evident the possible anatomical characters used in plant breeding to improve the passive resistance of the plant for field diseases attack and in the fruits storage. The transversal section was provided in the median leaves blade to establish the epidermis and mesophyll width and in the fruit epicarp to determine the wax layer and epidermis width. It was counted the stomata number on the lower epidermis. The variability of the width mesophyll and stomata numbers is high between the apple tree varieties and hybrids. The apple tree varieties and hybrids can be divided in four different groups taken into consideration the relation between the mesophyll width and stomata numbers. The hybrids have the biggest epidermis and wax layer than apple tree varieties. These characters indicate the better passive resistance of apple tree hybrids to scab attack (*Venturia inaequalis*), which it is the main disease in apple tree and the better fruit storage.

INTRODUCTION

The main frequent disease in apple tree is the scab, developed by *Venturia inaequalis* fungus. The morphological, anatomical and physiological particularities are taken into consideration in the plant breeding process to obtain the resistant varieties and hybrids.

The plant response to the pathogens attack can be passive and active. The passive response is determinate by the certain morphological and anatomical properties.

At the structural level, the types and size of tissue could have a significant influence on the resistance of the plant organs at the pathogen attacks. The leaf has the biggest ecological plasticity and it reflect the species particularities concerning the species adaptability and sensitivity to the environmental conditions and attack on or into leaf of the pathogens.

The extern part of the fruit, named epicarp, is covered from different size wax layer in term of the cultivated variety and hybrid properties and it represent a main barrier against pathogens attack.

MATERIALS AND METHODS

The biological materials used represented by the leaves and fruits were collected from R.D.S.T.F - Voineşti - Dâmbovița from 8 varieties and 12 hybrids in two years (2006, 2007). The leaves and fruits samples were collected at the end of August - begin of September.

At the leaves were make the observations and anatomical measurements regarding epidermis and mesophyll size and stomata number per square millimetre. The epidermis and wax layer size was determinate at the apple fruits.

The observations and measurement were making on the transversal sections performed by the median leaf blade. The fruits transversal sections was making through the epicarp. The anatomical sections were clarified 24 hours in chloral hydrate. The observations and measurements were making with the optical microscope - ML-4M.

RESULTS AND DISCUSSIONS

The upper epidermis done by only one cell layer covered by the cuticle didn't present the differences between the apple tree varieties and hybrids regarding the width epidermis. The average of the upper epidermis width was 18.8 μm.

The lower epidermis width is thinner than the upper epidermis, in average 15.6 μm with any differences between the apple tree varieties and hybrids. On the lower epidermis there are stomata and protective pills. The apple tree leaf is hypostomatic.

The stomata numbers are between 90 and 270 per mm^2 on the apple tree leaf (figure 1). The smallest stomata number was observe at Florina variety (90 stomata/mm^2) and the biggest at the Irisem variety and V97/192 hybrid (270 stomata/mm^2).

The width of the bifacial leaf mesophyll had between 164 and 235 μm (table 1).

Taken into consideration the width of the mesophyll and the number of stomata, the varieties and hybrids can be dividing in four different groups:

- varieties and hybrids with thin mesophyll (< 180 μm) and big number of stomata (> 240/mm^2), for the V97/192, V95/230 hybrids and Johnatan variety;
- varieties and hybrids with thin mesophyll (< 180 μm) and small number of stomata (< 140/mm^2), for the H1/18 hybrid and Florina variety;
- varieties and hybrids with medium mesophyll (180-220 μm) and medium number of stomata (140-240/mm^2), for the majority hybrids and variety;
- varieties and hybrids with wide mesophyll (> 220 μm) and medium number of stomata (140-240/mm^2), for the V38/72, H9/78 hybrids and Golden Delicious variety;

At the anatomical analysis of the fruits was any correlations observe between the epidermis and wax layer size of the apple fruits (figure 2).

The apple tree varieties with a thinnest epidermis (less than 8 μm) make a wide wax layer as it can see at Golden Delicious, Iris varieties and V95/230, H1/18 hybrids where the cuticle is two-three times widest than epidermis (>15 μm).

Some varieties are thin epidermis (8-10 μm) and they make a medium wax layer (10-15 μm) as there are Ciprian, Jonathan, Redix and Generos.

The majority varieties and hybrids are the width wax layer equal with the epidermis width, respectively medium size (10-15 μm).

Some varieties and hybrids with medium epidermis (10-15 μm) had thin wax layer (>10 μm) as it can see at the V1/26, V95/49 and V98/72 hybrids.

It was identify a distinct group composed by V98/72, V95/49 and H1/26 hybrids with the widest epidermis and wax layer (>15 μm). The protective layer formed by epidermis and wax layer is more than 30 μm. It is possible that those hybrids to demonstrate better passive resistance at scab infection and fruit storage.

CONCLUSIONS

The size of upper and lower epidermis has insignificant variations over the studied varieties and hybrids.

The size of the mesophyll and the number of stomata is very different between the varieties and hybrids and the hybrids and varieties could be grouped in four distinct classes take into consideration the both values.

The protective barrier formed by the wax layer and epidermis is every time more than 20 μm whatsoever the size of the both. At the majority of the varieties and hybrids the size of the wax layer decreases when the size of the epidermis increases.

The V98/72, V95/49 and H1/26 hybrids are possible to demonstrate the better passive resistance at scab infection and fruit storage with their gross protective barrier.

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Table 1. Variability of the epidermis and wax layer size of apple fruits (20 varieties and hybrids, average of two years)

Variety/hybrid	Size of the epidermis (µm)	Size of the wax layer (µm)	Size of the epidermis + wax layer (µm)
H1/26	22.5	18.1	40.6
V95/49	20.0	15.6	35.6
V98/72	16.9	15.6	32.5
V95/15	13.1	13.7	26.8
V95/12	12.5	12.5	25.0
V97/192	11.2	13.8	25.0
H9/78	10.6	12.5	23.1
V95/55	14.3	10.6	24.9
Irisem	12.5	11.3	23.8
V95/272	11.8	11.8	23.6
V95/52	14.3	9.4	23.7
Florina	11.9	9.4	21.3
Ciprian	10.0	15.0	25.0
Jonathan	10.0	15.0	25.0
Redix	8.8	15.0	23.8
Generos	8.1	13.7	21.8
Iris	7.5	16.2	23.7
Golden Delicious	6.3	18.8	25.1
V95/230	6.3	18.8	25.1
H1/18	6.3	16.2	22.5

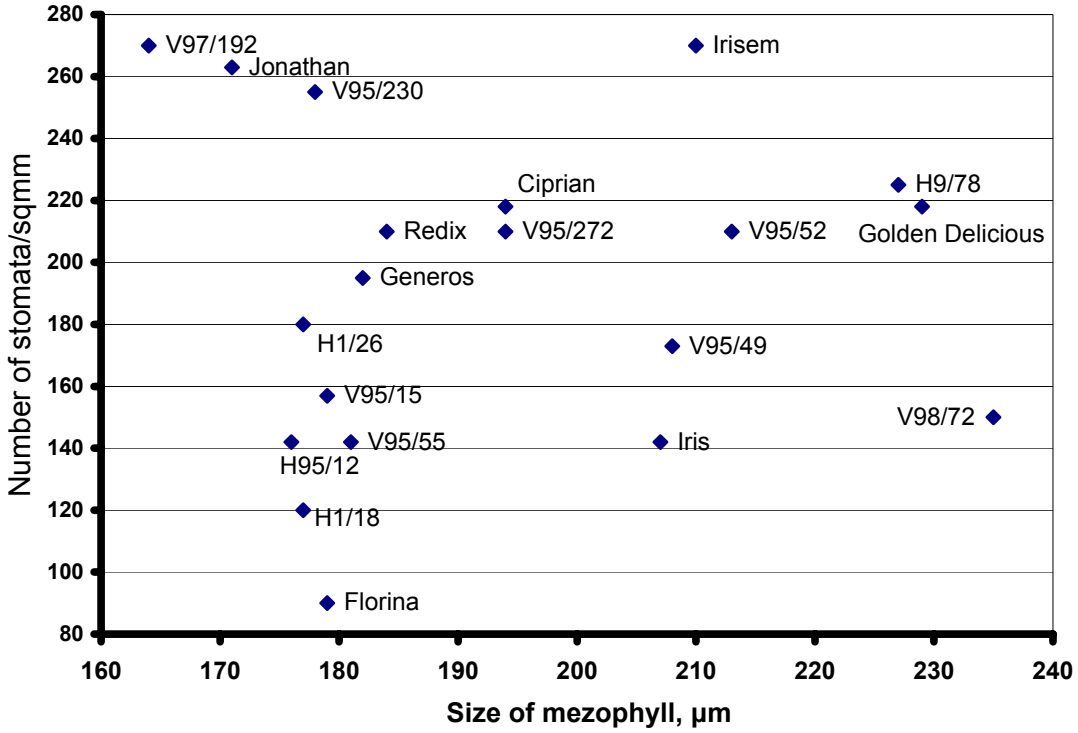

Fig. 1. Size of the mezophyll and number of stomata for 20 apple tree varieties and hybrides

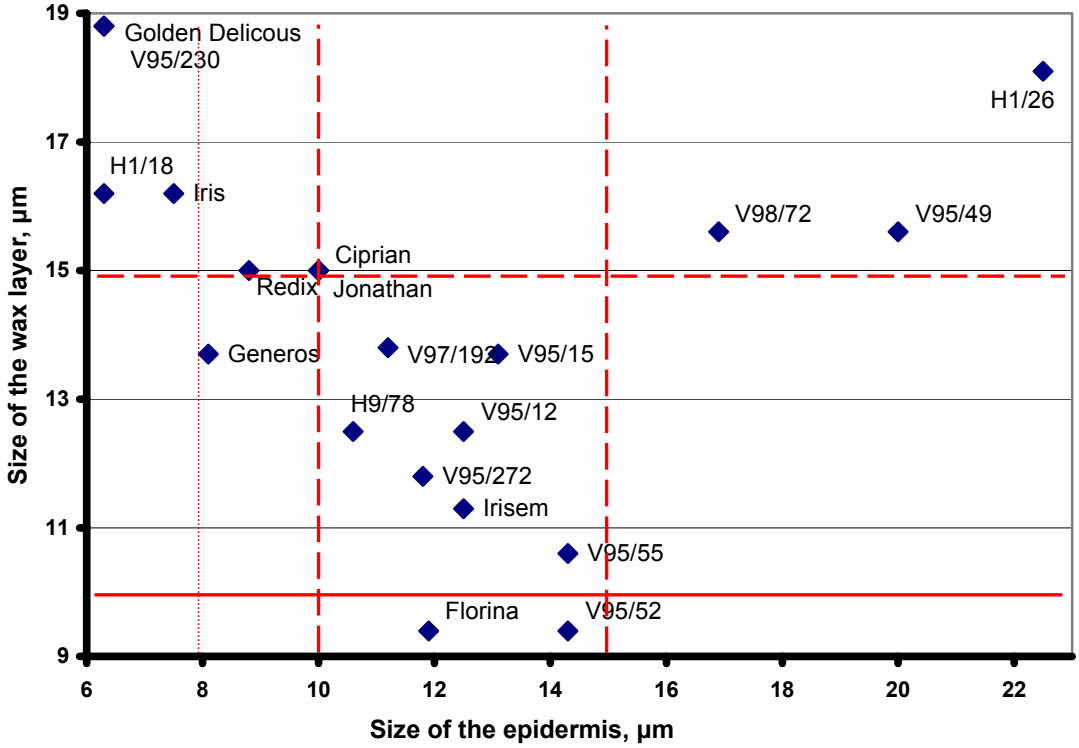

Fig. 2. Size of the apple fruit epidermis and wax layer for 20 apple tree varieties and hybrids

OTHER FIELDS

Characterization of the Molasses based culture media to obtain single cell protein, in order to optimize the medium composition

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Keywords: Saccharomyces, bacteriological evaluation, chemical-physical evaluation, fermentation, optimization

ABSTRACT

Molasses from sugar beet represents one of the main raw materials used for obtaining single cell protein biomass. Utilizing molasses as raw material is very convenient mainly from the economic approach (regarding the low costs and high accessibility).

Molasses were studied from a microbiological and physical-chemical point of view.

This paper presents the results from analytic tests performed to optimize the culture media used to grow selected yeasts, regarding the optimal composition of microelements. The selected yeasts belong to the Saccharomyces species and are part from the collection of microorganisms belonging to the fermentative technology lab, which is part of the Food Research Institute from Bucharest.

The researches are focused on studies regarding multiplication of yeasts on optimized growth media, in order to obtain single cell protein meant for nutrition.

INTRODUCTION

By-product resulted from sugar beet; molasses is the final syrup obtained trough the centrifugation of sugar crystals. From molasses there is no economical way to reform sugar trough crystallization. The sugar embedded in molasses represents a substantial lost, which is around 2% from the sugar from the processed sugar beet. Molasses have a 75-80 Brix degree concentration and contains approximately 48-50% saccharose, having a purity of approximately 69%.

The production of microbial protein is known in the specific literature under the term of single cell protein (SCP), term which shows that the protein has microbial origins, being obtained from single cell microorganisms (bacteria, yeasts, algae etc.).

The utilization of microorganisms, in our case yeasts from the Saccharomyces species, for obtaining SCP has some advantages, such as:

- the large content of microbial protein with similar profile to the amino acids contained in animal and vegetal proteins;
- trough selection we can obtain microbial yeasts with adequate properties to our goal;
- large development and production of biomass;
- micro-organisms can use as carbon source, un unlimited range of waste raw materials from other industries.

MATERIALS AND METHODS

The molasses used in experiments it is obtained from sugar beet, and it is being used to produce baking yeasts and for obtaining ethylic alcohol.

The general scheme for obtaining SCP using molasses is:

- Microorganism selection (high productivity and resistance);
- Selecting growth media (accessibility and effectiveness);

- Elaborating growth program (biosynthetic potential of the microorganism on the selected media);
- Developing and obtaining product, trough separation, concentration and purification (Zarnea, 1970).

During the experiments for obtaining SCP using molasses as growth media, and microorganisms from the Saccharomyces species, the following technological stages were used:

- Inoculum's preparation;
- Sterilization and preparation of the growth media for the selected microorganism;
- Actual fermentation;
- Separation and purification of the obtained biomass;
- Processing biomass in order to be used for food or feeding.

The used inoculums for multiplication start-up of the selected microorganism is prepared in a vessel (seeding vessel), from where it is passed in pilot plant equipped with stirring, heating and cooling devices. The nature, composition and work conditions of the growth media are related to the utilized microorganism.

To use them as growth media for SCP biomass, molasses were studied from a chemical, physical and microbiological point of view.

The physical and chemical evaluation of the molasses were made according to STAS 12871/90 – "Molasses", as well as with the specific methods used in fermentation technologies, owned by our laboratory (The 17th Edition of AOAC International; SR ISO 4833-2003; STAS 12871 / 90).

The objective of the microbiological exam, in raw molasses, was to establish the presence of harmful bacteria, in order to establish the subsequent treatment for sterilization, before beginning the fermentation (Begea et al., 2007).

The microbiological exam was according to SR ISO 4833-2003 – "General rules for establishing the number of microorganisms". "The method for counting colonies obtained at 30^0C" and SR 11499-2:1996 – "Sugar. Determination for microorganisms from Leuconostoc species".

RESULTS AND DISCUSSIONS

A. Physical and chemical studies

The determined values of the chemical and physical parameters will be the departure point, in order to establish and verify an analysis system for molasses at a pilot plant level, in order to optimize the growth media based on molasses used to obtain SCP.

The parameters taken into account for the used molasses used in the experiments are:

1. Determination of soluble substances in molasses
2. pH determination
3. Chemical determination of total sugars, trough Luff - Schoorl method
4. Direct reducing sugar determination
5. Calcium oxide determination
6. Colloid determination
7. Total sulphur dioxide determination
8. Nitrogen compounds determination
   - 8.1 Total nitrogen determination
   - 8.2 Assimilable nitrogen determination

The results for the physical and chemical analysis are detailed in table 1.

The chemical composition of the molasses is detailed in table 2.

B. Bacteriological studies and characterization

The bacteriological study has included the determination of the total mesophilic aerobe microorganisms (TPC) and bacteria from the Leuconostoc species.

The used growth media were:

- For determining the total mesophilic aerobe microorganisms (NTG) – Plate Count Agar (PCA) media
- For determining bacteria from the Leuconostoc species:
  - proteose-peptone enrichment medium;
  - selective media agar-sucrose;
  - biochemical confirmation media (media for the Voges-Proskauer reaction).

Based on performed analysis, for all molasses samples the Leuconostoc tests were negative.

The results of the bacteriological analysis (TPC value) for the molasses samples are presented in table 3.

Depending on the values of the analyzed parameters of the molasses, the technological diagrams will be settled and also the accordingly composition of the culture medium, especially as regards the addition of the growing factors (vitamins, nutrients, chemicals as traces).

CONCLUSIONS

For performed experiments within the current studies, we have reached to the following conclusions:

1. molasses characterization methods – raw material proposed for producing SCP biomass, are in accordance with the international standards and methods;
2. obtaining a data base regarding the raw materials category and their quality parameters will allow the developing of a tracking method for the evolution of certain parameters till obtaining the final product or sub products resulted from the basic technological process, as well as establishing optimum technologies , with maximum efficiency;
3. the registered values for the analyzed parameters, corresponding with the raw material taken into count, are within the limits specified by the technical and technological process, as well as with the existing legislation;
4. it's becoming more and more interesting the obtained results comparison of some raw materials with different quality characteristics used to obtain SCP and establishing the influence of the raw material as well, on the technological process itself.

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Tables

Table 1. Physical and chemical analysis results for the molasses samples

Parameter	Molasses samples									
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**
Soluble substances (%)	79	83.7	81.4	82.5	79.2	84.1	80.4	81.6	83.2	85.6
Total sugar (%)	49.16	51.45	53.17	50.31	48.62	55.51	52.39	50.47	49.93	50.68
Sucrose (%)	47.8	46.92	48.12	46.15	45.2	54.2	51.26	48.83	47.62	46.57
Direct reducing sugar (%)	1.18	0.96	1.04	0.73	0.60	1.36	1.27	1.12	1.06	0.81
Ash (%)	10.37	10.27	11.32	12.40	10.61	14.49	10.74	12.83	11.52	14.06
Total Nitrogen (%)	1.85	1.63	1.51	1.96	1.28	2.04	1.43	1.79	1.95	2.01
Assimilable nitrogen (%)	0.35	0.39	0.41	0.50	0.31	0.64	0.36	0.45	0.49	0.51
pH	7.5	7.2	7.0	8.2	6.9	8.8	8.4	7.7	7.5	8.0
Sulphur dioxide (%)	0.082	0.056	0.048	0.051	0.038	0.064	0.046	0.072	0.060	0.058
Colloidal substances (%)	0.07	0.09	0.10	0.07	0.06	0.11	0.06	0.07	0.09	0.09

Table 2. Chemical composition of molasses

Parameter	Average value
Soluble substances (%)	82.07
Total sugar (%)	51.17
Sucrose (%)	48.27
Direct reducing sugar(%)	1.013
Ash (%)	11.86
Total Nitrogen (%)	1.75
Assimilable nitrogen (%)	0.44
pH	7.7
Sulphur dioxide (%)	0.058
Colloidal substances (%)	0.08

Table 3. Bacteriological parameters of molasses

Sample no.	NTG ufc/g
M1	$1.8x10^4$
M2	$1.2x10^3$
M3	$7.8x10^3$
M4	$2.5x10^3$
M5	$9.6x10^3$
M6	$5.2x10^4$
M7	$4.3x10^4$
M8	$5.3x10^3$
M9	$3.1x10^4$
M10	$2.8x10^3$

AFLP markers as a powerful tool for fingerprinting and breeding *Tulipa* genus

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Keywords: *Tulipa* genus, AFLP genetic marker, DNA fingerprinting, *Li-cor* PCR, preamplification, selective nucleotides

ABSTRACT

The amplified fragment length polymorphism (AFLP) technique is one of a number of DNA fingerprinting procedures that takes advantage of the polymerase chain reaction (PCR) to amplify a limited set of DNA fragments from a specific DNA sample (Vos *et al.* 1995; Blears *et al* 1998). Typically the choice of which fingerprinting technique to use depends on 1) the application (e.g. DNA genotyping, genetic mapping, population genetics); 2) the organism under investigation (e.g., prokaryotes, plants, animals, humans); and 3) the resources (time and money) available. In most cases no one fingerprinting technique is ideal for all applications. However, AFLP's are quickly becoming the tool of choice for many applications and organisms. Potential applications include screening DNA markers linked to genetic traits, parentage analysis, forensic genotyping, diagnostic markers for pathogen borne diseases, and population genetics. Since the AFLP technique can be applied to a wide variety of organisms (and viral sources) with no prior sequence information this technique has the potential to become a universal DNA fingerprinting tool.

INTRODUCTION

The AFLP technique is a method, by which a selection of restriction fragments of a total genomic digest is detected by amplification using PCR. It is a very versatile method, able to detect the presence of restriction fragments in almost any DNA, regardless of its complexity. The technique allows efficient identification of DNA polymorphism's, because large numbers of restriction fragments may be detected simultaneously.

AFLP combines the advantages of RFLP and PCR to provide greatly enhanced performance in terms of reproducibility, resolution, time efficiency, and polymorphism detection at the whole-genome level (Scottdl et al., 1998), and no prior DNA sequence information is required, rendering it a particularly useful discovery tool.

The principle of the AFLP technique is basically quite simple. DNA is cut with restriction enzymes, and double stranded DNA adapters are ligated to the ends of the DNA fragments. In this way, a universal primer-binding site is created based on the sequence of the adapters and the adjacent recognition site of the restriction enzyme. This primer-binding site is used for subsequent PCR amplification of the restriction fragments. Extra nucleotides can be added to the 3' ends of the PCR primers. These extended primers will only then anneal properly to the genomic fragments, and allow PCR amplification, if the genomic nucleotide sequence (adjacent to the restriction recognition site) is matching the extra nucleotides. Consequently, core-primers with additional selective nucleotides will selectively amplify only a subset of the fragments.
Two restriction enzymes, a rare cutter and a frequent cutter generate the restriction fragments for amplification. The AFLP procedure results predominantly in amplification of those restriction fragments, which have a rare cutter sequence on one

end and a frequent cutter sequence on the other end. The rationale for using two restriction enzymes is the following:

1. The frequent cutter will generate small DNA fragments, which will amplify well and are in the optimal size range for separation on denaturing gels (sequencing gels).
2. Using the rare cutter reduces the number of amplified fragments, since only the rare cutter/frequent cutter fragments are amplified. This limits the number of selective nucleotides needed for the AFLP reaction. Most plant genomes can be analysed with E+3/M+3. However, to reduce the complexity of template from species with extremely large genomes, the adding of extra selective nucleotides could be replaced by using an eight-cutter such as *Sse*83871. Choosing other rare cutting enzymes, such as *Pst*I allows the targeting of other genomic regions, different in CG-content or methylation.
3. The use of two restriction enzymes makes it possible to label only one strand of the ds stranded PCR products, which prevents the occurrence of "doublets" on the denaturing gels due to unequal mobility of the two strands of the amplified fragments.
4. Using two different restriction enzymes gives the greatest flexibility in "tuning" the amount of fragments to be amplified. Apart from these technical considerations, the rare cutter sites may serve as landmarks in physical mapping studies.

The AFLP procedure consists of three main parts: template preparation, fragment amplification and gel analysis. An essential aspect is the quality of the DNA used for AFLP analysis. It is important that the DNA gives no partial restriction. When two DNA's are compared, which differ in completeness of restriction, polymorphic bands are likely to be detected not based on true DNA polymorphism.

Detection of AFLP fragments using infra-red dye detection technology offers several advantages over conventional detection using radiolabeled primers and autoradiography. The use of radioactivity is eliminated. The cost of infrared dye-labeled primers is less than the cost of corresponding amounts of radionucleotides for radiolabeling primers. Images are obtained in several hours rather than 2-4 days. And, finally, digital gel images can be scored more quickly with the use of specialized software, avoiding multiple data-entry steps in scoring markers and preparing data for use in mapping and phylogenetic analysis programs.

Digital images from the LI-COR sequencer are similar in appearance to the autoradiographs or phosphoimages produced with the conventional radiolabeling/standard sequencing gel protocol. A greater size range can generally be visualized and resolved with LI-COR automated sequencers, and the distance between bands 1 bp apart in size is much more nearly constant throughout the length of the image.

The procedures for conventional and automated AFLP analysis are virtually identical through the entire restriction, ligation and pre-amplification procedures. In the final (selective) amplification, IRD-labeled *Eco*RI adapter primers are substituted for the radiolabeled primers. The selective PCR profile is also modified slightly to increase the relative intensity of larger fragments. Quantities and concentrations of all reagents are basically the same as in the original protocol (Vos *et al.,* 1995).

Vos et al. (1995) tested the AFLP technique in middle-large human genomes and indicated that the AFLP technique could allow accurate amplification of subsets of restriction fragments, even in complex template mixtures generated from plant species

with very large genomes, just by increasing the number of selective nucleotides added to the core primers.

An example of a plant species with a very large genome is *Tulipa*. The genome of tulip is 5000x bigger as the genome of arabidopsis (120.000 Kb) and consists of 12 chromosomes. Little information about genes and their position on the chromosomes is available.

Tulips are very heterogeneous because they are cross-pollinated. Besides that, the period from seedling to flower producing bulb takes a long time, which makes research difficult.

MATERIAL AND METHODS

The AFLP method was performed as described by Vos *et al*. (1995) with some minor modifications. Hybriden Apeldoorn , 76261-24 Cantate x Verdi, Praestans x Albertii (030121), Kess Nelis x Cantate 9, Kess Nelis x Cantate 64, 89191 KN x Cantate and cultivars Ile de France, Christmas Marvel, Leen vd Mark, Bellona, Kees Nelis, Ballerina , Spring Green , Queen of Night were used to aquire general AFLP fingerprints with a wide genotypic background.

RESULTS AND DISCUSSION

The results indicated that obtain reproductible AFLP patterns, clear and labor saving fingerprints when seven selective nucleotides were used. We used en efficient enzyme primer combination M52G/E45 IRD 700(M+4/E+3).

In tulip, a plant with a very big genome it needs a lot of refinement of the non-radioactive procedure to get the required clear pictures.

AFLP markers can be applied for species with large genome as long as the preamplification step and the final selective nucleotides are well defined by the users.

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Quality assurance in education

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INTRODUCTION

Much has been debated about the management of learning, the quality of material provided for learning, the assessment of learning and the procedures in place to ensure the student learning experience is being continually reviewed with the object of ensuring the graduating young person is suitably qualified to meet the exacting requirements for the world of work.

Western Europe has systems in place for managing the learning environment; learning establishments within the United Kingdom maintain a Self Assessment Policy, which is regulated by the Education Funding Bodies.

A self assessment strategy requires a model for basis; the object of this presentation is to provide a potential structure for consideration, and carry the concept of self assessment and customer satisfaction further.

Customer satisfaction, student learning and continual improvement are integral to the concept of a Quality Learning Environment. The concept for the term Quality Assurance may be stated as: “fit for purpose”. We as Educational Practitioners have the responsibility for ensuring our graduates meet or preferably exceed the minimum requirements for successful achievement in the working environment.

This presentation suggests a suitable model, which can be adapted to individual organisational needs.

Reasons for seeking accreditation in education

- National and International recognition of a quality organisation.
- Major companies and businesses may only deal with accredited companies.
- A business which has accreditation is seen as providing a product which meets the needs of its customers

What is the Purpose of Quality Assurance?

- Accreditation proves the organisation has structure.
- Quality assurance can be used as a strategic management tool to manage an organisation or bring about change.
- The adoption of a quality management system should be a strategic decision made by senior management.
- Quality assurance developed in the manufacturing sector.
- A manufacturing company will make/produce a product.
- The product is sold to the customer.
- The product and the customer are easily identified.
- The Organisation is audited by an external body on an annual basis.

The Education Sector

- Education is not a manufacturing business.
- Education falls into the service sector.
- The challenge of applying a manufacturing standard to a service industry
- Need to interpret manufacturing terms if applying to education.

- Is the product the "student"?
- Is the product the course of study?

Customer and Product

- At this point if we consider the product as the student.
- The customer is the industry into which the student will work.
- We can now apply the concept of Quality Assurance.

Which Quality Assurance Model to Use?

- The work carried out in an educational institute is very procedural.
- EN ISO 9000:2000 is an internationally accepted and recognised quality standard, which uses procedures.
- Many service sector industries, in Europe, are accredited with ISO 9000.
- Adapting this manufacturing standard for the service sector has been accomplished

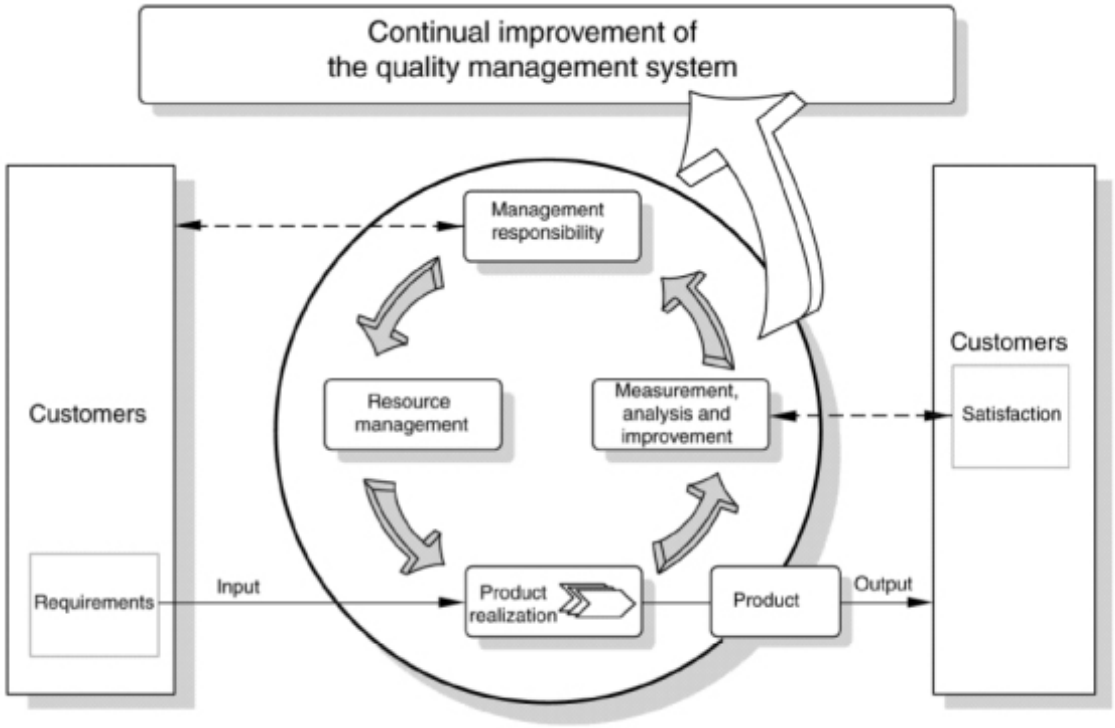

Fig taken from BSi ISO 9000/2000

Achieving Accreditation

- An enormous task; lucky to achieve accreditation within 12 months.
- Recommend the services of a consultant, or someone with expertise in gaining accreditation.
- Set up a dedicated team.
- Set up a framework for addressing the requirements of the quality standard
- Suggest developing a Gantt Chart.
- Decide which areas are to be accredited.
- Suggest running a pilot first (one department)

General requirements:

- Identify the processes needed for the quality management system and their application throughout the organisation.
- Determine the sequence and interaction of these processes
- Determine criteria and methods needed to ensure that both the operation and control of these processes are effective
- Ensure the availability of resources and information necessary to support the operation and monitoring of these processes
- Monitor, measure and analyse these processes
- Implement actions necessary to achieve planned results and continual improvement of these processes

Documentation requirements:

- Documented statements of a quality policy and quality objectives
- Quality Manual
- Documented procedures required by the International Standard
- Documents needed by the organisation to ensure the effective planning, operation and control of its processes
- Controlled records
- The main documents are contained within the Quality Manual, which describes how each requirement of the Quality Standard is to be addressed; how documentation is to be controlled and how records are to be controlled.

Management Responsibility

Management must be committed to:-

- communicating to the organisation the importance of meeting customer as well as statutory and regulatory requirements
- establishing the quality policy
- ensuring that quality objectives are established
- conducting management reviews
- ensuring the availability of resources

Customer focus

Top/Senior Management shall ensure that customer requirements are determined and are met with the aim of enhancing customer satisfaction

Quality policy

- Must be appropriate to the purpose of the organisation
- Includes a commitment to comply with requirements and continually improve the effectiveness of the quality management system
- Provides a framework for establishing and reviewing quality objectives
- Communicated and understood within the organisation
- Reviewed for continuing suitability

Planning

Quality objective:

Top/Senior Management shall ensure that quality objectives, including those needed to meet requirements for the product are established at relevant functions and levels within the organisation. The quality objectives shall be measurable and consistent with the quality policy

Quality management system planning

Top/Senior Management shall ensure that:

- the planning of the quality management system is carried out in order to meet the requirements outlined in the Quality manual
- the integrity of the quality management system is maintained when changes to the quality management system are planned and implemented

Responsibility, authority and communication

Top/Senior Management shall ensure that responsibilities and authorities are defined and communicated within the organisation

Management representative

Top/Senior Management shall appoint a member of management who, irrespective of other responsibilities, shall have responsibility and authority that includes:

- ensuring that processes needed for the quality management system are established, implemented and maintained
- reporting to senior management on the performance of the quality management system and any need for improvement
- ensuring the promotion of awareness of customer requirements throughout the organisation

Internal communication

Top/Senior Management shall ensure that appropriate communication processes are established within the organisation and that communication takes place regarding the effectiveness of the quality management system

Management review

Top/Senior Management shall review the organisations quality management system, at planned intervals, to ensure its continuing suitability and effectiveness. This review shall include assessing opportunities for improvement and the need for changes to the quality management system, including the quality policy and quality objectives. Records from management reviews shall be maintained.

Review input

The input to management review shall include:

- results of audits
- customer feedback
- process performance and product conformity
- status of preventative and corrective actions
- follow-up actions from previous management reviews

- changes that could affect the quality management system
- recommendations for improvement
- review output: the output from the management review shall include any decisions and actions related to:-
- improvement of the effectiveness of the quality management system and its processes
- improvement of the product related to customer requirements
- resource needs

Resource Management

The organisation shall determine and provide the resources needed:-

- To implement and maintain the quality management system and continually improve its effectiveness
- To enhance customer satisfaction by meeting customer requirements

Human resources

Personnel performing work affecting product quality shall be competent on the basis of appropriate education, training, skills and experience

Competence, awareness and training

The organisation shall:-

- determine the necessary competence for personnel performing work affecting product quality
- provide training or take other actions to satisfy these needs
- evaluate the effectiveness of the actions taken
- ensure that its personnel are aware of the relevance and importance of their activities and how they contribute to the achievement of the quality objectives and product
- maintain appropriate records of education, training, skills and experience

Infrastructure:

The organisation shall determine, provide and maintain the infrastructure needed to achieve conformity to product requirements. Infrastructure includes, as applicable:-

- Buildings, workspace and associated utilities
- Process equipment (hardware/software)
- Supporting services (transport, communication etc)

Work environment

The organisation shall determine and manage the work environment needed to achieve conformity to product requirements

Performing method for Patulin detection in apple juice, in order to food safety assuring

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Keywords: patulin, apples juice, high performance liquid chromatography

ABSTRACT

In Institute of Food Bioresources it was developed a method for patulin determination from apples juice by high performance liquid chromatography.

Extraction of patulin was made in acetonitrile from sample and purification of obtained extract, using C.U. Patulin columns (MycoSep®228). The obtained solution is evaporated to dryness under nitrogen and re-dissolved. Patulin is, after, separated on chromatographic column C18, 150 x 4 mm, 5 µm (high performance liquid chromatograph Thermo Finnigan), eluted in mobile phase and detected on 276 nm, using an UV-VIS „DIODE ARRAY" detector.

It was achieved an internal study for validation of method for patulin determination from apples juice, by high performance liquid chromatography. In concentration range 10 µg/l – 400 µg/l the average recuperation is 89.64 %. Detection limit (LOD) is 3.71 µg/l, and quantification limit (LOQ) is 7.42 µg/l.

INTRODUCTION

Patulin is a mycotoxin produced by moulds *Penicillium patulum, Penicillium expansum, Penicillium urticae, Penicillium claviforme, Aspergillus clavatus* and

Byssochlamys spp., with toxically effects on human and animal body. Patulin was identified, especially, in spontaneous damaged fruits and vegetables by micotoxigen fungus: apples, apricots, bananas, pineapples, grapes, black currants, raspberry, wild strawberries, cucumbers, tomatoes, green peppers, carrots (Pogosian J., 1983; Teresa Lipowaska, 1984). Patulin is stable, especially in processed products from apples: apples juice, apples concentrate juice, apples nectar, fruits nectar (which has as ingredient the apples juice also), apples piuree, apples in syrup, etc.

From chemical point of view, *patulin* is furo-piranone, with molecular mass 154, stable in acid medium, but unstable in alkaline medium.

It is presented as uncolored crystals form, rhombics or prismatics, with melting point 110.5°C, with absorption maximum at 276 nm, in alcoholic solution.

In Institute of Food Bioresources it was developed a method for patulin determination from apples juice by high performance liquid chromatography.

MATERIALS AND METHODS

For determination of patulin content were analyzed samples of clear apples juice, produced by: Santal, Pfanner, Tymbark, Rauch, Fruttia.

Determination of patulin from apples juice was performed by high performance liquid chromatography. Steps of method for patulin determination: preparation of test sample, patulin extraction, extract purification, extract evaporation to dryness, residue re-dissolvation, HPLC detection of patulin.

RESULTS AND DISCUSSIONS

For patulin determination from apples juice by high performance liquid chromatography, it was realized a calibration curve with 7 patulin standard levels (with each three repetitions), in concentrations range 0.0125 µg/ml – 0.5 µg/ml.

HPLC perform conditions:

- Chromatographic column C18, 150 x 4 mm, 5 μm
- Mobile phase: acetonitrile:water = 5:95 (v/v)
- Mobile phase volume: 1 ml/minute
- UV-VIS „DIODE ARRAY" detector, λ = 276 nm
- Injection volume: 25 μl
- Temperature: 25°C

For linearity domain establishment, of patulin determination method from apples juice, by high performance liquid chromatography, apples juice samples in what was not detected patulin, were "artificial" contaminated with patulin, using patulin standard solution for calibration, with patulin concentration 1 μg/ml.

Thus, they were obtained apples juice samples, with the following patulin concentrations: 10 μg/l, 15 μg/l, 25 μg/l, 50 μg/l, 60 μg/l, 80 μg/l, 100 μg/l, 125 μg/l, 200 μg/l and 400 μg/l. These samples were analyzed for patulin concentration determination.

By graphic representation of patulin concentration in injected sample, function peak area it obtains a right line with ecuation y = 0.0020x, and correlation coefficient (r^2) 0.9997. According to obtained results, linearity domain of patulin determination method from apples juice, by high performance chromatography, is 5 ppb – 218 ppb.

For *recuperation* establishment, in case of patulin determination method from apples juice, by high performance chromatography, apples juice samples in what was not detected patulin, were "artificial" contaminated with patulin, using patulin standard solution for calibration, with patulin concentration 1 μg/ml.

Thus, they were obtained apples juice samples, with the following patulin concentrations: 10 μg/l, 15 μg/l 25 μg/l, 50 μg/l, 125 μg/l, 200 μg/l, 315 μg/l and 400 μg/l. These samples were analyzed for patulin concentration determination.

In concentration range 10 μg/l – 400 μg/l, the average recuperation had the following values:

- 84.20 %, for patulin concentrations < 20 μg/l;
- 90.99 %, in patulin concentrations range 25 - 50 μg/l;
- 87.83 %, in patulin concentrations > 50 μg/l.

In concentration range 10 μg/l – 400 μg/l, the average recuperation is 89.64 %.

For repeatability estimation, they were analyzed parallel apples juice samples with patulin concentration 50 μg/l and 125 μg/l (apples juice in what was not detected patulin, it was „artificial" contaminated with patulin, using patulin standard solution for calibration, with patulin concentration 1 μg/ml) and they were calculated:

- relative standard deviation value RSD(r)**,** for determined patulin concentration;
- extended uncertainty;
- limit repeatability.

In case of 10 parallel samples of apples juice with patulin concentration 50 μg/l (apples juice in what was not detected patulin, it was "artificial" contaminated with patulin, using patulin standard solution for calibration, with patulin concentration 1 μg/ml), relative standard deviation RSD(r), for determined concentration is 2.66 %, and extended uncertainty is 7.80 μg/l. Confidence interval is 49.77 μg/l ± 7.80 μg/l. Limit repeatability is: 3.71 μg/l.

In case of 8 parallel samples of apples juice with patulin concentration 125 μg/l (apples juice in what was not detected patulin, it was "artificial" contaminated with patulin, using patulin standard solution for calibration, with patulin concentration 1

µg/ml), relative standard deviation RSD(r), for determined concentration is 4.89 %, and extended uncertainty is 18.91 µg/l. Confidence interval is 124.83 µg/l ± 18.91µg/l. Limit for repeatability is: 17.07 µg/l.

For intralaboratory reproducibility estimation, they were performed by three analysts, 8 apples juice samples (analyst A – 3 samples, analyst B – 2 samples, analyst C – 3 samples) with patulin concentration 50 µg/l (apples juice in what was not detected patulin, it was „artificial" contaminated with patulin, using patulin standard solution for calibration, with patulin concentration 1 µg/ml) and they were calculated:

- relative standard deviation value RSD(R), for determined patulin concentration;
- extended uncertainty;
- limit reproducibility.

In case of 8 samples of apples juice with patulin concentration 50 µg/l, analyzed by three analysts (analyst A – 3 samples, analyst B – 2 samples, analyst C – 3 samples), in the same laboratory, with the same equipment relative standard deviation RSD(R), for determined concentration is 4.75 and extended uncertainty is 9.10 µg/l.
Confidence interval is 49.77µg/l ± 9.10 µg/l. Limit reproducibility is: 6.62 µg/l.

Using *"signal – to-noise ratio"* canned by high performance liquid chromatograph, in case of patulin determination, as for patulin standard solutions, as for apples juice, they were calculated detection limit and quatification limit.

In case of proposed method for patulin determination by high performance liquid chromatography, detection limit (LOD) is 3.71 µg/l, and quantification limit (LOQ) is 7.42 µg/l.

Proposed method for patulin determination by high performance liquid chromatography is selective. The *patulin* adequate peak is separated by baseline and by other compounds peaks. Resolution (DAB) is min. 2.7.

CONCLUSIONS

In Institute of Food Bioresources it was developed a method for patulin determination from apples juice by high performance liquid chromatography.

Patulin is, after, separated on chromatographic column C18, 150 x 4 mm, 5 µm (high performance liquid chromatograph Thermo Finnigan), eluted in mobile phase and detected on 276 nm, using an UV-VIS „DIODE ARRAY" detector.

It was achieved an internal study for validation of method for patulin determination from apples juice, by high performance liquid chromatography.

In concentration range 10 µg/l – 400 µg/l the average recuperation is 89.64 %. Detection limit (LOD) is 3.71 µg/l, and quantification limit (LOQ) is 7.42 µg/l.

The *patulin* adequate peak is separated by baseline and by other compounds peaks. Resolution (DAB) is min. 2.7.

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Figures

Fig. 1. C.U. Patulin columns

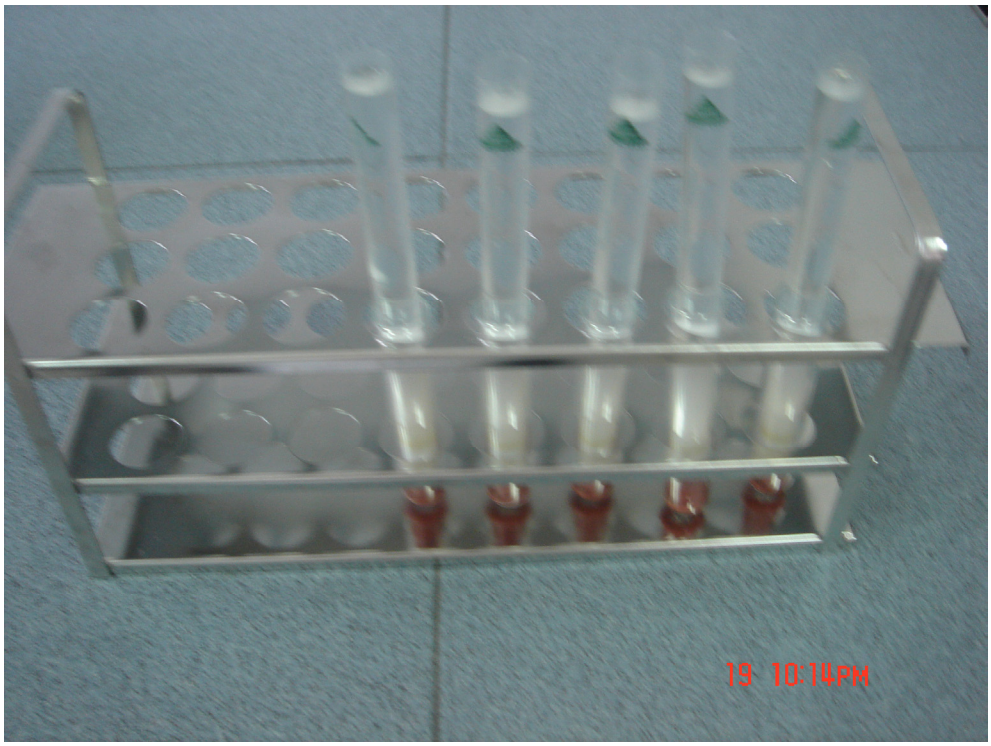

Fig. 2. Purified patulin extract

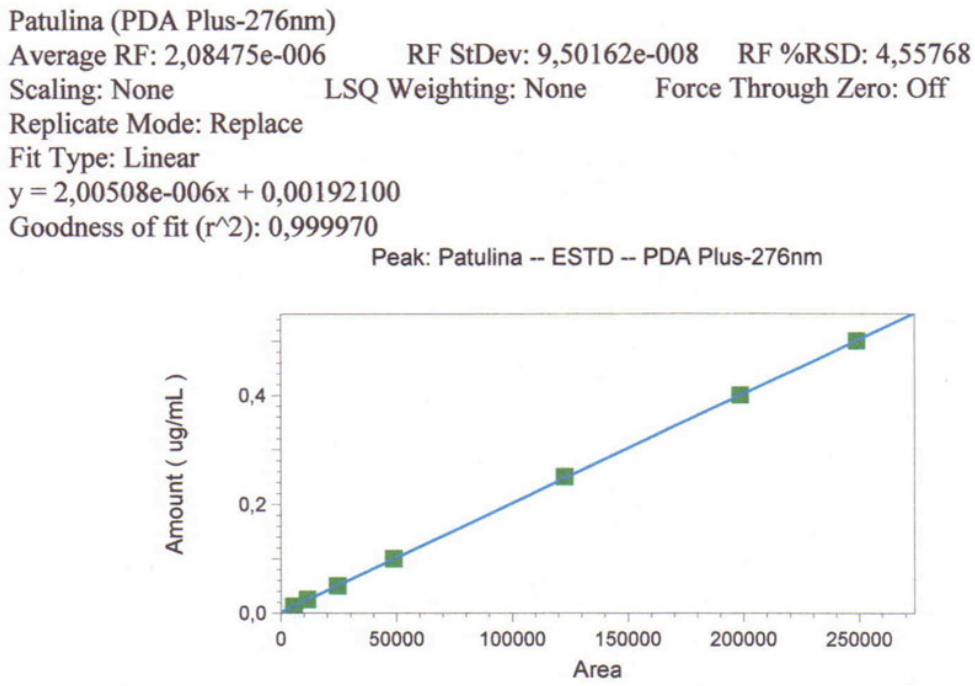

Fig. 3. Calibration curve

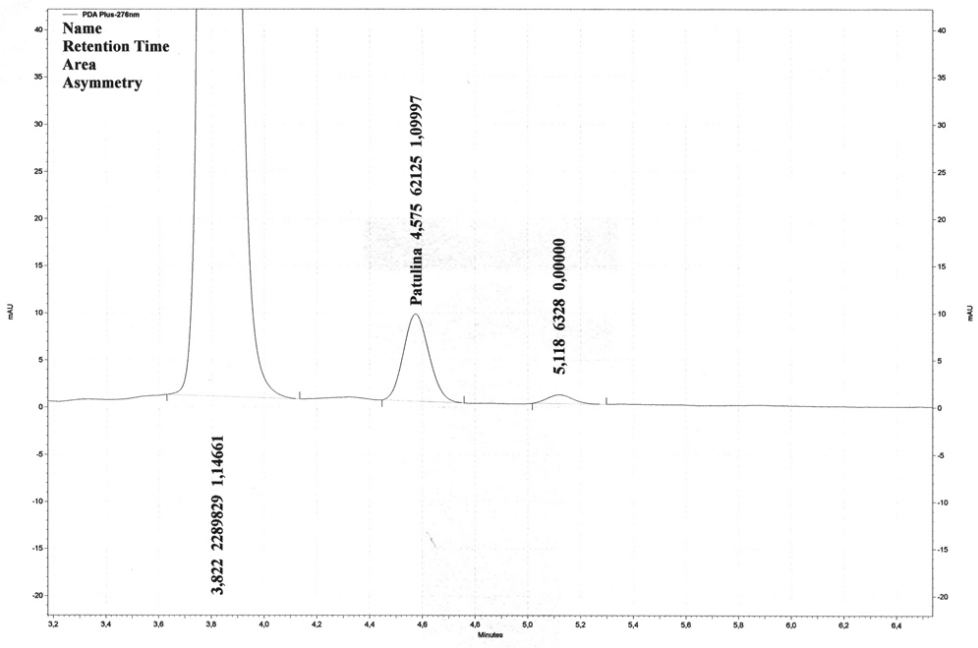

Fig. 4. Chromatogram with patulin peak

Phenological aspects of natural populations of *Helix pomatia* and *Helix lucorum* (Gastropoda-pulmonata-helicidae) in Romania

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Keywords: phenology, temperature transects, first deposition of eggs

ABSTRACT

The specific spreading area of *Helix pomantia* and *Helix lucorum* species is different in Romania; the first species is limited to the area within the Carpathians and to the West of the country, while the second species is limited to Moldavia, to the Outer Eastern Carpathians as well as to the South of Romania. Because of the mild climate in the West, the first deposition of eggs for *Helix Pomatia* species usually occurs two weeks earlier as compared to regions in Transylvania, within the Carpathians chains. In May, 2004, the air temperature throughout the country was higher than normal for this period of the year, both inside and outside the Carpathians chain, reaching 30 degrees, which influenced the first deposition of eggs for the two species, in the sense of its outrunning. However, the proper period of time fit the first deposition of eggs was maintained up to ten days for the species in the West, where there is a mild climate and those in Transylvania, where there is a harsh climate.

INTRODUCTION

Helix pomantia and *Helix lucorum* are two species of edible snails which, in Romania, are gathered and made profitable. To know their phenology is important both from an economic point of view and to establish some protection measures, taking into account that *Helix pomatia* is a protected species, according to the Berne Convention.

Temperature stands for an important ecological factor in starting and unfolding the phenological processes of natural populations. That is why, in 2004, researches followed the air temperature dynamics in different geographical areas and linked to the moment of eggs deposition on different transects, directed according to the temperature gradient.

While approaching this project, it was supposed that the term of the first deposition of eggs for the individuals of *Helix pomatia* was different, depending on general climate of the region; there were established transects whose temperature gradients were directed from the Mediterranean climate and warm areas in the South-West – West of Romania to the cold ones in Transylvania.

MATERIALS AND METHODS

Different observers have followed the temperatures in nearly the same periods, in all the areas of the country. They have also followed and put down the term of the first deposition of eggs as well as the number of eggs that were laid. A few observations were made in 21 counties throughout the country and in 50 places within these counties. The transects established were as follows:

- The first transect: Arad – Timis – Hunedoara – Alba – Sibiu – Brasov;
- The second transect: Caras-Severin – Gorj – Valcea - Arges – Prahova – Braila – Tulcea;
- The third transect: Giurgiu – Ialomita – Arges – Sibiu – Mures – Bistrita Nasaud.

The establishing of these transects was made according to the temperature gradients from areas with milder climate to areas with harsher climate (transects 1 and 3) and from areas with milder Mediterranean climate to areas with hot climate (transect 2).

RESULTS AND DISCUSSIONS

The analysis of the first chart and that of Romania Map draws into relief the fact that the deposition of eggs on all the surfaces of the first transect was accomplished early in June, between 1st and 17th of June. In the West Plain, known for its mild climate, relatively high temperatures and humidity in springtime, the first deposition of eggs occurred in one of the most advanced periods (3rd of June).

The type of habitat in which the observations were made is an important element as regards the first deposition of eggs. In Arad, the observations were made at the brink of a river where the best conditions for the activity of the snails were.

In Buteni, the situation is different. This is a colder area placed at the skirt of the forest, with low temperature of the soil, which caused the delay of the first deposition of eggs (14th of June). The last deposition was recorded on the 2nd of July, 20 days later as compared to the term of the latest deposition which was recorded in Arad County.

In Timis County, on grassland at Voiteg, the observers recorded the earliest deposition of eggs on the 1st of June while the last deposition was recorded on the 8th of June. It was thus confirmed the observation according to which the first deposition of *Helix pomatia* occurs in the West of the country, when spring sets in earlier than usual, as compared to the other areas of gathering in the country.

This observation is important as regards the setting up of some protection strategies of the species. The favorable condition in this area causes not only the early depositions of eggs, but also a more rapid maturity process of the individuals.

As long as Romania allows the gathering of snails belonging to this species, in order to make them profitable, the acquaintance with the moment of their maturity (the shell diameter is 28-30 mm) may be an indicator for the beginning of their gathering.

Thus, inside the Carpathians chain, in different areas of Transilvania Plain, the moment of the first deposition was delayed up to ten days; the first deposition of eggs in Brad and Simeria was recorded on the 11th -12th of June, while the last deposition occured on the 1st or 2nd of July. On all the surfaces of this transect where the observations were made, the first deposition of eggs in Alba, Sibiu and Brasov occurred in the second half of June, and the last one occurred early in July.

The habitats specific to these areas are favorable for *Helix pomatia* species, in some of them (Sibiu) people gathering the largest quantities of snails. But, because of the harsher climate than in West Plain These, the activity of snails and the first deposition of eggs are delayed up to 10-12 days.

The second transect, directed to the West –East, southwards the first transect covers Caras-Severin – Gorj – Arges – Prahova – Braila – Tulcea. This transect records the same trend similar to the other regions with the observation that the first deposition of eggs occurred in the latter half of June, till early in July.

The 3rd transect covering Giurgiu – Ialomita – Arges – Sibiu – Mures – Bistrita Nasaud, directed to the South – North has drawn up one of the most important conclusions of the analysis which refers to the temperature gradients.

One can notice from the data in the first chart that the first deposition of eggs on the surfaces phased on this route occurred at mid June while the last one was recorded early in July. We can conclude that the beginning of the snail's activity and the first deposition of eggs, after getting out of winter, took place according to the temperature gradients, directed to the West –East.

In Romania, the gathering of snails for economic purposes is still being practiced though this activity should certainly be stopped in the near future. In this

context, the cease of gathering of snails will be done either for the whole country or there will be established areas in order to alternate longer or shorter periods for the gathering of snails. Authorities should also establish certain periods of prohibition for some regions of the country. Following this observation, according to which snails begin their activity earlier in the West, as compared to other regions, one should establish different terms of gathering for each area.

CONCLUSIONS

As regards the edible snail's populations belonging to *Helix pomatia* and *Helix lucorum*, the observations recorded in 2004 outlined several conclusions:

1. The individuals of *Helix pomatia* species deposit their eggs at different terms in June, depending on the geographical areas, habitats and temperature dynamics.
2. In the West of Romania characterized by a wetter climate, high temperatures (due to the Mediterranean influence), the first eggs were laid early in June, while the last deposition was recorded nearly 10 days later.
3. On the transect whose temperature gradient had decreasing values from East to West, the first eggs (in the West habitats) were laid early in June, while in the other habitats, the deposition of eggs occurred in the latter half of June and early in July.
4. In May, 2004, temperatures throughout the country were higher than usual which caused the early activity of the snails and the outrunning of the first deposition of eggs.
5. The spreading area of *Helix lucorum* species lies outside the Carpathians, in the South – East and the depositions of eggs occurred in the latter half of June and at the beginning of July.

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Table 1. Temperature dinamics and eggs deposition of species *Helix pomatia* and *H. Lucorum*

DISTRICT Locality	Date of observation	air t°	First deposition	Last deposition	Maximum number of eggs	Minimum number of eggs
SIBIU						
Agnita	20 May	26	13 June	17 June	71	65
Birghiş	25 May	28	13 June	17 June	69	60
BRAŞOV						
Făgăraş	28 May	25	10 June	15 June	68	65
Făgăraş	16 June	13	19 June	3 June	75	62
Măieruş	27 May	27	11 June	15 June	71	68
Prejmer	14 June	16	19 June	2 iulie	80	65
TIMIŞ						
Voiteg	27 May	30	1 June	8 June	70	65
Ciacova	28 May	30	9 June	14 June	68	63
HARGHITA						
Secuieni	15 May	22	11 June	14 June	72	68
Avrameşti	18 May	24	15 June	20 June	73	65
Simeria	25 June	19	11 June	1 July	74	63
ARAD						
Arad	20 May	30	3 June	10 June	68	63
Sântana	20 May	30	5 June	10 June	71	66
Buteni	25 June	17,5	14 June	2 July	74	63
Lipova	10 April	19	10 June	15 June	62	40
MUREŞ						
Reghin	15 May	24	15 June	22 June	70	68
Batoş	18 May	25	12 June	16 June	72	67
Panet	20 May	28	11 June	16 June	71	69
Nazna	22 May	25	13 June	15 June	68	59
ALBA						
Alba Iulia	19 June	18	10 June	1 July	75	60
Aiud	19 June	18	10 June	1 July	80	66
Jidvei	5 May	23	2 June	15 June	75	51
ARGEŞ						
Stâlpeni	19 June	19	14 June	2 July	83	65
Clucereasa	24 June	17,5	14 June	2 July	78	66
Călineşti	25 April	20	14 June	29 June	75	55
BACĂU						
Târgu Ocna	25 June	18	16 June	3 July	75	62
Ungureni	19 June	15,5	16 June	3 July	72	65
BIHOR						
Marghita	24 June	17,5	13 June	2 July	70	67
Remetea	19 June	18	13 June	2 July	72	66
BISTRIŢA						
Livezile	25 June	17,5	16 June	3 July	74	63
Teaca	25 June	18	15 June	3 July	75	61
Văile Tecii	16 May	22	15 June	28 June	75	55
BUZĂU						
Suteşti*	25 June	18	16 June	3 July	85	65
CĂLĂRAŞI						
Lehliu*	19 June	17,5	9 June	1 July	78	66

DISTRICT **Locality**	**Date of observation**	**air t°**	**First deposition**	**Last deposition**	**Maximum number of eggs**	**Minimum number of eggs**
CARAŞ-SEVERIN						
Anina	25 June	18	15 June	3 July	83	60
Bocşa Română	25 June	19	14 June	2 July	83	66
Tigvaniu Mare	21 April	19	10 June	22 June	68	42
CLUJ						
Bonţida	25 June	18	12 June	2 July	74	61
Huedin	19 June	18	11 June	1 July	75	63
CONSTANŢA						
Basarabi*	24 June	17,5	12 June	2 July	72	66
Târguşor*	24 June	18	10 June	1 July	75	60
GIURGIU						
Bucşani	25 June	17,5	12 June	2 July	78	65
GORJ						
Leleşti	19 June	19	11 June	1 July	80	67
HUNEDOARA						
Brad	25 June	19	12 June	2 July	85	66
Simeria	25 June	19	11 June	1 July	74	63
Şoimuş	15 May	27	12 June	15 June	69	52
IALOMIŢA						
Gheorghe Doja*	25 June	18	11 June	1 July	83	66
IAŞI						
Bălceşti	24 June	19	14 June	2 July	85	67

**Helix lucorum*

Fig. 1. Map of Romania

Lucrări ştiinţifice U.Ş.A.M.V.B., Seria B, vol. LI, 2008

The effects of system management on soil carbon dynamics

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Keywords: soil respiration, carbon pools, management

ABSTRACT

Soil organic carbon plays an essential role in determining the soil quality, which is a central aspect to maintain in the long term sustainability and productivity in the agricultural ecosystems. Agriculture can be considered as a sink for atmospheric CO_2 through carbon sequestration into biomass product and soil organic matter, but it can also be a source for greenhouse gases, including CO_2. The impact of different agricultural managements (conventional and organic) and tillage level (deep and minimum tillage, DT and MT, respectively) on soil organic carbon pools content and CO_2 emission were investigated in this work. Both systems have a three-year crop rotation including pea – durum wheat – tomato; the organic system is implemented with the introduction of common vetch (*Vicia sativa* L.) and sorghum (*Sorghum vulgare bicolor*) as cover crops. The results, reported as average of three crops, showed highest values of total organic carbon (TOC) and total nitrogen (TN) in organic soil MT. Similar results were found for microbial biomass carbon (MBC) and nitrogen (MBN). The most labile carbon pools (water soluble carbon and labile carbon) showed higher values in the organic system than in the conventional one, with the lowest value in conventional system DT. Soil CO_2 emissions were also greater in the organic system than in the conventional one, with higher values in MT with respect to DT.

INTRODUCTION

Agriculture can be considered as a sink for CO_2 through carbon sequestration into biomass product and soil organic matter, but it can also be a source for greenhouse gases, including CO_2. Soil carbon sequestration benefits can be summarized on improved soil quality, increased soil productivity, reduced risk of soil erosion and sedimentation (Lal 2007). Soil organic carbon in permanently cropped fields can be increased trough a number of management practices including reduced tillage, cover crops and green manure (Paustian and Cole, 1998). These management options might enhance microbial function and promote soil organic carbon sequestration (Jareki and Lal, 2003). In particular, tillage determines the maximum depth to which plant residues are incorporated in soils and therefore it affects the vertical distribution of soil organic matter (Etana et al., 1999). In addition, conventional tillage induces rapid mineralization on SOM and potential loss of C and N from soil. On the contrary, the green manure of cover crops and incorporation of crop residues, related to organic management practices, are most effective to enhance soil structure by increasing its organic carbon content, size and stability of aggregates, water retention and infiltration (Singh et al., 2007).

The present work investigated the effects of different agricultural managements (conventional and organic) and tillage level (deep and minimum tillage) on soil organic carbon content and CO_2 emission.

MATERIALS AND METHODS

A long-term field study, established in 2001, is conducted at University of Tuscia experimental farm (Viterbo) on a volcanic soil (*Typic Xerofluvent*). In the experimental site organic and conventional system management are compared in a

randomized block design with three replications. Both systems have a three-years crop rotation :pea (*pisum sativum* L.) – winter durum wheat (*Triticum durum* Desf.) – tomato (*Licopersicum esculentum* Mill.). In the organic management, the rotation is implemented with common vetch (*Vicia sativa* L.) and sorghum (*Sorgum vulgare bicolor*) cover crops. For each management deep and minimum tillage levels (DT and MT, respectively) are adopted. The three crops are simultaneously present in the experimental field that includes 36 plots: 2 systems x 2 tillage levels x 3 crops x 3 field replicates.

Soil samples were collected in winter and in summer 2006. Two soil cores were taken in each plot and then pooled together, for a total of 36 soil samples at each sampling date. Soil samples were sieved (<2mm) and for biochemical analyses the moisture content adjusted to 60% of their water holding capacity (WHC). Total organic carbon (TOC) and total nitrogen (TN) were determined using an elemental analyzer (Thermo Soil NC - Flash EA1112). Total extractable carbon (TEC) was determined by by dichromate oxidation and titration techniques.

Microbial biomass carbon (MBC) was estimated following the Fumigation Extraction (FE) method (Vance et al., 1987). C extracted from non fumigated samples represents the labile pool of K_2SO_4 extractable C (ExC).

Microbial biomass nitrogen (MBN) was estimated on fumigated and non fumigated samples following Joergensen and Brookes (1990). N extracted from non fumigated samples represents the labile pool of K_2SO_4 extractable N (ExN). Water Soluble Carbon (WSC) was determined following the method as described for Burford and Bremner, 1975.

Soil CO_2 emission was measured in three randomized areas for each plot every 10 days from March to October 2006. The gas flux was measured using the non-steady-state through-flow chamber: EGM-4 instrument (PP Systems, Stotfold, UK), a portable infrared gas analyzer (IRGA), described in details by Pumpanen et al. (2004).

Data are presented as average of the two sampling dates and the three crops.

Analysis of variance (ANOVA) and LSD Post Hoc test were performed to evaluate the main effects of system (organic/conventional), tillage (conventional/minimum) and their interactions on the parameters analysed using Systat 11.0 statistical software package (SPSS Inc.).

RESULTS AND DISCUSSION

The physical and chemical characteristics of organic and conventional soil are reported in table 1. Total organic carbon (TOC) and total nitrogen (TN) content were positively affected by minimum tillage. The highest values for the two parameters were found in soil under organic MT management (15% and 25% increase with respect to organic DT respectively for TOC and TN). These findings are, in agreement with several reviews assessing the positive impact of conservation tillage on soil C sequestration (Franzluebbers and Follett, 2005; Lal, 2007; Six et al., 2004).

Similar results are reported in table 2a and 2b for C and N labile pools (microbial biomass carbon, nitrogen, and extractable nitrogen) ,that showed the highest values in soil under organic MT management. No significant changes were detected on passive carbon pool (TEC).

From these remarks we hypothesize that in the short period (5 years after beginning of experimental field) the reduced tillage, relative to conservative management (including green manure), improved labile carbon and nitrogen

availability, which consequently affected total carbon and nitrogen stock. The passive pool was less sensitive to soil management systems, according with other authors (Carvalho Leite et al., 2004).

The CO_2 production during the period of measurement (from March to October 2006) showed higher rates in soil under organic management in comparison with conventional one (figure 1). Consequently a higher cumulative amount of CO_2 produced in the organic system was observed (22.58 tons ha^{-1} and 21.93 tons ha^{-1}, respectively). This trend was probably a consequence of the green manure practice, which produced an increase of microbial biomass and therefore in respiration activities, as also reported by Tejada et al. (2008). Tillage practices induced as well differences in soil respiration rates, with higher values in organic MT with respect to organic DT (+ 12 %). This result, in agreement with the higher content of soil organic carbon, confirmed previous studies (Hendrix et al., 1988; Follet and Schimel, 1989) that related soil respiration to carbon availability for microbial biomass with a consequent greater biological activity.

CONCLUSIONS

The organic management and the minimum tillage practices enhanced in the short term the CO_2 emission from soil. This trend was related to the greater values of labile carbon pool in the organic system with minimum tillage, confirming that the management (mainly the green manure practice) and the tillage level had a positive effect on carbon sequestration, while the passive pool was less sensitive in short term.

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Tables

Table 1. Physical and chemical properties of organic and conventional soils.
DT = deep tillage; MT = minimum tillage; Data reported in brackets are the standard error (n = 9). Different letters indicate significant differences for each parameter.
* = $p \leq 0.05$.

Management	tillage	texture (USDA)	pH $_{H2O}$	pH $_{KCl}$	TOC %	TN %	C:N ratio	TEC %
Organic	DT	clay loam	6.91 ^{a} (0.05)	5.52 ^{a} (0.06)	1.10 ^{a} (0.05)	0.12 ^{a} (0.01)	9.55 ^{a} (0.31)	0.70 ^{a} (0.03)
	MT		6.81 ^{a} (0.04)	5.49 ^{a} (0.08)	1.27 ^{b*} (0.03)	0.15 ^{b*} (0.01)	9.10 ^{a} (0.44)	0.74 ^{a} (0.05)
Conventional	DT	sandy clay loam	6.84 ^{a} (0.05)	5.47 ^{a} (0.05)	1.15 ^{a} (0.06)	0.13 ^{ab} (0.01)	9.46 ^{a} (0.29)	0.69 ^{a} (0.03)
	MT		6.91 ^{a} (0.05)	5.62 ^{a} (0.06)	1.18 ^{a} (0.03)	0.13 ^{ab} (0.01)	9.28 ^{a} (0.33)	0.70 ^{a} (0.08)

Table 2a. Biochemical properties of organic and conventional soils.
DT = deep tillage; MT = minimum tillage; Data reported in brackets are the standard error (n = 9). Different letters indicate significant differences for each parameter.
** = $p \leq 0.01$; * = $p \leq 0.05$.

Management	tillage	MBC μg C g^{-1}	MBN μg N g^{-1}	MBC MBN ratio μg C μg N^{-1}
Organic	DT	245.21 ^{a} (23.95)	52.27 ^{a} (2.51)	5.91 ^{a} (0.58)
	MT	314.78 ^{b*} (22.92)	74.19 ^{b*} (10.31)	5.13 ^{a} (0.64)
Conventional	DT	210.15 ^{a} (16.15)	44.52 ^{c**} (2.52)	4.40 ^{a} (0.48)
	MT	217.78 ^{a} (26.83)	57.41 ^{a} (3.50)	4.80 ^{a} (0.77)

Table 2b. Biochemical properties of organic and conventional soils.
DT = deep tillage; MT = minimum tillage; Data reported in brackets are the standard error (n = 9). Different letters indicate significant differences for each parameter.
** = $p \leq 0.01$; * = $p \leq 0.05$.

Management	tillage	ExC μg C g^{-1}	ExN μg N g^{-1}	WSC μg C g^{-1}	LC μg C g^{-1}
Organic	DT	103.26 ^{a} (10.04)	21.17 ^{ab} (2.01)	44.55 ^{a} (4.44)	331.94 ^{a} (22.57)
	MT	92.08 ^{a} (7.30)	30.38 ^{b} (5.96)	42.07 ^{a} (4.49)	370.49 ^{a} (21.52)
Conventional	DT	78.49 ^{a} (8.21)	16.96 ^{a} (1.08)	31.23 ^{b*} (3.04)	274.16 ^{b*} (12.21)
	MT	90.65 ^{a} (5.37)	21.70 ^{ab} (3.02)	34.20 ^{ab} (3.45)	312.14 ^{ab} (23.30)

Figure

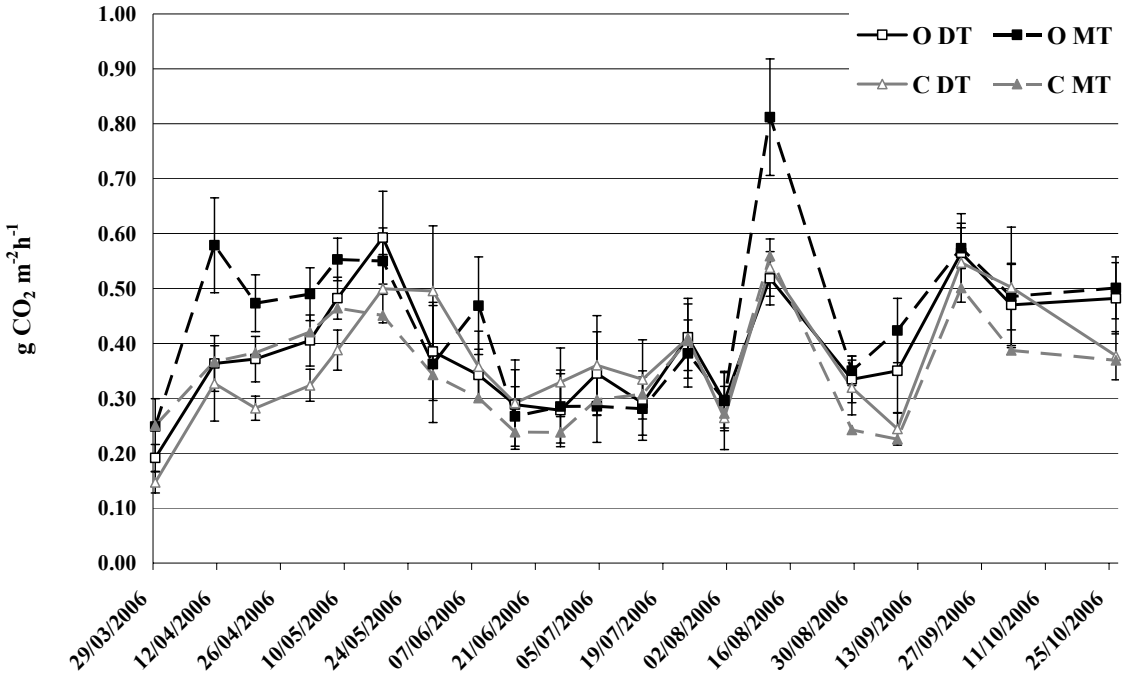

Fig. 1. CO_2 emission from soil measured with EGM-4 (IRGA) from March to October 2006. O = organic; C = conventional; DT = deep tillage; MT = minimum tillage. The bars represent the standard error (n = 9).

Tagging aphids with fluorescent dyes as a tool for epidemiological studies

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Key words: potato, virus, control, pattern of virus spread

ABSTRACT

An aphid tagging method with fluorescent dusts was investigated. The method is simple, no expensive and allows us to trace individual aphid's movement and flight. The method does not affect insect's longevity, its capability to transmit virus, movement, flight or response to color stimuli.

Thus the method allows detailed epidemiological studies. In the present work the model is PLRV (potato leaf roll virus) spread in potato by its vector *Myzus persicae* Sulz. – The peach aphid. The results will be discussed in the context of the present situation of PLRV- control.

INTRODUCTION

About 30% of the agricultural production is decimated by pests, bacteria, fungi and viruses. Control of these is essential in modern agriculture. In view of the present ecological trend of reducing or even banning chemical control, a better understanding of the epidemiology of those pests becomes a must. In recent years epidemiological studies got an impetus expecting that a better understanding of our plagues behavior will improve our control procedures.

Basically epidemiology relates to the dispersion of the different diseases or pests in space (special) and time (temporal).

It is clear that these processes, besides the organism characteristics, depend also on a large variety of environmental conditions and complex between the different pathogens. Yet it is possible to characterize specific trends of spread, by integration of the most factors we know. This is especially documented in the case of vector transmitted viruses where a close relation was found between vector presence and disease spread. (Shun'ichi, 1993)

In order to follow the pattern of spread, in practice, we need tools to enable us the observation of specific individuals or population.

One obvious possibility is to mark our specific individuals or specific population to be traced.

The present work was aimed at experimenting suitability of fluorescence dusts for tagging aphids, *Myzus persicae* Sulz., vector of two important viruses in potato- PLRV & PVY .Then the method was used for epidemiological and virus resistance studies.

MATERIALS AND METHODS

Adult aphids (specific numbers**)** were introduced with a painter brush into plastic vials and gently dusted with 11 different fluorescent dusts manufactured and supplied by Day-Glo Corp.Cleveland,OH, USA. Then the aphids were given to walk a few minutes in a plastic box to remove excessive dust and then placed on the target plants. The tagged aphids were easily observed under illumination of a portative UV lamp. All the tested dyes adhered to insect's body for life. The tagged aphids served for the following:

1. Comparison experiments in leaf cages placed on potato plants, with same numbers of untagged *aptera*, from the same culture as to: Longevity; Virus (PLRV) Transmission; Speed and Distance crawling. Similar experiments were conducted with *alata* in an aphid flight chamber as to Flight Behaviour and Reaction to Colors.
2. Investigating pattern of aphids spread in potatoes in: an environment controlled chamber; a screen house and in the field.
3. Investigating cultivars and genetically engineered clones for PLRV/APHID resistance. All experiments were done for at least four replications.

The plants used were potato plantlets grown uniformly from tissue cultures.

RESULTS AND DISCUSSIONS

A tagging method has to fulfill several requirements if it has to serve in practice. 1. The tag has to persist on the target object enough time in order to allow completion of the experiments. In the first tests done it was clear that all the dusts tested adhere to insect's body and persist till their death. 2. The tagging must not interfere with insect's normal life and behaviour. In 4 trials each one of 100 aphids no differences could be found in longevity of tagged *versus* non tagged aphids. 3. Tagged and control aphids were located in leaf cages on the same PLRV-infected plant and their subsequent transmission was assayed. No differences were found 4. For our epidemiological investigations is crucial that tagging should not interfere with aphid's movement or flight. Similarly to the previous experiments no differences were detected between tagged and non tagged aphids as to their crawling distance, flight takeoff or attractive to colour stimuli.

At this end the method was used to quantify speed and distance of *aptera* movement and understand the direction of movement along *versus* across the rows. The practical implications of the results will be discussed in relation to the present situation of virus control in potatoes

CONCLUSIONS

Tagging aphids by means of fluorescent dyes seems to be an efficient tagging method as it has not significant effects on aphid's life and behaviour.

The method is easy to perform and inexpensive. Using this method it was possible to evaluate speed and distance movement of aphid *aptera* in potato. As well it was established that wingless aphids move faster and to longer distances along than across the potato rows. This enables evaluation of the real infection time in the field and planning more reasonably aphid's control. Another approach was to assay in different cultivars and clones relative resistance to the virus *via* unsuitability to the vector.

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Research on isolation, characterization and testing the interaction between *Trichoderma harzianum* and *Botrytis cinerea* for biological control of gray mold in strawberry

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Keywords: plant pathogen, fungal isolates, antagonism, dual cultures

ABSTRACT

Many commercial strawberry cultivars are susceptible to *Botrytis cinerea,* the agent of gray mold. This microorganism can cause great damage if not controlled.

Research has been carried out in order to find new natural antagonists able to inhibit the pathogen development.

Botrytis cinerea F7 active pathogen isolated from infected strawberry fruits and an antagonistic fungal species *Trichoderma harzianum* P8 originated from soil were tested by dual culture technique for their interaction mechanisms. Biochemical products release was registered and *Trichoderma harzianum* showed hiperparasitism reactions on *Botrytis cinerea*, too.

INTRODUCTION

One of the most important diseases in strawberry crops is the gray mold caused by *Botrytis cinerea*.

Infection takes place at the receptacle through decaying stamens, but can also occur through the style. It causes major damages and loss of yield in many cultivars from Romania. The affected strawberries are not edible and are discarded.

To minimize infections, good ventilation around the berries is important to prevent moisture being trapped among leaves and berries. This is accomplished by slightly elevating the strawberry plants from the soil using straw rather than planting them directly on the ground.

The control of gray mold is yet a real challenge, related to the particularities of pathogen's biology, its adaptability to various environmental conditions, its destructive potential at harvest and the occurrence of fungicide resistance (Viret et al., 2004). Research for finding new biological control agent is motivated by the necessity to assure environment protection and healthy crops for consumers.

This paper presents the results obtained in the first phase of a research project that has as general objective to develop novel effective, environmental friendly and economically feasible agents for biological control of pathogens in strawberry crops.

MATERIALS AND METHODS

Infected fruits of strawberry (*Fragaria ananassa* Duch.) were placed in sterile Petri plates and let to develop abundant propagules from fungal natural contaminants. Conidia and hyphal structures belonging to *Botrytis cinerea* species picked with a sterile needle and plated on agar culture media (PDA) in order to obtain pure culture of pathogen.

Trichoderma was isolated from colonies developed on culture media in Petri plates according to soil decimal dilution method.

Species were identified according to Samson and van Reenen-Hoekstra (1988).

Interaction between the two fungal species was tested by dual culture technique (Phuoc, 1988).

Fungi inoculated opposite on both sites of a Petri plate of 10cm diameter on solid culture media PDA were incubated at 25^{o}C for 15 days and monitored for the evolution of growth rate and mechanisms of interaction.

Microphotographs were carried out to illustrate aspects of hiperparasitism revealed by examination of cultures at MC5A optic microscope.

RESULTS AND DISCUSSIONS

From infected berries incubated in sterile Petri plates (Fig. 1, A and B), a pure culture of *Botrytis cinerea* was isolated.

Its colony rapidly grew on PDA, covering with fungal structures the whole surface of Petri plate in four days. The colony (Fig. 2 A), white at first, became gray in time.

The margins of the colony were rich in tall conidiophores bearing apical heads of branches with light brown ovoid conidia (12.39-19.15 x 8.72-14.76 μm), smooth, with protuberant hilum.

Black sclerotia 2-5 mm diameter developed especially in the central zone of the colony.

The isolate was registered as *Botrytis cinerea* F7 in the fungal collection of the Institute.

A *Trichoderma* colony forming green cottony pustules was selected from plates with soil serial dilutions.

The colony grew rapidly on PDA, covering the entire surface of the Petri plate in four days with a lanose colony, odourless, white at first, with aspect of concentric circles varying in colour from yellow to green later (Fig. 2 B). The reverse of the colony was yellow pigmented.

Microscopical examination revealed the branched conidiophores 3.06 – 4.55 μm width, rough, bearing flask-shaped phialides in whorls of 2- 4 that arise in an angle of 90^{o} from the central axis.

Conidia smooth, subglobose to ovoidal, green, presented dimensions of 1.28 – 3.78 x 2.28 – 4.12 μm.

Hyaline subglobose to globose 5.9 – 6.12 μm diameter chlamydospores developed intercalary on hyphae.

The isolate was registered as *Trichoderma harzianum* P8 in the fungal collection of the Institute.

In dual cultures (Fig. 3, A and B), the two fungal isolates grew with relatively similar rates.

Pathogen development was restricted by *Trichoderma*, the percent inhibition radial growth % IRG (representing the growth of the pathogen in the presence of the antagonist comparatively to the control without antagonist) being 51%.

The antagonism between the two fungal isolates was illustrated by biochemical products released by both species and by hiperparasitism.

Figure 4 A represents a detailed image of the contact zone between the colonies on the reverse of the Petri plate. The inhibition zone is market by an intensified release of diffusible metabolites that conferred deeper colours of the agar comparatively with the rest of the colony. Thus, the pathogen releases toxins and *Trichoderma* inhibits its

development by creating a biochemical barrier and initiating a physical attack at contact line.

Microscopical photograph (Fig. 4 B) presents an aspect from this zone and has in the center a brown conidiophore of *Botrytis cinerea* F7 hiperparasitized by *Trichoderma harzianum* P8. The antagonist develops green conidiophores and conidial heads on pathogen, coiling a net of structures that will branch to form a hiperparasite pustule.

The pathogen growth is thus inhibited by the death of its structures caused by hiperparasitism which follows to the initial biochemical inhibition.

According to the scale of Rini and Sulochana (2007), *Trichoderma harzianum* P8 presented an antagonistic capacity of Class C to inhibit with over 50% radial growth of pathogen *Botrytis cinerea* F7. The authors reported inhibitory effect of *Trichoderma* isolates on *Rhizoctonia* and *Fusarium* infecting tomato. Differences in inhibitory properties may be due to differences in the quantity and the quality of the inhibitory substances (volatile or non-volatile) produced by antagonists.

Our research revealed the capacity of *Trichoderma harzianum* P8 to antagonize *Botrytis cinerea* by releasing biochemical products (diffusible metabolites) and to develop pustules on fungal structures of pathogen by coiling around them, penetrating the walls with the tips and absorbing the hyphal content for its own growth.

CONCLUSIONS

The results showed the capacity of *Trichoderma harzianum* P8 to inhibit the radial growth of *Botrytis cinerea* F7 with 51%.

Biocontrol mechanism is firstly biochemical by releasing non-volatile diffusible metabolites at contact zone.

Trichoderma harzianum P8 acts also as a hiperparasite by coiling and penetrating pathogen's hyphal structures with the tips and using their content as a nutrient source for developing its branched pustules.

Further, *Trichoderma harzianum* P8 could be used in controlling *Botrytis cinerea* effectively in strawberry.

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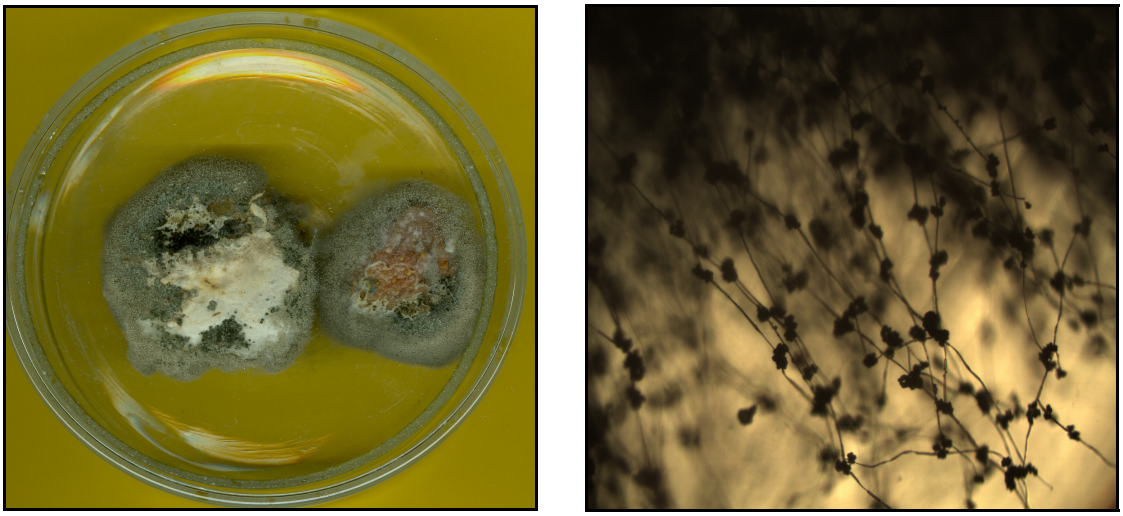
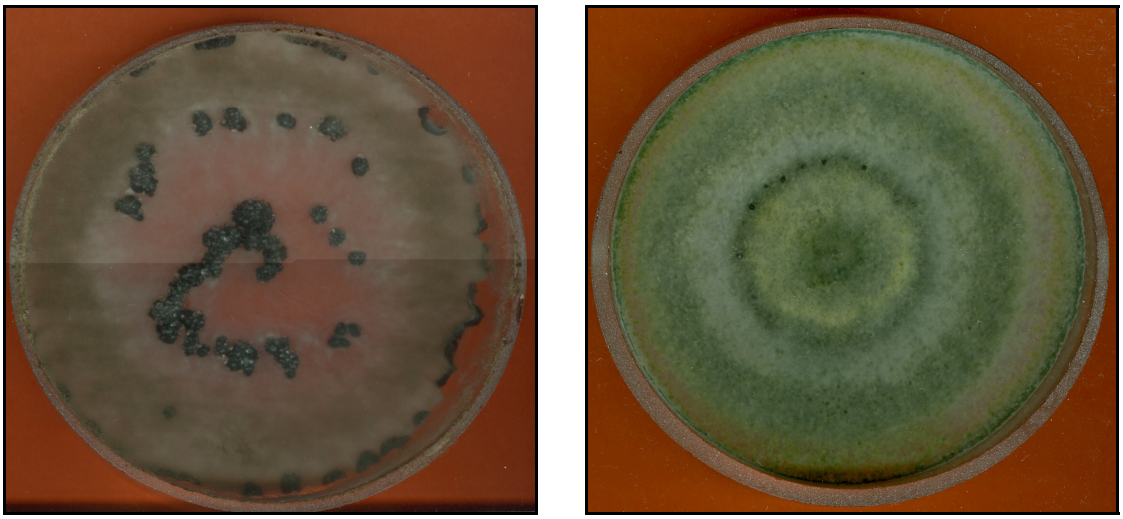
Figures



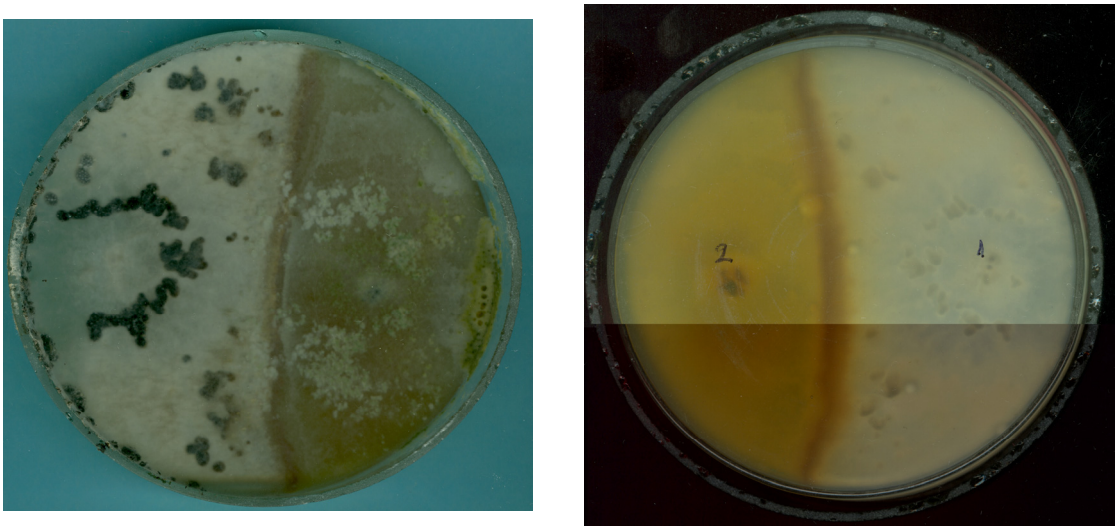
A **B**

Fig. 1. Strawberries infected with *Botrytis cinerea* A-berries in sterile Petri plate and B-microscopical detail with fungal structures (600x)



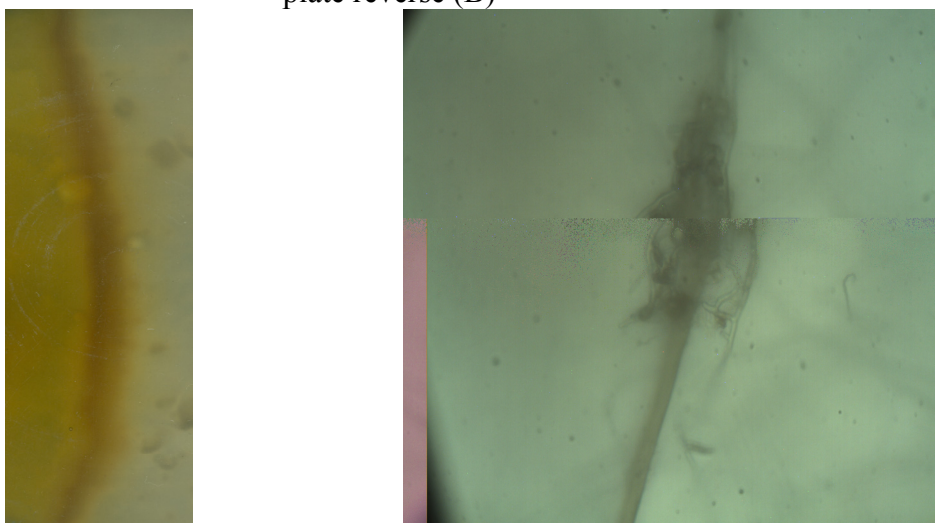
A B

Fig. 2. Pure cultures of A - *Botrytis cinerea* F7 and B – *Trichoderma harzianum* P8



A **B**

Fig.3. Dual cultures of *Botrytis cinerea* F7-*Trichoderma harzianum* P8 (A) and Petri plate reverse (B)



A **B**

Fig. 4. Biochemical interaction at contact zone (A) and hiperparasitism (B) between *Trichoderma harzianum* P8 and *Botrytis cinerea* F7

The study of the viticultural ecosystem biodiversity S.D. Banu Mărăcine-Craiova

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Keywords: biodiversity, weeds, pathogens agents, micolfora, entomofauna

ABSTRACT

The biodiversity of the viticultural ecosystem from S.D. Banu Maracine-Craiova, comprise, among the cultivated species *Vitis vinifera,* other spontaneous species (predominant being *Stellaria media* (16,9%), *Cardaria draba* (16%) and *Lamium purpureum* (12,2%)).

The key pathogens agents specific for the studied area are *Plasmopara viticola* (Berk. et Curt) Berl, et de Toni, *Uncinula necator* (Schw) Burr. f.c. *Oidium tuckerii* Berk. and *Botryotinia fuckeliana* (De Bary) Whetz f.c. *Botrytis fuckeliana* Pers.).

From the viticultural soil and from the grapes has been isolated 67 yeast strains with 45 strains of sporogenous species and 22 strains of nonsporogenous species.

From the total of 86 arthropods species 44 species are harmful to the vine (51,16%,), 14 beneficial species (16,28%) and 28 indiferent species (32,56%.).

INTRODUCTION

Due to the permanent deterioration of the environment, especially to the excessive use of the chemical products, the reorganization of the viticultural system applied in the present through technologies based on reducing the pollution risk constitute a logical alternative (Simeria, Gh., 2003).

In order to establish the technological controlling scheme in viticulture we consider that it must be studied the biodiversity of the viticultural ecosystem, the inventory and the monitoring of the pests and diseases evolution from the researched area in order to know their biological reserve (Simeria, Gh., 2002). In the same time it's neccesary to follow the climatic conditions from the vegetative repose, which modifies the prognosys established in autumn (Mirică, I. Mirică Afrodita., 1986).

MATERIALS AND METHODS

The research has been made in the vineyard from S.D. Banu Mărăcine belonging to the University of Craiova, during October 2006-September 2007 in production condition at the variety Fetească albă.

The weeding degree has been estimate by counting the weeds species on m^2 and framing in two groups: monocotyledonate weeds and dicotyledonate weeds.

The evolution of the phytopathogen agents has been followed through direct observations in the field and laboratory determination using the microscope ML 4, establishing the phytopathogen agents spectrum during the vegetation period. The studies of the yeast cells has focused on the aspect, shape, dimensions and their viability, the presence of the asci or hyphe, pseudo-hyphe and blastoconidia (fig. 1.).

The density of the acarians population has been estimated through visual observations during the vegetative repose and vegetation period, prevailing samples, field and laboratory analyze of the collected samples (fig. 2).

In order to collect different insect species there has been used food traps and yellow plates (fig. 3)., and the surveillance of the moths generations based on the capture recorded on the pheromones traps type *atraBOT* (fig. 3), for capturing the males of the

species *Lobesia botrana* Den et Schiff., *atraAMBIG*, for capturing the males of the species *Eupoecilia ambiguella* Hb. and *atraPil* for capturing the males of the species *Sparganotis pilleriana* Den et Schiff. The pheromone traps has been set at the end of April begining of May, 3 traps/ha. The reading of the traps has been made every 7 days, replacement of the capsule impregnated with synthetic pheromone as well the adhesive parts of the traps has been made every 4-5 weeks.

RESULTS AND DISCUSSIONS

1. The climatic characterization of the year 2007:

The year 2007 has been characterized through a very arid climate with high temperatures during winter-spring, spring-summer (table nr. 1). The air average temperature has presented values, with positive deviation given the multiannual average, the highest being recorded during January-March and May-August. Regarding the air relative humidity (%), during 2007, has presented very low values during April-September of 36%-66%, which favored the installation of a climate with a very accented hydric stress.

After the examination of the monthly climatic data (average/sum) during the research year we have to emphasize the droughty climate, with rainfall deficit of 42,8 mm respectively 48,6 mm. Besides the fact that has been recorded a long period of drougthness, during the research period the air average temperature has been higher than the multiannual average.

These climatic conditions have determined low evolutions of the phytopathogens agents; bur favored the development of some acarian species.

2. The weeding degree of the vineyard:

The weeds besides other factors exert a negative influence on the primary cultivated plants.

The weeding state of the vineyard from the Didactical Station Banu Mărăcine it's presented in table 2. Analyzing the florist composition of the weeds from the vineyard it come out that the monocotyledonate represent 27,4%, and the dicotyledonate 72,6%.

The most encountered species has been *Stellaria media* (16,9%) followed by *Cardaria draba* (16%) and *Lamium purpureum* (12,2%).

The covering degree of the soil with weeds has been of 80-85% (Fig.4).

3. The spectrum of the pathogen agents from the vineyard Banu Maracine:

The climatic conditions from 2006 in the Didactical Station Banu Mărăcine has been favourable to the evolution of the pathogens agents attack, ensuring for 2007, high biological reserve for the „key pathogen" (table nr. 3).

In 2007 the evolution of the climatic condition has been unfavorable, to the pathogen agent attack, the year being extremely drought. Thus, has been recorded attack degree with low values and a reduce number of the infection produced by the key pathogen (the grape vine downy mildew produce by *Plasmopara viticola* (Berk. et Curt) Berl, et de Toni, the powdery mildew produce by *Uncinula necator* (Schw) Burr. f.c. *Oidium tuckerii* Berk. And the grey mold rots produce by *Botryotinia fuckeliana* (De Bary) Whetz f.c. *Botrytis fuckeliana* Pers.).

4. Micoflora from the soil and grapes:

During 2007, from the viticultural soil and from the grapes has been isolated 67 yeast strain with 45 strains of sporogenous species and 22 strains of nonsporogenous species. (table 4). Following the assimilation physiological tests, fermentation and the morphological analyzes of the developed colonies, the isolated strain has been identified as belonging to 3 genres with 3 sporogenous species and to 2 genres with 2 unsporogenous species.

For their characterization we followed the habitat from which these were isolated and the results of the physiological tests, associating morphological and physiological features characteristic for the Banu Mărăcine ecotope.

5. Entomofauna plantației viticole de la S.D. Banu Mărăcine:

In 2007 within the viticultural ecosystem S.D. Banu Mărăcine there has been identified 86 Arthropods species, systematically framed in 11 orders (Panin L, 1951). The most numerous orders have been ***Coleoptera*** with 38 species, followed by ***Lepidoptera*** with 12 species and ***Heteroptera*** with 11 species.

From the 86 species of arthropods, 44 species are harmful for the vine, 14 species are beneficial and 28 species are indifferent (table 5).

The percentage express of the entomofauna from the viticultural ecosystem S.D. Banu Mărăcine during the research period has been: 51,16% harmful species, 16,28% beneficial species and 32,56% indifferent species.

CONCLUSIONS

The results show that: in the florist composition of the vineyard from the Didactical Station Banu Mărăcine prevail the dicotyledonate species. Due to the high weeding degree, the success of the vine crop in the Didactical Station Banu Mărăcine it's conditioned by the use of herbicides or the weeding at the right moment.

During October 2006-September 2007 the evolution of the climatic condition has been unfavorable to the attack of the pathogen agents, the year being extremely droughty.

During the research period, from the vitivultural soil and grapes has been isolated 67 strain of yeast.

In 2007 within the viticultural ecosystem S.D. Banu Mărăcine has been identified 86 Arthropods species, systematically framed in 11 order.

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Tables

Table 1. The climatic condition from the research period (2006-2007) at the Didactical Station Banu Mărăcine

Month	Average temperature (°C)		Rainfall (mm)		The air relative humidity (%)
	Monthly average	Multiannual monthly average	Monthly sum	Multiannual monthly sum	
2006					
October	12,4	11,4	13,0	39,2	75,0
November	7,4	5,6	7,0	47,0	73,0
December	1,6	0,2	32,0	45,0	90.0
2007					
January	5,5	-2,6	17,6	36,4	69,0
February	4,0	-0,2	36,9	31,4	72,0
March	7,6	4,8	51,3	35,0	64,0
April	12,9	11,4	0,0	42,8	46,0
May	18,7	16,8	93,6	61,7	60,0
June	23,0	20,9	57,6	63,8	57,0
July	26,5	22,1	5,6	54,6	36,0
August	23,0	22,0	148,6	43,6	66,0
September	15,6	17,5	65,6	38,0	71,0
Total/Average		**10,8**		**538,5**	

Table 2. The natural weeding state of the vineyard from S.D. Banu Mărăcine

The weed species	Nr. weed/m^2	% participation
Setaria pumila	8	7,5
Poa pratensis	6	5,7
Sorghum halepense	3	2,8
Elymus (Agropyron) repens	4	3,8
Hordeum sterilis	1	0,9
Bromus sterilis	2	1,9
Bromus arvensis	2	1,9
Bromus hordeoceus	2	1,9
TOTAL MONOCOTILEDONATE	**29**	**27,4**
Stellaria media (rocoină)	18	16,9
Cardaria draba (urda vacii)	17	16,0
Lamium purpureum (sugel puturos)	13	12,2
Vicia grandiflora (măzăriche)	12	11,3
Taraxacum officinale (păpădie)	8	7,5
Cirsium arvense (pălămidă)	6	5,7
Amaranthus retroflexus (ştir)	3	2,8
TOTAL DICOTYLEDONATE	**77**	**72,6**
MONOCOTYLEDONATE + DICOTYLEDONATE	**106**	**100**

Table 3. The evolution of the vine disease in 2006 and the biological reserve for 2007

Pathogen agent	Period	Treatment s number	Biological reserve	The degree attack (%)	Recorded damages
VIROSIS Fan- leaf	January -December	-	Growing at some varieties	0,5	Without economical importance
Stem pitting	January-December	-	Growing	0,5	Without economical importance
Vein clearing mosaic	January-December	-	Growing	0,2	Without economical importance
BACTERIOSIS Agrobacterium tumefaciens	January-December	-	Growing	1,5	1 - 1,5
MICOSIS Plasmopara viticola	May -October	6	Very high	7,71	1 - 5
Uncinula necator	April- October	4	high	4,3	1 - 5
Botryotinia fuckeliana	January- October	3	Very high	18,72	1 - 10
Elsinoë ampelina	April - August	-	reduced	0,2	Without economical importance
Phomopsis viticola	15 April - 15 September	-	reduced	1,0	Without economical importance
Pseudopeziza tracheiphilla	April, June, September, October	-	reduced	0,5	Without economical importance
Eutiopa armenicae	January - December	-	reduced	0,5	Without economical importance

Table 4. The number of yeast strain isolated from the soil and grapes in 2007, at Banu Mărăcine

Nr. crt.	SPECIES	Total strain 2007
1.	*Saccharomycodes ludwigii*	13
2.	*Saccharomyces elegans*	15
3.	*Hansenula anomala*	17
TOTAL SPOROGENOUS YEAST		**45**
4.	*Candida stellata*	12
5.	*Rhodotorula mucilaginosa*	10
TOTAL NONSPOROGENOUS		**22**

Table 5. The structure of the entomofauna at S.D. Banu Mărăcine in 2007

Order	No. of harmful species	No. of beneficial species	No. of indiferent species	TOTAL
Acari	2	0	0	2
Orthoptera	7	0	0	7
Dermaptera	1	0	0	1
Thysanoptera	1	0	1	2
Heteroptera	4	0	6	11
Homoptera	2	0	0	2
Hymenoptera	3	4	0	7
Neuroptera	0	2	0	2
Coleoptera	15	7	16	38
Lepidoptera	8	0	4	12
Diptera	1	1	0	2
TOTAL	**44**	**14**	**28**	**86**

Figures

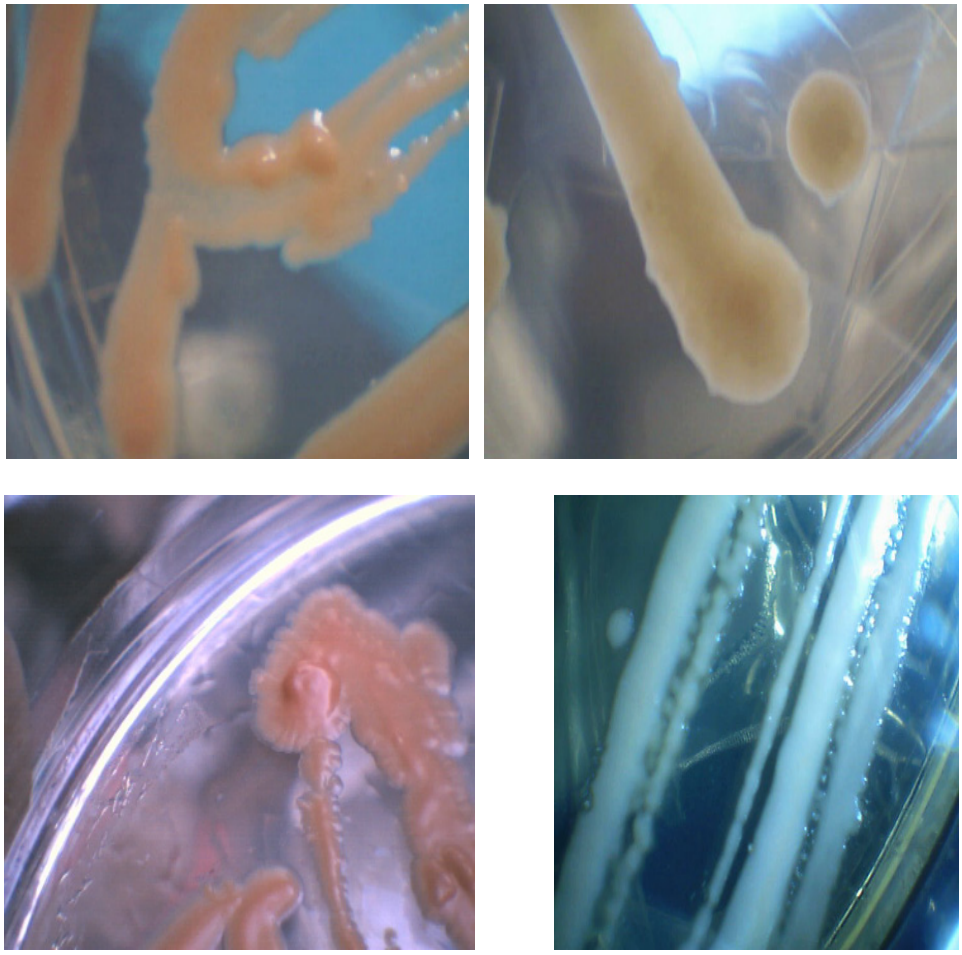

Fig. 1. Colonies developed on solid medium
(*Saccharomycodes ludwigii, S. Elegans, Hansenula anomala, Candida stellata)*

Fig. 2. Vine cords (Fetească albă) in different vegetation period

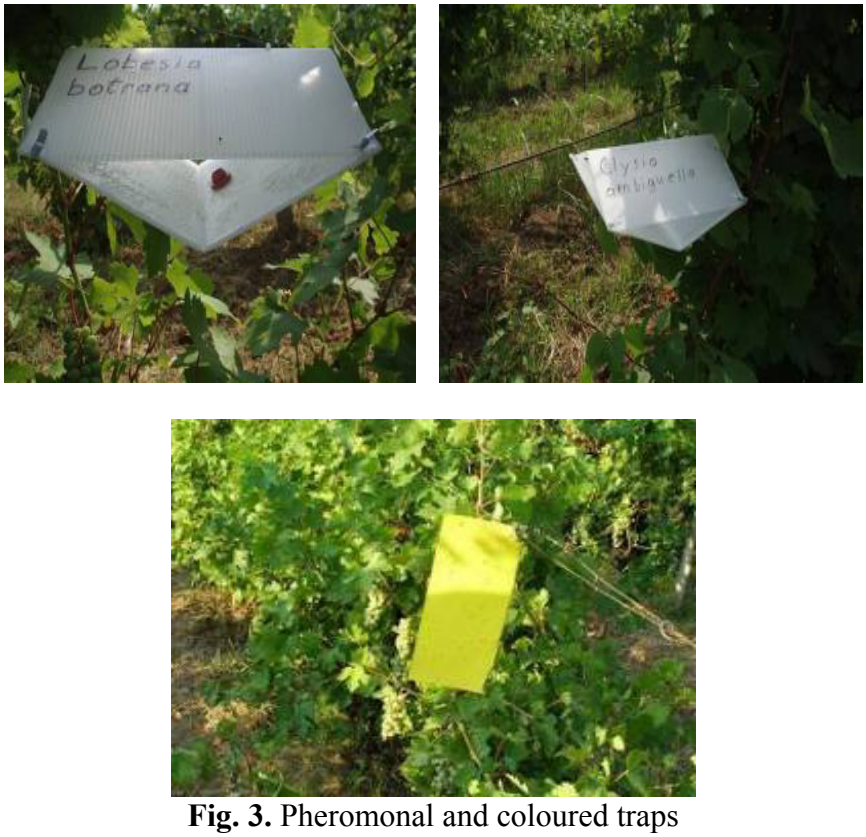

Fig. 3. Pheromonal and coloured traps

Fig. 4. *Stellaria media, Cirsium arvense, Cardaria draba, Lamium purpureum*

=

Histologic modification induced by the action of the insecticide Samurai on the skin and liver of *Rana Ridibunda*

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Keywords: acetamiprid, frog, gland mucose, hepatocytes, stratum corneum, lipofuscine

ABSTRACT

In our experiments we followed the histologic modifications induced by the action of the insecticide Samurai at the skin and liver level of Rana Ridibunda. The toxic substance used was the insecticide commercialized under the generic name of Samurai which has as an active substance the acetamiprid. This systemic, latest generation insecticide has a wide spectrum of action. The animals used in the experiment were divided in three experimental lots: one lot of control individuals and two experimental lots in which the frogs were kept in two acuaterrarios with a 0.01 ml/l and respectively 0.02 ml/l concentration of Samurai. The toxic water was changed daily and the animals were kept unfed for the duration of the experiment. We began sacrificing them at the beginning of each of the following three weeks from the start of the experiment. We observed a hypertrophied mucose gland of the tegument, an increased volume of hepatocytes as well as increased reserve of lipofuscin.

INTRODUCTION

Due to their thin and permeable skins, and prolonged exposure first to the aquatic environment and then to the terrestrial, amphibians may be particularly sensitive to environmental contaminants. Populations of many amphibians have declined and some species have disappeared from certain regions around the world, a phenomenon which appears to have accelerated during the last years. Agricultural pesticides may contribute to the decline in amphibian populations. Much of the amphibian life cycle occurs in ponds, streams, and temporary pools that are often associated with agricultural areas receiving pesticide applications (Têgowska E. et al., 2003). In addition, breeding and larval development of amphibians occur in spring and summer at the same time that heavy application of pesticides on agricultural lands occurs.

In our studies we followed the histologic modifications induced by the action of the insecticide Samurai at the skin and liver level of *Rana ridibunda*. This insecticide is considered to have a moderate level of toxicity on the aquatic organisms.

MATERIALS AND METHODS

In our experiment we utilized adult *Rana ridibuna* frogs (male and female) considering those, of the poikilotherms, the amphibians (especially the frogs) are the animals that are best suited for the work in the laboratory.

The animals were captured in the surrounding areas of the city Pitesti (Romania) and were kept in acuaterrarios filled with tap water. The water was changed daily to avoid the accumulation of toxic substances. Prior to starting the experiments, the amphibians were kept for seven days under normal conditions in order to test their health and accommodate them for the experiment.

The animal used in the experiment were selected into three experimental lots as follows: (1) one lot of control individuals, consisted of twelve untreated specimens of R*ana ridibunda* with an average weight of 22 grams, kept in the laboratory in containers filled with (daily changed) tap water, (2) a second lot consisting of twelve specimens of R*ana ridibunda* with an average weight of 43 grams which were kept in acuaterrarios filled with tap water and Samurai (0.01 ml/l), (3) a third lot which also had twelve

specimens (average weight 40 grams) kept in the same conditions as second lot but treated with a higher concentration of Samurai (0.02 ml/l).

The toxic water was changed daily, the animals were not fed though the experiment and the laboratory was kept at an average temperature of 25 degrees Celsius. The administered dosage of insecticide was not lethal as none of the subjects died through the experiment.

We began sacrificing the amphibians every seven days for three weeks after the first week of treatment and we kept for testing fragments of liver, dorsal and ventral tegument. Liver and skin pieces were fixed 4% buffered formaline and processed for the paraffin embedding, following the standard methods. Histological sections of 5µ were stained with hemalaum-eosin (HE).

The toxic substance used was the insecticide commercialized under the generic name „Samurai" which has as an active substance the acetamiprid. The acetamiprid is in the neonicotinoid chemical family, and controls sucking type insects on various food and ornamental crops by affecting their nervous system (Ponepal et al., 2006). This systemic, latest generation insecticide has wide spectrum of action and is compatible with a very large number of pesticides excepting the highly alkaline ones.

RESULTS AND DISCUSSION

The Samurai in a concentration of 0.01 ml/l after one week of action induces o light hypertrophy of the hepatocytes while at the same time changing the structures of the cellular chains. At the biliar pole of the hepatocytes we observed accumulations of lipofuscine (fig. 1.a) while at the dorsal (fig. 1.b.) and ventral (fig. 1.c.) skin level we noticed hypertrophic mucose glands. In parallel, we registered an intense secretion of the mucose. Finally, we concluded that the highly stratified epidermis was directly linked to the insecticide as this entered the organism through the skin.

A double concentration of Samurai determines, after one week of action, a volume increase of the hepatocytes which have a cytoplasm full of vacuole, with clear spaces and a nucleus centrally positioned (fig. 2.a.). We also observed an increase in the deposits of lipofuscine al through the hepatic parenchyma (Păunescu et al., 2005). The skin presents a more pronounced hypertrophy of the dorsal and ventral mucose glands (fig. 2.b.,c.). The stratum corneum is well represented.

After fourteen days of treatment with Samurai, 0.01 ml/l concentration, at the liver level we observed an increase in the lipofuscine deposits (pigmented connective tissue cells deterioration) which were frequently found in the hepatic parenchyma. The centerlobular venes were well defined with erythrocytes present in the lumen (fig. 3.a.). At the skin the big as well as the small dorsal glands were hypertrophic (fig. 3.b., c.). The stratum corneum is well represented.

After fourteen days of treatment with Samurai, 0.02 ml/l concentration, the amphibians' liver showed a hypertrophy of the hepatocytes and a deterioration of the pigmented connective tissue cells (fig. 4.a.). An enlargement of the Disse interspaces was also observed. The skin presented a high number of hypertrophied acinose glands (fig. 4.b, c.).

After 21 days from the beginning of the treatment, at 0.01% ml/l we registered a higher level of hepatocytes volume (fig. 5.a.); the skin also had a higher number of mucose glands found in hyperfunction (fig. 5.b., c.). After 21 days, using 0.02ml/l we recorded an even higher level of toxicity. The hepatocytes had a higher volume with their nucleus positioned centrally and higher deposits of lipofuscine in the Kuppfer cells

(Desmet, 1992). In certain zones of the hepatic parenchyma we noticed light necroses of the hepatocytes (fig. 6.a.) (Desmet and DeVos, 1983); the skin presents numerous dorsal and ventral mucose glands and the epithelial becomes stratified (fig. 6.b.,c.). All this changes explains the fact that the acetamiprid is quickly absorbed and that it enters the liver being subsequently eliminated through the renal system.

CONCLUSIONS

The toxic action of Samurai in a dose higher than 0.01 ml/l is shown as early as seven days from its' delivery (via the water from the acuaterrarios). At the liver's level, a concentration of 0.01 ml/l of Samurai increases the volume of hepatocytes and the deposits of lipofuscine while registering changes in the structural cellular chains. A concentration of 0.02 ml/l of Samurai determines a hepatocellular vacuolization as well as the formation of small necrosis areas in the hepatic parenchyma especially after the 21 days of exposure.

At the skin level we recorded a stratification of the epidermis with a well developed stratum corneum the dorsal and ventral mucose acinose glands hypertrophied and a more abundant mucose secretion. (The latest being the result of the defense system of the animals.) The same toxic dosage used for 14 respectively 21 days determined similar changes in the skin structure though cornification was stronger and the secretion of the mucose glands more abundant. The doubling of the Samurai concentrations determined an even more pronounced histologic modification of the skin (especially after 21 days of exposure) registering a loss of pigment at the dorsal and ventral tegument.

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Tables and Figures

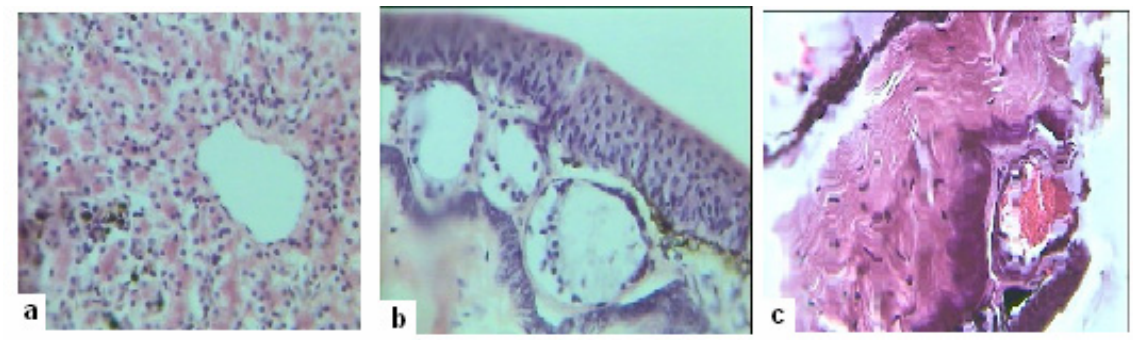

Fig. 1. Liver (a), dorsal tegument (b) and ventral tegument (c) of *Rana ridibunda* intoxicated with Samurai, 0,01 % concentration after 7 days action.(HE, 10X)

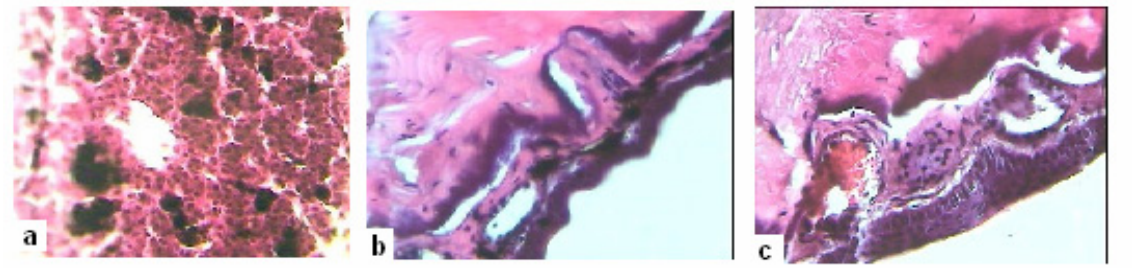

Fig. 2. Liver (a), dorsal tegument (b) and ventral tegument (c) of *Rana ridibunda* intoxicated with Samurai, 0,02 % concentration after 7 days action. (HE, 10X)

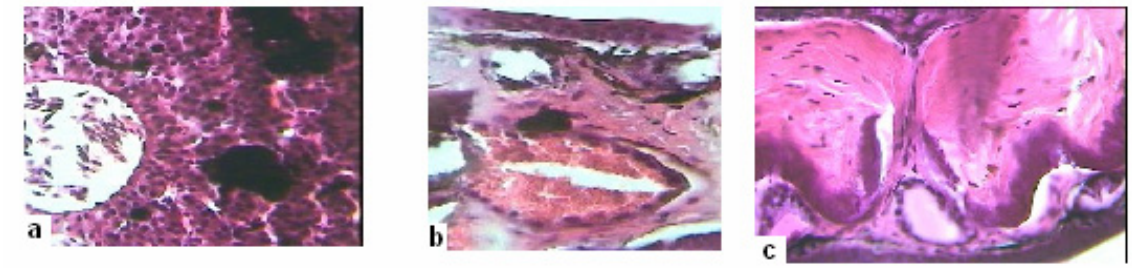

Fig. 3. Liver (a), dorsal tegument (b) and ventral tegument (c) of *Rana ridibunda* intoxicated with Samurai, 0,01 % concentration after 14 days action. (HE, 10X)

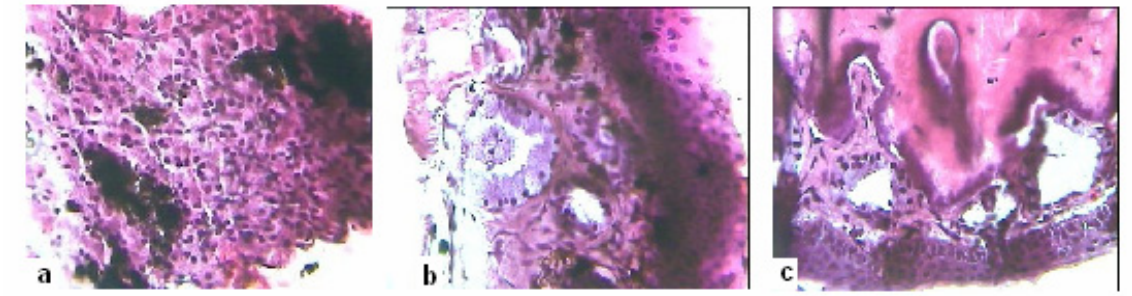

Fig. 4. Liver (a), dorsal tegument (b) and ventral tegument (c) of *Rana ridibunda* intoxicated with Samurai, 0,02 % concentration after 14 days action. (HE, 10X)

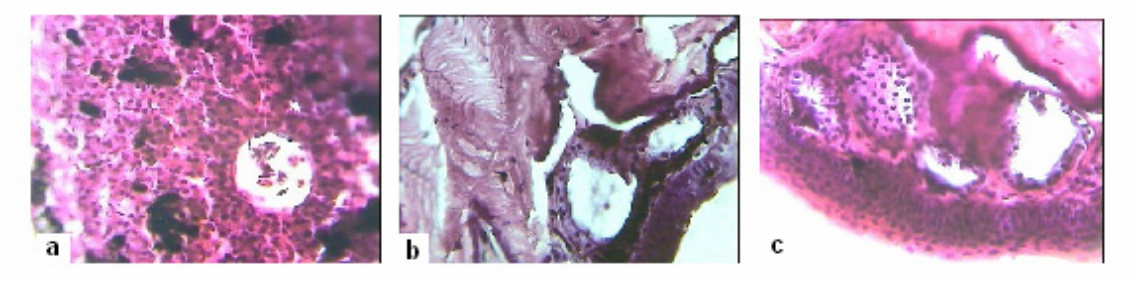

Fig. 5. Liver (a), dorsal tegument (b) and ventral tegument (c) of *Rana ridibunda* intoxicated with Samurai, 0,01 % concentration after 21 days action. (HE, 10X)

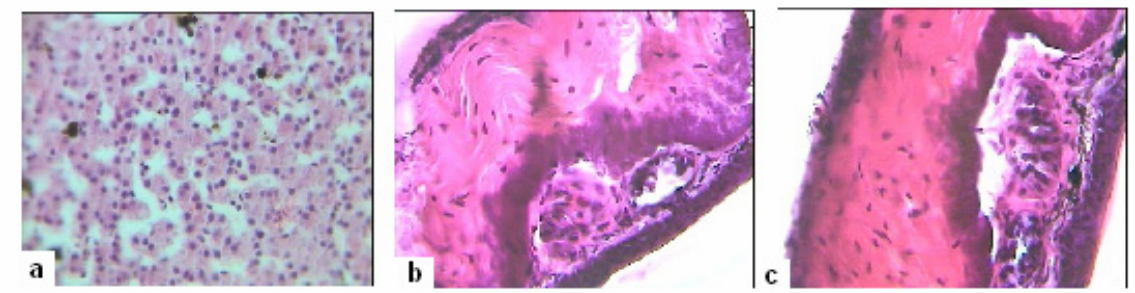

Fig. 6. Liver (a), dorsal tegument (b) and ventral tegument (c) of *Rana ridibunda* intoxicated with Samurai, 0,02 % concentration after 21 days action. (HE, 10X)

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Biotechnology for conversion of winery and vine waste into mushroom products

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Keywords: biotechnology, fungal conversion, edible and medicinal mushrooms, winery and vine wastes

ABSTRACT

The main aim of this work was to find out the best way to convert the vineyard and winery wastes into useful bioproducts by using them as a growing source for edible and medicinal mushrooms in order to extend the food chain in vineyard ecosystems. According to this purpose, three fungal species from Basidiomycetes, namely *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake) and *Pleurotus ostreatus* (Oyster Mushroom) were tested to determine their biological potential to grow on substrates made of vineyard and winery wastes which could be used in this way as culture composts. The experiments of this research work were achieved by growing all these fungal species in special culture rooms, where all the culture parameters were kept at optimal levels in order to get the highest production of fruit bodies. During the experiments, the effects of culture compost composition (carbon, nitrogen and mineral sources) as well as other physical and chemical factors (such as: temperature, inoculum size, pH level and incubation time, etc.) on mycelial net formation and especially, on fruit body induction, were investigated. From all these fungal species tested in our experiments, *Pleurotus ostreatus* was registered as the fastest mushroom culture, then *Lentinus edodes* and finally, *Ganoderma lucidum* as the longest mushroom culture. As control samples for each variant of culture composts used for the experimental growing of all these fungal species were used wood chops of oak and wheat straw.

INTRODUCTION

Agricultural works as well as industrial activities related to vine crops processing have generally been matched by a huge formation of wide range of waste products. Many of these lignocellulosic wastes cause serious environmental pollution effects, if they are allowed to accumulate in the vineyards or much worse to be burned on the soil. So far, the basis of most studies on lignocellulose-degrading fungi has been economic rather than ecological, with emphasize on the applied aspects of lignin and cellulose decomposition, including biodegradation and bioconversion (Smith, 1998).

In this respect, the main aim of this work was to find out the best way of recycling the vine and vineyard wastes by using them as a growing source for edible and medicinal mushrooms.

MATERIALS AND METHODS

Fungal Species and Culture Media

According to the main purpose of this work, three fungal species from Basidiomycetes, namely *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake) and *Pleurotus ostreatus* (Oyster Mushroom) were used as pure cultures in experiments.

The stock cultures were maintained on malt-extract agar (MEA) slants. Slants were incubated at 25°C for 5 - 7 d and then stored at 4°C. The seed cultures were grown in 250-ml flasks containing 100 ml of MEA medium (20 % malt extract, 2 % yeast extract, 20 % agar-agar) at 23°C on rotary shaker incubators at 110 rev min^{-1} for 5 - 7 d.

Methods Used in Experiments

The fungal cultures for experiments were grown by inoculating 100 ml of culture medium with 3-5% (v/v) of the seed culture and then cultivated at 23 - 25°C in

rotary shake flasks of 250 ml. The experiments were conducted under the following conditions: temperature, 25°C; agitation speed, 90 - 120 rev min $^{-1}$; initial pH, 4.5 – 5.5. The seed culture was transferred to the fungal culture medium and cultivated for 7-12 d.

After this stage of inoculum preparation, the seed cultures were cultivated on special culture media prepared from lignocellulosic wastes resulted from vine cuttings that were used as substrates for mushroom growing (Petre, 2002). These lignocellulosic materials were mechanical pre-treated to breakdown the lignin and cellulose structures in order to be more susceptible to the enzyme actions (Petre and Petre, 2003). All these pre-treated lignocellulosic wastes were disinfected by steam sterilization at 120^0 C for 60 min. The final composition of culture composts was improved by adding the following ingredients: grain seeds (wheat, rye, rice), $CaCO_3$, $NH_4H_2PO_4$, each kind of culture medium composition depending on the fungal species used to be grown.

The experiments of this research work were achieved by growing all these fungal species in special culture rooms, where all the culture parameters were kept at optimal levels in order to get the highest production of fruit bodies. During the experiments, the effects of culture compost composition (carbon, nitrogen and mineral sources) as well as other physical and chemical factors (such as: temperature, inoculum size and volume and incubation time) on mycelial net formation and especially, on fruit body induction were investigated.

All the culture composts for mushroom growing were inoculated using liquid inoculum with the age of 5 – 7 days and the volume size ranging between 3 - 7% (v/w). The optimal temperatures for incubation and mycelia growth were maintained between 23 – 25°C. The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30 – 60 days, depending on each fungal species used in experiments.

RESULTS AND DISCUSSION

The registered data revealed that lignocellulosic vine wastes has to be used as substrates for mushroom growing only after some mechanical pre-treatments that could breakdown the whole lignocellulose structure in order to be more susceptible to the fungal enzyme action (Petre and Petre, 2003). Due to their high content of carbohydrates and nitrogen, the variants of culture composts supplemented with wheat grains at the ratio 1:10 and rice grains at the ratio 1:5 as well as a water content of 60% were optimal for the fruit body production of *Pleurotus ostreatus* and, respectively, *Lentinus edodes*.

The Effect of Carbon Sources upon the Mushroom Mycelia Growth

In order to find a suitable carbon source for the mycelia growth and consequently for fungal biomass synthesis, the pure cultures of *Pleurotus ostreatus* (Oyster Mushroom)**,** as well as *Lentinus edodes* (Shiitake) and *Ganoderma viride* (Reishi) were cultivated in different nutritive culture media containing various carbon sources, and each carbon source was added to the basal medium at a concentration level of 1.5% (w/v) for 7-12 d. When the cells were grown in the maltose medium, the fungal biomass had the highest values (see Table 1).

What is very important to be noticed is that the maltose has a significant effect upon the increasing of mycelia growth and fungal biomass synthesis (Petre and Petre, 2003). The experiments were carried out for 12 days at 25 °C with the initial pH 5.5. Data are the means ± S.D. of triple determinations.

The Effect of Nitrogen Sources upon the Mushroom Mycelia Growth

To investigate the effect of nitrogen sources on mycelia growth and fungal biomass production, the pure cultures of these two fungal species were cultivated in media containing various nitrogen sources, where each nitrogen source was added to the basal medium at a concentration level of 10 g/l. Among five nitrogen sources examined, rice bran was the most efficient for mycelia growth and fungal biomass production (see Table 2). Malt extract was one of the best nitrogen sources for a high mycelia growth. Peptone, tryptone and yeast extract are also known as efficient nitrogen sources for fungal biomass production. In comparison to organic nitrogen sources, inorganic nitrogen sources gave rise to relatively lower mycelia growth and fungal biomass production (Petre and Borduz, 2004).

The Effect of Mineral Sources upon Mushroom Mycelia Growth

The influence of various mineral sources on fungal biomass production was examined at a standard concentration level of 5 mg. Among the various mineral sources examined, K_2HPO_4 yielded good mycelia growth as well as fungal biomass production and for this reason it was recognized as a favourable mineral source (Table 3).

Similar observations were made during the experiments concerning other mushroom fermentations Petre, 2002; Chang and Hayes, 1978). K_2HPO_4 could improve productivity through its buffering action, and essential phosphates were favourable for mycelia growth in submerged as well as in surface cultures of mushrooms.

The Effects of Initial pH and Temperature upon Fruit Body Formation

In order to study the effects of initial pH correlated with the incubation temperature upon fruit body formation, *G. lucidum*, *P. ostreatus* and *L. edodes* were cultivated on substrates made of vineyard wastes at different initial pH values (4.5 – 6.0). The optimal pH and temperature levels for fungal fruit body production were 5.0 – 5.5 and 21 – 23°C (Table 4).

The Effects of Inoculum Age and Inoculum Volume upon Fruit Body Formation

Amongst several fungal physiological properties, the age and volume of mycelia inoculum play an important role in fungal hyphae development as well as in fruit body formation [Petre and Petre, 2003)]. To examine the effect of inoculum age and inoculum volume, *G. lucidum*, *P. ostreatus* and *L. edodes* were grown on substrates made of vineyard wastes during different time periods between 30 and 60 days, varying the inoculum volume (5 - 7 v/w). As it is shown in Tables 5 - 6, the inoculum age of 120 h as well as an inoculum volume of 6.0 (v/w)have beneficial effects on the fungal biomass production (Stamets, 1993). All the experiments were carried out at 25°C and initial pH 5.5 and data are means ± S.D. of triple determinations.

The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30 – 60 days, depending on each fungal species used in experiments. As control samples for each variant of culture composts used for the experimental growing of all these fungal species were used wood chops of oak that were kept in water three days before the experiments and then they were steam sterilized to be disinfected. Due to their high content of carbohydrates and nitrogen, the variants of culture composts supplemented with wheat grains at the ratio 1:10 and rice grains at the ratio 1:5 as well as the water content of 60% were optimal for the fruit body production of *P. ostreatus* and *L. edodes* (Petre and Borduz, 2004).

The final fruit body production of these fungal species used in experiments was registered between 10 – 15 kg relative to 100 kg of compost.

CONCLUSIONS

1. From all these fungal species tested in our experiments, *Pleurotus ostreatus* was registered as the fastest mushroom culture (25 – 30 days), then *Lentinus edodes* (35 – 50 days) and finally, *Ganoderma lucidum* as the longest mushroom culture (50 – 60 days).
2. From those five nitrogen sources examined, rice bran was the most efficient for mycelia growth and fungal biomass production
3. Among the various mineral sources examined, K_2HPO_4 yielded good mycelia growth as well as fungal biomass production and for this reason it was recognized as a favourable mineral source.
4. The maltose has a significant effect upon the increasing of mycelia growth and fungal biomass synthesis
5. The inoculum age of 120 h as well as an inoculum volume of 6.0 (v/w) have beneficial effects on the fungal biomass production and the optimal pH and temperature levels for fungal fruit body production were 5.0 – 5.5 and 21 – 23 °C

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Tables

Table 1. The effect of carbon sources upon the mycelia growth of *Ganoderma lucidum (G.l.), Lentinus edodes* (L.e.) and *Pleurotus ostreatus*

Carbon Sources (w/v)	Fresh Fungal Biomass Weight (g/l)			Final pH (pH units)		
	G. l.	***L. e.***	***P. o.***	***G. l.***	***L. e.***	***P. o.***
Glucose	27±0.14	41±0.05	43±0.03	5.5	5.3	5.1
Maltose	27±0.10	45±0.12	49±0.05	5.8	5.4	5.3
Sucrose	25±0.23	35±0.03	37±0.09	5.1	5.1	5.7
Xylose	26±0.07	38±0.07	35±0.07	5.3	5.5	5.9

Table 2. The effect of nitrogen sources upon mycelia growth of *Ganoderma lucidum (G.l.), Lentinus edodes (L.e.)* and *Pleurotus ostreatus (P.o.)*

Nitrogen Sources (1%, w/v)	Fresh Fungal Biomass Weight (g/l)			Final pH (pH units)		
	G. l.	***L. e.***	***P. o.***	***G. l.***	***L. e.***	***P. o.***
Rice bran	37±0.10	57±0.05	73±0.23	5.5	5.5	5.1
Malt extract	36±0.12	55±0.03	69±0.20	5.3	5.2	5.7
Peptone	35±0.03	41±0.12	57±0.15	4.6	4.9	5.3
Tryptone	36±0.15	38±0.07	55±0.17	5.1	5.3	5.9
Yeast extract	37±0.21	30±0.01	61±0.14	4.3	5.1	5.1

Table 3. The effect of mineral sources upon mycelia growth of *Ganoderma lucidum (G.l.), Lentinus edodes (L.e.)* and *Pleurotus ostreatus (P.o.)*

Mineral Sources (5 mg)	Fresh Fungal Biomass Weight (g/l)			Final pH (pH units)		
	G. l.	***L. e.***	***P. o.***	***G. l.***	***L. e.***	***P. o.***
KH_2PO_4	37±0.15	45±0.07	53±0.12	5.5	5.3	5.9
K_2HPO_4	45±0.07	57±0.05	59±0.07	5.1	5.1	5.7
$MgSO_4 \cdot 5H_2O$	35±0.25	55±0.09	63±0.28	5.6	5.4	6.1

Table 4. The effects of initial pH and temperature upon fruit body formation of *Ganoderma lucidum (G.l.), Lentinus edodes (L.e.)* and *Pleurotus ostreatus (P.o.)*

Initial pH (pH units)	Initial temperature (t^0)	Final Weight of the Fresh Fruit Body (g/kg substratum)		
		G. l.	***L. e.***	***P. o.***
4.5	18	175±0.23	191±0.10	180±0.02
5.0	21	193±0.15	203±0.05	297±0.14
5.5	23	198±0.10	195±0.15	351±0.23
6.0	26	181±0.12	179±0.12	280±0.03
6.5	29	173±0.09	105±0.23	257±0.15

Table 5. The effect of inoculum age upon fruit body formation of *Ganoderma lucidum (G.l.), Lentinus edodes(L.e.)* and *Pleurotus ostreatus (P.o.)*

Inoculum age (h)	**Final Weight of the Fresh Fruit Body (g/kg substratum)**		
	G. l.	***L. e.***	***P. o.***
264	123±0.14	128±0.05	135±0.23
240	141±0.10	150±0.28	157±0.17
216	154±0.12	195±0.90	193±0.15
192	155±0.23	221±0.25	215±0.05
168	169±0.37	235±0.78	241±0.07
144	210±0.20	248±0.03	259±0.12
120	230±0.15	253±0.05	264±0.21
96	215±0.09	230±0.15	253±0.10
72	183±0.05	205±0.23	210±0.05

Table 6. The effect of inoculum volume upon fruit body formation of *Ganoderma lucidum (G.l.), Lentinus edodes (L.e.)* and *Pleurotus ostreatus (P.o.)*

Inoculum Volume (v/w)	**Final Weight of the Fresh Fruit Body (g/kg substratum)**		
	G. l.	***L. e.***	***P. o.***
7.0	234±0.12	215±0.20	220±0.05
6.5	245±0.15	248±0.23	251±0.20
6.0	253±0.15	257±0.07	280±0.15
5.5	243±0.12	235±0.03	247±0.07
5.0	255±0.23	215±0.15	235±0.03

Identification of *Plum Pox Virus* isolates from Transylvania Region, using RFLP method

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Keywords: sharka, plum, restriction enzymes, PCR, specific primers

ABSTRACT

This study was conducted to determine the presence of plum pox virus (PPV) (family Potyviridae, genus Potyvirus) in different regions of Transylvania. The disease mainly affects apricot, plum, and peach. The genus *Potyvirus* was first detected in Bulgaria in 1917; since then, it has spread to most of eastern and central Europe and the Mediterranean basin. We collected and investigated fifty seven PPV samples, who were molecular determined by RT-PCR (reverse-transcription polymerase chain reaction) targeting the genomic region (Cter)CP with specific markers and also with RFLP (restriction fragment length analysis). Analysis distinguished the two major strains, D and M, based on *Rsa I* polymorphism located in (Cter)CP. Results showed the existence of three groups of isolates belonging to D, M and PPV-rec (PPV recombinant) serotypes; PPV-D predominated from isolates studied (87,7%), while the remaining 5,3% isolates belonged to PPV-M and 7% belonged to PPV-rec.

INTRODUCTION

Sharka, caused by Plum pox virus (PPV), is an economically important disease of plum, apricot and peach in Romania. Sharka control is based on the use of tolerant and resistant varieties. This approach demands PPV strain identification, because isolates vary in their rate of spread by aphid, host range and pathogenicity (Markovic, 2006).

The genus Potyvirus was first detected in Bulgaria in 1917 (Atanassov, 1932); since then, it has spread to most of eastern and central Europe and the Mediterranean basin. In Pennsylvania, peach, plum, nectarine and apricot are the four commercial stone fruit susceptible to PPV, Strain D. In addition, certain native and ornamental Prunus and even some perennial weeds may become infected by PPV.

Potyviruses have a single-stranded plus-sense RNA genome of about 10 kb harboring a single open reading frame (ORF). Two main subgroups of PPV isolates have been recognized, PPV-M and PPV-D (Candresse et al., 1998).

It is the only recognized potyvirus infecting Prunus. The introduction of PPV to a new country or region is usually through propagative materials and the subsequent distribution of contaminated materials. The secondary spread can be rapid and results from aphid transmission.

The first evidence of homologous RNA recombination in PPV was reported in Yugoslavia, where the genome was derived from two wild PPV strains (Cervera et al., 1993).

In Romania, the PPV is widespread in plum orchards causing serious yield losses especially on sensitive cultivars (Minoiu, 1997, Zagrai et al, 2001). PPV-rec strain was identified for the fist time in natural infected plum tees from Bistrita area (Zagrai, et al, 2006).

PPV-D (Dideron) was originally described from apricot trees in southeastern France, PPV-M (Marcus) from peach trees in Greece. PPV-M isolates are more aggressive in peach, are aphid vectored more efficiently, and spread more rapidly in an

orchard than the D strain. PPV-M has been reported to be seed transmitted, while other PPV strains are known not to be transmitted through seeds. Both PPV strains M and D infest peach, plum, and apricot.

PPV symptoms can be present in young leaves in the spring and/or on developing fruit. Some trees show no symptoms on leaves or fruit. Plum Pox symptoms vary according to cultivar, age, and nutritional status of the host and may also vary with environmental factors, such as temperature. Severity of symptoms may differ according to the specific strain of PPV. The virus may be detected at the bottom of a branch but not at the tip or it may be detected on some fruit and leaves but not others. Symptoms of PPV often appear 3 years following initial infection. However, serological tests performed in the laboratory can detect the virus before visible symptoms occur (Minoiu, 1997).

Plum Pox and other plant viruses are difficult or impossible to control once they are in a field setting since the only way to eliminate the virus is to destroy the host. Therefore, disease prevention is the only practical management strategy to avoid virus problems.

This study was performed to determine the presence of PPV in different regions of Transylvania and to characterize PPV isolates by molecular (RT-PCR/RFLP) techniques.

MATERIAL AND METHODS

The PPV isolates were collected from different regions in Transylvania: Maramures, Satu-Mare, Salaj, Cluj, Mures and Hunedoara, based on typical symptoms on leaves. For RNA extraction was used Qiagen One Step Kit, and molecular characterization was confirmed by RT-PCR using pair of primer P1/P2.

Isolation of RNA. Total RNA was extracted from 100 mg fresh material plant using the RNeasy Plant Minikit (Qiagen, Hilton, Germany). The extract was homogenized by mortar and pestle and mix it RLT buffer (450 µl) which contain 2-mercapthoethanol. The extract was transferred into a 1.5 ml eppendorf tube and incubated at 56^0C for 3-5 min. The lysate was pipetted directly onto a QIA shredder spin column (lilac) placed in 2-ml collection tube, and centrifuged for 2 min at maximum speed (14,000 rpm for 2 min). After centrifugation, nucleic acids were precipitated from the supernatant with 0.5 volume ethanol (96-100%) and mixed immediately by pipetting. The pellet was washed in RNeasy mini column (pink) with 700 µl buffer RW1, 500µl of RPE, and centrifuged at 10,000 rpm for 2 min and 15 sec. Then the pellet was resuspended after drying in 50 µl sterile water.

Detection and characterization of PPV isolates by PCR. For the general detection of PPV, primers located near the 3` end of the coat protein (CP) gene were used to amplify a 243 bp fragment: P1: 5`-3` ACC GAG ACC ACT ACA CTC CC and P2: 5`-3` CAG ACT ACA GCC TCG CCA GA (Wetzel et al., 1991).

The reverse transcription (RT) and PCR assays were performed in a thermal cycling program (Qiagen). PCR products were amplified using Qiagen one Step kit in a 50 ml volume. The following conditions were used for amplification: reverstranscription 30 min at 50^0C, denaturation (RT inactivation) at 94^0C for 15 min, 35 cycles of 94^0C for 1 min, 61^0C for 1 min and 72^0C for 1 min and a final extension at 72^0 C for 10 min. RT-PCR products were size fractionated by migration on a 1,4% agarose gel in 1X TAE Buffer and visualized and photographed using an Alpha Innotech camera system under UV after ethidium bromide staining.

PCR/RFLP Analysis. For restriction fragment length polymorphism (RFLP) analysis of the 243 bp fragment, RT-PCR amplified products were incubated with 5 units of *Rsa*I or *Alu*I for 2 hours at 37^0C. Digested products were separated on an 8% polyacrylamide gel in vertical electrophoresis, then stained and photographed as above.

RFLP analysis of both fragments showed that fifty isolates were PPV-D, three were PPV-M and four isolates PPV-rec. The first report of naturally infected plum trees with PPV-rec strain in Romania was at Fruit Research and Development Station Bistrita (Zagrai et al, 2006).

RESULTS AND DISCUSSION

Fifty seven samples from different orchards in Transylvania were analyzed by RT-PCR with the specific primers (P1 and P2) and RFLP analysis using the restriction enzymes *Rsa*I and *Alu*I. These enzymes were previously described to be useful for strain differentiation.

RT-PCR analysis. PPV-infection was detected in all samples. The P1/P2 primers revealed and confirmed the presence of the virus by amplifying the expected 243-bp fragment from the CP gene (figure 1).

RFLP analysis. The amplified 243-bp fragment, derived from the C-terminal region of the CP coding region, was treated with the endonucleases *Alu*I and *Rsa*I.

Using RFLP analysis the D strain of PPV, considered the nonepidemic form of PPV, was detected in the fifty samples analyzed. The more aggressive M strain, considered the epidemic form, was detected only in three samples. Mixed infections by PPVM and PPV-D were found in the four tested *Prunus* samples infected by PPV-Rec. (table 1).

In case of PPV-D strain, following the digestion with the restriction enzyme *Rsa*I, the expected 182 pb and 61 pb fragments were amplified (for example: samples Calacea 2,3,4,6,7,8,9 and 10) (fig 2).

In case of PPV-M strain, following the digestion with the restriction enzyme *Rsa*I, the PCR product of 243 pb length was not digested and appeared as a single band after gel electrophoresis (for example: the sample Calacea 5) (fig. 2).

In case of mixed infections by PPV-M and PPV-D, the restriction enzyme cuts only PCR products from strain PPV-D, generating three expected fragments of 243 pb, 182pb and respectively 61 pb length (for example: the sample Calacea 1).

However, for accurate evaluation of the occurrence of different strains of PPV, it is necessary to investigate a larger number of isolates obtained from plum orchards from the Romanian area.

*Alu*I (figure 2) cut all of the RT-PCR products from the isolates. This result showed that no mutation occurred in the CP gene of the isolated strains, but also confirmed the RT-PCR product specificity (for example: the samples Reghin 1-9).

This study has shown that in some orchards, PPV-D or PPV-M isolates coexisted with PPV recombinants. The presence of several strains in the same orchard may originate from the nursery stock, or more likely is due to aphid-borne infections coming from neighboring PPV infected orchards.

CONCLUSIONS

1. PPV-Rec strain was identified for the second time in Romania in natural infected plum trees from Transylvania.
2. The combination of the PPV NIb RT-PCR with RFLP analysis will be valuable for studies of epidemiology and phylogeny.
3. Although mixed infections between conventional (M or D) and recombinant strains were not found in the same tree, this possibility could be investigated further in future studies.
4. These polymorphic spatial populations in the same location present the necessary conditions for potential new recombinants.

ACKNOWLEDGEMENTS

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Figures and table

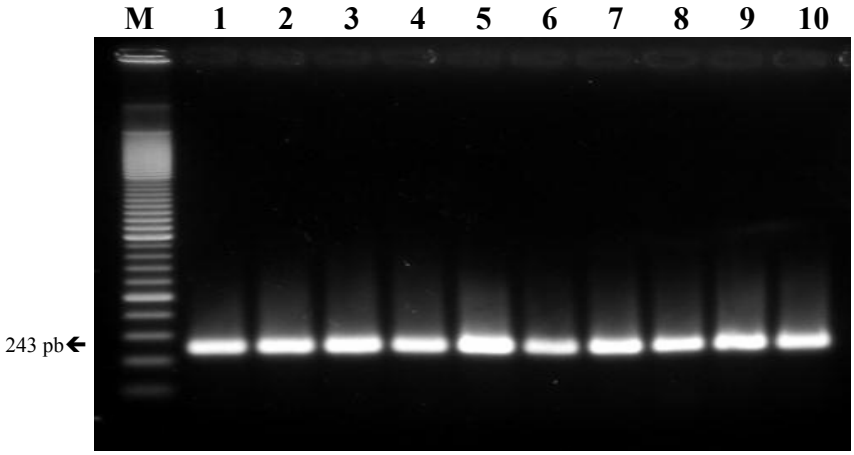

Fig. 1. The diagnosis of Plum Pox virus in 10 infected leaves, using P1 and P2 primers (samples Reghin 1-10)

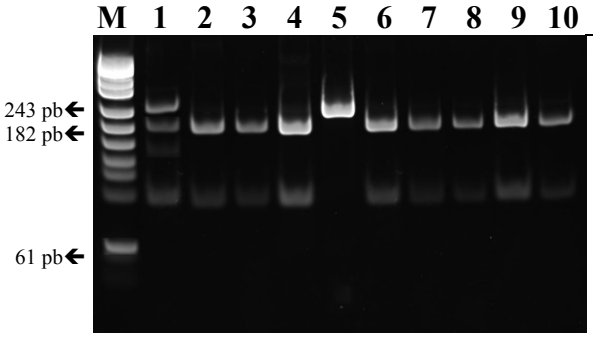

Fig. 2. Viral strain differentiation with *Rsa*I, using RFLP technique

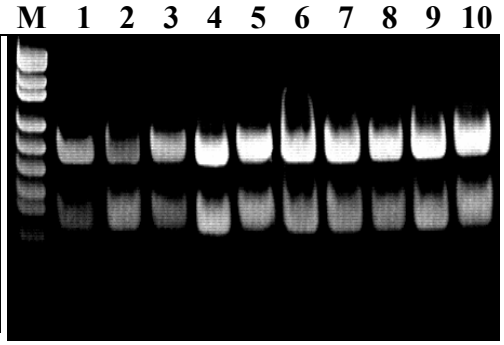

Fig. 3. Polyacrylamide gel separation of *Alu*I digested PCR products.

Table 1. Results of molecular analysis based on RT-PCR and RFLP metod

Samples	RT-PCR	RFLP				Samples	RT-PCR	RFLP			
	P1/P2	RsaI			AluI		P1/P2	RsaI			AluI
		D	M	D+M				D	M	D+M	
Dedrad 4	+	+	-	-	+	Săpânța 3	+	+	-	-	+
Dedrad 5	+	+	-	-	+	Săpânța 4	+	+	-	-	+
Dedrad 6	+	+	-	-	+	Săpânța 5	+	+	-	-	+
Dedrad 7	+	+	-	-	+	Tisa 6	+	+	-	-	+
Dedrad 8	+	+	-	-	+	Tisa 7	+	+	-	-	+
Dedrad 9	+	+	-	-	+	Tisa 8	+	+	-	-	+
Dedrad 10	+	+	-	-	+	Tisa 9	+	+	-	-	+
Reghin 1	+	+	-	-	+	Tisa 10	+	+	-	-	+
Reghin 2	+	+	-	-	+	Bixad 1	+	+	-	-	+
Reghin 3	+	-	-	+	+	Bixad 2	+	+	-	-	+
Reghin 4	+	-	-	+	+	Bixad 3	+	+	-	-	+
Reghin 5	+	-	+	-	+	Bixad 4	+	+	-	-	+
Reghin 6	+	-	+	-	+	Bixad 5	+	+	-	-	+
Reghin 7	+	-	-	+	+	Bixad 6	+	+	-	-	+
Reghin 8	+	+	-	-	+	Bixad 7	+	+	-	-	+
Reghin 9	+	+	-	-	+	Bixad 8	+	+	-	-	+
Reghin10	+	+	-	-	+	Bixad 9	+	+	-	-	+
Calacea 1	+	-	-	+	+	Bixad 10	+	+	-	-	+
Calacea 2	+	+	-	-	+	Geoagiu 1	+	+	-	-	+
Calacea 3	+	+	-	-	+	Geoagiu 2	+	+	-	-	+
Calacea 4	+	+	-	-	+	Geoagiu 3	+	+	-	-	+
Calacea 5	+	-	+	-	+	Geoagiu 4	+	+	-	-	+
Calacea 6	+	+	-	-	+	Geoagiu 5	+	+	-	-	+
Calacea 7	+	+	-	-	+	Cluj 6	+	+	-	-	+
Calacea 8	+	+	-	-	+	Cluj 7	+	+	-	-	+
Calacea 9	+	+	-	-	+	Cluj 8	+	+	-	-	+
Calacea 10	+	+	-	-	+	Cluj 9	+	+	-	-	+
Săpânța 1	+	+	-	-	+	Cluj 10	+	+	-	-	+
Săpânța 2	+	+	-	-	+	-	-	-	-	-	-
Total	**57**	**50**	**3**	**4**	**57**	**Total**	**57**	**50**	**3**	**4**	**57**
%	**100**	**87,7**	**5,26**	**7,01**	**100**	**%**	**100**	**87,7**	**5,26**	**7,01**	**100**

Genetic similarity assessment and molecular characterization of some *Castanea* genus genotypes using RAPD markers

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Keywords: genetic relatedness, chestnut, molecular markers, germplasm management, breeding programs

ABSTRACT

The potential use of RAPD technique for characterization and assessment of genetic relationships was investigated in ten *Castanea* genotypes. Twelve of the thirteen decamer primers yielded scorable amplification patterns. These primers generated polymorphic bands among the genotypes studied. Some of the primers produced no amplification or unreadable gel smears.

A dendrogram was built using neighbour joining analysis of Jaccard's coefficient of similarity. The accessions clustered into two main groups and the values of genetic distances between analysed data shows that there are some genetic differences.

The chestnut accessions held at S.C.D.P. Vâlcea come from different populations. In this context, the scientific interest for identification, evaluation and long time conservation in the national collections of valuable accessions for this species is growing.

RAPD is therefore a reliable technique for distinguishing among *Castanea* accessions cultivated at S.C.D.P. Vâlcea, and also for identifying the new cultivars as well as assessing the genetic similarity among different genotypes useful in fruit breeding selection programs.

INTRODUCTION

Chestnut (*Castanea sativa*) is a forest species of considerable agricultural and socio-economic importance spread over the southern part of Europe (Fineschi et al., 2000). The good quality fruits made chestnut an important source of food for human population, particular in rural area and the cultivation of chestnut in Europe reaching a peak at the beginning of 1940's. Since then, the fungal diseases (ink disease by *Phytophtora cinnamomi, Phytophtora cambivora* and canker blight by *Chryphonectria parasitica*) and the social-economic changes (the depopulation of the hillside and mountain areas and change of the food habits) determined a reduction of chestnut cultivation (Marinoni et al., 2003). At the beginning of the 1980's chestnut became of more interest for its fruit but also for the wood production (Buonous et al., 2000). In the present time, Turkey, Iberian Peninsula and Italy hold the leadership for chestnut production in Europe, collectively accounting for 55.3% of the 240000 tons world's annual production, the first two regions being also of particular biological value for sweet chestnut. Portugal only has an annual production of 20000 tons (Goulao et al., 2001). In the last thirty years the scientific community became more interested in the rescue, collection and exploitation of chestnut germplasm. Many Romanian populations of chestnut are of natural origin, composed of few trees separated by geographical natural obstacles. Genetic structures of such populations have been formed by natural selection and have not changed significantly due to the long reproductive life of trees – a hundred and more years and represent a valuable source of genetic variability. The chestnut accessions held at S.C.D.P. Vâlcea come from different populations. In this context, the scientific interest for identification, evaluation and long time conservation in the national collections of valuable accessions for this species is growing. Traditional methods of accession identification rely on morphological characteristics, which can be

influenced by environmental conditions, leading to erroneous characterization. Recently the development of DNA markers providing plenty of polymorphism has enabled the rapid identification of phenotypically extremely similar accessions. Furthermore, molecular identification techniques can be used at any stage of plant development and they are not affected by environmental factors. Among molecular markers used for identification of the chestnut accessions, RAPD markers are the most common (Fineschi et al. 1993, Botta et al. 1999, Seabra et al. 1998, Galderisi et al 1998, Santana et al. 1999, Oraguize et al. 1998). The preservation of the chestnut germplasm held at S.C.D.P. Vâlcea represents a real interest for Romania and in order to achieve this, genetic evaluation followed by vegetative regeneration of the accessions has to be carried out.

MATERIALS AND METHODS

Plant material The ten genotypes used in this study were obtained from the *Castanea* collection maintained at S.C.D.P. Vâlcea and labeled VL 302, VL 303, VL 304, VL 305, VL 307, VL 309, VL 312, VL 317, VL 318 and VL 319.

DNA extraction Young leaves from ten accessions (genotypes) were collected in spring and immediately stored at -80ºC prior to DNA extraction. Total DNA was extracted using the protocol developed by Lodhi *et al.* (1994) and modified by Pop *et al.* (2003). Two pieces of one cm^2 of leaf tissue were ground to fine powder in liquid nitrogen in an Eppendorf tube. 700 µL of 65ºC preheated extraction buffer (100 mM Tris-HCl, 20 mM sodium EDTA, pH=8, 1,4 M NaCl, 2 % (w/v) CTAB, 2% PVP, 5mM ascorbic acid and 4mM DIECA, the last three components being added to the extraction buffer just before the heating at 65^0C on the water bath) were added to the tube. The tube was then incubated at 65ºC for 25 minutes. The lysate was extracted with 700 µL of chloroform/isoamyl alcohol (24:1) and centrifuged for 15 min at 11000 rpm in a desktop centrifuge. In order to precipitate the nucleic acids, the aqueous fraction was mixed with an equal volume of 5M NaCl and then with 600 µL of ice cold 96% ethanol. The nucleic acid precipitate was washed two times in 76% ethanol and air dried before being resuspended in 50 µL TE buffer (10 mM Tris-HCl pH 8.0, 1mM disodium EDTA). The concentration and purity of extracted DNA were determined using a Nanodrop ND-1000 Spectrophotometer. DNA concentrations were between 300 ng/µL and 1870 ng/µL, and the A_{260}/A_{280} readings between 1,70-2,05. DNA was diluted to 50 ng/µL and used for PCR amplification.

DNA amplification and electrophoresis conditions PCR amplification reactions were carried out as described by Williams *et al.* (1990). Reaction mixtures (25 µL total volume) consisted of 250 ng DNA, 9,3 µL distilled H_20 for PCR reactions, 2 µL PVP (poly vinyl pyrrolidone), 5 µL GoTaq Flexi green buffer (Promega Corp., Madison, WI, USA), 2,5 µL $MgCl_2$ (Promega Corp., Madison, WI, USA), 0,5 µL dNTP mix (Promega Corp., Madison, WI, USA), 0,5 µL RAPD primer (Microsynth, Balgach, Switzerland), 0,2 µL GoTaq polymerase (Promega Corp., Madison, WI, USA). DNA amplification was carried out in a 96 Well Gradient Palm-Cycler CG1-96 (Corbett Research, Sydney, Australia) programmed for 1 cycle of 3 min at 95ºC, followed by 45 cycles of 1 min at 93ºC, 1 min at 34ºC and 1 min at 72ºC. After a final incubation for 10 min at 72ºC the samples were stored at 4ºC prior to analysis. The PCR amplified products were size fractionated by migration on a 1,4% agarose (Sigma-Aldrich) gel in 1X TAE Buffer (242 g Tris Base (MW=121.1), 57.1 mL Glacial Acetic Acid, 100 mL 0.5 M EDTA) at 0,29 V/cm^2 for 2 hours. The molecular marker used was 100bp DNA

Step Ladder (Promega Corp., Madison, WI, USA). Gels were visualized on a UV light Biospectrum AC Imaging System (UVP BioImaging Systems, Upland, CA) after staining with 0,5 μg/μl Ethidium Bromide for 25 min.

Data analysis Gel images were analyzed using TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) and the bands resulted after RAPD amplification were scored as present (1) or absent (0), data entered into a binary matrix. The genetic distance between accessions was calculated using Jaccard's coefficient of similarity. Cluster analysis was conducted with a neighbour-joining algorithm using FreeTree software (Hampl V. et al., 2001) and a dendrogram was constructed, using the TreeView software (Page 1996).

RESULTS AND DISCUSSIONS

DNA amplification with RAPD primers A total of 13 decamer primers were used to amplify DNA extracted from the ten *Castanea* genotypes used in this study. Almost all the primers yielded scorable amplification patterns (Table 1). Primer OPO-14 produced no amplification. The presence of the different patterns generated by RAPD primers shows variance between the accesions from the genetic point of view. This difference will be further analyzed using other types of molecular markers (Inter Simple Sequence Repeats-ISSR or Simple Sequence Repeats-SSR) in order to obtain a more precise molecular characterization of the studied genotypes. Figure 1 shows bands resulted from DNA amplification in the ten *Castanea* accessions using OPAL 20 primer. Primers OPD-20, OPF-04 and OPH-02 generated the most polymorphic bands, 21, respectively 17. Isolation of the polymorphic bands from gel followed by cloning and sequencing of the DNA fragments could lead to design specific primers. Our results agree with earlier studies using RAPD at *Castanea* species, RAPD markers revealing the genotypic diversity of *Castanea* (Goulao et al., 2001, Solar et al., 2005). RAPD is therefore a reliable procedure for distinguishing between different *Castanea* accessions cultivated at S.C.D.P. Vâlcea. The collected data will be useful in developing DNA fingerprinting techniques for routine use in the orchard, to distinguish the valuable genotypes used in selection.

Genetic assessment of accessions A dendrogram was built using neighbour joining analysis of Jaccard's coefficient of similarity (Fig.2). The dendrogram structure was not affected by the order in which samples were analyzed. The accessions clustered into two main groups. In the first group clustered accessions VL 302, VL 303, VL 309, VL 312, VL 317, VL 318 and VL 319, while in the second group clustered accessions VL 304, VL 307 and VL 305. The genetic distance between accession calculated using Jaccard's coefficient of similarity was the smallest between VL309 and VL318 - 0.96296 and the biggest between VL302 and VL317 - 0.33333 as it can be seen in Table 2.

CONCLUSIONS

Following agarose gels analysis it was certified that from the thirteen primers used, twelve yielded scorable amplification polymorphic bands.

The polymorphic pattern in the analyzed accessions shows that a genetic difference is present among the ten *Castanea* genotypes studied in this project. In order to certify these differences further studies using ISSR or SSR markers will take place.

Our results agree with earlier studies using RAPD with *Castanea* genus. RAPD is therefore a reliable procedure for distinguishing among the *Castanea* accessions

cultivated at S.C.D.P. Vâlcea. The data collected will be useful in developing DNA fingerprinting techniques for routine use in the orchard, to distinguish the valuable genotypes used in selection.

Using modern molecular analysis techniques for characterizing valuable chestnut accessions and vegetative regeneration of these genotypes will allow the conservation of the *Castanea* existent genetic resources available at S.C.D.P. Vâlcea.

The chestnut accessions held at S.C.D.P. Vâlcea come from different populations. In this context, the scientific interest for identification, evaluation and long time conservation in the national collections of valuable accessions for this species is growing.

ACKNOWLEDGEMENTS

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Tables

Table 1. Primers used for differentiation of the thirty nine analyzed *Prunus* accessions (+) - the presence of specific, polymorphic DNA fragment generated in RAPD reaction with described primer, (-) - the absence of specific, polymorphic DNA fragment generated in RAPD reaction with described primer

No.	Primer name	Primer sequence	Castanea	No. of polymorphic bands/primer
1.	OPA-01	5'- CAGGCCCTTC- 3'	+	8
2.	OPAL-20	5'-GAACCTGCGG-3'	+	9
3.	OPB-12	5'- GTAGACCCGT- 3'	+	10
4.	OPC 14	5'-AGGAGTCGGA- 3'	+	14
5.	OPF-04	5'- GGTGATCACC- 3'	+	17
6.	OPFIO	5'-GGAAGCTTGG-3'	+	6
7.	OPE 14	5'-GGTGCGGGAA-3'	+	14
8.	OPG 07	5'-GAACCTGCGG-3'	+	5
9.	OPO-14	5'- AGCATGGCTC- 3'	-	-
10.	OPO-16	5'- TCGGCGGTTC- 3'	+	14
11.	OPD-20	5'- ACCCGGTCAC- 3'	+	21
12.	OPH-02	5'-TCGGACGTGA-3'	+	17
13.	OPF 13	5'-GGCTGCAGAA-3'	+	6

Table 2 Genetic distance between *Castanea* accessions calculated using Jaccard's coefficient of similarity

	VL 318	VL 319	VL 317	VL 302	VL 305	VL 303	VL 312	VL 304	VL 307	VL 309
VL 318		0.89231	0.84848	0.9	0.91026	0.91026	0.925	0.88889	0.89706	0.96296
VL 319	0.89231		0.6875	0.6087	0.80769	0.74324	0.76316	0.775	0.89333	0.84211
VL 317	0.84848	0.6875		0.33333	0.62821	0.61842	0.54795	0.64634	0.75325	0.77586
VL 302	0.9	0.6087	0.33333		0.6	0.57317	0.50633	0.58621	0.71429	0.77273
VL 305	0.91026	0.80769	0.62821	0.6		0.43836	0.59756	0.4	0.58108	0.65
VL 303	0.91026	0.74324	0.61842	0.57317	0.43836		0.49333	0.46753	0.62667	0.61017
VL 312	0.925	0.76316	0.54795	0.50633	0.59756	0.49333		0.54878	0.68354	0.66102
VL 304	0.88889	0.775	0.64634	0.58621	0.4	0.46753	0.54878		0.54667	0.6875
VL 307	0.89706	0.89333	0.75325	0.71429	0.58108	0.62667	0.68354	0.54667		0.72222
VL 309	0.96296	0.84211	0.77586	0.77273	0.65	0.61017	0.66102	0.6875	0.72222	

Figures

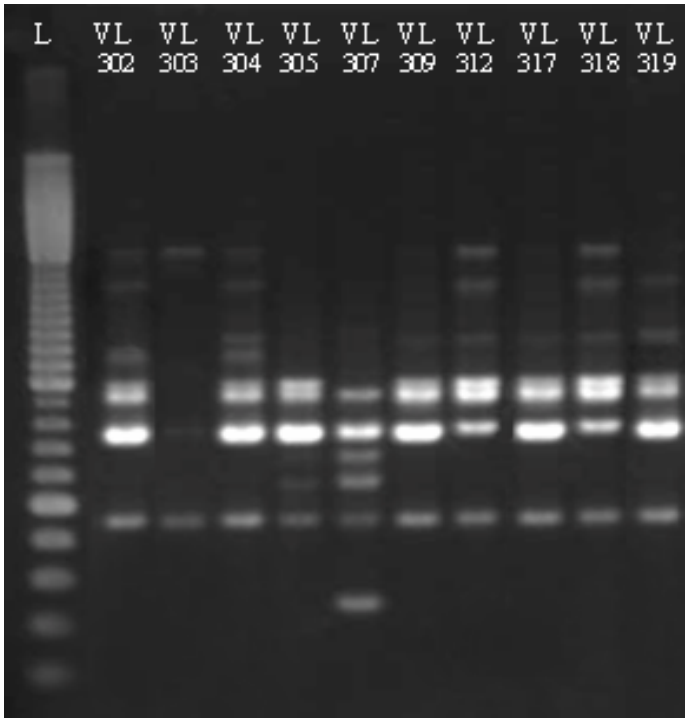

Fig. 1. Bands resulted after DNA amplification with OPAL 20 primer.
L – 100 bp Ladder, 1-39 – *Castanea* accessions VL 302, VL 303, VL 304, VL 305, VL 307, VL 309, VL 312, VL 317, VL 318 and VL

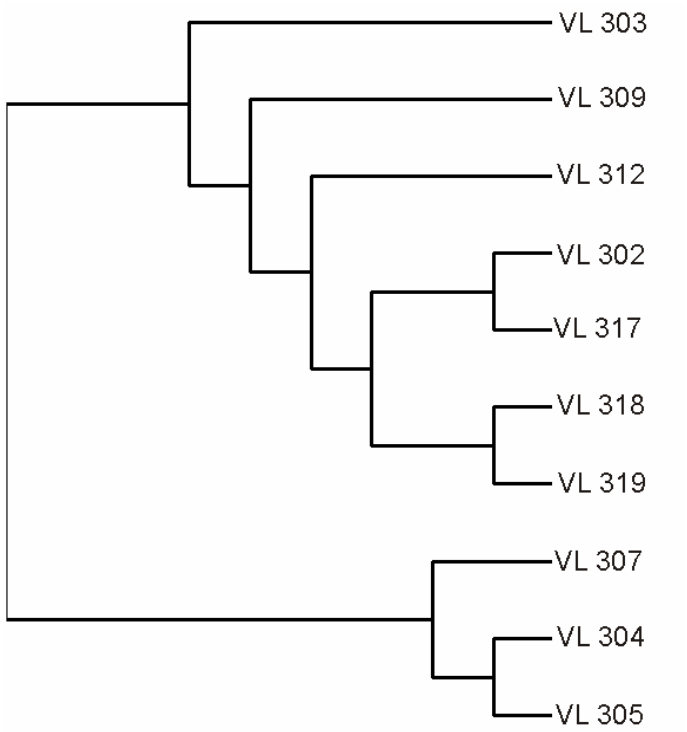

Fig. 2. Dendrogram built using neighbour joining analysis of Jaccard's coefficient of similarity between *Castanea* accessions

Analysis regarding the influence of non-conventional technologies on soil physical properties and corn yields

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Keywords: minimal tillage, physical properties, yields

ABSTRACT

In the present paper, it is presented the classical system comparatively with non-conventional technologies (conservative) for soil tillage referring to direct sowing as well as to the influence of corn cultivation method on soil physical properties and obtained yields.

The experiences were developed in the pedo-climatic conditions of Banat Field, within the Didactic Station of USAMVB Timişoara, during 2003-2005.

Elaboration of alternative technologies for soil tillage that may insure conservation and maintaining of its yield potential as well as the reduction of energy expenditures represent current basic needs in order to develop and perform sustainable agriculture.

INTRODUCTION

The flaws attributed to classical system for soil tillage, which tends to be an intensive system including undoubtedly plough works have determined the appearance and fast extension of soil conservation concept.

The main factor that determined coming out and development of soil tillage using non-conventional technologies rises from degradation and excessive erosion of soils, the main effect of an intensive, conventional agriculture. Elaboration of different types of agriculture was accelerated by petrol shortage during the '70 and high costs for fuels, numerous researches and studies demonstrating in most cases that it is possible to obtain crop yields that may compete with those of conventional agriculture.

MATERIALS AND METHODS

The data included in the present paper are underpinned by experimental results obtained at Didactic Research Station, USAMVB Timişoara, Timiş County.

The researches were performed considering a vertic chernozem strongly gleyed presenting salinization and alkalization processes in depth (below 100 m), extremely profound on bi-stratified parental materials, middle fine, medium clayed/ medium clayed.

The soil profile shows the following sequence of horizons: Ap - Ap - Amk - A/Cyk - CykG -CyGo - CcaGo - $CcaG_0$ - CcaGr

Climate conditions during the experimental period 2003-2005 were characterized by annual average temperatures between 10,8 ^oC and 11,3 ^oC while precipitation regime for the same period registered values between 577 mm and 791,1 mm.

The experimental setting up considered to study the influence of direct sowing system on soil physical properties and yield potential was organized as a stationary experience with the following variants: V_1 (control variant): ploughing using corman plough + harrow with discs; V_2 : ploughing using corman plough – two passages; V_3 :ploughing using corman plough + harrowing using harrow with discs; - two passages; V_4 :harrowing using combined rotating hoe; V_5 : harrowing using harrow with discs + soil preparation using vibroculture machine; V_6: direct sowing.

RESULTS AND DISCUSSIONS

The soil physical, physical-mechanical and hydro-physical properties are the main elements that define the limits of the physical and edaphic environment where occur physical-chemical phenomena that underpin and insure plant nutrition and porous-polyphasial environment combining all three phases (solid, liquid and gas) as well as intermediate phases including biological and physical-chemical activities.

The crop technologies may influence the main physical properties (apparent density Da, total porosity TP, compactness degree) as well as obtained yields.

Non-conventional soil tillage and particularly direct sowing are aiming the effectiveness of crop yields, preservation and increase of soil fertility. The obtaining of equal or reduced yields comparing the classical system was considered more lucrative, firstly due to reduction of expenditures for ploughing, this showing the highest rate in the classical system. Soil tillage system and climate conditions were the main factors that strongly influenced corn yields (table 4).

CONCLUSIONS

1. Evolution of apparent density considering depths of 0-20 cm during all three years with soil tillage applying direct sowing system, as well as for the rest of variants with non-conventional technologies showed significant changes only comparing the classical system and demonstrates that direct sowing increases soil compactness but without negative influences on crop development;
2. In accordance with soil tillage methods and correlated with clayed medium texture of a vertic chernozem that is found in the experimental plot, the values of total porosity ranging between normal limits, indicated moderate soil aeration;
3. During the experimental cycle, the values of compactness degree between 0-20 cm depth, indicated slight aerated to slight compact soil;
4. Corn yield registered values between 8190-8450 kg/ha for the variants with minimal soil tillage while for direct sowing was of 8400 kg/ha. Comparing the classical system (7536 kg/ha, control variant), yields are smaller (90,9-96,1 %) for the variants with minimal soil tillage and registered values of 89,2 % for direct sowing.

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Tables

Table 1. Evolution of bulk density (D_b, g/cm^3) depending on tillage system

Year	Depth (cm)	Tillage system					
		V_1 Plough+ Disk harrow	**V_2 Disk harrow x 2**	**V_3 Combined rotating harrow**	**V_4 Disk harrow + Combined rotating harrow**	**V_5 Disk harrow + Vibrocultor**	**V_6 Direct drill**
2003	0-10	1,27	1,35	1,34	1,33	1,31	1,38
	10-20	1,37	1,38	1,39	1,37	1,36	1,40
	20-30	1,47	1,48	1,42	1,41	1,41	1,43
	30-40	1,51	1,51	1,45	1,43	1,45	1,42
2004	0-10	1,31	1,34	1,37	1,33	1,33	1,39
	10-20	1,35	1,40	1,39	1,37	1,38	1,41
	20-30	1,40	1,42	1,41	1,39	1,39	1,43
	30-40	1,44	1,44	1,44	1,42	1,43	1,43
2005	0-10	1,32	1,35	1,38	1,33	1,34	1,40
	10-20	1,36	1,41	1,41	1,38	1,39	1,41
	20-30	1,41	1,43	1,43	1,40	1,40	1,43
	30-40	1,46	1,45	1,45	1,43	1,43	1,45

Table 2. Evolution of total porosity (TP, %) depending on tillage system

Year	Depth (cm)	Tillage system					
		V_1 Plough+ Disk harrow	**V_2 Disk harrow x 2**	**V_3 Combined rotating harrow**	**V_4 Disk harrow + Combined rotating harrow**	**V_5 Disk harrow + Vibrocultor**	**V_6 Direct drill**
2003	0-10	51	48	49	49	50	47
	10-20	49	49	48	49	49	48
	20-30	46	45	47	48	48	47
	30-40	44	44	46	47	46	47
2004	0-10	50	49	48	49	49	47
	10-20	50	48	48	49	49	47
	20-30	48	48	48	49	49	47
	30-40	47	47	47	47	47	47
2005	0-10	49	48	47	49	49	46
	10-20	49	47	47	49	48	47
	20-30	48	47	47	48	48	47
	30-40	46	46	46	47	47	46

Table 3. Evolution of the degree of settling (DS, %) depending on tillage system

Year	Depth (cm)	Tillage system					
		V_1 Plough+ Disk harrow	V_2 Disk harrow x 2	V_3 Combined rotating harrow	V_4 Disk harrow+ Combined rotating harrow	V_5 Disk harrow+ Vibrocultor	V_6 Direct drill
2003	0-10	0,8	6,7	5,7	4,9	3,5	8,6
	10-20	6,2	6,5	7,2	6,2	5,5	7,9
	20-30	12,6	13,5	9,2	8,3	8,3	10,0
	30-40	15,2	15,2	11,2	9,5	11,0	9,1
2004	0-10	3,5	5,9	8,1	4,7	5,2	9,6
	10-20	4,6	8,2	7,7	6,2	6,7	9,1
	20-30	7,3	8,8	8,5	6,9	6,6	9,7
	30-40	10,3	10,0	10,0	9,1	9,8	9,8
2005	0-10	4,5	6,7	8,9	5,2	5,7	10,4
	10-20	5,3	9,1	9,1	6,7	7,2	8,9
	20-30	8,3	10,0	9,7	7,3	7,8	10,0
	30-40	11,4	11,2	10,7	9,8	9,8	11,0

Table 4. Influence of tillage system on maize yield

No. crt.	Specification	Tillage system					
		V_1 Plough+ Disk harrow	V_2 Disk harrow x 2	V_3 Combined rotating harrow	V_4 Disk harrow+ Combined rotating harrow	V_5 Disk harrow+ Vibrocultor	V_6 Direct drill
1	Standard grain production (kg/ha)	7536	6854	6933	7141	7249	6725
2	Relative production (%)	100,00	90,9	91,9	94,7	96,1	89,2
3	Difference in production (kg/ha)	-	-682	-603	-395	-287	-811
4	Significance of differences	Mt	O	-	-	-	O

DL 5% =807,14 Kg/ha
DL 1% = 1152,11 Kg/ha
DL 0,1% =1487,32 Kg/ha

An ash dump's revegetation strategy, based on the management of *Rhizobium* and Arbuscular mycorrhizae

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Keywords: *Robinia pseudacacia* L., mycorrhizae process, biomass production, N-fixation.

ABSTRACT

The paper is presenting some partial results of a research programme, proposed for shrubland ecosystem recovery, to improve the ash dump stabilization, and to restore a stable and diversified matorral in a pilot-unit representative of this specific ecosystem. The research programme approach is based on enhancing the colonization ability of woody legumes belonging to the natural succession. In this context, legumes are of special importance since they are able to take advantage of their ability to fix N, in symbiosis with *Rhizobium,* and to form mycorrhizae. In this symbiosis the fungal partner (*Glomus* spp.) colonizes and links root with surrounding ash to play a critical role by improving plant rooting and establishment, helping plants to cope with stress situations such as nutrient deficiencies, drought, contamination with heavy metals and ash dump disturbance.

INTRODUCTION

It is known that the anthropic activities (e.g. coal burning) can create some special ecosystems, thus resulting in "degraded" ecosystem, depending upon the severity of the disturbance. Suitable strategies for their revegetation, based on ecological principles, could be, however, developed to recover vegetation. Many of the soil-borne microbes are bound to the surface of soil particles or found in soil aggregates, while others interact specifically with the plant root system (Glick, 1995); actually, a large number of microorganisms are living in the soil-plant interfaces where a microcosm system, the rhizosphere, develops. Soil microbial dynamics largely govern ecosystem functioning (Kennedy and Smith, 1995) through a number of activities, carried out mainly by rhizosphere micro biota constituents, which are known to enhance soil and plant quality. The functions include: improvement of plant establishment, increased availability of plant nutrients, enhancement of nutrient uptake, protection against cultural and environmental stresses, improvement of soil structure, etc. (Barea and Jeffries, 1995).

Interacting mycorrhizal and rhizobial symbioses seem to be, therefore, important to enhance revegetation.

MATERIALS AND METHODS

Black locust seedlings: A number of 120 acacia seedlings (*Robinia pseudacacia*), of approximately 40 cm height, harvested from the nursery of Starmina heals (Mehedinti county), were placed in plastic bags, with moisten ash, followed by an inoculation at the root system level, for a number of 60 plants, with 15 ml of diluted solution of *Glomus intraradices* spores (55 spores/ml). The prepared seedling were planted on the plateau of the ashes dump in 4 separate zones: V1 - zone with ash, without vegetation, non-infected with mycorrhiza; V2 - zone with grassed ash (*Lolium perene*), without mycorrhiza; V3 -zone with populated ash with mycorrhizal spores, without vegetation; V4 - zone with grassed ash, mycorrhizal inoculated.

Biomass determination: After 60 days of plantation, 15 seedlings from each non-mycorrhizal and mycorrhizal variant were harvested for biomass determination.

Leaves, stems, roots and nodules of each plant were separated and dried at 65°C to a constant mass for dry weight determination.

Total nitrogen content: It was colourimetrically estimated, using Nessler reagent (Delory, 1949; Humphries, 1956).

Determination of phosphorus: The plant material has been digested in nitric-perchloric acid mixture (5:3) and analyzed colourimetrically with malachite green reagent (Fernandez et al., 1985).

Mycorrhizal root infection: Subsamples of fresh root tissue (0.2 g) from each plant were boiled in 10% KOH for 5 min, rinsed in sterile tap water five times, and placed in 2% HCl for 5 min. Roots were stained in 0.05% Trypan blue (Phillips and Hayman, 1970). Mycorrhizal colonization was estimated using the gridline intersection method (Giovanetti and Mosse, 1980), in which the total number of mycorrhizal intersections in each weighed root sample was counted and divided by the total number of intersections in the sample.

Rhizobial root nodules: The root nodules were estimated for each plant, separated and collected to determine their fresh and dry mass (Vejsadova et al., 1992).

RESULTS AND DISCUSSION

Mycorrhizal installation was observed after 60 days of planting, by sampling plants from the 4 experimental variants (15 seedling /variant), with a view to analyze the obtained biomass. The average results are presented in tables 1 and 2. It can be observed that the biggest biomass values were obtained on the mycorrhized variants V3 (4.129 g/d.wt) and V4 (6.329 g/d.wt); a big surface of rizhosphere was created through the root system of *Lolium perene* inoculated in the previous year, leading to the development of the mycorrhiza installation process. The analysis of the simple correlation coefficients (table 3) is presenting a strong and significant influence between the assimilated nitrogen and phosphorus (r=0.98), demonstrating the establishment of a tripartite symbiosis between mycorrhizal fungi-plant-nodules of the N-fixing bacteria (figure 1). Benefits of N-fixing bacteria to AM fungi were previously proved by Johansson et al. (2004) and Rabie et al. (2005). These authors reported that in bacterial-vesicular mycorrhizas-legume tripartite symbiosis relationships nodulation of N-fixing bacteria and establishment of vesicular mycorrhizas often occur simultaneously and synergic ally. Besides, N-fixing bacteria provide fixed nitrogen not only to the plant, but also to the fungus (figure 3). Moreover N-fixing bacteria can also assist in mobilizing nutrients from the ash dump and improving the growth of infected plants (figure 2). Through the calculation of the determination coefficient ($d = r^2x100$), on this study was quantified the percentage of mycorrhiza influence on the N-fixing bacteria: d = 96.04. If so, we suggested that bacterial-AM-legume tripartite symbiosis could be a new approach to increase not only the nutrients but the heavy metal tolerance of legume plants under heavy metal pollution conditions. Nevertheless, the beneficial effects of the micro-symbionts, observed in this study, arouse an interest in considering the role of bacterial-AM-plant tripartite symbiosis in plant-based strategies of remediation and stabilization of ash dump. The isolated effect of the mycorrhiza, through the increase of the phosphorus accumulation, is especially oriented on the biochemical processes from the stem (r_t = 0.96), representing a percentage of 92.16 % and 7.84% on the roots. Without mycorrhizal fungi, the effect of the N-fixing bacteria became manifest mainly on roots and leaves (r_r = 0.99 si r_f = 0.98). The level of grassing the plateau of ash seems to be essential to the enlargement of the colonization rate, through the presence of

viable root systems; in their absence, the mycorrhizal fungi would not be viable. In the same time, the presence and the activity of the woody plants' exo-enzymes in the rizosphere is facilitating the dislocation and the mobilization of the mineral nutrients, which are needed both by plants and N-fixing bacteria's activity, which are present in the root nodules of the plants from the grassed variants. The obtained biomass with mycorrhizal share was of 2.124 g dry weight/plant, comparing with the control (V1). Comparing with the action of the N-fixing bacteria, naturally and constantly present in all the experimental variants, we could consider that the registered enhancements are integrally due to the activity of the mycorrhizal fungi, with a maximum density of the population in V4 – grasses variant - with 5.204 g dry weight/plant more than the control (V1) and with 3 g dry weight/plant more than the V3 (non-grassed).

CONCLUSIONS

Interacting mycorrhizal and rhizobial symbioses seem to be, therefore, important to enhance revegetation, but a selection of mycorrhizal fungi for symbiotic efficiency with the test legume must be first carried out;

From biotechnological/practical point of view, it must be stated that mycorrhizal biotechnology can be integrated into nursery and revegetation management, taking into account the fact that the mass production of mycorrhizal inocula is quite difficult, while rhizobial inocula can be easily obtained;

Woody legumes – *Robinia pseudacacia* – usually have a considerable degree of dependency on mycorrhiza to thrive in stressed situations;

The bacteria participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment; mycorrhizal fungi and N-fixing bacteria are among the most important members within the influential group of mutualistic symbionts. The mycorrhizal fungi, upon the biotrophic root colonization, develop an external mycelium which is in fact a bridge connecting the root with the surrounding soil microhabitats.

The beneficial effects of the micro-symbionts, observed in this study, arouse an interest in considering the role of bacterial – AM - plant tripartite symbiosis in plant-based strategies of remediation and stabilization of the ash dump.

ACKNOWLEDGEMENTS

We thank the AMCSIT Politehnica Bucharest for supporting the Research Program of Excellency 156/2006-2008.

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Tables

Table 1. Obtained biomass by the acacia plants in the experimental variants

Dry weight from	Without mycorrhiza		With mycorrhiza	
	non-grassed V1 (g/d.wt)	grassed V2 (g/d.wt)	non-grassed V3 (g/d.wt)	grassed V4 (g/d.wt)
Whole seedling	1.125±0.028	3.249±0.022	4.129±0.013	6.329±0.025
Leaf	0.534±0.014	1.715±0.012	1.757±0.017	3.427±0.038
Stem	0.259±0.008	0.762±0.004	1.413±0.026	1.533±0.027
Root	0.302±0.007	0.648±0.006	0.850±0.016	1.114±0.014
Nodule	0.060±0.002	0.117±0.004	0.108±0.007	0.256±0.025
Nodule number	18.6±0.5	37.2±0.5	29.8±0.6	45.6±0.5
Colonisation VAM	-	-	21.6 %	35.3%

Table 2. Plants phosphorus and nitrogen content

	Without mycorrhiza		With mycorrhiza	
	non-grassed V1	grassed V2	non-grassed V3	grassed V4
P (mg/plant)	3.56±0.05	5.23±0.06	12.13±0.09	15.6±0.07
N (mg/plant)	35.84±1.18	64.42±0.25	118.56±0.73	193.70±2.90

Table 3. Coefficients of simple correlation between the analyzed factors ($p<0.05$)

	Whole plant	Leaf	Stem	Root	Nodule	Phosphor	Nitrate
Whole plant							
Leaf	0.98*						
Stem	0.94ns	0.85ns					
Root	0.99*	0.96*	0.97*				
Nodule	0.93ns	0.98*	0.75ns	0.89ns			
Phosphor	0.94ns	0.88ns	0.96*	0.95ns	0.83ns		
Nitrate	0.97*	0.95ns	0.92ns	0.96*	0.93ns	0.98*	

Figures

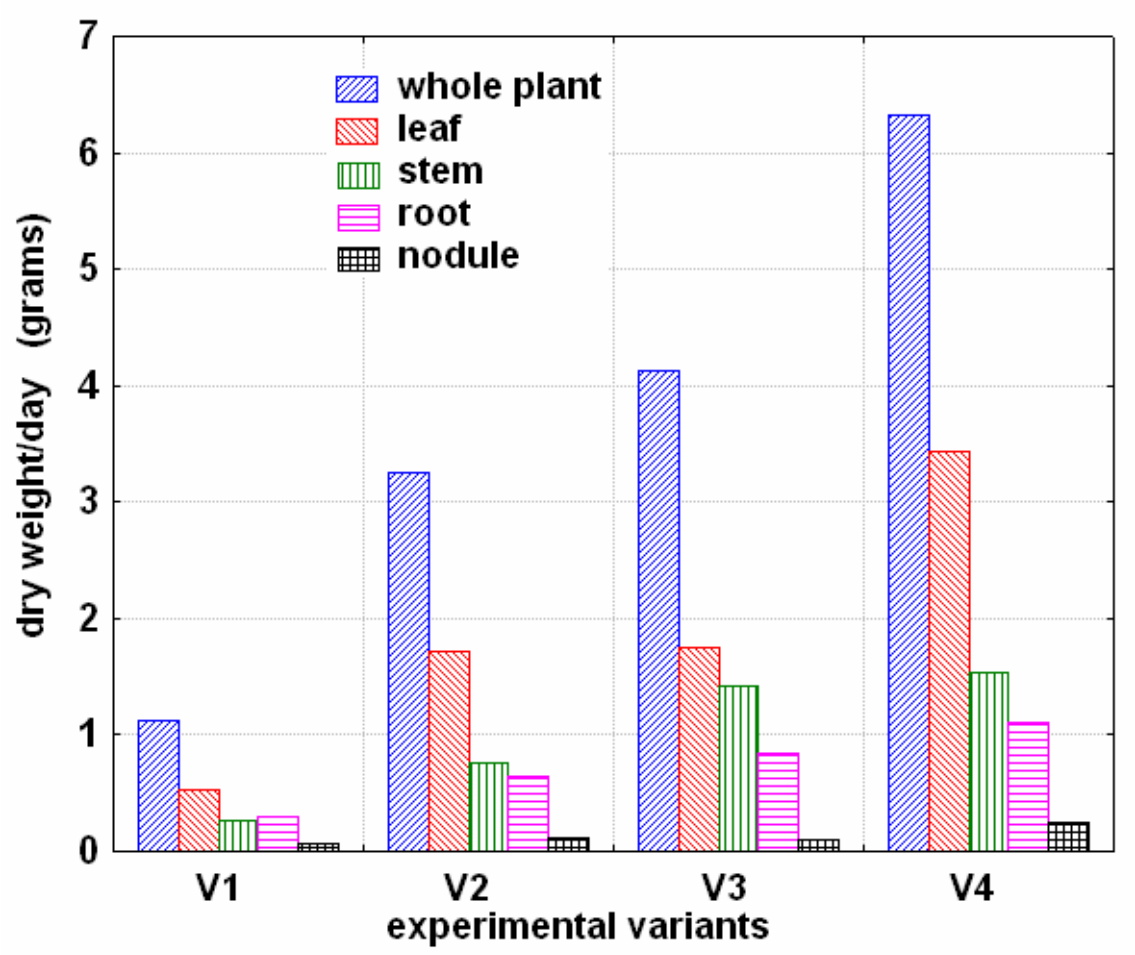
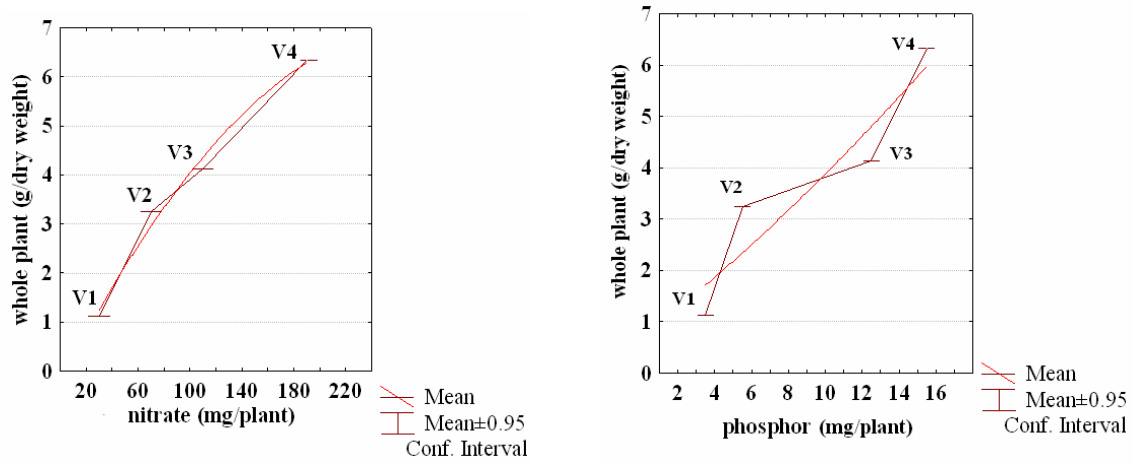


Fig. 1. Biomass evolution in the plants (60 days) in experimental variants (V1=Mt)



A **B**

Fig. 2. Influence of the tripartite simbioses arbuscular mycorrhizas-plant-nodule N-fixing bacteria on the development of the acacia biomass

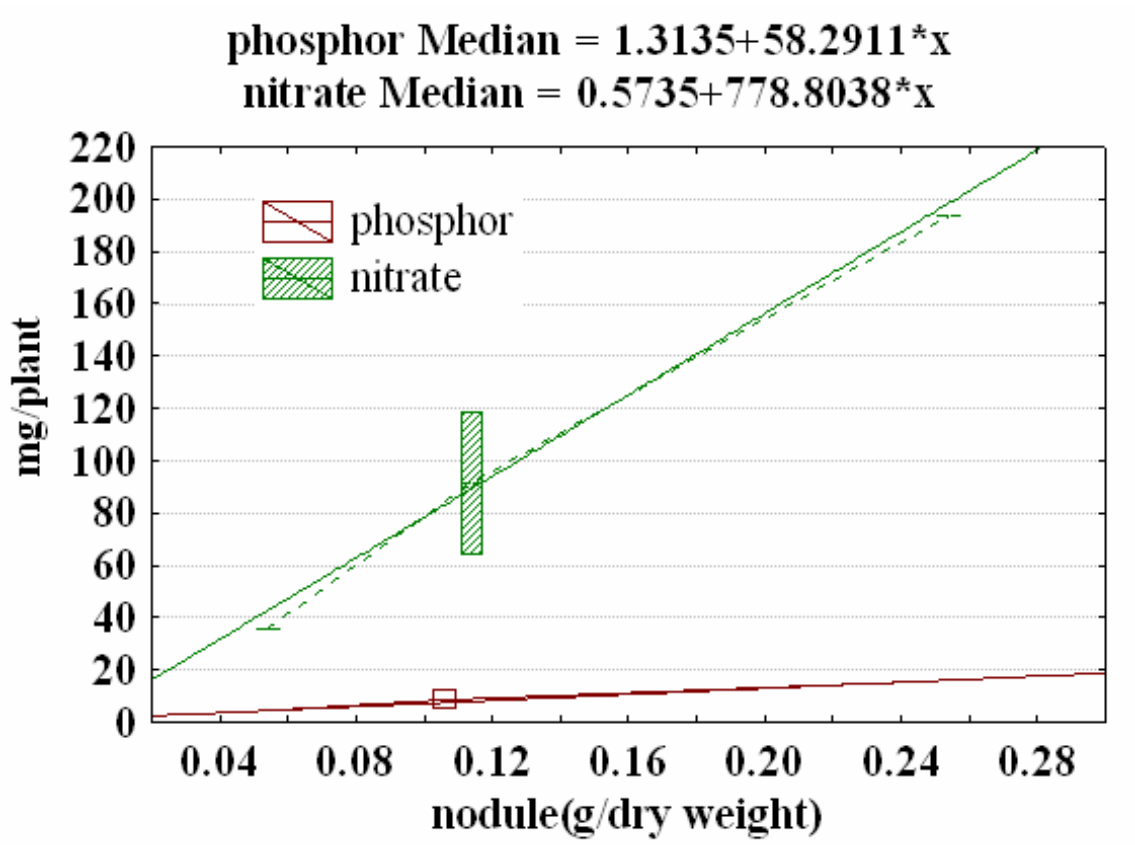


Fig. 3. Influence of N-fixing bacteria on mycorrhizal fungi's development and installation and on the phosphor accumulation in acacia seedlings

Microbial community structure and enzyme activities in fly ash cultivated with *Lolium perenne* in associations with *Glomus intraradices*

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Keywords: arbuscular-mycorrhizal fungi**,** microbial biotechnology, interactions.

ABSTRACT

Arbuscular-mycorrhizal (AM) symbiosis confers numerous benefits to host plants, including improved tolerance to abiotic and biotic stresses. Although the majority of grasses form an AM symbiosis, little is known of the mycorrhization of turf-grass species. This study was conducted to determine whether how one mycorrhizal species - *Glomus intraradices* affected the establishment of a lawn perennial ryegrass (*Lolium perenne* L.). It was pointed out the fact that the ryegrass inoculated with *G. intraradices* at rates of 10.000 spores L^{-1} was able to establish seeding, even with no irrigation or fertilization inputs. Further, as AM fungi coexist and interact with other present microorganisms, changes in microbial community structure may also affect the function of AM fungi. Considering the above aspects, the objectives of our work were to investigate the effects of single AM fungus and communities of AM fungi on the growth of *Lolium perenne* cultivated on the fly ash dumps from Isalnita – Craiova Thermo-Electric Power Station and the activities of phosphatase under unsterilized conditions. This paper highlights the ecological complexity and diversity plant-microbe-soil combinations, particularly AM. It could provide a starting point of a new appreciation of the AM symbiosis role on phytorestoration of degraded soils, i.e. mycorrhizo-remediation.

INTRODUCTION

Arbuscular mycorrhiza (AM) are beneficial, symbiotic fungal associations with plant roots which increase the effective absorbing zone of the root through the hyphae which explore the soil away from the root surface. AM are renowned for their ability to improve the P nutrition of plants. One estimate suggested that up to 80% of the P taken by a mycorrhizal plant was supplied by the fungus (Marschner and Dell, 1994). Study of plant roots and the diversity of soil micro biota, such as bacteria, fungi and microfauna associated with them, is important for understanding the ecological complexities between diverse plants, microbes, soil and climates and their role in phytoremediation of degraded soils. The arbuscular mycorrhizal fungi (AMF) are universal and ubiquitous rhizosphere microflora forming symbiosis with plant roots and acting as biofertilizers, bioprotactants, and biodegraders. In addition to AMF, soils also contain various antagonistic and beneficial bacteria. Their potential role in phytoremediation of contaminated/degraded soils is becoming evident although there is need to completely understand the ecological complexities of the plant-microbe-soil interactions and their better exploitation as consortia in soils remediation strategies. These multitrophic root microbial associations deserve multi-disciplinary investigations using molecular, biochemical, and physiological techniques. It could be appreciated that even the restoration of the fly ash ecosystems needs to incorporate microbial biotechnology research and development. There have been many reports that AM fungi can increase soil enzyme activities, such as phosphatase (Dodd et al., 1987; Kothari et al., 1990; Mar Vazquez et al., 2000). Soil phosphatase may play an important role in the P nutrition of plants because it mediates the release of inorganic phosphorus from organically bound phosphorus. Ecologists and evolutionary biologists are broadly interested in how the interactions among organisms influence their abundance, distribution, phenotypes, and

genotypic composition. Recently, we have seen a growing appreciation of how multi-species interactions can act synergistically or antagonistically to alter the ecological and evolutionary outcomes of interactions in ways that differ fundamentally from outcomes predicted by pairwise interactions. Here, we review the evidence for criteria identified to detect community-based, diffuse co-evolution. These criteria include: (*a*) the presence of genetic correlations between traits involved in multiple interactions, (*b*) interactions with one species that alter the likelihood or intensity of interactions with other species, and (*c*) non-additive combined effects of multiple interactors. In addition, we review the evidence that multi-species interactions have demographic consequences for populations, as well as evolutionary consequences. Finally, we explore the experimental and analytical techniques, and their limitations, used in the study of multi-species interactions. Throughout, we discuss areas in particular need of future research.

MATERIALS AND METHODS

AM fungal inocula: In this experiment the used inocula contained only one AM fungal strain, Glomus spp. These AM fungal species were identified morphologically using current taxonomic criteria (Schenck and Perez, 1990) and Internet information by INVAM (2008). Inocula was propagated on ryegrass (*Lolium perenne*) grown in an autoclaved (121^0C for 1h on three successive days), for three successive propagation cycles, each 4 months long. At the same time, the control non-mycorrhizal inoculum was also prepared with the same sterilized substratum on which ryegrass was cultivated. The inocula were air-dried and sieved (2 mm), and each consisted of a mixture of rhizospheric soil from pure pot culture containing spores, hyphae and mycorrhizal root fragments (the control without AM fungal propagules). Spores of the arbuscular mycorrhizal (AM) fungus, *Glomus intraradices*, were also applied at the recommended rate to achieve a spore density of ca. 10,000 spores L^{-1} in the upper 10 cm of ash. The spores of *Glomus intraradices* were inoculated into the ash after planting. This fungus has been demonstrated to be beneficial to a wide range of plant species and is commonly incorporated into nursery products to enhance plant establishment and growth.

Acidic and alkaline phosphatase: A spectrophotometrically method was applied (at 400 nm), using p-nitrophenol phosphate, a synthetic compound as substrate. A standard curve was made for calculation of the results. Statistical evaluations were conducted by means of the DUNCAN test, using STATISTICA Soft.

RESULTS AND DISCUSSION

The obtained results concerning the development of the mycorrhized plants cultivated on the fly ash, through the measurement of the dry substance and the plants height (table2), were quite the waited ones, having in view also the presence of a large number of other species of microorganisms (table1), with a competition-nutritional activity, which has positively influenced the enzymatic activity of the substrate. In contrast to the simplicity of the chemical control of fly ash, this system depends upon the inherent resistance of a mix of different genotypes (groups of other microorganisms), which thus reduce plant to plant spread of the disease, the enhanced resistance given by a significant level of arbuscular mycorrhyzal (AM) infection and the effect of the plants rotation on ash chemical and microbiological properties. There was a positive interaction between AM fungi and the activities of phosphatase enzymes. The highest activity of acidic and alkaline phosphatase was found on the ash dump's cliff,

which is considered to be under the effects of the rhizosphere created by the invasive flora (figure 3). Further more, it can be considered that the highest activity of phosphatase was correlated at a highest degree with the activity of the bacteria and fungi (figure1). A negative interaction of mycorrizas with Actinomycetes activity (fig. 2) was found to be developed under the fly ash conditions. Taking into account the percentage, the AM activity demonstrated to have a positive influence on the fungi activity (6.76%); a negative influence was observed on the bacteria activities (17.64%) and on the Actinomycetes (60.84%).

CONCLUSIONS

This paper highlights the ecological complexity and diversity plant-microbe-soil combinations, particularly arbuscular mycorrhiza (AM). It could provide a starting point of a new appreciation of the AM symbiosis role on phytorestoration of degraded soils, i.e. ashes' mycorrhizo-remediation;

A complet study of the mycorrhizal types could be realized just through the implication of an interdisciplinary research team, considering that the mycorrhiza process is a complex, but especially, an extremely fragile phenomenon;

Taking into consideration the obtained results, it could be appreciated that the herbal plant species (i.e. *Lolium perenne)*, which can offer a spatial-temporal sustainability for the mycorrhizal processes, could be recommended to be planted on the ashes dumps, in order to improve the ecosystem's quality. The huge quantity of biomass that could be obtained, is leading us to decide to realize a parallel study related to the kinetics of nutrients' absorption, benefits and costs of installation and how to optimize the conversion of this biomass in "ecological energy".

ACKNOWLEDGEMENTS

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Tables

Table 1. Presence of the microorganisms on the studied ashes dumps (without and with mycorrhiza)

	Control without mycorrhiza	Dumps'plateau with mycorrhiza	Dumps'cliff with mycorrhiza
Nr. VAM viable spores	-	187,6	299,5
Fungi ($x10^3$/g ash)	12,5	9,5	14,5
Bacteria ($x10^4$/g ash)	136,5	56,8	113,0
Actinomycete ($x10^4$/g ash)	21,0	8,0	12,0
Nitrosomonas ($x10^4$/g ash)	0,52	0,27	0,35

Table 2. Plant biometrics and biochemical analysis of *Lolium perenne* cultivated on the fly ash

	Control without mycorrhiza	Dumps'plateau with mycorrhiza	Dumps'cliff with mycorrhiza
Plants average hight (cm)	33,5	89,7	107,7
Dry substance (g/plant)	1,7	4,1	5,0
Acid phosphatase (n Kat 100/g ash)	0,63	0,74	0,92
Alcaline phosphatase (n Kat 100/g ash)	0,52	1,19	1,55
Organic matter (%)	0,29	0,36	0,38

Figures

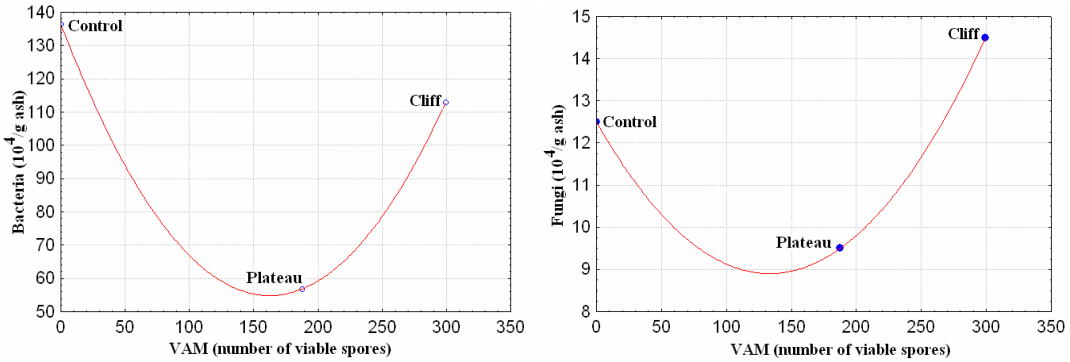

Fig. 1. Influence of the mycorrhizal symbiosis on bacteria and fungi's activities

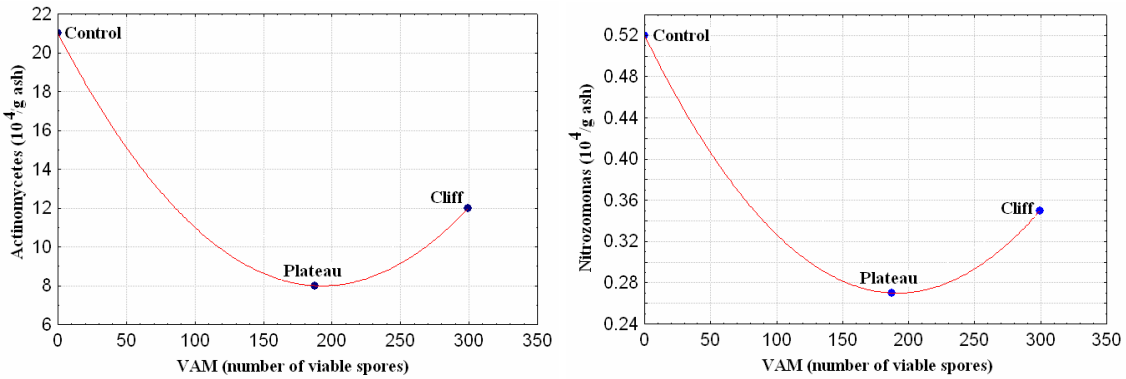

Fig. 2. Influence of the mycorrhizal symbiosis on actinomycetes and nitrosomonas activities

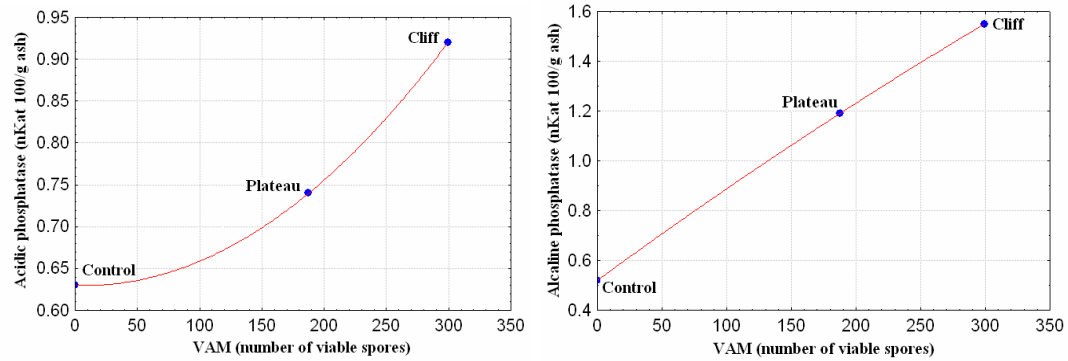

Fig.3. Positive interaction between mycorrhizal fungi and phosphatase activity (acidic, $r = 0.96^{xx}$; alkaline, $r = 0.99^{xx}$)

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The promotion and building of associative farms in horticultural field, in Teleorman District

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Keywords: Co-operative farms, vegetable growing, consultation, co-operation, agriculture

ABSTRACT

The Law of co-operative farm promulgated in December 2004, opened the gates to the initiative groups in Romania, the gates of the reorganization of agriculture based on co-operative grounds. The measure was requested by the adhering to European Union, -union that does not see with good eyes the structuring of the agricultural area in millions of lots/parcels and practicing a rudimentary agriculture on most of the area. In the country, as both in Teleorman district the first consultants appeared (for this particular purpose), the way the Japanese International Cooperation Agency – JICA, but also the first forms of cooperation, though the idea of co-operative farm still sounds like "collectivization" for many people. The mentality is very hard to beat and because the agricultural associations starting with the year 1989 ended terribly in mot cases. With the support of the Japanese International Cooperation Agency – JICA, the National Consultancy Agency started the last autumn a program of training for the agricultural producers in order to create a co-operative farm system that would produce advantageousness (high levels). These co-operative farms, that guaranties the property deals with the taking over of the production, standardization, respecting the laws of quality as well as valuing the production.

INTRODUCTION

The association in Co-operative farms gives the chance of developing the agriculture, allows loans necessary for the acquisition of modern machines – everything for the development of horticultural production, packing and delivery on EU market and other markets.

After a complex information campaign some trends of associating appeared – trends that after a while transformed into co-operative farms or cooperative societies, Teleorman district being the first where a co-operative farm appeared according to the law no 566/2004.

The main role of the groups of producers is the one of commercializing in one place the products of individual farmers. The groups of producers that respect certain conditions can obtain subvention from the European Union. The groups of producers are considered a chance for the farmers to strengthen the economic power by negotiating the prices of products, diminishing the costs of transport, obtaining the profit that the intermediaries used to get.

The success of a group of producers can be determined by: a good and stabile market strategy, innovation, ability of the members of the group to learn, applying the rules and penalties in the group and homogeneity of members. The association doesn't only mean a simple registration- it must also be participative, each of them must contribute to the development of the patrimony- which would lead to incomes for the co-operative farm and at the same time would lead to profit for farmers. The groups of producers take into consideration a statute of their own approved by the general association and are recognized due to a request of recognizing and according to the group of products for which they ask the recognition the Ministry of Agriculture and Rural Development offers a notice of recognition to that group of producers.

The present work was made for the purpose of emphasizing the importance of the foundation of the groups of producers in co-operative farms or associations that would allow a growth of the productivity of labor in agriculture.

MATERIALS AND METHODS

For the present work a series of normative acts related to forms of associations in agriculture were first studied and after the collaboration with the Regional Office of Agricultural Consultancy information were obtained and studied. Thus, we make a series of legislative observations.

There may be recognized as groups of producers or as organizations of producers the following juridical forms: commercial societies, according to law no 31/1990, republished, with later changes, agricultural societies and other forms of association in agriculture , according to law no 36/1991, associations and foundations, according to Government Order no 26/2000 regarding association and foundations, approved with changes and completions by law no 246/2005, co-operative farms according to the law of agricultural cooperation no 566/2004, any other juridical type of association, according to the present legislation.

The groups of producers are formed and function to free initiative of producers, based on interest and action of the group in the following conditions: are formed of at least 5 members, prove by bookkeeping evidence a minimum value of the commercialized production, for the group of product that a recognition is requested, of at least 10 000 Euro (the same amount in lei); for the fruit vegetable group the amount is of minimum 100 000 Euro (the same amount in lei), respects the rules of art.6 paragraph(1) in Government Order no 37/2005, with the later changes and completions, have a centralized system of bookkeeping, invoice, registration and quantity and quality observations of the member's production.

RESULTS AND DISCUSSIONS

The position of member of a group of producers can be obtained by any agricultural or forest producer who legally owns a production base and declares in written the intention of commercializing his own agricultural and forest production in a group of producers and pays the share, according to his statute.

The groups of producers can exist for the commercialization of the following categories of horticultural products: fruit and vegetables, fruit, vegetables; horticultural products for certain destinations, peel fruits; mushrooms, early and summer potatoes; whine grapes; flowers; ornamental plants; dendrology plants.

During 2004-2007, in Teleorman district there were 9 co-operative farms constituted (5 for vegetables and 4 apiarian), one being in process of developing, having as subject of activity vegetable growing.

Regarding the associations organized according to G.O. no 26/2000 their number is 4, the subject of activity being in vegetable growing.

CONCLUSIONS

From the entire presented information one can see the following general conclusions:

1. In rural communities numerous forms of associations can be formed –like participating with machines for doing the seasonal agricultural works in campaigns and of other works including during winter (the removing of snow, transport of wood

material and construction material, etc, association for buying the inputs and the valuing of the agricultural production, association for producing nursery transplant, association for valuing vegetables and fruit.

2. It is important to give a support to the agricultural producers starting with informing the land owners, presenting the results of the production in the area, of the ways of making the technologies work, expenses, the final financial result related to the work processes and using in a more efficient way the machines and the human resources in order to convince them about the importance of the associative forms.
3. After the setting up of the association it is necessary the involvement of the Consultancy Office for giving technical assistance for negotiating the contracts, for buying the inputs as well as valuing the products. The improvement of the access to information in the case of the new created associations can be made through mass-media and through specialists from Local Centers of Agricultural Consultancy.

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Tables

Table 1. The situation of the promotion of groups of producers in Teleorman district

The name of the group of producers organized with the support of OJCA	The for of association of the group of producers (name, law no.)	The subject of activity of the producers group	Place (location)	Groups of producers in process of recognition
The Association of vegetable gardeners from Teleorman	G.O. no 26/ 2000 associations	The valuing of vegetables	Contesti	Gave up
Cartotimp	Co-operatives Law 566/2004	The valuing of vegetables	Peretu	A file at DADR
Tobacco	G.O. no 26/2000 Associations	The commercialization of tobacco	Zimnicea	done

Table 2. The situation of the co-operative farms from the horticultural field formed according to the law 566/2004

District	The name of the Cooperative farm, location and the date of the constitution	The subject of activity of the cooperative farm	Number of members	Number of cooperative farms in progress of constitution according to the law 566/2004
TR	The vegetable gardener Peretu 07.06.2005	The growth and valuing of vegetables and greenhouse products	127	Done
TR	Berceanu M. Contesti 21.01.2007	The growth and valuing of vegetables and greenhouse products	5	Done
TR	Cartotimp Peretu 29.03.2007	The valuing of potatoes and of greenhouse and field products	14	Done
TR	Viisoara C.A	The growth and valuing of vegetables and greenhouse products	5	In progress
TR	Progresul C.A	The growth and valuing of vegetables and greenhouse products	6	Done

Table 3. The situation of the associations of vegetable gardeners already functioning and those in progress of developing, in Teleorman district during 2004-2007

No	Name	Subject of activity	Number of members	Associations in progress of developing
1	The Association of vegetable gardeners Teleorman, constituted in 2005	Producing and valuing vegetables	40	
2		Vegetable growth	5	The Association of vegetable gardeners "Tarancuta Romanca"
3		Vegetable growth	12	Horticola Bragadiru
4		Producing and valuing vegetables	5	HIRAMF 2006

The modernization of agricultural exploitations in the Teleorman District

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Keywords: agriculture, finance, efficiency, productivity, machines

ABSTRACT

Romania's European integration brings for agriculture a series of advantages expressed in real life by the growth of agriculture's production, the encouragement of the development of middle (not more than 50 hectares) and high (more than 100 ha) agricultural exploitations, the improvement of the access of the agricultural products on UE market and the disappearance of all commercial barriers, as well as the slowing down of the migration of the manpower from agriculture. The private agriculture is characterized in present by a excessive splitting of the infield and also of the system of agricole production, with negative impact over the product's market. The support for the foundation of producers groups will contribute to the growth of the economical efficiency of agricultural exploitations, having an important role in the improvement of the quality of the products, of environment protection, of web provision and marketplace as well as in establishing a balance between consumption and offer.

INTRODUCTION

The structural changes that took place in the Romanian agriculture, having as effect the process of changing to private property of over 96% of the infields have determined small and moderate exploitations. The main form of agricultural exploitation is represented by the small rural farm, with an entire surface of 1.8 hectares of infield and a percentage of 53% of the entire infield of the country.

Agricultural exploitations, especially those from the vegetal field, are dealing with an insufficient endowment as well as with the existence of a high degree of physical and moral dilapidation of the already existing materials. Thus, the medium charge on a physical tractor is of 58.6 ha infield (arable soil) and the medium charge on a cropper is of 80-90 ha infield. From the land under crop, a high share has the cereals with over 60% and the technical plants over 16.3%. Vine crops, with noble breed in bearing have diminished at 16.3% and the surfaces of orchards and nursery gardens have diminished at 13.7% as a consequence of the land clearing of the land under crop.

The characteristic objectives for the modernisation of agriculture refer to the modernization of the specific technologies, the reduction of production costs, the reduction of production loses, the growth of efficiency, a better using of manpower in agriculture, the value of agriculture potential of every area and encouraging the rivals.

MATERIALS AND METHODS

This study takes into consideration the establishment of the viability of some measures that stood at the base of the modernization of the agricultural exploitations, and one of them refers at the Sapard Programme as well as the measures that are imposed to be taken in the immediate period of the integration.

With the help of the Sapard Programme, in the programme "Investments in the agricultural exploitations" in 2003-2006 a support for 612 projects of modernization of vegetal and zootechnic farms was given.

The support given to this programme is complementary to the actions mentioned in other programmes from Axe I: "The growth of the added value of agricultural products", "The support of founding producers groups", "The improvement and the development of infrastructure important for the development and the adaptation of agriculture and forestry", "the installation of the new people responsible for agriculture", " The early retirement of the people responsible with the agriculture and the workers", "The professional forming and actins of information, including spreading new specific knowledge also in practice, innovative for farmers" with rules from Axe II: "Support given to the people responsible with agriculture in areas with problems:, "Support for the welfare of the animals", "Agro-ecological support", with rules from Axe III: "Base services for economy and rural population", as well as with measures that regard the implementation of local development strategies from Axe IV.

RESULTS AND DISCUSSIONS

The content of the mentioned rule for the modernization of the agricultural exploitations is about a series of aspects that would allow the improvement of the general performances of the agricultural exploitations, respecting the standards of the Community- applicable to the respective investments. These aspects refer to:

- the acquisition of the necessary equipment for the modernization of the exploitations of the vegetal by endowment with tractors, croppers, machines, installations and agricole equipments, including for calibration, selecting, conditioning and storing of the resulted agricole products obtained and used by the farm, irrigation systems, special equipment for dealing with the vegetable remains.
- buildings new and/or modernization of the buildings (offices, places to store and select, warehouse of fuel, detop for the equipment, the surroundings and so on) and utilities (water feeding, electrical energy, thermic energy, throwing the waste, sewerage and so on) in vegetal farms.
- building and/or the modernization of the greenhouses, including the necessary thermic equipment and irrigation system, assuring the utilities in order to respect the conditions to protect the environment.
- costs representing the architect's, engineer's, consultant's salary, the legal taxes of feasibility studies, the endowment of licences, for preparing and/or implementing the project, directly associated by the rule that does not pass 12%of the total eligible cost of the project.
- the endowment of new, specialised ways of transport – necessary as a result of their identification in the feasibility study;
- costs regarding the endowment of land for specific buildings, including the main building, when the agricultural exploitations are moved in a different place, but not more than 10% from the value of the project;
- soft - materials for the use of the computer, including the costs of installation and assembling.
- the acquisition of animals (reproductive- male) with high genetic potential, mentioning the origin (pedigree), as well as the acquisition of seminal material of good quality;
- the endowment with tractors, equipment, machines, installations and equipments for the activities from the animal farms ;
- investments that promote the production of biogas from the manure, obtained from their own farms or collected from other farms.

- new buildings and/or the modernization of the buildings from the farms where there are animals and birds, adding other buildings or utilities: paddocks, the place where the hay is kept, the sewerage system, platforms for evacuation, purifying systems, special equipments for producing and measuring complex fodders, rooms for milking and collecting the milk, thermic installation, warehouses for fuel, water feeding installation, sewerage, electrical installations, surroundings (fence in), and so on.

For the modernization of agricultural processes from Teleorman district all the involved elements searched to realize the financing of farmers with modern and advanced agricultural machines, in order to work in time the areas that were leased and for doing more works with a single pass, thus, saving money, time and work labor.

The endowing with technical systems the agricultural institutions in Romania is still precarious and for these reasons a great part of the absorbed founds with the Sapard and The Farmer (Fermierul) programs have followed this direction.

CONCLUSIONS

In conclusion, we can say that the development of the private agriculture especially the improvement of economical efficiency of agriculture exploitations in Romania, is the main element that makes us different from the evolved agriculture of the EU counties.

Thus, so that Romania can be successful in agriculture, including with the help of the implementation of the programme ruled until 2006 – Sapard, (the country) must have numerous programs post adhering which could lead to an efficient agriculture, based on equal chances in competition with the countries which already have an evolved agriculture.

The continuity of implementing programs in agriculture and the rural development must become a priority for Romania, in order to reduce the already existent difference between countries with high potential and our country.

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Tables

Table 1. The endowing with technical systems in Romanian agriculture

Agricultural machines	2005		2006	
	Total	Of which entirely private	Total	Of which entirely private
Physical agricultural tractors	169240	163711	169177	165375
Plough for tractor	131252	127829	132142	129907
Mechanical farmer	27433	26578	27366	26770
Mechanical sower	62061	60555	63149	62142
Machines for spreading the chemical manures	9656	9000	9525	9098
Spraying and dusting machines with mechanical traction	7191	5676	6814	5721
Auto propelling combines for gathering the stalky cereals	24231	23703	23935	23641
Auto propelling combines for gathering the corn	1084	1052	1113	1089
Auto propelling combines for gathering the fodder	1091	951	910	804
Auto propelling for gathering the fodder	1512	1331	1408	1298

Table 2. The situation regarding the projects financed by Sapard and The Farmer (Fermierul) programs, in Teleorman district

No	Title	The beneficiary/ address	The amount RON	The program
1	Acquisitions – agricultural machines	A.F. Şerban Ştefan, from Purani commune, Teleorman district	292610	The Farmer
2	Acquisitions – tractor and agricultural machines	A.F. Şerban Nicuşor and Elena, Purani commune	287793	The Farmer
3	Bee keeping foundation	A.F. Martinescu Marius. Orbeasca commune, Teleorman district	36649	The Farmer
4	Bee keeping modernization	P.F.A. Matei Doru , loc. Roşiorii de Vede , Teleorman district	36960	The Farmer
5	Bee keeping modernization	P.F.A. Pantelimon Gabriel, Călmăţuiul de Sus, Teleorman District	93721	Sapard
6	Bee keeping modernization	P.F.A. Timoftei Vladimir, Uda Păciurea , Teleorman district	33123	Sapard
7	Acquisitions – agricultural machines	A.F. Mina Ion, Pietroşani, Teleorman district	72181	Sapard
8	Acquisitions – agricultural machines	P.F.A. Buică Dorian, Ţigăneşti, Teleorman district	32305	The Farmer
9	Acquisitions – agricultural machines	A.F. Mocanu Nicolae, Nanov, Teleorman district	48852	Sapard
10	Acquisitions – agricultural machines	A.F.Colea Marin Alexandru, Zimnicea, Teleorman district	343532	The Farmer
11	Farm foundation for snails growth	P.F.A. Neagu Valentina, Zimnicele, Teleorman district	39689	Sapard
12	Foundation of a cows farm for milk production	SC M&G FERM AGRO SRL, Plopii Slăviteşti, Teleorman district	235466	The Farmer
13	Acquisition of Celtis 446 tractor	SC KISS SRL, from Băneasa commune,Teleorman district	146944	The Farmer
14	Acquisitions – agricultural machines	AF Boagiu,Comuna Plopii Slăviteşti, Teleorman district	650611	The Farmer
15	Acquisitions – tractor and agricultural machines	A.F. Dobre Florin, Schitu Poienari , Teleorman district	176555	The Farmer
16	Acquisitions – tractor and agricultural machines	BRATUT F., from Tatarastii de Sus comnube,Teleorman district	236194	The Farmer
17	Acquisitions – tractor and agricultural machines	P.F.A. Nedelcu, Siliştea, Teleorman district	102660	The Farmer
18	The modernization of vegetable farm	SC AGROALEXMAR SRL , Plopii Slăviteşti, Teleormandistrict	43373	Sapard

Preparation of DNA samples for GMO analysis of soybean - derived foodstuffs

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Keywords: genetically modified organism, RoundUp Ready® soybean, DNA extraction, PCR, real-time PCR.

ABSTRACT

As part of an integrated protocol for GMO detection, DNA extraction is very important for getting accurate final results. Four methods for DNA purification were tested: a CTAB extraction protocol; one automated extraction kits using magnetic beads; and two kits based on column separation. DNA was extracted from different types of food matrices derived from or containing soybean. Spectrophotometer measurements and agarose gel electrophoresis indicated relatively low quality of extracts, especially for high processed matrices. Next, all extracts were tested for PCR using specific primers for plant, soybean and transformation event, respectively. All extracts showed the expected results for the plant and soybean specific primers. In the case of GM specific primers only the positive controls and one unknown sample showed the expected PCR products. The negative results for the other samples is due to either the absence of GM derived DNA or to its presence below the LOD. The best cost per sample was obtained with the CTAB method, while the automated extraction was the easiest and quickest to perform. The experiment showed that all extraction methods are good candidates for further testing and optimization. We concluded that the automated extraction kit is best suited for raw or low processed matrices while the other methods are recommended for all types of samples.

INTRODUCTION

The first commercial release of a GM crop took place in 1992, in China (Zhou *et al.*, 1995; Hails and Kinderlerer, 2003). Since then transgenic crops are spreading more rapidly than any other agricultural technology in history (Raney, 2006). Soybean is the most widespread and cultivated GM crop around the globe (Figure 1). Different views on this subject caused intense controversy.

In order to ensure transparency and to meet consumers' needs, EU legislation (*e.g.* Regulation EC No. 1829/2003 and Regulation EC No. 1830/2003) established new policies such as labeling, traceability and post-market monitoring of GMO derived food products.

Labeling of foodstuffs is based on molecular qualitative and quantitative analyses performed by accredited laboratories. Qualitative analysis is usually based on PCR techniques, while quantitative measurement is archived using real-time PCR, technique considered to be the most powerful tool for quantitative nucleic acids analysis (Kubista *et al.*, 2006). DNA extraction represents the first step of an integrated GMO analysis protocol and it plays an important role in getting accurate and correct results.

The implementation of these policies and methods is important to Romania, especially after joining the EU on January 1, 2007.

MATERIALS AND METHODS

Samples. All samples used are derived from soybean or contain it as an ingredient (Table 1).

DNA extraction. Genomic DNA was extracted using different methods: a CTAB-based protocol; MagNA Pure LC DNA Isolation Kit I (Roche) which is based on magnetic beads and used with the MagNA Pure LC system (Roche) for automated DNA

isolation; High Pure GMO Sample Preparation Kit (Roche Diagnostics) and Qiagen DNeasy Plant Mini Kit (Qiagen), both based on column separation.
MagNA Pure LC DNA Isolation Kit I is not specially designed for plant derived matrices DNA extraction so a pretreatment stage was added to the basic protocol in order to facilitate tissue rupture (Sakai *et al.*, 2002).

The CTAB method was designed after the protocols published by Somma (2004) and the International Organization for Standardization in EN ISO 21751:2005. It is intended to be used on raw and processed plant derived foodstuffs.

DNA concentration, purity and fragmentation state. Extracted DNA was first quantified using the NanoDrop Nd-1000 UV/Vis 1µl Spectrophotometer (Labtech International LTD).

Quality characteristics of the extracted DNA were further checked by electrophoresis on 1% (w/v) agarose gel (TAE buffer system) and EtBr staining (0.5 µg/ml). The results were visualized on a BioSpectrum® AC Imaging System (UVP).

PCR. The extracts were also tested for PCR using different primers specific for the chloroplast DNA, the soybean lectin gene *Le1*, the CaMV 35S promoter, the nos terminator and the GTS 40-3-2 transformation event (Table 2). One PCR reaction contained 1X Green GoTaq® Reaction Buffer (Promega), 2.5 mM MgCl2 (Promega), 0.2 mM of each dNTP (Promega), 0.5 µM of each primer, 0.03 U/µl of GoTaq® DNA Polymerase (Promega), 2µl of DNA solution with a concentration of less than 100 ng/µl and nuclease free water up to a final volume of 25 µl. The reactions were performed on a Palm Cycler™ thermalcycler (Corbett Research) using the amplification profile from Table 3.

PCR results were evaluated by electrophoresis (TAE buffer system) on 2% (w/v) agarose gel and EtBr staining. Stained amplicons were visualized on a BioSpectrum® AC Imaging System (UVP).

Nested PCR (with primers GM05/GM09 and GM07/GM08) was also employed for some of the samples that showed negative amplification results, in order to exclude the possibility of false negative results due to low copy numbers.

RESULTS AND DISCUSSIONS

DNA extraction, concentration, purity and fragmentation state. Overall, spectrophotometer and electrophoresis results indicated relatively good concentration and quality of extracts. Also, all extraction protocols performed significantly better in the case of seed, flour and textured soybean samples, compared to the highly processed ones.

The best spectrophotometer readings were obtained for the High Pure GMO Sample Preparation Kit. In this case, concentrations were much higher compared to the other extraction methods. A260/A280 values indicated good purity of all samples, regardless of the extraction method.

Electrophoresis (Figure 2) results were consistent with those obtained by spectrophotometer measurement. In the case highly processed matrices absence of the analyte was suggested. For raw and low processed samples however, extracts held DNA of high molecular weight. DNA solutions obtained with High Pure GMO Sample Preparation Kit held high concentrations of nucleic acid, as indicated by spectrophotometer readings, but most of the molecules were highly fragmented. DNA obtained with the MagNA Pure LC DNA Isolation Kit I was also degraded.

PCR (Figure 3). PCR tests gave good results for all sample types when using plant and species specific primers. For GMO specific primers, only the positive controls and the flour unknown sample showed the expected amplicons, the others being negative. We concluded that in these samples the GM derived DNA was either absent or in quantities below the LOD.

Low copy numbers of target molecules could be overcome by using a nested PCR protocol (Figure 3E). This strategy enabled the detection of GM derived DNA in samples which at first we thought to be negative.

CONCLUSIONS

For validation of results, absence of PCR inhibitors should also be checked in a real-time PCR amplification experiment using serial dilutions of the samples.

Some of the positive control samples extracts were quantified by real-time PCR, on a Rotor-Gene™ 3000 instrument (Corbett Research) using the Biogenics RoundUp™ Ready Soya QT Kit (Biotools), specially designed to be used with this apparatus. Calculated values indicated a good performance of the integrated protocol (data not shown).

We concluded that all methods are suited for further optimization, in-house validation and implementation in our laboratory as part of an integrated protocol for GMO analysis of foodstuffs. The CTAB-based method is the one needing most refinements. However, there are other aspects to be considered when selecting an extraction method: the cost per analyzed sample and the ease of use (Figure 4). Although High Pure GMO Sample Preparation Kit and MagNA Pure LC DNA Isolation Kit I gave good results, the cost per sample is relatively high in both cases, compared to the other two methods. In turn, the CTAB method is much more difficult to perform. We recommend the automated extraction kit to be used with raw or low processed matrices while the other methods are suited for all types of samples.

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Tables

Table 1. Samples used to test the performance of extraction methods

Sample	Type	Degree of processing
Roundup Ready® soybeans	Positive control	Raw
CRM ERM-BF410f 5% RRS (IRMM)	Positive control	Low processed
Flour	Unknown	Low processed
Textured soybeans	Unknown	Highly processed
Pate	Unknown	Highly processed
Cheese	Unknown	Highly processed
Chocolate bar	Unknown	Highly processed

Table 2. Primers used for amplification of DNA extracts

Primers	Target	Size (bp)	Reference
CP3/CP4	*trnL* chloroplast intron	> 500	Thion *et al*., 2002
GM03/GM04	*Le1* gene	118	Meyer *et al*., 1996 Queeci and Mazzarra, 2004
Lektin1/Lektin6	*Le1* gene	318	Tengel *et al*., 2001
p35S-cf3/ p35S-cr4	35S promoter	123	Lipp *et al*., 2001 Queeci and Mazzarra, 2004
HA-*nos* r/ HA-*nos* f	*nos* terminator	118	Lipp *et al*., 2001 Queeci and Mazzarra, 2004
RR01/RR04	GTS 40-3-2 transf. event	356	Studer *et al*., 1998 Tengel *et al*., 2001
GM05/GM09	GTS 40-3-2 transf. event	447	Meyer and Jaccaud, 1997 Querci and Mazzarra, 2004
GM07/GM08	GTS 40-3-2 transf. event	169	Meyer and Jaccaud, 1997 Querci and Mazzarra, 2004

Table 3. Amplification profile for PCR tests

Step	Temperature (°C)	Time (seconds)	Repetition
Initial denaturation	95	180	1
Denaturation	95	30	40X
Annealing	60 / 62 / 63[1]	30	
Extension	72	30	
Final extension	72	180	1

[1]60 for RR01/RR02, GM05/GM09, Lektin1/Lektin6 and GM07/GM08; 62 for HA-nos r/HA-nos f; 63 for CP3/CP4, GM03/GM04 and p35S-cf3/p35S-cr4.

Figures

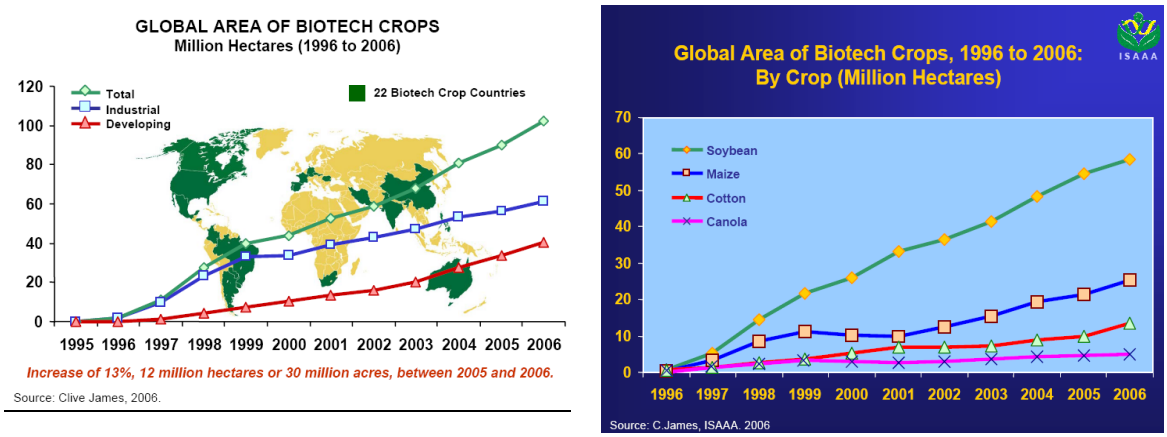

Fig 1. Cultivation of GM crops.

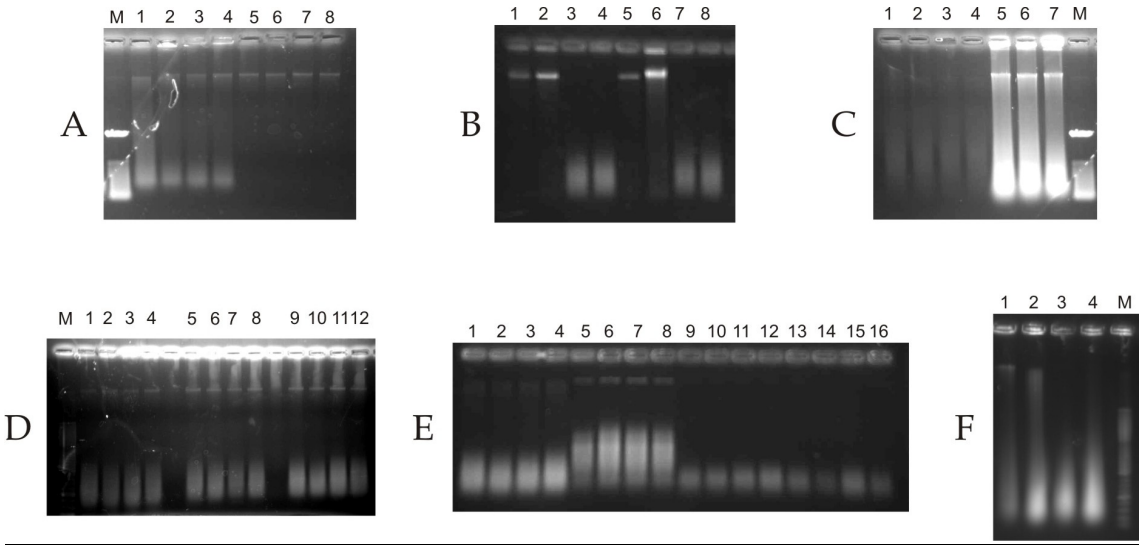

Fig 2. Agarose gel elecrophoresis of DNA extracts. A) DNeasy Plant Mini Kit extracts: 1-4 RR soybean, 5-8 soya flour; B) CTAB method extracts: 1,2,5,6 RR soybean; 3,4,7,8 textured soya; C) MagNA Pure LC DNA Isolation Kit I extracts: 1-4 chocolate bar; 5-7 RR soybean; D) MagNA Pure LC DNA Isolation Kit I extracts: 1-12 RR soybean; E) High Pure GMO Sample Preparation Kit extracts: 1-4 ERM-BF410, 5-8 RR soybean, 9-12 soya flour, 13-16 textured soya; F) High Pure GMO Sample Preparation Kit extracts: 1 RR soybean, 2 ERM-BF410, 3 soya flour, 4 textured soya.

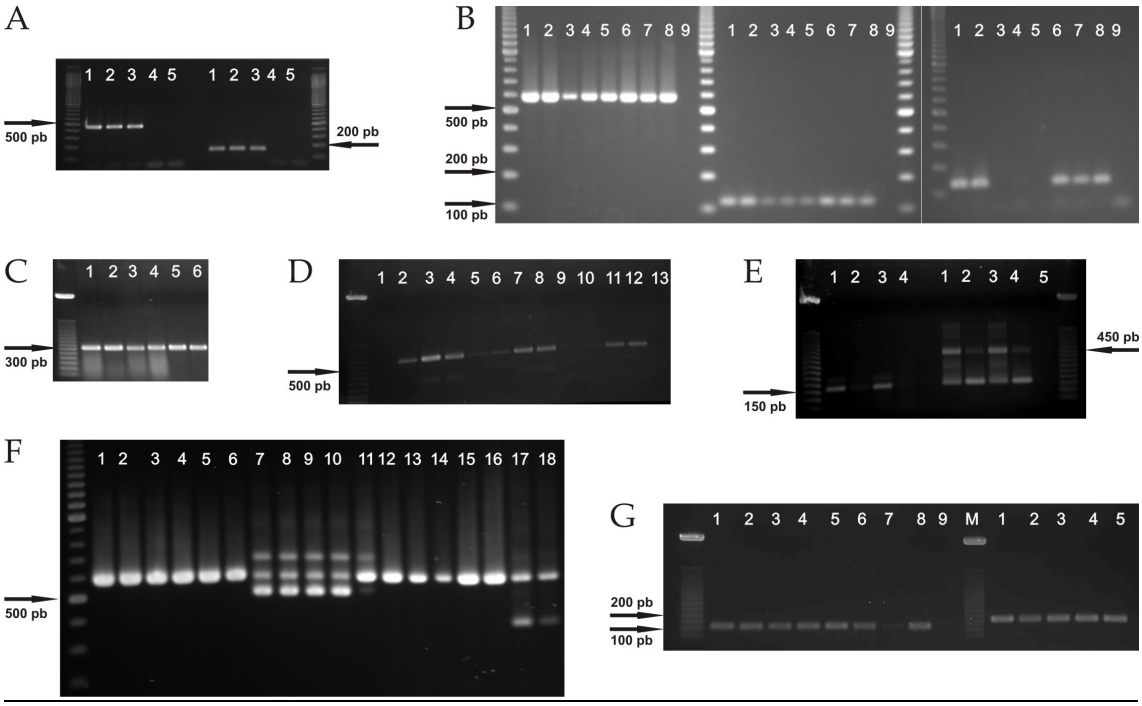

Fig 3. PCR amplification of DNA extracts. A) MagNA Pure LC extracts: 1-3 RR soybean, 4,5 textured soya; amplification with GM05/GM09 (447 bp) (left) and GM07/GM08 (169 bp) (right); B) MagNA Pure LC extracts: 1,2 RR soybean,3-5 textured soya, 6-8 ERM-BF410f, 9 NTC; amplification with CP3/CP4 (500-600 bp) (left), GM03/GM04 (118 bp) (middle) and HA-nos r/HA-nos f (118 bp) (right); C) High Pure GMO Sample Preparation Kit extracts: 1-4 RR soybean, 5,6 chocolate bar; amplification with Lektin1/Lektin6 (318 pb); D) DNeasy Plant Mini Kit extracts: 2 ERM-BF410f, 3,4 RR soybean, 5,6 soya flour #1, 7,8 soya flour #2, 9,10 textured soya #1, 11,12 textured soya #2, 1,13 NTC; amplification with CP4/CP3 (500-600 bp); E) nested PCR with CTAB extracts: 1,3 RR soybean, 2,4 textured soya; amplification with GM05/GM09 (447 bp) (left) and GM07/GM08 (169 bp) (right); F) CTAB extracts: 1-4 RR soybean, 5,6 ERM-BF410f, 7-11 cheese, 12-16 textured soya; amplification with CP4/CP3 (500-600 bp); G) CTAB extracts: 1-4 RR soybean; amplification with GM03/GM04 (118 bp) (left) and GM07/GM08 (169 bp).

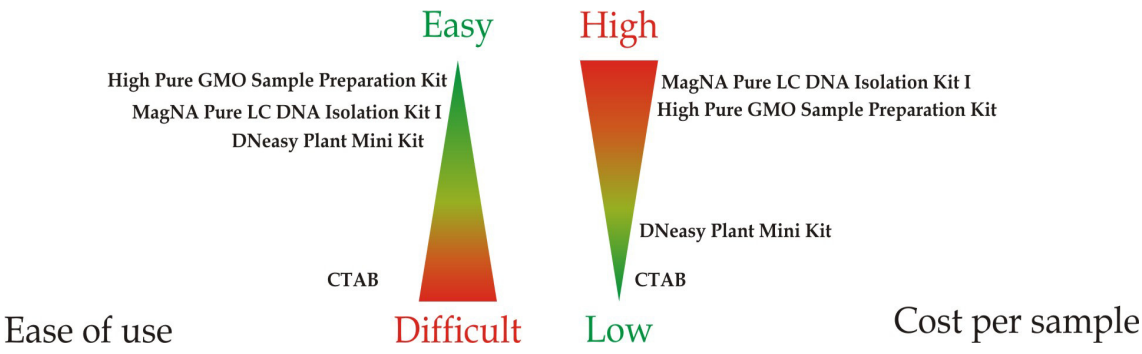

Fig 4. Comparison of method characteristics: ease of use and cost per sample.

Phytophthora Infestans the agent of late blight of potato and tomato: mechanisms of pathogenicity

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Keywords: potato, tomato, *Phytophthora infestans*, effectors, pathogenicity

ABSTRACT

Phytophthora (plant destroyer) *infestans* is an important disease in tomato crop, and it is the most devastating disease in potato crop. The use of molecular methods in the study of *P. infestans:* gene silencing, genetic mapping... has clarified many aspects of *P. infestans* pathogenicity and avirulence mechanisms.

To accomplish parasitic colonization *P. infestans* use many disease proteins known as effectors. Effectors are molecules that manipulate host cell structure and function, thereby facilitating infection (virulence factors or toxin) and/or triggering defense responses (avirulence factors or elicitors) (Kamoun, 2006).

In this paper we will review the recent development accumulated in the pathogencity and avirulence mechanisms used by late blight agent.

INTRODUCTION

Phytophthora (Plant destroyer) *infestans* is the most important and devastating disease in potato crop. It is also very destructive to tomatoes and some other members of the family *Solanaceae* (**Agrios, 1997**). In the years 1840, it caused the famous Irish famine. The direct monetary costs of control efforts and lost production are estimated at > $3 billion/year worldwide (CIP, **1996, cited in Fry, 2008**). Today, although chemicals used against *P. infestans* provide some level of disease control, worldwide losses due to late blight and control measures, are estimated to exceed 5$ billions annually, *P. infestans* is thus regarded as a threat to global food security (**Duncan, 1999**).

Biology of *P. infestans*

The oomycetes include a unique group of eukaryotic plant pathogens, which evolved the ability to infect plants independently from true fungi (**Kamoun, 2000**). *Phytophthora infestans* (Mont.) de Bary is an oomycete pathogen, Kingdom of *Chromista*, Phylum *Oomycota*, Order *Peronosporales*, Family *Peronosporaceae* (**Birch & Whisson, 2001**). It is a specialized pathogen, primarily causes disease on foliage and fruits of a range of solanaceous species (**Erwin & Ribero, 1996**). It is generally considered a specialized pathogen that causes disease on the aerial parts (fruits and leaves) of potato and tomato crop (**Kamoun, 2000**). In potato tuber infection occurs frequently.

It is a heterothallic pathogen, for sexual reproduction needs both mating type A1 and A2. Oomycetes share with many bacterial, fungal and nematode plant pathogens the requirement for living host tissue for at least part of the infection cycle (**Birch et al., 2006**). *P. infestans* exhibits hemibiotrophic lifestyle under natural and agricultural conditions.

The life cycle of *P. infestans* is illustrated in Figs. 1. It starts when zoospores or oospores reach a leaf surface. The zoospores are motile. They locate host tissues by several mechanisms, including recognition of the charge on the plant surface and recognition of chemical stimuli exuded by the plant as both non-specific (amino acids) and specific (isoflavones) chemoattractants (**Haldar et al., 2006**). Zoospores can also exhibit autoattraction (autoaggregation), a phenomenon that might increase the

frequency of successful infection (**Haldar et al., 2006**). They encyst and germinate. The resulting germ tubes bear an appressorium and penetration peg which facilitates the penetration process. The optimal temperature for zoospores germination is around 12°C (**Birch & Whisson, 2001**). Disease development is favored by cool (16 to 21°C), cloudy, moist weather (**Erwin & Ribero, 1996**). With *Solanum tuberosum* as a host, the asexual life cycle of late blight agent can be completed rapidly with production of massive numbers of sporangia that are readily dispersed (**Fry, 2008**). After 2 days, symptoms are visible, whole fields can be transformed from slightly diseased to nearly completely destroyed within just a few days (**Fry, 2008**). The sexual cycle occurs when both mating type are present. The female mating type participates to sexual reproduction by oogonium, and the male mating type by the antheridium. The fertilization of oognium by antheridium bears oospores. The sexual reproduction offers to *P. infestans* more genetic variability. In general, the races resulting from sexual reproduction are more aggressive than those resulted from vegetative multiplication. For instance, the races occurred in Mexico where are present both mating type are the most virulent, and the disease occurs although in cultivars that are demonstrated resistant in the other regions of the world (**Fry, 2008**)

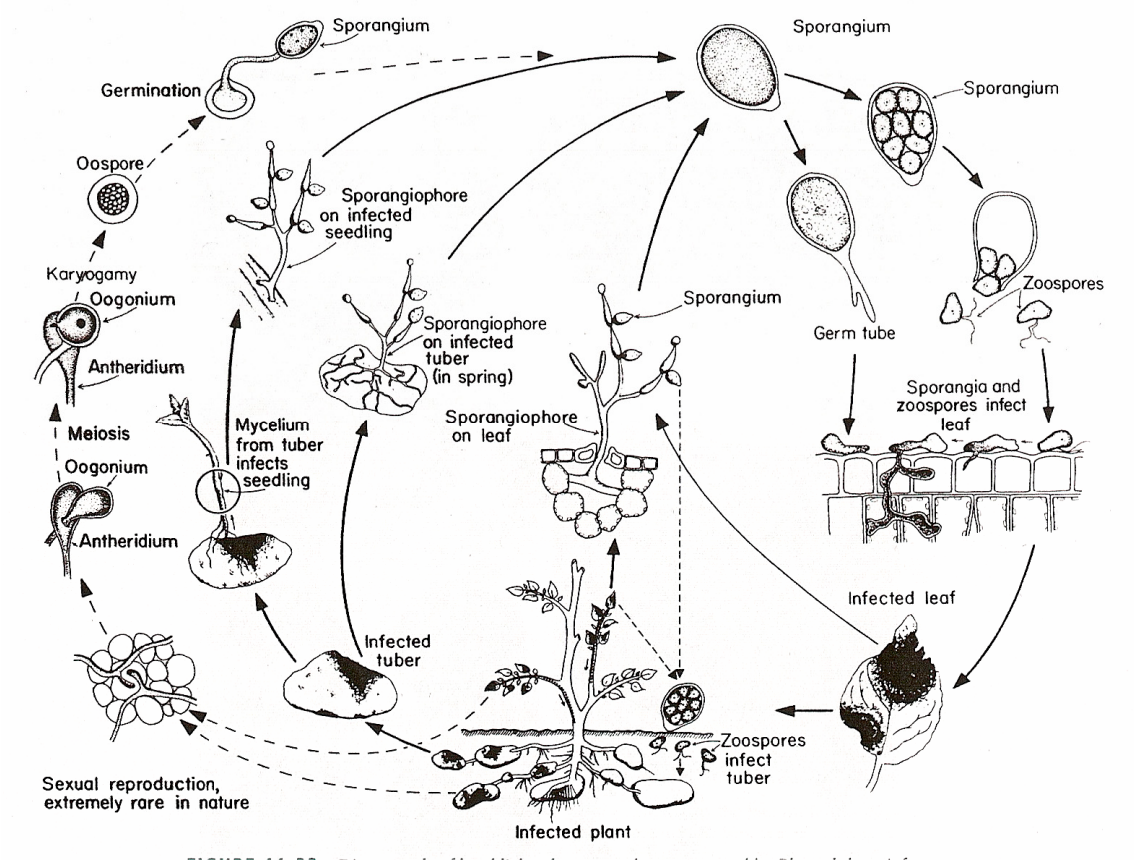

Fig. 1. *Phytophthora infestans* life cycle (Agrios, 1997)

Mechanisms of pathogenicity

Plant pathogens have developed sophisticated molecular strategies to evade and manipulate the plant immune system (**Qutob et al., 2006**).

P. infestans adopts a two-step infection style typical of hemibiotrophs: the first infection stage, in which the pathogen needs living host cells, is followed by extensive

necrosis resulting in colonization and sporulation (**Kamoun and Smart, 2005**). For a successfully infection and colonization, a series of processes are needed. They include adhesion to plant surface, penetration and colonization (**Huitema et al., 2004**). It involves the secretion of proteins and other molecules (**Huitema et al., 2004**). Some of these molecules participate to the pathogen attachment to plant surface, others to breaking physical obstacles to infection (cell wall and membranes), and several modify the plant physiology by suppressing plant defense (**Kamoun and Smart, 2005**). The suppression of host defenses can occur through the production of inhibitory proteins that target host enzyme (**Kamoun, 2003**). Effectors are molecules that manipulate host cell structure and function, thereby facilitating infection (virulence factors or toxin) and/or triggering defense responses (avirulence factors or elicitors) (**Kamoun, 2006**). A classification of *P. infestans* effectors is presented in table 1. It is based on the **Kamoun**'s (**2006**) oomycete effector classification.

Table 1. *P. infestans* effectors and their function.

Localisation	Group	Effector	Function	References
Apoplastic	Enzyme Inhibitors	EPI1 and EPI10	Inhibit and interact with P69B, protect proteins from degradation by P69B	Kamoun, 2006, and references in
		EPIC1 and EPIC2	Target an apoplastic papain-like protease	Kamoun 2006
	Small Cysteine-rich protein	INF1, INF2A and INF2B	Condition avirulence in *Nicotiana benthamiana*	Kamoun 2006 and references in
		SCR74 and SCR91	unknown	
	Nep1-like (*NLP*) family	PiNPP1	Induce defense reponses in both susceptible and resistant plants	Kamoun, 2006
Cytoplasmic	RxLR protein	AVR3a	Induces HR in potato carrying R3a gene Cell death suppressor	Kamoun, 2007
	CRN protein	CRN1, CRN2 and CRN8	Elicit cell death	Kamoun 2007 and references in

Kamoun (2006) divided oomycete effectors into two groups: cytoplasmic effectors are translocated inside the plant cell through specialized structures (haustoria and infection vesicle) and the apoplastic effectors which are secreted into the plant extracellular space where they interact with extracellular targets and surface receptors (**Kamoun, 2006**).

Apoplastic effectors

1. Enzyme inhibitors

Their function is to neutralize plant pathogenesis-related (PR) protein: plant enzymes, such as glucanases, chitinases, and proteases.

1.1. Serine protease inhibitors EPI1 and EPI10:

In tomato, the P69B is a serine protease. The *epi1, epi10,* and *P69B* genes are concurrently expressed and up-regulated during tomato infection (**Kamoun, 2006**). **Tian and Kamoun, in Kamoun,** (**2006**) have found that EPI1 protects several secreted proteins in *P. infestans* from degradation by P69B thereby directly contributing to virulence.

1.2. Cysteine protease inhibitors EPIC1 and EPIC2

In tomato, they target an apoplastic papain-like cysteine protease (**Tian et al., 2007**).

2. Small cyteine-rich protein

2.1. Elicitins: INF1, INF2A and INF2B

In *Nicotiana* sp. Elicitins induce defense responses (hypersensitive response, ...). *P. infestans* strains deficient in the INF1 induce disease lesions in *Nicotiana benthamiana* (**Kamoun et al., 1998**) all elicitin genes encode a putative extracellular proteins that share a 98 amino-acid elicitin domain with 6 conserved cysteines (**Kamoun, 2006**).

2.2. PcF-like SCR74 and SCR91

PcF is a peptide formed the 52 amino-acids secreted by *P. cactorum* (**Orsamando et al., 2001**). In *P. infestans* two genes are known *scr74* and *scr91* that code for this type of proteins. The *scr74* expression is up-regulated approximately 60 fold two-four days after tomato inoculation, in potato it is also induced during early stage of colonization (**Liu et al., 2005 in Kamoun, 2006**). The function of these proteins in pathogenicity is until now unknown.

3. NEP1-Like family

Or NLPs are widely distributed proteins particularly in plant associated species (fungi, oomycetes and bacteria) with about 25 kDa (**Pemberton and Salmond, 2004**). NLPs induce defense response in both susceptible and resistant plant (**Kamoun, 2006**). In *P. infestans* PiNPP1 is described. The NLP genes are expressed late during infectionand thus may function in triggering the host tissue necrosis observed during the necrotrophic infection phase thereby facilitating colonization (**Kanneganti and Kamoun, in Kamoun, 2006**).

Cytoplasmic effectors

1. RXLR protein family

This family is represented in *P. infestans* by Avr3a. Which are at least two secreted proteins of 147 amino acids (**Armstrong et al., 2005 in Kamoun, 2006**). Potato cultivars carry the *R3a* are resistant to *P. infestans* races which carry the *Avr3a. P. infestans* races with recessive allele (*avr3a*) are virulent on potato cultivars carried the *R3a*. The *avr3a* is conserved among all *P. infestans* isolates examined, suggesting that it plays an important function in the pathogen (**Kamoun, 2006**). The Avr3a can suppress the hypersensitive response induced by elicitin (INF1), pointing to a possible virulence function (**Bos et al., 2006**). Avr proteins are similar to a host-cell targeting signal in virulence protein of *Plasmodium falciporum* (malaria parasite), which suggested a conserved role in pathogenicity (**Birch et al., 2006; Haldar et al., 2006**).

2. CRN protein family

The expression of both *crn1*and *crn2* in *Nicotiana* sp. and in tomato results in a leaf-crinkling and cell death phenotype accompanied by an induction of defense related genes (**Kamoun, 2006**). The genes *crn1*, *crn2* and *crn8* induce cell death in host plants (**Torto et al., 2003**; **Win et al., 2006**).

CONCLUSION

Phytophthora infestans uses many protein effectors to accomplish its parasitic colonization. The host resistance is based on the recognition of these effectors. The mechanisms of action of the majority of effectors are unclear. More study in this domain, and in the host responses to infection are necessary to well understanding the relationship *Phytophthora infestans*-host, for more efficiency in resistance breeding.

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