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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

EX·TERRA·AVRVM

FRUIT GROWING

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FERTILIZATION IMPACT ON THE GROWTH AND NUTRITIONAL STATUS OF PEACH PLANTING MATERIAL FROM REDHAVEN CULTIVAR ON GF677 ROOTSTOCK, GROWN IN CONTAINERS

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Abstract

A pot experiment was conducted to investigate the impact of different fertilizer rates on growth characteristics and nutritional status of plants from Redhaven cultivar, grafted on GF677 (P. amygdalus x P. persica) rootstock and grown in containers. Variants of the experiment were: Variant I - Control (non-fertilizer), Variant II - $N_1P_{0.25}K_{0.5}Mg_{0.1}$*, Variant III -* $N_2P_{0.5}K_1Mg_{0.2}$ *and Variant IV -* $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$*. The results show that, at the three fertilized variants are obtained plants with a height of 164 to 177.26 cm and a stem diameter of 12.10 to 12.65 mm. The highest average values for stem diameter (12.65 mm) were found in low-fertilized plants. The control plants reached average values of 59.13 cm in height and 5.86 mm in stem diameter and in comparison to all fertilized variants the differences were statistically proven. The results obtained show that the fertilization influenced the content of N, P, K, Ca and Mg. It is concluded that fertilization with all three fertilizer rates (*$N_1P_{0.25}K_{0.5}Mg_{0.1}$*;* $N_2P_{0.5}K_1Mg_{0.2}$*;* $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$*) are suitable for the container production of peach planting material for establishing fruit orchards.*

Key words*: peach, container growing, fertilization, vegetative behaviour, planting material.*

INTRODUCTION

A new approach in the production of fruit planting material is the container growing. Its advantages are the easier controlling of the cultivation conditions, such as pH of the nutrient substrate, water and nutrient requirements, diseases and pests (Ruter, 1993). Container grown plants have a greater fine root mass compared to field-grown plants (Gilman & Beeson, 1996), show much less stress when planted in the orchard, due to their undamaged root system (Harris, J. R. and E. F. Gilman, 1993), resulting in lower rate of dying after being planted in the field (Mathers et al., 2007). Fertilization is one of the most important practices for the quality of container grown plants, because they are grown in a limited nutritional volume which prevents their growth (Landis, 1989). The production of a qualitative fruit planting material is of great importance as it determines the future behaviour of fruit trees in the orchards. According to Oliet et al. (2004) fertilization can increase the growth of plants, improve their nutrient supply and increase the resistance to water stress, low temperatures and diseases. Often fertilizers used in plant nurseries with container cultivation exceed the required rates for optimal growth (Maust & Williamson, 1991). Improving the efficiency of fertilizer application is one of the ways of reducing production costs and obtaining fruit planting material suitable for establishing fruit orchards.

The aim of the study was to assess the impact of fertilization on the growth and nutritional status of peach planting material from Redhaven cultivar on GF677, produced in containers.

MATERIALS AND METHODS

The study was conducted in the period 2017-2018 at the Fruit Growing Institute in Plovdiv, Bulgaria. Peach plants from Redhaven cultivar, grafted on GF677 (*P. amygdalus* x *P. persica*) rootstock under conditions of container growing experiment were studied.

The GF677 rootstock was produced in 2017 at the Production laboratory for in vitro propagation in the Fruit Growing Institute - Plovdiv. The micropropagated plants were grown in plastic containers of 7.5 litters capacity. A mixture of peat and perlite in a 2:1 ratio was used for the substrate. The plants were grown outdoors in a shaded field and fertilized with ammonium nitrate (NH_4NO_3). In August the rootstocks were grafted with

Redhaven cultivar. The fertilization experiment was set in 2018 in four variants with twenty five replications, each plant considered a separate replicate.
Variants of the experiment:
I. Control (non-fertilizer);
II. $N_1P_{0.25}K_{0.5}Mg_{0.1}$/container;
III. $N_2P_{0.5}K_1Mg_{0.2}$/container;
IV. $N_{3.2}P_{0.8}K_{1.6}$ $Mg_{0.32}$/container

Fertilization with increasing nutrient rates was applied on the surface four times. The fertilization was carried out with a combined Kristalon compound fertilizer by YARA - N(20%)-P_2O_5(5%)-K_2O(10%)-MgO(2%), applied every 20 days, the first introduce being made at the beginning of May.
The soil moisture in the containers was maintained to a field capacity, with the number of waterings complied with the specific temperature conditions and the amount of precipitated rainfall.
The following parameters were recorded: plant height (cm), stem diameter (mm), leaf area (cm^2), root system volume (cm^3) and content of photosynthetic pigments. The leaf area was measured by scanning the leaves and analysing the resulting images with specialized software (Gao et al., 2011). The volume of the root system was measured by the Burdett method (1979). The content of chlorophyll (a, b, a+b) and carotenoids was determined spectrophotometrically in 95% ethyl alcohol extract (Skazkin et al., 1958).
In order to determine the nutritional status of the plants, a chemical analysis of the leaves was carried out. Samples of 15 fully developed leaves from each replication of the variants were taken. Total concentrations of nitrogen, phosphorus, potassium, calcium, and magnesium were determined by standard methodologies (Tomov et al.,1999; Campbell & Plank, 1998; Karageorgiev, 1977; Stoilov, 1968).
The results obtained are subjected to mathematical analysis using the method developed by David B. Duncan (Duncan, 1955; Harter, 1960). Software used in the study are "R-3.1.3" in combination with "RStudio-0.98" and installed package "agricolae 1.2-2" (Mendiburu, 2015).

RESULTS AND DISCUSSIONS

The results show that the YARA Kristalon mineral fertilization has a significant impact on the growth characteristics of peach plants (Table 1). The fertilized variants (var. II, var. III and var. IV) had higher values in all measured parameters compared to the control (var. I), the differences being statistically proven.
The obtained results show that there are no significant differences between the fertilized variants (var II, var. III and var. IV)) on the growth of stem diameter and plant height.

Table 1. Impact of the fertilization on the growth characteristics of peach planting material, at the end of vegetation

Variant	Plant height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Volume of root system (cm^3)
I (Control)	59.13 b	5.86 b	32.36 c	78.33 c
II	170.40 a	12.65 a	51.86 b	320.00 a
III	177.27 a	12.31 a	56.74 b	213.33 b
IV	164.00 a	12.10 a	65.58 a	206.67 b

The nourished plants (var. II, var. III and var. IV) had a height from 164 to 170.40 cm, and those of the control variant had lower average height values (59.13 cm) (Table 1).
The average stem diameter of the control plants was 5.86 mm. The fertilized plants (var. II, var. III and var. IV) had higher average values for stem diameter - from 12.10 to 12.65 mm. The differences are statistically proven.
Significant differences were observed in the leaf area of all fertilized variants compared to the control. The plants of fertilized variants (var. II, var. III and var. IV) had leaf area from 50.06 to 65.58 cm^2, and those of the control variant were characterized by lower average values (32.36 cm^2) (Table 1).
The data shows that fertilization affects both the aboveground part of the plants and the root system. The average value of the volume of the root system of the control plants was 78.33 cm^3 The low-fertilized plants (var. II) have a higher root system volume - 320.00 cm^3, which is approximately 4 times higher than the non-fertilized plants (var. I). Medium and high fertilizer rates (var. III and var. IV) have been found to stimulate the growth of the root, but compared to the low fertilizer rate (var. II) the

effect of the fertilization on the root system volume is lower. A number of authors point to a positive correlation between the volume of the root system and the subsequent crop development under field conditions, planting material with larger root system having higher survival rates. (Rose et al., 1991a, 1991b, 1992, 1997; Jacobs et al., 2005).s


Figure 1. Peach planting material at the beginning and at the end of vegetation

Chlorophyll is the basic catalyst of photosynthesis, as the green pigments exists in all plant tissues that do photosynthesis (Masinovsky et al., 1992). Although the content of photosynthetic pigments is not the only criterion for photosynthesis of plants, their content can be considered as a indicator of the photosynthetic competence of the plants. The results obtained for the photosynthetic pigments in the leaves (mg/g fresh weight) are presented in Table 2.

Table 2. Content of photosynthetic pigments in leaves (mg/g fresh weight) of peach plants with different rates of fertilization

Variant	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a+b (mg/g)	Carotenoids (mg/g)
I (Control)	0.88 b	0.33 b	1.21 b	1.19 b
II	1.91 a	0.87 a	2.94 a	2.21 a
III	2.06 a	0.93 a	2.96 a	2.33 a
IV	2.09 a	1.03 a	2.98 a	2.36 a

The results obtained show that the content of chlorophyll a, b, a+b and carotenoids increases when applied increasing rates of the used fertilizer Kristalon, as the differences with the control plants are statistically proven. In all tested variants the values of chlorophyll a are higher than those obtained for chlorophyll b. The content of chlorophyll a increases from 0.88 mg/g (var. I) to 2.09 mg/g (var. IV), and that of chlorophyll b increases from 0.33 mg/g (var. I) to 1.03 mg/g (var. IV). The highest values of total chlorophyll a+b (2.98 mg/g) and carotenoids (2.36 mg/g) are obtained at the high fertilization rate (var. IV) As the fertilizer rate increases the content of photosynthetic pigments in leaves also increases, but there are no significant differences between the fertilized variants (var. II, var. III and var. IV). Considering the obtained results we can therefore conclude that the applied fertilizer is beneficial to the content of photosynthetic pigments in leaves, which is a condition for good physiological status of peach plants.

The influence of the various fertilizer rates on the content of macroelements in the leaves is presented in Table 3.

Table 3. Contents of the macroelements in the leaves

Variant	%N	%K	%P	%Ca	%Mg
I (Control)	1.88 d	3.30 b	0.47 ab	2.68 a	0.44 b
II	3.26 c	3.32 b	0.36 b	2.33 a	0.59 ab
III	3.63 b	3.63 a	0.41 ab	2.74 a	0.73 a
IV	4.13 a	3.36 b	0.51 a	2.30 a	0.56 ab

The results obtained show that the fertilization influenced the content of macroelements in the leaves of peach plants.

It was found that with the applied fertilizing rates the content of N in the leaves increases straightforward to the fertilizer rate. The content of N in the leaves was approximately twice higher in the fertilized plants than those of the control. Foliar nitrogen fluctuates from 1.88% at the control to 4.13% for variant IV. The differences are statistically proven.

The values for the K content in the leaves were in the range from 3.30% to 3.63%. Only in variant III there is a statistically proven difference with the control.

The values obtained for the Mg in all variants of fertilization were in the range 0.44% - 0.73%. The highest content of 0.73% was in variant III and the difference with the control variant being significant.

The content of P in the leaves was in the range from 0.36% (var. II) to 0.51%. (var. IV). The results regarding the content of P did not show a clear tendency.

The values for the Ca content in the leaves varied in range 2.30-2.74%. The fertilization rate did not significantly affect the calcium content in the leaves of the peach plants.

The fertilization applied has a favourable effect on the content of the macroelements in the leaves of peach trees. Fertilization leads to a better nutritional supply compared to the control.

CONCLUSIONS

Fertilization with Kristalon by YARA in fertilization rates $N_1P_{0.25}K_{0.5}Mg_{0.1}$; $N_2P_{0.5}K_1Mg_{0.2}$ and $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$ leads to the production of peach planting material of larger sizes than the control (not-fertilized) trees.

The content of macroelements in the leaves of the grafted plants of the Redhaven cultivar on GF677 (*P. amygdalus* x *P. persica*) rootstock are effected from fertilization with the combined fertilizer Kristalon by YARA. Fertilization leads to a better nutritional supply compared to the control.

All three fertilizer rates ($N_1P_{0.25}K_{0.5}Mg_{0.1}$; $N_2P_{0.5}K_1Mg_{0.2}$ and $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$) are effective, stimulate plant growth and are suitable for the container production of peach planting material for establishing fruit orchards. In container production of peach plants, nutrition should be a mandatory practice.

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FIRE BLIGHT SUSCEPTIBILITY OF PEAR CULTIVARS GRAFTED ON OHF 333 ROOTSTOCK

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Abstract

The rootstock OHF 333 ('Old Home' × 'Farmingdale') is characterized by high yield efficiency and moderate resistance to fire blight (Erwinia amylovora) in Bulgarian environmental conditions. The aim of this study was to examine the reaction of 10 pear cultivars grafted on OHF 333 rootstock to Erwinia amylovora. Their susceptibility was determined after artificial inoculation of leaves in a greenhouse. Several vigorously growing shoots per tree were inoculated with two pathogenic local isolates of Erwinia amylovora by cutting the leaves. The two bacterial suspensions of Bulgarian strains were used single and mixed inoculum (3 x 10^8 cfu/ml). Based on the artificial inoculation the degree of attack of leaves and shoots was calculated. All cultivars were grouped into 5 categories - very low susceptible, low susceptible, moderately susceptible, susceptible and very susceptible. In twenty-five days after inoculation with isolate Ea3325, 6 of the studied cultivars reacted as very low susceptible showing less than 20% shoot necrosis. Two of the cultivars, artificially inoculated, with mixed inoculum showed terminal shoots necrosis over 60%.

Key words: *bacteria, Erwinia amylovora, pear rootstock, single strain, mixture of two strains*.

INTRODUCTION

During the recent 15 years, the Bulgarian pear production has collapsed. In 1980 Bulgaria has produced 96 thousand tons pears and was on 18th place in the world. In 2005 our pear fruit production reached its lowest level - 750 tons, and today Bulgarian pear production is only 1,974 tons. The areas occupied by pear orchards are much reduced and the fruit production is limited. The main reasons for that are the use of old and inefficient technologies for growing them and low efficiency of the applied plant protection products against the major pest *Cacopsilla pyri* L. and bad health condition of the orchards (Gandev et al., 2014).
The most economically important disease for the pears is fire blight. This disease is caused by the bacterium *Erwinia amylovora*. Fire blight is apparently indigenous to North America and it was first noticed in the late 18th century in New York state. Later during 1921 and 1970 fire blight was included in the list of the plant diseases in Central and South America, Europe, Oceania and Africa. In Bulgaria, it was discovered for the first time 17 years ago (Bobev et al., 1999).

Erwinia amylovora is a pathogen of more than 130 plant species belonging to 40 genera, mainly from the family Rosaceae (Van der Zwet and Keil, 1979). The disease occurs on pear (*Pyrus* spp.), apple (*Malus* spp.) and quince (*Cydonia* spp.) trees as well as on hawthorn (*Crataegus* spp.), sorb (*Sorbus* spp.), cotoneaster (*Cotoneaster* spp.). It is generally believed that *E. amylovora* is a homogeneous species and does not show pathogenic specialization. This means that each isolate of the pathogen is potentially able to infect any of the known host plants (Momol and Aldwinckle 2000). However, in an artificial inoculation experiment, De Ley et al. (1983) have shown that different isolates may exhibit some variations in host range. Isolates from plants of the genus *Rubus* are particularly noteworthy because they are incapable of infecting apple and pear trees (Asselin et al., 2008; De Ley et al., 1983; Evans, 1995; Ries and Otterbacher, 1977; Starr et al., 1951).
Biodiversity of bacterial plant pathogens is a result of their variability. This variability is caused by both environmental conditions leading to not fully hereditary changes in phenotype, and variation in the genetic material

(Agrios,1988; Puławska and Sobiczewski, 2012).
The pear breeding for resistance to fire blight is a priority in obtaining new cultivars (Gunen et al., 2003). For this reason, it is necessary to identify resistant genotypes which can be used as genitors in artificial hybridisations (Zwet et al., 1974; Zwet and Bell, 1990; Sestras et al., 2008).
The cultivar performance on different rootstocks particularly on OH x F clones, including effects on the size, vigour, compatibility and precocity, have been studied in the USA and Canada (Westwood et al., 1976; Lombard and Westwood, 1976; Larsen and Fritts, 1984; Kappel and Quamme, 1988)
The pear rootstock OH × F (*P. communis* L.), bred in the USA, shows 10% more vigorous growth compared to BA29. The rootstock is characterized by high yield efficiency and moderate resistance to fire blight (*Erwinia amylovora*) (Dzhuvinov et al., 2008).
The aim of the following study was to examine the reaction of pear cultivars when grafted on OHF 333 to *Erwinia amylovora* and to compare the reaction of the cultivars when inoculated with different strains.

MATERIALS AND METHODS

This study was conducted in 2014-2017 at the Fruit Growing Institute - Plovdiv. The cultivars 'Beurré Bosc', 'Williams', 'Red Williams', 'Abate Fetel', 'Packham's Triumph', 'Starkrimson', 'Highland', 'Carmen', 'Tosca', 'Etrusca' were grafted on the pear rootstock Old Home x Fermingdale (OHF333) and grown in an experimental greenhouse as potted one-year-old trees.
Bacteria of two *Erwinia amylovora* strain were cultivated on a King's B medium for 24 h at 25°C then washed off the medium with sterile distilated water. The concentration of the bacterial suspension was regulated to 3 x 10^8 cfu/ml (Mc Farland). The artificial inoculation of the cultivars was done by the application of two different isolates of bacteria and a combination of them in suspension mixture:

- Ea 3325 strain - isolated from apple on 16.05.2013 in Petrich (Bulgaria);
- Ea 3345 strain - isolated from pear on 27.06.2013 in Botevgrad (Bulgaria).

The artificial inoculation was done by cutting 1/3 of the blade of three leaves with scissors dipped in the bacterial suspension. The reaction of the leaves was classified by the grading scale of disease severity of Zeller and Wolf (1996), on the 10^{th} and 15^{th} day after the artificial inoculation:

- Class 0 - no visible symptoms of an infection;
- Class 1 - the place where the cut was done is in black;
- Class 2 - visible symptoms on the place of the cut and leaf veins;
- Class 3 - necrosis on the leaf blade;
- Class 4 - necrosis on petiole;
- Class 5 - necrosis spread on the whole shoot tip.

To determine the disease attack index of leaves, the results obtained by the scale were transformed by the Mc Kiney formula.
Twenty-five days after the inoculation susceptibility level of the shoots to fire blight, was calculated by the formula below (Thomson et al., 1975):
Length of the infected part (cm)= Susceptibility of shoots/Total shoots length (cm) x 100.
The tested cultivars were classified in 5 susceptibility classes according to the calculated susceptibility level of the terminal shoots (Le Lezec et al., 1997):

1 Very Low Susceptible - 0-20
2 Low Susceptible - > 20-40
3 Moderately Susceptible - > 40-60
4 Susceptible - > 60-80
5 Very Susceptible - > 80-100

On the 45^{th} day after the artificial inoculation, on all pear cultivars, fire blight symptoms development to stem and rootstock was observed. To prove the damage was caused by *Erwinia amylovora* a reisolation of infected tissue was done.

RESULTS AND DISCUSSIONS

OHF 333 is a pear rootstock tolerant to fire blight, drought, chloroses and nematode (Erdal & NazlI, 2019). In Bulgarian environmental conditions it is also characterized by high yield efficiency and moderate resistance to fire blight. The experiments with artificial inoculation of grafted on OHF 333 cultivars give us information about their susceptibility to fire blight when a tolerant rootstock is used for producing the planting material.

Ten days after artificial inoculation with Ea 3325 strain the lowest disease severity index was recorded for the cultivars ‘Williams’ and ‘Red Williams’ (Table 1). The differences in the disease severity index with ‘Carmen’, ‘Tosca’, ‘Abate Fetel’ and ‘Packham’s Triumph’ were statistically significant. The highes pecentage of infected with the single Ea3325 strain leaves was recorded for ‘Carmen’ cultivar.

Fifteen days after the artificial inoculation the calculated indexes for the cultivars ‘Williams’ and ‘Red Williams’ were still the lowest.

Table 1. Pear leaves infection to fire blight after inoculation with a single *Erwinia amylovora* strain or a mixture of 2 strains

Cultivar/Day	Strain Ea3325		Strain Ea3345		Mixture of both strains	
	10	15	10	15	10	15
Beurré Bosc	15.77 bc	50.94 abc	48.26 ab	83.57 a	49.93 a	100.00 a
Williams	7.50 c	14.33 c	31.17 bc	71.00 a	25.00 a	62.67 ab
Red Williams	8.67 c	12.92 c	34.33 bc	55.00 ab	32.67 a	58.67 ab
Abate Fetel	34.89 ab	51.78 ab	27.84 bc	54.71 ab	45.67 a	66.97 ab
Packham's Triumph	29.18 ab	59.37 a	34.17 bc	85.00 a	43.16 a	92.37 ab
Starkrimson	21.02 abc	65.00 a	23.56 c	56.40 ab	20.51 a	73.33 ab
Highland	14.93 bc	23.50 bc	13.82 c	28.96 b	20.66 a	54.60 ab
Carmen	37.43 a	44.13 abc	20.50 c	33.71 b	20.00 a	36.77 b
Tosca	29.37 ab	41.32 abc	61.73 a	89.80 a	35.56 a	73.33 ab
Etrusca	17.87 abc	38.93 abc	22.65 c	55.23 ab	25.57 a	82.50 ab

After artificial inoculation with Ea3345 strain the lowest percentage infected leaves was recorded for the cultivars ‘Highland’ and ‘Carmen’. This was observed and 15 days after the inoculation. When infected with Ea3345 the cultivars ‘Williams’ and ‘Red Williams’ reacted with a higher percentage of necrosis and 15 das after the inoculation ‘Williams’ is even one of the most affected cultivars.

When infected with a mixture of both *Erwinia amylovora* strains non-significant differences between the ractions of all pear cultivars were observed on the 10th day. Fifteen days after the artificial inoculation the differences were statistically significant between the least and the most affected cultivars - resp. ‘Carmen’ and ‘Beurré Bosc’.

Twenty-five days after the artificial inoculation was recorded the spread of the disease from the cultivars leaves to the shoots. The result shown in Table 2 express the average length of the shoots lessions calculated for the 3 years of experiments. When ‘Red Williams’ was inoculated with Ea3325 the infection was localized in the leaves.

Figure 1. Infected leaves of ‘Packham's Triumph’ cultivar after inoculation with mixture of strains

Table 2. Length of the observed lesions on shoots after inoculation with single or mixture of *Erwinia amylovora* strains (%)

Cultivar	Ea3325	Ea3345	Mixture of both strains
Beurré Bosc	63.08 a	37.27 abc	86.4 a
Williams	6.33 ef	23.01 cd	23.66 c
Red Williams	0 f	12.1 de	48.24 b
Abate Fetel	22.19 bcd	5 e	13.12 c
Packham's Triumph	34.52 b	48.55 a	72.86 a
Starkrimson	19.74 cde	14.53 de	47.74 b
Highland	8.48 def	15.68 de	29.57 c
Carmen	6.95 ef	4.4 e	28.61 c
Tosca	28.05 bc	43.91 ab	77.65 a
Etrusca	16.45 cde	28.84 bcd	53.66 b

The result for this cultivar's reaction were statistically significant with 'Beurré Bosc', 'Abate Fetel', 'Packham's Triumph', 'Starkrimson', 'Tosca' and 'Etrusca'. The longest lesions were measured on the 'Beurré Bosc' cultivar - 63.08% of the total length of the shoot.
For two of the tested cultivars ('Carmen' and 'Abate Fetel') inoculated with Ea3345 strain the infection was in the lowest percentage. The highest percentage of shoots with lesions was observed for 'Packham's Triumph' fllowed by 'Tosca' and 'Beurré Bosc'.

Figure 2. Infected shoot of 'Red Williams' cultivar inoculation with sigle strain Ea3325

When for the inoculation was used mixure of both *Erwinia amylovora* strains were observed distinct differences between the cultivars. The most damaged cultivars were 'Beurré Bosc', 'Tosca' and 'Packham's Triumph'. The cultivars with the lowest reaction to the mixture were - 'Abate Fetel', 'Williams', 'Carmen' and 'Highland'. 'Starkrimson', 'Red Williams' and 'Etrusca' had a medium value of the length of infected part of the shoots.
The results described, show that a variation in the reaction of each pear cultivar was observed when the inoculation was done using the separate strains. Some of them are susceptible to Ea3325. For example 'Williams' and 'Red Williams'. When both strains cause mixed infection they are more virulent. For screening genotype's susceptibility to fire blight some authors consider using inoculum containing a mixture of several highly virulent strains originating from various hosts as more reliable and more reflective of the situation occurring in nature where cross infections are common (Lespinasse and Aldwinckle, 2000; Norelli et al., 1987, 2003).
The five grade scale of Le Lezec et al. (1997) gave us the opportunity to classify the tested cultivars according to their terminal shoots susceptibility level. According to their susceptibility to Ea3325 strain, 6 of the cultivars were classified as very low susceptible, 3 - as low susceptible and 'Beurré Bosc' was the only cultivar showing moderate susceptibility. The five cultivars 'Red Williams', 'Abate Fetel', 'Starkrimson', 'Highland' and 'Carmen' were classified as very low susceptible to Ea3345 strain. Moderately susceptible to this strain were 'Tosca' and 'Packham's Triumph'. In a combination as mixture both strains are more aggressive and the susceptibility of the cultivars increased. The moderately and low susceptible to the single strains cultivars were classified as susceptible to the mixture - 'Beurré Bosc', 'Packham's Triumph' and 'Tosca'. The cultivars with best results were 'Williams', 'Abate Fetel', 'Highland' and 'Carmen'. Their susceptibility was very low to low when inoculated with the single strains or the mixture (Table 3).

Table 3. Susceptibility classes according to calculated susceptibility level of the terminal shoots

Cultivar	Susceptibility class Ea3325	Susceptibility class Ea3345	Susceptibility class Mix
Beurré Bosc	Moderately Susceptible	Low Susceptible	Susceptible
Williams	Very Low Susceptible	Low Susceptible	Low Susceptible
Red Williams	Very Low Susceptible	Very Low Susceptible	Moderately Susceptible
Abate Fetel	Low Susceptible	Very Low Susceptible	Very Low Susceptible
Packham's Triumph	Low Susceptible	Moderately Susceptible	Susceptible
Starkrimson	Very Low Susceptible	Very Low Susceptible	Moderately Susceptible
Highland	Very Low Susceptible	Very Low Susceptible	Low Susceptible
Carmen	Very Low Susceptible	Very Low Susceptible	Low Susceptible
Tosca	Low Susceptible	Moderately Susceptible	Susceptible
Etrusca	Very Low Susceptible	Low Susceptible	Moderately Susceptible

Fourty five days after the artificial inoculation the bacteria development was monitored from shoot to rootstock (Table 4).

Necrosis on shoots was observed for each of the tested cultivars. For 'Beurré Bosc' and 'Tosca' necrosis was detected on the stem. For all of the other pear cultivars, the bacteria has localized in the shoots. No symptoms were observed on the rootstock.

Table 4. Bacteria development in shoots, stem and rootstock

Cultivar	Shoots	Stem	Rootstock
Beurré Bosc	+	+	-
Williams	+	-	-
Red Williams	+	-	-
Abate Fetel	+	-	-
Packham's Triumph	+	-	-
Starkrimson	+	-	-
Highland	+	-	-
Carmen	+	-	-
Tosca	+	+	-
Etrusca	+	-	-

CONCLUSIONS

The cultivars with best results were 'Carmen', 'Williams', 'Abate Fetel' and 'Highland'. Their susceptibility to the bacterial disease was low when inoculated with the single strains and the mixture of both. These cultivars could be recommended for establishing production orachards.

After this experiment, 'Beurré Bosc' cultivar grafted on OHF 333 could be classified as the most susceptible among the 10 tested.

The mixture of *Erwinia amylovora* strains is more virulent than the single strains and caused the highest percentage of damages of all studied cultivars.

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SUSCEPTIBILITY OF PEAR CULTIVARS AFTER INOCULATION WITH BULGARIAN STRAINS OF *ERWINIA AMYLOVORA*

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Abstract

Pear production in Bulgaria was limited because of the occurrence of fire blight. There is no certain chemical control of this disease. In terms of integrated management of bacterial diseases, it is very important to grow resistant cultivars. The aim of this study was to examine the reaction of 15 pear cultivars grown in Bulgaria. Terminal shoots of these cultivars grown in a greenhouse and grafted on quince BA29 rootstock were inoculated with two Erwinia amylovora isolates. The bacteria were isolated from apple and pear orchards in various regions of Bulgaria. After artificial inoculation degree of severity of diseases on the leaves and terminal shoots were recorded. For all tested cultivars the degree of infection on the leaves varied from 11.0% for 'Alexander Lucas' to 82.3% for 'Packham's Triumph'. Twenty-five days after the inoculation the tested cultivars were distributed in five susceptibility classes by their degree of severity of diseases on terminal shoots. 'Beurré Bosc' was considered as susceptible with 61.1% necrosis on terminal shoots, 'Dr. Jules Guyot' was considered as very low susceptible with 18.4% terminal shoots showing symptoms. Six of the tested cultivars were low susceptible with recorded degree of infection 20-40%. The very low susceptible and low susceptible cultivars could be recommended for commercial pear orchards establishment.

Key words*: bacteria, Erwinia amylovora, quince rootstock, single strain.*

INTRODUCTION

Fire blight, caused by *Erwinia amylovora* is one of the most destructive diseases affecting pome fruit trees (Sobiczewski et al., 2015; van der Zwet 2006; Thomson 2000; Bonn and van der Zwet, 2000; Vanneste, 2000). In 1989, the pathogen causing fire blight was recorded for the first time in Bulgaria (Bobev et al., 1999). Now the disease spread in all parts of the country. Up to now, control measures used in the fire blight contaminated areas consisted of removal of diseased host plants or their parts (orchard sanitation), adherence to cultural practices, and application of chemical sprays (Bobev, 2010).

One of the most promising approaches for sustainable fire blight management is the planting of tolerant or highly resistant cultivars and rootstocks (Van der Zwet et al., 2012; Korba et al., 2008). The pear breeding for resistance to fire blight is a priority in obtaining new cultivars (Gunen and Misirli, 2003). For this reason, it is necessary to identify resistant genotypes which can be used as genitors in artificial hybridisations (Zwet et al., 1974; Zwet and Bell, 1990; Sestras et al., 2008).

The quince rootstock BA29 is commonly used rootstock in European pear. It is a vegetative (*Cydonia oblonga* L.), suitable for intensive pear orchards, with a possibility of intensive fruiting of pear cultivars. This rootstock has some serious disadvantages - it is highly susceptible to fire blight, often attacked by *Psylla pyri* and shows graft incompatibility with some pear cultivars (Dondini, L. and Sansavini, S., 2012).

The aim of this study was to determine for a first time in Bulgaria the reaction of fifteen pear cultivars grafted on BA29 after inoculation with *E amylovora* strains.

MATERIALS AND METHODS

The studies were conducted in 2014-2017 at the Fruit Growing Institute Plovdiv, Bulgaria. Pear cultivars were grafted on a quince rootstock (BA29) and grown in an experimental greenhouse as potted one-year-old pear trees. The cultivars 'Dr. Jules Guyot', 'Beurré Hardy', 'Beurré Giffard', 'Santa Maria', 'Curé', 'Ranna Bolyarka', 'Passe Crassane', 'Beurré Hardenpont' 'Conference', 'Beurré Bosc', 'Alexander Lucas', 'Packham's

‘Triumph’, ‘Red Williams’, ‘Williams’, ‘Abate Fetel’. Each of pear cultivar was planted in seven potted in repetition.

Bacteria of two *E. amylovora* strains were cultivated on a King’s B medium for 24 h at 25°C, then washed off the medium with sterile distilled water. The concentration of the bacteria suspension was regulated to 3×10^8 cfu/ml (Mc Farland). The artificial inoculation of the pear cultivars was done by the application of two different bacterial isolates and a combination of them:

- Ea 3325 - isolated from apple trees on 16.05.2013 in Petrich (Bulgaria);
- Ea 3345 - isolated from pear trees on 27.06.2013 in Botevgrad (Bulgaria).

The artificial inoculation was done by cutting 1/3 of the leaf blade of three leaves on each terminal shoot with scissors dipped in the bacterial suspension. On the 10th and 15th day after the artificial inoculations, the reaction of the leaves was classified using the grading scale of disease severity of Zeller and Wolf (1996):

Class 0 - no visible symptoms of an infection;
Class 1 - the place of the cut is black;
Class 2 - visible symptoms on the place of the cut and leaf veins;
Class 3 - necrosis observed on the leaf blade;
Class 4 - necrosis observed on petiole;
Class 5 - necrosis is spread on the whole shoot tip.

To determine the severity of diseases of leaves, the results were transformed by the McKinney formula.

$$I=\frac{\sum (n\times k)\times 100}{N\times K}$$

I - percentage Index of attack (%);
E (n.k) - the sum of the number of infected plants or organs (n) in the corresponding attack class (k);
N - the total number of plants examined (organs);
K - the highest grade in the corresponding scale.

Twenty-five days after the inoculation susceptibility level of the shoots to fire blight, was calculated by the formula below (Thomson et al., 1975):

Length of infected part (cm) = Susceptibility of shoots/Total shoots length (cm) × 100

The tested cultivars were classified in 5 susceptibility classes according to the calculated susceptibility level of the terminal shoots (Le Lezec et al. 1997):

1 Very Low Susceptible: 0-20;
2 Low Susceptible: > 20-40;
3 Moderately Susceptible: > 40-60;
4 Susceptible: > 60-80;
5 Very Susceptible: > 80-100.

On the 45th day after the artificial inoculation, on all pear cultivars, *E. amylovora* development to stem and rootstock was observed.

To prove the damage is causal by *E. amylovora* was taken the samples of infected tissue for resolution of bacteria in each cultivar.

The results were statistically analyzed by variance analysis of the SPSS 19 / column shows a significant difference ($p < 0.05$). The mean values were compared by using Dunkan test.

RESULTS AND DISCUSSIONS

The infection of the leaves of the tested pear cultivars was evaluated in dynamics through the 10th and 15th day after the inoculation was done.

The inoculated leaves with strain Ea3325 on the 10th days with the lowest percentage of infection was registered the cultivar ‘Alexander Lucas’ (11.0%) on the 15th day, the value of the infection with the same strain increased to 30.0% (Table 1) in 2016 year. The cultivars ‘Santa Maria’ (11.5%) and ‘Beurré Giffard’ (12.0%) had a similar severity of diseases. The most affected after inoculation with this strain were the cultivars ‘Beurré Bosc’ (76.5%) in 2015 year and ‘Packham's Triumph’ (60.4%) in 2017 year. The increased of value on the 15th day reached a 100% infected leaves.

The results after artificial inoculation with strains Ea3345 showed that with the lowest values respectively on 10th and 15th day after the infection were the cultivars ‘Beurré Hardy’ (from 13.6% to 30.9%) in 2017 year, ‘Santa Maria’ (from 13.0% to 30.8%) in 2016, and ‘Alexander Lucas’(from 10.6% to 30.6) in 2016 year. The most percentage of necrosis of leaves was calculated in cultivars ‘Beurré Bosc’ (from 52.1% to 100.0%) in 2017 year, ‘Williams’ (from 43.0% to 100.0%),‘Red Williams’ (from 67.4% to 100.0%) in 2016 year and ‘Packhams Triumph’ (from 54.6% to 100.0%) in 2016 year.

When the leaves were inoculated with mixture of strains the severity of diseases on the 15th day after inoculation is more than 50% of the tested pear cultivars were calculated 100.0% infected leaves. The lowest percentage of necrosis on 10th day were determine in cultivars Beurré Hardy (23.6%), Conference (30.0%), and Beurré Giffard (30.0%). From the result after the artificial infection was determined the difference in the percentage of infection witch depend on tested cultivars, this result was confirmed by other authors.

The leaves infection can be used for reliable rapid screening for the sensitivity of the varieties (Fazio et al., 2006, 2008), which can be used for early diagnostics.

Other researchers support this view by explaining different pathogenic susceptibility due to different structural and biochemical features in the leaves (Viljevac et al., 2009).

Table 1. Fire blight development (%) on pear leaves, inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Cultivar	Year	Strain Ea3325		Strain Ea3345		Mixture of Ea3325 and Ea3345 strains	
		Percentage of leaves infection after (days)					
		10	15	10	15	10	15
Dr. Jules Guyot	2015	20.2	87.9	26.0	51.5	55.0	80.0
	2016	20.4	50.7	30.0	43.1	33.3	50.0
	2017	16.7	40.5	21.1	40.0	54.9	96.1
Beurré Hardy	2015	17.4	34.8	23.2	42.5	31.7	67.4
	2016	18.4	38.1	20.5	50.1	23.6	63.6
	2017	22.2	49.8	13.6	30.9	30.7	63.8
Ranna Bolyarka	2015	30.0	70.8	20.0	37.8	50.0	100.0
	2016	24.7	60.4	36.2	73.1	46.7	100.0
	2017	37.6	70.4	42.3	83.9	48.1	100.0
Beurré Giffard	2015	33.3	69.8	21.2	47.1	57.1	93.3
	2016	12.0	30.8	27.5	63.2	30.0	85.0
	2017	32.8	60.4	40.0	60.1	41.3	87.0
Santa Maria	2015	40.3	80.0	34.0	78.4	64.7	100.0
	2016	31.9	79.6	13.0	30.8	43.3	100.0
	2017	11.5	34.9	43.0	87.2	62.6	100.0
Passe Crassane	2015	33.3	78.1	23.5	68.2	53.8	100.0
	2016	37.6	76.3	34.9	73.4	53.3	100.0
	2017	23.3	55.3	30.3	70.0	52.7	100.0
Beurré Hardenpont	2015	43.3	89.1	46.7	70.0	51.7	82.6
	2016	27.1	66.3	30.2	68,1	48.6	100.0
	2017	21.9	42.9	25.1	46.8	49,3	100.0
Alexander Lucas	2015	33.3	40.2	38.0	54.0	40.0	60.0
	2016	11.0	30.0	10.7	30.6	30.3	56.8
	2017	38.2	40.5	21.5	30.1	42.8	65.7
Curé	2015	20.0	48.9	24.0	69.4	43.3	100.0
	2016	50.3	90.5	36.4	89.0	86.7	100.0
	2017	48.1	87.5	43.2	90.3	91.0	100.0
Conference	2015	30.0	55.6	30.0	51.0	40.0	85.9
	2016	25.7	53.0	23.0	41.1	30.0	40.0
	2017	12.8	39.9	27.0	37.8	43.5	88.5
Beurré Bosc	2015	76.5	100.0	48.3	97.5	64.0	100.0
	2016	70.2	100.0	51.2	100.0	24.4	73.3
	2017	56.3	100.0	52.1	100.0	54.6	86.5
Williams	2015	44.0	76.4	20.0	40.0	53.3	100.0
	2016	35.0	100.0	43.0	100.0	50.0	100.0
	2017	40.5	97.0	31.6	65.4	51.8	100.0
Red Williams	2015	56.9	83.0	53.3	76.0	86.7	100.0
	2016	42.5	100.0	67.4	100.0	50.0	100.0
	2017	50.0	87.1	65.0	80.0	43.9	100.0
Abate Fetel	2015	46.0	54.3	37.8	60.0	75.0	100.0
	2016	51.1	100.0	32.0	89.9	24.4	46.7
	2017	48.7	68.5	45.0	79.5	62.2	89.6
Packham's Triumph	2015	30.0	68.7	41.1	65.0	80.0	100.0
	2016	40.0	100.0	54.7	100.0	37.5	100.0
	2017	60.4	100.0	64.6	100.0	76.5	100.0

In our study in each studied year, the experiment was conducted with the same cultivars, the artificial inoculations were done with the same strains and inoculation plant were maintained in similar conditionals.

The result showed that in different years were reported a variance in the percentage of infection of pear leaves.

The differences were explained by other authors. Following infection, the activity of phytoalexins becomes increasingly important in host plant resistance (Kuć et al., 1999).

Phytoalexins are low-molecular-weight antimicrobial compounds that are synthesized and accumulate in host tissue following exposure to microorganisms (Ebel, 1986).

The same author noted that some phytoalexins accumulate in both resistant and susceptible cultivars (Kuć et.al., 1999).

However, the accumulation of higher concentrations and the timing of their synthesis is critical for them to be effective (Bell, 2004).

The reacted of each plant is different after inoculation and the accumulation of phytoalexins in the plants may be different.

Figure 1. Infected leaves of Beurré Hardy cultivar

On the 10th after the inoculation six of the tested cultivars had different reaction which was statistically significant between inoculation with single or a mixture of two *E. amylovora* strains - ‘Dr. Jules Guyot’, ‘Beurré Hardy’, ‘Ranna Bolyarka’, ‘Passe Crassane’, ‘Conference’ and ‘Williams’ (Table 2). For the rest pear cultivars were observed non-significant differences. As a more virulent from both single strain in 10th days after inoculation is Ea3345. The averages rate for three studied years in all tested cultivar were reported the less damage after inoculation with Ea3325 but the differences non-significant.

Table 2.The average value of fire blight development (%) on the pear leaves after 10th days from inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Type of inoculum	**Cultivar**				
	Dr. Jules Guyot	**Beurré Hardy**	**Ranna Bolyarka**	**Beurré Giffard**	**Santa Maria**
Ea3325	19.1* b	19.1* b	30.8*b	26.1*a	27.9*a
Ea3345	25.7 b	19.3 b	32.8 b	29.6 a	30.0 a
Mixture	47.5 a	28.7 a	48.3 a	42.8 a	56.9 a
Type of inoculum	**Passe Crassane**	**Beurré Hardenpont**	**Alexander Lucas**	**Curé**	**Conference**
Ea3325	29.6*b	30.8* a	23.4* a	34.5* a	22.8 *b
Ea3345	31.4 b	34.0 a	27.5 a	39.5 a	26.7 ab
Mixture	53.3 a	49.8 a	37.7 a	73.7 a	37.8 a
Type of inoculum	**Beurré Bosc**	**Williams**	**Red Williams**	**Abate Fetel**	**Packham's Triumph**
Ea3325	47.7* a	31.5* a	49.8* a	38.3* a	43.5* a
Ea3345	50.5 a	39.8 ab	60.2 a	48.6 a	53.4 a
Mixture	67.7 a	51.7 b	61.9 a	53.9 a	64.7 a

*Different letters in the same column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

Table 3. The average value of fire blight development (%) on the pear leaves after 15th days from inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Type of inoculum	**Dr. Jules Guyot**	**Beurré Hardy**	**Ranna Bolyarka**	**Beurré Giffard**	**Santa Maria**
Ea3325	44.9* a	40.9* b	65.0* b	53.7* b	64.8* a
Ea3345	59.7 a	41.2 b	67.2 b	56.8 b	65.4 a
Mixture	75.4 a	64.9 a	100.0 a	88.4 a	100.0 a
Type of inoculum	**Passe Crassane**	**Beurré Hardenpont**	**Alexander Lucas**	**Curé**	**Conference**
Ea3325	69.9* b	61.64* a	36.9* b	75.6* a	43.3* a
Ea3345	70.5 b	66.1 a	38.2 b	82.9 a	49.5 a
Mixture	100.0 a	94.2 a	60.8 a	100.0 a	71.5 a
Type of inoculum	**Beurré Bosc**	**Williams**	**R. Williams**	**Abate Fetel**	**Packham's Triumph**
Ea3325	86.6* a	68.5* a	85.3* a	74.3* a	88.3* a
Ea3345	99.2 a	91.1 a	90.1 a	76.5 a	89.6 a
Mixture	100.0 a	100.0 a	100.0 a	78.8 a	100.0 a

*Different letters in the same row/column indicated significant difference ($p<0.05$)) were compared by using Dunkan test.

On the 15th day, five of tested cultivars had statistically significant differences – ‘Beurré Hardy’, ‘Ranna Bolyarka’, ‘Beurré Giffard’, ‘Passe Crassan’ and ‘Alexander Lucas’ (Table 3). The difference in the reaction of the ten cultivars: ‘Dr. Jules Guyot’, ‘Santa Maria’, ‘Beurré Hardenpont’, ‘Curé’, ‘Conference’, ‘Beurré Bosc’, ‘Williams’, ‘Red Williams’, ‘Abate’ ‘Fetel’ and ‘Packham's Triumph’ was statistically non-significant. As a more virulent from both single strain in 15th days after inoculation is again strain Ea3345. The averages rate for three studied years in all tested cultivar were reported the less damage after inoculation with Ea3325 but the differences non-significant. These results confirmed earlier findings (Sobiczewski et al., 2004; 2006) from experiments with application of single strain inoculum or inoculated either with a mixture of 4 strains or with each of them separately showed some differences in strain virulence, which was reflected in the severity of fire blight caused by mixed inoculum. Other studies (Norelli et al., 1984) clearly indicate that differential interactions occur between apple cultivars and strains of *E. amylovora*. On the other hand, (Paulin and Lespinasse, 1990) pointed that using the mixture of strains for inoculation of apple shoots did not always give a higher overall disease incidence and severity than the most virulent strain alone.

On the 25th day after the inoculation, the length of the lesions on shoots was measured for each cultivar. The inoculated pear cultivars with the mixture of the two strains or with each of them separately showed some differences in strain virulence, which was reflected in the severity of fire blight (Table 4). Both single strains showed a lower virulence level. The percentage of infected shoots after inoculation with single strain Ea3325 was lower for ‘Dr. Jules Guyot’ and ‘Beurré Giffard’. Similar results on these cultivars were observed and after the artificial inoculation with single strain Ea3345. After artificial inoculations with the mixture of both *E. amylovora* strains only ‘Dr. Jules Guyot’ reacted with low percentage of necrosis. In each artificial inoculation variants, the cultivar ‘Beurré Bosc’ reacted with the highest percentage of necrosis. After inoculation with single Ea3325 strain the cultivar ‘Dr. Jules Guyot’ showed severity of disease with statistically significant difference with ‘Beurré Hardy’, ‘Ranna Bolyarka’, ‘Santa Maria’, ‘Passe Crassane’, ‘Beurré Hardenpont’, ‘Conference’, ‘Beurré Bosc’, ‘Red Williams’, ‘Abate Fetel’ and ‘Packham's Triumph’.

Table 4. Pears shoots susceptibility to fire blight after 25th days from inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Pear shoots susceptibility to fire blight (%)			
Cultivar	Strain Ea3325	Strain Ea3345	Mixture of Ea3325 and Ea3345 strains
Dr. Jules Guyot	13.3*g	6.6* g	18.4*f
Beurré Hardy	30.3 b-e	20.8 c-g	32.9 d-f
Ranna Bolyarka	32,76 b-d	8.4 fg	30.1 d-f
Beurré Giffard	12.4* g	6.9* g	26.1 ef
Santa Maria	28.4 b-e	28.4 b-e	45.0 a-d
Passe Crassane	37.5 a-c	25.1 c-f	45.5 a-d
Beurré Hardenpont	39.7 a-c	31.6 b-d	45.3 a-d
Alexander Lucas	18.9 e-g	11.7 e-g	36.4 c-e
Curé	15.3 fg	18.4 d-g	34.8 c-e
Conference	27.9 c-e	28.3 b-e	45.8 a-d
Beurré Bosc	45.1 a	93.4 a	61.2 a
Williams	20.2 e-g	36.9 bc	43.2 b-d
Red Williams	36.9 a-d	33.3 b-d	50.5 a-c
Abate Fetel	25.4 d-f	21.1 c-g	39.8 c-e
Packham's Triumph	33.4 b-d	43.0 b	57.4 ab

*Different letters in the same row/column indicated significant difference ($p<0.05$)) were compared by using Dunkan test.

The most affected cultivar after inoculation with the single strain Ea3345 was ‘Beurré Bosc’. Its difference in the severity of diseases was statistically significant compared to all other cultivars. When inoculated with the mixture of both strains the lowest level of infection showed again ‘Dr. Jules Guyot’. The differences were statistically significant with ‘Santa Maria’, ‘Passe Crassane’, ‘Beurré Hardenpont’, ‘Alexander Lucas’, ‘Curé’, ‘Conference’, ‘Beurré Bosc’, ‘Williams’, ‘Red Williams’, ‘Abate Fetel’ and ‘Packham's Triumph’ (Table 4).

Compared to the outlined trend of leaves infection the results obtained of respect to the virulence of the spread strain of *E. amylovora* on the twenty-fifth day after inoculation, the results showed a different away.

The differences were reported in ten cultivars 'Beurré Hardy', 'Dr. Jules Guyot', 'Beurré Giffard', 'Ranna Bolyarka',' Passe Crassane', 'Beurré Hardenpont', 'Alexander Lucas', 'Red Williams'and 'Abate Fete'l to compared in both single strains, the result showed strain Ea3325 as a more virulence. From the results was conclude that after infection with a single or mixture of bacterial suspension, the susceptibility of individual plant organs is different in depends on the used strain, this comment was confirmed by other authors (Zwet and Beer, 1992).

According the reactions to inoculation with a single or mixture of strains the cultivars were classified in a different class of susceptibility. The distribution of each cultivar among susceptibility classes reflected their general susceptibility of fire blight (Table 5). In all variants of artificial inoculation, 'Dr. Jules Guyot' was classified as a very low susceptible. When inoculated with single strain Ea3325 ten of the cultivars had similar results. The necrosis observed on their shoots was 20-40% and they were also classified as low susceptible. The similar results were observed and when these cultivars were infected by Ea3345. For seven of the tested cultivars the observed necrosis was 40-60% and they were classified as moderately susceptible. As the most susceptible was evaluated 'Beurré Bosc' cultivar.

Similar results were reported by other authors (Toth et al., 2004) after artificial infection with the *E. amylovora*. The cultivar 'Dr. Jules Guillot', was classified as very sensitive and in our study as a very low susceptible. Greek researchers (Tsiantos and Psallidas, 2004) studied the cultivars 'Williams' and 'Santa Maria' were determine as highly susceptible after artificial infection with a mixture of six local strains of *E. amylovora*, in our study the cultivars were identified with the same degree of susceptibility after infection with a mixture of both the strains. The cultivars 'Williams', 'Santa Maria', 'Abate Fetel', 'Passe Crassane', Beurré Hardy were classified as susceptible (Demir and Gündogdu, 1992) in our result were determine as a moderately susceptible..

Table 5. Distribution of the pear cultivars in five susceptibility classes, in 25th days from inoculation.

Type of inoculum	Susceptibility classes				
	Very Low Susceptible*	Low Susceptible	Moderately Susceptible	Susceptible	Very Susceptible
	0-20%	20-40%	40-60%	60-80%	80-100%
Mixture of 2 strains	Dr. Jules Guyot	Beurré Hardy Beurré Giffard Ranna Bolyarka Alexander Lucas Curé Abate Fetel	Santa Maria Passe Crassane Beurré Hardenpont Conference Williams Red Williams Packham's Triumph	Beurré Bosc	-
Ea3325	Dr. Jules Guyot Beurré Giffard Alexander Lucas Curé	Beurré Hardy Ranna Bolyarka Santa Maria Passe Crassane Beurré Hardenpont Conference Williams Red Williams Abate Fetel Packham's Triumph	Beurré Bosc	-	-
Ea3345	Dr. Jules Guyot Ranna Bolyarka Beurré Giffard Alexander Lucas Curé	Beurré Hardy Santa Maria Passe Crassane Beurré Hardenpont Conference Williams Red Williams Abate Fetel	Packham's Triumph	-	Beurré Bosc

*Susceptibility classes (Le Lezec, 1997)

Figure 2. Infected shoot of Beurré Bosc cultivar

In 45th days after inoculation was researched to spread of necrosis distribution to shoots and stem was observed in all cultivars. Symptoms of the infection of rootstock were observed in each pear, the result showed five of the tested cultivars -'Red Williams', 'Abate Fetel', 'Curé' 'Alexander Lucas' and 'Dr. Jules Guyot' less to symptoms of infection. For the rest of pears cultivar the disease was spread to the rootstock.

Table 6. Fire blight infection of shoots, stem and rootstock after inoculation with mixture of two *Erwinia amylovora* strains

Cultivar	Shoots	Stem	Rootstock
Dr. Jules Guyot	+	+	-
Beurré Hardy	+	+	+
Ranna Bolyarka	+	+	+
Beurré Giffard	+	+	+
Santa Maria	+	+	+
Passe Crassane	+	+	+
Beurré Hardenpont	+	+	+
Alexander Lucas	+	+	-
Curé	+	+	-
Conference	+	+	+
Beurré Bosc	+	+	+
Williams	+	+	+
Red Williams	+	+	-
Abate Fetel	+	+	-
Packham's Triumph	+	+	+

Figure 3. Infection on a stem on 'Dr. Jules Guyot'

The fire blight has recognized as the most dangerous disease of pear orchards in the world. Timely diagnosis is very important for the detection of this disease. Visual assessment and reaction of the pear cultivar after the infection is important for limiting the diseases.
Under conditions of heavy infection during bloom, the yield can be considerably reduced or even destroyed. The need to remove fire blight infected parts from the plant forces the grower to prune away large portions of the tree, which has a long-term severe impact on the plant itself (Al-Dahmashi and Khlaif, 2004; Kuflik et al., 2008).

CONCLUSIONS

Using the mixture of two strains of *Erwinia amylovora* for inoculation of pear leaves always gives a higher percentage of disease incidence and severity than the single strain of bacteria.
The cultivar 'Dr. Jules Guyot' in all variants of artificial inoculation give the lowest percentage of necrosis on leaves and shoots. 'Beurré Bosc' reacted with the highest – 93.36% infected shoots.
The distribution of susceptibility classes depends on inoculation with single or mixed *Erwinia amylovora* strains.
On a 45th after inoculation development of bacteria was reported on shoots, stem and rootstock. Only five cultivars reacted with localization of the infection to the stem.

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REPRODUCTIVE POTENTIAL OF *IN VITRO* RASPBERRY CULTIVARS GROWN ON POORLY PRODUCTIVE SOILS

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Abstract

Biotechnological approaches for the production of raspberry planting material is a fundamental technology to obtain healthy, homogeneous and cultivar authentic plants.
The objectives of the present study were raspberry cultivars, such as: 'Willamette', 'Meeker', 'Samodiva' and 'Magdalena' - a candidate cultivar. The experiment was carried out in the collection plantation of the Research Institute of Stockbreeding and Agriculture, Troyan, in the period of 2018/2019. The field experiment was set with various intra row spacing (0.30 and 0.50) and inter row spacing (3.00 m).
The highest average fruit weight was found in 'Meeker' (2.91 g) at a planting distance of 0.50 m and 'Magdalena' - a candidate cultivar (2.87 g) at a planting distance of 0.30 m. The highest average yield per 1 m² was registered for 'Willamette' (1707.84 g), 'Magdalena' - a candidate cultivar (1605.92 g) and 'Meeker' (1469.17 g) at intra row spacing of 0.30 m.

Key words: *raspberry, cultivars, agrotechnics, fruit weight, yield.*

INTRODUCTION

The distribution area of raspberry is from the temperate climate zone to the polar regions of Siberia, but it still does not tolerate heavy colds and heat (Dinkova et al., 2000)
The global warming of the Earth's climate has a significant impact on the phenological and reproductive manifestations of small-sized fruit species, including raspberries. Phenological calendar and reproductive characteristics are directly dependent on latitude, agro-meteorological conditions of the area, cultivar characteristics and applied agrotechnics.
A number of researchers have focused their studies on testing the fertility of raspberry cultivars (Hristov, 1991; Stanisavljevic et al., 1996; Boicheva, 1999; Buskiene, 1999; Koron, 2004; Georgiev, 2006; Leposavić et al., 2015). Clozza et al. (1994) found that 'Meeker' and 'Schonemann' cultivars show better fruit quality and strength, unlike 'Glen Clova' and 'Heritage' cultivar, which are smaller and of unsatisfactory taste. Leposavić et al., 2006, compared 6 raspberry cultivar ('Willamette', 'Meeker', a yellow branch of 'Meeker', 'Polana', 'Polka', 'Poranna Rossa' and 'Samodiva') and one raspberry branch and found that 'Willamette' had the largest fruit (4.22 g) followed by 'Meeker' (4.13 g), yellow fruit branch of 'Meeker' (3.95 g) and 'Samodiva' (3.55 g).
The purpose of this study is to follow the occurrence of different phases of the phenological calendar and the effect of different intra row spacing (0.30 m and 0.50 m) in cultivation on fruit weight and the average yield of cultivars.

MATERIALS AND METHODS

The survey was conducted during the period of 2018-2019. The experiment included raspberry cultivars from the collection plantation of RIMSA: 'Willamette', 'Meeker', 'Samodiva' and 'Magdalena' - a candidate cultivar. The plants were grown under irrigation conditions, the area was maintained in black fallow in the intra row spacing and with naturally grassed row spacing. The planting distances were 3.00/0.30 m и 3.00/0.50 m. The necessary agrotechnical events for cultivating were applied.
The following cultivation variants were used for plants:
- planting at 0.30 m intra row spacing;
- planting at 0.50 m intra row spacing;

The following indicators were observed:
- phenological calendar;
Reproductive indicators:
- average yield of $1m^2$ (kg);
- average fruit weight (g).

The indicators were meausred according to the methodology of plant resources (Nedev et al., 1979).

Data processing was performed by a two-way dispersion analysis (Lidanski, 1988), using MS Excel software - 2010.

RESULTS AND DISCUSSION

During the study period, the beginning of the vegetation of raspberry cultivars began in March. It was first registered in candidate cultivar 'Magdalena' - 04.03 (2019), and at the latest for 'Samodiva' - 30.03 (2018).

Flowering began from the end of April in the first year of the experiment until about the middle of May ('Meeker' 16.05) the following year. The mass flowering of all cultivars took place in May to early June. It ended with 'Meeker' on 06.06 (2019).

It is noteworthy that in 2018, the phenophases of flowering, fruit ripening and harvesting occurred earlier than in the following 2019.

The fruits began to ripen in 2018 about 29.05 (candidate cultivar 'Magdalena') until 01.06 ('Willamette').

The next year, the phase continued until the middle of June. Similarly, the fruit harvesting began from 05.06 and 06.06 in 2018 and continued until 25.06. In 2019 it began from 13.06 (candidate cultivar 'Magdalena') and 18.06 ('Willamette' and 'Meeker') until the middle of July. The end of the vegetation occurred in November-December.

Higher average fruit weight was measured in the first year of the experiment for all cultivars and variants (Table 2).

Table 1. Phenological calendar of raspberry cultivars for the period 2018-2019

Cultivars	Bud sprouting	Beginning of blossoming	Mass blosso-ming	End of blosso-ming	Beginning of fruit ripening	Harves-ting	End of harvesting	End of vegeta-tion
2018								
'Willamette'	25.03.	30.04	4.05.	14.05.	01.06.	06.06.	25.06	09.12
'Meeker'	27.03.	30.04	5.05.	15.05.	30.05.	05.06.	25.06	07.12
'Samodiva'	30.03	30.04	6.05.	14.05.	31.05.	05.06.	25.06	02.12
Candidate cultivar 'Magdalena'	22.03.	30.04	4.05.	15.05.	29.05.	05.06.	25.06	23.12
2019								
'Willamette'	29.03	11.05	18.05	03.06	14.06	18.06	15.07	12.12
'Meeker'	29.03	16.05	27.05	06.06	13.06	18.06	15.07	18.11
'Samodiva'	16.03	08.05	15.05	30.05	12.06	16.06	15.07	18.11
Candidate cultivar 'Magdalena'	04.03	07.05	15.05	03.06	10.06	13.06	15.07	30.11

Table 2. Average weight (g) of raspberry fruit from different cultivars and variants for 2018 and 2019

Cultivars	'Willamette' (50 cm)	'Willamette' (30 cm)	'Meeker' (50 cm)	'Meeker' (30 cm)	'Samodiva' (50 cm)	'Samodiva' (30 cm)	Candidate cultivar 'Magdalena' (50 cm)	Candidate cultivar 'Magdalena' (30 cm)
2018								
	2.6	2.74	3.07	2.81	2.89	2.66	3.30	3.26
2019								
	2.08	2.42	2.74	2.57	2.28	2.07	2.32	2.48
Average for the period	2.34	2.58	2.91	2.69	2.59	2.37	2.81	2.87

The largest fruit weight was measured in the first year in candidate cultivar 'Magdalena' in both variants (3.30 g at 0.50 m; 3.26 g at 0.30 m) and 'Meeker' (3.07 g at 0.50 m).

In the second year, the values ranged from 2.07 g ('Samodiva' at 0.30 m to 2.74 g for 'Meeker' at 0.50 m).

The highest average weight of 2.91 g for the two-year period was reported for 'Meeker' (0.50 m) and candidate cultivar 'Magdalena' - 2.87 g (0.30 m) and 2.81 g (0.50 m).

It can be stated that fruits with higher average weight predominated at planting distances of 0.50 m in 'Meeker' and 'Samodiva'.

For 'Willamette' and candidate cultivar 'Magdalena' higher average weight was registered at 0.30 m planting distance.

Table 3. Yield of raspberry cultivars per 1 m^2 (g) for different variant in 2018-2019

Cultivars	'Willamette' (50 cm)	'Willamette' (30 cm)	'Meeker' (50 cm)	'Meeker' (30 cm)	'Samodiva' (50 cm)	'Samodiva' (30 cm)	Candidate cultivar 'Magdalena' (50 cm)	Candidate cultivar 'Magdalena' (30 cm)
2018 г.								
	896.5	1767.5	873.17	1620.33	1422.67	1161.17	821.17	944.5
LSD $_{0,001}$ Among variants	460.89							
LSD $_{0,01}$ Among cultivars	351.02							
2019 г.								
	1138	1648.17	806.17	1318	782	1048.5	1843.5	2267.33
LSD $_{0,01}$ Among variants	346.17							
LSD $_{0,001}$ Among cultivars	454.53							
Average for the period	1017.25	1707.84	839.67	1469.17	1235.59	1104.84	1332.34	1605.92

In the first year, the highest yield per 1 m^2 was gathered from 'Willamette' 1767.5 g (30 cm), followed by 'Meeker' 1620.33 g (30 cm), 'Samodiva' 1422.67 g (50 cm) and 'Magdalena' 944.5 g (30 cm). The smaller the planting distances, the higher the yield in the variants (with the exception of 'Samodiva'). The lowest yield was gathered from 'Magdalena' candidate cultivar 821.17 g (50 cm). The differences were mathematically very well proven among variants, while the distinctions among cultivars were well proven. The highest yield in the second experimental year was obtained from candidate-cultivar 'Magdalena' 2267.33 g (30 cm planting distance). It was lower in another variant of the same cultivar (1843.5). The third place was for 'Willamette' 1648.17 g (30 cm). Higher results were registered for all variants at a planting distances of 30 cm. The lowest yield was registered in 'Samodiva' 782 g (50 cm). The differences among variants were statistically well proven and very well proven among cultivars.

The highest average yield for the experimental period was registered for 'Willamette' 1707.84 g (30 cm), candidate-cultivar 'Magdalena' 1605.92 g (30 cm) and 'Meeker' 1469.17 g (30 cm), i.e. the smallest planting distances. Only for 'Samodiva', the average yield was higher than the other variant (1235.59 g) (Table 3).

CONCLUSIONS

The study conducted significantly determines the relationship between cultivars and the impact of planting distances on reproductive performance.

The phases in the phenological calendar indicate that fruit ripening can be registered significantly early, at the end of May, which is a special case that is not characteristic of raspberry crop.

The highest average weight was found in 'Meeker' and 'Magdalena' candidate cultivar in both variants.

The reported average yield shows higher results mainly from smaller planting distances. The most fertile cultivars were 'Willamette', 'Magdalena' candidate cultivar and 'Meeker'. There was an exception for 'Samodiva' with higher yield from a planting distance of 0.50 m.

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SENSORIAL ANALYSIS OF APPLES BEFORE AND AFTER STORAGE IN CONTROLLED ATMOSPHERE CONDITIONS

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Abstract

Apples are the most consumed climacteric fruits in the cold season, due their biochemical contribution to the health of the human body. In this study, the main purpose is to compare the organoleptic attributes, provided through trained evaluators, like: exterior appearance and color, smell, taste and texture (crispness) for four apples cultivars (Topaz, Redix, Florina and Rubinola) stored for 350 days, in three different controlled atmosphere conditions with the following parameters: T: 1°C, RH: 95%, O_2: 3%, CO_2: 0%, 2% and 5%, in two different years. Taste is the most important sensorial indicator and is high correlated with the total soluble solids/acidity ratio of the apples. The quality indicators variations present specific behavior when apples are stored in different concentrations of carbon dioxide. Evaluators noted lower, the exterior appearance, for Topaz (in first year), Florina and Rubinola (in both years) apples stored without CO_2 compared to those stored in both CO_2 concentrations. Moreover, apples stored in both CO_2 concentrations, shown healthier appearance, without being affected by the specific storage diseases.

***Key words**: apple, crispness, appearance, sensorial analysis, quality.*

INTRODUCTION

Apple is one of the most consumed fruit (Francini et al., 2013; Mureşan et al., 2012) all over the world (Yildirim et al., 2017), due to health benefits (Brovelli, 2006; Mitić et al., 2013) and good sweet-sour taste.

The main objective in postharvest technologies is maintaining the best organoleptic qualities of the agriculture products (Oltenacu et al., 2015).

The two most important attributes in sensory analysis are fruits firmness, considered by the consumers as a freshness indicator (Cortellino et al. 2017; Péneau et al., 2006) and the taste which is high correlated with the total soluble solids/acidity ratio of the apples (Weibel et al., 2004).

An important factor of consumer acceptability of fruits is the maturity index (Yoon et al., 2005).

One of the storage method used in postharvest for agricultural products is represented by controlled atmosphere (CA) conditions which can prevent quality loss of the fruit (Bessemans et al., 2016) and maintaining the same organoleptic characteristics during storage (Oltenacu et al., 2013).

It was observed that during storage there are changes in fruit quality indicators, dry matter and content and maturity index (TSS/TA) increase and firmness decreases (Jan et al., 2012), ascorbic acid and aroma volatiles decreases, the final values depending on the cultivar (Lemmens et al., 2020; Tough and Hewett, 2001). The total phenol content also decreases, depending on storage conditions and fruit origins (Lysiak et al., 2020).

To maintain better fruit firmness and to inhibit ethylene production, 0.625 μL L^{-1} 1-methylcyclopropene (1-MCP) can be used, which also reduces the oxidative activity of ACC oxidase enzyme (Schmidt et al., 2020).

During the fruit storage period, depending on the storage temperature, ethylene is released, which helps ripen the fruits, but also are synthetized a various compounds that contribute through the volatiles to develop specific apple flavor (Qi et al., 2020).

The main purpose of this study was to compare the organoleptic qualities changes during postharvest storage, for apples varieties like Topaz, Redix, Florina and Rubinola in two different years.

MATERIALS AND METHODS

The four apple cultivars: Topaz, Redix, Florina, and Rubinola, uniform in size and colour, were stored bulk in plastic boxes. The samples were stored and monitored in both years, under the same controlled atmosphere conditions, as follows: temperature (T): 1°C, O_2: 3%, CO_2: 0%, 2% and 5%, relative humidity (RH): 95%, in the Research Center for Studies of Food Quality and Agricultural Products, of the USAMV Bucharest.
Before sensorial analyses sesions the evaluators were trained to recognize the basic characteristics of each variety. The sensorial analyses questionaire contain questions in order to evaluate organoleptic attributes like: exterior appearance, color, smell, taste, and texture (crispness). The apples total titratable acidity was determined by titration with NaOH 0.1N to 8.2 pH, using the automatic titrator TitroLine. The firmness of the apples was determined using a piston of 1.1 cm diameter (Bessemans, 2016; Rizzolo, 2010) of an electronic penetrometer Turoni TR and the results were expressed in kg/cm^2. The total soluble solids content of the apples juice was obtained with refractive device Kruss DR301-95 (%Brix).
The the total soluble solids/acidity ratio, known also as maturity index, was calculated using the formula: TSS/TA, and it was correlated with the values of the organoleptic attribute: taste. The firmness of the samples was correlated with the values of the organoleptic attribute: texture. Statistical analyses were performed using Excel, for the samples results like: mean, standard deviation, correlations, T Test and ANOVA single factor (Pomohaci, 2017). In this study, the main purpose is to compare the organoleptic attributes, provided through trained evaluators, for the four apples cultivars stored for 350 days in CA conditions in two different years.

RESULTS AND DISCUSSIONS

Topaz cv. registered scores like: 3.6 points for the first year (Figure 1) and 4.27 points for the second year (Figure 2) out of maximum 5 points, for the initial moment, without statistical differences between years for all five organoleptic attributes.

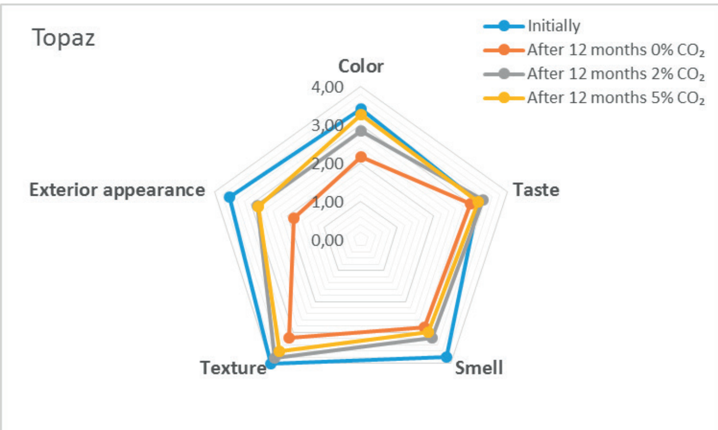

Figure 1. Sensory analysis for Topaz apple variety, during storage period in CA for the first year (october 2016- september 2017

After 350 days of storage in controlled atmosphere without additional injection of CO_2, the scores drop at: 2.6 points in the first year, and 3.77 points in second year, with semnificant differences (*P<0.05*) between years at exterior appearance and color. In this conditions, in both years, the apples presented dehydrated peel after storage. Vanzo et al. (2013) obtained the similar results for sensorial evaluation of Topaz. According to Bessemans

(2016), apple fruit cortex tissues are highly sensitive even to subtle differences in oxygen concentrations close to the anaerobic compensation point. That could explain the decrease of the exterior appearance scor of Topaz apples after storage.

In 2% CO_2, the scores drop at: 3.2 points in the first year, and 3.4 points in second year, without semnificant differences between years for all five organoleptic attributes.

Interesting fact is that the apples behavior stored in 5% CO_2 was different. In the first year obtained 3.23 points comparing with second year when obtained only 2.79 points.

Semnificant differences ($P<0.05$) were observed between years in taste and texture.

No significant correlations were obtained for Topaz in the first year (R = -0.0955), and also for the second year (R = -0.3874) between the apples taste and their maturity index (TSS/TA) (Table 1).

Significant positive correlations (R^2 = 0.7936), with linear regression equation y =–0.3432x + 1.3743 were obtained for Topaz in the first year and positive correlations (R^2 = 0.2831), with linear regression equation y = 0.5144x + 0.673 for the second year between the apples firmness (Table 1) and their texture (Figures 1 and 2).

Table 1. Variation of the taste, texture, firmness and maturity index during storage period for Topaz cultivar

		Taste		*TSS/TA*		*Texture*		*Firmness*	
		Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Year 1	Topaz initially	3.20	1.30	20.884	1.74	4.00	1.00	7.55	0.92
	Topaz - 0% CO_2	3.00	1.10	30.721	2.22	3.17	1.17	5.36	1.17
	Topaz - 2% CO_2	3.33	0.82	30.869	1.11	3.83	0.75	6.57	0.61
	Topaz - 5% CO_2	3.20	0.52	28.053	2.75	3.60	0.82	7.04	1.80
Year 2	Topaz initially	4.22	0.67	22.267	2.13	4.00	0.71	5.94	0.56
	Topaz - 0% CO_2	3.57	0.98	40.097	2.57	3.43	0.98	4.34	0.43
	Topaz - 2% CO_2	3.43	0.53	40.091	2.71	3.00	0.82	4.37	0.25
	Topaz - 5% CO_2	2.36	0.75	32.820	3.10	2.29	0.76	4.84	0.50

Figure 2. Sensory analysis for Topaz apple variety, during storage period in CA for the second year (october 2017- september 2018)

For the initial moment, Redix cv. were registered 4.2 points for the first year (Figure 3) and 3.73 points for the second year (Figure 4) out of maximum 5 points. Statistical differences were observed when color values were compared between years. For the other four organoleptic attributes, no statistical differences between years were observed, for the initial moment.

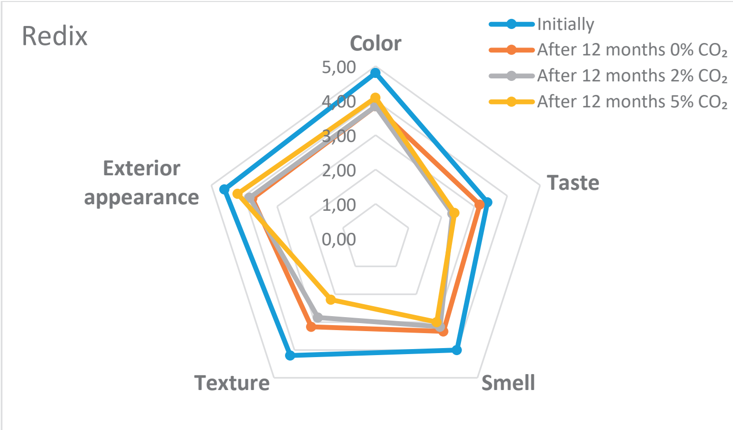

Figure 3. Sensory analysis for Redix apple variety, during storage period in CA for the first year (october 2016- september 2017)

After 350 days of storage in controlled atmosphere without additional injection of CO_2 the scores drop at 3.5 points in the first year, and 3.11 points in second year, with semnificant differences (*P<0.05*) between years at texture. In 2% CO_2, the scores drop at 3.2 points in the first year, and 3.47 points in second year, with semnificant differences between years for the taste.

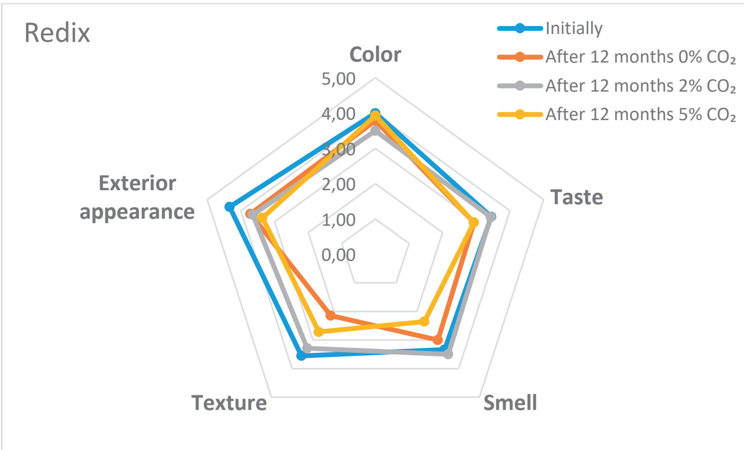

Figure 4. Sensory analysis for Redix apple variety, during storage period in CA for the second year (october 2017- september 2018)

In 5% CO_2, the scores drop at 3.2 points in the first year, and 3.06 points in second year, without semnificant differences between both years, for all five organoleptic attributes.

Negative significant correlations ($R^2 = 0.6447$), with linear regression equation $y = -0.0353x + 4.4138$ were reported for Redix, for the first year, between the taste of the apples and their maturity index (TSS/TA) (Table 2). No significant correlations were reported for Redix in the second year ($R = -0.338$).

Significant positive correlations ($R^2 = 0.6606$, with linear regression equation $y = 0.5944x + 0.5961$ were reported for Redix in the first year and positive correlations ($R^2 = 0.3255$), with linear regression equation $y = 0.5146x + 0.4872$ for the second year between the fermity of the apples (Table 2) and their texture (Figures 3 and 4).

Table 2. Variation of the taste, texture, firmness and maturity index during storage period for Redix cultivar

		Taste		*TSS/TA*		*Texture*		*Firmness*	
		Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Year 1	Redix - initially	3.40	0.55	27.142	2.27	4.20	0.84	7.91	1.43
	Redix - 0% CO_2	3.17	0.98	48.042	7.38	3.17	0.41	5.65	0.93
	Redix - 2% CO_2	2.33	0.82	54.986	5.85	2.83	0.41	5.42	1.64
	Redix - 5% CO_2	2.40	0,84	49.438	2.51	2.20	1.21	5.89	0.48
Year 2	Redix - initially	3.44	0.73	34.757	1.88	3.56	0.73	5.74	0.95
	Redix - 0% CO_2	2.93	0.84	54.938	7.52	2.14	0.38	4.62	0.54
	Redix - 2% CO_2	3.43	0.79	65.049	2.87	3.29	0.49	4.45	0.69
	Redix - 5% CO_2	2.93	1.30	60.676	3.27	2.71	0.95	4.14	0.38

Florina cv. registered scores like: 3.88 points out of maximum 5 points for the first year (Figure 5) and 4.42 points out of maximum 5 points for the second year (Figure 6), for the initial moment, with statistical differences between years for the color and exterior appearance.

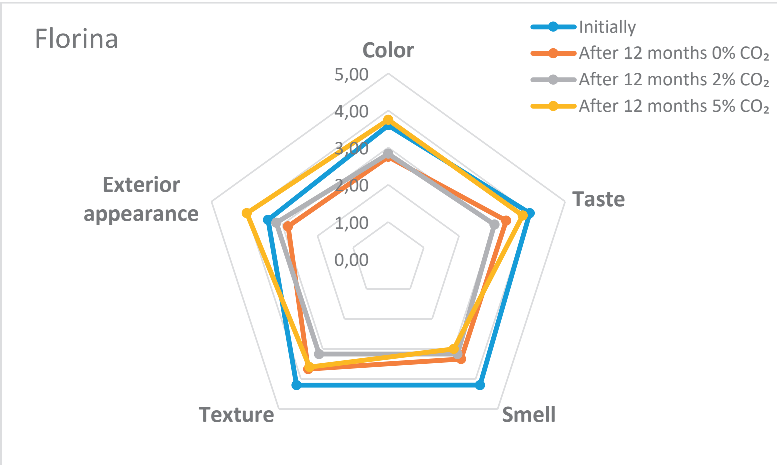

Figure 5. Sensory analysis for Florina apple variety, during storage period in CA for the first year (october 2016 – september 2017)

After 350 days of storage in controlled atmosphere without additional injection of CO_2, the scores drop at 3.18 points in the first year, and 2.97 points in second year, with semnificant differences ($P<0.05$) between years at texture. In 2% CO_2, the scores drop at 3.07 points in the first year, and 4.03 points in second year, with semnificant differences between years for color and exterior appearance.
In 5% CO_2, the scores drop at 3.63 points in the first year, and 3.54 points in second year, without semnificant differences between both years, for all five organoleptic attributes.

According to Brizzolara et al. (2017) apples respond differently to low oxygen storage protocols, and the genetic background plays a key role in determining and modulating the metabolic changes under postharvest hypoxic conditions, mainly with a selective reconfiguration of the primary C/N metabolism. Cukrov et al. (2016) concluded that specific molecular and metabolic changes occur at the earliest stages of the imposed stress conditions and some of them appear to be transient. Some of these represent a sort of a rapid adaptation response to the stress.

Table 3. Variation of the taste, texture, firmness and maturity index during storage period for Florina cultivar

		Taste		*TSS/TA*		*Texture*		*Firmness*	
		Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Year 1	Florina - initially	4.00	1.00	27.960	2.03	4.20	0.84	8.24	0.83
	Florina - 0% CO_2	3.33	0.82	36.920	1.85	3.67	1.03	6.19	1.28
	Florina - 2% CO_2	3.00	1.41	44.668	4.34	3.17	1.17	6.67	1.62
	Florina - 5% CO_2	3.80	0.75	38.131	5.77	3.60	1.03	6.56	1.41
Year 2	Florina - initially	4.56	0.53	38.019	1.08	4.33	0.71	6.36	0.76
	Florina - 0% CO_2	2.79	0.70	59.726	3.04	2.43	0.79	5.03	0.39
	Florina - 2% CO_2	3.71	0.95	58.025	3.44	3.43	0.79	4.63	0.50
	Florina - 5% CO_2	3.17	1.13	56.873	2.16	3.42	0.85	4.16	0.70

Negative significant correlations ($R^2 = 0.7334$), with linear regression equation y = -0.0563x + 5.613 were reported for Florina, for the first year, between the taste of the apples and their maturity index (TSS/TA) (Table 3).

For the second year negative significant correlations ($R^2 = 0.7538$), with linear regression equation y = -0.0681x + 7.1503 were reported for Florina, between the taste of the apples and their maturity index (TSS/TA) (Table 3).

Significant positive correlations ($R^2 = 0.5473$, with linear regression equation y = 0.3443x + 1.2776 were reported for Florina in the first year and positive correlations ($R^2 = 0.3036$), with linear regression equation y = 0.4535x + 1.1145 for the second year between the fermity of the apples (Table 3) and their texture (Figures 5 and 6).

Figure 6. Sensory analysis for Florina apple variety, during storage period in CA for the second year (october 2017- september 2018)

Rubinola cv. registered scores like 3.64 points out of maximum 5 points for the first year (Figure 7) and 4.09 points out of maximum 5 points for the second year (Figure 8), for the initial moment, with statistical differences between years for the exterior appearance. For the other four organoleptic attributes, no statistical differences between years was observed, for the initial moment.

Figure 7. Sensory analysis for Rubinola apple variety, during storage period in CA for the first year (october 2016- september 2017)

After 350 days of storage in controlled atmosphere without additional injection of CO_2, the fruits could't be evalueted, and the score drop at 1.8 points in second year. In 2% CO_2, the scores drop at 3.05 points in the first year, and 2.84 points in second year, with no semnificant differences between year. In 5% CO_2, the scores drop at 2.97 points in the first year, and 2.19 points in second year, with semnificant differences ($P<0.05$) between years, in color and exterior appearace. Iglesias et. al (2012) showed a high correlation between consumer acceptability and some of the volatile compounds emitted, especially 2-methylbutyl acetate. Sensory analysis for Rubinola apple variety shows high scores for smell and taste initially that was lost during long storage in hypoxia conditions.

Table 4. Variation of the taste, texture, firmness and maturity index during storage period for Rubinola cultivar

		Taste		*TSS/TA*		*Texture*		*Firmness*	
		Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Year 1	Rubinola initially	4.00	1.00	35.061	1.91	4.00	1.00	6.156	0.89
	Rubinola - 0% CO_2	-	-	40.052	2.10	-	-	4.922	1.00
	Rubinola - 2% CO_2	3.17	1.33	40.311	4.42	3.50	1.05	5.864	1.31
	Rubinola - 5% CO_2	2.20	1.21	38.304	3.06	3.00	0.98	5.316	0.91
Year 2	Rubinola initially	3.78	0.67	33.964	1.13	3.89	0.93	4.894	0.77
	Rubinola - 0% CO_2	1.86	0.69	38.044	2.91	1.57	0.98	2.907	0.74
	Rubinola - 2% CO_2	3.14	1.07	39.417	4.28	2.86	1.21	2.858	0.89
	Rubinola - 5% CO_2	2.07	1.17	42.813	6.98	1.93	1.10	3.376	0.83

Negative correlations ($R^2 = 0.3239$), with linear regression equation $y = -0.1908x + 10.36$ were reported for Rubinola, for the first year, between the taste of the apples and their maturity index (TSS/TA) (Table 4). For the second year, for Rubinola, negative significant correlations ($R^2 = 0.6785$), with linear regression equation $y= -0.2013x + 10.553$. Significant positive correlations ($R^2 = 0.97$), with linear regression equation $y =-1.1547x - 3.1728$, were reported for Rubinola in the first year and positive correlations ($R^2 = 0.6154$), with linear regression equation $y = 0.8545x + 0.4369$ for the second year between the fermity of the apples (Table 4) and their texture (Figures 7 and 8).

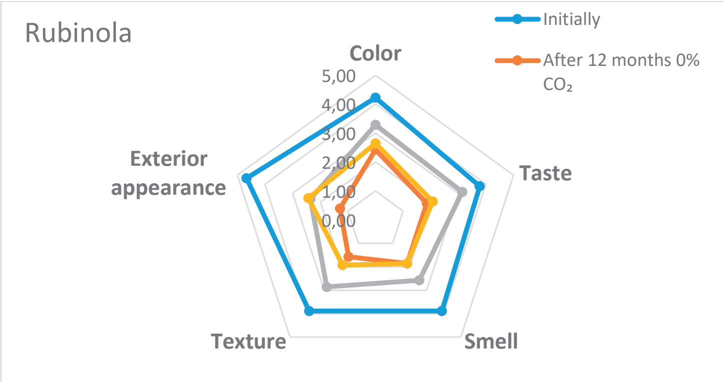

Figure 8. Sensory analysis for Rubinola apple variety, during storage period in CA for the second year (october 2017- september 2018)

CONCLUSIONS

The present study showed that the yearly climatic conditions and the cultivar influenced the fruit quality during long storage. Thus, the Topaz variety, in the first year the fruits storage in a 2% CO_2 CA conditions had higher values at 4 analyzed indicators, except for the firmness, and for the second year, the best storage option was the one without CO_2. For the Redix variety, the values obtained for the two years were similar. For the first year, the storage conditions didn't influence quality parameters, but in the second year, for storage conditions with 2% CO_2, most of the analyzed parameters had slightly higher values. For the Florina variety, in the first year, the maturity index and firmness were positively influenced by the 2% CO_2 atmosphere. In the second year, the CO_2-free atmosphere influenced these two parameters, too. The fruits of the Rubinola variety in the second year had higher values for the maturity and firmness index in atmosphere with 5% CO_2, but in the first year at a concentration of 2%. The texture of the fruit varied greatly between years within varieties and from one variant of storage to another without being able to establish a direct correlation between these parameters.

The quality indicators variations present specific behavior if apples are stored in different concentrations of carbon dioxide. The exterior appearance had lower values for Topaz (in first year), Florina and Rubinola (in both years) apples stored without CO_2 compared to those stored in 2% and 5% CO_2 conditions. Apples stored in controled atmosphere with 2% and 5% CO_2 shown healthier appearance, without being affected by the specific storage diseases.

Further investigations are needed to clarify to how the pre-harvest climatic conditions influence the storage of the 4 studied apples varieties, so that recommendations can be made.

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EFFECT OF BIOSTIMULATOR REGOPLANT ON ACCLIMATIZATION OF MICROPROPAGATED GiSelA 6 CHERRY ROOTSTOCK IN FLOATING SYSTEM

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Abstract

Acclimatization is one of the key steps in the success of micropropagation of woody plants. Improvement of acclimatization of micropropagated woody fruit plants could be achieved in different ways. The purpose of this study was to optimize the acclimatization of micropropagated sweet cherry rootstock GiSelA 6 (Prunus cerasus 'Schattenmorelle' × Prunus canescens) through application of biostimulators of natural origin in floating system. A new generation of plant growth biostimulators Regoplant and Charkor (Agrobiotech, Ukraine) contain metabolism products of symbiotic fungus-endophyte of ginseng roots, cultivated in vitro. Micropropagated and rooted plantlets from cherry rootstock GiSelA 6 (Prunus cerasus 'Schattenmorelle' × Prunus canescens) were acclimatized in a floating system with 100 µl L^{-1} Regoplant or Charkor. As control plantlets with no additional treatments served. Multi-cell bedding plant trays filled with peat: perlite 1:1 (v:v) were used for acclimatization. Plants were grown in a greenhouse under high humidity conditions for one week and then humidity was gradually decreased. Data on some growth, physiological and biochemical parameters were collected 45 days after transplanting to ex vitro conditions. Regoplant (100 µl L^{-1}) led the highest plants survival rate (86%), also the greatest values for the growth determined parameters.

Key words*: acclimatization, pears, Regoplant, Charkor, nutrient solutions.*

INTRODUCTION

Micropropagation *in vitro* has shown promises for rapid and large scale clonal multiplication of disease-free planting material all year round. But woody plants are often recalcitrant to *in vitro* cultivation and this process is highly genotype dependent. During *in vitro* cultivation, plantlets grow under specific conditions - in small tightly closed vessels with high air humidity, low gas exchange and thus a CO_2-shortage during almost the whole photoperiod, ethylene production and relatively low light intensity, in a culture medium with a large concentration of sugar (Ziv, 1991). These special conditions result in the formation of plants with abnormal morphology, anatomy and physiology. After the transfer from *in vitro* to *ex vitro* conditions, plants have to correct the abnormalities and to acclimatize to the new environment in the greenhouse or in the field. Acclimatization is one of the key steps in the success of micropropagation of woody plants and most losses of *in vitro* plants occur when the plantlets are moved from *in vitro* to the *ex vitro* conditions.

Natural light shading and antitranspirants application for reducing plant transpiration are some of the approaches which are often used to increase plant survival rate after transplanting.

GiSelA 6 rootstock (*Prunus cerasus* 'Schattenmorelle' × *Prunus canescens*) is the most popular rootstock for new plantings in the northwest Pacific, but already sought after in other parts of the world, including in Bulgaria. GiSelA 6 is not demanding for soils and although it is a relatively vigorous rootstock, it can easily be controlled. The production of GiSelA 6 by rooting cuttings by conventional methods is a rather difficult and slow process and do not always deliver high-quality, healthy and uniform propagating material. The scale and rapid multiplication of quality plants can be achieved through biotechnological approaches through micropropagation (Nacheva & Gercheva, 2008).

Floating system is one of the most simplistic cultivation systems composed by a tank containing the nutrient solution and floating panels where the plants are sown and grown (Pardossi et al., 2006). On the surface of the nutritive solution, there are floats made of polystyrene or other materials that sustain the plants (Sheikh, 2006). Floating systems are increasingly used for greenhouse production of fresh-cut leafy vegetables and for the cultivation of medicinal plants (Dorais et al., 2001). Several reports suggest that float hydroculture could be successfully applied in acclimatisation of *in vitro* produced plantlets, such as *Solanum tuberosum* L. (Nhut et al., 2006), *Grammatophyllum speciosum* Blume (Sutthinon et al., 2015), *Lycium barbarum* L. and other species, including cherry rootstock GiSelA 5 (Clapa et al., 2013). In these studies, hydroponics were proved to be a feasible alternative to acclimatise *in vitro* plantlets in a clean, convenient and water-saving way. However, there has been little information about an efficient method for acclimatization of *in vitro* cherry plantlets using a hydroponic system.

A new generation of plant growth biostimulators Regoplant and Charkor (Agrobiotech, Ukraine, http://www.agrobiotech.com.ua) contain metabolism products of symbiotic fungus-endophyte of ginseng roots, cultivated *in vitro*.

Regoplant is composite natural plant biostimulator. Its mode of action is based on synergic effect of products of biotechnological cultivation of fungi-micromycetes from root system of ginseng and aversectin - biological product with antiparasitic action. Charkor contains a complex of amino acids, fatty acids, sugars, macro- and microelements and analogs of phytohormones. According to the authors, Charkor is more effective than indolyl-acetic and indolyl-butyric acid in rooting cuttings of a number of ornamental trees and shrubs (Ponomarenko et al., 2010). It was successfully applied for rooting of micropropagated magnolia plantlets (Gercheva et al., 2015). According to our previous results, Charkor and Regoplant stimulate growth and improve acclimatization of micropropagated pear plantlets (Dimitrova et al., 2017; Dimitrova et al., 2019).

The purpose of this study was to optimize the acclimatization of micropropagated sweet cherry rootstock GiSelA 6 (*Prunus cerasus* 'Schattenmorelle' × *Prunus canescens*) through application of biostimulators of natural origin in floating system.

MATERIALS AND METHODS

Plant material and experimental conditions

The experiment was carried out on micropropagated plantlets of sweet cherry rootstock GiSelA 6 (*Prunus cerasus* 'Schattenmorelle' × *Prunus canescens*).

The research was done in September - October, 2019 in the greenhouse at the Fruit Growing Institute - Plovdiv, Bulgaria.

Well-rooted plantlets were potted in styrofoam form pads (528x308x60 mm) filled with peat-perlite 1:1 (v:v).

The pads were placed in a plastic tank containing Knop's nutrient solution, supplemented with 36.7 mg L^{-1} iron sodium ethylenediaminotetraacetate (FeNaEDTA). Regoplant and Charkor at concentration 100 µL L^{-1} were added to the nutrient solution. Thus, three variants were formed:

1. Knop's nutrient solution - Control (C);
2. Knop's nutrient solution, supplemented with 100 µl L^{-1} Regoplant (R);
3. Knop's nutrient solution, supplemented with 100 µl L^{-1} Charkor (CH).

The nutrient solution was replaced weekly. The top of the plastic tank was covered with transparent plastic. Plants were grown in a floating system at high humidity conditions for 2 weeks and then humidity was gradually decreased.

For each experimental treatment three replications, each containing 40 plants, were tested. For biometrical analysis ten representative plants were studied. For gas-exchange and chlorophyll *a* fluorescence analysis at least 5 measurements were performed.

After 45 days in *ex vitro* conditions growth parameters, physiological and biochemical analysis have been performed.

Growth parameters

The leaves, stem and roots were separated and a specific fresh weight (FW) (g) of the relevant botanical organs, also leaf area (cm^2) were determined immediately after removing the plants from the soil. The dry weight (DW) (g) of of the relevant botanical organs was measured after drying the material at 80°C for 48 h (Beadle, 1993).

Physiological and biochemical parameters

Gas-exchange analysis

Gas-exchange analysis was performed on the youngest fully developed leaves of 3 randomly selected plants of the respective variant. Measurements were taken with a LCpro + portable photosynthesis system (ADC, UK) on a sunny day at a light intensity of about 180 μmol m^{-2} s^{-1} Photosynthetic Photon Flux Density (PPFD) and a temperature of 25°C. Net photosynthesis rate (A, μmol CO_2 m^{-2} s^{-1}), transpiration intensity (E, mmol H_2O m^{-2} s^{-1}), stomatal conductivity (gs, mol H_2O m^{-2} s^{-1}) were determined.

Chlorophyll a fluorescence

Chlorophyll *a* fluorescence analysis was performed on the youngest fully developed leaves of 5 representative plants of the respective variant. The basic parameters of rapid chlorophyll a fluorescence (JIP test) were taken with a HandyPEA portable system (Hansatech Instruments, UK). The leaves were dark adapted for 40 minutes with special clips. The main parameters of chlorophyll fluorescence were measured - minimal (F_0), maximal (Fm), and variable (Fv) fluorescence, Fv/Fm, as well as HandyPEA-specific indicators - Performance index (PI_{ABS}) on the absorption basis and total PI (PI_{total}) measuring the performance up to the photosystem I (PSI) end electron acceptors (Goltsev et al., 2010).

Photosynthetic pigments

The photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) were extracted in 80% acetone, the extracts absor-bances were determined spectrophotometrically and the content (mg g^{-1} FW) calculated according to the formulae of (Lichtenthaler & Wellburn, 1983).

Determination of Total Antioxidant Activity

Free radical scavenging activity of plant samples against stable 2, 2- diphenyl-2-picrylhydrazyl hydrate (DPPH, Sigma-Aldrich Chemie, Germany) was determined spectro-photometrically. The change in colour (from deep-violet to light- yellow) was measured at 517 nm using UV-1600PC Spectrophotometer (VWR International, Europe).

Radical scavenging activity of plants was measured by method of Yen & Chen (1995). Briefly, 100mg fresh leaves were extracted with 50 ml of methanol (HPLC grade) in the ultrasonic bath for 15 minutes at 10°C.

The extract was centrifugated at 10 000 RPM for 5 minutes at 10°C. One ml of this of plant extract was mixed with 1.5 ml freshly prepared solution of DPPH in methanol (0.3 M) and 3.5 ml methanol.

The samples were kept in the dark for 15 minutes at room temperature and then the decrease in absorbance at 517 nm was measured.

The reference cuvette contained DPPH blank. The radical scavenging activity of the samples was calculated according to Rossi et al. (2003) and was expressed as percent inhibition of DPPH radical as following:

Inhibition% = $[(A_{control}-A_{sample})/ A_{control}] \times 100$,

Where: $A_{control}$ was the absorbance of the control (the blank solution without plant extract) and A_{sample} was the absorbance of the sample.

Statistical analysis

Data of different parameters were analyzed statistically by using one-way ANOVA in SPSS statistical software (version 13 for Windows) and significant differences between the means were evaluated using Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

In the floating systems, GiSelA 6 (*Prunus cerasus* 'Schattenmorelle' × *Prunus canescens)* cherry plants grew vigorously, but the differences between the plants grown with the biostimulator Regoplant were visible two weeks after planting. A relatively low survival rate (40%) was reported in the control variant (Table 1). Enrichment of the nutrient solution with Charkor led to a higher survival rate of cherry plants (56%) and the highest survival (86%) was reported in plants treated with the Regoplant. The plants of this variant were also distinguished by the greatest stem length

(111.01 mm), number of leaves, leaf area, fresh and dry mass of leaves and stems (Table 1, Figure 1). For the plants cultivated with Charkor, the biometric parameters were similar to those of the control, with the exception of the smaller leaf area.

Table 1. Growth parameters, survival rate (%) and antioxidant activity (% DPPH) of cherry plants 45 days after acclimatization on floating system with biostimulators Regoplant or Charkor

Variants	Control	Regoplant	Charkor
Stem lenght (mm)	55.32 b	111.01 a	43.09 b
Number of leaves	10.00 ab	12.60 a	9.00 b
Leaf area (cm^2)	6.48 b	8..82 a	4.33 c
FW leaves (g $plant^{-1}$)	0.611 ab	1.1991 a	0.373 b
FW roots (g $plant^{-1}$)	0.048 a	0.047 a	0.050 a
FW stem (g $plant^{-1}$)	0.025 b	0.074 a	0.029 b
DW leaves (g $plant^{-1}$)	0.1098 ab	0.2145 a	0.0793 b
DW roots (g $plant^{-1}$)	0.038 a	0.038 a	0.040 a
DW stem (g $plant^{-1}$)	0.018 b	0.076 a	0.022 b
Survival rate (%)	40	86	56
DPPH (%)	42.75 c	57.43 b	88.69 a

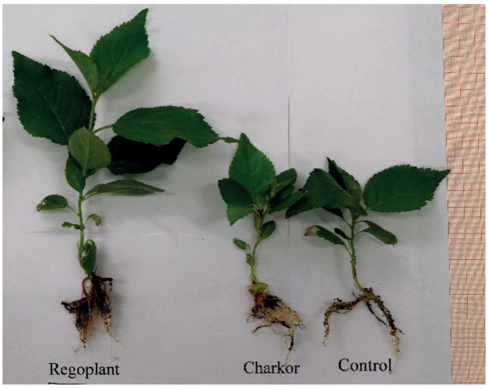

Figure 1. Sweet cherry rootstock GiSelA 6 plants (*Prunus cerasus* 'Schattenmorelle'× *Prunus canescens*) at acclimatization in floating system with biostimulators Regoplant or Charkor

The content of photosynthetic pigments in the leaves of the control plants and in those with the Regoplant did not differ significantly (Table 2). However, statistically lower values were reported for plants grown with Charkor.

Table 2. Content of photosynthetic pigments in leaves (mg g^{-1} fresh weight) of GiSelA 6 plants at acclimatization on floating system with biostimulators Regoplant or Charkor

Variants	Chloro-phyll a (mg g^{-1}FW)	Chloro-phyll b (mg g^{-1} FW)	Chloro-phyll (a + b) (mg g^{-1} FW)	Carotenoids (mg g^{-1}FW)	Chl a/b ratio	Chl (a+b)/ Carotenoids ratio
Control	1.246 a	0.360 a	1.603 a	0.467 ab	3.461 a	3.433 a
Regoplant	1.373 a	0.397 a	1.768 a	0.496 a	3.459 a	3.568 a
Charkor	0.656 b	0.194 b	0.849 b	0.275 b	3.379 a	3.087 b

The results were similar for net photosynthetic rate, transpiration and stomatal conductance, in which no significant differences were observed between the plants cultured with Regoplant and the control variant (Table 3).
In the variant with Charkor, lower values of gas exchange parameters were reported.

Table 3. Effect of biostimulators Regoplant and Charkor on net photosynthesis rate - (A) (µmol CO_2 m^{-2} s^{-1}); transpiration intensity - (E) (mmol H_2O $m^{-2}s^{-1}$) and stomatal conductance - (gs) (mol H_2O $m^{-2}s^{-1}$) at 180 µmol m^{-2} s^{-1} PPFD

Variants	Net photosynthetic rate (A) (µmol CO_2 $m^{-2}s^{-1}$)	Transpiration (E) (mmol H_2O $m^{-2}s^{-1}$)	Stomatal conductance (gs) (mol$H_2O$$m^{-2}s^{-1}$)
Control	**4.250 ± 0.28 a**	**1.032 ± 0.21 a**	**0.062 ± 0.017 a**
Regoplant	**4.233 ± 0.62 a**	**0.934 ± 0.11 a**	**0.067 ± 0.014 a**
Charkor	**2.568 ± 0.67 b**	**0.531 ± 0.10 b**	**0.025 ± 0.005 b**

Chlorophyll *a* fluorescence is another indicator of the functional activity of the photosynthetic apparatus of plants along with the intensity of photosynthesis.
The analysis of the induction curves of rapid chlorophyll fluorescence (OJIP test) links the structure and functionality of the photosynthetic apparatus and allows for rapid assessment of plant viability, especially in stress conditions (Strasser et al., 2000, 2004).
In the three variants studied, the rapid chlorophyll fluorescence curves had a typical OJIP shape from F_0 to F_m level with clearly separated J and I phases (Figure 2), indicating that the cherry plants, included in the experiment, were photosynthetically active (Yusuf et al., 2010).

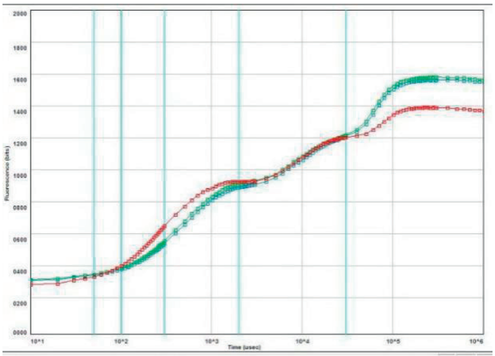

Figure 2. Induction curves of rapid chlorophyll a fluorescence (OJIP test);
(----) Control without biostimulators; (----) nutrient solution, supplemented with100 µl L^{-1} Regoplant (R); (----) nutrient solution, supplemented with100 µl L^{-1} Charkor (CH)

The minimal (F_0) and maximal (F_m) fluorescence of the control plants and plants treated with Regoplant did not differ significantly (Table 4).

In plants grown with Charkor in the nutrient solution F_0 and F_m were the lowest, and the difference was statistically proven (Table 4).

Table 4. Basic parameters of the JIP test of cherry plants 45 days after acclimatization on floating system with biostimulators Regoplant or Charkor

Variants	Control	Regoplant	Charkor
t for Fm	313.33 a	280.00 a	240.00 a
Area	30079.67 a	29691.00 a	19002.00 b
Fo	331.33 a	335.00 a	290.33 b
Fm	1573.00 a	1590.00 a	1404.67 b
Fv	1241.67 ab	1255.00 a	1114.33 b
Fo/Fm	0.211 a	0.210 a	0.207 a
Fv/Fm	0.789 a	0.789 a	0.792 a
PI Inst.	2.134 a	1.959 a	0.923 b
Fv/Fo	3.760 a	3.747 a	3.839 a
Sm	24.123 a	23.693 a	17.000 b
N	32.443 a	32.346 a	33.075 a
psi(Eo)	0.530 a	0.520 a	0.408 b
phi(Eo)	0.418 a	0.410 a	0.324 b
delta(Ro)	0.528 a	0.551 a	0.406 b
PI abs	2.561 a	2.351 a	1.108 b
PI total	2.850 a	2.882 a	0.776 b

Lower F_m values may indicate that the photosynthetic object is in a state of stress and not all electron acceptors in PS II can be completely reduced. Maximal fluorescence is a complex parameter that is determined by a number of factors but also depends on the chlorophyll content of the tissues examined. Indeed, the lower Fm values for Charkor treated plants correspond to the measured lower content of total chlorophyll a, total chlorophyll and net photosynthetic rate in these plants (Tables 2,3 and 4).

Despite fluctuations in the initial, maximum, and variable fluorescence, the quantum yield (Yield = F_v/F_m), reflecting the potential of photochemical activity of PS II, ranges from 0.789-0.792 and corresponds to normal (0.750-0.830) in healthy, unstressed leaves (Bolharnordenkampfh & Oquist, 1993). This indicates that in all three variants studied, a normally developed photosynthetic apparatus was functioning. This is confirmed by the slight differences in the measured values of the rate of net photosynthesis in control and Regoplant treated plants. However, a more in-depth analysis of the parameters of the JIP test revealed some characteristic features of the potential of the photosynthetic apparatus in plants treated with biostimulators and control plants. Characteristic differences between plants grown with and without Regoplant were not reported in the other three important parameters of the JIP test - psi (Eo), phi (ψEo), the performance index (Plabs) and the total performance index (Pl total). For plants grown with Charkor, the four parameters were statistically lower than the control plants and those cultivated with Regoplant. Parameter ψEo reflects the probability of electron transport outside Q_A. The performance index (PIabs) shows the functional activity of the PS II relative to the energy absorbed, and the total performance index (PI total) reflects the functional activity of the PS II, PS I and the electron transport chain between them. PI_{total} is closely related to the overall plant growth and survival under stress and is considered to be a very sensitive indicator of the JIP test. The highest PI_{total} of the GiSelA 6 plants treated with Regoplant clearly showed the effectiveness of the applied treatment. Treatment with Regoplant contributed to the more active development and structuring of the photosynthetic apparatus, which in turn is a prerequisite for more intensive photoassimilation and biomass accumulation (Table 1).

The results reported by other authors regarding the acclimatization of cherry rootstocks of the GiSelA series are quite controversial. Vujovic et al. (2012) reported a high acclimatization rate (up to 100%) in GiSelA 6 plants in plastic pots containing sterile soil substrate on a "mist' bench in greenhouse, while in GiSelA 5 (*Prunus cerasus 'Schattenmorelle'* × *Prunus canescens*) 61.8% acclimatization was reported under the same conditions. The results obtained by classical acclimatization method (by using solid substrates and high air humidity) of GiSelA 5 were similar with low survival percentages-36.4% (Šiško, 2011).

In experiment for *ex vitro* acclimatization in float hydroculture of GiSelA 5 plantlets rooted *in vitro*, Clapa et al. (2013) reported the lowest survival percentages obtained by this method (58%) among studied species. They achieved higher survival rate (67.24%) when planting the *in vitro*-rooted GiSelA 5 plantlets into the layer of floating perlite.

According to Yepes and Adwinckle (1994), lack of vascular connections between roots and shoots was implicated in low survival of *in vitro* rooted apple plantlets after transfer to the soil. Similarly, indirect *in vitro* rhizogenesis through callus formation can be one of the reasons for low percentage of acclimatization in GiSelA 6 in control variant, as was shown in pear cultivar Bartlett (Bommineni et al., 2001).

Results indicate that during the acclimatization of micropropagated GiSelA 6 (*Prunus cerasus* 'Schattenmorelle' × *Prunus calescent*) plants in floating system conditions, 100 µl L^{-1} Charkor increased plant survival compared to the control, although showing some depressing effect on leaf development and photosynthetic pigment content.

The floating system provides easier maintenance of the air humidity, especially in the conditions of autumn acclimatization and is a valuable approach, especially in the conditions of hot and dry autumn as reported in Bulgaria in 2019. The results of the presented experiment would serve as a basis for the acclimatization of other woody species under floating system conditions.

CONCLUSIONS

Enrichment of the nutrient solution with biostimulator Regoplant (100 µl L^{-1}) in floating system led to the highest survival rate (86%) of GiSelA 6 (*Prunus cerasus* 'Schattenmorelle' × *Prunus canescens*) plants, the greatest stem length, number of leaves, leaf area, fresh and dry mass of leaves and stems.

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DRYING BLACKCURRANT FRUIT IN A DRYER WITH AN ALTERNATIVE SOURCE OF ENERGY

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Abstract

The alternative sources of energy for fruit drying preserve to some extent the ecological purity of the final product and its cost-effectiveness. The following cultivars were included in the present study: 'Ometa', 'Titania', 'Neosaipayushtasya', 'Bogatir', 'Byurlovska', 'Ben Lomond' and 'Ben Sarek' in the collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The experiment on drying blackcurrant fruit, without any direct sun light, was conducted in 2019.
The aim of the present study was to observe the suitability of this type of drying of blackcurrant fruit as well as the changes in their biochemical composition. The highest refractometric substance in fresh fruit was found in 'Ometa' (19.0%). The highest content of total (6.85%) and inverted (5.50) sugar was found in 'Neosaipayushtasya'. Dry matter in dried fruit was within the range of 81.43% ('Neosaipayushtasya') to 87.74% ('Ometa'). The highest values of total and inverted sugar were found in fruit of 'Titania' (6.10%) and sucrose in 'Ometa' (3.04%).

Key words: *blackcurrant, biochemical composition, cultivars, drying, fruit.*

INTRODUCTION

The advantages of blackcurrant over other fruit crops are due to its biological and economic qualities: high plasticity, easy reproduction, rapid return on capital investments, cost-effectiveness, application of modern cultivation technologies, high nutritional and dietary properties (Vater and Arena, 2002; Kampuss and Strautina, 2004; Madry et al., 2010; Sasnauskas et al., 2012).
Its fruits have an attractive appearance, superb aroma and great taste. They are particularly valuable because of their rich and varied mineral composition, including potassium, phosphorus, calcium, magnesium, iron, copper, manganese, zinc, cobalt. Their high levels of ascorbic acid and anthocyanins contribute to the normal course of physiological processes in the human body (Topchiiski, 1968; Häkkanen et al., 2002; Georgiev et al., 2007; Pantelidis et al., 2007).
Except for healing purposes, blackcurrant fruits are used to meet the needs of the population for fresh fruits and processed products. They are very suitable for drying, freezing and processing (Brashlyanova et al., 2014). Fruit drying is one of the most ancient methods of conservation and year-round preservation in order to maintain and restore the human body (Karabadzhov et al., 2011; Georgiev et al., 2014).
The purpose of the present scientific experiment is to monitor changes in the biochemical composition of fresh and dried blackcurrant fruits in a sun drier of different cultivars in order to have fruit all over the year.

MATERIALS AND METHODS

Fruit drying was conducted in the period from 02.07 to 11.07. 2019 in an outdoor dryer using daytime temperatures as the solar energy is transformed within the heat collector. The warm air flow passes through fruits and takes the moisture outside. The drying process in the experiment took ten days.
The scientific experiment included the following cultivars: 'Ometa', 'Titania', 'Neosaipayushtasya', 'Bogatir', 'Byurlovska', 'Ben Lomond' and 'Ben Sarek' from the Institute's collection plantation.
The collection plantation is located on a slope with an eastern exposure and at an altitude of 460 m. The soils are gray forest. The planting schemes are as follows: 1.00 m in the intra row

area and 3.00 m in the row spacing of plantation. The agrotechnics involved naturally grassed row spacings and black fallow in the intra row spacing.

The change in the biochemical composition of fresh and dried fruits of cultivars of different origins on heavy, moist soil in the region of Troyan were observed.

The following biochemical characteristics of selected fruit cultivars were investigated:

- dry weight matter (%) - 5 to 10 g were taken from the sample, using glass weight, glass rod and quartz sand;
- dry matter (DM) according to (refractometer) Re (%);
- Determination of sugars (total, invert and sucrose) and acid, according to the method of Schoorl (Donchev et al., 2001), 25 g of sugars were taken from the sample. Chemicals: 10% $NaCO_3$, $NaHPO_4$, Fehling's solution I, Fehling's solution II (made in the laboratory), 30% KJ, 1:6 H_2SO_4, titrated by 0.1 n $Na_2S_2O_3$ and starch indicator - sugars; 5 ml of acids were taken from the primary filtrate (as malic) by titration with 0.1-n NaOH - (%) and phenolphthalein indicator - acids;
- tanning substances according to the method of Levental (Donchev et al., 2000), 25 g of tannins were taken from the sample. Chemicals: 1:4 H_2SO_4, titrated by 0.1 KM_4O_4 and an indicator (indigo carmine);
- anthocyanins mg/% according to the method of Fuleki and Francis (1968), 2 g were taken from the sample . Chemicals: 96% spirt, buffer with pH - 1.0; buffer with pH - 4.5;
- pectin according to the method of Melitz (Donchev et al., 2000), 12.5 g were taken from the sample. Chemicals: 0.1 n NaOH, 1 n CH_3COOH, $CaCl_2$, $AgNO_3$;

Data processing was performed by the Statistical methods in biology and in agriculture (Lidanski, 1988), using MS Excel software - 2010.

RESULTS AND DISCUSSIONS

Fresh blackcurrant fruit

The diverse biochemical composition of blackcurrant fruits has been repeatedly studied. The individual indicators vary widely. Most often researchers have looked for the reasons in cultivar characteristics, not infrequently in the impacts of growing conditions. Different relationships have been found in the accumulation of anthocyanins, ascorbic acid, mineral salts, etc. (Topchiiski et al., 1968) with the content of flavonols (Häkkanen et al., 2002), ferments and enzymes.

The dry weight matter in the examined cultivars ranged from 17.41% ('Ben Sarek') to 23.43% ('Byurlovska'), that is, with a difference of approximately six units (Table 1).

The blackcurrant fruits have a relatively high content of dry refractometric substances, which for 'Titania' and 'Ometa' cultivars reached respectively: 17.3% and 19.0%.

The highest content of total sugars were reported for 'Neosaipayushtasya' (6.85%) and the lowest in 'Ben Sarek' (5.85%). The lowest content was observed is 'Bogatir' (4.20%). Inverted sugar was in approximate values to total sugars. The highest content of it again was found in 'Neosaipayushtasya' (5.50%) and 'Ben Sarek' (5.35%) and the lowest in 'Lisil' (3.70%). Sucrose was available in some cultivars. It was 1.28% for 'Neosaipayushtasya' and slightly lower for 'Lisil' - 0.95%. There is a strong variation of the indicator among the cultivars.

The highest content of organic acids was found in 'Ben Sarek' with a value of 1.40%, lower for 'Titania' (1.15%) and the lowest in 'Byurlovska' with 0.57%.

The tannins were high in blackcurrant fruits, ranging from 0.145% ('Ometa' and 'Ben Lomond') to 0.253% ('Byurlovska').

Pectin levels were high in 'Neosaipayushtasya' up to 4.17%. Fruits of 'Byurlovska' also had high content (3.16%). The lowest pectin level was found in 'Ben Lomond' (1.00%). The variation among cultivars was considerable.

High variation was registered in the values of total sugars, sucrose, organic acids, tannins and pectin among fruit cultivars.

Dried blackcurrant fruit

The biochemical composition of dried fruits indicates that dry weight matter values among cultivars are very close. The highest dry weight content was found in 'Ometa' (87.74%) and for the other cultivars was 81-85% (Table 1).

The content of total sugars is close to fresh fruits. The highest content was found in

'Titania' (6.10%) and significantly lower in 'Ben Lomond' (2.20%).
The content of inverted sugar was lower, compared to fresh fruits.
The highest content was found again in 'Titania' (6.10%) and the lowest in 'Ometa' (1.60%) with four point five units. The decrease in total and inverted sugar towards absolute dry units in fruit of 'Titania' cultivar was 3.1 times compared to fresh fruit. The indicator was highly variable among cultivars.
The highest content of sucrose was found in 'Ometa' (3.04%) and the lowest in 'Neosaipayushtasya' and 'Ben Sarek' (0.57%). It should be noted that 'Titania', 'Byurlovska' and 'Ben Lomond' do not have any sucrose.

Table 1. Biochemical composition of fresh and dried fruits of different blackcurrant cultivars in 2019

Cultivar	Dry weight (%)	DM according to Re (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Acids (as malic) (%)	Tannins (%)	Pectin (%)
Fresh fruit								
'Ometa'	20.71	19.0	4.35	4.35	-	0.70	0.145	2.05
'Titania'	19.57	17.3	4.50	4.50	-	1.15	0.199	2.35
'Lisil'	23.43	12.0	4.70	3.70	0.95	0.64	0.181	2.35
'Neosaipayushtasya'	19.47	15.5	6.85	5.50	1.28	0.89	0.199	4.17
'Bogatir'	20.17	14.0	4.20	4.20	-	0.64	0.163	2.99
'Byurlovska'	20.75	15.0	4.50	3.85	0.62	0.57	0.253	3.16
'Ben Lomond'	20.08	14.5	5.00	4.50	0.48	0.70	0.145	1.00
'Ben Sarek'	17.41	12.5	5.85	5.35	0.48	1.40	0.199	1.27
ẋ ±SE	0.59	0.82	0.32	0.23	0.17	0.10	0.01	0.36
St Dev	1.68	2.33	0.91	0.64	0.47	0.29	0.04	1.03
VC %	8.32	15.55	22.02	14.25	97.92	34.52	21.05	42.56
Dried fruit								
'Ometa'	87.74	-	4.80	1.60	3.04	0.94	0.208	0.80
'Titania'	83.08	-	6.10	6.10	-	2.01	0.291	1.53
'Lisil'	84.60	-	5.70	3.80	1.81	1.34	0.291	0.53
'Neosaipayushtasya'	81.43	-	3.80	3.20	0.57	1.34	0.208	0.96
'Bogatir'	84.56	-	5.70	3.80	1.81	1.34	0.291	1.51
'Byurlovska'	84.37	-	3.20	3.20	-	1.74	0.208	1.01
'Ben Lomond'	84.61	-	2.20	2.20	-	1.34	0.249	1.06
'Ben Sarek'	84.10	-	3.20	2.60	0.57	2.01	0.291	1.22
ẋ ±SE	0.62		0.51	0.48	0.40	0.13	0.01	0.12
St Dev	1.77		1.44	1.36	1.12	0.38	0.04	0.34
VC %	2.1		33.18	41.09	114.29	25.17	16	31.48

Interesting results have been obtained regarding the sucrose content of dried fruits. 'Ometa' has registered values of 3.04% and was much lower in other cultivars. A high variation coefficient was reported among cultivars in total, invert sugar and sucrose.
The content of organic acids was close to fresh fruits, which were in the range of 0.94% ('Ometa') to 2.01% (in 'Titania' and 'Ben Sarek').
Higher tannin values were found in dried fruit over 0.200% in all cultivars. The higher concentration of cell sap might be the reason for the greater preservation degree of tannins.
The pectin content in dried fruits was lower than fresh ones in almost all cultivars. The highest was found in 'Titania' (1.53%) and 'Bogatir' (1.51%). The highest values of sugars (total sugars, invert and sucrose) and organic acids were found in dried fruit.

CONCLUSIONS

An experiment was carried out on the opportunity of applying a technology for drying of blackcurrant fruits in a drier with an alternative energy source.
The results show that the blackcurrant is high in refractometric substance, tannins and pectin. The highest DM according to Re was found in 'Ometa' (19.0%). The highest content of tannins (0.253%) was found in 'Byurlovska' and pectin (3.16%).
In dried fruits, the values of tannins are increased in all cultivars, while pectin was greatly reduced in most of them.

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PRELIMINARY RESULTS REGARDING THE POSTHARVEST PATHOLOGY OF PAWPAW (*ASIMINA TRILOBA* DUNAL) FRUITS

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Abstract

Asimina triloba, the pawpaw, is one of the few fruit tree species native to the United States. The plant is native to eastern North America, growing spontaneously from the Gulf of Mexico till the Great Lakes. The tree produces large fruits, yellow-green colour, sweet and flavored. In Romania, the first plants were introduced in Transylvania in 1926 and have been grown locally, remaining unknown to the rest of the country. Starting with the year 2000, within the Faculty of Horticulture from Bucharest, there were cultivated several varieties and hybrids of pawpaw, in order to determine the suitability of this species for the establishment of new plantations in Romania. The fruits were harvested manually and stored in controlled atmosphere rooms with 1.5% CO_2*, at 4°C temperature and 90% relative humidity. During the 2 months storage period were identified several pathogens affecting the paw paw fruit. The micromycetes identification was carried out by isolation and successive subculture on PDA medium culture (Potato Dextrose Agar), followed by incubation at 22°C in the thermostated chamber.*

Key words: *Asimina triloba, storage pathogens, postharvest.*

INTRODUCTION

The fruits are rich in active ingredients, vitamins and minerals. The fruits have an excellent exotic taste, that evolves during the storage from the taste of vanilla cream at crème brûlée, and finally, to chocolate cream taste.

Asimina triloba (L. Dunal), is part of the family *Annonaceae* being known in North America - its area of origin, as pawpaw (Stanica, F. et al., 2008). The genus includes the largest edible fruits originating in North America (Darrow, 1975). It is a large, thin, leathery fruit with two rows of almond-sized seeds. Its shape can vary from oval to elongated, peanut-shaped and can vary between 3 and 6 mm in length (McGrath M., 1994). Of the approximately 2300 species and 130 genera in the *Annonaceae* family, only the genus *Asimina* grows in temperate climates. All other genera of the family *Annonaceae* grow in the tropics. In south-eastern Florida and Georgia there are eight species of the genus *Asimina*: *Asimina angustifolia* Raf. - pawpaw with narrow leaves, *Asimina incana* (W. Bartram)- pawpaw with wool, *Asimina obovata* (Willd.) Nash - pawpaw with large flowers, *Asimina parviflora* (Michx.) Dunal - pawpaw with small flowers, *Asimina pygmea* (W. Bartram) Dunal - pawpaw dwarf, *Asimina reticulata* Shuttlw. ex. Chapman - Netted pawpaw, *Asimina tetramera* Small- pawpaw with four petals, *Asimina triloba* (L.) Dunal - Common pawpaw (Callaway, 1993).

Pawpaw grows at the edge of forests and is noted for its high frost resistance, surviving winters at -25-30°C, however, it is grown as an orchard crop in several states, including Alabama, California, Maryland, Michigan, Missouri., North Carolina, Kentucky, West Virginia, and Ohio. It has also been planted in Italy, China, Japan, Israel, Belgium, Portugal and Romania (Brannan, R. G et al., 2015). In Romania, it was introduced around 1900 in Pianu Nou, Alba County, by an emigrant returning from America. From there, some plants arrived in the locality of Geoagiu, where they are still found in the present of some locals (Stănică F. et.al., 2004).

In 2000, at the Faculty of Horticulture in Bucharest, 7 varieties and 3 hybrids of *Asimina triloba* (L.) Dunal imported from Italy were introduced, with which three collections were established. One collection exists within the Bucharest Faculty of Horticulture, and the other two collections are in private gardens in Argeş and Ilfov counties (Stănică F., 2002; Stănică F. et al., 2004; Stănică F., 2012). Today, the northern banana is one of the most exotic plants that have been acclimatized in Romania with a huge potential on the domestic market, especially due to the special taste of vanilla and chocolate. Pawpaw fruits have a special taste, are rich in nutrients, dietary fiber, protein, minerals and vitamin C, they can be used as ornamental and medicinal species (Layne, D.R., 1996; Picchioni, G.A. et al., 2004; Pomper, K.W. et al., 2002; Pomper and Layne, 2005).

The harvest season for pawpaw fruit is from mid-August to the end of September. A color change often occurs later in the ripening period. On the other hand, a decrease in fruit firmness is relatively obvious and a detectable indicator of maturity (Pomper et al., 2008). It is also recommended to harvest the fruits during the early ripening period, in order to increase the storage time (Archbold et al., 2003; Pomper & Layne, 2005; Szilagyi B.A., 2015).

In Bucharest, the fruits of *Asimina triloba* (L.) Dunal ripen only at the beginning of October (Cepoiu N. et al., 2003).

During ripening, the loss of firmness is extremely fast, the fruits soften quickly, at ambient temperature and this can be an obstacle in the development of a wider development market (Galii et al., 2008).

Monilinia spp. is the plant pathogen responsible for the occurrence of grey mold and fruit rot in stone fruits species, and it is present in all cultivated areas (Cristea S. et al., 2017)

Agricultural production is vulnerable to contamination and infection with various microorganisms during storage and the safety of agri-food products can be achieved by maintaining climatic factors in the stored areas, thus limiting the population level of contaminating microorganisms. The most common genera of fungi identified in storage room are *Aspergillus*, *Penicillium* and *Fusarium* (Dudoiu, R. et al., 2016)

Micromycetes' development on storage room is conditioned by temperature and atmospheric humidity present in stored areas and by its fluctuations in time (Cristea S., 2004)

The results showed that Romanian natural conditions are suitable for growing this species as one of the most exotic plants that were ever acclimated in Romania. The pawpaw has an huge potential on the domestic market.

MATERIALS AND METHODS

The present paper presents the results of research conducted between 2016-2017, a period in which no phytosanytary treatements have been applied, the plants proving their suitability to the organic production systems

Research has sought to identify the presence of mycoflora on the fruits of *Asimina triloba* (L.). The biological material consisted of samples of pawpaw fruit, stored in the room with a controlled atmosphere, from which samples were taken for analysis. The fruits were harvested by hand and stored in rooms with a controlled atmosphere with 1.5% CO_2 at 4°C and a relative humidity of 90%.

The samples were taken immediately after collection and stored for 60 days. The batch sampling was performed on three levels, respectively the base, the middle and the upper surface, then a sample of 100 fruits was constituted (Chira L., 2008).

PDA culture medium was used to isolate and identify microorganisms associated with the disintegration of pawpaw fruits.

The fruits were washed from the ground with tap water and finally rinsed with sterile water. No pesticides have been used as they can also affect pathogenic fungi. Since the aim was to isolate the fungi that spore on the surface, the introduction of the samples in the humid chamber was preceded by a day or two (Severin, V et al., 2009).

Segments of 1-2 mm of tissue from the edge of the affected area are passed, with the help of a sterile scalpel and a repeating needle, in Petri dishes with a diameter of 70 mm, on the PDA culture medium. The vessels were incubated at 22°C for 9 days. Observations were made at 3, 6 and 9 days. The Euromex stereo microscope and the Euromex Ox Range microscope were used to identify fungi, based on the scientific literature (Raicu C., 1978; Hulea A., et al., 1986; Hulea A., 1969)

RESULTS AND DISCUSSIONS

Studying the spectrum of pathogens on the fruits of the "northern banana" it was found that the mycoflora present in the analyzed samples was composed of fungal species belonging to the genera *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp. (Table 1).

The analysis of the 2016 samples shows that the fruits from the studied genotypes showed fructifications of the micromycetes *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp.

In the samples of pawpaw fruits, from the 2016 harvest, micromycetes belonging to the genera *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp. Were identified were detected on the analyzed genotype R2P3, and the fungus *Alternaria* spp. was present on the skin of genotype R1P2, R2P3. The pathogen *Penicillium* spp. was identified on the genotypes R1P8, R2P1, R2P3, 10344, 10836. The fungus *Fusarium* spp. was present on the genotypes R2P1, R2P3, 10836 (Table 2).

Table 1. Mycoflora detected on pawpaw fruits

Genotypes	The pathogen agent				
	Alternaria spp.	*Verticillium* spp.	*Monilinia* spp.	*Penicillium* spp.	*Fusarium* spp.
R1P2	+	+	+	-	-
R1P8	-	-	-	+	-
R2P1	-	-	+	+	+
R2P3	+	+	+	+	+
10344	-	-	+	+	-
10836	-	-	+	+	+

Table 2. Incidence of mycoflora detected on pawpaw fruits expressed as a percentage (2016)

Genotypes	The pathogen agent (after 9 days)				
	Alternaria spp.	*Verticillium* spp.	*Monilinia* spp.	*Penicillium* spp.	*Fusarium* spp.
R1P2	70	10	20	-	-
R1P8	-	-	-	100	-
R2P1	-	-	45	20	35
R2P3	10	4	66	17	3
10344	-	-	56	44	-
10836	-	-	35	48	7

Micromycetes of the genus *Monilinia* spp. were identified on genotypes R1P2, R2P1, R2P3, 10344 and 10836.

Observations made on the incidence of mycoflora detected on “northern banana” fruits in 2016 show that the micromycete *Alternaria* spp., present on genotypes R1P2, R2P3, had the highest frequency value, in the variety R1P2 with F = 70%.

Pathogens of the genus *Monilinia* spp, showed the highest incidence values in the variety R2P3 with 66%, 10344 (56%) and R2P1 with 45%. The lowest incidence value was noted in the R1P2 genotype (20%).

In the analyzed samples, the micromycete *Penicillium* spp. was identified on the genotypes R1P8, R2P1, 10836, 10344, R2P3, with frequency values of 100%, 48%, 44% and F = 17%, respectively.

The results showed that *Alternaria* spp., *Verticillium* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp. were found with pawpaw fruits studied. All five isolated organisms were confirmed to be pathogenic on pawpaw fruits, but in different percentages. Gupta and Pathak (1986) previously reported that pathogens were responsible for post-harvest pawpaw losses in southwestern Nigeria.

CONCLUSIONS

Research has shown that fruit rot and the development of pathogens depend on various factors. The optimum temperature range for the development of pathogenic fungi was 22° C.

Isolation of these pathogens confirmed the studies of Baiyewu and Amusa (1999), Baiyewu et al. (2007), Gupta and Pathak (1986).

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STORAGE AND QUALITY OF AUTUMN PEARS, DEPENDING ON THE DOSE OF POST-HARVEST TREATMENT WITH ETHYLENE INHIBITOR 1-MCP

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Abstract

The aim of the research was to determine the effect of post-harvest SmartFresh treatment of autumn pears (various 1-MCP doses) on the natural weight losses, skin browning, senescent breakdown and ethylene-production, firmness, soluble solids content, titratable acidity and taste during fruit storage. Autumn pears, known in Ukraine as Delbarau RX 12/47 (the local name is also Snizhynka) were cooled to 5°C and treated with 1-MCP at the dose of 1000 ppb recommended for apples (SmartFresh^{TM}) and experimental doses of 750 and 500 ppb. Fruits were stored at 2±1°C and relative air humidity 85-90%. With the losses lower than 10%, autumn pears without treatment of 1-MCP can be stored at 2±1°C for no longer than three months and those treated with an ethylene inhibitor - for no more than four months. A high efficiency of post-harvest treatment of autumn pears with an ethylene inhibitor is achieved at doses of 500, 750 and 1000 ppb 1-MCP. A more harmonious taste of fruits is achieved after the use of smaller doses of 1-MCP.

Key words: *1-MCP, ethylene, pears, physical-chemical parameters, taste.*

INTRODUCTION

Market demand for pear fruit is determined by its harmonious taste, aroma and low calorie content. Unlike apple, pears are less resistant to mechanical damage and physiological disorders and they require more careful storage conditions (Konopacka et al., 2014).

Controlled atmosphere and low temperature are the main storage practices for pears. Post-harvest ripening of pears is initiated by ethylene, which has a negative impact on storage duration (Watkins, 2006). An ethylene inhibitor 1-methylcyclopropene (1-MCP) reduces the sensitivity of the fruits to the action of ethylene, thus controlling the rate of maturity and loss of firmness. It also limits skin browning and internal decay and improves product stability during sales (Baritelle et al., 2001; Defilippi et al., 2011).

During storage, the firmness of pears varies considerably, so fruits with a flesh firmness of at least 4.0 kG should be brought to the market and that of 0.8-1.2 kG - for consumption (Ma and Chen, 2003; Blaszczyk, 2011).

The effect of 1-MCP depends on the pomological variety, the degree of harvesting ripeness and the duration of fruit storage (Gamrasni et al., 2010). However, post-harvest treatment of pears with a full dose of 1-MCP, which is recommended for apples, results in the loss of ability to ripen, the fruits remain too firm and do not yellow without acquiring the organoleptic characteristics desired by consumers (Villalobos-Acuna and Mitcham, 2008). Lower doses of 1-MCP delay the onset of climacteric rise and fruit partially restores sensitivity to ethylene (Jeong et al., 2002). Therefore, pear fruits are treated with a half dose of 1-MCP, as compared with apples (Cucchi and Regiroli, 2011).

The aim of this study was to improve the consumer properties of autumn pears by post-harvest treatment with a different dose of 1-MCP, as well as to identify the level and nature of losses, changes of ethylene activity, physical and chemical parameters and tasting evaluation during conventional cold storage.

MATERIALS AND METHODS

The research was conducted in the storage season of 2016/2017 at the Department of fruit growing and viticulture of Uman National University of Horticulture. Common autumn pears, known in Ukraine as Delbarau RX 12/47 (the local name is also Snizhynka), from trees on the rootstock of quince A were selected in the irrigated fruitful orchard with grass in the inter-rows and herbicide strips under trees in Chernivtsi region, Ukraine. Conducting the experiment and processing of the results were performed by standard methods.

The pears were collected in the stage of harvest maturity. Fruits of uniform maturity were selected with a diameter at least 70 mm and they were put into 15 kg boxes with chess stacking. Also, polyethylene nets with fruits were put there to record natural weight losses.

On the day of collection, the fruits were cooled at 5±1°C and relative air humidity of 85-90%, avoiding the presence of an external source of ethylene - fruits not intended for research. The following day, pears were treated with 1-MCP with experimental doses of 500 (0.034 g/m^3), 750 (0.051 g/m^3) and a recommended dose for apples - 1000 ppb (0.068 g/m^3) of SmartFresh; untreated fruits were the control. For this purpose, boxes with fruits were placed in a gas-tight container of a polyethylene film of 200 microns thick, where a glass of distilled water and a powdered preparation, calculated per volume unit, were placed. The circulation of air in a container was carried out by the fan. After 24-hour exposure, the film container was removed, treated and control fruits were stored at 2±0.5°C and air humidity of 85-90%.

At harvest, flesh firmness of pears, the content of soluble solids, titratable acidity, iodine/starch test (on the CTIFL scale) and Streif index were determined. The Streif index (SI) was calculated as the ratio of firmness (F, kG) to the soluble solids content (SSC, Brix %) and iodine/starch (S) test (Streif, 1996):

$$SI = F/(SSC \times S)$$

The estimation of weight loss during storage was periodically done by weighing polyethylene nets with fruit before and after storage. The number of fruits affected by skin browning and senescent breakdown was determined in comparison with the total number of fruits.

The intensity of fruit ethylene production (μl·kg^{-1}·hr^{-1}) was measured with analyzer ICA-56 (International Controlled Atmosphere Ltd) after removing from the cold store and 24-hour warming of fruits, the first measurement was done at 18-20°C and the others were conducted during shelf-life at the same temperature and relative humidity of 55-60%. Measurements were made on separate batches of fruits. A sample of three or four fruits of a weight approximately 0.5 kg was placed in a 4 liter airtight jar and maintained for 0.5-1 h at 18-20°C (Melnyk, 2010).

In a 20-fruit sample flesh firmness was determined with penetrometer FT-327 with an 8-mm plunger mounted on a tripod, with two measurements on each pear (skin was removed before the measurement). The content of soluble solids (Brix %) was determined with a refractometer RHB-32ATC and titratable acidity (malic acid, %) was determined by dissolving a known weight of sample in distilled water and titration against 0.01 N NaOH using phenolphthalein as indicator.

Pear organoleptic evaluation was carried out by a permanent panel of 10 people after four months of storage and a week shelf-life at 18-20°C and relative humidity of 55-60%. Samples of three fruits were blind, marked with numbers. Aroma, hardness, crispiness, juiciness, oiliness, sweet taste, sour taste and overall assessment were evaluated as 10 points - perfect and 1 point - unsatisfactory. Sweet/sour index as the ratio of sweet taste to the sour taste was determined. The effect of the studied factors was evaluated with a multivariate analysis of variance by Statistica 6 with LSD_{05}, $P<0.05$.

RESULTS AND DISCUSSIONS

During the harvesting, flesh firmness of pears was 9.0 kG, the content of soluble solids was 11.3%, titratable acidity - 0.29%, iodine/starch test - 6 points and 0.13 - Streif index.

1. Physiological disorders

During the three-month storage, there were no losses from the skin browning in the untreated products, and with 1-MCP treatment, they were at the level of 5.0-8.5% (insignificant difference, Figure 1).

Losses from the scald increased as the duration of storage increased. After four months of storage, only processed fruits were affected, regardless of a doses. Senescent breakdown losses (9.2%) were detected only for untreated fruits after four months of storage, and after treatment with 1-MCP they were not recorded. Similar results were obtained by Chen and

Spotts (2005) for d'Anjou pears where, after four months of storage at -1°C, the fruits treated with an ethylene inhibitor were affected.

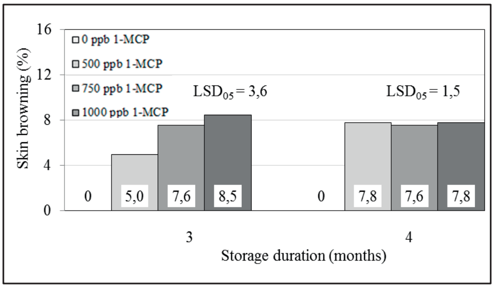

Figure 1. Damage of pears by the skin browning after post-harvest treatment with different doses of 1-MCP during refrigeration storage

2. Natural weight losses

Natural weight losses during storage were steadily increasing (Figure 2). After two months of storage, the rate of fruits treated with a dose of 500 ppb reached 1.5%, which was 1.3-1.4 times lower, as compared with treated ones with doses of 750 and 1000 ppb. After three months of storage, the weight losses of untreated fruits were 1.5-2.0 times higher as compared with the processed ones.

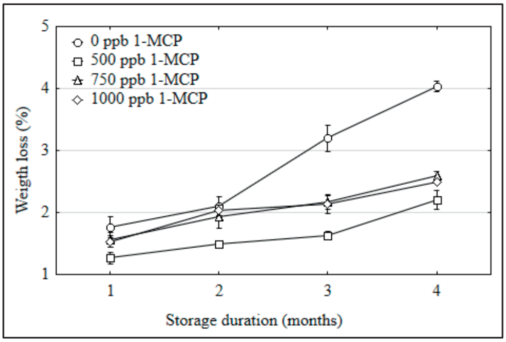

Figure 2. Change of natural weight losses of pears treated with different doses of 1-MCP, during storage

After four months, the natural weight losses of untreated fruits were 1.6-1.8 times higher than that of 1-MCP treated, whereas after treatment with a dose of 500 ppb, the indicator was 1.1-1.2 times lower as compared with the 750 and 1000 ppb doses. Similar results were obtained by Mahajan et al. (2010) during storage of pears cv. Patharnakh.

3. Ethylene activity

Post-harvest treatment with 1-methylcyclopropene significantly inhibited an ethylene production rate of recently harvested fruits (Figure 3).

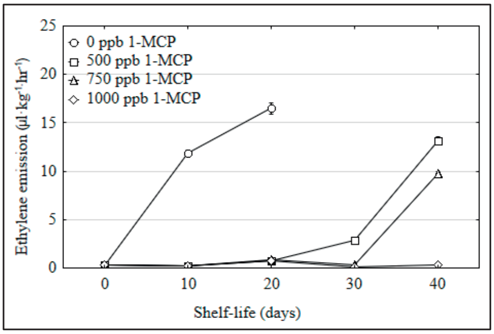

Figure 3. Ethylene production at a temperature of 20°C by freshly harvested pears, depending on the doses of post-harvest treatment 1-MCP

Ethylene production of untreated fruits steadily increased, and it reached a level of 16.5 µl·kg^{-1}·hr^{-1} on the 20th day of shelf-life at 20°C. Regardless of the 1-MCP doses, during first 20 days, the activity of treated fruit ranged within 0.8-0.9 µl·kg^{-1}·hr^{-1}, which was less than the value of untreated pears by 18.3-20.6 times. After 30 days, the ethylene production of fruits treated with a dose of 500 ppb was 2.9 µl·kg^{-1}·hr^{-1}, which was 9.6-29.6 times lower than those with the treatment with doses of 750 and 1000 ppb (lower ethylene production of pear fruit for higher dose of 1-MCP). After 40 days of shelf life at 20°C, the highest ethylene production of 13,2 µl·kg^{-1}·hr^{-1} was reached by 500 ppb treated fruits, it was 1.3 times lower for 750 ppb and 44 times lower for 1000 ppb dose.

Untreated 1-MCP fruits also exhibited the highest ethylene production during four months of storage (Figure 4). After three months of common cold storage, untreated fruits have the highest level of 39.0 µl·kg^{-1}·hr^{-1}, it is 13 times lower for 500 ppb dose treatment and at 0.1-0.5 µl·kg^{-1}·hr^{-1} for fruits treated with doses of 750 and 1000 ppb. After four months of storage, the ethylene production of untreated fruits was 4.8 times higher, as compared with fruits treated with a dose of 500 ppb, and the pear rate treated with doses of 750 and 1000 ppb did not exceed the level of 0.6-0.9 µl·kg^{-1}·hr^{-1}.

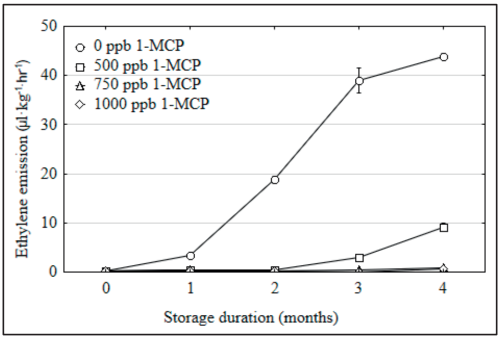

Figure 4. Ethylene production at a temperature of 20 °C by pears during the four-month cold storage, depending on the doses of post-harvest treatment with 1-MCP

A similar pattern was found by Folchi et al. (2014) during storage of pears cv. Abate Fetel

4. Flesh firmness

Flesh firmness of non-treated fruits was actively reduced, especially in the first three months of storage (Figure 5). The index level of the untreated fruits, which was necessary for shipment to the market, was kept not less than 4.0 kG only during the first three months.

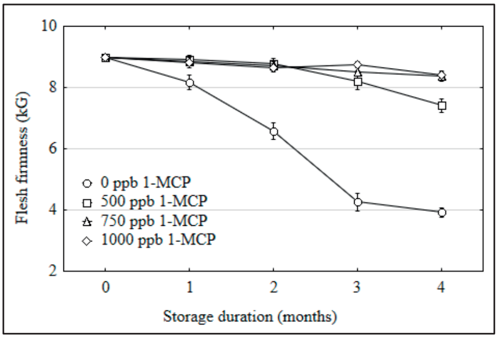

Figure 5. Change in the flesh firmness of pears treated with different doses of 1-MCP, during cold storage

Therefore, without post-harvest treatment with 1-MCP, autumn pears are suitable for sale only during the first three months storage at 2±1°C, whereas post-harvest treatment provided a 1.9-2.0 times higher level of the indicator, regardless of the dose 1-MCP.
After four months of cold storage, the firmness of the treated fruits was 1.9-2.2 times higher than the untreated ones. After treatment with a dose of 500 ppb the firmness decreased faster and reached 1.1 times lower level than the results of the application of doses 750 and 1000 ppb. Similar results were obtained by Calvo et al. (2004) during storage of pears cv. Red Clapps.

5. Soluble solids content and titratable acidity

In the initial period of storage, the content of soluble solids of all investigated variants increased to a certain extent, substantially decreasing later (Figure 6).

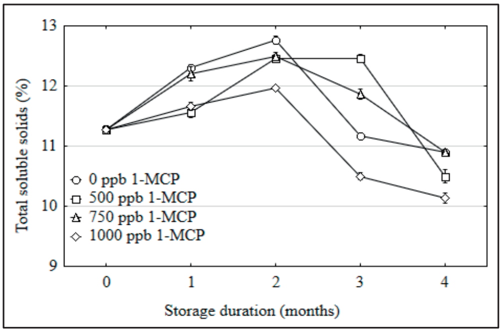

Figure 6. Change in the content of soluble solids in pears treated with different doses of 1-MCP, during storage

During the first two months (three were treated with a dose of 500 ppb), the level of soluble solids increased, decreasing significantly later on. After four months, the highest content of soluble solids was found in untreated fruits and those treated with a 750 ppb dose, it was 0.4% lower at 500 ppb and 0.7% lower at 1000 ppb.
During two months of storage, the content of total soluble solids increased to a certain extent (three months for 500 ppb treatment), decreasing significantly after that. After four months, the higher soluble solids content is found in untreated fruits and fruits treated with a 750 ppb dose, it is 0.4% lower for a 500 ppb dose and it is 0.7% lower for a 1000 ppb treatment.
The content of titratable acidity decreased steadily during pear storage of all the studied variants (Figure 7). After the first 30 days of storage, a significant effect of post-harvest treatment on the change in the content of titratable acidity for pears from all tested doses of 1-MCP was observed. After four months of storage, the acid content of pears treated with 1-MCP was 1.2-1.4 times higher, as compared with untreated fruits.

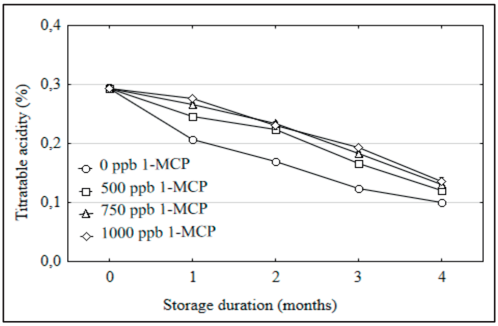

Figure 7. Change in the titratable acidity content in pears treated with different doses of 1-MCP, during storage

At a higher dose of 1-MCP, the content of titratable acidity was also slightly higher. A similar pattern was found by Kurubas et al. (2018) during storage of pears cv Ankara under the treatment with 250 and 500 ppb 1-MCP.

After four months, the content of titratable acidity in the treated pears is higher by 1.2-1.4 times, as compared with non-treated fruits. At higher doses of 1-MCP, the content of titratable acidity is slightly higher.

6. Tasting score

During storage, the ripening of pears treated with 1-MCP is significantly slower (Table).

Table. Organoleptic evaluation of pears with post-harvest treatment at different doses of 1-MCP, after four months of storage and a week shelf life at 20°C (crop 2016)

Dose of SmartFresh, ppb	Aroma	Hardness	Crispness	Juiciness	Oiliness	Sweet taste	Sour taste	Sweet/sour index	Overall score
90 days storage + 7 days shelf-life									
0	8.9	2.9	2.6	9.1	6.5	5.9	3.5	2.8	7.1
500	4.3	7.4	7.3	5.9	2.5	3.8	3.6	1.4	5.0
750	3.2	7.4	7.8	6.0	2.3	3.9	3.4	1.5	5.4
1000	2.6	8.3	8.3	5.1	1.9	2.7	2.8	1.2	3.8
LSD_{05}*	0.8	1.1	1.1	1.0	1.0	0.9	NS	1.1	0.8
120 days storage + 7 days shelf-life									
500	6.0	3.5	3.5	8.2	8.0	5.7	4.5	1.9	7.8
750	2.8	7.5	7.3	5.3	3.2	3.7	4.8	1.2	5.5
1000	2.7	7.8	7.7	4.5	2.5	3.8	4.0	1.1	4.8
LSD_{05}*	0.6	1.8	1.2	1.8	1.8	1.3	NS	NS	1.1

*NS - not significantly

After three months, the aroma estimate of treated fruits was 2.1-3.4 times lower than untreated ones; however, for a dose of 500 ppb, this estimate was 1.3-1.6 times higher than those for 750 and 1000 ppb.

After three months, the untreated fruits have a higher juiciness - by 3.1-4.0 times, oiliness - by 4.0-4.6 times, sweetness - by 1.5-2.2 times, sweet/sour index - by 1.9-1.3 times and total tasting score was higher by 1.9-1.3 times, as compared with treated fruits.

Regardless of the dose, the treated pears were 2.5-2.8 times hardness and 2.8-3.1 times crispness as compared with the untreated fruits. The juiciness and oiliness of the untreated fruits were 3.1-4.0 and 4.0-4.6 points higher, respectively, as compared with the treated fruits. Untreated pears (5.9 points) were the sweetest, and the index of fruits with 1-MCP treatment was 1.5-2.2 times lower.

No significant effect of treatment with 1-MCP on the degree of sour taste was recorded.

The sweet/sour index of the untreated fruits was 1.5-2.2 times higher as compared with the 1-MCP treated ones. Due to higher indicators of aroma, juiciness, oiliness and sweetness, the untreated fruits received 1.3-1.9 times higher total score than the treated ones, and for the 500 and 750 ppb dose treatments, the scores were 1.3-1.4 times higher than at 1000 ppb.

As compared with the treatment with doses of 750 and 1000 ppb, after four months of storage, the fruits treated with the dose of 500 ppb had higher flavor - by 2.1-2.2 times, lower hardness - by 2.2 times and lower crispness - by 2.1-2.2 times. These fruits had 1.5-1.8 times higher juiciness, 2.5-3.2 higher oiliness and 1.5 times higher sweetness.

There was no significant difference between the doses of 1-MCP in terms of sour taste and sweet/sour index. Due to higher other indicators, fruits treated with a dose of 500 ppb received a 1.4-1.6 times higher overall score as compared with the treatment with doses of 750 and 1000 ppb.

CONCLUSIONS

Post-harvest SmartFresh treatment has a significant effect on the storage results of autumn pears, known in Ukraine as Delbarau RX 12/47 (the local name is also Snizhynka), in particular on the natural weight losses, skin browning, senescent breakdown and ethylene-production, firmness, soluble solids content, titratable acidity and taste during fruit storage.

With the losses lower than 10%, autumn pears without treatment of 1-MCP can be stored at 2±1°C and relative air humidity 85-90% for no longer than three months and those treated with an ethylene inhibitor - for no more than four months. For this purpose, after harvesting, the fruits must be immediately cooled to 5°C and treated with 1-MCP.

The sale-permissible flesh firmness of the untreated fruit at a level of 4.0 kG is formed when pears are stored at 2±1°C for not longer than three months, and at this time, post-harvest treatment results in a 1.9-2.0 times higher level of the index, regardless of the 1-MCP dose.

A high efficiency of post-harvest treatment of autumn pears with an ethylene inhibitor is ensured in a wide range of 1-MCP doses - 500-1000 ppb. At lower doses, a more harmonious taste of pears is achieved without reducing storage ability.

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INVESTIGATION OF THE BIOCHEMICAL COMPOSITION OF FRUITS FROM TWO GENOTYPES SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.)

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Abstract

The aim of present study is to make a comparative analysis of biochemical composition of fruits of selected genotypes and their suitability for food products.
The experiment was conducted between RIMSA-Troyan and FRDI - Plovdiv. Fruits of two perspective genotypes of sea buckthorn were included in the experiment in the collection plantation of the institute. Biochemical composition (dry matter refractometric (%), total sugars (%), sucrose (%), inverted sugar (%), organic acids (%) tannins (%), pectin (%), ascorbic acid (mg%)), antioxidant activity, total polyphenols of fresh fruits and were studied.
Data analyses show that the measured dry matter has higher values for the Siberian shed - 13.25%. The total polyphenols defined in the Siberian berries are three times more than the total Caucasian berries. Antioxidant activity was comparatively with similar values for both genotype due to the content of total polyphenols in fruits. The data were statistically distinguishable due to genotypes difference (p <0.05).

***Key words**: antioxidant activity, biochemical compounds, Hippophae rhamnoides L., total polyphenols.*

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides*), is a hardy thorny bush or small tree of family *Eleagnaceae.* It is commonly known as sea buckthorn, , sandthorn distributed on a sandy soil near the rivers, the dunes and the coastline in Europe, Japan, Himalayas, Altai, Tibet. (Li, 2003; Li & Schroeder, 1996). The female plants produce ripe sea buckthorn berries yellow, orange, or red , are spherical in shape, and range in size between 3 and 8 mm in diameter weighting from 0.2 g to 1 g, (Li, 2003).
The sea buckthorn berries has been used for medicinal and nutritional purposes in Russia, Europe, and Asia for many centuries. As an agricultural plant is grown in Germany, France, Finland, India and China, which is the largest agricultural producer of sea buckthorn. Many of the substances that are found in the sea buckthorn are known to have beneficial effects on health (Li and Wang, 1998). It has been well established in the literature that berries and seeds contain high amounts of natural antioxidants including ascorbic acid, tocopherols, carotenoids, flavonoids, as well as health beneficial fatty acids (Gao et al., 2000; Kallio et al., 2002; Rosch et al., 2003; Mondeshka, 2005).
The chemical and nutritional composition of sea buckthorn berries vary considerably between different subspecies, with the origin, climate, time of harvesting and method of processing. The chemical composition of *H. rhamnoides* ssp. sinensis varies greatly with growth locations (variation in latitudes and altitudes). It is also reported that sea buckthorn fruit berries and seed oil contain various kinds of bioactive substances. Clinically proven are the regenerative properties of the oil on gallbladder, duodenal and epithelial cells in skin burns. It acts favorably in colitis, gastritis and ulcers, as well as on lipid exchange in the liver. Its antioxidant properties protect the body from cardiovascular disease, hypertension, atherosclerosis and lower blood cholesterol

levels (Mingyu et al., 2001; Yang, 2001; Yang, 2009).

In taste the fruits resemble the gums - sweet, slightly acidic but with a specific flavor. All food products in the diet normalize the functioning of the gastrointestinal tract Products on the market from sea buckthorn range from oil, juice, and food additives to candies, jellies, cosmetics, and shampoos. Sea-buckthorn fruit can be used to make pies, jams, lotions and liquors. The juice or pulp has other potential applications in foods or beverages. For example, in Finland, it is used as a nutritional ingredient in baby food. It provides a nutritious multi-vitamin beverage, rich in ascorbic acid and carotenes (Dharmananda, 2004; Schroeder & Yao, 1995; Mondeshka, 2005; Cenkowski et al., 2006).

MATERIALS AND METHODS

Fruit materials

The fruits of the Siberian variety have been grown and supplied by IPJZ-Troyan. Siberian is early spring - end of July - until mid-August, the bushes are up to 2.50 m high.

Siberian is early, fruits are large and oval, mature from the end of July to mid-August, the height of the bushes is up to 2.50 m.

The Caucasus has small berries, ripening from October to November, woody in appearance, with a height of 5.00 m.

Methods

- Determination of dry matter, % - BDS EN 12143-00;
- Determination of active acidity (pH) - BDS 11688-93;
- Total titrable acidity, % - BSS 6996-93;
- Active acidity (pH) - BDS 11688;
- Total sugars, % - BDS 7169-89;
- Ascorbic acid, mg% - BDS 11812-91;
- Pectin, % - BDS 16491-86;
- Tanning substances, % - Levental-Nibbaur method by (Bukharina et al., 2015).

Chemical compounds

For the analytical purposes, the following reagents were used: DPPH (2,2-diphenyl-1-picrylhydrazide) and Trolox [(±) -6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); Folin-Ciocalteau reagent (FC-reagent) (Merck, Darmstadt, Germany); gallic acid monohydrate (Fluka, Buchs, Switzerland). All other reagents and solvents analytical purity.

Preparation of samples for chemical analysis

5 g of each fruit are placed in a 50 mL volumetric flask. The contents of the flask were adjusted to ~ 2/3 of the volume with acidified methanol. After standing overnight under refrigeration conditions (10°C), the contents of the flask were added to the mark. The resulting methanol extracts were filtered through a crimped filter and analyzed.

All measurements were performed with a Helios Omega UV-Vis Spectrophotometer with VISIONlite software installed (Thermo Fisher Scientific, Madison, WI, USA) using 1 cm optical paths.

Total polyphenols (TPP)

The content of total polyphenols was determined by the Singleton and Rossi method in the following modification: In a 10 ml measuring tube, 0.1 ml of extract (base solution or fraction), ~ 7 ml of distilled water, 0.5 ml of FC reagent (diluted 1: 4 with distilled water) and 1.5 ml of a 7.5% (w / v) aqueous solution of sodium carbonate. After shaking, the tubes are poured to the mark with distilled water. After standing at rest for 2 hours at room temperature, absorbance of the reaction mixture was measured at 750 nm. An analogous blank was prepared using distilled water instead of extract. The results obtained are presented as gallic acid equivalents (GAE).

Antioxidant capacity

Radical scoring ability is determined by the method of Brand-Williams et al 2005. in the following modification: In a cuvette, 2250 μL of DPPH solution (2.4 mg DPPH in 100 mL of methanol) and 250 μL of extract (base extract or fraction) previously diluted with distilled water in a 1:3 volume ratio was dispensed sequentially prepared blank sample using methanol instead of extract. After the closed cuvettes were kept for 15 minutes in the dark at room temperature, the absorption of the reaction mixture at 515 nm was measured. The results obtained are presented as Trolox equivalents (TE).

Color measurements

Color measurements were performed instrumentally with COLORGARD SYSTEM 2000 colorimetry of BYK-GARDNER JNC., USA. The indicators are accounted for by the CIELab system. Three color coordinates are taken: L - luminosity (L = 0 - black, L = 100 - white), + a - red color, -a - green color, + b - yellow color Five measurements were performed on each sample. The color coordinates of each sample represent the arithmetic average of the measured coordinates.

Mathematical and statistical processing

The results presented are arithmetic mean values of at least three parallel definitions, with coefficients of variation less than 5%. Statistical data processing was performed with ANOVA, Microsoft Excel programs.

RESULTS AND DISCUSSION

Table 1 presents the data from the conducted studies of two genotypes (Siberian and Caucasian), grown and supplied by IPJZ - Troyan - differing in terms.

The data from Table 1 presents the studies carried out on the two genotypes sea buckthorn (Siberian and Caucasian) grown and provided by RIMSA - Troyan - differing in terms of ripening and form of the fruits.

Table. 1. Physico-chemical indicators of two genotypes of sea buckthorn (*Hippophae rhamnoides*. L.)

Genotype	Dry matter %	pH	L	a	b	mg GAE/ 100 g	µmol TE/ 100 g
Caucasian	9.25	3.24	55.94	31.46	65.59	55.00	579.17
Siberian	13.25	3.07	55.28	22.88	56.18	185.00	525.00

Data show that the measured dry matter has higher values for Siberian pepper - 13.25% as reported by (Lougas et al., 2006; Dhyani et al., 2007). Data are statistically distinct due to genotypic difference ($p<0.05$).

The total polyphenols defined in fruit Siberian sea buckthorn are 3 times more than the total content in the fruits of the Caucasian sea buckthorn.

Antioxidant activity was comparatively with similar values for both genotype due to the content of total polyphenols in fruits. The data were statistically distinguishable due to genotypes difference ($p<0.05$). A positive linear relationship with a high coefficient of determination $R^2 = 0.930$ between the total polyphenols and antioxidant activity in both genotypes of sea buckthorn. The data are presented in Figure 1.

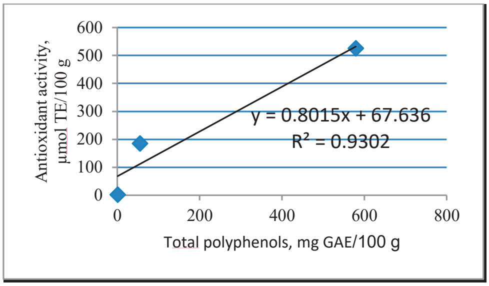

Figure 1. Linear relationship between the content of total polyphenols and antioxidant activity in the studied genotypes sea buckthorn (*Hippophae rhamnoides* L.)

The data from the conducted biochemical analyzes show that the fruits of the Caucasian sea buckthorn have higher values indicators of total sugars, sucrose and pectic substances. The fruits from Siberian genotypes have a higher percentage of invert sugar, total acidity, and tanning substances (Figure 2). The results were statistically distinguishable due to the difference of the genotypes selected raw materials ($p<0.05$).

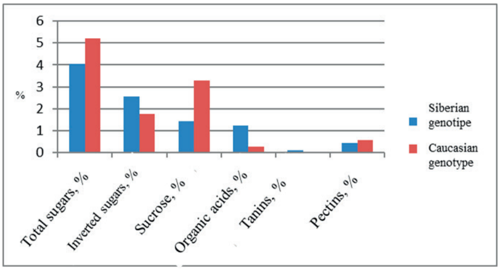

Figure .2. Biochemical indicators of genotypes of *Hippophae rhamnoides*. L.

The measured color characteristics in both genotypes have shown that indicator brightness of the color values is not close statistically distinguishable differences ($p> 0.05$). The predominant color tone is yellow for both genotypes, with the Caucasian genotype having higher values for this indicator and the measured red color component compared to the Siberian one.

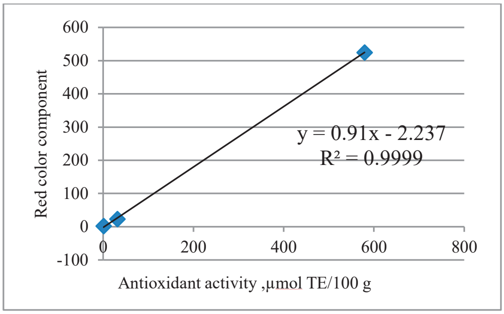

Figure 3. Linear relationship between antioxidant activity and a red component color genotypes *Hippophae rhamnoides* L.

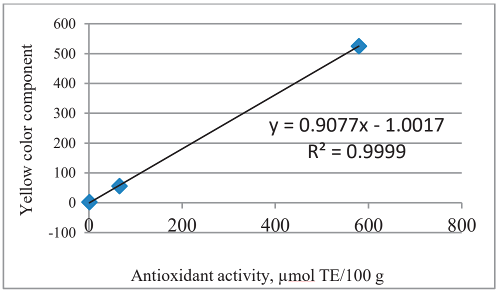

Figure 4. Linear relationship between antioxidant activity and a yellow component color genotypes sea buckthorn

Statistical processing showed that the values for both indicators were statistically distinguishable due to genotypes difference ($p < 0.05$). For both genotypes establish a positive linear relationship with a high coefficient of determination $R^2 = 0.99$ between antioxidant activity and pigments which impart yellow and red color in the fruit. The data are presented in Figures 3 and 4.

CONCLUSIONS

The biochemical composition ~~of~~ two genotypes sea buckthorn has been studied.

It was were reported higher values for dry matter and polyphenols in the Siberian genotypes.

The Caucasian fruits are distinguished by a higher percentage of invert sugar, total acidity and tanning substances.

Due to high nutritional value and its growing demand, it could be suggested that sea buckthorn berries might be explored for uses in different food commodities such as ready-to-serve beverage, squash, syrup, jam and jellies, etc. It is growing the variety of assortment list with a non-traditional raw material not well known to Bulgaria.

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LOCAL PLANT RESOURCES OF GENUS *MALUS* IN THE REGION OF TROYAN

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Abstract

A survey was carried out in the region of Troyan and Apriltsi and its adjacent villages and neighborhoods from the Central Balkan Mountain region of Bulgaria. Because of its specific climate and intersected relief, it has favorable conditions for growing of orchards. The development of fruit-growing and the traditions of lovers of fruit trees in the region have led to the creation of a wide variety of local cultivars of fruit crops and especially apple cultivars and forms. A wide range of members of genus Malus have been selected for the investigations. Most of the selected forms belong to the group of autumn and winter varieties according to their ripening period. Some of them are distributed across all parts of the surveyed areas, while others are found in single sites.
The most common are: 'Cheshka momina', 'Jonathan', 'Troyanka', 'English Green Renet', 'Renet du Canada', 'Limonka', 'Manastirka', 'Shekerka', 'Perusha', 'Yellow Bellflower', 'Ayvaniya', 'Tsiganka'.
The following cultivars are also marked: 'Stefanka', 'Bozhechka', 'Amerikanka', 'Medena abalka', 'Ostreshka reneta CM', 'Marishnitsa 8/10', 'Winter green', 'Yovovka', 'Kojesta reneta' presented as single trees in separate habitats.
The fruit of the group of Petrovka apple had the earliest ripening period - they start to ripen from the first half of July and have a weight of 28.8 g to 80.6 g.
Fruits of 'Cheshka momina', 'Jonathan', 'Troyanka', 'Ayvaniya', 'Limonka' are long lasting and are preserved and kept in ordinary cellars until the end of March and early April.
Valuable forms are selected and marked for reproduction and preservation.

Key words: *plant genetic resources, apples, varieties.*

INTRODUCTION

Apple is one of the most valuable fruit crops. Despite of the specific climate and soil requirements, it has a large range of distribution. From all the fruit species of temperate climate, the most economic importance is the apple. World apple production exceeds the production of any of the other fruit species by several times. It is highly fertile compared to other perennial crops and produces high yields. Its fruits have a long shelf life and good transportability. They are rich in pectin and important biochemical ingredients, which determines their importance and high demand. Apple is among the most traded fruits on the world market (Dzhuvinov, 2016; Radomirska, 2007; Wagner et al., 2014).

Unformer fruit growers have played an important role in enriching the diversity of varieties and forms of the genus Malus in Europe, Asia and America. (Bostan, 2009; Bozovic et al., 2013; Chavlesh et al., 2019; Giovannoni, 2010; Dzhangaliev, 2003; Gradinariu et al., 2003; Ercisli, 2004; Forsline et al., 2003). Apple fruits are a valuable diet food. They are rich in pectin and vitamins (Denkov, 1998; Denkova, 1998; Dzhuvinov, 2016).

The requirements of the world market production of fruit grown without using pesticides. In practice this can be achieved by reclaiming old varieties that have shown increased resistance to disease. One reason for the growing of local varieties and forms apples is their good adaptability to habitats, and their resistance to diseases and pests (Bozhkova et al., 2006; Iliev, 1985; Stoyanova et al., 2014).

The aim of this study to select and study valuable apple varieties and forms from the local plant resources of the Malus genus in the Troyan region, possessing good economic qualities and low susceptibility to diseases suitable for storage and inclusion in organic production.

MATERIALS AND METHODS

Expeditionary studies of local plant resources of the genus Malus in the Troyan region have been carried out. Growing conditions are not irrigated. The altitude is from 380 to 600 m. The soils are gray forested. Selected genotypes are with high valuable agricultural qualities. The region has a cool and humid climate, due to the location. Studied varieties are found in habitats near rivers and riverbeds, where they found conditions for good growth and fruiting.
Forms with valuable economic qualities were selected. Trees are marked against a natural background of contagion with manifested tolerance and low sensitivity to economically important diseases of the apple. The biological and morphological features of the fruits have been established. The peculiarities of the fruits are taken into account: their size, mass, shape, taste and color. The studies were performed according to the Methodology for the Study of Plant Resources in Fruit Plants (Nedev et al., 1979).

RESULTS AND DISCUSSIONS

A study of the Malus gene pool in the study areas revealed a wide variety of shapes and the proven old varieties of apples. They cover a very long harvest period - from July to October. The established apple trees are in preserved orchards of cooperative land use and single trees, some of which are 80-100 years old in private estates and yards.
Apple is a water-loving crop and grows best in mountainous areas, at the foothills of mountains, in valleys of non-drying rivers, and in places with northern exposure.
During the expeditionary studies and the laboratory tests, are selected representatives from the group of Shekerki and 14 local genotypes were discovered and studied. They cover a 30-40 day harvest period. In early ripening genotypes, the fruits reach the harvest ripening of the second half of September, and in the late ripening genotypes, known as winter Shekerka, the second half of October. Yields are significant, reaching 200-280 kg per tree in some years. The most important characteristic of this group of apples are the sweet fruits with low acid content.
Most fruits are yellowish in color with intense red blush and darker streaks with a relatively small size. The weight of the studied genotpes varied from 27.4 g to form 1 / 25.09. to 124.6 g in the form of Petran / 24.09., in most forms the weight of the fruit is 40-60 g. The most promising are the forms of Dyadovata Hristova winter Shekerka and Petran / 24.09.
The advantages of the Shekerka genotypes are their fertility, relatively low sensitivity to diseases and pests and good preservation of their fruits (Table 1).

Table 1. Measurement of samples of apple fruits of the type Sekerki collected in the Troyan region

Genotype	Weight, g	Length of the fruit stem, mm	Height, mm	Average diameter, mm
1/25.09.	27.4	8.3	34.1	38.0
Petran/24.09.	124.6	11.7	59.6	65.6
4/24.09.	40.9	14.2	40.0	47.2
6/24.09.	85.3	13.3	49.6	59.0
8/25.09.	54.7	9.2	46.1	49.8
9/25.09.	51.2	5.9	43.1	50.0
11/24.09.	43.6	14.5	40.8	45.2
14/24.09.	44.8	19.8	44.6	46.3
14/25.09.	53.2	9.2	43.3	51.6
15/25.09.	63.9	10.2	48.4	54.3
1/10.10.	56.8	7.8	45.5	50.8
16/10.10.	40.6	8.2	42.5	44.8
18/10.10.	62.4	8.0	46.3	53.2
19/10.10. Dyadovata Hristova	71.0	10.8	49.1	56.7
Average	58.60	10.79	45.21	50.89
STDEV	23.85	3.64	5.81	6.83

In the surveyed areas of the group of Rene registered following varieties: Skinny Reinnette, Canadian Reinnette, Banana Reinnette, Dutch Reinnette, Blenhaymska Reinnette de Blenheim, Cox Orange Reinnette, English green Reinnette.
The main color of the fruit skin is green in shades, while in the Skinny and Canadian Reinnette it is brownish. Their fruits begin to ripen from the end of September, and at the latest ripen the fruits of the English Green Reinnette - the end of October. Fruit in all of the tested Reinnette is sweet and sour. Their fruit weight ranges from 58 g in Cox Orange Reinnette to 172 g in English Green Reinnette (Table 2).
They usually begin to ripen in the second half of July. At full maturity it is greenish-yellowish, covered with fuzzy redness in some forms, with well-visible red streaks with blush.

Table 2. Fruit sizes

Genotype	Weight, g	Length of the fruit stem, mm	Height, mm	Average diameter, mm
Skinny Reinnette	95.7	11.0	44.6	51.0
Canadian Reinnette	145.6	8.0	58.8	73.4
Banana Reinnette	100.0	22.4	48.4	63.4
Dutch Reinnette	90.0	4.2	53.3	62.2
Reinnette de Blenheim	117.8	15.5	57.5	63.6
Cox Orange Reinnette	58.0	17.2	42.3	53.4
English green Reinnette	172.7	8.0	61.1	79.6

CONCLUSIONS

This allowed to select the varieties of Czech Momina, Jonathan, Troyanka, English Green Reinnette, Canadian Reinnette, Limonka, Manastirka, Shekerka, Perusha, Yellow Bellflower, Ayvania, Tsiganka, which are widespread in many areas of the area.
Within as single trees in separate locations and as single trees, were found the local varieties - Stefanka, Bozhechka, American Honey apple, Ostreshka Reinnette, Marishnitsa 8/10, Winter green, Jovovka, Skinny Reinnette. The larger-fruited of these, are Perusha with a fruit weight of 124.6 g, Canadian Reinnette - 145.6 g and English green Reinnette - 172.7 g. Their fruits ripen from the second ten days in September to the second ten days in October, which defines them as winter varieties.

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DETERMINATION OF THE SENSORY QUALITY OF APRICOTS DEPENDING ON THE BIOLOGICAL TREATMENTS IN ORCHARD

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Abstract

Biological control is a method of controlling pests such as insects, mites and plant diseases using products that don’t harm human health. Biological control may be an option in preventing crop disease and fungicide resistance. This work present influence of biological treatments on sensory quality of the apricots of Orizont variety. In orchard it was fertilized with the biological fertilizer Cropmax which was applied together with some biological fungicides, insecticides and/or acaricides in four variants: v1= Cropmax + Konflic + Funres; v2= Cropmax + Oleorgan + Canelys; v3= Cropmax + Canelys + Mimoten. After harvest, the fruits were stored in different storage conditions: ambient temperature (20-22°C), for 7 days, temperature of 10-12°C (refrigerant conditions, for 15 days) and temperature of 3-5°C, with and without modified atmosphere (cold storage), for 30 days. At harvest and at the end of the storage the sensorial quality of the fruits (appearance, firmness and taste) was determinated. The V1 variant highlighted because its fruits presented the greatest values of these parameters at the moment of the harvest and it have maintained their high values during storage in all technological preservation variants.

Key words: *biological fertilizers, pesticides, storage conditions*

INTRODUCTION

Fresh apricots *(Prunus armeniaca* L.) are in high demand, but are available for only a short period during the Summer.

Fragrant and sweet, with a low caloric intake of just 47 kcal per 100 grams, apricots are some of the most loved fruits. The apricots are above all a true pro-vitamin A battery, proven by the cheerful and beautiful orange colour. Four large apricots are sufficient to ensure the daily requirement of vitamin A, a valuable and vital antioxidant to the body. The apricots are rich in C vitamin and B vitamins complex but also in minerals and oligo-elements (600 mg/100 g). They are a generous source of potassium (315 mg/100 g), which makes apricots the best ally of athletes. Potassium is the mineral that has the property to stimulate the metabolic and natural elimination of toxins. The presence of potassium gives fruit colour and retains the aroma (Ion, 2010). Also the apricots are an excellent source of fiber. The apricots act gently in the intestine, stimulate the transit, and the mild acidity make them easily digestible, being very well assimilated by the body, which is why they are considered ideal food for babies and for those who have problems in the digestive tract. Consumed frequently the apricots fight asthenia and depressive states, stop memory loss due to high phosphorus content, treat insomnia and fight against infections in the body.

The organic crops contribute to the presser-vation of precious natural resources, species diversity and soil quality and, out of respect for Planet, we have a duty to extend their surfaces.

Even for those who believed that "organic nutrition" is a whim of the modernity, it is becoming increasingly clear that we must pay special attention to where our food comes from and how it is produced. Plant protection without chemicals is of interest to most fruit growers (but not only - Singh, 2017, 2019) due to the increasing demand for the organic products. The fruits produced without additives and chemicals are required in both fresh and, in particular, canned form.

The sensory evaluation of several varieties of apricots led Valentini et al. (2006) at the conclusion that the overall quality is positively related with flavor, sweetness and juiciness. In general, the most appreciated apricots had a well balanced ratio sugars/acids, although the different proportion between the main acids can influence the fruit acceptance.
In a study on the sensory quality of apricots and peaches Bassi and Selli (1990) also suggests that sugar and acid patterns of fruit flesh may be useful in cultivar characterization.
Not only the biological treatments in orchard influence the sensory quality of apricot after harvest, but also harvest maturity stage. There is more information on the correct harvest maturity stage that should be chosen to ensure a long post-harvest life and high sensory quality (Infante et al., 2008; Chira et al., 2018).
In order to obtain highly qualitative fruits as far as the organoleptic aspect is concerned, their temporarily storage must be under certain conditions, defined for each species of fruit separately.
The temporary storage of the fruit depends in particular on temperature. The damage caused by very high or low temperatures results in tissue decomposition (Alexe et al., 2014, Chira et al., 2016).
Cold storage makes it possible to market perishable fruits and vegetables outside their harvest season. The main purpose of storage is to control the rate of sweating, respiration, disease and insect infestation and to keep the vegetable products in optimum condition for the consumer (Spadoni et al., 2015).
In most cases, the maintaining of a defined temperature and humidity regime is not enough to prolong the period of fruit storage and reduce fruit losses. A defined gas composition of the atmosphere, strictly differentiated for each type of fruit and for different varieties of the same species, is also required (Rao, 2015).
Our previous research has focused on the influence of foliar biological treatments on the preserving capacity after harvesting of the apricots (Moale, 2019), evaluating the quantitative and qualitative losses of the fruits during the storage under different technological conditions.

The purpose of this paper is to determine the effect of the biological treatments applied to apricot trees of Orizont variety on the sensory qualities of apricots at the moment of the harvest and their evolution post-harvest.
Also, the influence of the technological factors from the storage areas (temperature, humidity, gaseous composition of air) on the maintenance of these qualities was evaluated.

MATERIALS AND METHODS

The apricots of Romanian variety Orizont (Figure 1) were harvested from the experimental culture of Research Station for Fruit Growing Constanta. The researches were conducted in years 2017 and 2018 at the Research and Development Institute for Processing and Marketing of the Horticultural Products "Horting" Bucharest.

Figure 1. Apricots of the Orizont cultivar

In orchard the experimentation consisted of the foliar application, on the apricot trees, of the fertilizer Cropmax together with some biological fungicides, insecticides and/or acaricides, in three variants, as follows:
- V1 - Cropmax 0.15% + Konflic 0.3% + Funres 0.3% (Figure 2);
- V2 - Cropmax 0.15% + Oleorgan 0.3% + Canelys 0.3% (Figure 3);
- V3 - Cropmax 0.15% + Canelys 0.3% + Mimoten 0.3% (Figure 4);
- V4 - control - untreated

For this purpose the STIHL 400 SR atomizer was used.

Figure 2. Biological products for V1 variant

Figure 3. Biological products for V2 variant

Figure 4. Biological products for V3 variant

The products which were used are described below:
- Cropmax - super-concentrated, foliar fertilizer (100% natural);
- Konflic - organic insecticide; it is a natural product used against the pest population (white fly, trips, aphids);
- Oleorgan - insecticide; it is a natural product which contains vegetable oils, used to combat and reduce the population of pests (white fly, trips, aphids) from horticultural crops;
- Funres - fungicide; it is a natural herbaceous extract intended to combat diseases of the horticultural crops, such as *Botrytis* sp., *Sclerotinia* sp., *Peronospora* sp., *Phytophthora* sp.;
- Mimoten - fungicide, with a preventive and curative effect on most fungi and bacteria that attack the crops and fruit trees, by inhibiting their growth and development (*Botrytis* sp., *Septoria* sp., *Sphaeroteca* sp.);
- Canelys - acaricide, a natural product successfully used to control the population of mite spiders *(Tetranichus* sp.) and of some pathogen fungi, as *Oidium* sp.

After harvesting, as soon as it was possible, the fruits were transported to the Research and Development Institute for Processing and Marketing of the Horticultural Products Bucharest. There the apricots were examined organoleptic, in order to estimate the appearance (size, shape, colour), the taste (sweetness, flavour) and the firmness (texture) of the fruits.

To find out what are the factors on which the maintaining of the sensory quality of apricots depends, we have placed the fruits in storage under different technological conditions, as follows:
- ambient temperature - warm (T = 24-27°C, RH = 69-71%), for 7 days;
- fridge storage (T = 10-12°C, RH = 75-78%); for 15 days;
- cold storage (T = 3-5°C, RH = 82-86%), for 30 days;
- cold + modified atmosphere (MA) storage (T = 3-5°C, RH = 92-96%) - in hermetic packages, so that the composition of the atmosphere inside was modified, by the reducing of the O_2 amount and the increasing the CO_2 amount - storage in modified atmosphere - MA, for 30 and 45 days;

After storage, the fruits were re-examined to determinate the effect of culture technology on quality preserving capacity after harvesting of the apricots.

The evaluation of the organoleptic quality was achieved by carrying out the fruits sensory testing, using a grading scale from 1 to 100. Tasting sheets were used which included a number of three criteria of appreciation (aspect, firmness, taste), with different share in the general notation, depending on their importance: the aspect represents 15%, firmness 35%, and taste 50%. Depending on the score obtained, five quality classes are distinguished, as follows: very good (80-100 points), good (60-79 points), satisfactory (40-59 points), sufficient (20-39 points) and insufficient (0-19 points).

RESULTS AND DISCUSSIONS

The results shown in Table 1 indicate that, in the moment of the harvest, the apricots got high marks because of their attractive appearance, firmness and taste.

The apricots of V1 variant (Cropmax 0.15% + Konflic 0.3% + Funres 0.3%) got the highest score (91.20 point) and the apricots of V4 variant (control - untreated), the lowest (81.20 points).

The tasters accorded the same rating for all four variants of biological treatments, "very good" qualifying.

Table 1. The organoleptic appreciation of apricots at harvest (points)

Criteria for assessment	Variant			
	V1	V2	V3	V4
Aspect	13.20	12,60	13.20	11.40
Firmness	31.00	30,80	32.20	28.00
Taste	47.00	38,00	38.00	41.60
Total score	91.20	81,40	83.40	81.20
Qualifying	very good	very good	very good	very good

After 7 days of storage in an ambient temperature, the organoleptic quality of the apricots deteriorated drastically, the score dropped due to the diminishing of the aspect, firmness and taste, so that the final grade was “good” for V1-V3 variant, at inferior limit of the class for V1 and V2 variant (Table 2).

Table 2. The organoleptic appreciation of apricots after storage in ambient temperature -T = 24-27°C for 7 days (points)

Criteria for assessment	Variant			
	V1	V2	V3	V4
Aspect	10.80	10.20	10.80	6.00
Firmness	23.40	23.80	25.20	20.80
Taste	40.00	30.00	30.00	27.00
Total score	74.20	64.00	66.00	53.80
Qualifying	good	good	good	satisfactory

In the V4 variant one of the repetitions presented a strong attack of diseases (Figure 5), fact for which some tasters gave very few points, and the rating obtained by this variant was “acceptable”.
On the first place, with 74.20 points, it is ranked V1 variant and on the last, with 53.80 points, it is located V4 variant.

Figure 5. Warm storage of apricot - infested fruits

After 15 days of storage in temperature of 10-12°C (Figure 6) the parameters of the organoleptic properties of the apricots in V1 variant remained fairly reasonable, the score being of 84.50 points and the grade “very good” (Table 3).

Table 3. The organoleptic appreciation of apricots after storage in fridge storage - T = 10-12°C for 15 days (points)

Criteria for assessment	Variant			
	V1	V2	V3	V4
Aspect	12.00	12.20	12.60	11.60
Firmness	30.50	26.60	30.50	27.20
Taste	42.00	36.00	34.00	32.40
Total score	84.50	74.80	77.10	71.20
Qualifying	very good	good	good	good

At the apricots from the other three variants, the organoleptic quality was degraded, but not very much, which is why they received the “good” rating.

Figure 6. Fridge storage of apricot

During storage at a temperature of 3-5°C for 30 days (Figure 7) the metabolic processes of the fruits slowed down, so that the sensory quality of the fruits was better preserved (Table 4).

Table 4. The organoleptic appreciation of apricots after storage in cold storage - T = 3-5°C for 30 days (points)

Criteria for assessment	Variant			
	V1	V2	V3	V4
Aspect	13.80	13.20	9.60	9.60
Firmness	28.00	22.60	26.60	26.60
Taste	42.00	34.00	34.00	30.00
Total score	83.80	69.80	70.20	66.20
Qualifying	very good	good	good	good

Figure 7. Cold storage of apricot

Variant V1 maintained its 1st place in the ranking (83.80 points), and the other variants kept their positions from previous experiments.

During MA storage for 30 days, the apricots, though are maintaining their pleasant aspect and their firmness, the quality of the taste has decreased a lot so that the tasters degraded V2 variant and V4 variant in the quality class “good” (Table 5).

Table 5. The organoleptic appreciation of apricots after storage in cold + modified atmosphere storage - T = 3-5°C for 30 days (points)

Criteria for assessment	Variant			
	V1	V2	V3	V4
Aspect	13.00	12.60	13.20	11.00
Firmness	31.00	30.60	31.20	26.80
Taste	42.20	34.80	36.00	39.20
Total score	86.20	78.00	80.40	77.00
Qualifying	very good	good	very good	good

The V1 variant, with a score of 86.20 points received, together V3 variant (80.40 points), grading “very good”.

CONCLUSIONS

The organoleptic quality of the apricots at harvest and its evolution post-harvest varies according to type of applied culture technology.

The use of the different biological treatment schemes applied to apricots trees against pathogens is reflected in the level of organoleptic parameters of the fruit at harvest, but also in their evolution during storage.

The greatest values of these parameters at harvest is recorded by the fruits obtained from the trees that were treated with Cropmax 0.15% + Konflic 0.3% + Funres 0.3% (V1 variant), followed by V3 variant: Cropmax 0.15% + Canelys 0.3% + Mimoten 0.3%.

During storage the V1 variant was also particularly highlighted because its fruits have maintained their high values of the sensory quality in all technological preservation variants.

The cold + modified atmosphere (MA) storage recorded the best results regarding the slowing down of the rhythm of the metabolic processes and inhibition or slowing down the fungi and molds growing rate, which has been reflected in the maintaining of the sensory quality for a longer period of time.

Because low temperatures inhibit or slow down the fungi and molds growing rate - specific to each species of vegetables and slow down the rate of biochemical processes during their storage. Therefore, the losses through depreciation are greatly reduced.

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SIRMA AND PAGANE - NEW BULGARIAN PLUM CULTIVARS

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Abstract

Plum is a traditional fruit crop grown in Bulgaria. Fruit Growing Institute has launched its plum breeding program in 1987 in order to improve the range of available plum cultivars for this region. In 2019 were registered two new plum cultivars - 'Sirma' and 'Pagane'. In this study are presented their main tree and fruit characteristics observed from 2014 to 2018. The standard cultivar 'Stanley' was used for comparison. The flower development of all three cultivars starts in the third decade of March. 'Sirma' had a moderately early flowering period which starts 2-3 days before 'Stanley'. 'Sirma' outperforms the standard cultivar in yield – 170.30 kg per tree (from 7 years old, not pruned trees) and average fruit weight of 44.70 g. Compared to 'Stanley', 'Pagane' had lower yield (78 kg/per tree) but much larger fruits with an average weight of 63.68 g. After sensory evaluation, both cultivars had higher marks than 'Stanley' and their taste qualities were rated as very good. 'Sirma' cv. had a smaller tree size. Between 'Stanley' and 'Pagane' was not observed significant difference for their tree dimensions. The new cultivars are tolerant to Sharka like 'Stanley'.

***Key words**: breeding, fruit, new cultivars, plum.*

INTRODUCTION

Plum is a traditional fruit crop for Bulgaria. Due to its high productivity and good adaptability to the agro-climatic conditions it is widespread in the country (Vitanova et al., 2014). According to Agrostatistics (2018), 18.5% of the harvested areas are occupied by plums. This puts it on second place after the cherry with harvested area of 4876 ha and fourth in fruit production - 24 640 t. Sharka disease caused by Plum Pox Virus (PPV) is a major limiting factor for the plum production (Milusheva et al., 2013). The most secure way for limiting the disease spread is growing tolerant or resistant cultivars. In the past, in our country the most commonly grown plum cultivar was the highly susceptible 'Kyustendilska sinya sliva'.

This led to endemic disease spread through infected planting material in the 70's. Later this highly susceptible cultivar was replaced by the tolerant 'Stanley' (Bozhkova et al., 2004; Dragoyski et al., 2009).

This cultivar combines tolerance to PPV with high fertility and attractive, suitable for canning fruits (Vitanova et al., 2014). *Prunus domestica* is a hexaploid and breeding new cultivars and clarifying the mechanisms of inheritance of resistance is extremely difficult (Neumüller et al., 2007). That is why 'Stanley' is officially registered as a plum cultivar back in 1926, and in the 80's of the 20th century it became the main grown cultivar for our country, occupying about 80% of the cultivated area (Dzhuvinov et al., 2012). Afterwards in the past years the resistant to sharka disease 'Jojo' cultivar has been widely planted in production orchards. Unfortunaly, this cultivar manifests itself to be sensitive to the late spring frosts occurring in the country.

For the new plum orchards in Bulgaria are needed cultivars resistant or tolerant to Plum Pox Virus wich are providing high yield and good fruit quality early. In general these are the main objectives of the plum breeding programs developed in Bulgaria.

The first plum breeding program of the Fruit Growing Institute - Plovdiv, started in 1987. F1 hybrid progeny consisted of populations obtained by controlled hybridization and by open pollination of the tolerant cultivars 'Stanley', 'President', 'Green gage', 'Scoldush', etc. (Zhivondov, 1994; Zhivondov and Djouvinov, 2001). The first successful final results of the breeding program were the new plum cultivars 'Plovdivska renkloda' (Zhivondov, 2008), 'Sineva' and 'Ulpiya' (Zhivondov and Bozhkova, 2008), 'Ostromila' (Zhivondov and Milusheva, 2016).

As a result of the ongoing plum breeding program of the Fruit Growing Institute, the two new cultivars 'Sirma' and 'Pagane' were registered in 2019. The aim of the present study was to describe the major pomological and fruit quality characteristics of two new Bulgarian plum cultivars and to compare them with the standard 'Stanley' cv.

MATERIALS AND METHODS

The current study was conducted in the period 2014-2018 in a collection orchard at the Fruit Growing Institute of Plovdiv, Bulgaria planted in 2011. The orchard was grown on humus-carbonate soil, maintained as black fallow, under non-irrigation conditions, at a planting distance of 5 x 4 m, applying conventional plant protection practices. The flower development was traced according the stone fruits BBCH (2001) scale. Yield per tree was measured in 2018. For determining the fruit quality biometric analyses and sensory evaluation were performed. An average sample of fruits was taken and biometric data was measured with Mitutoyo 500-196-30 Digimatic Absolute Caliper 150 mm. Total soluble solid content (Brix°) in juice using a handheld Sper Scientific 300019 Digital Refractometer was determined. For the sensory evaluation a total number of 8 characteristics determining the fruits appearance and taste qualities were scored using the following scale - 1-3 very bad to bad; 3-4-satisfactory; 5-6 better and 7-9 - excellent. Each characteristic was multiplied by a coefficient depending on its importance: 0.175 for appearance, 0.050 for flavour attractiveness and 0.225 for flavour intensity, 0.200 for sweetness, 0.125 for sour taste, 0.125 for bitterness, 0.200 for flavour balance, and 0.150 for fruit flesh texture and juiciness. The final result was obtained based on the average grade of the total evaluation grades by each consumer. According to the final grade, the fruits were classified as fruits with bad (1-3), medium (3-5), good (5-7), very good (7-8) and excellent (above 8) qualities. After drying the fruits the sensory evaluation was repeated. Trunk diameters, tree height, canopy width of both sides (east-west and north-south) were also measured and canopy volume was calculated using the formula $V=(3.14*d^2*h)/12$. For statistical data processing Duncan's multiple range test (Steele and Torrie, 1980) of the IBM SPSS Statistics 19 statistical software was used.

RESULTS AND DISCUSSIONS

'Sirma' cv. was obtained from open pollination of 'Stanley' cv. The stones were stratified in moist sand and grown in a nursery and a breeding orchard where from the population Elite № 3-12 was selected. 'Pagane' was obtained after open pollination of the 'Altan's gage' cultivar. The collected stones were irradiated with 1000 Re. The stones were stratified in moist sand and plants were grown in a nursery and breeding orchard, where from the resulting population Elite № 1-53 was selected. The development of principal growth stages inflorescence emergence and flowering was traced in 2014-2016 period (Table 1). The earliest flowering cultivar is 'Sirma'. The flowering of this cultivar was moderately early - 2-3 days before 'Stanley'. This difference is negligible when compared to the standard cultivar. All stages of the flower development of 'Pagane' run a day after 'Sirma' and 0 to 3 days before 'Stanley'. The duration of full flowering phase typically varies over the years (Dumitru et al., 2009). Its longer duration is a prerequisite for better pollination (Tsonev, 1991). The average duration of the whole flowering phenophase of 'Sirma' and 'Pagane' was 10.3 and 10.0 days resp. which was a day longer compared to 'Stanley'. Manipulation of tree architecture is the cornerstone of horticultural management and has continued to evolve as the development of intensive planting systems (Tustin, 2014). The reduced compact tree habit allows creating intensive orchards.

The tree growth of 'Pagane' is vigorous. Out of the studied cultivars, it had the smallest measured trunk diameter. 'Pagane' trees were the shortest and the difference between its canopy height and the one measured for 'Stanley' was statistically significant (Table 2). Due to its dense and widely spread canopy this cultivar had higher value of the calculated canopy volume than 'Sirma'. Compared to 'Stanley', 'Pagane' had smaller canopy volume but the difference was statistically non-significant.

Table 1. Development of principal growth stages inflorescence emergence and flowering

Cultivar	Year	BBCH code						Flowering period (days)
		55	57	61	65	67	69	
		Stage						
		Single flower buds visible	Single flowers with white petals	Beginning of flowering	Full flowering	Flowers fading	End of flowering	
Pagane	2014	20.03	21.03	24.03	26.03	29.03	2.04	10
	2015	05.04	9.04	11.04	13.04	17.04	20.04	10
	2016	26.03	26.03	28.03	29.03	6.04	7.04	10
	average	**29.03**	**29.03**	**29.03**	**2.04**	**7.04**	**10.04**	**10**
Sirma	2014	20.03	21.03	24.03	26.03	28.03	2.04	10
	2015	2.04	6.04	8.04	10.04	14.04	17.04	10
	2016	24.03	26.03	28.03	29.03	7.04	8.04	11
	average	**28.03**	**28.03**	**30.03**	**1.04**	**6.04**	**9.04**	**10.3**
Stanley	2014	20.03	24.03	26.03	28.03	31.03	3.04	9
	2015	7.04	9.04	10.04	11.04	14.04	17.04	8
	2016	28.03	30.03	1.04	2.04	9.04	10.04	10
	average	**29.03**	**31.03**	**2.04**	**3.04**	**5.04**	**10.04**	**9**

Table 2. Tree dimensions

Cultivar	Trunk diameter	Tree height	Canopy hight	Canopy width 1	Canopy width 2	Canopy volume
Sirma	36.38 a	3.96 a	3.45 ab	2.56 b	2.39 a	5.52 b
Pagane	33.75 a	3.86 a	3.26 b	3.31 a	2.81 a	8.01 a
Stanley	37.33 a	4.35 a	4.00 a	2.77 b	2.87 a	8.32 a

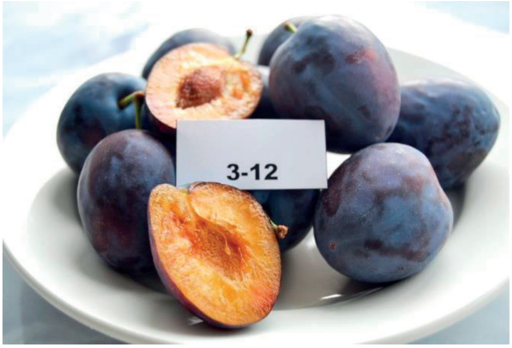
The tree growth of ‘Sirma’ is also vigorous. This cultivar’s tree is tall but with compact, moderately dense and spherical crown. ‘Sirma’ had the smallest calculated canopy volume and the statistical difference with the other studied cultivars was significant. ‘Pagane’ has fruits with very attractive appearance and good quality. ‘Pagane’s fruits were categorized as large, with an average fruit weight of 63.68 g. (Table 3). The bigger fruits have bigger stones, but the stone relative share compared to the whole fruit was very good. It’s fruits also have and obovate, asymmetrical shape and deeply sunken suture, they are violet-blue in color with strong wax bloom (Picture 1). The fruits ripen at the end of August - 20.08 on average. The fruiting is regular and abundant. In 2018 were obtained 78 kg fruits from 7-years-old trees grown without pruning (Figure 1).

‘Sirma’ also has larger fruits than the standard cultivar, with an average fruit weight 44.70 g. The stone is medium sized and the stone relative share was also better compared to the standard. ‘Sirma’s fruits are oval in shape, symmetrical with dark violet-blue color, with strong bloom (Picture 2). Out of the three studied cultivars ‘Sirma’ had the highest yield in 2018 (Figure 1). Its yield was 52.7 kg more than the amount of fruits picked from ‘Stanley’.

Table 3. Fruit biometry analyses

Cultivar	Rippening time	Fruit				Stone weight (g)	Relative share (%)
		Lenght (mm)	Width (mm)	Thickness (mm)	Weight (g)		
Pagane	22.08.2014	49.84	43.76	45.28	57.98	1.87	3.22
	20.08.2015	53.38	44.91	48.59	68.48	1.98	2.89
	18.08.2016	49.02	46.58	44.93	64.59	2.05	3.18
average	**20.08.**	**50.75 a**	**45.08 a**	**46.27 a**	**63.68 a**	**1.97 a**	**3.10 b**
Sirma	18.07.2014	45.49	40.16	39.88	43.52	1.42	3.26
	20.07.2015	49.56	42.41	44.15	55.91	1.50	2.68
	23.07.2016	43.14	35.68	37.29	34.67	1.38	3.98
average		**46.06 a**	**39.42 b**	**40.44 b**	**44.70 b**	**1.43 b**	**3.31 b**
Stanley	2.09.2014	48.93	35.7	36.17	35.88	1.74	4.84
	21.08.2015	50.34	33.96	37.7	36.46	2.02	5.54
	30.08.2016	48.23	39.62	37.73	41.04	1.64	3.99
average		**49.17 a**	**36.43 b**	**37.20 b**	**37.79 b**	**1.80 a**	**4.79 a**

Picture 1. Fruits of ‘Pagane’cv.



Picture 2. Fruits of ‘Sirma’cv.

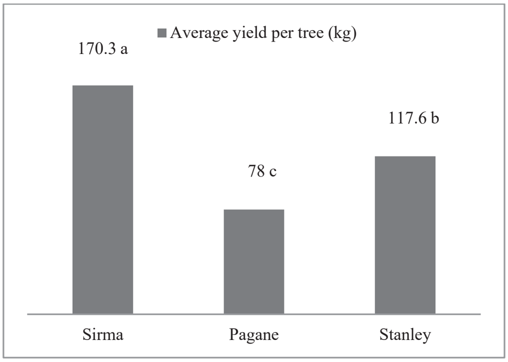


Figure 1. Average yield per tree obtained in 2018

The fruit quality is a combination of their physical and chemical characteristics - appearance, consistency, taste and aroma (Velisek and Cejpek, 2007). Consumers in our country prefer attractive fruits - medium to large, with a dark purple-blue skin color. Usually, a well-informed consumer prefers fruits with good taste, and when evaluated in sensory analyzes, the taste qualities are the most important ones (Bozhkova and Nesheva, 2016). After the fruits of the three studied cultivars were picked from the tree their appearance and taste qualities were evaluated by a group of trained consumers (Table 4). ‘Pagane’ has the most attractive and sweet fruits with balanced taste and very good texture of the fruit flesh. ‘Sirma’ has sweet fruits with moderate intensiveness of the aroma. They have the highest grade for sourness but anyway the taste is balanced and the final grade for this cultivars fruits is the highes. Both new cultivars do not have that intensive and attractive aroma as ‘Stanley’ but according their total score and final grades, their fruits are as good as the fruits of the standard and their taste qualities were evaluated as very good.

Total soluble solid content is important characteristic for the dried fruits production. Its high content is related to the high amount of sugars and increases the quality and yield of the dried product (Akin et al., 2008). Dry fruits producers prefer to use cultivars with high TSS content. ‘Pagane’ has a little bit higher TSS content in the fruits compared to ‘Sirma’ and the standard.

After sensory evaluation of the dried fruits both cultivars had equally good results. The trained consumers evaluated their dried fruits with very good qualities. ‘Sirma’s dried fruits had a little higher grade for their sweetness and consistency. ‘Pagane’ has better appearance and taste balance of its dried fruits (Table 5).

Table 4. Sensory evaluation of fresh fruits

Cultivar	TSS	Appearance	Aroma		Taste qualities				Fruit flesh texture and juicesness	Total score/ Final grade	
			Attractiveness	Intensiveness	Sweetness	Sowerness	Bitter taste	Taste balance			
Pagane	20	1.44	0.43	0.45	1.6	0.38	-0.03	1.23	1.65	7.15	Very good
Sirma	18.6	1.28	0.5	0.53	1.53	0.71	-0.03	1.23	1.47	7.22	Very good
Stanley	18.2	1.36	0.6	0.64	1.35	0.47	-0.02	1.27	1.45	7.11	Very good

Table 5. Sensory evaluation of dried fruits

Cultivar	Appearance	Color	Skin tickness	Sweetness	Sourness	Bitter taste/ presence of mold	Taste balance	Consistency	Total score/ Final grade	
Pagane	1.56	0.9	-0.17	1.44	0.43	-0.04	1.56	1.48	7.16	Very good
Sirma	1.52	0.95	-0.15	1.64	0.38	-0.03	1.52	1.52	7.35	Very good

CONCLUSIONS

The two new plum cultivars, described in that study, are suitable for diversifying the variety list in the new orchards. The most valuable features of 'Sirma' are its early ripening and good fruit taste. 'Pagane' has markedly dessert qualities of the fruit.

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A PERSPECTIVE OF APRICOT SELECTION FOR THE DOBROGEA AREA

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Abstract

In the conditions of Romania, and in particular in Dobrogea Region, there are two limiting factors in apricot growing: flower damage caused by late spring frosts and insufficient knowledge of the biology of Monilinia laxa disease. The annual production in Romania in the past years varied from 43,606 t in 2014 to 35,704 t in 2018 (Romania National Institute of Statistics, 2020). In the last 25 years, at Research Station for Fruit Growing Constanta were created and realesed more then 10 apricot cultivars. Climate change requires the introduction of new varieties of apricot better adapted to new challenges. The apricot selection "R8P22" has medium vigor, the blooming occurs in the last decade of March is abundant and lasts 10 days. The fruit is medium size, with an average weight of 55 grams. The flesh is yellow-orange with good smoothness and succulence, with sweet-acid taste and flavor.

***Key words**: Prunus armeniaca, cultivars, yields.*

INTRODUCTION

Apricot is a thermophiles, heat-sensitive species whose flowering and fruiting phenophases are recorded when air temperature exceeds 10-12^{0}C. Some of the limiting factors that maintain the yield to a relatively low level are the absence of varieties that can better adapt to various ecological conditions (Balan V. et al., 2008).

In the agroecological conditions of Dobrogea, there are two limiting factors in apricot growing: flower damage caused by late spring frosts and the intensity of climatic accidents.

The purpose of this study is to provide data on the new apricot cultivars widen the fresh consumption that lasts from 10 period of June to August 15 in Dobrogea area.

MATERIALS AND METHODS

The study was carried out at Research Station for Fruit Growing Constanta, located in south-eastern Romania, near the Black Sea.

The site is located at 44°10' Northern latitude and 28°29' Eastern longitude, and 70 m above sea level. Climate is continental with warm and droughty summers, frequent dry winds all the year round and temperate winter generally without snow. The mean annual temperature is 12.0°C and the total active temperature is 3988°C, out of which 3170°C during the growing season; the annual precipitation amount is 400 mm, out of which during the growing season (April 1 to September 30), 240.7 mm.

The lowest winter temperatures below -20°C are not very often: 1 out of 10-15 years and so are the spring frosts susceptible to cause apricot yield damage.

The climatic water deficit reaches as much as 400 mm/year, so irrigation application is needed for apricot.

The zonal soil type is a calcaro-calcic chernozem formed on loess, with loam texture and a proper capacity of water preserving, holding and circulation. The humus content ranges between 2.5 and 4%; pH of the soil is poor alkaline (7.0-8.1).

The cultivars planted at 4 m × 4 m scheme in 2009 with north-south row orientation and the crown shape is improved vase. The apricot trees are grafted on Constanta 14 described by Indreias et al. (2010).

The floral, agronomic and fruit-quality characteristics were checked for four years (2016 to 2019). The beginning of flowering was considered when the first open flower was visible and its end was noticed when the last petals of the flowers fell.

The blooming intensity was noted from 0 (absent) to 5 (abundant), according to the research methodology of fruit tree breeding (Cociu and Oprea, 1989).

Determination of dry matter was conducted by reading it directly from the refractometric scale (Zeiss, Germany) and the determination of acidity was based on potentiometric titration with the solution of sodium hydroxide (AOAC, 1995).

The trees and fruit characteristics were evaluated according to the Methodology for trying new varieties of fruit trees, shrubs and rootstock in order to approve the homologation and International Union for the Protection of New Varieties of Plants (UPOV, 2007) guidelines.

During 2016-2019 the fruit yield was recorded starting with the 7th year after planting, when fruit production was considered stable. The average yield was evaluated by weighing the fruit of three apricot trees of each cultivar (kg/tree) and then as kg/ha.

RESULTS AND DISCUSSIONS

The swelling of the vegetative buds (Table 1) began on the 11th of March and lasted until the 4th of April, while the blossoming began on the 17rd of March and lasted until the 30th of March.

Table 1. Main vegetative phenophases and active thermal sum (2016 - 2019)

Variety	Swelling of the buds		Blossoming		Beginning of sprout growth		Ending of sprout growth	Ending of the vegetative period		Duration of the veg. period (days)
	Data	tºC	Data	tºC	Data	tºC	Data	Data	tºC	
R8P22	11.03-25.03	125-135	17.03-30.03	240-255	04.04-26.04	340-377	20.07-30.07	19.10-03.11	3467-4109	210-248
Traian (control)	16.03-04.04	120-143	22.03-30.03	190-237	01.04-20.04	340-381	10.07-25.07	25.10-10.11	3653-4100	210-239

The beginning of sprout growth for the R8P22 selection was almost in the same period with witness variety. The beginning of blossoming (Figure 1) for the R8P22 selection (Table 2). occurred between the 20nd of March and the 3th of April, while the ending of the blossoming occurred between the 28st of March and the 12th of April.

Figure 1. R8P22 blossoming

Table 2. Observations and determinations concerning the fructification phenophases (average 2016-2019)

Variety	Beginning of blossoming		Ending of blossoming		Duration (days)	Ripening of the fruit		Duration of the fruct. stage (days)	Average tºC
	Data	tºC	Data	tºC		Data	tºC		
R8P22	20.03-03.04	149-190	28.03-12.04	261-310	8-14	25.06-02.07	1468-1545	70 - 90	1506
Traian (witness)	18.03-30.03	140-228	28.03-05.04	264-300	8-12	28.06-08.07	1534-1700	98 - 105	1617

The blossoming takes up to 8-13 days, which is enough for the realisation of the pollination and the fertilisation.

As concerns the ripening of the fruit, the R8P22 proved to be early than the Traian variety, which ripens in the same climatic and soil conditions with 3-6 days later. In the 4 studied years, the R8P22 reached the ripening stage between the 25th of June and the 2nd of July, the earliest being in 2018 (25th of June) and the latest in 2017 (8nd of July).

The R8P22 selection has a high coefficient of natural fertility of 30.5% (Table 3), being superior to the control variety Traian (27.4%). The fruit production of this hybrid is positively influenced by the fertility percentage.

As concerns the fruit production (Table 4), it has been observed that in favourable years, its values are higher than those of the witness variety.

Table 3. Behaviour during the pollination and fertilisation process (average 2016-2019)

Variety	Autofertility %					Natural fertility %				
	2016	2017	2018	2019	Average	2016	2017	2018	2019	Average
R8P22	4.8	10.6	11.9	9.8	9.27	29.4	32.9	28.7	31.3	30.5
Traian (control)	1.8	3.7	2.8	1.2	2.3	22.4	31.4	28.3	27.6	27.4

Table 4. Fruit production between 2016 and 2019 (year X of vegetation) (4 m x 4 m = 625 tree/ha)

Period		R8P22	Traian
2016	kg/tree	20.9	18.7
	t/ha	13.06	11.6
2017	kg/tree	30.1	22.3
	t/ha	18.8	13.9
2018	kg/tree	12.8	7.5
	t/ha	8.0	4.6
2019	kg/tree	16.7	12.4
	t/ha	10.4	7.7
Average 2016-2019	kg/tree	20.1	15.2
	t/ha	12,6	9.4

The year 2017 is considered to have been favourable for the fruit production from a climatic point of view, the lower temperatures during the blossoming (5.7-12.6º C) leading to a delay in this phenophase and implicitly, to a good thing, thus realizing a greater production (18.8 t/ha), the difference being significantly positive when compared to the witness variety.

The year 2018 was unfavourable to the cultivation of apricot trees because of the climatic accidents that occurred during the blossoming stage (-2.9ºC in the air and -5°C on the ground), which led to the destruction of the tied fruit; however, this hybrid proved to be more resistant, the production being of 8.0 t/ha.

The year 2019 was not quite favourable for the apricot tree because of the extended draught from the previous year, thus determining a production of 10.4 t/ha of the R8P22.

In 2016, the R8P22 realised an average production of 13.06 t/ha and it is safe to say that it has remade its productive potential faster than the witness variety.

Analysing the average of the fruit production over a period of 4 years and taking into account the year 2019, when the production was quite low, we can state that this hybrid realized an average production of over 12.6 t/ha.

A criterion based on which a variety in promoted into the assortment is the appreciation of the resistance to cold and variations in temperature (Figure 2).

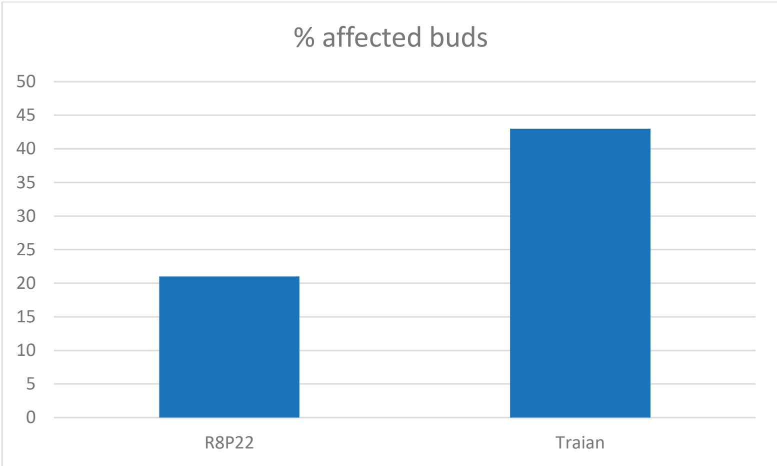

Figure 2. Resistance to cold of flowering buds

Between 2016 and 2019, before the beginning of the vegetative stage, observations for this hybrid were performed on over 760 flowering buds. We can state that the P8R22 is highly resistant to low temperatures and fairly resistant to comeback cold periods, the average percentage of destroyed buds over a period of 4 years being of 21.6%, as compared to 43.1% for the control variety.

CONCLUSIONS

The R8P22 hybrid can be considered a variety with early ripening stage and it can improve the structure of the current assortment, which is still lacking in early varieties (in the area).

This hybrid is constant in terms of fruiting, having good results even in unfavourable years for apricot cultivation.

The hybrid has a semi-early ripening period, which makes it an economically hybrid.

The guarantee of this variety's value is also given by its adaptability to local climatic and soil conditions, expressed through its high resistance to the extreme temperatures specific to this area, to diseases and pests, which recommends its homologation as variety and its extension within crops.

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PERFORMANCE IN BREEDING SCAB RESISTANT APPLE VARIETIES AT RESEARCH AND DEVELOPMENT STATION FOR FRUIT GROWING VOINEȘTI

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Abstract

The last fifteen years of research activity at Research and Development Station for Fruit Growing Voinesti, conclude in a large number of patented apple varieties with genetic resistance to diseases which proved high quality and performance in the orchards. Therefore, the aim of the paper is to present and highlight the wide range of achievements in terms of apple resilient national breeds. The trees vigour defined by the circumference of the trunk measured in the 10th year apple varieties, grafted on the M9 rootstock recorded values between 14.9 cm for 'Cezar' variety and 18.4 cm for 'Redix' variety. The crown volume calculated per unit area, was of 6,800 m^3/ha for the 'Iris' variety and 8,800 m^3/ha for the 'Redix' variety, which proved to be more vigorous. The productivity during the full fruiting period of the nine scab resistant apple varieties, grown in a high-density system, was between 28-45 t/ha, the most productive being considered 'Iris', 'Real', 'Remar', 'Valery' and 'Cezar' with constant yields over 35 t/ha. The fruit size varied between 155 g and 180 g, smaller ones remarked at the varieties 'Iris', 'Inedit', 'Voinicel' and 'Revidar' and larger fruits at the varieties 'Cezar', 'Valery', 'Remar' and 'Redix' with fruits over 170 g that match to the market requirements.

Key words: *scab resistant variety, productivity, quality, crossing, genotypes.*

INTRODUCTION

Last decades were marked by the trend of the global apple market to focus of performant apple varieties but with disease-resistant traits (Luo F. et al., 2020). Nevertheless, new breeding techniques have been experimented and driven in the same direction for apple breeding (Kellerhals M. et al., 2014, 2020)

The Research and Development Station for Fruit Growing Voinești has been a great contributor in breed apple scab resistant apple varieties and demonstrated a prolific and successful activity (Petre, V. and Petre, Gh., 2014). Also, steadily promote such apple varieties with genetic resistance to diseases for the new plantations with higher economic performance (Comanescu et al., 2012).

Latest achievements (Petre V. et al., 2017) named 'Valery', 'Cezar' and 'Revidar' already gained a well-deserved respectfully place in the Romanian apple assortment and looking forward for an extensive spread in the new orchards.

The mainstream of apple varieties apart from the disease resistant traits is to assure quality attributes requested by the consumers. Appearance and excellent eating quality are demanded to all the varieties and independent of the maturity class.

Present paper objectives are to emphasize the outcomes of the breeding activity over the past years at the RDSFG Voinesti and to highlight the performances of apple resistant varieties in the national frame.

MATERIALS AND METHODS

A multiannual evaluation program of apple varieties bred at the RDSFG Voinesti has been organized in order to point out the valuable and particular qualities.

The research was organized in the experimental fields of RDSFG Voinesti. The genetic bio-base is composed by selection plots, hybrid nursery fields, higher level plots for elite testing etc. Also, all the patented varieties were tested and validated by the ISTIS protocols.

In the trial fields, the trees have been planted at a distance of 3.5 m x 1.0 m corresponding to a density of 2,857 trees/ha. Varieties were grafted on M9 rootstock and the training system of the crown was slender spindle.
Research conducted during last ten years reveal the great growing and fruiting potential of apple varieties. Biometrical measurements of the tree growth were done according to the experimental protocol and took into consideration the trunks dynamic growth, the size of the crown and completed with observations on the specific behaviour of growing. Regarding the productivity of the trees, the indicator considered the annual yield, fruit quality, content of dry soluble substance etc.
Nine apple varieties were studied: ‘Redix’, ‘Iris’, ‘Real’, ‘Remar’, ‘Inedit’, ‘Voinicel’, ‘Valery’, ‘Cezar’, ‘Revidar’.

RESULTS AND DISCUSSIONS

The apple variety obtaining procedure is time consuming and of a high complexity especially when one major objective is disease resistance.
For a new variety many traits have to comply with the breeder expectations and market demand.
Besides common breeding goals such as productivity, high quality of fruits, resistance to pest and diseases, a lot of complementary requirements have to be achieved:

- ecological adaptation of the new cultivar;
- destination of the production according to the inner particularities;
- consumer preferences;
- cost/efficiency ratio for the growing technology;
- suitability for nursery and propagation.

Tree vigour was estimated combining four indicators: trunk circumferences, heigh of the tree, fruiting wall thickness and the crown volume.
According to the data presented in the Table 1, the trunk diameter oscillated from 14.9 cm (‘Cezar’) to 18.4 cm (‘Redix’) with an average value of 16.27 cm.
Looking to the tree heigh, there was no big differences seized. The smallest trees were measured at ‘Iris’ (220 cm) with 33 cm less than the average tree heigh. ‘Redix’ confirm again as a vigorous variety (270 cm).
Crown volume express similar ranked values as tree heigh and the thickness of the slender spindle showed unsignificant differences.
The blossom behaviour of the apple varieties indicated the early start of flowering of ‘Iris’ variety and ‘Voinicel’ and a late start of the blossom at the ‘Valery’ variety.
The yield of the nine apple varieties in a high-density system varied from 28 t/ha to 45 t/ha. Varieties which exceed 35 t/ha are the following: ‘Iris’, ‘Real’, ‘Remar’, ‘Valery’ and ‘Cezar’.
Most of the apple varieties are producing fruits around 155-180 g such as ‘Cezar’, ‘Valery’ and ‘Remar’ that overpass 180 g/fruit while smaller fruits have been registered for ‘Iris’, ‘Inedit’ and ‘Voinicel’.
The soluble dry substance (SDS) has a different distribution among the varieties that the size of the fruits. Only ‘Valery’ variety proved to be also rich in SDS.
Concerning the harvest date for the scab resistant apple varieties combined with the fruit’s storability group the varieties in two classes.
First group (‘Revidar’, ‘Real’, ‘Remar’, ‘Voinicel’, ‘Iris’) is represented by the apples with only one to maximum 2-3 months storability and the harvest date in late August or September.
The second group (‘Valery’, ‘Cezar’, ‘Inedit’, ‘Redix’) has a longer shelf life and the harvest date at the end of September in the growing area conditions.
Below there are presented the main apple varieties characteristics and superior value of the forementioned nine varieties bred at the RDSFG Voinesti.

Table 1. Growth and fruiting particularities of the new scab resistant apple varieties created at RDSFG Voinesti

Item	Variety								
	Redix	Iris	Real	Remar	Inedit	Voinicel	Valery	Cezar	Revidar
I. Growth (vigour)									
Trunck circumference (cm)	18.4	15.2	17.2	17.9	16.2	15.2	16.5	14.9	15.0
Tree height (cm)	270	220	250	260	240	240	280	260	260
Thickness of the fruiting wall (cm)	140	140	130	140	130	130	130	130	130
Crown volume (mc/ha)	8,800	6,800	7,430	8,400	7,060	7,060	8,550	7,800	7,800
II. Fructification									
Yielding phenophases									
Beginning of flowering	20 - 26.04	15 - 20.04	20 - 24.04	20 - 26.04	18 - 22.04	16 - 20.04	24 - 29.04	22 - 28.04	22 - 28.04
End of flowering	28-04-03.05	26 - 30.04	28.04 - 04.05	28.04 - 03.05	28.04 - 02.05	24 - 30.04	01 - 06.05	30.04 - 05.05	30.04 - 05.05
Blossom period (days)	9-10	9-12	9-10	10-11	9-12	11-12	7-9	7-9	7-9
Rippening period	26 - 30.09	15 - 20.09	26 - 31.08	01 - 10.09	25.09 - 01.10	10 - 15.09	25.09 - 01.10	15 - 20.09	25 - 31.08
Consumption period	oct. - ian.	sept. - nov.	sept.	sept. - oct.	oct. - febr.	sept. - nov.	oct. - martie	oct. - dec.	sept.
Storability (days)	125 - 130	70 - 75	25 - 30	40 - 50	145 - 150	70 - 75	145 - 150	80 - 86	30 - 35
Yield (t/ha)	30-35	40 - 45	35 - 40	38 - 42	30 - 35	28 - 30	35 - 40	35 - 40	28 - 30
Production quality									
Fruit weight (g)	170	155	180	170	155	155	185	190	160
Dry matter content (%)	14.5	14.2	13.5	13.8	15.0	14.5	16.5	13.8	13.0

Redix sin. H 3/73-83 Voineşti

The variety has been approved in 2004.

The fruits are medium to large (170 g) and very similar to ‘Starkrimson’ in shape, with a violet-red skin which became lighter during the storage period.

The firmness is good and help during the harvest and transportation process.

The flesh is white-yellowish at the consumption right time, juicy and well-balanced sweetness and acidity.

The tree is resistant to scab and mildew tolerant.

It is recommended for the great appearance of the fruits and for the excellent dry chips that can be obtained through dehydratation.

Figure 1. ‘Redix’ apple at the harvest time

Iris sin. H 8/94-82 Voineşti
Apple variety is present in the ISTIS catalog from 2005.
The fruits are medium in size (155 g), rounded and slightly compressed at the extremities with a green-yellow base colour covered by carmin-red colour over 2/3 of the fruit surface. The flesh is white-yellowish, sweet and with a pleasant acidity. The fruit are crunchy at the harvest time and excellent in taste. Tree is very precious and never affected by biannual production even the yield is high. Is scab resistant and tolerant to mildew.

Figure 2. 'Iris' apple scab variety

Real sin. H 9/78-82 Voineşti
The variety is patented in 2007 and was spread in production mainly in the Voinesti basin.
The fruits are big (180 g) with ridges near the calyx area and waved profile. Flesh is firm, juicy, white-yellowish and with great taste. It is a scab resistant variety and tolerant to *Podoshaera leucotricha*. Some more advantages are related to the precocity and economic stability due to its annual production of high-quality fruits.

Figure 3. 'Real' apple scab variety

Remar sin. H 1/26-90 Voineşti
The variety is patented in 2008.
Fruits are medium in size (170 g), truncated with a green-yellow behind colour. The skin is covered almost entirely in dark-shiny red. Flesh is white, juicy and with a very good taste and pleasant aroma. Is immune to *Venturia inaequalis* and rarely attacked by mildew. It is precocious and with a high yield each year.

Figure 4. ‘Remar’ apple scab variety

Figure 5. ‘Inedit’ apple scab variety

Inedit sin. H 3/5-90 Voineşti
The variety is patented in 2009.
Fruits are medium is size (155 g), round-cone-shaped with a greenish-yellow under overlay vivid red colour that cover 2/3 of the fruit surface.
Flesh is crunchy, very juicy, white to yellowish in colour, very good in taste.
Trees are immune to scab and tolerant to mildew. Is precocious and the storability is good. The fruits could be consumed by February in cold condition.

Voinicel sin. H 2/5-90 Voineşti
The variety is patented in 2009.
Fruits are medium (155 g), globulous and slightly flattened at the poles, nice coloured in red over most of the skin. Taste is good.
Manifest very strong resistance to scab and mildew and is precocious.

Figure 6. ‘Voinicel’ apple scab variety

Valery sin. H 4/37- 04 Voineşti

The variety was patented in 2016.

The fruit is large (185 g) of conical shape and with visible ridges on the surface. The skin is yellow with blush of orange on the sunny side. The flesh is crunchy, white-yellowish and with a very good taste. It is scab resistant and demonstrate a high yearly productivity. Apples can be ready for consumption until March due to a very good storability and shelf life.

Figure 7. ‘Valery’ apple scab variety

Cezar sin H 1/78-90 Voineşti

The variety was patented in 2016.

Fruits are large (190 g) round-conical in shape, with irregular outline. The covering colour is shiny red over almost entirely surface of the skin. Flesh is white, sweet and delicate acidic taste, crunchy and fine texture. Tree is medium to small in size, precocious and with economic yields.

Figure 8. ‘Cezar’ apple scab variety

Revidar sin H 1/16-90 Voineşti
The variety was patented in 2016.
Fruits are medium in size (160 g) round-conical in shape, with a shiny red over 2/3 of the fruit surface. Flesh is white, sourish, crunchy and fine texture. Tree is medium to small in size, precocious and with constant yields.

Figure 9. ‘Revidar’ apple scab variety

CONCLUSIONS

The apple assortment bred at the Research and Development Station for Fruit Growing Voineşti between 2004-2016 overlap a big part of the consumption season and offer a great opportunity for growers to start new orchards with very well adapted and performant scab resistant apple conveyer.

Using resistant to scab and tolerant to mildew varieties such as ones created at the RDSFG Voinesti, there are good premises in higher economic benefits and healthier apple production along with a smaller risk for the environment.

Each variety highlights specific and particular performances that indicate the right option for new investments in apple plantations.

In the context of winter variety demand, 'Valery' and 'Inedit' are the most suitable for this, matching in the same time the fruit quality major parameters.

The apple breeding activity must continue in order to better valorize the genetic heritage of the Romanian achievements.

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POST-HARVEST TECHNOLOGIES INFLUENCES IN ORGANIC 'TITA' PLUMS QUALITY

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Abstract

The paper aims to present the influences of post-harvest technologies on organic 'Tita' plums during storage period, taking in consideration the variation of quality indicators, physiological parameters, and bioactive compounds. Organic 'Tita' plums were harvested in 2019, at the end of July and stored in three different condition, i.e.: 1) normal atmosphere (NA) with 1˚C and 95% relative humidity (RH), 2) controlled atmosphere (CA) conditions with 1˚C, 95% RH, 3% O_2 and 5% CO_2, and 3) CA conditions with 1˚C, 95% RH, 1.5% O_2, and 10% CO_2. Organic plum samples were evaluated at 4 moments: initially (before storage), after 3, 5, and 7 weeks of storage. Obtained results showed that total phenolic content and antioxidant activity registered the same variation trend during storage period for all samples. Differences were observed during storage period, which was shorter with 2 weeks for plums stored in NA than for those stored in both controlled conditions. Taking into consideration these results, present work suggests that plums stored in both controlled atmosphere conditions were better preserved than those stored in NA, but further trials and studies are required.

Key words*: CA, storage, respiration, transpiration, antioxidant activity.*

INTRODUCTION

In Romania, plums represent the major fruit species with highest diversity in native cultivars and are very well adapted to climatic conditions and soils (Butac et al., 2019). Moreover, almost all cultivated plums belong to *Prunus domestica* L. species (Butac et al., 2019). Beside *P. domestica* cultivars like 'Carpatin', 'Roman', 'Romanta', 'Agent', and 'Andreea', 'Tita' variety is part of Romanian plums breeding program (Butac et al., 2019).

Generally, plums (*Prunus domestica* L.) are climacteric fruits which means that respiration and transpiration processes continue after harvesting (Rozo-Romero et al., 2015) and lose their nutritional value in short time after harvesting (Hussain et al., 2015; Panahirad et al., 2020). Due to these, early harvesting is necessary in order to withstand transport which often causes the fruits to not reach the consumption maturity required by consumers (Rozo-Romero et al., 2015). As consequences of climacteric behaviour of plums are reduced shelf life, commercial problems and decreased quality parameters like color, firmness, fruit turgidity and finaly the decay occurrence and off-flavours (Peano et al., 2010), total soluble solids and total tritatable acidity (Zapata et al., 2014; Valero and Serrano, 2010). Storage conditions like 0-5°C and 80-95% RH were reported to delay softening, but also may promote disorders development like translucency, flesh browning, bleeding, and ripen failure (Manganaris & Crisosto, 2020; Manganaris et al., 2008). Many postharvest techniques were tested and reported to reduce the losses and to extend the shelf life of plums (Martínez-Romero et al., 2017) such as cold storage (Serrano et al., 2009), force air-cooling (Martínez-Romero et al., 2003), cold storage combined with 1-MCP (methylcyclopropene) (Minas et al., 2013; Valero et al., 2004), alginate edible-coating (Valero et al., 2013), polyamines (Serrano et al., 2003), calcium and heat (Valero et al., 2002), and modified atmosphere packaging (Díaz-Mula et al., 2011). In order to reduce the losses and to extend the postharvest life of organic plums, the controlled atmosphere conditions as postharvest technologies are more and more used. As many authors reported, cold storage at 0°C combined with controlled atmosphere (CA) conditions are beneficial in extending postharvest life of plums (Peano et al., 2010; Crisosto et al., 2004). But storage technologies like CA and modified atmospheres are not widely used commercially because the benefits are not as pronounced as in other fruit species, moreover the contradictory results about the cost/benefit of plums CA storage are open (Manganaris & Crisosto, 2020).

The paper aims to present the influences of post-harvest technologies based on cold storage and controlled atmosphere conditions on organic 'Tita' plums during storage period, taking in consideration the variation of quality indicators, physiological parameters, and bioactive compounds.

MATERIALS AND METHODS

Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl) AND Folin & Ciocalteu's reagent were purchased from Sigma-Aldrich Chemie GmbH (Riedstrasse, Steinheim). Gallic acid was purchased from Carl Roth and Trolox (6 - hydroxy - 2, 5, 7, 8 - tetramethylchroman - 2 - carboxylic acid) from Acros Organics, Fisher Scientific (Geel, Belgium). Anhydrous sodium carbonate was purchased from Lach-Ner, s.r.o. (Neratovice, Czech Republic). Methanol used in experiments was bought from Honeywell (Riedel-de Haën, Seelze, Germany) and sodium hydroxide 0.1N was from Cristal R Chim S.R.L. (Bucharest, Romania). Ultrapure water used it was made with a Milli-Q equipment (Millipore, Bedford, MA).

Samples

Organic plums from 'Tita' variety were harvested in July 2019, from Research Institute for Fruit Growing Pitesti, Romania and stored for one day at 2°C, 90% relative humidity (RH). Then were transported to Postharvest Technologies Laboratory from Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest. Plums were stored in Cold Rooms with normal atmosphere, 1°C and 95% RH, 24 hours, until initially analyses were performed. After these, organic plums were equally divided and stored in three different conditions, i.e.: 1) normal atmosphere (NA) with 1˚C and 95% RH, 2) controlled atmosphere conditions with 1˚C, 95% RH, 3% and 5% CO_2 (CA 5% CO_2), and 3) controlled atmosphere conditions with 1˚C, 95% RH, 1.5% O_2, and 10% CO_2 (CA 10% CO_2). Organic plum samples were analysed in 4 moments, first one being realised before storage (noted with 0 - zero), after 3, 5, and 7 weeks of storage (noted with 3, 5, and 7).

Quality indicators

Quality parameters were represented by pH, total titratable acidity (TTA), total soluble solids (TSS), dry matter (DM) and firmness, their methods of analyse being described forward.

The pH and TTA analysis were realised using the TitroLine automatic system, equipped with pH electrode. The analysis consist in mixing 5 g of fresh sample with 25 mL of distillate water, measuring the initially pH values and then titration with 0.1N NaOH up to a 8.1 pH according with Saad et al. (2014) and AOAC Official Method 942.15. For TTA, results were expressed in g malic acid /100 g of fresh fruit (Stan et al., 2020, Gherghi et al., 2001). The total soluble solids (TSS) analysis were performed similar as Turmanidze et al. (2017), using Kruss DR301-95 digital refractometer, in accordance with Brix reading. Dry matter results were obtain using UN110 Memmert oven and drying approximately 1 g of sample at 105°C (Ticha, 2015) until constant weight. Firmness results were obtained and expressed in N/cm^2 using a digital penetrometer (53205 TR Italy) equipped with an 8 mm piston (Stan et al., 2019).

Physiological parameters

The respiration rate was performed using Lambda T NDIR Monitor, ADC BioScientific LTd. and results were expressed in mg CO_2/kg FW/hour (Rozo-Romero et al., 2015). Similar methods were used and described by Popa et al.

(2019), Bezdadea-Cătuneanu et al. (2019) and Farcuh et al. (2019).
The transpiration rate was determined by gravimetric measurements (Fante, 2014) before and after 30 minutes and results were expressed in g water/100 g FW/hour similar with those presented by Bezdadea-Cătuneanu et al. (2019) in their work.

Bioactive compounds
For total polyphenol content (TPC) quantitative determination was used the Folin - Ciocâlteu method adapted after Georgé et al. (2005) protocol. Samples extraction consist in trituration of 1 g fresh sample with 10 mL of 70% methanol and incubated overnight at room temperature (aprox. 21°C) and dark. Extraction continue next day through 1 h and 500 rpm homogenization, then centrifugation for 5 min at 4°C and 7000 rpm. The supernatant was recovered and the residue re-extracted two more times and the final volume of extract was 30 mL. By mixing 0.5 mL of extract with 2.5 mL of Folin - Ciocâlteu reagent and incubated for 2 minutes at room temperature (aprox. 21°C) is the first step in total polyphenol content determination. Second step is represented by adding 2 mL of 7.5% sodium carbonate solution (Na_2CO_3) and incubate the obtained mix for another 15 minutes at 50°C. Third and final step is based on the absorbance read at Specord 210 Plus UV-VIS spectrophotometer (Analytik Jena, Jena, Germany) at the 760 nm wavelength. Results are expressed in mg GAE/100 g fresh weight and Gallic acid is used as standard solution.
For antioxidant activity determination was used the DPPH (2,2-diphenyl- 1-picrylhydrazyl) method, similar as described Bujor et al. (2016) with modifications presented forward. Mixing 0.2 mL of extract with 2 mL of 0.2 mM solution of DPPH in methanol and incubated for 30 minutes, in dark with continuous homogenising. The absorbance was measured at 515 nm wavelength. Results were expressed as mg Trolox/100 g FW and blank reference was realised with methanol.

Statistical analysis
Statistical analysis of obtained data was performed using Microsoft Excel for standard deviation. Standard deviation represent the average of three replicates with independent sample preparation.

RESULTS AND DISCUSSIONS

Quality indicators
During storage period organic 'Tita' plums registered quality indicators variations for all three post-harvest technologies used. Experiments were performed during 7 weeks of storage, but physiological disorders were observed after 3 weeks of NA conditions storage and after 5 weeks for those stored in CA 5% CO_2 and CA 10% CO_2, 1˚C and 95% RH. Candan et al., 2008, reported flesh translucency as being first symptom of chilling injury, which appeared after 30 days of storage at 0˚C. In our case (Table 1) it can be observed that physiological disorders as translucency appear after 3 weeks of storage for organic 'Tita' plums stored in CA 5% CO_2 and CA 10% CO_2 conditions. Comparing with organic 'Tita' plums stored in NA conditions were the overripe disorder is already installed after 3 weeks of storage, those stored in CA 5% CO_2 and CA 10% CO_2 conditions the overripe disorder appear after 5 weeks of storage. These physiological disorders observed in our work are related with chilling temperatures, and similar behavior was described by Manganaris & Crisosto (2020) in their study which present that commercial storage conditions (0-5°C and 80-95% RH) are delaying softening, but may promote the storage disorders development, manifested as translucency, bleeding, flesh browning, and/or failure to ripen. The delayed onset of physiological disorders for plums stored in CA conditions was due to low O_2 and increased CO_2 concentrations which slowed metabolic process and consequently the respiration (Figure 1) and transpiration (Figure 2) processes.
CA conditions should be monitored in different ways, by measuring the respiration rate (Díaz-Mula et al., 2011), intensity (Wang et al., 2016) and measurements of the stored fruit respiratory quotient (Bessemans et al., 2016).
The initially TTA values (Tabel 2) of organic 'Tita' plums were 1.16 ± 0.01 g malic acid/100 g FW, similar with results presented by Bozhkova (2013). During storage the TTA values decreased up to 0.95 ± 0.01 malic

acid/100 g FW for organic 'Tita' plums after 3 weeks and 0.74 ± 0.04 malic acid/100 g FW after 5 weeks of NA storage, which means that plums acidity increased. Majeed & Jawandha (2016) observed similar behavior in their study. For plums stored in CA 5%CO_2 and CA 10% CO_2 conditions were observed smaller decreases of TTA results. Important decreases of firmness were registered for organic 'Tita' plums stored in NA conditions, while for those stored in CA 5% CO_2 and CA 10% CO_2 conditions firmness presented smaller decreases. Correlation between firmness decreases and storage period were described also by Majeed & Jawandha (2016). Manganaris & Crisosto (2020) explain in their work that most studies indicate that fruits wich were stored at temperatures above 0°C and CA were firmer than those air-stored.

Table 1. Influences of post-harvest technologies based on cold storage and controlled atmosphere conditions on organic 'Tita' plums appearance

Moment of analysis (weeks) / Storage conditions	0	3	5	7
Before storage		n/a	n/a	n/a
NA with 1˚C, 95% RH	n/a			After 5 weeks analysis, no healthy fruits remained
1˚C 95% RH 3% O_2 5% CO_2	n/a			
1˚C 95% RH 1.5% O_2 10% CO_2	n/a			

Table 2. Variation of pH, total titratable acidity (TAA), total soluble solids (TSS), and dry matter (DM) content during storage of 'Tita' plums

Variety	Storage conditions	Analysis moment (weeks)	pH	TAA (g malic acid/100 g FW)	TSS %	DM %	Firmmnes (N/cm²)
'Tita' Organic	NA with 1˚C, 95% RH	0	3.42±0.06	1.16±0.01	17.85±1.10	8.13±1.47	15.14±1.86
		3	3.34±0.05	0.95±0.01	15.93±2.29	14.38±0.36	7.54±1.73
		5	3.60±0.20	0.74±0.04	16.87±2.14	15.74±0.87	6.50±2.31
		7	After 5 weeks analysis, no healthy fruits remained				
	1˚C, 95% RH, 3% O_2, 5% CO_2	3	3.29±0.09	1.00±0.01	15.91±2.30	13.86±0.24	15.22±4.81
		5	3.46±0.07	0.98±0.005	14.48±1.49	13.48±0.59	16.29±3.79
		7	3.50±0.25	0.87±0.03	16.79±1.62	15.49±4.27	10.59±1.19
	1˚C, 95% RH 1.5% O_2, 10% CO_2	3	3.38±0.05	1.11±0.01	12.89±1.07	11.97±0.49	18.97±2.17
		5	3.45±0.01	1.07±0.02	14.07±1.62	12.73±1.50	19.07±8.89
		7	3.36±0.10	0.99±0.02	15.23±1.64	14.57±1.53	16.17±3.93

Data represent mean ± standard deviation of three replicates.

Also, Manganaris & Crisosto (2020) explain that CA has a rather limited use for plums storage for periods longer than 4 weeks. TSS results shown variation in all three storage conditions experimented in our work and were similar with those obtained by Butac et al., (2019).

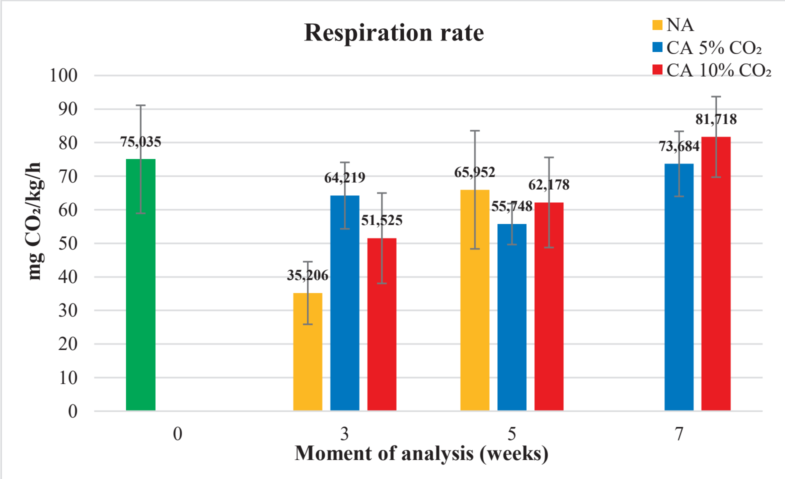

Figure 1. Respiration rate results for organic ‘Tita’ plums, registered during storage period

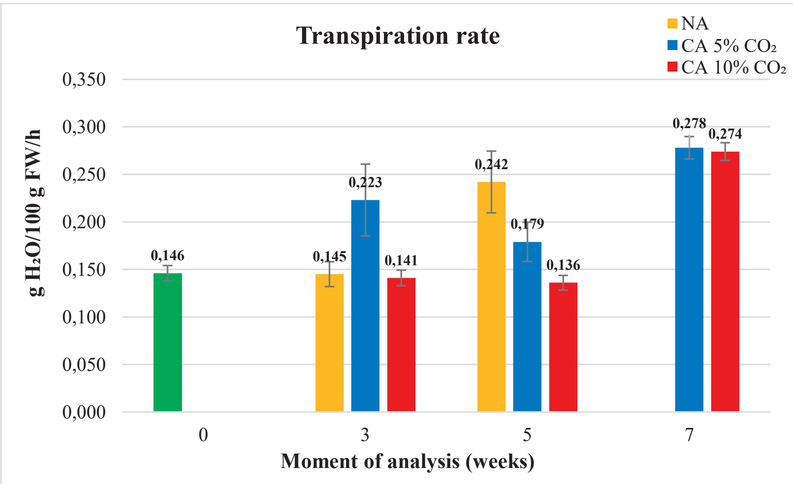

Figure 2. Transpiration rate results for organic ‘Tita’ plums, registered during storage period

Bioactive compounds

The content of bioactive compounds were determined from whole plum and showed similar behavior for all tested storage conditions. For organic ‘Tita’ plums stored in NA conditions with TPC values 129.9 mg GAE/100 g FW demonstrate small decreases after 3 and 5 weeks of storage up to 120.3 mg GAE/100 g FW, respectively 117.3 mg GAE/100 g FW. Organic ‘Tita’ plums stored in CA 5% CO_2 and CA 10% CO_2 conditions also presented decreased TPC values in comparison with those from the initially moment of analyses. After 7 weeks of storage the TPC values were smaller than those obtained after 5 weeks, which indicate that organic ‘Tita’ plums were no longer safe to be consumed.

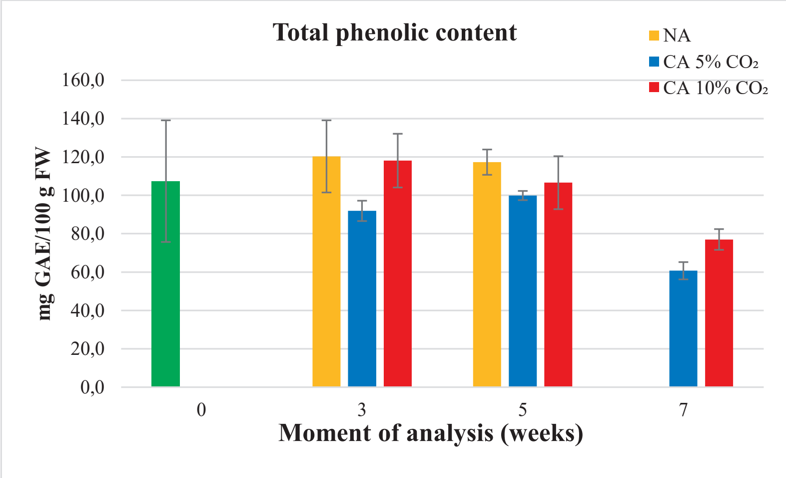

Figure 3. Total phenolic content variations for organic ‘Tita’ plums, registered during storage period

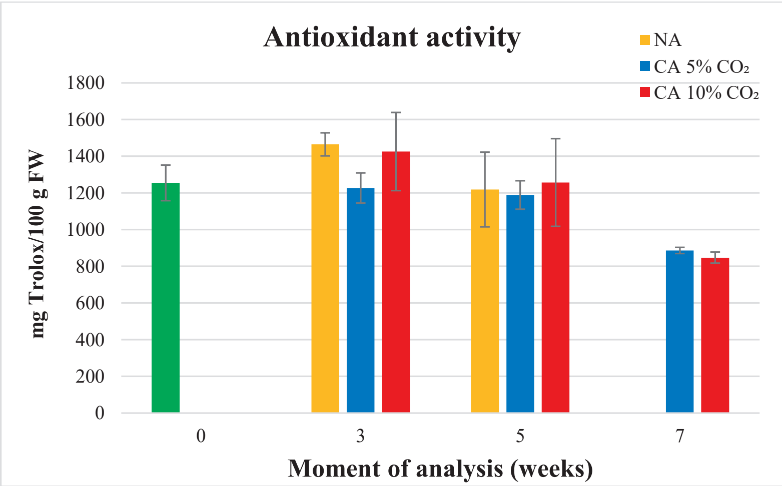

Figure 4. Antioxidant activity variations for organic ‘Tita’ plums, registered during storage period

All analyzed samples present an increased antioxidant activity during storage period for all three different applied conditions. Similar behavior of antioxidant activity increasing during storage in plums was observed by Martínez-Romero et al. (2017), in both peel and pulp, when they analyzed the hydrophilic antioxidant activity (H-TAA) and the lipophilic antioxidant activity (L-TAA). As Puerta-Gomez & Cisneros-Zevallos (2011) observed in their study, in case of present study, the antioxidant activity trolox, mg/100 g FW, did not registered major changes during storage, presenting similar behaviour as total phenolic content.

CONCLUSIONS

Results showed that total phenolic content and antioxidant activity registered the same variation trend during storage period for all samples. Differences were observed during storage period, which was shorter with 2 weeks for plums stored in NA than for those stored in CA 5% CO_2 and CA 10% CO_2 conditions. Physiological disorders as translucency appear after only 3 weeks of storage in CA 5% CO_2 and CA 10% CO_2 conditions, and after 5 weeks appear the overripe disorder. For organic ‘Tita’ plums stored in NA conditions the overripe disorder was already installed after 3 weeks of storage. Physiological disorders observed in our work are related with chilling temperatures. The delayed onset of physiological disorders for plums stored in CA conditions was due to low O_2 and increased CO_2 concentrations which slowed metabolic process. Taking these results in consideration, present work suggests that plums stored in both controlled atmosphere conditions were better preserved than those stored in NA, but further trials and studies are required.

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THE INFLUENCE OF SOME BIOPRODUCTS ON THE YIELD AND CHEMICAL COMPOSITION OF THE PEACHES UNDER THE CONDITIONS OF INTEGRATED PLANT PRODUCTION

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Abstract

Studies were carried out in the period 2018-2019 in a fruit-bearing peach orchard on the territory of the Fruit-Growing Institute, Bulgaria. The influence of different fertilizer norms of Lumbreco, Agrifull and Humustim bioproducts on the yield and chemical composition of the 'Glohaven' cultivar grafted on vegetative pad GF677 was studied. The highest yield was obtained after applying Agrifull with an average yield of 32.16 t/ha followed by Lumbreko with 29.64 t/ha. With a yield close to the control plants, the variant is fertilized with Humustim 22.42t/ha. The best results are reported by Brix of 12.7% and 12.0% were established in the variants treated with Lumbreco at all the applied rates and those with Agrifull at the rate of 1 L/da - 12.2%. Sugar content ranged from 4.7% in the untreated control to 10.44% in the variant with the application of Lumbrco soil nutrition. Peaches are rich in K, Mg, P and Ca. Imported bioproducts do not significantly affect the content of the main macro elements. The higher fertilizer norms had a positive effect on the content of N, P and Mg, and the differences with the fertilizer control for the element P and Mg were statistically proven.

Key words: *peach, bioproducts fertilization, yield, macro elements.*

INTRODUCTION

The major problem in integrated production systems is that when mineral fertilization is reduced, it is difficult to maintain the nutrient balance in the soil-plant system, required to produce high yields (Reganold et al., 2001). Chang et al. (2010) announced that the quality and quantity of the fertilizers applied are the key factors affecting growth, yield and fruit quality. However, the long-term use of chemical fertilizers leads to a deterioration of soil characteristics and fertility. The excessive use of inorganic fertilizers creates environmental problems and the situation can be improved by the use of organic products (Von-Bennewitz & Hlousek, 2006; Marzouka, & Kassem, 2011; Liu, C. H. & Liu Y., 2012). Biofertilizers contain useful microorganisms instead of synthetic chemicals, which improve plant growth on the one hand and protect the environment and maintain soil fertility, on the other (Grzyb et al., 2014). Organic fertilization is based on the use of natural products: manure extracts, a fertilizer from red California worms, natural resources rich in biologically active substances, compost derived from wood and paper waste (Edwards & Bohlen, 1996; Leroy et al., 2007). Along with those products, new sources are being sought. Biofertilizers, produced entirely from natural products that do not pollute the environment and the production itself, play a decisive and successful role. There are no harmful residual substances and cause no harm to human health. Despite the growing interest in bioproducts as substitutes for synthetic agrochemicals, knowledge about how the different levels of fertilization affect nutritional components is still limited (Carbonaro et al., 2002).

The aim of the present study was to evaluate the effect of the different rates of the bioproducts (Lumbreco, Agriful and Humustim) on the yield and chemical composition of 'Glohaven' peach fruits in integrated production.

MATERIALS AND METHODS

The study was carried out in 2018-2019 in a fruit-bearing peach plantation on the territory of the Fruit-Growing Institute - Plovdiv, Bulgaria. The subject of the study was 'Glohaven' cultivar grafted on the vegetative rootstock GF677. The soil was alluvial-meadow with a neutral soil reaction of pH 7.10 and well supplied with phosphorus (22 mg/100 g) and potassium (26 mg/100 g). Planting distance was 5 × 3 m (670 trees/ha).

Agriful was applied to soil as water solution. Two rates of 1.0 and 2.0 L/da were studied. Humustim was used as a foliar fertilizer at three rates of 200, 240 and 300 ml/da. Lumbreco was applied in three variants: **I** – foliar nutrition (1 L/da); **II** – soil application (2 L/da) and **III** – combined foliar nutrition (360 ml/da) + soil application (2 L/da). Each of the variants was in three replications. The control was untreated, without foliar or soil nutrition.

The fertilization rates were introduced four times during vegetation, every 15-20 days from April to July inclusive.

The yield was reported at the fruit maturity stage by replications and variants in kg/tree. Statistical evaluation of the differences between the yields obtained by variants was carried out by Duncan's test. The average samples for chemical analyses were taken randomly at the time of fruit maturity by fertilization variants. The chemical analysis of the fruit flesh included determining the N, P, K, Ca, Mg, Fe contents, the dry matter content - refractometrically by Brix%; sugars - by Schoorl - Regenbogen; the acid content - titrimetrically and the active acidity (pH) - by potentiometric titration.

RESULTS AND DISCUSSIONS

All the bioproducts had a better effect on the yield compared to the untreated control variant. In 2019 the results obtained for the yield in all the three variants of fertilization showed the same tendency, with significantly higher yields compared to 2018.

Data on rainfall and the average, maximum and minimum daily temperatures during the growing season in the years of the study are presented in Figure 1.

Experimental year 2018 was characterized by humid weather, rainfall reaching 480 mm and 34% probability. The year was very hot: the average daily temperature was 20ºC and 2% probability. The maximum temperatures exceeded 35ºC during the second and third decades of July, as well as throughout August, which was not favourable for the peach crop, since that is the period of fruit growth and ripening. Precipitation during the vegetation period of 2019 was 431 mm and the year was characterized as moderately humid and 16% probability. However, with the exception of the heavy rainfall in May, the precipitation probability was 63%. The year was hot: the average daily temperature was 19.6º C and 13.5% probability. The maximum temperatures exceeded 35ºC mainly in the second and third decades of August (Figure 1).

In terms of their climatic characteristics, the years of the study covered a wide enough spectrum, which makes the results representative.

The yields obtained in 2018 after soil and foliar application of Lumbreco showed no statistically significant difference between the two variants and they amounted to 29.5 kg/tree and 28.2 kg/tree, respectively. The highest yield (43 kg/tree) was reported in the variant of combined application of the fertilizer, the differences to the other variants and to the untreated control being statistically significant (Figure 2). In 2019 the highest yield was reported in the variant with soil application of Lumbreco - 71 kg/tree, followed by foliar + soil application - 61 kg/tree, the differences to the control being statistically significant.

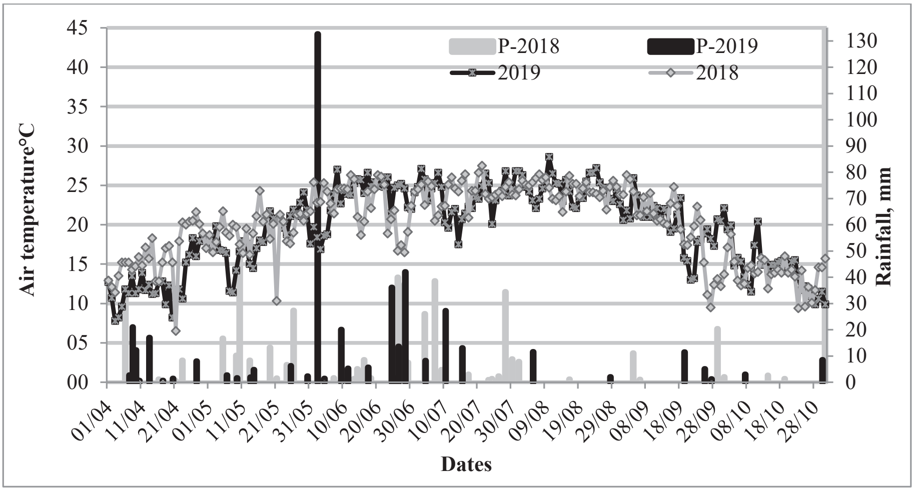

Figure 1. Rainfall and course of the average and the maximum daily temperatures during the vegetation period of 2018 and 2019

The results obtained for the yields are presented in Figures 2, 3 and 4.

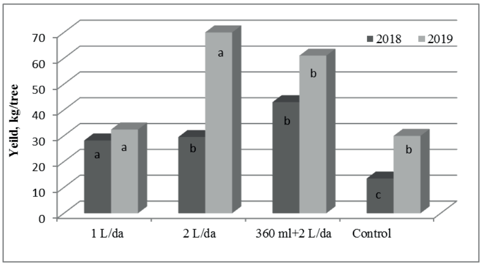

Figure 2. Yield per tree from 'Glohaven' cv. in different variants with application of Lumbreco during the period 2018-2019

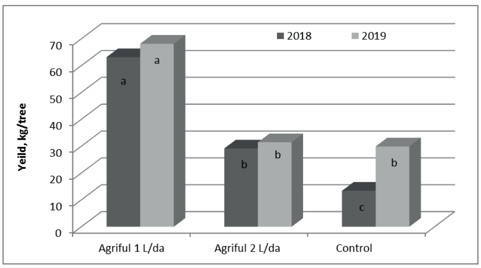

Figure 3. Yield per tree from 'Glohaven' cv. in different variants with application of Agrifull during the period 2018-2019

For Agriful, the rate of 1 L/da showed the best results for both years of the study, the yields being 63 kg/tree (2018) and 68 kg/tree (2019) versus 29 and 31 kg/tree at the rate of 2 L/da. The higher fertilization rate depressed the yields and resulted in a yield comparable with that of the control trees (Figure 3).

The results obtained after the application of Humustim were quite controversial over the two years. In 2018, the lowest fertilization rate of 200 ml/da gave a yield comparable with the control - 14.83 kg/tree. The yields obtained with increasing rates of fertilization also increased, the differences to the control being statistically significant (Figure 4).

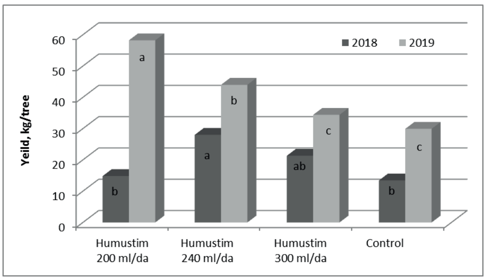

Figure 4. Yield per tree from 'Glohaven' cv. in different variants with application of Humustim during the period 2018-2019

It was not the case in 2019, when by far the highest yield was reported at the rate of 200 ml/da, but with the rate increase the average yield per tree decreased and at the rate of 300 ml/da the yield was 34.4 kg/tree and it was similar to that in the control - 29.93 kg/tree.

The differences between the variants are even more obvious when calculating the average yield per ha. Agriful resulted in the highest average yield of 32.16 t/ha, followed by Lumbreco with 29.64 t/ha. The yield was close to the control plants in the variant treated with Humustim (22.42 t/ha) (Table 1).

Table 1. Average yields of 'Glohaven' cv. after treatment with different bioproducts for the period 2018-2019

Variants	Yeild, t/ha 2018	2019	Average yeild, t/ha
Lumbreco	22,48	36,79	29,64 b
Agriful	30,93	33,38	32,16 b
Humustim	14,33	30,51	22,42 a
Control	9,00	20,05	14,53 a

The yields obtained after treatment with Agriful and Lumbreco bioproducts were optimal for the peach crop and can successfully replace the application of chemical fertilizers. The recommended rate of Agriful is 1 L/da and of Lumbreco - soil application of 2 L/da, as well as the combined application (soil + foliar) of the bioproduct.

With regard to the biochemical parameters of the peach fruits, differences were found between the years of the study. In 2018, lower levels of total sugars were observed for all the variants with fertilization. The values were about a unit lower compared to 2019. In 2019 the characteristics Brix, % and total sugars, % were higher in all the treated variants (Table 2). The highest values 12.7% were reported in 2019, when the climatic factors were more favourable for the peach crop. The precipitation sum during the vegetation season was 431 mm, i.e. the year is characterized as average.

More pronounced differences were found between the applied fertilization rates of a given bioproduct than between the bioproducts. Increasing the fertilization rates increased the dry matter content (Brix, %) and the total sugars content. Brix soluble solids content ranged within 8.7% to 12.7%. The best values of 12.7% and 12.0% were reported in the variants treated with Lumbreco at all the rates applied and those with Agriful at a rate of 1 L/da - 12.2%. The sugar content varied from 4.7% in the untreated control to 10.44% in the variant with Lumbreco nutrition. Increasing Lumbreco rates resulted in an increase in total sugars by 2.74%.

Table 2. Biochemical composition of peach fruits of 'Glohaven' cultivar at different treatment variants

Variants/Rate	Lumbreco 1 L/da	Lumbreco 2 L/da	Lumbreco 360 ml+2L/da	Agriful 1.0 L/da	Agriful 2.0 L/da	Humustim 200 ml/da	Humustim 240 ml/da	Humustim 300 ml/da	Control 0
2018									
Brix, %	11.40	11.80	11.8	11.4	10.8	8.70	9.60	10.30	8.30
Total sugars, %	6.88	7.08	7.44	6.96	6.60	4.84	5.18	6.60	4.70
Inverted sugar, %	1.68	1.74	2.20	1.80	1.80	1.94	1.54	1.62	0.96
Sucrose, %	4.94	5.07	4.98	4.90	4.56	2.76	3.46	4.73	3.55
Total acids, %	0.42	0.39	0.40	0.49	0.39	0.51	0.42	0.44	0.50
pH	3.82	3.65	3.66	3.48	3.61	3.57	3.54	3.53	3.57
Acidimetritic coefficient	16.36	18.15	18.75	14.08	16.77	9.47	12.42	14.87	9.38
2019									
Brix, %	12.00	12.00	1270	12.2	10.9	11.6	11.2	11.40	10.6
Total sugars, %	8.14	10.44	9.68	8.48	9.04	7.58	8.48	9.74	7.70
Inverted sugar, %	2.20	2.60	2.54	2.54	1.94	1.94	2.00	1.80	2.20
Sucrose, %	5.64	7.45	6.78	5.64	6.75	5.36	6.16	7.30	6.18
Total acids, %	0.42	0.35	0.39	0.43	0.38	0.38	0.42	0.39	0.43
pH	3.70	3.68	3.64	3.79	3.81	3.79	3.62	3.69	3.68
Acidimetritic coefficient	19.21	30.14	24.81	19.54	23.79	19.77	20.33	24.96	17.89

Sucrose is the dominant sugar in peaches. Fruits are characterized by high values of its content. Sucrose values are on average about 2.7 times higher than the values of inverted sugars. The best sucrose values of 7.45% and

6.78% were found in the variants of soil and combined treatment with Lumbreco.

The acid content was not significantly affected by the fertilizers applied. Acids ranged from 0.39% to 0.50% in the different fertilization variants.

The sugar-acid ratio is an indicator that characterizes the taste qualities of fruits. It gives information about the balance between total sugars and acids in them. In the studied variants, the sugar-acid ratio ranged from 9.38 to 30.14. The results showed different tendencies during the years of the study, but the values were optimal in all the variants. In the two experimental years the control plants yielded a sugar-acid ratio that was lower compared to the variants with the application of bioproducts.

Elemental analysis of fruits is not a common practice to estimate the sufficiency of a fertilization programme and the nutritional value of fruit (Basar, 2006). Peach fruits are rich in potassium, magnesium, phosphorus and calcium. The major nutrient found in the mesocarp of peaches is potassium, where it is accumulated with fruit ripening (Tagliavini et al., 2000). Optimal K content leads to a high degree of photosynthesis and translocation of soluble sugars and organic acids, thus improving fruit quality (Crisosto & Costa, 2008).

The results obtained for the chemical composition of the fruits of 'Glohaven' cultivar are presented in Table 3.

Table 3. Concentrations of the elements in the fruit flesh samples of the variants

	Percentage					mg kg^{-1}
Variants	N	P	K	Ca	Mg	Fe
Lumbreco I	0.67 b	0.192 a	1.58 d	0.132 d	0.704 a	69.85 c
Lumbreco II	0.67 b	0.194 a	1.67 c	0.639 a	0.608 b	97.88 a
Lumbreco III	0.99 a	0.112 b	1.84 b	0.256 c	0.578 c	38.96 d
Control	0.66 b	0.062 c	1.96 a	0.400 b	0.305 d	81.25 b
Agriful 1 L/da	0.91 a	0.176 b	1.72 c	0.390 a	0.419 a	73.90 b
Agriful 2 L/da	0.77 b	0.234 a	1.91 b	0.118 c	0.424 a	70.93 c
Control	0.66 c	0.062 c	1.96 a	0.400 a	0.305 b	81.25 a
Humustim 200 ml/da	0.64 c	0.103 b	1.77 b	0.256 d	0.303 c	64.37 c
Humustim 240 ml/da	0.76 b	0.128 a	1.96 a	0.390 c	0.508 a	71.12 b
Humustim 300 ml/da	0.88 a	0.138 a	2.00 a	0.517 a	0.450 b	72.07 b
Control	0.66 c	0.062 c	1.96 a	0.400 b	0.305 c	81.25 a

The results obtained show that the imported bioproducts did not significantly influence the content of the major macro elements. The higher fertilization rates had a positive effect on the nitrogen, phosphorus and magnesium content, the differences to the untreated control being statistically significant for phosphorus and magnesium (Table 3). Potassium and calcium contents in fruits were optimal in all the variants. The opposite tendency was observed for those elements - the application of bioproducts had a negative effect on the content of those elements in the fruit flesh. The control plants had higher values than the treated plants, the differences being statistically significant. Probably that can be explained by the good soil supply with potassium.

CONCLUSIONS

The yields obtained after treatment with Agriful and Lumbreco bioproducts were optimal for the peach crop and the bioproducts can successfully replace the application of chemical fertilizers. The rate of 1 L/da is recommended for Agriful and for Lumbreco - soil treatment at a rate of 2 L/da, as well as a combined application (soil treatment with 2 L/da + foliar application of 360 ml/da).

The biochemical parameters of peach fruits showed more pronounced differences between the applied fertilization rates of a given bioproduct than between the separate organic products used. The best Brix % values of 12.7% and 12.0% were established in the variants treated with Lumbreco at all the rates applied and those with Agriful - at the rate of 1 L/da - 12.2%.

The bioproducts used (Agriful, Lumbreco and Humustim) did not significantly affect the content of the major macroelements. The higher rates had a positive effect on nitrogen, phosphorus and magnesium contents.

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THE GRAFTING BEHAVIOR OF 'ANDREEA' PLUM CULTIVAR GRAFTED ON GENERATIVE ROOTSTOCK WITH INTERMEDIARY

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Abstract

The behaviour of 'Andreea' plum variety was studied, on different rootstocks and with different grafting variants. As rootstock, selections of Prunus cerassifera and armeniaca vulgaris were used. And the graft variants were with the intermediary (Rival) and without the intermediary, by different grafting methods (improved copulation and budding chip). The diameter was analyzed at the grafting point, 5 cm above and below the grafting point, graft height, length and number of shoots. The results show that regardless of the rootstock used, the intermediary influences the growth force in a negative way.

Key words: *grafting, rootstock, intermediary.*

INTRODUCTION

Grafting is a common practice for fruit tree propagation. The rootstocks used for a specific culture are close relatives of the culture or wild selections (especially within the genera), but grafting between different species has also been observed (Warschefsky et al., 2016). The use of rootstocks that control the vigour is a method used to promote precociousness, reduce vigour and increase productivity (Webster, 2004). Also, grafting with intermediary is used to induce tolerance to cold, resistance to disease and vigour reducing (Rogers and Beakbane, 1957). The rootstock selection is a powerful tool for sustainable intensification of fruit production, as while graft can be used to induce fruit properties, adaptation to water deficit and high salinity, tolerance to alkaline soils and susceptibility to agents can be influenced by rootstock choice. (Jensen et al., 2012; Marguerit et al., 2012; Tamura, 2012). Necas and Krska (2013) evaluated the propagation potential and efficacy of fruit tree rootstocks using different phytohormone concentrations.
Koepke and Dhingra (2013) stated that rootstock controls many aspects of graft growth and physiology, including quality production and attributes, as well as biotic and abiotic stress tolerance, and the study of somatic genetic interactions between rootstock and graft is an area that can bring vast improvements in the next decade in today's agricultural environment, where sustainable production practices are needed, the rootstock offering a non-transgenic approach to respond quickly to the changing environment and to expand agricultural production of annual and perennial crops, where grafting is possible to responds to the needs of global food, fiber and fuel.
The plum cultivar 'Andreea' was obtained at SCDP Vâlcea, Romania, in 2000. The tree has medium vigour, with semi-erect bearing resistant to moniliosis and red spots of leaves, tolerant to Plum pox. The middle blossoming timing, partially self-fertilizing, requiring pollinators: Stanley, Anna Spath. The fruits are ripening at the end of August, showing a staggering ripening.
As generative rootstocks for grafting the 'Andreea' plum cultivar, the cherry plum (*Prunus cerasifera*) and the apricot tree (*Armeniaca vulgaris*) were used, and the Rival vegetative rootstock was used as an intermediary.
Specific to culture technology, the use of grafted material brings many advantages (Ercisli et al., 2006). Studies conducted on the influence of intermediary on growth parameters of grafted trees, have shown that the intermediary, depending on its length, has a greater or lesser impact on precocity and vigour, noting that the length of intermediary caused the reduction of trees vigour and fruit

production (DI VAIO et al., 2009). Moreover, it seems that the diameter and the number of wood vessels are the main characteristics of rootstock that influence the vigour of grafted trees, the absolute values of measured parameters being higher in the grafted trees with intermediary (Tombesi et al., 2010).
Hernández et al. (2010) analyzed the influence of rootstocks on flowering, cross-section area of trunk, yield and quality parameters of *Prunus armeniaca* L. fruits, grown in a Mediterranean agro-climatic environment, in an experimental orchard in the southeast Spain, also showed that the rootstock had no significant influence on the number of flowers, but induced a higher weight of fruits, the fruit weight being positively correlated with pulp production and negatively with the TCSA.
Also, the precocity and colour of fruits was influenced by rootstocks. Zhilong (2016) analyzed the effects of rootstocks on the increase of absorption, accumulation and use of nutrients, as well as the mechanism involved.
The aim of this study was to reduce the vigour of grafted variety for setting up superintensive plantations; for inducing the fruit-setting precocity and quantitative and qualitative improvement of fruits.

MATERIALS AND METHODS

The experiment regarding the studied material was established at the Didactic Station, Banu Mărăcine of the University of Craiova, located in the southern extremity of Getic Plateau between the coordinates 44º19'' north latitude and 23º48'' east longitude, at a distance of 6 km from the town of Craiova.
The grafting was performed at the table, both of the intermediary on the two rootstocks by the improved copulation method, and of cultivar on the intermediary using the chip budding method. The rootstock seedlings were harvested in autumn after the fall of leaves from the seedling and stratified nursery, and before grafting it was put to pre-forcing. Scions were harvested in autumn and kept in the refrigerator at 4°C. Both methods of grafting, both of the intermediary on the two rootstocks (cherry plum and apricot tree) in improved copulation and of the cultivar on the intermediary in chip budding, were performed in the first ten days of March. The grafting of intermediary on rootstocks and grafting of cultivar on the intermediary were carried out at the same time. The length of the intermediary was 20 cm. The same rootstocks and cultivars were used as control but without the Rival intermediary. The experiment is of a bifactorial type, located in a linear way.
Maintenance operations were carried out at regular intervals, such as removal of shoots started from rootstocks, irrigation, fertilization, phytosanitary treatments. At the end of vegetation period, the following observations and determinations were made: diameter (mm) at grafting point, diameter (mm) 5 cm above grafting point, diameter (mm) at 5 cm below grafting point, graft height. For determination of diameter, the electronic caliper with an accuracy of 0.01 mm was used. The determinations were made for both intermediate and non-intermediate variants. The data obtained were processed using the statistical analysis program (Stat Point Technologies, Warrenton, VA, USA).

RESULTS AND DISCUSSIONS

Rootstock improves the vigour of plants, extends the vegetation period (Lee et al., 2010), productivity and fruit quality (Huang et al., 2011; Rouphael et al., 2010; Tsaballa et al., 2013), extends the quality of fruits after harvest. (Zhao et al., 2011), increases tolerance to low and high temperatures (López-Marín et al., 2013; Li et al., 2016), reduces stress caused by salinity and heavy metals (Santa-Cruz et al., 2002; Estañ et al., 2005; Albacete et al., 2009; Schwarz et al., 2010; Huang et al., 2013a; Wahb-Allah, 2014; Penella et al., 2015, 2016), increases resistance to floods (Bhatt et al., 2015), improves efficiency of water usage (Cantero-Navarro et al., 2016), manages resistance to soil pathogens (Arwiyanto et al., 2015), manages nematode resistance (Lee et al. ., 2010), gets the weed control (Dor et al., 2010; Louws et al lab., 2010) and produces new plant species (Fuentes et al., 2014).
The grafting with intermediary was carried out in order to reduce the vigour of graft in order to intensify the plum crops, the earliness of the fruit-setting, to increase the productivity and quality of fruits. The obtained results regarding

Table 1. Characteristics of studied material

Variants	Descriptive Statistics	Diameter (mm)			Graft height
		At grafting point	At 5 cm above grafting point	At 5 cm below grafting point	
Andreea – Cherry plum	Average ± SE	18.25 ± 0.48	10.73 ± 0.43	11.98±0.46	113.79 ± 3.54
	Standard Deviation	1.79	1.62	1.75	13.26
	Minim/ Maxim	14.11/20.63	6.65/12.91	8.99/16.31	95/148
	Variation coeff. (CV %)	9.80	15.09	14.60	11.65
Andreea – Rival - Cherry plum	Average ± SE	12.62 ± 0.69	7.86 ± 0.35	9.58 ± 0.23	69.06 ± 3.64
	Standard Deviation	2.77	1.41	0.94	14.58
	Minim/Maxim	9.35/19.09	5.29/10.49	7.48/10.83	50/99
	Variation coeff.	21.94	17.96	9.82	21.11
Andreea – Apricot	Average ± SE	21.22 ± 0.51	12.12 ± 0.38	12.62 ± 0.32	130.03 ± 5.96
	Standard Deviation	2.79	2.11	1.80	32.65
	Minim/Maxim	13.33/26.34	6.21/16.53	9.17/16.61	84/211
	Variation coeff.	13.15	17.42	14.27	25.11
Andreea – Rival - Apricot	Average ± SE	13.57 ± 0.54	8.72 ± 0.25	10.21 ± 0.20	84.43 ±3.05
	Standard Deviation	2.16	1.22	0.96	14.65
	Minim/Maxim	10.47/18.13	6.17/11.03	8.76/12.30	60/117
	Variation coeff.	15.92	13.99	9.40	17.35

the characteristics of the studied material are presented in Table 1.

The average values regarding the height of the studied material show significant differences in the four variants studied, namely in the variants grafted with intermediary, the average height of graft was 69.06 cm in Andreea-Rival-cherry plum variant and 84.43 cm in Andreea- Rival-apricot, compared to the variants grafted without intermediary, namely: Andreea-cherry plum where the average height was 113.79 cm, and for the Andreea-apricot variant the average height was 130.03 cm.

Variation range regarding the height of graft had values between 50 cm and 99 cm for Andreea-Rival-plum cherry variant, 60 cm and 117 cm in Andreea-Rival-apricot variant and in variants without intermediary the limits were between 95 cm and 148 cm in Andreea-plum cherry and 84 cm and 211 cm in Andreea-apricot.

With regard to graft growth, major differences were observed in grafted variants without intermediary compared to the grafted variants with intermediary, highlighting the role of the intermediary in grafting. The coefficient of variation regarding the height of graft had the following values 21.11% in Andreea-Rival-plum cherry variant, respectively 17.35% in Andreea-Rival-apricot variant, and in the non-intermediate variants the values were 11.65% in Andreea-plum cherry and 25.11% in Andreea-apricot. The largest length of shoots growth was recorded in Andreea-apricot variant (211 cm).

The vigour of fruit trees and the vegetative growth is generally given by the size of trunk diameter. The determinations made on the values of diameter at the grafting point revealed that the variants grafted with intermediary had lower values, 12.62 mm in Andreea-Rival-plum cherry variant, respectively, 13.57 mm in Andrea-Rival-apricot variant, compared to the grafted variants without intermediary, which had values of 18.25 mm in Andreea-plum cherry variant and of 21.22 mm in Andreea-apricot variant.

Regarding the material studied, the limits were significant regarding the diameter at the grafting point being between 9.35 / 19.09 mm in Andreea-Rival-cherry plum variant and 10.47/18.13 mm in Andreea-Rival-apricot variant, and in variants without intermediary the limits were between 14.11/20.63 mm in Andreea-cherry plum and 13.33/26.34 mm in Andreea-apricot. The values of variation coefficient on this feature were between 21.94% in Andreea-Rival-cherry plum variant and 15.92% in Andreea-Rival-apricot variant, and for non-intermediary variants they were 9.80% in Andreea-cherry plum and 13.15% in Andreea-apricot. The values of variation coefficient indicate a specific average variability for diameter at grafting point. The diameter at 5 cm above the grafting point for the variants grafted with intermediary had the average value between 7.86 mm in Andreea-Rival-cherry plum respectively 8.72 mm in Andreea-Rival-apricot and in grafted variants without intermediate the average value was 10.73 mm in Andreea-cherry plum and 12.12 mm in Andreea-apricot. Variation range for variants grafted with intermediate had values between 5.29/10.49 mm (Andreea-Rival-cherry plum) and 6.17/11.03 mm (Andreea-Rival-cherry plum), and for variants without intermediary they were 6.65/12.91 mm (Andreea-plum cherry) and 6.21/16.53 mm in Andreea-apricot. Variation coefficient had the following values, 17.96% (Andreea-Rival-cherry plum), respectively, 13.99% (Andreea-Rival-apricot) and 15.09% in Andreea-cherry plum, respectively, 17.42% in Andreea-apricot.

The average values of diameter at 5 cm below grafting point in the 2 variants grafted with intermediary were 9.58 mm (Andreea-Rival-cherry plum), respectively, 10.21 mm (Andreea-Rival-apricot), and for grafted variants without intermediate were in Andreea-cherry plum variant of 11.98 mm, respectively, 12.62 mm Andreea/apricot. Variation coefficient had values of 9.82% (Andreea-Rival-cherry plum), and 9.40% (Andreea-Rival-apricot), and in variants without intermediary the values of variation coefficient were 14.60% in Andreea-cherry plum, and 14.27% in Andreea-apricot. The values of variation coefficient for this characteristic show an average variability, indicating a good uniformity within the studied variants.

Di Vaio et al. (2009) showed that in apple tree the intermediate determined a vegetative growth of less than 50-80% compared to control plants, an increase of fruit production and the average weight of fruits and the increase of length of the intermediate caused the reduction of growth vigour of plants and the reduction of fruit production, the length of 10 cm of the intermediary being the one that gave the best results.

Many other scientific studies confirm the intermediary's effectiveness in controlling the vigour of trees and, in some cases, in inducing early fruiting and increased productive efficiency and fruit quality (Vercammen et al., 2007; Samad et al., 1999; Webster, 1995).

It is recommended to choose the intermediary based on agronomic factors, such as distance from the graft point and the length of intermediary (Beakbane and Rogers, 1956; Rufato et al., 2001).

Other experiments have shown that the decrease of growth induced by the intermediary depends on its vigour (Lockard and Lasheen, 1971), the rootstock and the cultivar used (Tukey, 1943; Carlson, 1965).

In order to verify criteria between the characteristics of material studied, the correlation coefficient between: diameter at grafting point and the height of graft, the diameter at 5 cm above grafting point and the height of graft, the diameter at 5 cm below grafting point and the height of graft, was determined, the correlation between the three diameters and the number of shoots, the correlation between the three diameters (at the grafting point, 5 cm above and 5 cm below the grafting point) and the total length of shoots (Table 2).

From the data obtained it is found that there are positive correlations between the three diameters, at the grafting point, 5 cm above and 5 cm below the grafting point and the graft growth in all the analyzed variants, the highest values being in Andreea-apricot (r = 0.76), and Andreea-Rival-apricot (r = 0.75), between the three diameters and the total length of shoots, in Andreea-apricot (r = 0.70), at Andreea-Rival-apricot (r = 0.81).

Table 2. Correlation coefficient of characteristics in studies material*

Characteristics/ Variants	H				No. of shoots				Total length of shoots			
	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
DP	0.54	0.59	0.54	0.56	-0.10	0.23	-0.06	0.39	-0.28	0.50	0.06	0.59
DDP	0.52	0.76	0.64	0,75	0.38	0.23	0.12	0.57	0.23	0.56	0.27	0.81
DSP	0.17	0.57	0.34	0.40	0.38	0.49	0.08	0.44	0.57	0.70	0.39	0.61
No of shots/total length of shoots	V1			V2			V3			V4		
	0.91			0.77			0.77			0.76		

*DP: Diameter at grafting point; DDP: Diameter at 5 cm above grafting point; DSP: Diameter at 5 cm below grafting point; V1: Andreea-cherry plum; V2: Andreea-apricot; V3: Andreea-Rival-cherry plum; V4: Andreea -Rival-apricot.

Significant correlations were also calculated between the number of shoots and the total length of shoots, for all four variants analyzed (r = 0.91; r = 0.77; r = 0.76). The rootstock has a higher vigour to increase the graft compared to the rootstock on both graft variants (with and without intermediary).

The role of the intermediary used in the grafting process is to ensure the compatibility between graft and rootstock; to reduce the growth vigour of grafted cultivar; induces the precociousness of fruit-setting; ensures adaptability to environmental conditions of grafted cultivar it influences the increase of productivity and fruit size.

CONCLUSIONS

Resulting from the study, it was noted that grafting with an intermediary has a key influence on reducing the growth vigour in order to intensify the plum crops.

Apricot graft has imprinted a higher growth vigour of the graft compared to the cherry plum rootstock in both grafting variants (with and without intermediary).

The largest growth length of anticipated shoots was recorded in Andreea-apricot variant.

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STUDY ON THE MATURITY EVOLUTION OF SOME GRAPE VARIETIES FOR ESTABLISHING THE CORRECT HARVEST TIME IN THE CERNAVODA VINE CENTER

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Abstract

The paper's aim was to present the maturity evolution for three grapes varieties, for establishing the correct harvest time in 2019 in Cernavoda vine Center. These were: 'Victoria' - grapes for table, 'Riesling Italian' - grapes for white wine and 'Merlot' - grapes for red wine, for which there were determined the moments of commercial, full and technological maturity, settling the optimum time of the grapes harvest according to the degree of ripen, which is an important factor in the quality of future wine. The data obtained indicated that the grapes 'Victoria' had reached commercial maturity in August 24, with the caracteristics: sugars - 156.7 g/l and total acidity - 3.5 g/l H_2SO_4. The grapes, 'Riesling Italian' had reached full maturity in September 25, with the follow characteristics: sugars - 230 g/l and total acidity - 5.2 g/l H_2SO_4, and the black grapes 'Merlot' had reached in the same time full and phenolic maturity in October 3, with the characteristics: sugars - 235 g/l, total acidity - 6.02 g/l H_2SO_4 and anthocyanins 1680 mg/kg. The mechanical composition of the grapes was analyzed and the uvological indices were calculated, and the conclusion was that these are adequate to each variety.

Key words: *commercial maturity, full and phenolic maturity, total acidity, uvological indices.*

INTRODUCTION

Establishing the optimum harvesting time, consisted in knowing and choosing that data during the maturity (ripening) period in which the grapes correspond qualitative to their purpose and destination. The factor that determines the quality of the grapes is their maturity. This is influenced by: the geographical position of the vineyard, the forms of relief, climate, soil, agrotechnical works applied to the soil and the plants. The maturity of the grapes is also appreciated according to their destination, that they are for direct consumption or processing. Those destined for processing, are evaluated according to the category and the type of wine expected to be obtained. As an evolutionary phase, maturation is a genetically coordinated physiological process characterized by complex morpho-anatomical and chemical changes (Pomohaci, 2000). During maturation, the processes of accumulation in sugars, organic acids, nitrogenous substances, vitamins, aromatic substances, a.s.on, are considerably increased (Târdea et al., 2010).

The epicarp changes its green color, becomes translucent and softens due to pectin hydrolysis.

In this stage the grapes increase their volume due to the cells elongation and accumulations of assimilated, especially sugars (Antoce, 2007).

The grape varieties for wine have a greater capacity for sugars accumulation compared to those for the table. Other important processes that occur in grapes during this period are: reduction of acidity, hydrolysis of pectic substances, accumulation of color (anthocyanins) and aromatic compounds (Cotea et al., 2009). The grapes maturation process is influenced by the vine variety and the climatic conditions and therefore it is very important to follow the dynamics of grape maturity in order to determine the optimal moment of grape harvest (Beleniuc and Beleniuc, 2015).

The grapes maturity is divided in: - physiological maturity; full maturity; technological maturity, over maturity (over ripening), commercial maturity (Cotea, 1985).

MATERIALS AND METHODS

The working material was represented by three vine varieties: 'Victoria'-grapes for table, 'Riesling Italian'-grapes for white wine and 'Merlot'- grapes for red wine, to which were determined the moments of commercial maturity, full and technological maturity, settling the optimum time of the grapes harvest according to the degree of ripping, which is an important factor in the quality of future wine. (Figure 1)

Figure 1. Grapes variety: 'Victoria', 'Riesling Italian', 'Merlot'

These three varieties are grafted on the rootstock of the Berlandieri x Riparia Oppenheim Selection 4 and planted at 2.2 m distances between rows and 1.1 m between plants in a row; the area of a vine nutrition is 2.42 m^2; the vine number/ha - 4132; rows orientation N-S; support system: concrete pillars; number of wires: 6 (two load-bearing wires and two double rows for off shoots directing); trunk highness: semi-tall with 70 cm; driving form: double Guyot. The paper's aim was to present the maturity evolution of these three grapes varieties, for establishing the correct harvest time in 2019 year in Cernavoda vine Center.

METHODS OF STUDY

The study included observations in the vine plantations and laboratory analyzes to determine the characteristics of the grapes according to the time of ripening. Observations and determinations made in plantations are referring to: - the ecological relations between the vine and the local conditions; - the effective heliothermic resources; - the dynamics of the grape's maturity, starting from the beginning of maturity to the full or technological maturity of the grapes and then to the harvest.

In 2019, from the beginning of the maturation, grapes samples were taken from 5 to 5 days, and as the maturation process advanced, from 3 to 3 days. The sample consists of grapes from 20 vines, located at different points in the plot. With a scissors, small portions of bunches of 3-5 grape berries were harvested from the grapes located in the center of the vine and from those on the sunny or shaded side. Very ripped grapes or those with a large number of grape berries affected by mold were avoided. Grapes of all sizes have been harvested, because large grapes are high in sugar and have low acidity while small grapes are poorer in sugar and have high acidity. Samples were put in plastic bags labeled with variety, date of harvest and plot number. The sample taken weighed 1kg/variety. The samples were worked on the same day, but until the analysis started, they were kept in the refrigerator at 40ºC.

In laboratory were analyzed in dynamic:

- the weight of 100 grapes berry (g) starting from the beginning of maturity and to harvesting, was determined gravimetrically using a technical balance (Figure 2);

Figure 2. The separation of 100 grapes from clusters and weighing them, from the varieties: 'Victoria', 'Merlot'

- sugar content (g/l), by refractometric method;
- total acidity (g/l H_2SO_4), by titrimetric method using a NaOH solution of a certain normality; These have been determined from the musts. 'Victoria', 'Riesling Italian' and 'Merlot' grapes varieties, have been crushed well and the resulting musts have been placed in tubes that were stored in the refrigerator for a few hours to clear them. Sugar and total acidity have been determined from the clear musts (Figure 3).

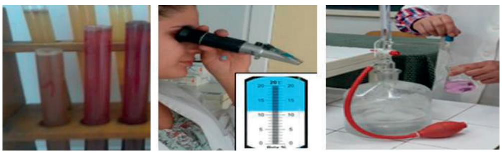

Figure 3. Musts in the tubes for clearing; sugars evaluation; total acidity evaluation

The data obtained have been recorded and helped us to realize the maturation graphs of the varieties;

- anthocyans content (for black grapes only for determining the phenolic maturity), by spectrophotometric method. After weighing 100 Merlot grape berries, they were crushed and treated with strongly acidic HCl solution (pH = 1) and macerated for 4 hours. Then the colored extract was filtered and using the spectrophotometer the optical density (DO280) of the extracts was measured and the content in anthocyanins (A) was determined. In addition, the mechanical composition of the grapes was analyzed and the uvological indices have been calculated. All of these have been made in the wine technology and chemistry laboratory of the Cernavoda vine center.

RESULTS AND DISCUSSIONS

The climatic factors evolution in the ecosystem of Cernavoda viticol centre was monitored using the WS-GP1 performance meteorological station (air temperature value, precipitation, hygroscopicity and sun shine time). In the Figure 4 and Table 1, it can be observed that during the nonvegetative vine period (November - February) 2018-2019 was slightly warmer than normal, the average temperatures of January and February were higher than the multiannual averages (5.9°C compared to 0.5°C normal value for January, and 7.9°C in February compared to 1.3°C).

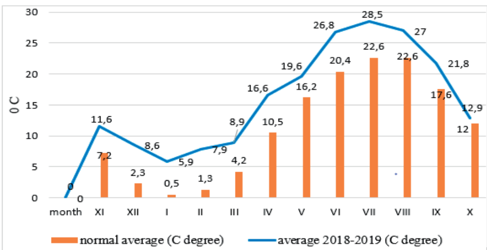

Figure 4. The evolution of the average monthly temperatures in the viticol year 2018-2019

The absolute minimum temperatures recorded at the beginning of January of - 14.0°C (02.01.2019) did not affect the vines winter buds. This is because the minimum temperatures were recorded in one day only. In the spring months, the temperatures were higher than normal (March 8.9°C versus 4.2°C multi-year average, and in April with 6.1°C higher than normal values). Air temperatures in the summer months were higher than normal (in August the absolute maximum was 38.7°C.

Table 1. Average monthly temperatures in the vine year 2018-2019 compared to the multiannual (normal)

Month	Average temperature ^{0}C		Absolute maximum temp ^{0}C 2018-2019
	Normal	2018-2019	
XI	7.2	11.6	23.9
XII	2.3	8.6	17.9
I	0.5	5.9	18.0
II	1.3	7.9	24.9
III	4.2	8.9	27.1
IV	10.5	16.6	36.0
V	16.2	19.6	34.1
VI	20.4	26.8	36.3
VII	22.6	28.5	38.1
VIII	22.6	27.0	38.7
IX	17.6	21.8	36.3
X	12.0	12.9	26.4
average	11.45	16.34	

During the vegetation period, the amounts of global temperature levels, (Σ^0C global), active (Σ^0C active) and useful (Σ^0C useful), have been much higher than the multiannual values (Table 2). Thus, the global thermal balance was 4,234.4^{0}C compared to 3422,0^{0}C multiannual value, the active thermal balance was 4,234.4^{0}C with 903,8^{0}C compared to the normal, and the useful thermal balance of 2,435.4^{0}C compared to 1,615.6^{0}C multi-year average by almost 820^{0}C more.

Table 2. Rainfall in the viticol year 2018-2019

Month	Global (Σ°C)		Active (Σ°C)		Useful (Σ°C)	
	Normal	2019	Normal	2019	Normal	2019
IV	369.7	477.5	219.8	477.5	53.8	187.4
V	513.7	608.1	513.6	608.1	203.7	297.0
VI	620.1	810.0	620.1	810.0	328.1	504.0
VII	726.3	863.9	726.3	863.9	416.3	564.9
VIII	671.0	825.3	671.0	825.3	361.0	523.4
IX	521.2	649.6	552.7	649.6	252.7	358.7
Σ°C	3422	4,234.4	3,303.6	4,234.4	1,615.6	2,435.4

In the viticol year 2018-2019 the rainfall recorded 47.9 mm higher than normal, and for this reason the year was considered to be a normal one. During the vegetation period (April - September), the amount of rainfall recorded was 26.0 mm less than the multiannual average (245.7 mm). In May, there was a precipitation surplus, 99.9 mm, and in the

other months a deficit between 1.5-32.4 mm. In July the deficit was a big one of 32.4 mm (Table 3). At that moment the grapes were between the growing and compacting grapes phenophase -and the beginning of the ripening process.
Referring to the air relative humidity, its value, during the vegetation period was close to the multiannual averages, between 60 and 77% (Table 3). The lowest amount of insolation was in May, when it totaled 163.4 hours compared to the normal of 261.8 hours. Total hours of sunshine exceeded the normal value (Table 3).

Table 3. Rainfall in the viticol year 2018-2019

Month	Rainfall (mm)		Higroscopicity %		Sunshine (hour)	
	Nor-mal	2018-2019	Normal	2018-2019	Normal	2018-2019
XI	40.4	70.0	82	83	87.2	135
XII	34.0	3.5	85.0	89	66.1	120
I	31.0	63.4	83.0	91	63.5	120.2
II	33.0	31.5	81.0	88	84.8	137
III	21.7	34.3	75.0	87	111.7	122
IV	33.5	22.5	72.0	73	160.7	197.1
V	50.2	99.9	68.0	77	261.8	163.4
VI	53.2	41.6	65.0	67	314.5	222
VII	35.6	3.2	61.0	60	323.7	356.9
VIII	31.6	19.9	60.0	62	305.5	309
IX	41.6	32.6	68.0	67	221.0	286.7
X	30.2	61.5	70.0	81	176,0	170.7
Ave-rage	436	483.9			2,176.5	2,340.0

- The evolution of the grapes maturity

The evolution of the maturation process for ‚Victoria' variety was carried out starting with 10.07.2019 (Table 4 and Figure 5) when the grapes characteristics were: weight of 100 grapes, 180.3 g; sugars, 52.2 g/l, total acidity, 10.5 g/l H_2SO_4. At harvest the grapes weight and acidity slightly decreased and sugars slightly increased.

Table 4. The evolution of the table grapes maturity in the Cernavoda vine center in 2019

Grapes variety	Date of sample harvest and analysis	Analised element		
		Weigh of 100 grapes berries (g)	Sugars (g)	Total acidity (g/l H2SO4)
'Victoria'	10.07. 2019	180.3	52.2	10.5
	15.07. 2019	199.0	58.1	9.4
	20.07. 2019	215.7	65.1	8.5
	25.07. 2019	240.0	79.6	7.4
	30.07. 2019	290.3	92.5	6.9
	04.08.2019	312.5	110.9	6.1
	09. 08.2019	337.4	129.6	5.6
	14. 08.2019	350.1	140.8	4.8
	21.08.2019	380.1	154.9	3.8
	24.08.2019	405.5	156.7 Commercial maturity	3.5
	26. 08.2019	395.2	157.1 Harvest	3.1

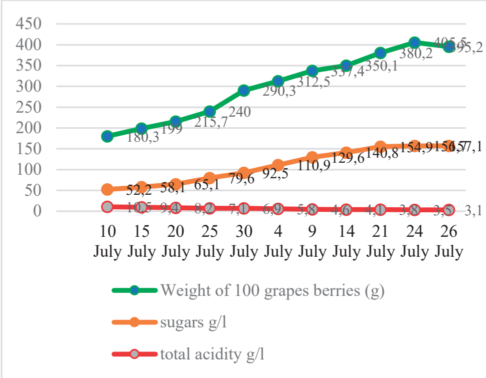

Figure 5. The evolution of the table grapes maturity in the Cernavoda vine center in 2019

From the Table 4 and Figure 5, it can be seen that the commercial maturity of the Victoria variety was reached on 24.08.2019, and the harvest started on 26.08.2019, the grapes having the characteristics: weight of 100 grapes, 405.5 g; sugars, 157.1 g/l, total acidity, 3.1 g/l H_2SO_4.
For the others variety, respectivly 'Riesling Italian' and 'Merlot' (both grapes for wine) the evolution of the full maturity of the grapes, started with 15.08. 2019. The data obtained are presented in the Tables 5 and 6.

Table 5. The evolution of the grapes full maturity for 'Riesling Italian' variety

Grapes variety	Date of sample harvest and analysis	Analised element		
		Weigh of 100 grapes berries (g)	Sugars (g)	Total acidity (g/l H_2SO_4)
'Riesling Italian'	15. 08.2019	94.5	168.5	9.4
	20. 08.2019	99.9	174.9	8.9
	25. 08.2019	106.3	180.7	8.2
	30. 08.2019	111.5	188.6	7.8
	05.09.2019	115.1	202.3	7.0
	10.09.2019	120.7	210.7	6.5
	15.09.2019	128.5	217.0	6.1
	20.09.2019	134.8	224.5	5.7
	25.09.2019	141.5	230.8 Full maturity	5.2
	28.09.2019	137.6	232.8 Harvest	4.7

From the Table 5, it can be seen that the grapes of 'Riesling Italian' variety reached full maturity in 25.09.2019, when the grapes had 230.8 g/l sugars and the weight of 100 grapes berries had 141.5 g, and a balanced acidity, than the last two parameters start to decrease. From the Table 6, the grapes of 'Merlot' variety reached full maturity in 03.10.2019, when the grapes had 235 g/l sugars, the weight of 100 grapes berries had 148 g and acidity 6.02 g/l H_2SO_4.

Table 6. The evolution of the grapes full and phenolic maturity for 'Merlot' variety

Grapes variety	Date of sample harvest and analysis	Analised element			
		Weigh of 100 grapes berries (g)	Sugars (g)	Total acidity (g/l H_2SO_4	Antho-cians mg/kg
'Merlot'	15. 08.2019	72	129.0	12.9	72
	20. 08.2019	78	137.5	12.1	190
	25. 08.2019	85	147.1	11.5	310
	30. 08.2019	98	161.9	10.6	420
	05.09.2019	105	169.7	9.7	580
	10.09.2019	112	182.6	9.1	750
	15.09.2019	120	194.7	8.5	890
	20.09.2019	128	207.8	7.9	1,070
	25.09.2019	136	217.1	7.0	1,360
	30.09.2019	140	228.7	6.5	1,560
	03.10.2019	148	235.0	6.02	1,680
		Full & phenolic maturity			
	06.10	143	236.1 Harvest	6	1,610

In this case the full maturity was reached in the same time with phenolic maturity (specific to black varieties) when the anthocyanins accumulated in the grape skin reached the maximum value of 1680 mg/kg (Figure 6), then these values started to decrease also.

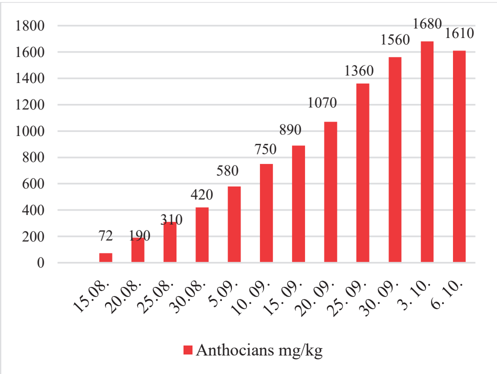

Figure 6. 'Merlot' - the quantity of anthocyanins in the grape skin (2019)

The data from Figure 6 show that the 'Merlot' grapes contain sufficient amounts of anthocyans, that according to the wine technology will ensure a specific color of the future 'Merlot' red wine.

At the beginning of the grapes maturity period (15.08.2019), the weight of 100 grapes was different according to variety, having values between 72-94.5 g. In the interval 15.08 - 31.08 2019, the increase of weight of the grapes was ascending, growing relatively little and differently depending on the variety (Figure 7).

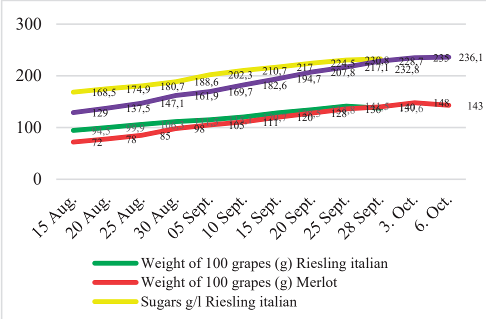

Figure 7. Evolution of grape weight and sugar content in 'Riesling Italian' and 'Melot' varieties

In the first decade of September due to precipitation, there was a slight stagnation of grapes growth. After this period due to the increased influx of leaf sugars and moderate amounts of water absorbed by plants from the soil, in order to achieve osmotic balance, the weight of the grapes increased, so that at harvest date they reached values of: 137.6 g - 'Riesling Italian' and 143 g - 'Merlot'. Referring to the sugars evolution (Figure 7), at the first determination they had values between 129 ('Merlot') and 168.5 g/l ('Riesling Italian') and than increased to 232.8 g/l ('Riesling Italian') and 236.1 g/l ('Merlot') at harvest.

The total acidity parameter at the beginning of the grape maturity, was 9.4 g/l H_2SO_4 ('Riesling Italian') and 12.9 g/l H_2SO_4 ('Merlot'). The total acidity decreased in both grapes varieties and at the end of the analyzed period (at harvest), its values registered between 4.7 g/l H_2SO_4 ('Riesling Italian') and 6.0 g/l H_2SO_4 ('Merlot') (Figure 8).

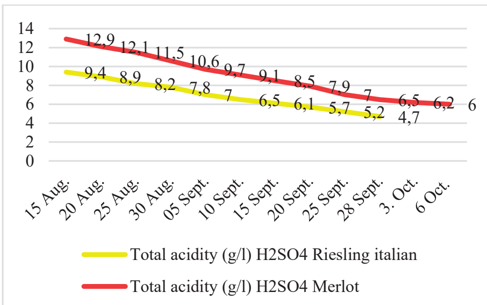

Figure 8. Total acidity evolution of 'Riesling Italian' and 'Merlot'

From the obtained data it appears that the evolution of the grapes weight and the concentration in sugars are the main indicators which help us to establishe the moment of full maturity. The study of the evolution of total acidity is important because, together with the concentration in sugars, provide us data on the grapes quality side.

The mechanical composition was determined for each grapes variety. This consist in the analysis of 1 kg of grapes, the analysis of 100 grapes berries and the calculation of the uvological indices (Table 7).

Table 7. Mechanical composition of the three grapes variety in 2019 year (average data)

Variety / Analized elements	'Victoria'	'Resling Italian'	'Merlot'
1 kg of grapes contains:			
Bunches, g	23.5	36.0	53.0
Grape berries, g	930.5	952.0	921.0
Grape berries, no.	180.0	943.0	943.0
Must, ml	670.0	699.0	680.0
Must, g	770.0	763.0	755.0
Skin and hard parts of the pulp, g	175.0	155.9	150.0
Seeds, g	48,1	40.0	46.0
Total husks of grapes, g	241.6	231.9	249.0
100 grapes berries have:			
Total weight, g	580.0	147.0	138.2
Skin, g	29.0	23.0	22.2
Pulp, g	570.4	125.6	130.0
Seeds, g	9.0	6.0	6.0
Number of seeds	220.0	180.0	179.0
Seeds weight of 100 g	4.0	3.9	3.6
Uvological indices:			
Grape structure index	39.6	26.4	17.37
Grape berrie index	29.0	96.0	94.0
Grape berrie composition index	15.01	4.33	4.60
Efficiency index	3.18	3.29	3.03

From the table 7, it can observed that the values of the analyzed parameters are specific to the studied varieties. The values of the efficienty index are close to the three varieties (3.03-3.29). In the specialized literature this index is not considered as a criterion in distinguishing the wine varieties, those for the table (Cotea, 1985). In the case of wines grapes varieties, the uvological characterization allows the varieties association in technological assortments and the establishment the best technologies for obtaining different categories and types of wines.

CONCLUSIONS

Grapes maturity from Cernavoda vine centre is a complex process, dependent of many factors.

The complexity process was different from one variety to another, depending of the grapes destination and the quality of the products obtained from them.

The climatic factors (heat, light and humidity), influenced the ripening process in 2019.

The studied varieties reached the corresponding maturity at different dates, the ripening time being a criterion in their classification.

All the results presented in the paper (grapes weight, sugars, acidity) correspond to the studied varieties.

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VRANCEA - NEW VARIETY OF VINE FOR OBTAINING WHITE WINES OF HIGHER QUALITY CREATED AT S.C.D.V.V. ODOBEŞTI

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Abstract

The scientific research in the field of improvement of vines has as main objective the diversification of the wine variety by creating new varieties with high quantitative and qualitative potential, with tolerance to diseases, pests and stress factors. Responding to this purpose, at the SCDVV Odobeşti was created the 'Vrancea' variety, obtained from the sexual crossing of the hybrid combination (Traminer x Armaş) with the 'Fetească regală' variety, approved in 2018. The new creation is characterized by grapes of small to medium size (153 g) , small to medium grains (1.9 g), uniform, globular, with thin skin, pink - yellow, succulent pulp, slightly firm, without anthocyanin coloration. The average grape production is 6.7 kg/ha, respectively 23.7 t/ha. It presents good tolerance to the main cryptogamic diseases and stressors. The grapes reach maturity in their V age. Higher quality white wines are obtained, with an average alcoholic potential (12.0-12.6% vol alcohol), good total acidity (6.2 g/l tartaric acid) and medium to high values of the extract non-reducing dry (21.0 g/l).

***Key words**: sexual hybridization, tolerance, variety.*

INTRODUCTION

Productivity, quality and adaptability of vine varieties are very complex characteristics that depend on the genetic traits (inherited genotype or dowry) of each variety, the environmental conditions and the interaction between genotype and environment (Sestraș, 2004).
The obtaining of vines varieties with higher productive and qualitative traits, adapted to climate change, which exploit local natural resources in the context of sustainable viticulture, is the result of research in the field of vine improvement.
Through works of sexual hybridization enter and interspecific, at the S.C.D.V.V. Odobești have been approved in the last three years, three varieties: 'Măgura' and 'Remus' - varieties for wine grapes and 'Putna' variety for table grapes (Bosoi et al., 2017; Puşcalău et al., 2018).
To this genetic dowry is added the new 'Vrancea' variety, intended for the production of high quality white wines approved in 2018 (Mihu et al., 2016; Puşcalău et al., 2017).

MATERIALS AND METHODS

The study was carried out in the period 2016 - 2017, on a 34-year-old plantation, set up on a soil of leached chernozem type, located in the biological field of S.C.D.V.V. Odobeşti.
The 'Vrancea' variety was obtained from the sexual crossing of the hybrid combination (Traminer x Armaş) with the 'Fetească regală' variety, named during the study - the hybrid elite 8-5-1.
Studied and verified in all the stages imposed by the improvement scheme by sexual hybridization, according to the norms of experimental technique, it was compared with one of the basic varieties from the assortment of the Odobeşti vineyard - 'Fetească regală', which represents the paternal parent and is similar as the production direction.
The varieties 'Vrancea' and 'Fetească regală' (the control) were grafted on to the Kobber 5 BB rootstock, with Dr. Guyot cutting system. The fruit load was 44 buds/vine, distributed on 9-eye fruiting cane and 2-eye spur.

The planting distance is 2.2 m x 1.2 m, returning 3787 vine/ha. Were studied the main ampelo-graphic characters, determinations were made regarding the elements of fertility and productivity, the quantity and quality of grape production, physico-mechanical composition of grapes and technological indices, the physico-chemical characteristics of the wine.

RESULTS AND DISCUSSIONS

Climate condition. The research period was characterized by very high heliotermic availability (2451.7 hours in 2016 and 2536.3 hours in 2017) compared to the multiannual value of 2137.2 hours (Table 1). Also, the average annual air temperatures recorded in the two years of study (12.7ºC, respectively 12.3ºC) were above the multiannual average value (10.6ºC).

Table 1. The main climatic data from the study period (Odobeşti, 2016-2017)

Climate indicator	Multiannual (1946-2015)	Year		Average 2016 - 2017
		2016	2017	
Annual				
The average temp.,ºC	10.6	12.7	12.3	12.5
Temp. max. abs. ºC	39.4	35.4	37.9	36.7
Temp. min. abs., ºC	-22.8	-14.0	-15.6	-14.9
Amount degrees usuful temp (Σºtu), ºC	1624.9	1952.8	1926.0	1939.4
The amount heatstroke, hours	2143.0	2451.7	2536.3	2494.0
Precipitation amount, mm	615.5	1049.0	655.2	852.1
On the vegetation period				
The average temp.,ºC	17.1	18.7	18.5	18.6
Temp. max. abs. ºC	39.4	35.4	36.8	36.1
Temp. min. abs., ºC	-8.2	-0.1	1.2	0.6
Amount degrees usuful temp (Σºtu), ºC	1609.7	1938.0	1892.5	1915.3
The amount heatstroke, hours	1655.0	1912.5	1917.8	1915.2
Precipitation amount, mm	436.9	909.4	492.4	700.9

The rainfall regime was surplus in 2016 (1049.0 mm) and close to normal in 2017 (655.2 mm). The sum of the useful temperature degrees during the vegetation period (1938.0ºC, respectively 1892.5ºC) was well above the multiannual value (1609.7ºC).

The ampelographic characters. At the budburst (Stages C, Baggiolin), the rosette is greenish-brown. The tip of the shoot is completely open with bristles and anthocyanic pigmentation absent or very small (Figure 1).

Figure 1. ‘Vrancea’ variety (young shoots)

The young leaves (4th leaf) are pentalobate with deep sinuses, intense green color. The adult leaves are medium, intensely green, pentalobate, with the upper lateral sinuses of the tongue are open, elliptical, with a "V" base, and the petiole sinus is wide open in the U or V shape (rarely in the shape braces).

The petiole is slightly longer in relation to the median rib length. The shoots have vigor of medium growth, green-red color on the sunny side and reddish on the shaded side, with streaks on both sides and middle merits.

The flowers are normal hermaphrodites, on type 5, with fertile pollen. The grapes are small to medium in size, cylindrical in shape, uniaxial, sometimes double-winged, with a length of 12.0-14.0 cm (Figure 2).

Figure 2. ‘Vrancea’ variety (leaf, grape, berry)

The grains are small to medium, uniform, globular, with a thin skin, pink - yellow - more intense on the sunny side, and the pulp is succulent, slightly firm, without anthocyanin coloration and no specific taste.

The vegetation phases. Under the climatic conditions of the Odobeşti vineyard, the new variety carried out the annual cycle of vegetation in 182 to 193 days, the beginning and the end of the vegetation taking place at calendar dates close to the control variety – ‘Fetească regală’ (Table 2).

Table 2. The phenological spectrum of the ‘Vrancea’ variety (Odobeşti, 2016 -2017)

Variety	Phenological phases					During the vegetation days
	Disbudding	Flowering	Grapes ripping	Technological maturity	Fall leaves	
‘Vrancea’	10 - 15.IV	2 - 10.VI	2 - 5.VIII	14 - 20.IX	14 -20.X	182-193
‘Fetească regală’ (Mt.)	9 -14.IV	29.V- 2.VI	4 - 6.VIII	14 - 16.IX	13 - 19.X	182-194

The full ripening of the grapes at the new variety was carried out in the second decade of september, approximately with ‘Fetească regală’ variety (Mt.), falling within the 5th age of ripening.

Fertility and productivity (Figure 3). The main elements of fertility and productivity of the new variety, appreciated by the percentage of fertile shoots, the coefficients of fertility (absolute and relative), the average weight of the grape and the productivity indices (absolute and relative), show higher values compared with the control variety - ‘Fetească regală’ (Table 3).

Table 3. Comparative fertility and productivity of the ‘Vrancea’ variety

Variety	Year	Fertile shoots (%)	Fertility coefficients		Productivity indices		Average weight grapes (g)
			relative	absolute	relative	absolute	
‘Vrancea’	2016	91	1.63	1.79	243	267	149
	2017	74	1.04	1.41	163	221	157
	Average	83	1.34	1.60	203	244	153
‘Fetească regală’ (Mt.)	2016	85	1.25	1.46	158	184	126
	2017	72	1.10	1.57	171	212	155
	Average	79	1.18	1.52	165	198	141

Figure 3. The variety ‘Vrancea’

The biological resistance to the main cryptogamic diseases was established by assessing the grades from 1 to 9 according to the resistance scale developed by O.I.V (International Organisation of Vine and Wine). In the year 2016 (year favorable to the attack of cryptogamic diseases), under the application of the treatment scheme, the variety Vrancea showed resistance close to or even superior to the control variety Fetească regală (Table 4).

Table 4. Behavior of the ‘Vrancea’ variety at the main diseases of vines (after O.I.V. descriptor list for grape varieties and *Vitis* species, 2nd edition - 2009)

Variety	Downy mildew (*Plasmopara viticola)*		Powdery mildew *(Uncinula necator)*		Gray rot *(Botrytis cinerea)*	
	Leaf OIV 452	Grape OIV 453	Leaf OIV 455	Grape OIV 456	Leaf OIV 458	Grape OIV 459
‘Vrancea’	7	7-9	7	7-9	7-9	5
‘Feteasca regală’ (Mt.)	7	7	7	7	7	5

Resistance to stressors and growth force. In the climatic conditions recorded in Odobeşti vineyard (years 2016 and 2017), the new ‘Vrancea’ variety showed good frost resistance comparable to the ‘Fetească regală’ variety and a high drought tolerance (Table 5).

Table 5. Behavior of the 'Vrancea' variety to stress factors (after OIV descriptor list for grape varieties and *Vitis* species, 2nd edition - 2009)

Variety	Frost resistant (% dead buds)	Drought resistant	Growth vigor
'Vrancea'	9.8	7	5
'Fetească regală' (Mt.)	10.2	5	7

The vigor of growth of the buds expressed by the length of the shoots is an average one.

Quantity and quality of production. The study of the technological characteristics of the grape production completed the knowledge elements for the new 'Vrancea' variety (Table 6 and Figure 4).

The number of grapes per vine and the production obtained (kg / vine) confirm the data on the fertility of the vine for the 'Vrancea' variety compared to the control variety.

Table 6. The quantitative and qualitative characteristics of the grapes in the 'Vrancea' (2016 - 2017)

Variety	Year	No. grapes/ vine	Grape production		Sugar (g/l)	Total acidity (g/l H_2SO_4)
			kg/vine	t/ha		
'Vrancea'	2016	48	7.22	25.27	192	3.58
	2017	40	6.33	22.15	212	3.35
	Average	44	6.77	23.71	202	3.47
'Fetească regală' (Mt.)	2016	35	4.55	17.23	188	3.62
	2017	33	5.20	18.19	191	4.36
	Average	34	4.88	17.71	189	3.99

The grape production made by the new variety, as an element that defines the opportunity of its approval and extension in culture, was superior to the control variety.

The average value of grape production over the two years of study was 6.77 kg/ha, respectively 23.71 t/ha in the 'Vrancea' variety compared to 4.88 kg/ha respectively 17.71 t /ha in the control variety - 'Fetească regală'.

The production increase achieved by the new 'Vrancea' variety is 39% compared to the control variety being provided statistically ($p < 0.05$ - 0.0039), and representing a production increase of 1.89 kg/vine, respectively 6.0 t /ha.

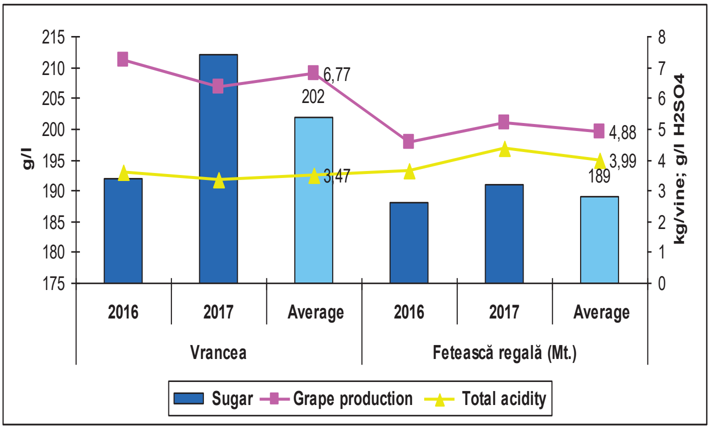

Figure 4. Quantity and quality of production (Odobeşti, 2016-2017)

The quality of the production appreciated by its sugar content and acidity of the juice, shows a potential for accumulation of sugars higher than the control variety (average values of 202 g/l in the 'Vrancea' variety, respectively 189 g/l in 'Fetească regală' variety), under the conditions of an average acidity of 3.47 g/l H_2SO_4 in the 'Vrancea' variety, respectively 3.99 g/l H_2SO_4 in the control variety.

The values of the technological indices resulted.

from the physical-mechanical analysis of one kilogram of grapes, complete the qualitative properties of the new 'Vrancea' variety (Table 7).

Table 7. Physico-mechanical composition of 1 kg grapes and technological indices of 'Vrancea' variety compared with control

Elements determined	'Vrancea'	'Fetească regală' (Mt.)
1 kg grapes:		
Berry, g	960	956
Bunch, g	39	46
Must weight, g	735	758
Volume of must, cm^3	662	725
Seeds, g	38.4	27.3
Skin and core weight, g	187	171
Marc weight, g	225	198
100 berry:		
Average weight, g	198	189
Volume, cm^3	176	168
Number of seeds	181	161
Seeds weight, g	6.6	5.8
Skin weight , g	23.5	18.3
Core weight, g	145	165
Technological indices:		
Berry index	51	55
Structure of the grape index	25	28
Composition of berry index	5.62	9.15

The oenological parameters of the wines obtained from the 'Vrancea' variety were comparable to those of the control variety - 'Fetească regală', namely an alcoholic concentration of 12.65% vol., a total acidity of 6.85 g/l tartaric acid and a non-reducing dry extract of 19.90 g/l (Table 8).

Table 8. The physico-chemical characteristics of wines (average data 2016-2017)

Soiul	Alcohol vol. %	Total acidity g/l tartaric acid	Dry extract unred-ucible g/l	Residual sugar g/l
'Vrancea'	12.65	6.15	19.90	1.29
'Fetească regală' (Mt.)	12.49	5.92	17.98	3.04

The physico-chemical characteristics of the obtained wine classify the 'Vrancea' variety in the assortment of white wines of superior quality.

CONCLUSIONS

The new variety, called 'Vrancea', is an original and valuable creation, which enriches the source of germplasm, completes and diversifies the varietal assortment of grapes for white wines, with an increased resistance to diseases and abiotic factors.

It has a high fertility potential (83% fertile vines), reflected in the high and constant productions, from 22 to 25 t/ha.

The quality of the production appreciated by its sugar content and acidity of the must, shows a potential for accumulation of sugars higher than the control variety (average values of 202 g/l in the 'Vrancea' variety, respectively 189 g/l in the 'Fetească regală' variety).

The physico-chemical characteristics of the obtained wine classify the 'Vrancea' variety in the assortment of white wines of superior.

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STUDY CONCERNING THE INFLUENCE OF SULPHUR DIOXIDE AND DIMETHYL DICARBONATE TREATMENTS IN WINE

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Abstract

One of the current main challenges of modern enology is the effect of some preservative treatments on the final quality of wine regarding the chromatic and physical-chemical parameters. SO_2 is the most commonly used product in winemaking due to its antioxidant and antimicrobial effect. Nowadays, attempts are concentrated towards reducing sulphur dioxide and substituting it with other substances that play a significant role in wines stabilization.
For this study, twentyone variants were obtained from a blend of 'Muscat Ottonel' and 'Fetească regală' grape varieties at the experimental wine cellar of the Oenology Laboratory of the Faculty of Horticulture from Iași. The wines were treated with 6% SO_2 solution and dimethyl dicarbonate liquid solution, in various ratios.
This research aims to analyse the effect of stabilisation treatments with sulphur dioxide and dimethyl dicarbonate on physical-chemical and chromatic parameters of wines.
To carry out this experiment, Schizosaccharomyces spp. and Brettanomyces spp. were inoculated and the evolution of physical-chemical and chromatic parameters of wines was recorded.
The applied treatments have shown a synergic activity on quality, physical-chemical and chromatic parameters of wine.

***Key words**: antiseptic, antimicrobial, colour parameters, conditioning treatments, quality wines.*

INTRODUCTION

In the last years, many studies have been focused on the impact of conditioning treatments on wine composition (Reynolds, 2010; Malfeito - Ferreira, 2010; Ough 1975). The conditioning treatments aim to eliminate or reduce the chemical, physical, microbiological and enzymatic degradation (Pomohaci *et. al*., 2001). A stabilized wine should not change its clarity after it has been bottled and sent out for consumption. In practice, stability is achieved by subjecting the wine to treatments and operations which, as a whole, form the conditioning process (Pomohaci *et al.*, 2001).
Modern researchers focus on the action of the oenological substances used for wines stabilization and their influence on physical-chemical composition and chromatic parameters. Many substances can be used to protect wine's composition and colour. This group includes those products which inactivate or eliminate microorganisms (Cotea, 1985). Sulphur dioxide is the most commonly used product in winemaking due to its antioxidant and antimicrobial effect, which is considered a necessity in winemaking (Țârdea, 2007). Wines can also be obtained without addition of sulphur dioxide, but in order to obtain a good quality product, they require special management and strict conservation conditions (Reynolds, 2010).
Nowadays, many studies focus on decreasing the total quantity of SO_2 in wines (Santos *et al*., 2011; Tambora *et al*., 2013). Producers are trying to decrease the added SO_2 by strictly managing its addition on grapes, must and wine, looking for new alternatives. Factors such as grapes health, chemical composition of musts, present microorganisms, temperature, and humidity must be monitored to prevent alteration of wines (Țârdea *et al*., 2000). Commission delegated regulation (EU) 2019/934 indicates the following maximum concentrations to be respected: red wines: 150 mg/L; white wines: 200 mg/L; sweet wines: up

to 350 mg/L total SO_2. In the last years, another substance used from modern winemakers was dimethyl dicarbonate (DMDC), known as yeast inhibitor and preservative for alcoholic beverages, especially for low alcohol wines.
DMDC is used as antimicrobial agent, its efficacy depending on the pH (lower pH requires less DMDC for equivalent antimicrobial action) (Ough *et al*., 1978). Wine pH plays a critical role in many aspects of winemaking, ie. microbiological stability of wines (Ribéreau-Gayon *et al.*, 1972).
The action of DMDC depends on numerous factors such as wine composition (ethanol, pH), temperature, yeast strains and initial inocculum (Bartowsky, 2009; Costa *et al*., 2008). After its addition in wines, it is immediately decomposed into alcohol and carbon dioxide. This compound has been proposed to be used instead of SO_2 in winemaking (Divol *et al*., 2005). DMDC was approved in the European Union for winemaking at a maximum of 200 mg/L at wines that contain more than 5 g/L of residual sugar (Regulation (EC) No 643/2006).
Nowadays, the researchers focus on a key parameter in winemaking: wine's colour (Basalekou *et al.*, 2017), one of the most important visual characteristics available since it provides a considerable amount of highly relevant information about its quality (Dobrei, 2017). Microorganisms can spoil wines' quality; therefore a continuous challenge to inhibit their growth is under study worldwide. It is now confirmed that *non-Saccharomyces* yeasts, considered in the past less important for the winemaking industry, can improve the composition and aroma profile in wines. Their contribution is represented by the ability to secrete enzymes and produce secondary metabolites, glycerol and ethanol, the release of mannoproteins. Moreover, they contribute to the colour stability of wine (Padilla *et al*., 2016).
In this study, a blend of 70% 'Muscat Ottonel' must and 30% 'Fetească regală' must from Iași vineyard was obtained. 'Muscat Ottonel' is known as an aromatic grape variety often used to obtain different blends due to its strong and fruity aromas. 'Fetească regală' is a semi-aromatic grape variety, appreciated for its fruity, wildflowers odor and high acidity. (Dobrei, 2017).

MATERIALS AND METHODS

Winemaking practices

'Muscat Ottonel' and 'Fetească regală' grapes were manually harvested in autumn of 2018 and processed in the Oenology Laboratory of the Faculty of Horticulture from Iași. Experimental samples were obtained by using the classic method for producing white wines. After the quantitative and qualitative reception, the grapes were crushed, destemmed and pressed using a hydraulic press. The resulted grape juice was collected in a stainless steel tank for the fermentation phase.
After this stage, white wine was divided into three aliquots in which different amounts of sulphur dioxide were administered: 40, 80, and 160 ppm. *Schizosaccharomyces pombe* spp. and *Brettanomyces* spp. were inoculated in various amounts (30 mg/L and 100 mg/L). *Schizosaccharomyces pombe* spp.yeast is able to metabolize malic acid causing an increase in alcohol concentration, favoring the formation of stable pigments in wine. It also has certain disadvantages such as low fermentation speed, the development of undesirable flavors and aromas (Loira *et al*., 2018). *Brettanomyces* spp. yeasts typically grow in wines with residual sugar content, appearing after completion of the alcoholic and malolactic fermentation, during aging of wine in barrels or bottles. The aroma characteristics of their spoilage-causing metabolites were described as burnt plastic, smoky, barnyard, horse sweat, leather and wet wool (Henick-Kling *et al*., 2000).
In this research, this type of yeasts was used to highlight their possible microbiological activity through the sensory, physical-chemical and chromatic changes of samples.
The resulted mixture was filtered (using sterile filters), bottled into 750 mL glass bottles and then different amounts of dimethyl dicarbonate (100 or 200 mg/750 mL) were added (Table 1). This substance plays a significant role in the wine stabilization process and prevents the growth of harmful microorganisms. Bottles were stored under controlled temperature conditions until physical-chemical analyses were performed.
For each aliquot, the control sample was represented by the wine mixture treated with

different concentrations of SO_2 (40 ppm. - V0; 80 ppm. - V0′; 160 ppm. - V0″).

Standard chemical analyses were realized according to the International Organization of Vine and Wine methods (OIV, 2019).

Colour parameters were studied under the OIV standards and regulations. Evaluation of chromatic characteristics was made by the CIELab76 method using a Specord UV-Vis spectrophotometer.
This procedure measures different parameters, such as clarity, tonality, chromaticity, saturation, luminosity and hue (tint). The measurements were made at a wavelength of 300 and 800 nm (OIV-MA-AS2-11).
CIELab system characterizes colour variations as perceived by the human eye, representing a uniform 3-dimensional space defined by colorimetric coordinates L*, a* and b*.
L* represents the vertical axis, measured from 0 that means completely opaque to 100 (totally transparent). Parameters "+a*" red, "-a*" green, "+b*" yellow, "-b" blue were also registered (Rolle & Guidoni, 2007).

Statistical analyses were performed using Statgraphics Centurion XVI® software, (StatPoint Technologies, Inc, U.S.A.).

The alcoholic strength is one of the most important parameters defining wine's quality (Moreno & Peinado, 2012), which has an essential role in wine preservation (Jordão *et al*., 2015). Ethanol level influences the stability, taste and aroma profiles of wine. Its concentration depends on the accumulated sugar in grapes, climate changes and winemaking practices (Albertin *et. al*., 2017).
Wine's *total acidity* (TA) normally ranges from 5 to 7 g/L tartaric acid (Zoecklein *et al.* 1995).
Many factors can influence this parameter, such as grape variety, ripeness, wine-making technology, storage conditions and climatic conditions (Samoticha *et al.,* 2017).
pH has an essential influence on wine stabilization. Optimum pH levels in wines are between 3.2-3.6.
Volatile acidity refers to the total content of short-chain volatile acids removed from wine by steam distillation (Țârdea *et al*., 2000).

Table 1. Samples

Samples	Yeasts	Sulphur dioxide doses (ppm)	DMDC doses (mg/L)
V1	*Brettanomyces* spp.	40	0
V2			100
V3			200
V4	*Schizosaccharomyces pombe* spp.		0
V5			100
V6			200
V0	-		-
V7	*Brettanomyces* spp.	80	0
Vf8			100
V9			200
V10	-		-
V11	*Schizosaccharomyces pombe* spp.		100
V12			200
V0'	-		-
V13	*Brettanomyces* spp.	160	0
V14			100
V15			200
V16	*Schizosaccharomyces pombe* spp.		0
V17			100
V18			200
V0''	-		-

V0 - control sample with 40 ppm SO_2;
V0' - control sample with 80 ppm SO_2;
V0'' - control sample with 160 ppm SO_2.

Volatile acidity refers to the total content of short-chain volatile acids removed from wine by steam distillation (Țârdea *et al*., 2000).
Acetic acid accounts for 95-99 % of the total volatile acidity and the rest is due to small quantities of lactic acid, propionic acid, butyric and formic acid.
Volatile acidity represents an important parameter in assessing the quality and health state of the final product (Gardner, 2015).
Malic and tartaric acids play a crucial role in winemaking, influencing organoleptic quality, physical-chemical parameters and microbial stability of wines (Volschenkla *et al*., 2006).
Malic acid is found in L-acid form in grapes, musts and wines. Its concentrations vary between 2-4 g/L, being influenced by grape varieties and climatic conditions.
During alcoholic fermentation, 10-15% of malic acid can be transformed by the

Saccharomyces spp. yeasts into ethyl alcohol and carbon dioxide.

Also, *Schizosaccharomyces species* metabolize malic acid up to 70-80%. Lactic bacteria can metabolize malic acid completely, resulting lactic acid and carbon dioxide (Redzepovic *et al.*, 2003).

RESULTS AND DISCUSSIONS

Physical-chemical assays

Physical-chemical parameters of samples were performed according to the International Organization of Vine and Wine Compendium methods of analysis (OIV, 2019). For each sample, the analyses was realised in triplicate for: ethanol content (% vol.), total acidity (g/L tartaric acid), volatile acidity (g/L acetic acid), pH (real acidity), density, total sugar (g/L), malic acid (g/L) and lactic acid (g/L).

The means and standard deviations of the physical-chemical parameters of wines are represented in Table 2.

Statistical analysis of physical-chemical and chromatic parameters of wines

In this study, ANOVA multifactor test was applied to construct a statistical model describing the impact of three categorical factors, such as X_j (sulphur dioxide, yeast type and dimethyl dicarbonate) on a dependent variable Y (physical-chemical parameters and CIE Lab parameters of wines). The results of the multifactor ANOVA statistical test on the physical-chemical parameters of wines were represented in Table 5. Dependent variables in this experiment are represented by the identified physical-chemical parameters while three factors influence their concentration (sulphur dioxide, yeast type and dimethyl dicarbonate content). In this sense, the contribution of each factor was statistically interpreted independent to the effects of all other factors.

The results showed that both treatments and inoculated yeasts have statistically significant influence (p-value < 0.05) on all physical-chemical parameters except for total acidity (p-value = 0.1084) and density (p-value = 0.0984).

The means and standard deviations of the chromatic parameters of wines are represented in Table 3. Wine colour measurements of each sample were performed in triplicate.

Dependent variables were represented by the identified chromatic parameters while three factors influence their values (sulphur dioxide, yeast type and dimethyl dicarbonate).

SO_2 content factor showed a statistical significant influence on the clarity and chromaticity “a” parameters (p-value < 0.05).

Synergic action of SO_2 content - yeast type (A-B) showed a statistical significant influence on all parameters except chromaticity “b” and chroma (saturation).

CONCLUSIONS

The statistical results suggest a synergic action of administrated treatments (sulphur dioxide and dimethyl dicarbonate) having important effects on chromatic and physical-chemical parameters. Regarding the physical-chemical parameters, only total acidity and density are not statistically significant influenced.

The inoculated microorganisms also played an important role in the development of the final colour and stability of wines.

Since this article aimed to evaluate the influence of sulphur dioxide and DMDC on the evolution of chromatic and chemical parameters in white wines, the results confirm that the treatments have a significant effect on wine’s quality.

Table 2. The means and standard deviations of the physical-chemical parameters of samples

Samples	Ethanol % vol. alc.	Total acidity (g tartaric acid/L)	Volatile acidity (g acetic acid/L)	Total sugar g/L	Density	pH	Malic acid g/L	Lactic acid g/L
V0	14.20±0.17	4.90±0.16	0.39±0.01	20.70±0.01	0.9968±0.0001	3.42±0.02	0.10±0.03	1.10±0.01
V1	14.80±0.15	4.90±0.13	0.23±0.02	22.00±0.02	0.9966±0.0002	3.39±0.02	0.20±0.02	0.90±0.03
V2	14.70±0.19	5.66±0.12	0.21±0.01	21.00±0.02	0.9966±0.0001	3.30±0.03	1.70±0.02	0.00±0
V3	14.90±0.15	5.97±0.15	0.18±0.03	20.50±0.01	0.9970±0.0002	3.23±0.02	2.30±0.03	0.00±0
V4	15.00±0.17	4.90±0.16	0.21±0.02	21.70±0.02	0.9967±0.0003	3.38±0.02	0.40±0.03	0.60±0.04
V5	14.90±0.19	6.12±0.18	0.20±0.02	21.20±0.01	0.9972±0.0003	3.25±0.01	2.50±0.02	0.00±0
V6	14.80±0.18	6.43±0.17	0.16±0.01	19.20±0.02	0.9961±0.0002	3.26±0.02	2.90±0.03	0.00±0
V0’	14.90±0.17	6.28±0.15	0.17±0.03	20.70±0.04	0.9969±0.0004	3.25±0.03	2.60±0.02	0.00±0
V7	14.90±0.18	6.12±0.14	0.18±0.02	21.00±0.01	0.9971±0.0003	3.21±0.02	2.40±0.02	0.00±0
V8	14.80±0.16	6.43±0.15	0.16±0.04	19.60±0.02	0.9969±0.0002	3.27±0.02	2.80±0.02	0.00±0
V9	15.10±0.14	6.12±0.17	0.20±0.02	21.40±0.02	0.9967±0.0001	3.21±0.01	2.40±0.03	0.00±0
V10	14.90±0.15	6.43±0.16	0.13±0.01	20.10±0.01	0.9971±0.0001	3.27±0.02	2.90±0.03	0.00±0
V11	15.00±0.17	6.12±0.18	0.17±0.02	20.80±0.01	0.9968±0.0002	3.22±0.03	2.40±0.02	0.00±0
V12	14.90±0.16	6.12±0.19	0.13±0.03	19.70±0.04	0.9965±0.0003	3.22±0.01	2.50±0.02	0.00±0
V0’’	15.20±0.18	6.12±0.17	0.17±0.04	17.50±0.03	0.9954±0.0004	3.19±0.01	2.40±0.03	0.00±0
V13	15.20±0.19	6.28±0.19	0.16±0.02	19.50±0.01	0.9959±0.0002	3.18±0.02	2.40±0.02	0.00±0
V14	15.20±0.16	6.28±0.20	0.16±0.01	19.20±0.02	0.9958±0.0003	3.18±0.03	2.50±0.02	0.00±0
V15	15.00±0.17	6.43±0.18	0.13±0.03	18.40±0.04	0.9954±0.0003	3.22±0.02	2.70±0.02	0.00±0
V16	15.00±0.18	6.28±0.17	0.14±0.02	20.60±0.03	0.9966±0.0002	3.20±0.01	2.60±0.02	0.00±0
V17	15.10±0.16	6.12±0.19	0.17±0.01	20.50±0.02	0.9966±0.0003	3.17±0.03	2.40±0.02	0.00±0
V18	15.10±0.17	6.12±0.20	0.17±0.02	20.80±0.03	0.9967±0.0002	3.17±0.03	2.50±0.02	0.00±0

Table 3. The means and standard deviations of the chromatic parameters of samples

Sample	Clarity L*	Chromaticity		Chroma C	Tonality H	Luminosity	Tint	ΔE	ΔH
		a*	b*						
V0	55.33±0.24	8.86±0.18	31.29±0.12	32.52±0.14	74.18±0.22	2.44±0.05	1.68±0.03	64.18±0.20	8.86±0.24
V1	63.47±0.22	8.44±0.19	32.4±0.11	33.47±0.12	75.04±0.20	1.97±0.05	1.78±0.04	71.76±0.18	8.44±023
V2	60.89±0.20	8.74±0.17	32.46±0.13	33.62±0.11	74.93±0.19	2.12±0.04	1.75±0.02	69.55±0.20	8.74±0.23
V3	64.18±0.22	8.38±0.18	31.98±0.12	33.06±0.14	75.32±0.20	1.92±0.02	1.77±0.05	72.19±0.19	8.38±0.22
V4	59.76±0.20	9.00±0.16	32.82±0.12	34.04±0.15	74.68±0.21	2.19±0.03	1.74±0.03	68.77±018	9.00±0.23
V5	67.38±0.19	7.95±0.16	32.15±0.12	33.12±0.15	76.1±0.18	1.75±0.03	1.83±0.02	75.08±0.20	7.95±0.22
V6	60.21±0.17	9.33±0.18	30.65±0.13	32.04±0.13	73.06±0.15	2.12±0.05	1.68±0.04	68.20±0.19	9.33±0.23
V0'	96.62±0.18	1.17±0.19	7.39±0.14	7.49±0.15	80.97±0.19	0.19±0.02	2.4±0.06	96.91±0.22	1.17±0.20
V7	96.54±0.19	1.29±0.17	7.48±0.14	7.59±0.14	80.18±0.18	0.19±0.02	2.37±0.07	96.84±0.21	1.29±0.21
V8	96.66±0.17	1.09±0.17	7.37±0.13	7.45±0.13	81.63±0.20	0.19±0.03	2.42±0.03	96.95±0.19	1.09±0.20
V9	96.66±0.16	1.13±0.16	7.29±0.11	7.37±0.13	81.17±0.16	0.19±0.05	2.4±0.05	96.94±0.19	1.13±0.19
V10	96.86±0.18	0.96±0.17	7.17±0.12	7.23±0.12	82.35±0.17	0.18±0.09	2.49±0.03	97.13±0.20	0.96±0.21
V11	96.66±0.19	1.15±0.18	7.41±0.11	7.5±0.14	81.19±0.18	0.19±0.07	2.41±0.04	96.95±0.19	1.15±0.19
V12	96.66±0.17	1.15±0.17	7.4±0.11	7.49±0.13	81.18±0.17	0.19±0.08	2.41±0.05	96.95±0.18	1.15±0.19
V0''	98.15±0.16	-0.25±0.17	5.67±0.1	5.7±0.14	-87.5±0.20	0.12±0.05	3.48±0.02	98.31±0.20	0.25±0.20
V13	98.21±0.15	-0.36±0.16	5.79±0.09	5.8±0.13	-86.39±0.16	0.13±0.07	3.66±0.02	98.38±0.17	0.36±0.21
V14	98.22±0.13	-0.34±0.15	5.8±0.1	5.81±0.12	-86.62±0.18	0.13±0.07	3.63±0.04	98.39±0.18	0.34±0.21
V15	98.09±0.14	-0.28±0.15	5.85±0.11	5.86±0.13	-87.27±0.19	0.13±0.05	3.46±0.05	98.26±0.18	0.28±0.19
V16	98.35±0.15	-0.31±0.14	5.54±0.09	5.55±0.12	-86.81±0.20	0.12±0.04	3.69±0.05	98.51±0.17	0.31±0.20
V17	98.2±0.16	-0.29±0.13	5.61±0.08	5.62±0.13	-87.09±0.19	0.12±0.05	3.52±0.04	98.36±0.19	0.29±0.19
V18	98.3±0.16	-0.31±0.12	5.51±0.08	5.52±0.11	-86.79±0.20	0.12±0.04	3.63±0.03	98.45±0.19	0.31±0.18

"ΔE" represents colorimetric difference; "ΔH" represents tonality difference.

Table 4. Results of the multifactor ANOVA statistical test on the physical-chemical parameters of wines

FACTORS	ETHANOL CONTENT		TOTAL ACIDITY		VOLATILE ACIDITY		TOTAL SUGAR		DENSITY		pH		MALIC ACID		LACTIC ACID	
	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value
A- SO_2 CONTENT	34.85*	0.0000	26.22*	0.0000	74.50*	0.0000	34.40*	0.0000	39.18*	0.0000	49.83*	0.0000	29.79*	0.0000	35.37*	0.0000
B-YEAST TYPE	5.01*	0.0101	2.32^{ns}	0.1084	29.93*	0.0000	6.02*	0.0044	5.45*	0.0070	4.24*	0.0194	4.01*	0.0237	7.30*	0.0016
C-DMDC CONTENT	3.24*	0.0294	15.21*	0.0000	19.1*	0.0000	7.64*	0.0000	2.21^{ns}	0.0984	19.31*	0.0000	55.72*	0.0000	166.03*	0.0000
A-B	7.57*	0.0001	2.64*	0.0438	20.85*	0.0000	13.08*	0.0000	9.75*	0.0000	2.70*	0.0402	3.48*	0.0134	7.30*	0.0001
A-C	5.06*	0.0004	19.04*	0.0000	13.96*	0.0000	6.21*	0.0001	6.21*	0.0001	13.61*	0.0000	60.09*	0.0000	166.03*	0.0000

Table 5. Results of the multifactor ANOVA statistical test on the chromatic parameters of wines

FACTORS	CLARITY		CHROMATICITY (±a)		CHROMATICITY (±b)		CHROMA	
	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value
A- SO_2 CONTENT	3130.89*	0.0000	4418.17*	0.0000	21906.18*	0.0000	27292.76*	0.0000
B-YEAST TYPE	9.95*	0.0002	0.82^{ns}	0.4477	4.04*	0.0233	3.51*	0.0368
C-DMDC CONTENT	8.44*	0.0001	1.68^{ns}	0.1830	9.71*	0.0000	8.53*	0.0001
A-B	9.50*	0.0000	0.85^{ns}	0.4987	2.18^{ns}	0.0835	1.96^{ns}	0.1138
A-C	8.15*	0.0000	1.21^{ns}	0.3179	9.94*	0.0000	9.21*	0.0000

The superscript symbol * indicates that these factors with *p*-value less than 0.05 have a statistically significant effect on the parameter at the 95.0 % confidence level.
Data are means of triplicate determination ± standard deviation over the three replications in wine sample. The superscript letters *n.s.* indicates that the factor does not have a statistically significant influence. All F-ratios are based on the residual mean square error.

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concerning the documents accompanying the carriage of wine products and the records to be kept in the wine sector.

***Commission delegated regulation (EU) 2019/934 of 12 March 2019 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards wine-growing areas where the alcoholic strength may be increased, authorised oenological practices and restrictions applicable to the production and conservation of grapevine products, the minimum percentage of alcohol for by-products and their disposal, and publication of OIV files.

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LONG-TERM EFFECTS OF ORGANIC FERTILIZERS ON MACROELEMENTS STATUS IN GRAPEVINE LEAF ON CALCAREOUS SOIL

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Abstract

Growing grapevines on calcareous soils often leads to plant nutrition dissorders. Vineyard fertilization practices on such soils differ significantly from the fertilization on acid soils because of influence of free calcium and high pH on the availability of nutrients.Three year trial was set up according to randomize complete block design with 6 treatments (unfertilized, farmyard manure 20 t ha^{-1} and 40 t ha^{-1}, peat 20 000 L ha^{-1} and 40 000 L ha^{-1}, NPK (5-20-30 500 kg ha^{-1}+200 kg UREA kg ha^{-1}) in 4 repetitions. Samples of grapevine leaves were taken three times during the growing period: at the flowering, two weeks after flowering and at the veraison. Total nitrogen was determined by the modified Kjeldahl method, phosphorus was determined spectrophotometrically, potassium flamephotometricaly and calcium and magnesium atomic absorption spectrometricaly. Significant effect of fertilization was determined in the first year of the study on the amount of calcium (two weeks after flowering) and in the third year on the amount of potassium (flowering). Fertilization did not significantly affect the amount of nitrogen, phosphorus and magnesium in grapevine leaves.

Key words: *grapevine, nitrogen, phosphorus, potassium, organic fertilizers.*

INTRODUCTION

Soils are conditionally renewable natural resources and their sustainable use is possible only with appropriate agrotechnics (cultivation and fertilization), regulation of the air regime, decomposition of plant residues, and prevention of degradation and pollution (Varallay, 2007). In the last few decades intensive agriculture showed negative effects on soil and environment (loss of organic matter, soil erosion, pollution of groundwater, etc. (Zhao et al., 2009). Calcareous soils are characterized by a high proportion of carbonates with high pH values and thus high concentrations of HCO_3^- ions in soil solution (Mengel & Kirkby, 2001). Soil carbonates are derived from calcium and magnesium minerals (calcite $CaCO_3$ and dolomite $CaCO_3$ x $MgCO_3$). Their decomposition releases calcium and magnesium, which are important plant nutrients. In contrast, calcareous soils are associated with a soil reaction higher than 7.0 pH (pH_{KCl}), which leads to disruption in the uptake of some plant nutrients (Čoga et al., 2011).

Soil organic matter is a basic indicator of soil quality (Bouajila and Sanaa, 2011) and has two basic roles: nutritional, which arises from the mineralization of organic nitrogen, sulfur and phosphorus (Kaur et al., 2005) and physical, because improves soil properties such as are: water capacity, drainage, structure. In sandy soils, it is the only stable source of most avaialble plant nutrients (Gladstones, 1994).

Use of modern agricultural techniques, with the use of grapevine cultivars with high-yielding, potential causes numerous problems in grapevine nutrition. So, fertilization of vineyards is an agro-technical practice that has a great impact on the yield and quality of must and wine. Fertilizer application increases yields, however excessive or unbalanced fertilization can have a negative impact on quality (Delgado, 2004). On the other hand, the use of organic fertilizers is a traditional agricultural practice (Tagliavini, 2001), and in the last few decades their influence on the physical, chemical and biological properties of soil has been updated. This is the result of increased production and the desire to safely dispose of organic waste (manure from fattening yards and farms, composted municipal waste and sludge), at higher doses, which far exceed the application rates in traditional agronomic practice (Haynes i Naidu, 1998). According to Herencia et al. (2008), application of organic materials affects the distribution of biogenic elements and has positive effect on availability of microelements to plant. Furthermore, the use of organic fertilizers is associated with desirable soil characteristics, including higher water capacity, cation exchange complex, and lower soil bulk density. It also promotes the growth and development of beneficial microorganisms (Bulluck et al., 2002). The beneficial effect of organic fertilizers on the amount of nitrogen, phosphorus and potassium in grapevine leaves is probably result of an increase in soil organic matter (El-Wahab, 2011). Plant nutrition based on organic fertilizers as a source of plant nutrients has a different dynamics of nutrient availability compared to plant nutrition involving the use of mineral fertilizers, because the biomass of microorganisms in organic matter is relatively small, but very important in the circulation of nutrients in soil (Ladd et al., 2004; Kaur et al., 2005). According to El-Wahab (2011) fertilization of vineyards with organic fertilizers is a good alternative for supplying macro and microelements. However, only a small fraction of mineralized nitrogen, phosphorus and sulfur from organic matter is absorbed from depth more than 50 cm. However, some authors consider that distinctive character of wines obtained from older vineyards is the result of absorption of calcium, magnesium, iron and manganese from greater depths (layers) of the soil (White, 2009). On the other hand, the positive effects of the combination of organic and mineral fertilizers on increasing the yield, mass and length of the cluster are probably the result of meeting needs of the vines on nutrients from different sources over a longer period, as well as their impact on better availability of soil nutrients, resulting in better nutrient status in the vine (El-Wahab, 2011). Therefore, according to some authors (Efthimiado, 2010; Islam et al., 2011) integrated plant nutrition represents the best approach for improving or maintaining soil fertility and productivity on a sustainable basis.

Soils of Plešivica wine region have heavy mechanical composition, low air capacity, and a high content of activated lime (CaO), which favors the formation of HCO_3^- ions, which can decrease or inhibit absorption of plant nutrients. Under such conditions, various chlorosis often occurs (Petek et al., 2008). Methods of fertilization of vineyards on these soils vary considerably from fertilization on acid soils due to the significant impact of free calcium and high pH values on the availability of nutrients, and chemical reactions that encourage permanent loss or fixation of plant nutrients (Ksouri et al., 2005).

Nitrogen is one of the main plant nutrients, as part of proteins, enzymes, amino acids, polypeptides and many biochemical compounds in the plant system. It is essential for cell division and development of meristem tissue (Mengel & Kirkby, 1987; El-Wahab, 2011). Nitrogen leaf concentration is often not correlated with increased growth and yield. Lack of other nutrients with water or temperature stress can cause an increase in total nitrogen in the tissue with a simultaneous decline in yields (Barker & Pilbeam, 2007). Decreasing of nitrogen leaf concentration begins at the beginning of ripening (veraison), when starts decomposition of proteins into amino acids and their transfer into the berries of clusters (Wermelinger, 1991). Excessive nitrogen supply causes increased vegetative growth, which competes with sugar translocation and pigment accumulation in grapes. It also interferes with the metabolic pathways of synthesis of compounds responsible for aroma (Bravdo & Hepner, 1987; Delgado, 2004). According to (El-Wahab, 2011), organic fertilizers are considered as more desirable source of nitrogen due to mineralization that allows slower release of nitrogen over a longer period. Moreover, the use of organic fertilizers (slow available nitrogen) has the effect on reducing the accumulation of nitrates in the plant compared to mineral fertilizers (fast available nitrogen) (EL-Sisy, 2000).

Phosphorus, along with nitrogen and potassium, is the most important plant nutrient. For high yields it is often lacking in the soil, so it is added by fertilizers. Phosphorus plays an important role in the process of plant growth. It is a constituent of phosphatides, nucleotides, nucleic acids, enzymes, and as a reserve in the plant, phosphorus is most commonly bound in phytic acid (Coga et al., 2011). According to Čoga et al. (2008), the basic factor that determines the solubility and availability of phosphorus to plant is the soil pH or saturation of the adsorption complex by the bases. Under neutral and alkaline pH conditions, various forms of Ca-phosphates are dominated, which are more readily soluble than aluminum and ferric phosphates in acid soils. According to (Eghball et al., 2002), a large proportion of phosphorus (> 75%) in organic fertilizers is in inorganic form, which indicating high utilization after application.

Potassium is the only monovalent cation essental for plant growth. It is dominant inorganic cation in the plant cell, and makes 0.5 to 6% weight of plant dry matter (Pavalek-Kozlina, 2003). Unlike nitrogen and phosphorus, potassium does not a constituent of organic matter. Its importance arises from its activity in ionic form and high mobility in the plant (Bergman, 1992). Grapevine is a large consumer of potassium, and it is most widely uptake from the beginning of the vegetation to flowering, 5/8, and the rest of from veraison to harvest (Mirošević and Karlogan Kontić, 2008). According to Brancadoro et al. (1994) the highest amount of potassium in leaves and musts was determined on rootstocks 44-53 M and SO4, and the lowest amount of potassium on rootstocks 140 Ru, 420 A and 1202 C. This effect is especially pronounced in the dry years. The effect of potassium fertilization on the status of potassium in the grapevinevine depends on the type of soil, as well. In soils with a heavy mechanical composition, its fixation is greater and the risk of leaching is reduced, while the water retention capacity is much higher (Kuchenbuch, 1986). According to Čoga et al. (2011), the highest concentrations of K in grapevine leaves were determined on strongly acidic soil at flowering stage, while the lowest concentration was determined on calcareous soil at havest.

Magnesium is required for a large number of processes for organic matter synthesis. It has a positive effect on the metabolism of carbohydrates, proteins and fats, Together with other cations, influences protoplasmic colloids and activates a large number of enzymes (Vukadinović & Vukadinović, 2011). Although in calcareous soils the amounts of total magnesium are sufficient to supply the plants, due to the high amount of calcium in the soil, an imbalance of the Ca-Mg cation is often obtained. Furthermore, in calcareous soils more than 80% of exchangeable cations is calcium, while the amount of exchangeable magnesium is less than 4%, which often leads to magnesium deficiency in the plant (Hugin & Tucker, 1982).

Calcium is a component of a small number of organic molecules, but it participates in the structure of the cell membrane, reducing the hydration of the protoplasm and increasing its viscosity, and in cell division. In the central lamella, calcium is bound to the pectate, which is essential for cell wall strength (Bergman, 1992).

MATERIALS AND METHODS

Three years fertilization trial was set up on Plešivica wine-growing region, Borička location (northwestern Croatia), in a 10-year old vineyard, cv. Sauvignon White grafted on Kobber 5BB rootstock, planted on soil with quite high pH for grapevine growing (pH_{H2O} 8.02), containing 2 mg P_2O_5 100 g^{-1} soil, 14 mg K_2O 100 g^{-1} soil and 13.5% CaO. The trial was set up according to randomize complete block design with 6 treatments: unfertilized (C), farmyard manure 20 t ha^{-1} (FM 1) and 40 t ha^{-1} (FM 2) , peat 20 000 L ha^{-1}(P 1) and 40 000 L ha^{-1} (P 2), NPK (5-20-30 500 kg ha^{-1} + 2 x 100 kg UREA kg ha^{-1}) in 4 repetitions. Samples of grapevine leaves were taken three times during the growing period: at the flowering, two weeks after flowering and at the veraison. Average leaf samples were formed from 80 healthy, fully developed and undamaged leaves, taken opposite to clusters from 40 vinestocks (4 replicates x 10 vinestocks). Dried (105ºC) homogenized grapevine leaf samples were analyzed in triplicate and the results are presented as mean values.

Total nitrogen was determined by the Kjeldahl method. After digestion with concetrated HNO_3

phosphorus was determined spectrophotometrically, potassium flamephotometricaly and calcium and magnesium atomic absorption spectrometricaly (AOAC, 1995).
Statistical data analyses were performed using the SAS 8.2 System (2002-2003).

RESULTS AND DISCUSSIONS

Nitrogen leaf content (Table 1) is affected by a large number of factors, however, fertilization with organic fertilizers had no a significant effect in any year of research by individual sampling (flowering, two weeks after flowering, veraison).

Table 1. Amount of nitrogen (% N in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

% N DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	2.47	1.61	1.46	1.85
FM 1	2.52	1.58	1.31	1.80
FM 2	2.53	1.63	1.56	1.90
P 1	2.42	1.50	1.29	1.74
P 2	2.49	1.58	1.45	1.84
NPK	2.35	1.55	1.45	1.78
Average	2.46 a	1.57 b	1.42 b	
	2009			
C	3.63	2.36	1.90	2.63 b
FM 1	3.70	2.46	1.93	2.69 b
FM 2	3.93	2.71	2.11	2.91 a
P 1	3.65	2.46	1.98	2.70 b
P 2	3.76	2.45	1.91	2.70 b
NPK	3.72	2.51	2.03	2.75 ab
Average	3.73 a	2.49 b	1.97 c	
	2010			
C	3.51	2.32	2.26	2.70 ab
FM 1	3.39	2.28	2.18	2.61 b
FM 2	3.47	2.61	2.33	2.80 ab
P 1	3.59	2.43	2.31	2.77 ab
P 2	3.74	2.47	2.26	2.82 ab
NPK	3.73	2.59	2.50	2.94 a
Average	3.57 a	2.45 b	2.30 b	

Factor level means accompanied by different letters are significantly different, with error $p \leq 0.05$ according to Tukey's HSD test. Means without any letter indicate no significant differences.

In the first year of the study, nitrogen concentrations (% N) in leaf dry matter ranged from 2.35 to 2.53% N, in 2008 from 3.63 to 3.93% N, and in 2009 from 3.39 to 3.74% N.
The average values of nitrogen in the veraison stage ranged from 1.29 to 1.56% N in 2008, from 1.90 to 2.11% in 2009, and from 2.18 to 2.50% in 2010. In the first year of the study, the average values in the verasion stage were below the optimal range, because according to Fregoni (1998), the optimal concentrations of nitrogen in the veraison is between 1.60 and 2.65% N. This is probably due to the early ripening of the grapes (remobilization of nitrogen from the leaves into the berries) as explained by Wermelinger (1991). However, according to the average annual values of the nitrogen leaf content of individual treatments, in 2009 and 2010 statistically significant differences were determined. The highest annual average values in 2009 were determined on treatment with 40 t ha^{-1} farmyard manure (2.91% N) and in 2010 on treatment with NPK fertilization (2.94% N), although according to Barker and Pilbeam (2007), nitrogen concentration in leaves is often not correlated with the increase in growth and yield, and lack of other nutrients with water or temperature stress can cause an increase in total tissue nitrogen. In all three years (Figure 1), the average concentration values decreased from the first to the third sampling, as confirmed by some authors (Löhnertz et al., 1988; Wermelinger, 1991).

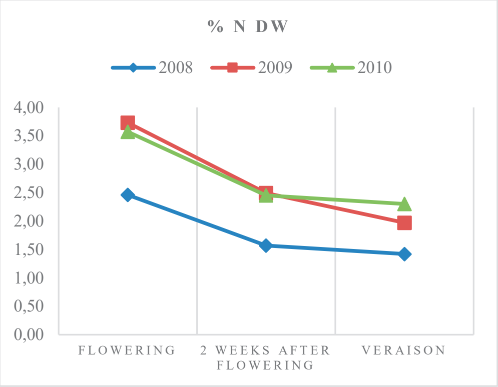

Figure 1. Average nitrogen content (% N in DW) in grapevine leaves by sampling

Grapevine is a crop that requires large quantities in the annual production cycle potassium and nitrogen, and significantly less phosphorus (Villa, 2005).
Dynamics of phosphorus in grapevine leaves during the growing season (Figure 2) is similar to the dynamics of nitrogen, i.e. the amount in leaves falls towards the end of the growing season (Petek et al., 2008), while Skinner and William (1988) were found the highest

amounts of phosphorus at the harvest stage and the lowest at the flowering stage.

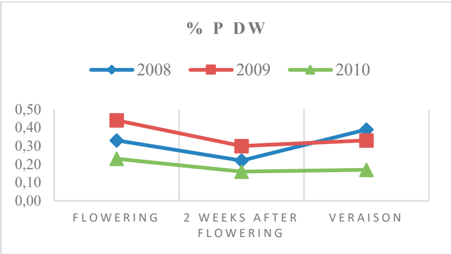

Figure 2. Average phosphorus content (% P in DW) in grapevine leaves by sampling

The results in this study varies (Table 2), in the second and third year of research, the highest average amount of phosphorus in the grapevine vine leaves according to the sampling was determined at flowering stage (0.44 and 0.23% P), while in the first year, the highest amount was recorded in the verasion stage (0.33% P).

Table 2. Amount of phosphorus (% P in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

% P DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	0.31	0.22	0.39	0.31
FM 1	0.33	0.22	0.39	0.31
FM 2	0.32	0.22	0.42	0.32
P 1	0.33	0.22	0.40	0.32
P 2	0.34	0.22	0.38	0.31
NPK	0.32	0.21	0.37	0.30
Average	0.33 b	0.22 c	0.39 a	
	2009			
C	0.45	0.31	0.33	0.36 ab
FM 1	0.42	0.26	0.27	0.31 b
FM 2	0.48	0.33	0.38	0.40 a
P 1	0.42	0.28	0.32	0.34 ab
P 2	0.46	0.32	0.37	0.39 a
NPK	0.42	0.30	0.32	0.34 ab
Average	0.44 a	0.30 b	0.33 b	
	2010			
C	0.24	0.16	0.16	0.19
FM 1	0.23	0.15	0.18	0.19
FM 2	0.24	0.16	0.16	0.18
P 1	0.23	0.16	0.16	0.18
P 2	0.22	0.17	0.19	0.19
NPK	0.22	0.16	0.16	0.18
Average	0.23 a	0.16 b	0.17 b	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

According to Fregoni (1998), the optimum phosphorus content in leaves is 0.15 to 0.38% P at flowering stage, while (Bergmann, 1992) states a general range of 0.25-0.45% P/DW.

The percentage of phosphorus in the leaf at flowering stage ranged from 0.31 to 0.34% in 2008, then from 0.42 to 0.48% in 2009, and from 0.22 to 0.24 in 2010. So, average values in the first and third years are in the range of optimal, while in 2010 they were slightly higher. In the second year of research in all sampling, the relatively highest amount of phosphorus in leaves was determined on treatment with 40 t ha-1 farmyard manure (0.48, 0.33, and 0.38% P), which is similar to Masoud (2012), who found the highest average percentage of phosphorus in grapevine leaves on treatment with compost.

In the this research (Table 3), fertilization with organic fertilizers had significantly influenced the amount of potassium in the third year at flowering stage, when the highest amount of potassium was determined with fertilization of 40 t ha^{-1} of farmyard manure (1.22% K), while the lowest amount was recorded on control treatment (0.79% K).

Table 3. Amount of potassium (% K in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

% K DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	0.74	0.75	0.79	0.76
FM 1	0.71	0.65	0.76	0.71
FM 2	0.72	0.69	0.68	0.70
P 1	0.72	0.65	0.73	0.70
P 2	0.72	0.71	0.73	0.72
NPK	0.82	0.91	0.77	0.83
Average	0.74	0.73	0.75	
	2009			
C	0.79	0.76	0.63	0.72 b
FM 1	0.85	0.74	0.64	0.74 b
FM 2	0.82	0.87	0.74	0.81 ab
P 1	0.85	0.77	0.69	0.77 ab
P 2	0.80	0.81	0.69	0.77 ab
NPK	0.89	0.94	0.84	0.89 a
Average	0.83 a	0.81 a	0.73 b	
	2010			
C	0.79 b	0.87	0.78	0.81 b
FM 1	0.85 b	0.80	0.72	0.79 b
FM 2	1.22 a	1.20	0.95	1.12 a
P 1	0.97 ab	1.04	0.79	0.93 ab
P 2	1.00 ab	0.96	0.78	0.92 ab
NPK	0.87 ab	1.10	0.82	0.93 ab
Average	0.95 a	0.99 a	0.80 b	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

The same trend was observed in the second and third sampling, although no statistically signi-ficant differences were found. Therefore, the average annual value was statistically significantly highest for fertilization with 40 t ha^{-1} of manure (1.12% K).

According to Fregoni (1998), the optimum amount of potassium in grapevinevine leaves during development of berries ranges between 0.65 and 1.70% K and during the veraison from 0.50 to 1.60% K, while Vercesi et al. (1993) state that potassium deficiency occurs when the concentration in leaves is lower than 0.57% K, while a toxic effect can be expected when concentration is higher than 1.46% K.

Determined potassium values for treatment with 40 t ha-1 of farmyard manure in Sauvignon Blanc grafted on SO4 were in accordance Herak Ćustić et al. (2008), for calcareous soils.

Results are also in line with studies (Morlat & Saymoneaux, 2008), which state that the highest amount of potassium in a multi-year fertilization experiment is regularly determined on the treatment with the highest amount of organic fertilizer (20 t ha^{-1} of manure).

According to the average values of each sampling in 2009 and 2010 (Figure 3), statistically significant higest values of potassium were determined at flowering stage (0.83 and 0.82% K), respectively, and two weeks after flowering (0.81 and 0.99% K).The lowest values were determined at veraison stage (0.70 and 0.80% K). This is in accordance with Čoga et al. (2011) who found the highest concentrations of K at flowering stage on very acidic soil, while the lowest concentration on calcareous soil was determined at harvest.

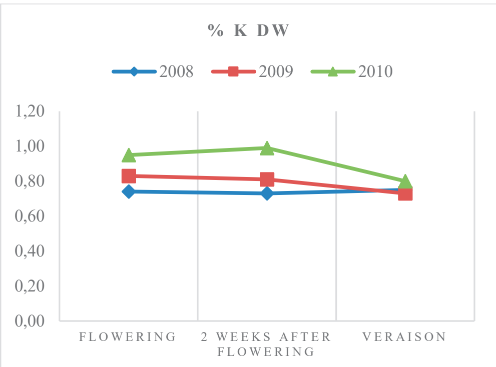

Figure 3. Average potassium content (% K in DW) in grapevine leaves by sampling

On the other hand, the results are in contrary to Schreiner and Scagel (2006) because potassium concentrations in leaves of Pinot noir cultivar increased from the beginning of the vegetation up to the harvest during the two-year trial.

Results show that fertilization with organic fertilizers did not significantly affect the change in the percentage of calcium in leaves (Table 4).

According to Fregoni (1998), the optimal percentage of calcium in grapevine leaves is 1.70% of 3.80% Ca in dry matter, and a deficit occurs when it falls below 1.41% Ca, as reported by Vercesi et al. (1993) who state that calcium deficiency occurs when the concentration in leaves is less than 1.41% Ca.

Table 4. Amount of calcium (% Ca in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

% Ca DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	1.88	1.78 ab	2.25	1.97
FM 1	1.97	1.84 a	2.91	2.24
FM 2	1.88	1.73 ab	2.80	2.13
P 1	1.96	1.80 ab	2.69	2.15
P 2	1.81	1.51 ab	3.09	2.13
NPK	1.92	1.44 b	2.78	2.05
Average	1.90 b	1.68 c	2.75 a	
	2009			
C	2.98	3.73	6.04	4.30
FM 1	2.82	4.04	5.51	4.10
FM 2	2.99	3.84	6.10	4.30
P 1	2.77	3.97	5.50	4.01
P 2	2.88	4.02	6.06	4.30
NPK	3.06	4.17	5.90	4.40
Average	2.92 c	3.96 b	5.85 a	
	2010			
C	1.56	1.67	2.89	2.04
FM 1	1.55	1.73	2.86	2.05
FM 2	1.61	1.74	2.74	2.03
P 1	1.55	1.73	2.87	2.05
P 2	1.58	1.55	2.81	1.98
NPK	1.79	1.66	2.87	2.11
Average	1.60 b	1.68 b	2.84 a	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

In these study, at the flowering stage the percentage of calcium in leaves in 2008 ranged from 1.81 to 1.96% Ca, in 2009 from 2.77 to 3.06% Ca, and in 2010 from 1.55 to 1.79% Ca, which means that in the first and second years the values were in the range of optimal values.

According to average values of individual sampling (Figure 4), in all three years of research statistically significant lowest amounts were determined at flowering stage (1.90, 2.92 and 1.60% Ca), while statistically significant highest values were determined at veraison stage (2.75, 5.85, 2.84% Ca), which is in accordance to Gluhić et al. (2009), who state that amount of calcium in grapevine leaves depends of amount of active lime in the soil and sampling time, so that the amount in leaves increases through the vegetation and reaches very high values at the end of the vegetation (first sampling 1.66% Ca - fourth sampling 4.85% Ca).

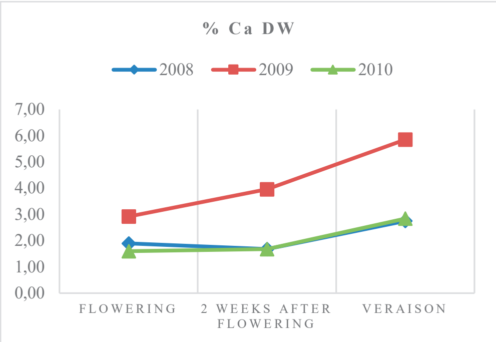

Figure 4. Average calcium content (% Ca in DW) in grapevine leaves by sampling

Although fertilization did not significantly affect the magnesium content in grapevinevine leaves, according to annual average values, in all three years of research, the relatively highest magnesium content (annual average of treatment) was determined on treatment with 40 t ha^{-1} of farmyard manure (0.36; 0.47 and 0.41% Mg).

In this study (Table 5), at the flowering stage, the percentage of magnesium in leaves in 2008 ranged from 0.25 to 0.29% Mg, in 2009 from 0.21 to 0.31% Mg, and in 2010 from 0.28 to 0.34% Mg, which is in line with Fregoni (1998), who states that for optimal plant growth magnesium requirements range from 0.15 to 0.35% Mg in the dry matter of vegetative part of the plant, and for grapevine at flowering stage 0.15 to 0.45% Mg and 0.17 to 0.60% Mg at verasion stage.

Furthermore, according to average sampling values, in the second and third year statistically significant the lowest values were determined at flowering stage (0.25 and 0.30% Mg), and the higest values at veraison stage (0.60 and 0.55%).

Average values have increased from the beginning of the vegetation to ripening (Figure 5), which is in accordance with Conradie (1981), who concludes that magnesium uptake increases around flowering, when is translocated to growth sites, and continues until ripening when increase magnesium reserves in roots, shoots and leaves.

Table 5. Amount of magnesium (% Mg in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

% Mg DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	0.25	0.26	0.26	0.26
FM 1	0.29	0.31	0.34	0.31
FM 2	0.29	0.32	0.48	0.36
P 1	0.29	0.37	0.35	0.33
P 2	0.29	0.31	0.32	0.30
NPK	0.27	0.36	0.37	0.33
Average	0.28	0.32	0.35	
	2009			
C	0.21	0.39	0.59	0.40
FM 1	0.23	0.45	0.56	0.41
FM 2	0.31	0.45	0.66	0.47
P 1	0.25	0.44	0.60	0.43
P 2	0.26	0.47	0.62	0.43
NPK	0.26	0.44	0.56	0.42
Average	0.25 c	0.44 b	0.60 a	
	2010			
C	0.30	0.34	0.54	0.39
FM 1	0.28	0.34	0.52	0.38
FM 2	0.31	0.31	0.60	0.41
P 1	0.29	0.33	0.52	0.38
P 2	0.28	0.31	0.56	0.38
NPK	0.34	0.31	0.55	0.40
Average	0.30 b	0.32 b	0.55 a	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

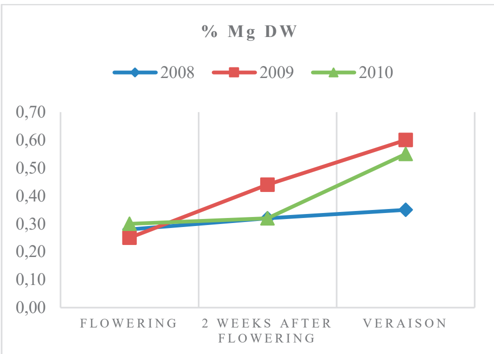

Figure 5. Average magnesium content (% Mg in DW) in grapevine leaves by sampling

Results are also in accordance with Gluhić et al. (2009) who states that magnesium concentration in grapevinevine leaves ranges between 0.25% at the beginning of vegetation and 0.64% Mg at the end of vegetation. However, the results obtained are in collision to

Schreiner and Scagel (2006), who states that magnesium concentration in leaves of Pinot noir cultivars decreases during vegetation.

CONCLUSIONS

Significant effect of fertilization was determined in the first year of the study on the amount of calcium (two weeks after flowering) and in the third year on the amount of potassium (flowering). Fertilization did not significantly affect the amount of nitrogen, phosphorus and magnesium in grapevine leaves.

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STUDIES ON THE AGROBIOLOGICAL AND TECHNOLOGICAL VALUE OF GRAPE VARIETIES FOR WHITE WINES GROWN IN STEFANESTI WINE CENTRE

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Abstract

The purpose of this work is to study the climatic conditions for three years (2017-2019) and to correlate with the quality of the grapes on varieties, for white wines (Feteasca Regala, Riesling, Sauvignon, Muscat Ottonel and Sarba) from the Stefanesti wine center. Were made determinations to the climatic conditions specific to the years of study and their influence on the production and quality of the grapes (grape mass, 100 grains mass, total acidity, pH). The study helps us to observe the direction of production and quality followed by the white wine grape varieties in the current climatic conditions of the Stefanesti wine center.

Key words*: grapes, climatic conditions, varieties.*

INTRODUCTION

The global warming of the climate has caused many disturbances in the vineyard ecosystems, the vine varieties being forced to modify the annual cycle of vegetation, with consequences most often negative for the quality and quantity of grape production, including on the resulting wines. But this global warming could have a remarkable influence on vineyards producing high quality wines on the verge of vine cultivation. For northern vineyards, this heating will be beneficial, while for southern ones it will be disadvantageous due to the too hot climate. (Popescu, 2011)

Already negative effects of climate change have been observed in some vineyards on the Globe, such as: earlier ripening of grapes, loss of acidity through respiration and a greater accumulation of sugar. Also, if the harvest takes place earlier than usual (August or September instead of October in the northern hemisphere), and the harvest is not irrigated, dehydrated grapes will result. Given that global warming affects the normal development of vine phenophases at both the continental and regional levels, a reassessment of land use in these areas is necessary based on a detailed pedo-climatic study, as well as the application of a strategy viable wineries in the face of future climate change. (Popescu, 2011).

Climate change has led to significant changes in the development and spread of diseases and pests that affect the vineyards, causing the growers to focus on new varieties and treatment schemes (Onache P.A., 2018).

The main criteria that a horticulturist should consider should be the uniformity of the variety, the quality and quantity of the production, the resistance to freezing diseases and pests, as well as to the drought, which is becoming increasingly felt in us in the country. Vines react differently to climate and soil conditions and exhibit specific adaptations to ecosystem conditions. The different mode of reactions and adaptations results from the determination of the production potential and quality of the different genotypes appreciated by phenotype (Onea C.M., 2012).

Grape varieties for wine have a shorter period of ripening of grapes, from the third to the sixth, with a maximum frequency in the fourth and fifth growing stage. In this way, the winemaking campaign runs for about 30 days (September 15 - October 15). In order to determine the quality of wine grapes, priority is given to biochemical criteria, respectively the content of the must in sugar and acidity, to which is added the content in anthocyanic substances for red wines (Stoian M., 2010).

Grape ripening represents the stage in which the grapes synthesize and accumulate oenological value compounds (carbohydrates, organic acids, flavors and phenolic compounds) through specific metabolic processes. Grape ripening is evaluated on the basis of technological maturity and phenolic maturity (Cotea V.D., 1985).
The technological maturity represents that evolutionary moment when the grapes present an optimal composition for the production of a certain type of wine and a quality category. It is defined by the following parameters: weight of 100 grains, volume of 100 grains, sugars, total acidity and pH.
Phenolic maturity is a specific notion of red wine wine-making. It defines the time when grapes for red wines have maximum anthocyanins and soft and slightly astringent tannins (Ţârdea C., 2007).
In order to determine the quality of the wines from the Stefanesti Vineyard, determinations and analyzes were carried out on grapes starting from the ripening stage to full maturity. Thus, the optimal harvesting period could be established, when the grapes accumulated a significant amount of sugars.

MATERIALS AND METHODS

For climate records, an automatic weather station was used in the area of the experimental pilot station Golesca with coordinates data 44°51'N and 24°57' E, 300 m, exposure S. The climate data collected were in the period 2017-2019 using grape varieties grown in this research station.
The soil is of the class Umbrisoils of Eutricambosoil type, brown, typical eumezobasic with clay *in situ*; the texture of the soil is clay-sandy, clay-clay, without skeleton, and the soil tillage is done with plows, the mechanical presses at intervals, the manual hoeing in a row (Radulescu I., 2010).
Sugar content (g/l) was determined with refractive method. Total acidity was determined (g/l tartaric acid) with titrimetric method. For the physico - chemical determinations of grapes and wine, laboratory equipment, equipment and glassware were used. For the statistical interpretation of the results, the data were included in an Excel database and then statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple t test) for a 5% statistical significance.

RESULTS AND DISCUSSIONS

The values registered in Stefanesti wine centre are influenced by the deficient climatic data of the wine year 2017, and the years 2018-2019 presented a series of particularities whose effects were found both in the unfolding of the vegetative cycle, and especially in the level and the quality of the grape harvest. Of these particularities we mention the most significant ones (Tables 1 and 2):
- In 2017, average temperatures (0^oC) between -3.9^oC (January) and 23.8^oC (July) were presented and with low rainfall in January-February-March (7.0; 26.8; 26.2 mm) and June (38.40 mm). Starting with the months of June - July - August, temperatures of five days were among consecutively (40.1^oC/June 29; 43.2^oC/ August 28; 41.6^oC/ August 4; 42.8^oC/August 5; 41.0^oC/ August 6). The unfavorable weather conditions of 2017 (April 24 frost and August 08 hail) have contributed to diminishing the qualitative value of the vine varieties. The effect of the prolonged drought during the formation of the berries, the crop and the ripening, alternating with the few precipitations of June-July, favored the appearance of the cryptogamic diseases, and in particular downy mildew (*Plasmopara viticola*).
- At the beginning of 2018 it was thermally balanced with high rainfall in March (108.8 mm) and freezing temperatures in March (-8.8^0C/March 01), with high rainfall starting in April until in August (this month it rained almost every day), and from May there were temperatures of 30 degrees until September, which led to the diminution of the qualitative value of the vine varieties. The year 2018 continued with a late autumn (12.4^oC November 02), starting with 09th it starts to cool -1.1^oC, with difficult winter conditions 2018-2019, low temperatures between December and January 2019 (-7.8^0C/ December 01; -12.7^0C January 08) and freezing temperatures in January (-12.7^0C/08) alternating with periods of warming (13.4^0C/January 17).

- In 2019 low temperatures during April - May leading to a delay of the flowering of the vines by one week (3^0C/April 01, 5.9^0C/May 01, 6.1^0C/May 7), June does not exceed the temperature of 35^0C only in 2 days (37^0C/June 26, 35.6^0C/ June 27), also in July there were no very high temperatures (maximum 37.8^0C/21 and 26), but in the two months the temperatures during the night being quite low (14^0C/June between 03 and 10, 8.2^0C/July 11) led to the prolongation of the ripening period of the grapes (Tables 1 and 2).

- Higher temperatures recorded in August but did not exceed 40^0C (39.4°C/22.08, 23.09) (Table 2).

- Low precipitation during the resting period of the vines and high during the harvesting process (193.6 mm in June) of the grapes - high hygroscopicity at the level of the vine trunk in August (Table 1).

Table 1. The viticultural climate from the vegetation period in the years 2017-2019

Year	Month	Air temperature			Air temperature	Index Huglin	Nr. days with precipitation >10 mm	Σ °t global (°C)	Σ °t active (°C)	Σ °tusable (°C)
		T med (°C)	Media T min (°C)	Media T max (°C)						
2017	April	10,7	3,4	19,5	83,6	153,0	3	321,1	244,5	64,7
2018		16,1	-0,4	32,7	4,6	330	-	484,0	484,0	190,0
2019		11,2	5,4	19,1	42,2	171	1	338,1	244,4	53,4
2017	May	16,0	10,0	25,3	155,2	330,1	5	497,2	490,3	196,1
2018		18,6	7,7	33,5	121,6	422	4	577,1	577,1	267,1
2019		16,3	9,8	24,6	93,6	323,95	2	504,1	494,5	194,5
2017	June	22,5	14,5	32,2	38,4	520,5	2	674,6	674,6	375,6
2018		21,6	7,9	35,8	171,2	475	6	649,4	649,4	327,5
2019		22,1	15,5	32,0	193,6	511,5	6	664,6	664,6	364,6
2017	July	22,8	15,4	33,0	96,6	399,9	3	708,3	708,3	394,0
2018		24,7	9,4	35,4	111,8	575	3	765,6	765,6	369,0
2019		22,2	14,5	32,2	70,6	531,95	1	687,1	687,1	377,1
2017	August	23,8	16,0	34,8	83,4	598,3	2	738,7	738,7	428,7
2018		23,6	12,9	37,7	63,0	592	2	732,8	732,8	422,8
2019		24,6	15,7	35,8	6,6	626,2	0	762,1	762,1	452,1
2017	September	18,1	11,2	30,0	74,4	421,5	8	544,4	544,4	382,4
2018		18,9	-1,4	37,8	6,0	550	0	565,8	547,2	267,2
2019		19.3	10.5	30.5	5.2	447	0	578.0	578,0	278.0

Huglin Index =[(T_{med} – 10)+(T_{max} - 10)]/2 x no. days of the month
Σ°t global = the sum of positive daily average temperatures
Σ°t active = the sum of daily average temperatures> 10^0C
Σ°tusable = the sum of the differences between the average daily temperature> 10^0C and the biological threshold of starting in the vegetation of the vines (10^0C)

Table 2. The climate of the ripening stage in the year 2017-2019

	Month	Temperature					Hygroscopicity (U%)	Nr. Days with T>30°C	Night coolness index *
		T med (°C)	T min(°C)		T max(°C)				
			average	absolute	average	absolute			
2017	July	22,8	15,4	18,9	33,0	39,8	66	25	-
2018		24,7	15,0	9,4	32,4	35,4	76	25	-
2019		22.2	14.5	8.2	32.1	36.9	68	21	-
2017	August	23,8	16,0	21,3	34,8	43,2	61	26	-
2018		23,6	17,6	12,9	34,8	37,7	66	31	-
2019		24.6	15.7	10.8	35.8	39.9	57	27	-
2017	September	18,1	11,2	4,1	30,0	36,4	63	15	1,5
2018		18,9	10,5	-1,4	30,4	37,8	62	-	2,5
2019		19.3	10.5	1.6	30.5	37.5	60	19	10.5

* it is calculated only for September

These conditions influenced the development of the vegetative cycle as follows: starting in the vegetation, the release of 14-20.04 in 2017, 18.04.

In 2018 and later in 2019 between June 27 and 30 starting 10 days later, compared to 2018. It flowering in 2019 (June 14) later with almost 2 weeks compared to 2018 (June 03) and compared to 2017 (June 05), due to the minimum temperatures in the second half of May (5.9^{0}C/May 1 in 2019) and average (9.6^{0}C/ Mai 07 in 2019), but also the abundant rainfall between May 29 - June 24 in 2019 (193.6 mm). The ripening phenophase was achieved later than the year 2019 with approximately 2 weeks (between August 10 and 30), being influenced by the average temperatures (15.7^{0}C/June 4 and 17.2^{0}C/July 15), maximum (35.6^{0}C/June 27 and 37.8^{0}C/July 21), and minimum (11.1^{0}C/ June 30 and 8.2^{0}C/July 11). The high temperatures in July and August of 2019 and rainfall increased in June and low from August, up to the drought threshold, they led to slower grape ripening in the Stefanesti wine-growing center, and this year was a very good one for the vineyards. Of the useful components existing in grapes, sugars, along with acids, are some of the most important quality elements in vines. The two biochemical components are particularly important in terms of sugar/acidity ratio, which decisively influences the taste of grapes (Cotea V., 1985).

In the three years of study 2017-2019 determinations were made on seven white grape varieties: Sauvignon, Muscat Ottonel, Chardonnay, Feteasca Regala, Feteasca Alba, Riesling Italian, Sarba. The dynamics of the ripening of grapes for each year 2017-2019 was made, determining the weight of 100 berries, the sugar content and the total acidity. For the statistical interpretation of the results, the data were included in an Excel data base and then statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple t test) for 5% statistical significance.

Table 3. The indicators of the central tendency of the sample (mean, median and mode), as well as the indicators for spreading the values around the average (maximum amplitude, extreme values, standard deviation and asymmetry coefficient).

Year			Year	Analysis time Factor A	Total sugar content (g/l)	Cultivars	Total acidity	Mean fruit weight-100 grape beans (g)
2017	N	Valid	42	42	42	42	42	42
	Mean		2017	3,50	134,8095	4,00	6,7614	118,5238
	Median		2017	3,50	134,5000	4,00	5,9100	116,0000
	Mode		2017	1(a)	78,00	1(a)	4,79(a)	81,00(a)
	Std. Deviation		,00000	1,729	37,60399	2,024	2,51570	31,57201
	Std. Error of Skewness		,365	,365	,365	,365	,365	,365
	Std. Error of Kurtosis		,717	,717	,717	,717	,717	,717
	Range		,00	5	119,00	6	8,61	106,00
	Skewness			,000	-,085	,000	1,130	,156
	Kurtosis			-1,276	-1,307	-1,255	,051	-1,261
	Minimum		2017	1	72,00	1	3,93	67,00
	Maximum		2017	6	191,00	7	12,54	173,00
2018	N	Valid	42	42	42	42	42	42
	Mean		2018	3,50	134,9524	4,00	5,7752	131,2857
	Median		2018	3,50	136,5000	4,00	5,8750	133,5000
	Mode		2018	1(a)	81,00(a)	1(a)	3,76(a)	83,00(a)
	Std. Deviation		,00000	1,729	37,84819	2,024	1,53741	31,52025
	Std. Error of Skewness		,365	,365	,365	,365	,365	,365
	Std. Error of Kurtosis		,717	,717	,717	,717	,717	,717
	Range		,00	5	119,00	6	5,70	96,00
	Skewness		,00	,000	-,080	,000	,075	-,197
	Kurtosis		,00	-1,276	-1,337	-1,255	-,927	-1,528
	Minimum		2018	1	75,00	1	3,10	80,00
	Maximum		2018	6	194,00	7	8,80	176,00
2019	N	Valid	42	42	42	42	42	42
	Mean		2019	3,50	157,6905	4,00	6,4695	135,2381
	Median		2019	3,50	169,0000	4,00	6,5050	142,0000
	Mode		2019	1(a)	119,00(a)	1(a)	3,66(a)	172,00
	Std. Deviation		,00000	1,729	34,64840	2,024	2,63791	35,72784
	Std. Error of Skewness		,365	,365	,365	,365	,365	,365
	Std. Error of Kurtosis		,717	,717	,717	,717	,717	,717
	Range		,00	5	121,00	6	11,59	117,00
	Skewness			,000	-,456	,000	,726	-,359
	Kurtosis			-1,276	-1,050	-1,255	,279	-1250
	Minimum		2019	1	90,00	1	2,44	71,00
	Maximum		2019	6	211,00	7	14,03	188,00

a Multiple modes exist. The smallest value is shown

In general, a normal distribution is symmetrical when the value of the asymmetry coefficient is equal to zero.

Analyzing the indicators of dispersion or genetic and experimental diversity in 2017, with regard to the average mass of 100 grapes, the average sample size was 118.5238, the average mass values being between the minimum value 71.0 and the maximum value 188.0. A negative asymmetry is observed (Skewness - 0.359) which means that the values above the average predominate and indicate a normal distribution (Figure 1).

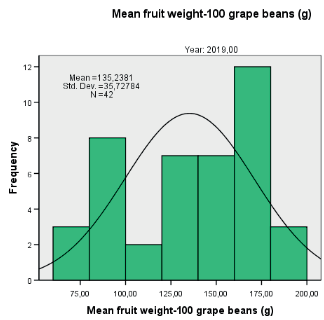


Figure 1. Histogram of the distribution by absolute frequency classes of the average mass of 100 berries

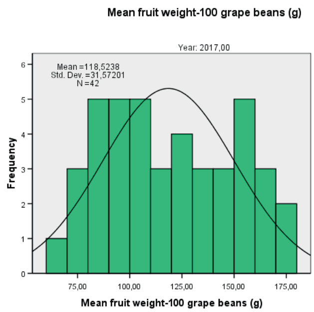


Figure 2. Histogram of the distribution by absolute frequency classes of the average mass of 100 berries in the studied varieties (2018)

In 2018, the average was 131.2857 with a standard deviation of 31.52025. In 2019, the histogram of the mass distribution of the average of 100 berries showed a standard deviation of 35.72784. The overturning coefficient has a negative value (Kurtosis -1.025) which shows the appearance of a large number of values far from the average (Figure 3).

In the case of the content of grapes in sugar in 2017 the average was 134.8095 with a standard deviation of 37.60399, the values being between the minimum value 90.0 and the maximum value 211.0.

In 2019, a negative asymmetry (-1,050) is observed, which means that the values above the average prevail and indicates a normal distribution (Figure 5). Figures 7, 8 and 9 show the histograms of distribution by absolute frequency classes of grape acidity in the 7 varieties studied. In the case of total acidity, in 2017 the average was 5.7752, with a standard deviation of 1.537413, the values being between the minimum value 2.44 and the maximum value 14.03. Analyzing Figure 8 e, we observe a positive asymmetry (Skewness +0.726), which means that the values lower than the average predominate and indicate an abnormal distribution.

In 2019, the average total acidity sample was 157.6905, with a deviation of 37.84819.

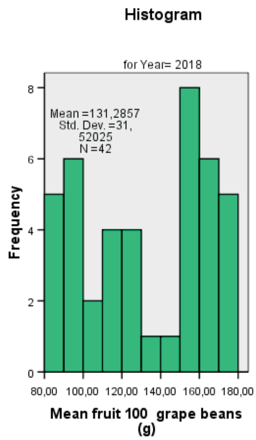


Figure 3. Histogram of the distribution by absolute frequency classes of the average mass of 100 berries (2019)

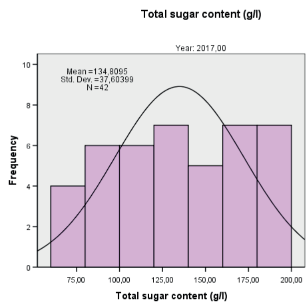


Figure 4. Histogram of distribution by absolute frequency classes of grape content in total sugar, in the studied varieties (2017)

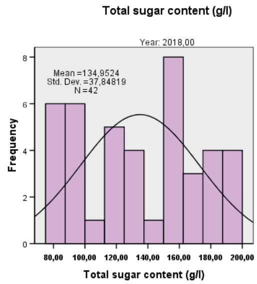


Figure 5. Histogram of distribution by absolute frequency classes of fruit content in total sugar, in the studied varieties (2018)

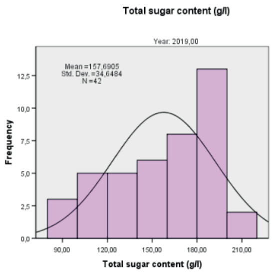


Figure 6. Histogram of distribution by absolute frequency classes of grape content in total sugar, studied varieties (2019)

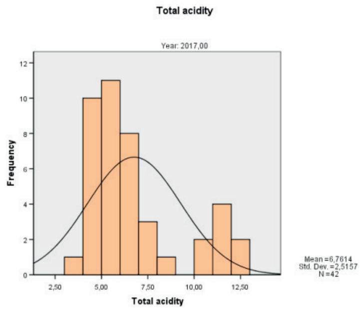


Figure 7. Histogram of distribution by frequency classes absolute of the total acidity, to the 7 varieties (2017)

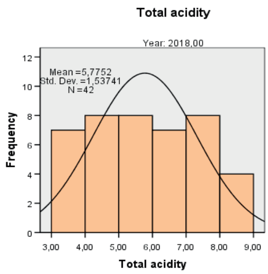


Figure 8. Histogram of the distribution by absolute frequency classes of the total acidity, in the studied varieties (2018)

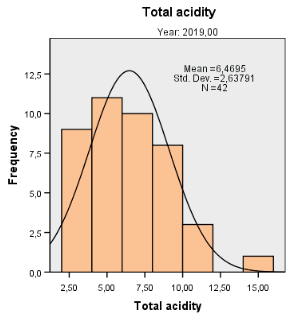


Figure 9. The histogram of the distribution by frequency classes absolute of the total acidity, to the 7 varieties (2019)

The values of sugar content in the 7 varieties studied differ from variety to variety. In the case of varieties Sauvignon (83.66 g/l) and Feteasca Alba presents the lowest values (83.00 g/l), and the variety Sarba the highest value of the sugar content (104 g/l) at the beginning of the dynamics of grape ripening. At the average of the moments, on varieties, the lowest value is observed for the Sauvignon variety (Figure 10). Also, it is important to determine the sugar for each variety, within 40 days, a higher growth is observed in the variety Sarba (198.33 g/l) (Figure 11).

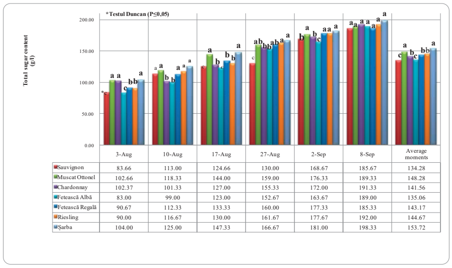


Figure 10. Influence of the variety on the sugar content of grapes, depending on the moment of determination (A to B)

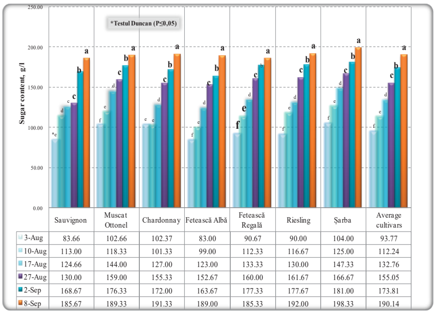
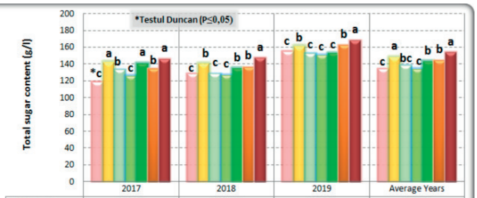


Fig 11. The influence of the moment of determination on the sugar in the grapes, depending on the variety (B to A)

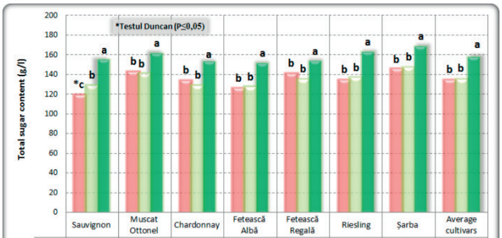
The variety with the highest sugar content is Sarba with 168,17g/l in 2019 and the lowest variety is Sauvignon with 119,16g/l in 2017 (fig 12). It is observed in all varieties, a much higher total sugar content in 2019, due to favourable conditions.



	2017	2018	2019	Average Years
Sauvignon	119,16	129,00	154,67	134,28
Muscat Ottonel	142,83	140,67	161,50	148,33
Chardonnay	134,00	128,67	152,67	138,45
Fetească Albă	126,00	127,33	151,00	134,78
Fetească Regală	141,17	135,00	153,33	143,17
Riesling	134,67	136,83	162,50	144,67
Șarba	145,83	147,17	168,17	153,72

Fig 12. The influence of the variety on the total fruit sugar, according to the year of study (A to C)

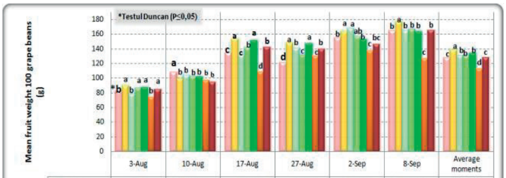
And the variety has an impact on the sugar content depending on the climatic conditions of the study year, the lowest sugar content of the Feteasca Alba variety is in 2017 with 126g/l, followed by the one of 2018 with 127,33g/l. Muscat Ottonel variety in 2017 and 2018 has a sugar content of around 140g/l, and in 2019 of 161,5g/l. The average sugar content of varieties per year is 134,8080g/l in 2017, 134,9529g/l in 2018 and in 2019 it is 157,6914g/l. (Figure 13)



	Sauvignon	Muscat Ottonel	Chardonnay	Fetească Albă	Fetească Regală	Riesling	Șarba	Average cultivars
2017	119,16	142,83	134,00	126,00	141,17	134,67	145,83	134,8080
2018	129,00	140,67	128,67	127,33	135,00	136,83	147,17	134,9529
2019	154,67	161,50	152,67	151,00	153,33	162,50	168,17	157,6914

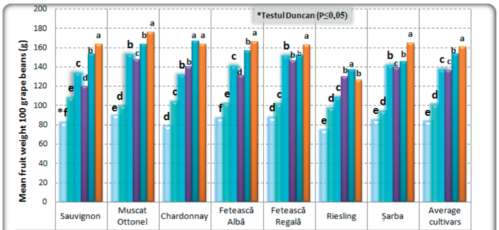
Fig. 13. The influence of the variety on the total sugar from the grapes, according to the year of study (C to A)

In Figure 14 we can trace the influence of the genotype on the average mass of 100 grape berries, depending on the moment of the determinations and we can see in Muscat Ottonel a significant increase of the weight from (89 g) on August 3rd to (176 g) on September 8th. In the case of the average berries weight there is not a very large increase between varieties, the smallest is in Riesling (111.6 g) and the highest in Muscat Ottonel (137.83 g). The influence of the moment on the average mass of 100 berries (Figure 15) it can be observed differences between cultivars, for example Riesling (126.33 g) compared to Muscat Ottonel (176 g).



	3-Aug	10-Aug	17-Aug	27-Aug	2-Sep	8-Sep	Average moments
Sauvignon	81.33	107.33	133.33	120.00	153.67	163.67	126.56
Muscat Ottonel	89	99.00	152.33	147.33	163.33	176.00	137.83
Chardonnay	77.67	103.33	131.00	140.33	167.00	163.67	130.50
Fetească Albă	86.00	101.33	140.67	131.00	156.67	166.33	130.33
Fetească Regală	86.67	101.00	151.00	146.33	152.33	162.67	133.33
Riesling	73.67	96.67	108.00	129.67	136.67	126.33	111.84
Șarba	84.00	93.67	141.33	139.00	145.67	164.67	128.06

Fig14. The influence of the genotype on the average mass of 100 berries, according to the moment of determination (A to B)



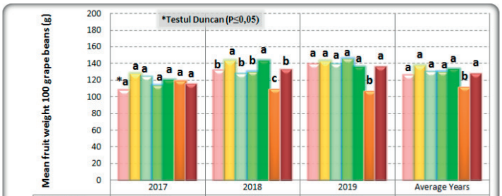
	Sauvignon	Muscat Ottonel	Chardonnay	Fetească Albă	Fetească Regală	Riesling	Șarba	Average cultivars
3-Aug	81.33	89	77.67	86.00	86.67	73.67	84.00	82.62
10-Aug	107.33	99.00	103.33	101.33	101.00	96.67	93.67	100.33
17-Aug	133.33	152.33	131.00	140.67	151.00	108.00	141.33	136.81
27-Aug	120.00	147.33	140.33	131.00	146.33	129.67	139.00	136.24
2-Sep	153.67	163.33	167.00	156.67	152.33	136.67	145.67	153.62
8-Sep	163.67	176.00	163.67	166.33	162.67	126.33	164.67	160.48

Figure 15. The influence of the moment on the average mass of 100 berries, according to variety (B to A)

Figure 16 shows the influence of varieties per year on the average weight of 100 berries, the lowest average weight was registered by Riesling (111.83 g), followed by Sauvignon (126.56 g) and the largest Muscat Ottonel variety (137.83 g).

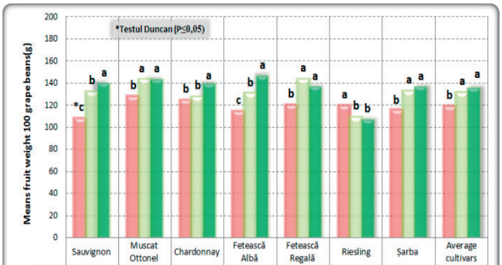
In Figure 17, the mass of 100 berries does not differ much from one variety to another in 2017, as can be seen in Sauvignon (108.17) compared to Feteasca Regala (120.17), or to Sarba (115.33)

Differences from one variety to another are observed in 2018, such as Riesling (109.17), compared to the Royal Feteasca with an increase in weight of 100 berries (143.67).



	2017	2018	2019	Average Years
Sauvignon	108,17	132,17	139,33	126,56
Muscat Ottonel	127,50	143,17	142,83	137,83
Chardonnay	124,67	127,67	139,17	130,50
Fetească Albă	114,33	130,50	146,17	130,33
Fetească Regală	120,17	143,67	136,17	133,34
Riesling	119,50	109,17	106,83	111,83
Șarba	115,33	132,67	136,17	128,06

Figure 16. The influence of the variety on the average mass of 100 berries, according to the year of study (A to C)

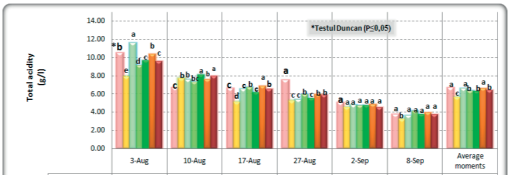


	Sauvignon	Muscat Ottonel	Chardonnay	Fetească Albă	Fetească Regală	Riesling	Șarba	Average cultivars
2017	108,17	127,50	124,67	114,33	120,17	119,50	115,33	118,5243
2018	132,17	143,17	127,67	130,50	143,67	109,17	132,67	131,2886
2019	139,33	142,83	139,17	146,17	136,17	106,83	136,17	135,2386

Figure 17 The influence of the study year on the average mass of 100 berries, according to variety (C to A)

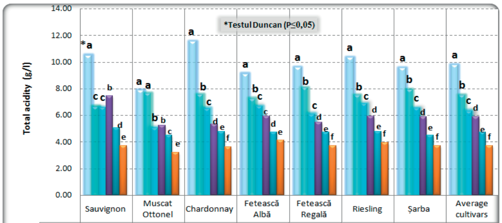
In 2019, the average weight of 100 berries per variety no longer increases with great differences, except Riesling (106.83 g) compared to the other Chardonnay or Sauvignon varieties that have a 139 g increase. Performing an analysis of the content of grapes in total acidity, on varieties and at the moment of determination (Figure 18), the highest value of total titratable acidity was recorded on August 03, at the Chardonnay variety (11.56 g/l sulfuric acid) and lowest in Muscat Ottonel variety (7.9 g/l sulfuric acid)

The total acidity is influenced by the variety and the moment of determination (Figure 18), Muscat Ottonel at the beginning of the dynamics of maturation has the lowest total acidity (7.9 g/l), and the highest is at Chardonnay (11.56 g/l). At the end of the grapes ripening, the highest acidity is Feteasca Alba variety with 4.11 g/l sulfuric acid.



	3-Aug	10-Aug	17-Aug	27-Aug	2-Sep	8-Sep	Average moments
Sauvignon	10.50	6.67	6.58	7.48	5.04	3.73	6.67
Muscat Ottonel	7.9	7.65	5.07	5.23	4.51	3.20	5.59
Chardonnay	11.56	7.56	6.52	5.33	4.79	3.61	6.56
Fetească Albă	9.11	7.26	6.69	5.92	4.71	4.11	6.30
Fetească Regală	9.60	8.06	6.11	5.46	4.71	3.73	6.28
Riesling	10.33	7.52	6.87	5.93	4.80	3.95	6.57
Șarba	9.57	7.93	6.54	5.87	4.52	3.72	6.36

Figure 18. The influence of the variety on the total acidity of the grapes, depending on the moment of determination (A to B)



	Sauvignon	Muscat Ottonel	Chardonnay	Fetească Albă	Fetească Regală	Riesling	Șarba	Average cultivars
3-Aug	10.50	7.9	11.56	9.11	9.60	10.33	9.57	9.80
10-Aug	6.67	7.65	7.56	7.26	8.06	7.52	7.93	7.52
17-Aug	6.58	5.07	6.52	6.69	6.11	6.87	6.54	6.34
27-Aug	7.48	5.23	5.33	5.92	5.46	5.93	5.87	5.89
2-Sep	5.04	4.51	4.79	4.71	4.71	4.80	4.52	4.73
8-Sep	3.73	3.20	3.61	4.11	3.73	3.95	3.72	3.72

Figure 19 The momentary influence on the total acidity of grapes, depending on the variety (B to A)

The total acidity at the time of grape maturation stage, at the Sauvignon variety was 10.5 g/l sulfuric acid, and finally 3.73 g/l sulfuric acid, at the Feteasca Regala at the beginning 9.6 g/l sulfuric acid, and the final 3.73 g/l. On average of the varieties at different moment of determination the highest total acidity was recorded on August 3.98 g/l sulfuric acid and at the last moment decrease of 3.72 g/l (Figure 19).

The total acidity is also influenced by the years of study, not only by the variety (Figure 20). In 2017, the grape varieties registered a high acidity Sauvigon (7.94 g/l), Sarba (7.05 g/l), Riesling (6.81 g/l).

In 2018, the total high acidity was registered at Feteasca Regala (6.17 g/l), followed by that of the Sauvignon variety with 6.06 g/l.

In 2019, the highest total acidity was at Chardonnay (7.46 g/l) followed by the Riesling variety (7.04 g/l). The average total acidity per year is 5.6 g/l at Muscat Ottonel, 6.68 g/l Sauvignon, 6.58 g/l Riesling variety.

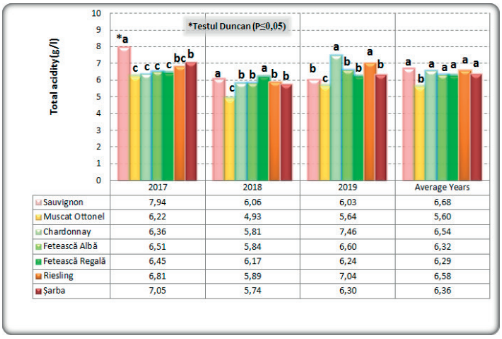


Figure 20. The influence of thevariety on the total acidity of thegrapes, according to the years of study (A la C)

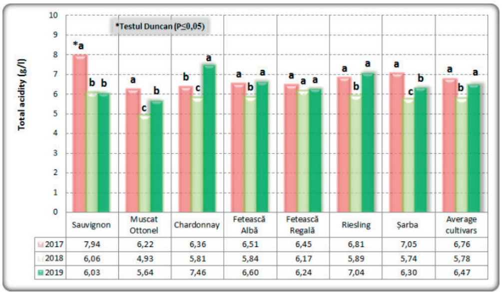


Figure 21. Influence of the study year on the total acidity of grape berries, according to variety (C to A)

Trying to compare the hierarchy between varieties with the situation existing each year in particular, we note that the order of varieties on the average is maintained in terms of total acidity by the Riesling, Feteasca Regala and Feteasca Alba varieties. There is, however, a slight interaction between the 2017 study year and varieties, in the case of Muscat Ottonel, Sauvignon and Sarba varieties, and to a lesser extent in the case of Chardonnay and Riesling varieties, in which the differences between varieties are smaller (Figure 21).

The highest values of total titrable acidity were registered by the Sauvignon variety, for 2017, for the year 2018 is the Feteasca Regala variety (6.28 g/l) and for 2019 it was registered at the Chardonnay variety (7.46 g/l sulfuric acid).

The most important component of the determinations made in grape seeds is the maturity index or the acidic-glycemic index, which represents the ratio between the sugar content and the total acidity of the grapes expressed in sulfuric acid. Depending on the variety, at full maturity this index can be between 15 and 50. (Cotea, V.D., 1985).

In 2017, due to the high temperatures in July and August, the ripening stage starts in late July the beginning of August and ends on September 15th. The lowest sugar-acidity index has Chardonnay at the beginning of the ripening stage, and at the end of the ripening of grapes the lowest index had grapes from the Sarba variety and the highest from the Muscat Ottonel variety (Figure 22).

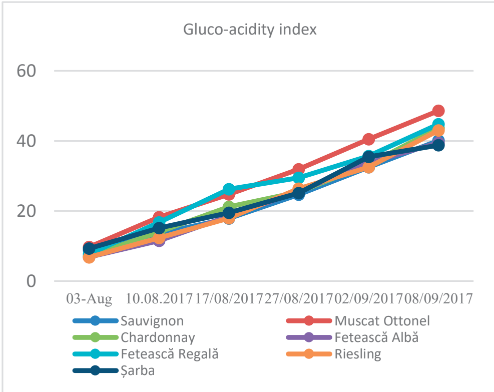


Figure 22. Evolution of grape ripening using the gluco-acidic index in the year 2017

In 2018, the ripening of grapes started later, at the beginning of August, due to the long spring of that year and ended on September 15th. The lowest maturity index is Chardonnay 6.47 and the highest, at the end of maturing, is Sauvignon (49.72) (Figure 23).

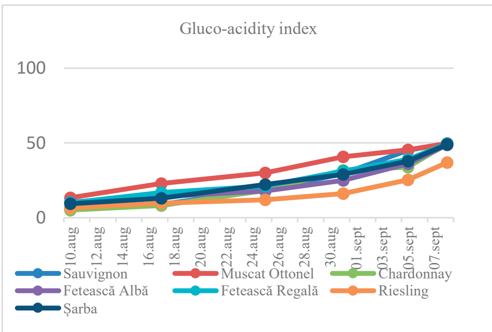


Figure 23. Evolution of grape ripening using the gluco-acidic index in the year 2018

In the year 2019, due to the high spring temperatures, the ripening phenophases started earlier, and due to the low temperatures in July and August, as well as the rains during the ripening stage, it led to a delay in grape ripening and the beginning of the harvest was one week later than 2017- 2018 (Figure 24).

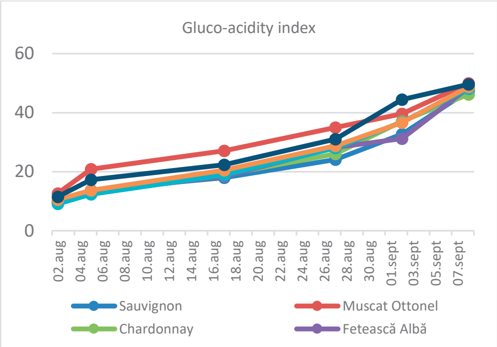


Figure 24. Evolution of grape ripening using the gluco-acidic index in the year 2019

CONCLUSIONS

The unfavorable weather conditions of 2017 (April 24 frost and August 08 hail) have contributed to diminishing the qualitative value of the vine varieties. In 2018 the temperatures were balanced with high rainfall from April to August, leading to a year similar to 2017 in terms of heat and the harvesting period.
The high temperatures in July and August of 2019 and the high precipitations in June and low from August, to the drought threshold, led to the slower maturation of grapes in the Stefanesti wine-growing center, delaying harvesting for a week, but having qualitative values and quantities higher than previous years.
In order to evaluate the dynamics of the ripening of grapes in each year of the study were determinate the fallowing indicators: the weight of 100 berries the sugar content and the total acidity. In order to evaluate the dynamics of the ripening of grapes for in each year of the study were determinate the following indicators: the weight of 100 berries, the sugar content and the total acidity.
The average sugar content, at the time of determination depends on the grape variety, the highest is in Sarba (153.72 g/l) and the lowest in Sauvignon (134.28 g/l). The average sugar content per variety at the time of harvest is 190.14 g/l. The average of the sugar content per variety in the three years is the highest in Sarba (153.72 g/l) and the lowest in Sauvignon (134.28 g/l).
The average weight of 100 berries of grapes per variety according to the moment of determination was between the values 126.56 g -137.83 g. The average mass of 100 berries of grapes per year and varieties, was quite different, at Riesling the average was of 111.83 g, while at Feteasca Regala it is 133.34 g.
The average total acidity per year according to each variety is close to the total acidity for the years 2018 and 2019, but for 2017 it is non-specific for each variety.
The most important component for determining the ripening of grapes is the sugar-acidity index. The lowest sugar-acidity index are in 2017, Sarba having 38.77 and the highest Muscat Ottonel with 48.6. In 2018, the lowest sugar - acidity index was Riesling (36.88) and the highest was Sauvignon variety (49.72). For the year 2019, the values of the sugar-acidity index have been balanced falling in 46.23 and 49.96.

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APPLICATION OF PHEROMON TRAPS FOR THE DYNAMIC TRACKING OF *LOBESIA BOTRANA* FLYING IN VINEYARDS

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Abstract

The colorful grape moth Lobesia botrana is found in all wine-growing regions of Europe, as well as in countries such as Asia and Africa. It is one of the most dangerous enemies in the vine and in Bulgaria especially in the south central part. (Stalev, B., 2013). The purpose of our study was to determine when it is most appropriate to combat flying forms. To accomplish this task, namely to track the flight dynamics of the colorful grape moth (Lobesia botrana). In the territory of the training and experimental base of the Department of Viticulture near the village of Brestnik, flying was carried out and monitored by pheromone traps type C1. This was done during the two growing seasons of 2017-2018 from experience.

Key words*: viticulture, pheromone traps, Mavrud variety, Lobesia botrana.*

INTRODUCTION

With the development of viticulture as one of the important sub-sectors of agriculture in many countries around the world and the introduction of technical progress in the cultivation of vine varieties, the need to study their biology has increased.

Climatically, the region belongs to the transitional continental climate region and is included in the sub-region of the Plovdiv field, which occupies part of the Upper-Thracian lowland. The temperature regime is characterized by relatively hot summers and mild winters. The freezing time is an average of 211 days. The last spring frost occurs between 5 and 20 April, and the first autumn frost occurs between 27 October and 8 November. With regard to the duration of the freezing weather during the year, the region does not differ from the plains of southern Bulgaria. The temperature sum for the period with average daily temperatures higher than 10ºC is in the range 3800-4000°C (3900°C). The average annual rainfall for a multi-year period is 515 mm (Angelov, L., 2006). It is about the annual rainfall in the plains regions of southern Bulgaria and at the same time is about 250 mm smaller than the area of transitional Mediterranean climate in the South-Eastern part of the country. Nowadays, a great deal of attention is paid to wine-making, with the majority of that production being red wines. The Mavrud variety, which is an old local variety and typical of the Plovdiv and Asenovgrad districts, also deserves attention in this line of thought (Babrikov D., 2000; Babrikov D., 1977; Babrikov D., 1997).

The integration of Bulgaria into the EU opens up opportunities, but at the same time also presents serious challenges to the development of the agricultural sector in the conditions of increased competitive pressure. Especially when quality raw material for wine production has to be produced. Following the restriction of the range of chemicals such as chlorpyrifos and dimethoate from EU Member States to combat *Lobesia botrana*, pheromone traps will increasingly need to be used to control the density and control of the moth grape moths. (Gordon, D., L., 2003; Louis, F., 2001). Mating One of the most dangerous enemies of the vine in Europe, including the colorful grape moth *Lobesia botrana* Den in Bulgaria et Schiff. (Galet, 1982). Extensive research on morphology, biology, its harmful activity and its fight in Bulgaria is conducted by Harizanov (1981). In various countries of Europe, at present, the method of sexual pheromone disorientation is applied over an area of 90,000

ha (Charmillot & Pasquier, 2001). Natural populations of parasitic and predatory insects limiting the density of mossy grape moth have been reported (Galet., 1981; Kreiter, S., 2000; Wahl, T., 1988) and other. In Bulgaria this method is not yet widely used, but in recent years it has been worked in this direction (Papanikulau et al., 2009; Atanasova et al., 2010; Stalev et al., 2012; Braikov, 2006; Harizanov, A., 1981).
The purpose of the present development is to apply pheromone traps type C1 and to track the flight dynamics of the moth grape moth (*Lobesia botrana*) in the Mavrud variety grafted and grown on the Kober 5BB pad.

MATERIALS AND METHODS

The Mavrud variety planted in 2013 in the educational and experimental base of the Vineyard Department near the Brestnik village was used as the object of the study. The study itself was conducted during the period 2016-2017. Planting distances are 3.0/1.0 m, the vines are formed on a stepped double sided border. The loading of the vines was accomplished by short fruiting units. The variety is grafted on a Berlandieri x Riparia Teleki sel Kober 5BB pad. The experimental design included variants with load flowering in the flowering phase, grain growth and 5-cluster layering. To track the flight dynamics of the colorful grape moth (*Lobesia botrana*). On the territory of the training and experimental base of the Department of Viticulture near the village of Brestnik, flying was performed and monitored by pheromone traps type C1.
Ampelographic characteristics of the Mavrud variety under study.
Name - Causeni (Moldova), Kachivela (Pomorie, Burgas - Bulgaria).
Origin and distribution - The Mavrud variety is an old local variety grown in our lands from ancient times.
The areas occupied by this variety as of 1981 are 1.18%.
And by 2015, the area is 1663 ha with Mavrud, which is just under 2% of the total vineyards in Bulgaria.
Areas with Mavrud in the area of Asenovgrad and Plovdiv are about 781.4 ha.

Botanical description
Normally developed leaves - large, five-lobed, the upper incisions are deep, mostly closed at the bottom, once there is a tooth at the base.
The lower incisions are medium-deep, open-shaped, with a narrow mouth and rounded bottom. The caudal incision is open, arched wide.
The upper teeth are triangular with a curved tip on one side and the posterior and intermediate teeth are triangular and triangular.
The upper surface of the leaf blade is mesh-like with webs of moss, with flesh and skin strongly frayed below.
The nerves are pink-wine-red at the base, even-mossy.
Autumn color is violet red.
Mature shoots - medium long and thick internodes with slightly convex nodes.
Color - bisexual.
Cluster - large, winged, branched, broadly expanded at its base half-compacted to loose.
Grain - medium spherical, slightly trapped on the handle, juicy, with a sweet taste.
The skin is thick, wiry, blue - black, with an abundant waxy coating.
Climatic characteristics of the area and relief
The length of the growing season in the days for the climatic indicators is taken from the Climate Directory of the Republic of Bulgaria.
Mechanical properties of the grains - skins %, meat %, seeds %, theoretical yield, killed grains %, dry grains.
Of each variant, 45 vines (three replicates of 15 vines) were included in the experiment.

Figure 1. Formulation of the experiment

Figure 2. V1 - Felizeno at 30 cm long shoots and ruffled, normalized during flowering with 5 fissures

Figure 3. V2 - Felizeno at 30 cm long shoots, brittle and scabrous, normalized during the IV phase of 5-grape grain growth

RESULTS AND DISCUSSIONS

The quality of the grapes depends on the content of sugars, titratable acids, dyestuffs, mineral salts, amino acids, but these indicators will be in the necessary condition only if the grape is protected by enemies and, above all, by Lobesia botrana. The average annual air temperature for station Plovdiv is 12.4°C, and for the region of the city Brestnik - 12.6°C and varies from 10.9 to 13.5 °C. In the spring, small for loos are possible, which sometimes slow down the development of the vine in the beginning.

Figure 4. Pheromone traps type C1

Figure 4. Carrying out a visual control by counting

This characteristic of the region phenomenon is almost always observed during the month of April. Monthly air temperatures for the winter and summer months are relatively constant. The annual temperature amplitude is in the range of 20.5°C to 22.5°C and does not differ from the amplitude for the entire south-western, transitional-continental climatic area. The frequency of late spring cooling in studies area is not different from that in the rest of the lowland territories of southern Bulgaria. As a result of the study conducted in the period 2016-20117 in the conventional cultivation of vines with the use of pheromone traps type C1. Figures 4 and 5 to track flight dynamics. Butterflies in pheromone traps have been identified, indicating their efficacy. In the conventional cultivation of the vines, the seasonal dynamics of the flight of the mulled grape moth were monitored in the experiment, with the density of the enemy significant. The flight dynamics and the number of butterflies attracted are presented in Figure 5. They show that in the vineyard that was monitored in 2016, the largest number of butterflies of the colorful moth was identified and caught at the beginning of the first generation of vegetation, with the number of butterflies caught over 110. The chart also notes that this trend continues for future generations. 2017 data in Figure 6 again showed the beginning of the flight in April by about 45, and in July and August again the flight found a large number of butterflies per colorful grape moth, similar to the results of 2016. Their number reaches 100 in the third generation.

From the experience gained during the two years we can conclude that in the land of the village.

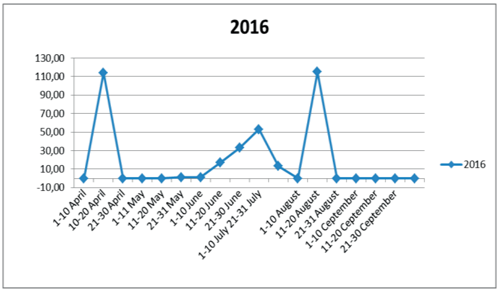

Figure 5. The dynamics of flying butterflies of the colorful grape moth in the vineyards of the village Brestnik in 2016

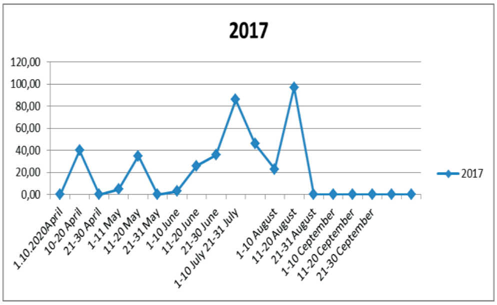

Figure 6. The dynamics of flying butterflies of the colorful grape moth in the vineyards of the village Brestnik in 2017

Brestnik is in a high density area and it is necessary to systematically combat the colorful grape moth throughout the growing season, for this purpose it is necessary to carry out annual monitoring using pheromone traps. The purpose is to determine the beginning of a flight in order to adequately combat the caterpillars of this enemy. Otherwise, it would lead to damage and deterioration of both the appearance of the grape and its technological in terms of fermentation parameters on the one hand and on the other hand the production of poor quality wines as All this is confirmed by the results presented in Table 1.

The data from the mechanical analysis of the grapes show that the percentage of normal grains is very high in the two variants involved in the experiment - from 94.71% in V1 to 95.83%. V2. 29.96% were reported at V1 to 18.46% at V2 grains, proving that a high percentage of grape samples and grains could be obtained by failure to follow the forecast given by the *Lobesia botrana* flight haul before the grape harvest. Regarding the construction of the grain, the meat occupies from 74.43% at V1 to 80.78% at V2, respectively, the skins are 19.29-14.01%, respectively for V1 and V2.

Table 1. Mechanical analysis of the grapes of the Mavrud variety

	GRAPES						***RAIN***		
Variant	**Year**	**Clings, %**	**Nipples, %**	**Rot, %**	**Milere, %**	**Staff, %**	**Of skins, %**	**Seedof, %**	**Meat, %**
V_1	2017	5.29	94.71	29.96	4.60	1.10	19.29	6.27	74.43
V_2	2017	4.17	95.83	18.46	3.56	0.32	14.01	5.21	80.78

CONCLUSIONS

The results of the studies on the dynamics of flight and the possibilities of using pheromone traps for monitoring *Lobesia botrana* in the production of grapes of the variety Mavrud in the village Brestnik, allow us to draw the following conclusion:

• The terrain of which is planted the Mavrud variety is characterized by favorable climatic conditions for growing varieties for red table wines. The total temperature amount is 4000°C. The average 24-hour temperature of the warmest month of July is 22.8°C the duration of the frost-free period is 211 days. The amount of precipitation is 515 mm. The area is characterized as moderately dry. The average multi-annual deficit in the balance of atmospheric hydration for the period June-August is within the range 200-300 mm. This creates a prerequisite for the normal wintering and development of *Lobesia botrana*.

• In the area of Brestnik village, the colorful grape moth *Lobesia botrana* was found to be very high density (over 110 pieces per catch), using C1 type pheromone trays, and during the two years of the experiment a high density of the enemy was recorded.

• Used on pheromone traps type C1. for the determination of the density of the colorful grape moth have shown high efficiency and must be placed in the land of Brestnik to signal to wine producers when insecticidal treatments should be carried out against the host.

• Mechanical analysis shows that the normalization of the yield leads to a change in the ratio of the clump of grain and, in particular, the ratio of meat to skin on the one hand, and from the other to the reading of a high % of rotten grains, which is evidence that failure to follow the forecast submitted by the *Lobesia botrana* flight catch could lead to a high percentage of rotten grains leading to a poor harvester quality of grape harvest and wine.

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ASSESSMENT OF STEVIA (*STEVIA REBAUDIANA* B.) SEEDS VITALITY IN THE CONDITIONS OF BULGARIA

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Abstract

In the conditions of Bulgaria stevia multiplies by storage of the rhizomes and obtainment of seedlings of cuttings and from in vitro maintained clones. Seeds are obtained episodically in conditions of short day at the end of vegetation with a warm and continuous autumn. Aiming art gene fund enrichment, in 2018-2019 are harvested seeds by gathering and cleaning of tied seeds from selected plants of the Selection Field of Agricultural Institute - Shumen. For the seeds progenies in laboratory conditions the germination energy on the 4th day reaches up to 46%. On the seventh day the mean germination increases to 40.9%. In a soil mixture the average value for the seventh day falls back insignificantly to that in laboratory conditions, and the variation between the origins is significantly higher. On the 14-th day the germination reaches mean of 47.5%, as for separate genotypes it exceeds 75%. The results of the seeds obtained from regenerants show a decrease of the mean values of germination to 36.2%in laboratory, and to 31.1% - in external conditions. A bigger variation is registered in the germination energy in laboratory conditions. The one year storage of the seeds decreases the germination to the average of 15.8%. It is impressive the influence of the genotype for the germination preservation, the variation increases to 52.1%, and the lack of significant correlation between the germination before and after storage.

Key words: *stevia, seeds, germina.tion, gene fund*

INTRODUCTION

Stevia (*Stevia rebadudiana* Bertoni) is a perennial, cross pollinated plant from the Asteraea (Compositae) family. It originates from the mountains of Paraguay and Brasil (Lewis, 1992). In the last years there is an increased interest in its growing as a source for natural, non-caloric sweeteners. They are from the group of the diterpene glycosides like Rebaudioside A, B, C, E and stevioside and have up to 300 times the sweetness of sugcrose (Geuns, 2004).
Because of its sensitivity to low temperature in the moderate climate countries stevia is grown as an annual plant, and it could be maintained by storage of the rhizomes (Lankes and Pude, 2008). The *in vitro* propagation and maintenance are alternative methods, whichallow the preservation of the initial genotype (Krumov et al., 1984).
Back in the 80's in the Sugar beet Institute - Shumen started researches on the introduction and growing of stevia, and they have been established the optimal technological conditions and methods of in vitro propagation (Varbanov et al.,1996). Now in the Agricultural Institute - Shumen the researches are directed in the selection of new forms of stevia (Kikindonov, 2012). In 2012 the first Bulgarian variety of Stevia is certified, with excellent economical qualities and adopted to the conditions of Bulgaria (Uchkunov, 2016). Some aspects of the obtainment and sprouting of seeds *in vitro* and in external conditions (Bojimirov and Slavova, 2011; Kikindonov and Enchev, 2012).
In natural conditions it is propagated by seeds. Stevia is a short-day plant, and a certain light and temperature regime is required for insemination (Zaidan et al., 1980). As a cross-pollinated culture, mechanisms of self-incompatibility impede the self-pollination and the production of homozygous off springs (Nakamura et al., 1985). The flowering is not simultaneous and is long lasting. In our conditions, after *in vitro* propagation and continuous adaptation in greenhouse individual origins and plants begin to bloom in late July. The mass flowering is from September to the harvesting of the rhizomes before the formation of autumn frosts (Bojimirov and Slavova,

2011). The mass flowering is from September to the harvesting of the rhizomes before the formation of autumn frosts (Bojimirov and Slavova, 2011). The great genetic diversity of the seeds progeny is the basis for enriching the gene pool for the selection of high-productivity forms and content of stevioside (Caneiro et al., 1997; Costel et al., 2019).

The study reports some results from the assessment of seeds germination of some origins as initial material for the selection.

MATERIALS AND METHODS

The survey was conducted in 2018-2019 at Shumen Agricultural Institute. The seeds were harvested from selected plants of certain origins from the breeding program of the Institute. Individual seed progeny that are cloned in vitro as well as regenerants from isolated in vitro meristems are included. The plants obtained from the Laboratory for Tissue Culture are rooted and adapted initially in a thermostatic room and then exported to the greenhouse from the end of February to the end of April. At least 10 plants of each origin are planted in the field in early May.

Individual origins and plants begin to bloom in late July. The mass flowering is from September to the harvesting of the rhizomes before the formation of autumn frosts. In our study, unlike previous practice, plants with mature seeds are harvested and dried whole. Later the seeds are separated without the empty and parachute shoots being dropped out.

To determine laboratory germination, seeds are plated in harmonic filter paper, in four replicates of 20 each, for each origin. For preliminary determination of the optimum dates, a mixed sample of all origins was plotted in 6 repetitions and germinated sprouts were counted on days 3, 5, 7, 10, and 14. For origin assessment, 4 and 7 days for laboratory germination were selected.

In January, in a thermostatic room at 20-25°C and illumination for 18 hours in pots (repetition) with 10 cm diameter, with a mixture of 4/1/1 of peat, perlite and sand, 50 seeds are sown in 4 repetitions for each origin. Seeds are evenly distributed over the surface of the pre-moistened mixture and pressed tightly against it. Optimal humidification is maintained with a polyethylene chamber for the first 4-7 days, avoiding over moisturizing.

For estimation of the viability is studied the germination after harvest of the 2018 crop seeds and after one year of storage of seeds from the same crop. The statistical processing involves variational analysis to determine mean, mean error, coefficient of variation, and accuracy of the experiment.

RESULTS AND DISCUSSIONS

The impossibility of propagation of stevia by seeds in our severe for the crop conditions is a limiting factor for the actual entry of stevia into practice. The study assesses the germination of seeds harvested under real mass production conditions.

On Figure 1 could be traced the dynamics of seed germination under laboratory and controlled external conditions. As early as on the fifth day, the germinating energy of the seeds is manifested in optimal laboratory conditions, and between the seventh and the tenth day the final germination parameters of the seeds are reached. The later germinated sprouts are of low vitality and usually do not develop normal plants. A slight delay in germination processes is observed in the soil mixture. Optimal for germination and energy of initial growth readings are those made on the 7th and on the 14th day.

From 85 of more than 212 planted origins in 2017 are harvested seeds. Tables 1 and 2 show the results of a total of 48 origins, of which there were sufficient seeds to place four replicates under laboratory and controlled external conditions. This is a testament to the fact that more than half of the genotypes with harvested seeds undergo normal binding and ripening of the seeds. Over 22% of the tested origins give viable seed progeny.

The results are interpreted separately for the offspring and for the regenerants in order to determine the effect of the way of reproduction. For seed offspring under laboratory conditions, germination energy on the fourth day reaches 46%. The variation is relatively high with a CV of 25.8%. On the seventh day, the average germination is increased to 40.9% with an identical high variation of 16.6 to 66.6% and CV-31.7%.

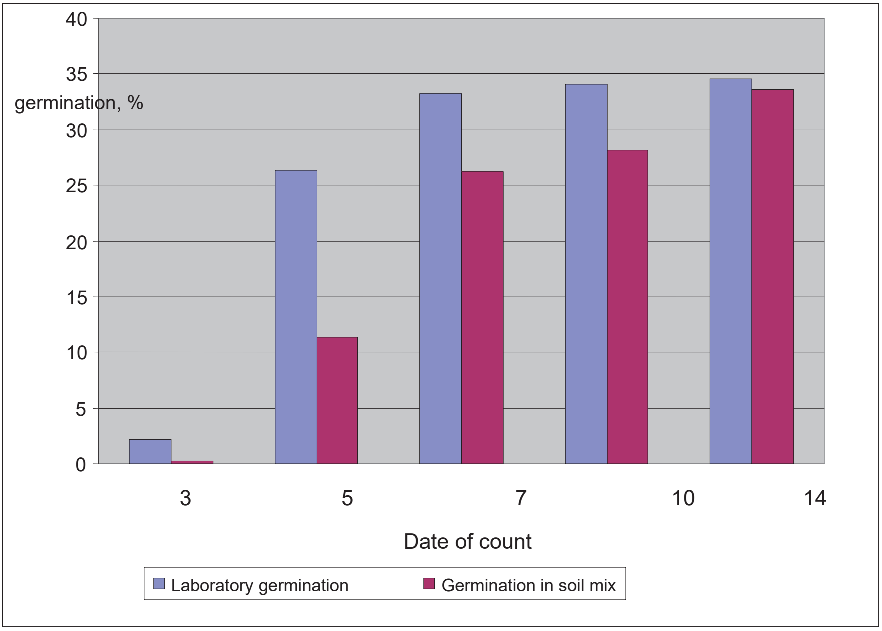

Figure 1. Dynamics of germination of stevia (*Stevia rebaudiana*) seeds in laboratory conditions and in soil mix under controlled conditions, 2018

Table 1. Germination of seeds from seed progeny plants of stevia (*Stevia rebaudiana*), 2018

Variant	Germination (%) in:			
	Laboratory conditions		External controlled conditions	
	4th day	7th day	7th day	14th day
1. IV-30	22.0	33.1	20.0	34.0
2. IV-39	28.0	50.0	28.0	44.1
3. IV-43	32.4	48.0	70.3	78.7
4. IV-46	23.7	43.0	34.2	40.0
5. IV-50	28.0	62.1	40.4	50.1
6. IV-64	26.1	45.0	32.0	36.3
7. IV-65	28.2	66.6	68.0	78.3
8. IV-66	6.6	16.6	6.0	6.0
9. V-79	13.0	22.0	28.3	50.0
10. V-91	18.5	40.3	40.0	50.6
11. V-102	33.3	50.0	46.6	72.0
12. V-116	20.2	52.0	62.0	82.0
13. V-124	23.3	45.5	36.0	44.0
14. IV-66	15.5	21.2	10.7	24.0
15. V-96	15.0	31.6	24.0	26.6
16. IV-67	26.6	45.0	30.0	44.0
17. IV-16	45.7	48.3	38.1	46.9
18. IV-32	46.2	50.0	50.0	52.1
19. IV-54	21.6	38.3	32.0	42.0
20. V-84	30.0	41.6	36.3	46.7
21. VI-20	33.7	38.3	34.0	42.3
22. IV-44	20.0	20.0	28.8	54.0
23. IV-83	26.7	40.1	38.0	44.0
24. IV-33	33.3	33.3	40.0	46.7
$\bar{X}$	**25.8**	**40.9**	**36.8**	**47.5**
Sx	**1.88**	**1.67**	**3.11**	**3.57**
CV	**35.6**	**31.7**	**41.4**	**36.8**
P %	**4.73**	**3.64**	**6.85**	**3.75**

In soil mixture, the average value for the seventh day is slightly inferior to those under laboratory conditions, with the variation between the origins being much higher - from 6.0% to 70.3% and CV - 41.4%. With the increase of the weight of environmental factors in external conditions, the genotypic differences appear to be stronger. The germination on day 14 is increased to 47.5% on average, reaching over 75% for some of the genotypes.

Table 2. Germination of seeds from stevia (*Stevia rebaudiana*) plants regenerated *in vitro*, 2018

Variant	Germination (%) in:			
	Laboratory conditions		External controlled conditions	
	4th day	7th day	7th day	14th day
1. VI-17	33.0	48.0	32.3	38.0
2. V-137	13.3	33.3	24.0	34.6
3. V-150	25.0	36.0	17.6	23.0
4. VI-18	32.3	35.0	28.0	34.0
5. V-143	25.0	33.3	14.6	16.0
6. III-168	35.3	38.6	30.0	46.0
7. III-165	36.0	46.6	30.6	36.6
8. III-180	21.6	30.0	12.0	20.0
9. III-192	3.3	21.6	14.7	32.0
10. III-162	46.7	50.0	34.1	42.7
11. V-153	13.3	31.7	20.0	34.3
12. III-166	20.7	66.0	14.7	14.0
13. III-163	13.3	40.0	22.3	32.0
14. V-147	13.0	33.0	24.7	27.7
15. III-167	8.3	36.6	32.0	52.3
16. III-170	13.0	13.0	4.0	8.7
17. V-158	20.7	20.7	17.8	23.4
18. III-191	13.0	13.0	30.3	38.0
19. III-161	13.3	53.0	30.7	34.0
20. III-175	13.3	20.0	14.7	32.3
21. III-185	40.0	40.0	24.3	24.3
22. III-195	26.7	60.0	42.3	54.0
23. V-173	32.0	35.3	28.0	34.7
24. VI-20	25.1	33.0	12.7	16.0
$\bar{X}$	**22.5**	**36.2**	**23.2**	**31.1**
Sx	**2.28**	**7.39**	**4.74**	**2.36**
CV	**49.4**	**36.8**	**38.2**	**37.0**
P %	**4.01**	**7.04**	**5.84**	**3.76**

The results for regenerants' seeds indicate a decrease in the average germination values of 36.2% in laboratory and 31.1% in external conditions. Greater variation is recorded in the germination energy under laboratory conditions. The correlation between laboratory germination and the germinated plants in soil mixture is r = 0.688, which makes the laboratory germination a reliable parameter for assessing the viability of seed progeny for the stevia.

Table 3 shows the results of laboratory germination of seeds of 12 origins immediately upon their harvest in the fall of 2018 and after one year of storage. The vitality parameters of seeds harvested in 2018 are down compared to those in 2017. The average laboratory germination on day 7 was 33.1%, with a variation of 45.2%. The one-year seed storage reduces germination by half to 15.8% on average. Obvious is the influence of the genotype on the retention of germination, and the variation is increased to 52.1%. It is impressive also that there is no significant correlation between germination before and after storage.

Table 3. Seeds germination of stevia in laboratory conditions and after one-year storage, 2019

Variant	Laboratory conditions, %		After 1 year storage, %	
	4thday	7thday	4th day	7th day
1. IV-30	6.7	26.7	12.0	12.0
2. IV-39	15.0	15.0	10.0	20.7
3. IV-43	13.3	33.3	12.7	20.1
4. IV-46	13.3	26.6	10.0	20.2
5. IV-50	6.7	6.7	10.0	24.0
6. IV-64	53.3	53.3	0	0
7. III-165	6.7	33.7	0	12.7
8. III-180	20.0	20.0	21.0	30.0
9. III-192	53.3	60.0	0	0
10. III-162	33.7	33.7	21.7	21.0
11. V-153	46.7	46.7	40.6	42.0
12. III-166	26.7	40.0	0	0
$\bar{X}$	**24.7**	**33.1**	**10.9**	**15.8**
Sx	2.32	2.62	3.21	3.42
CV	41.0	45.2	48.7	52.1
P %	5.21	5.72	7.21	8.72

Figure 2. Blooming plants

Figure 3. Stevia seeds

Figure 4. Germination of Stevia seeds

CONCLUSIONS

For seed offspring under laboratory conditions, the germination energy on the fourth day reaches 46%. On the seventh day, the average germination is increased to 40.9%. In the soil mixture, the average value for the seventh day was slightly inferior to those under laboratory conditions, with the variation between the origins being much higher. The germination on day 14 is increased to 47.5% on average, reaching over 75% for individual genotypes.

The results for regenerants' seeds show a decrease in average germination values of up to 36.2% in laboratory and up to 31.1% in external conditions. Greater variation is recorded in the germination energy under laboratory conditions

One-year seed storage reduces germination by half to 15.8% on average. The influence of the genotype on germination retention is impressive and the variation is increased to 52.1%. Impressive is also the lack of significant correlation between germination before and after storage.

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SILICON TREATMENT EFFECTS ON *TETRANYCHUS URTICAE* AND PHYSIOLOGY OF ZUCCHINI PLANTS

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Abstract

The aim of the study was to examine the effect of Silicon in the form of stabilized orthosilicic acid (H_4SiO_4) on the population of the two-spotted spider mite Tetranychus urticae Koch. on zucchini (Cucurbita pepo L.) cv. Izobilna and on the physiology of the infested plants. The experiment was conducted in a laboratory under controlled conditions at the Department of Entomology, Agricultural University - Plovdiv, Bulgaria. The plants were grown hydroponically and separated into four variants consisting of 1 - control, 2 - mite-infested plants, 3 - Silicon-treated plants and 4 - mite-infested plants, treated with Silicon. The results obtained suggest that Si could be successfully used for reducing the population density of the two-spotted spider mite on zucchini cv. Izobilna and to improve the physiological status of the plants. Several biochemical parameters concerning the main physiological processes in the infested plants were also measured. The results showed a decrease in the population density of T. urticae on the Si-treated leaves. The net photosynthetic rate and the content of the main photosynthetic pigments increased in the Si-supplied infested plants compared to the Si-unsupplied.

Key words: *mites, photosynthesis, pigments, silicon, zucchini.*

INTRODUCTION

Silicon (Si) is the second most abundant elements on Earth`s crust after Oxygen. Most of the Si is present in the soil in the form of insoluble oxides and silicates, but there are also water-soluble forms. Si is the only element that does not damage plants when it is accumulated in excess due to its non-dissociative properties at physical pH and polymerization (Mahdieh et al., 2015). The influence of silicon on growth-enhancing and plant-resistance metabolic processes is recorded under stress conditions mainly. Several studies have shown that silicon is able to reduce the effect of stress caused by abiotic (Zhu et al., 2004; Feng et al., 2010; Harizanova et al., 2014; Harizanova and Koleva-Valkova, 2019) and biotic factors (plant pests and diseases). There are several studies on the effect of silicon on herbivore insect pests and noninsect pests (Massey et al., 2006; Massey and Hartley, 2009; Kvedaras et al., 2010; Harizanova et al., 2019).

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most harmful pests in the world and belongs to the group of the spider mites (Van Leeuwen et al., 2010; Tehri et al., 2014a). The mite damages crops directly by feeding which reduces the photosynthetic leaf area and biomass of the plants. This leads to disturbance of water balace, reduction of dry matter, CO_2 gas exchange, reduction of chlorophyll content and necrosis (Gorman et al., 2001; Park and Lee, 2002; Alatawi et al., 2007; Tehri et al., 2014b).

Different studies have shown that silicon is involved in the defense system of plants in several ways. According to some authors silicon provides an indirect protection, based on the increased release of volatile substances that attract natural enemies of pests (Kvedaras et al., 2010). Other researchers report that silicon`s positive effect is based on a direct protection through increased (passive) resistance due to the deposition of amorphous silicon in the tissues of the cells. Silicon acts as a mechanical barrier (Guntzer et al., 2012) and reduces the digestibility and/or increase the hardness and abrasion of plants (Massey et al., 2006; Massey and Hartley, 2009). Some hypothesis researches the regulation of genes observed in the infection with powdery mildew in *Arabidopsis* sp. (Fauteux et al., 2005) and infection of rice with *Magnaporthe oryzae*

(Brunings et al., 2009). One of the possible ways of action is also the activation of plant defense system. This triggers protective mechanisms including the accumulation of lignin, phenolic compounds and phytoalexins (Epstein, 1999; Ma and Yamaji, 2006), formation of papillae, deposition of callose and H_2O_2 and stimulation of system stress signals (salicylic acid, jasmonic acid and ethylene) (Shetty et al., 2012) and activation of enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), which are key enzymes regulating the formation and accumulation of secondary metabolites, phytoalexins and momilactones (Gomes et al., 2005; Ye et al., 2013).

There is scarce information about the effect of Si on plants which are infested by non-insect pests like spider mites. Most of the results obtained are connected to the silicon effect on the pest. The authors study the longevity and mortality of the herbivore enemy and there is a lack of information about the physiological status of the infested plants. The aim of the current study was to investigate the effect of silicon in form of orthosilicic acid on photosynthesis and the biomass of laboratory grown zucchini plants infested by the two-spotted spider mite *Tetranychus urticae*.

MATERIALS AND METHODS

Laboratory tests were conducted to study the effect of H_4SiO_4 on the physiological status of zucchini plants and on the polulation of the two-spotted spider mite were carried out in the phytostatic boxes of the Department of Entomology, Agricultural University - Plovdiv. Plants were grown in inert substrate under controlled environmental conditions: photoperiod 14/10 hours (light/dark), photosynthetically active radiation (PhAR) – 250 µmol/m/s/, air temperature 24±2°C/17±2°C (day/night) and relative air humidity 65±5%. After 24 hours in a thermostat germinated seeds from zucchini (*Cucurbita pepo* L.), cv. Izobilna were transferred to plastic pots filled with inert material (perlite) for a period of 5-7 days. Then, plants with the same developmental phase were selected and transferred to plastic containers. The experimental design consisted of four variants: 1 - control plants; 2 - plants infested with *Tetranychus urticae* Koch; 3 - plants treated with silicon if form of H_4SiO_4; 4 - plants infested with mites and treated with H_4SiO_4. All test plants were grown in an inert substrate (perlite) supplied with a nutrient solution containing all the necessary macro- and microelements. Ten days after germination, which coincided with the appearance of a third mature leaf, the plants were infested with 50 adult mites per plant. The first foliar Si treatment was 2 days after the infestation. Plants were treated with ones a week for four weeks and analyzed at the end of the period. Small leaf discs with 50 females in total were placed on the second and/or third true (mature) leaf of each zucchini plant. The population density was checked 30 days after infestation. Infested leaves from each plant were removed and checked under the stereomicroscope. The number of eggs, larvae, nymphs and adults of *T. urticae* was counted and recorded on twenty-four leaf discks (2 cm in diameter).

Experiment for study the duration of developmental stages of *T. urticae* was carried out under the same laboratory conditions. Small leaf discs (3 cm in diameter each) of zucchini plants from two variants (treated and untreated with Si respectively) were placed on wet cotton in Petri dishes (9 cm in diameter). Five females from the plants, treated and untreated respectively, were individually isolated and transferred to each leaf disc for egg laying. The discs containing adult females were checked every two hours after mite transfer. The mites were removed if at least one egg was found. Immediately after the new egg deposition females were transferred to new leaf discs. The discs were checked twice a day and the duration of developmental stages was recorded. The immature stages were transferred to new discs very carefully with the help of a tin hair brush.

Analysis

Guaiacol Peroxidase (GPOD)

The activity of GPOD was determined spectrophotometrically by the method of Bergmeyer et al. (1974). First 2.3 ml of KH_2PO_4 buffer (pH 7.0), 300 µl of H_2O_2, 300 µl of 8 mM guaiacol and 100 µl of extract are placed in the cuvette. Absorbance was measured at 436 nm against a blank with the same components without enzyme extract (E = 26.6 mm/cm^2). The values obtained are expressed as U mg/g FW.

Leaf gas exchange parameters

The rate of net photosynthesis (P_N), stomatal conductance (g_s) and transpiration rate (E) were determined with LCpro+ portable photosynthetic system [Analytical Development Company Ltd., Hoddesdon, England]. Measurements were performed under the following conditions: Photosynthetically active radiation (PhAR) of 500 μmol/m/s, temperature 25°C and natural external CO_2 concentration of about 400 vpm.

Content of photosynthetic pigments

The content of photosynthetic pigments was determined spectrophotometrically by the Lichtenthaler method (1987) and was expressed as mg/g fresh leaf material.

Statistical Analysis

The data were presented as mean ± SD of 3 replicates. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis using Duncan's comparative method, with the validity of the differences determined at a 95% significance level. The different letters (a, b, c, d) after the average show statistically significant differences between the analyzed variants. In the analysis of the mite population the differences between two variants with mites were analyzed and the significance was determined by independent samples t-test with a $p<0.05$. All data were analyzed using IBM SPSS Statistics 20 software.

RESULTS AND DISCUSSIONS

In order to analyze the effect of the silicon treatment on the mite population, the number of the different developmental stages of *T. urticae* was counted and recorded (Table 1). It is obvious that the Si treatment affects the number of almost all developmental stages of the mite, especially the number of mobile immature stages - their number is reduced by 29%. The reduction of the number of larvae on the plants treated with silicon is 33% and for nymphs these values are between 70 and 73%.

Table 1. Number of developmental stages of *T. urticae*, on Si-treated and Si-untreated plants

Developmental stages of *T. urticae*	Variant	Mean	Std. Error Mean
Eggs	Mite+Si	105.00	2.97
	Mite	82.25	3.23
Larvae	Mite+Si	31.75	3.72
	Mite	47.13	2.42
Protonymjphs	Mite+Si	3.25	0.73
	Mite	12.13	1.20
Deutonymphs	Mite+Si	9.25	1.18
	Mite	30.50	3.81
All mobile immature stages – larvae and nymphs	Mite+Si	44.25	4.47
	Mite	77.25	7.22
Females	Mite+Si	20.50	4.01
	Mite	14.13	1.57
Males	Mite+Si	9.25	0.81
	Mite	9.38	1.12
Adults (females and males)	Mite+Si	29.75	3.98
	Mite	23.50	1.31

The results of the independent samples t-test show that the difference between mean number of immature mobile stages from two variants (Mite and Mite+Si) is statistically significant ($t_{31.541}$=4.090; p=0.000).

Larvae and nymphs, as actively feeding mobile stages of the pest, are smaller than adults and this could explain some possible difficulties in their feeding and development and the reduction of their number. As it is mentioned by many authors (Reynolds et al., 2016; Alhousari and Greger, 2018) silicon treatment provides mechanical barrier and could reduce the tissues digestibility to some vertebrates.

Table 2. Duration of the different developmental stages (in days) of the two-spotted spider mite *T. urticae* on Si-treated and Si-untreated zucchini plants

Developmental stage	Variant	N	Mean	Std. Deviation	Std. Error Mean
Egg	Mite+Si	647	4.48	0.66	0.03
	Mite	307	4.19	0.78	0.04
Larvae	Mite+Si	131	2.76	0.67	0.06
	Mite	119	3.35	0.55	0.05
Protonymph	Mite+Si	103	2.17	0.51	0.05
	Mite	112	2.04	0.64	0.06
Deutonymph	Mite+Si	97	2.44	0.90	0.09
	Mite	96	2.44	0.65	0.07
Life cycle – egg to adult	Mite+Si	75	10.47	1.11	0.13
	Mite	95	10.44	1.18	0.12

The Si-treatment does not affect significantly the life cycle of the pest, although there are some differences between two variants regarding the duration of each developmental stage of the pest (Table 2).

The number of mobile immatures, reaching the next stage is lower on Si-treated plants (Figure 1). Eighty percent of the larvae on Si-untreated plants reach adulthood compared to 57% on Si-treated ones.

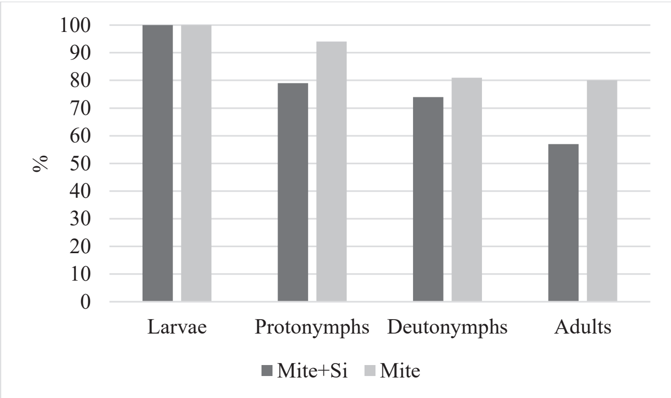

Figure 1. Percentage of the mobile immatures of *T. urticae*, reaching next developmental stage on Si-treated and Si-untreated plants zucchini plants after 30 days mite infestation and treatment with Si

In order to determine the degree of damage by the pest, the activity of guaiacol peroxidase (GPOD) was analyzed. The results show that the mite infestation increased the activity of GPOD in the leaves of the mite-infested plants more than five times compared to the control plants (Table 3). It is related to the higher number of the pest on Si-untreated plants. In the leaves of the Si-supplied mite infested plants the GPOD activity is reduced by 35%. The sole Si application led to a slight increase by only 13% compared to the control.

Table 3. Activity of guaiacol peroxidise GPOD (U mg/g FW) in the leaves of zucchini plants (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	Control	Si	Mite	Mite+Si
GPOD	6.302[c]	7.145[c]	35.57[a]	23.16[b]

The parameters of the leaf gas exchange are presented in Table 4. The results show a significant decrease of the net photosynthetic rate (P_N) of the infested plants compared to the control (by 55%). The transpiration rate is also reduced (by 44% compared to the control). The Si application increased P_N of the mite-infested Si-treated plants by 132% in comparison with the infested plants which were not treated with Si. There is also an enhancement of the transpiration rate by 49%. There are some differences in the rate of the stomatal conductance, although they were not statistically proven.

Table 4. Leaf gas exchange parameters of zucchini plants (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	P_N[1]	g_s[2]	E[3]
Control	6.47[ab]	0.1[a]	1.12[ab]
Mite	2.88[b]	0.05[a]	0.74[b]
Si	5.04[ab]	0.13[a]	1.72[a]
Mite+Si	6.68[a]	0.08[a]	1.1[ab]

[1]P_N = net photosynthetic rate (µmol/m^2/s); [2]g_s = stomatal conductance (mol/m^2/s); [3]E = transpiration rate (mmol/m^2/s).

The content of the main photosynthetic pigments is presented in Table 5. The infested plants were dramatically affected by the feeding of the pest. A reduction of chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoids in the mite-infested plants by 27%, 40%, 30%, and 22% respectively was observed. Silicon supply to the infested plants led to an increase of the pigment content by 28%, 16%, 26%, and 35% respectively. In a previous research Harizanova et al. (2019) reported about a significant increasment of the main photosynthetic pigment content in cucumber plants infested by the two-spotted spider mite *T. urticae*. The net photosynthetic rate was enhanced too.

Table 5. Content and ratio of photosynthetic pigments (mg/g) in zucchini leaves (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	Chl[1] a	Chl b	Chl (a+b)	Carotenoids	Chl a/b	Chl (a+b)/ carotenoids
Control	1.71[b]	0.7[b]	2.4[b]	0.56[ab]	2.46[b]	4.3[a]
Mite	1.35[c]	0.5[c]	1.84[c]	0.46[c]	2.7[ab]	4[b]
Si	2.5[a]	1.02[a]	3.52[a]	0.81[a]	2.46[b]	4.37[a]
Mite+Si	1.74[b]	0.58[c]	2.33[b]	0.62[b]	3.03[a]	3.75[c]

[1]Chl = chlorophyll.

The biomass of the analyzed plants was also measured. The fresh weights of the leaves, stems and the roots of the infested plants were strongly affected. The decrease in leaf biomass was by 51%. Stems were 25% lighter than the control and the weight of the roots was reduced by 52%. The mite infested and Si-treated plants demonstrated a significant increase in the biomass of all the plant organs. There is evidence of the positive relationship between high silica concentrations and plant resistance to herbivores (Gatarayiha et al., 2010; Yavas and Unay, 2017). The authors applied potassium silicate of different doses on the nutrient solutions of maize, eggplant, beans, and cucumber which were infested by the two-spotted spider mite. During their experiment, they observed that the Si-supplied plants suffer less damage than the untreated infested plants which is in line with the observations of the current study. Toledo and Reis (2018) examined the effect of foliar spraying with potassium silicate on coffee plants infested with red mite *Oligonychus ilicis*.

Table 6. Biometry of zucchini (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	Leaves		Stems		Roots	
	FW[1] (g)	LA[2] (cm^2)	FW (g)	L[3] (cm)	FW (g)	L (cm)
Control	21.6[a]	1003.4[a]	6.13[bc]	39.8[ab]	4.97[a]	29.5[ab]
Mite	14.27[b]	671.2[b]	4.9[c]	27.4[c]	2.39[b]	25.3[b]
Si	24.9[b]	1079.8[a]	8.6[a]	43.6[a]	4.16[ab]	35.7[a]
Mite+Si	21.4[b]	949.7[a]	6.81[b]	34.6[b]	2.47[b]	30.6[ab]

[1]FW = fresh weight; [2]LA = leaf area; [3]L = length.

The authors report about the reduced population of red mite and suggest that this effect may be due to chemical and physical changes in the plant tissues. They also observed the increased content of lignin and tannins which probably makes plant tissues less attractive to herbivores because of the increased hardness and toxic compounds in the plant tissues. About the same effect of the silicon treatment reported Yavas and Unay (2017). They suggest that Si could improve plant growth and resistance under stress conditions as it acts as a mechanical barrier for pests and diseases and prevents of oxidative stress via enzyme activation. The increased resistance to herbivore pests caused by silicon treatment has been reported also in various sensitive varieties of cereal crops (Keeping and Meyer, 2006, Hou and Han, 2010, Sidhu et al., 2013) and grasses (Massey and Hartley, 2009). He et al. (2015) found that increased levels of silicic acid in plants shortened the stay of cicadas on them and reduced the fertility of the pest. In another research it was established that after treatment with $CaSiO_3$, the mortality of *Bemisia tabaci* nymphs increased (Correa et al., 2005).

Higher silicon concentration in the soil or in the nutrient medium causes a decrease in the number of insect and non-insect pests of crop plants (Liang et al., 2005). The positive effect of silicon application has been reported by various pests including *spider mites* (*Tetranychus* spp.) (Gatarayiha et al., 2010) or mites (Savant et al., 1999; Nikpay et al., 2014). In the case of silicon feeding, the number of pests in the infested plants was reduced (Gomes et al., 2005). It is reported that pests with piercing-sucking mouth organs and herbivore caterpillars prefer silicon-poor tissues. Silicon reduces food intake, growth, fertility, and ultimately the population of *Sogatella frucifera* (Salim and Saxena, 1992). Silicon also reduces the reproductive capacity of the phloem-feeding species (*Myzus persicae*) on potatoes and whitefly (*Bemisia tabaci*) in cucumber.

CONCLUSIONS

Silicon supply in form of H_4SiO_4 is able to alleviate the negative effect of mite infestation in zucchini plants.

The number of the larvae and nymphs on the silicon supplied plants is reduced and the duration of some developmental stages of the two-spotted spider mite increased.

The net photosynthetic rate, the pigment content and the biomass accumulation of Si-treated mite-infested plants increased.

The positive effect of the Si-treatment is probably due to activation of the enzymatic defense system of the plants or to the increased hardness and abrasiveness of the plant tissues which makes plants tissues less attractive to the herbivores.

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INFLUENCE OF BIOCHAR AND MANURE FERTILIZATION ON THE MICROBIOLOGICAL ACTIVITY OF AGRICULTURAL SOIL

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Abstract

Fertilization with biochar (carbonized wood chips) and compost (well decomposed cow manure) was carried out on the following crops: zucchini (Cucurbita pepo), broccoli (Brassica oleracea), broad bean (Vicia faba) and leeks (Allium ampeloprasum). The introduction of organic fertilizers increases the development of all groups of microorganisms studied most strongly in zucchini, followed by broccoli and less in cultures of broad bean and leek. In zucchini, the application standalone of manure and biochar has a more microbial-stimulating effect, while in broccoli, broad bean and leek, the combined fertilizing variants increase the activity of soil microorganisms to a greater extent. As sampling days increase after application of organic fertilizers the biogenicity decreases, more noticeably after 200 days of application. The highest percentage in the composition of the total microflora is occupied by the ammonifying bacteria (non-spore-forming bacteria and bacilli), and the least represented are micromycetes and actinomycetes.

***Key words**: biochar, manure, crops, soil microorganisms.*

INTRODUCTION

Organic fertilization improves the development and activity of soil microorganisms, which helps the soil fertility to be improved by using of harmless ameliorants. A number of authors have investigated the organic fertilization effects on microbial comunities development and their activity in the soil (Li et al., 2004; Dinesh et al., 2010; Qiu et al., 2012; Muñoz et al., 2015). Partanen et al. (2010) has found that Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Deinococcus-Thermus bacteria and more than 2,000 different filotypes have participated in the composting process. Moulds and actinomycetes have been developing in both mesophilic and thermophilic composting phases, in the amount of 0.01 to 1 million on a gram of compost (Kowalik, 2015). The biochar is a carbon-rich product obtained by biomass heating in a closed container with little or no air available (Lehmann and Joseph, 2009).

In present study well decomposed cow manure and biochar from wood chips was used.

The manure contains a high amount of organic carbon, well stored with total N and medium stored with P and K, the values of the mobile forms being approximately equal to the reported total amounts. The ratio of ammonium to nitrate forms indicates that the mineralization process is not fully complete. The pH reaction of manure is slightly alkaline.

While the pH reaction in the analysed sample from biochar is highly alkaline. It contains a large amount of carbon, which confirms the ability of biochar to deposit carbon into the soil, reducing its release into the atmosphere. The mineral content of NPK is minimal. The presence of $CaCO_3$ is one of the causes of the highly alkaline reaction of the substrate.

Reducing the amount of green and brown plant materials and addition of rabbit fertilizer slows the development of microorganisms in the beginning of experiment, but in the following period they have been activated, and non-spore-forming bacteria have a significant role in the composting process, followed by the bacilli (Malcheva et al., 2018). The rabbit manure inclusion to compostable plant residues leads to an increase of soil nutrients upon matured compost application and, consequently, to an increase soil microbial activity (Malcheva et al., 2019). Micromycetes and bacteria exhibit different dynamics in the composition of soil microbiocenosis during mulching (Baldrian et al., 2012; Prewitt et al., 2014). It has been established that upon organic fertilizers using (Yankova et al., 2016) the fertilized soils show a greater amount of total microflora and higher activity of the microorganisms compared to the non-organic fertilized control sample. Non-spore-forming bacteria, followed by actinomycetes and bacilli occupy the major percentage share of the microbiocenosis composition.

Applying biochar leads to soil fertility and agricultural productivity augmentation, as well as it provides protection against certain plant diseases. Its application significantly improves soil quality (Petrova et al., 2019). It has been reported that soil microbial biomass and activity increase when it is used, especially when biochar was prepared at a lower temperature (350°C) and it was applied in the early stages of plant development (Luo et al., 2010). Higher temperature biochar production has eliminated potential substrates for germs. Produced at 700°C, the biochars, regardless of the raw material source, do not increase soil microbial biomass or activity (Zhang et al., 2014a). Sagrilo et al. (2014) demonstrate that large soil biochar additions significantly increase CO_2 emissions, while lower fertilization norms do not significantly affect emissions. Fabbri et al. (2012) investigated the degree of mineralization of the soil organic matter upon 20 types of biochar applying, depending on their chemical composition, and it has been found that biochar with higher concentrations of proteins and sugars (of incomplete transformation by pyrolysis) have increased the mineralization rate in its highest degree. In contrast, the biochar produced at higher-temperature result in lower CO_2 emissions. The soil conditions can strongly interact with the impact of biochar on soil biological activity (Blackwell et al., 2010). In arable soils, that are often fertilized and altered, the biochar long-term effect on the soil microbiota depends on the soil tillage. The properties of biochar must be considered in conjunction with soil conditions in order to be made properly design of its positive soil impact (Hardy et al., 2019).

According to Xu et al. (2014) studies, the use of biochar for fertilization increases plant growth, soil pH, total carbon and nitrogen forms, C/N ratio and cations exchange capacity. Their results show that its application significantly increases the diversity and alters the relative abundance of some microbes which are associated with the carbon and nitrogen cycle. Nitrification and denitrification processes are stimulated while reducing N_2O emissions overall. According to these authors, the use of biochar will reduce the soil acidification caused by the nitrogen fertilizers using, as well as reduce hothouse gas emissions. Other authors have also argued for the ecological role of biochar applying for fertilization. Song et al. (2017) have found that wheat straw biochar significantly reduce the toxicity of polycyclic aromatic hydrocarbons (PAHs), it helps to preserve bacterial diversity in PAH contaminated soil, and it significantly affects the structure of the bacterial community after 12 weeks of biochar use, and the effects depend on the type of biochar - the reproduction of rare bacterial genera (relative abundance of 0.01-1%) in the investigated soil increases. Therefore, the use of wheat straw biochar can reduce the environmental risks of PAH and it helps in soil microbial ecology.

Chintala et al. (2014) have established that upon applying corn biochar there is a negative effect on the activity of some soil enzymes - esterase (fluorescein diacetate hydrolysis), dehydrogenase, β-glucosidase and protease. According to them, the biochar materials used are highly hydrophobic, with a high fragrance, regardless of the biomass raw materials and the pyrolytic process. Wang et al. (2015) have investigated the combined effect of different biochar amounts (0, 0.5, 1.0, 2.5 and 5.0% by mass) on enzyme activities and the microbial community in soil. Increased addition of corn biochar leads to a significant increase in soil organic carbon, total nitrogen and digestible forms of potassium and calcium. According to their opinion, nitrogen and calcium are dominant factors affecting the activity of soil enzymes. The activities of soil extracellular enzymes involved in carbon (C) and sulfur (S) cycles (except for β-xylosidase) suggest smaller amounts of biochar (0.5% by mass) to increase the activity of enzymes in the soil. However, the activity of 1-leucine aminopeptidase and urease involved in the nitrogen cycle increases with the biochar amount raising. The total phospholipid fatty acids content and the relative abundance of bacteria decrease significantly with the biochar amount added. The relative abundance of fungus in the soil with added urea is significantly higher than in other treated soils, and the abundance of actinomycetes does not show a clear response from the biochar addition. The microbial community composition changes are mainly related to the organic carbon content and the total nitrogen content with a significant negative correlation. Therefore, the effect of biochar addition on soil enzymes and the composition of the microbial community are highly variable. Liao et al. (2016) have found in their investigation that fertilization with cotton straw biochar enhances soil microbial biomass, gram-positive and gram-negative bacteria, actinomycetes and enzyme activity associated with C and N transformation. The use of rice straw biochar may improve nutrient status in soil (increase in pH, C, N, K, P readings) and affect the structure of the microbial community - the composition of soil bacteria consists mainly of proteobacteria, actinobacteria and acidobacteria (Gao et al., 2017). These authors have found that by increasing amounts of biochar applied, the proteobacteria and acidobacteria increased, while actinobacteria decreased. With respect to the fungal population, Ascomycota species decrease, and Zygomycota and Basidiomycota species increase with increasing biochar. Pursuant to a study by Zhang et al. (2014b), the microbial biomass ratio of C:N is significantly increased upon biochar applying and it causes a less extreme environment for microorganisms throughout the winter wheat development season. Application of palm core and rice husk biochar in soils increases pH, moisture, organic carbon, microbial C and N biomass, activity of β-glucosidase and xylanase enzymes (Simarani et al., 2018). The synergistic effect of biochar and addition of nutrients to the composition of the microbial community increases the content of carbon and nitrogen, the amount of soil microorganisms and the index of dehydrogenase activity in the soil (Mierzwa-Hersztek et al., 2019).

There is a need to be further evaluated both the positive and the negative long-term effects of biochar on soil quality and crop productivity. Biochar may have a positive or negative effect on beneficial microorganisms in the soil, for which should be taken into account the temperature of pyrolysis when BC is prepared (Ajema, 2018). The researches on the long-term effects of biochar are insufficient (Maestrini et al., 2014; Sagrilo et al., 2014), although the biochar has existed in soil for centuries (Singh et al., 2012). New tools in molecular biology allow the identification of specific microbial groups associated with the presence of biochar, or because of the inherent ability to decompose biochar or specific microhabitats provided by the biochar porosity (Hardy et al., 2019).

The purpose of this research has been intended to investigate the effect of biochar of wood chips and cow manure alone and in combination on the soils microbiological activity with tested crops: zucchini (*Cucurbita pepo*), broccoli (*Brassica oleracea*), broad beans (*Vicia faba*) and leek (*Allium ampeloprasum*).

MATERIALS AND METHODS

The experiment has been carried out at the Vrazhdebna Training Center - Sofia on alluvial meadow soil (*Fluvisol*), on an area of 150 m^2 with an irrigation rate of 50 l/m^2. After the soil pre-treatment, the experimental plots were resized and on 31.03.2017 biochar (BC) was put in - BC (from pyrolysed wood chips) and well decomposed cow manure according to the following scheme:

► Variant 1 – Control (without ameliorants);
► Variant 2 - Manure 4 t/decare;
► Variant3 - Biochar 500 kg/decare;
► Variant4 - Manure 4 t/decare + Biochar 250 kg/decare;
► Variant 5 – Manure 4 t/decare + Biochar 500 kg/decare;
► Variant 6 - Manure 4 t/decare + Biochar 750 kg/decare.

The variants have been applied to the following crops: zucchini, broccoli, broad beans and leek. Sampling of soil samples has been carried out in sterile paper bags at a depth of 0-30 cm, at the maturity stage of the crop development – 80 Day After Incorporation of Ameliorants (DAIA) (zucchini), 102 DAIA (zucchini), 200 DAIA (broccoli), 445 DAIA (broad beans) and

557 DAIA (leek). A control, non-fertilized soil sample has been also examined for each piece. For microbiological analysis, the limit dilution method, solid medium culture (ordinary agar for non-spore bacteria and bacilli; Czapek-Dox agar for molds and bacteria absorbing mineral nitrogen; Actinomycete isolation agar for actinomycetes and bacteria absorbing mineral nitrogen), cultivation and determination of colony forming units (CFU) in 1 g of absolutely dry substrate. Statistical data processing involves the calculation of an average of three repetitions and a coefficient of variation (C.V.).

RESULTS AND DISCUSSIONS

The microbiological analysis for soil samples in zucchini culture (before the beginning of harvesting - 80 days after application of ameliorants) is presented in Table 1.

Table 1. Qualitative composition and amount of soil microorganisms under zucchini crop at the beginning of harvest (x 10^3 CFU/g abs. dry substrate); ± C.V.

Variant	Total microflora	Non-spore-for-ming bacte-ria	Bacilli	Micro-mycetes	Bacteria absorbed min. N	Coefficient of minerali-zation
V1	3200	2600± 0.314	200± 0.816	400± 0.408	6800	2.43
V2	26200	22200± 0.074	3000± 0.544	1000± 1.633	16800	0.67
V3	15600	11600± 0.141	3600± 0.227	400± 0.816	17200	1.13
V4	7420	4600± 0.177	1800± 0.454	1020± 0.800	18000	2.81
V5	12200	8400± 0.194	3200± 0.255	600± 0.544	17800	1.54
V6	10800	9600± 0.085	1200± 1.361	0	18800	1.74

The lowest microbial number, i.e. degree of microbial population or soil biogenicity has been established in the control soil (V1). This was observed both in terms of the overall microflora and the quantitative development of the individual microbial groups studied - non-spore-forming bacteria, bacilli, micromycetes (molds), bacteria absorbing mineral forms of nitrogen.

The biogenicity of fertilized soils with manure and biochar is increased more than 8 times compared to the control (non-fertilized) soil. The addition of nutrients to the soil obviously improves the conditions for the development and multiplication of soil microorganisms, with microbes imported with the organic fertilizers themselves likely to have a significant role for increase in soil biogenicity. For the investigation phase, the microbial mineralization activity did not reach that of the control soils- the rate of the organics decompose was lower at 80 days after fertilization (except for variant 4 - manure 4t/decare + BC 250 kg/decare).

In a comparative aspect for imported fertilizer variants (Variants 2-7), the total microflora has the highest amount of manure fertilization at 4t/decare (Variant 2) and the lowest for combined manure fertilization 4 t/decare + BC 250 kg/decare (Variant 4). However, it has been found that in Variant 4, the rate of the organics decompose is the highest, including for control soils with a mineralization factor of 2.81. It is proved by the fact that the lower amount of microorganisms does not always implies their lower activity.

As per other variants of the combined fertilization experiment (Variants 5 and 6), it was observed that at the same amount of manure, by increasing the amount of biochar, the amount of microorganisms also increases.

The investigations also show differences in the composition of the microbiocenosis in the different variants of the experimental scheme. A regrouping of bacilli and micromycetes was observed - the amount of bacilli was higher in fertilized variants than in the control. Whereas in the non-fertilized variant, micromycetes occupy a higher percentage (13% in V1) in the composition of the total microflora than in the other variants.

The highest percentage in the composition of the total microflora in all investigated sites is occupied by non-spore-forming bacteria (62-89%), followed by bacilli in fertilized variants (11-26%), i.e. the group of ammonification that are involved in the initial stages of organic matter decompose. Their amount is highest in soil fertilized only with manure (V1) or only with biochar (V3) and lower in the combined fertilization with both products (Variants 4, 5 and 6). In all variants of the experiment, the amount of non-spore-forming bacteria increased from 1.8 (V4) to 8.5 (V2) times compared to the control untreated soil. This is also the trend in the development of bacillary microflora. The number of this group of microbes has an even more pronounced upward trend of development - it exceeds the same in the control samples of 6 times in V6 to 18 times in V3.

The microflora in the composition of the microflora is the least micromycetes, which are even missing in the combined variant with the highest concentration of biochar (V6). Obviously the highest amount of biochar (750 kg/decare) used in combination with manure inhibits their development.

The fertilization increases the development of bacteria that absorb mineral nitrogen - their amount is increased up to 3 times compared to the control sample. The highest is the amount at V6 and V4, where the coefficient of mineralization is also highest. Combined manure fertilization and biochar fertilization have been found to increase the activity of this group of microorganisms against samples using either manure or biochar alone.

The results for the quantity and quality composition of soil microorganisms in the zucchini culture at the end of harvest (102 days after application of ameliorants) are presented in the Table 2.

Table 2. Qualitative composition and amount of soil microorganisms under zucchini crop at the end of harvest (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Micro-mycetes	Bacteria absorbed min. N	Coeffi-cient of minera-lization
V 1	30800	16000± 1.617	14200± 0.575	600± 0.272	62400	2.07
V 2	165400	147200± 0.022	16000± 0.612	2200± 0.742	59200	0.36
V 3	183400	169600± 0.019	13200± 0.680	600± 0.816	19200	0.11
V 4	174000	168000± 0.097	5800± 0.282	200± 1.633	185600	1.07
V 5	34600	25600± 0.128	7400± 0.221	1600± 1.531	160000	4.85
V 6	32400	24000± 0.340	6800± 0.240	1600± 1.021	168000	5.45

The fertilization increases the biogenicity of all the variants tested. It was highest after the biochar adding only (V3 - BC 500 kg/decare) and lowest in the control sample (V1), as the difference was 6 times. In the variant with the addition of both ameliorants - Manure 4 t/decare + BC 250 kg/decare (V4), the total amount of microorganisms is 5.6 times higher than the control sample. The only use of manure (4 t/decare - V2) increased biogenicity by 5.4 times towards the control samples. However, by increasing the amount of biochar from 250 kg/decare to 750 kg/decare in combination with 4 t/decare of manure (V6), the total amount of micro-organisms decreases, as this decrease is smooth upon increasing from 250 kg/decare to 500 kg/decare biochar (1 time) and it is significant when 750 kg/decare biochar is added - 5 times. The introduction of carbonized plant residues as soil improver (biochar) after a certain amount results to delay the development of the microorganisms - their quantity, but not to slow their activity - the rate of degradation of the organic matter is highest in the variants with the lowest amount of total microflora. The bacteria that absorb mineral nitrogen develop most actively during fertilization with V4, V6 and V5, which causes higher values of the mineralization coefficient in these variants.

The highest percentage in the composition of the total microflora is occupied by ammonify-cation non-spore-forming bacteria and bacilli, which initiate the processes of organic matter decomposition. The development of non-spore-forming bacteria follows the trends found in the total amount of micro-organisms analysis. While in bacilli only fertilization with manure V2 alone is higher than in control sample. The least represented in the composition of the total microflora are micromycetes, as the fertilization increasing the amount of molds by 3.7 times at V2 and 2.7 times at V5 and V6.

The microbiological analysis after fertilization with the same ameliorants in broccoli culture (200 days after application of ameliorants) is presented in Table 3.

Table 3. Qualitative composition and amount of soil microorganisms under broccoli crop (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Micro-myce-tes	Bacteria absorbed min. N	Coefficient of mineralization
V 1	47400	38400± 0.213	6800± 0.120	2200± 0.742	83200	1.84
V 2	67200	52800± 0.155	10400± 0.785	4000± 1.225	57600	0.91
V 3	50200	38400± 0.213	10000± 0.816	1800± 0.907	73600	1.52
V 4	85800	70400± 0.162	12800± 0.638	2600± 0.628	62400	0.75
V 5	172000	160000± 0.102	9000± 1.633	3000± 0.544	48000	0.28
V 6	81200	62400± 0.151	10800± 0.756	8000± 0.408	40000	0.55

It has been also established for this culture that the fertilization increases the total amount of microorganisms in all variants - 3.6 times (V5), 2 times (V4, V6), 1.4 times (V2), 1 time (V3) compared to control sample (V1). The results show that the use of the same enhancers and norms in broccoli increases the amount of microorganisms less than in the the zucchini experiment. Again, the highest percentage in the composition of the total microflora is occupied by non-spore-forming bacteria, followed by germs, and the least represented are micromycetes. In all researched groups, the fertilization increased the amount of microorganisms, with the exception of bacteria that absorb mineral nitrogen - their lower amount towards to the control sample determines a lower rate of mineralization of organic substances after fertilization.

The biogenicity decreased in the test broad beans culture (445 days after application of the amendments) after fertilization with the same amendments compared to the previous two cultures, but remained higher than the control sample, except for the use of V6 (Table 4):

Table 4. Qualitative composition and amount of soil microorganisms under broad bean crop (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria absorbed min.N	Coefficient of mineralization
V 1	4060	3100± 0.263	360± 0.454	240± 0.680	360± 0.454	5700	1.65
V 2	4240	3160± 1.034	620± 0.527	260± 0.628	200± 0.816	5640	1.49
V 3	4140	3200± 1.021	540± 0.605	220± 0.742	180± 0.907	5540	1.48
V 4	4200	3300± 0.495	500± 0.327	200± 0.816	200± 1.633	5700	1.50
V 5	4580	2700± 0.605	660± 0.247	240± 0.680	980± 0.333	5660	1.68
V 6	3940	2940± 0.278	660± 0.495	180± 0.907	160± 1.021	5720	1.59

The total amount of microorganisms is highest upon using manure 4 t/decare + BC 500 kg/decare (V5), but the values of the total microflora are close - 1 times higher in variants 2 to 5 than in the untreated control sample. The tendency for increased participation in the composition of the total ammonia microflora and lower presentation of actinomycetes and micromycetes persists, except for V5, where the amount of micromycetes is higher than that of bacilli. The activity of the micro-organisms after fertilization is close to that of the non-fertilized control sample, comparing the values of the mineralization coefficient.

Such trends, similar to those for broad beans, were also observed in the tested leek experiment - 557 days after the ameliorants introduction (Table 5):

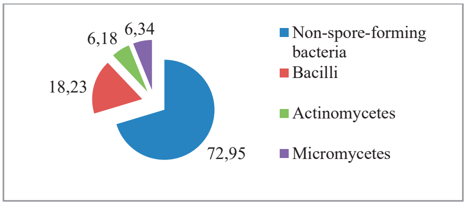
Table 5. Qualitative composition and amount of microorganisms under leek crop (x 10^3 CFU/g abs. dry substrate); ± CV

Variant	Total Microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria absorbed min. N	Coefficient of mineralization
V 1	3720	2760± 0.296	580± 0.282	240± 1.361	140± 1.166	3600	1.08
V 2	4140	3200± 0.510	540± 0.302	260± 1.256	140± 0.583	4000	1.07
V 3	3880	2760± 0.171	620± 0.263	320± 0.510	180± 0.907	3900	1.15
V 4	4280	3000± 0.544	580± 0.563	340± 0.480	360± 0.907	3760	1.05
V 5	4040	3020± 0.541	600± 0.544	240± 0.000	180± 0.454	3580	0.99
V 6	4820	3760± 0.217	540± 0.907	360± 0.454	160± 0.510	4740	1.10

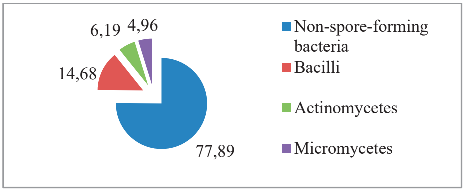
Similarly, the biogenicity for this crop increased after fertilization (about 1 time in all variants), but less than in the testing of zucchini and broccoli. The fertilization redistributes the relative participation of individual microbial groups in the composition of the microflora by degree of dominance, but in all variants of the experiment the composition of the common microflora is dominated by ammoniating bacteria (non-spore bacteria and bacilli), and micromycetes and actinomycetes are the least represented.

The biggest difference in the redistribution of individual groups of microorganisms towards the control has been established in zucchini crop 102 days after fertilization. In summary, the percentage of microorganisms in the composition of the total microflora in all experiments is presented in the Figure 1: A (control variants) and B (fertilized variants).

As a summarized trend for all crops and all variants, Figure 1 A and B established that fertilization increases the development of non-spore bacteria by 5% towards the control samples.



A.



B.

Figure 1. Percentage distribution of microorganisms under control samples (A) and fertilized variants (B)

While the bacilli group and the micromycetes group are respectively about 3% and 1% less represented after fertilization than in the control samples. The actinomycetes group shows close values in control samples and fertilized variants. Some authors also reported that the application of organic fertilizers, including and biochar in soils mainly increases the amount and diversity of bacteria in the composition of total microflora (Partanen et al., 2010; Xu et al., 2014; Yankova et al., 2016; Song et al., 2017). While other studies have indicated that increasing the biochar concentration inhibits bacterial reproduction, the development of micromycetes is activated, and the abundance of actinomycetes does not show a clear response from the addition of biochar (Wang et al., 2015). Gao et al. (2017) establish that with increasing amount of applied biochar, the development of proteobacteria and acidobacteria increased, while actinobacteria decreased. In their study, Liao et al. (2016) reported that fertilizing with biochar increases the amount of bacteria and actinomycetes. Therefore, the effect of biochar addition on the microbial community composition is highly variable.

CONCLUSIONS

Fertilization with manure and biochar increases the soil biogenicity. It is increased up to 8 times in zucchini, up to 4 times in broccoli and up to 1 time in broad beans and leek. Not only the different organic material as a crop type is relevant to this trend, but also the test time, humidity and nutritional status of the soils investigated. As sampling days increase after ameliorants applying, biogenicity decreases, more significantly after 200 days of applying.

As a summarized tendency for the zucchini crop, single applying of manure and biochar has a more microbial stimulating effect. For broccoli and broad bean, the introduction of Variant 5 (Manure 4 t/decare + BC 500 kg/decare) increases the total amount of micro-organisms to the highest extent. Only in leek, the addition of the highest amount of biochar - 750 kg/decare in combination with 4 t/decare of manure increases soil biogenicity the most.

The fertilization redistributes the relative participation of individual microbial groups in the common microflora composition by degree of control, but in all variants of the experiment ammoniating bacteria (non-spore bacteria and bacilli) prevail, and micromycetes and actinomycetes are the least represented.

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MANIFESTATION OF RESISTANCE TO SOME PATHOGENIC FUNGI AND PRODUCTIVITY CHARACTERISTICS IN TOMATOES

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Abstract

As a result of the analysis of the reaction of tomato genotypes to the culture filtrates (CF) of Alternaria alternata, Fusarium oxysporum, F. solani fungi, it was found that in the most cases they had a negative influence on seed germination. An inhibition of less than 15% in all FCs studied was recorded in the Flacăra, Mary Gratefully and L 10 B varieties, thus showing interest in the breeding process as possible donors of complex resistance to the seed germination stage. Root and stem growth in the evaluated varieties and lines was most strongly influenced by F. solani and A. alternata. The lowest sensitivity of root and stem to FC was recorded at L 66. The climatic conditions of the 2019 year have significantly influenced the general harvest of tomatoes. The highest productivity was recorded in the varieties Mary Gratefully (31.5 t/ha), Rome (30.3 t/ha) and L 71 lines (32.2 t/ha), that can be successfully used in the breeding when creating new varieties with high adaptability to the pedoclimatic conditions.

Key words: *Alternaria alternata, Fusarium spp., productivity, resistance, tomatoes.*

INTRODUCTION

Among the factors that greatly affect the productivity and quality of tomatoes fruit can be mentioned the low temperature, especially at the early stages of ontogenesis (Foolad and Lin 2000; Bralewski et al., 2004; Mihnea, 2006), burning in summer (Sato et al., 2006; Bita, Gerats, 2013; Mihnea and Lupașcu, 2018), root rot and alternariosis fungal diseases (Lupaşcu, Rotaru and Mihnea, 2009; Mihnea, Lupașcu and Gavzer, 2018).

Under the conditions of the Republic of Moldova, lately, in tomatoes are recorded the fungal pathogens *Fusarium* spp. which cause root rot at different stages of development, weakening and wilting of plants and *Alternaria* spp., which is manifested by brown stains of leaves, shoots, fruit and root rot (Lupaşcu et al., 2008; Lupascu et al., 2015; Mihnea, 2017).

The use of advanced technologies, resistant varieties, chemical treatments are considered as basic factors among the effective measures for the management of diseases in tomatoes. The lifespan of resistant varieties, usually recommended for production, is often limited due to the emergence of different pathogens new races that exceed the genes resistance of the cultivated varieties (Amini and Sidovich, 2010).

Varieties and hybrids that are characterized by high productivity stability under different ecological conditions are of great importance for agriculture. Thus, obtaining stable crops at vegetable plants, including tomatoes, reducing losses due to diseases and environmental unfavorable factors can be achieved by creating resistant varieties, with ecological stability and high plasticity (Mihnea, Lupașcu and Gavzer, 2018).

Creating resistant tomato varieties is one of the most effective strategies for controlling fungal diseases (Zhang et al., 2003; Çalış and Topkaya, 2011; Mihnea, Lupascu and Grigorcea, 2017).

The tomatoes genofond of the Institute of Genetics, Physiology and Plant Protection, currently has over 900 samples and consists of samples of local origin, collected from amateur growers; varieties of IGFP involved in tomato selection; varieties purchased from retail sales, which belonged to different seed companies abroad. Currently, the genofond is completed with samples created in the institute, through scientific cooperation and material exchange between scientific institutions and gene banks abroad.

The assessment of plant genetic resources is based on their differentiation according to the most important characteristics, highlighting the forms that have the capacity of hereditary

The aim of our research was to identify the level of resistance of some varieties and tomato lines to the fungal pathogens *Fusarium* spp., *A. alternata* in laboratory conditions and genotypes with high productivity under field conditions.

MATERIAL AND RESEARCH METHODS

As a research material, 6 varieties and 4 tomato lines were served. The lines and varieties Kristina, Florina, Măriuca, Darsirius, are of romanian origin from the Vegetable Research and Development Station Buzau. The experiments were carried out in laboratory and field conditions, on the experimental field of the Institute of Genetics, Physiology and Plant Protection, Republic of Moldova. Resistance to fungal diseases was carried out under laboratory conditions.

The culture filtrates (CF) of the fungi *Fusarium oxysporum, F. solani* and *Alternaria alternata* (isolated from diseased tomato plants) were used, prepared by inoculation of the mycelium in Czapek-Dox liquid medium and further cultivated at 22-24°C for a time for 21 days.

The tomato seeds were treated with CF of the fungi for 18 hours. The seeds kept in distilled water served as a control. Seedlings were grown in Petri dishes on filter paper moistened with distilled water at 22-24ºC for 6 days. As a test index of the plant reaction, important growth and development characteristics of tomatoes were used at the early stage of ontogenesis – germination, root length and stem length.

Cluster analyzes by constructing dendrograms (agglomerative-iterational algorithm, the Ward method), and the *k*-media method were performed (Savary, S. et al., 2010). Within the k-means method, 3 clusters were programmed according to the possible values of the characters: small, medium and high.

Tomatoes were grown by seedling culture in three repetitions according to the standard method (Erşova, 1978). Planting in the field took place in the third decade of May. The data obtained were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

The varieties and lines in the collection that showed a complex of useful characters in 2018 were tested under laboratory conditions to determine the reaction to the culture filtrates of pathogens *F. oxysporum, F. solani* and *A. alternata* based on seeds germination, embryo root and stem length. There was a strong inhibition of seeds germination, growth and development of the embryonic root and stem. It is worth mentioning that the reaction of the plants depended on the genotype, the analyzed character and the fungal species.

As shown from the data presented, the influence of CF on seeds germination in tomato genotypes included in the study was different (Figure 1).

For example, *F. oxysporum* CF inhibited seeds germination by -1.6% ... -30.3%, and *F. solani* by -5.8% ... -46.4%. Under the influence of *A. alternata* CF inhibition was -10.0% ... -44.9%. Significant inhibition was recorded in Florina variety (-30.3%) and lines L 66 (-19.4%), L 11 (-16.4%) under the influence of *F. oxysporum* CF.

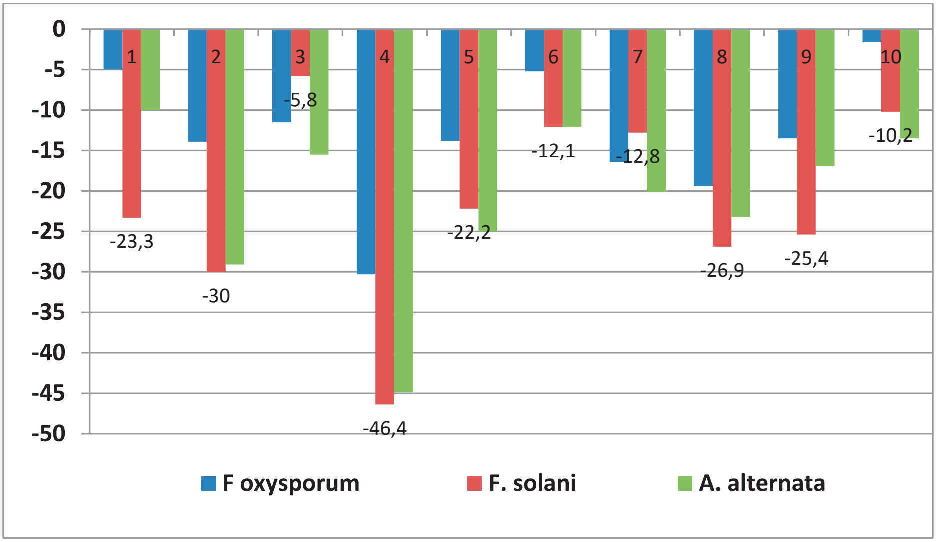

Figure 1. The influence of culture filtrates on seeds germination (%) of perspective lines and tomato varieties:

1. Roma; 2. Pontina; 3. Flacara; 4. Florina; 5. Mariuca; 6. L 10 B; 7. L 11; 8. L 66; 9. L 71; 10. Mary Gratefully

In the variant with *F. solani* CF strong character inhibition was observed in Florina, Pontina, Roma varieties, L 66, L 71 - 46.4, 30.0, 23.3, 26.9, 25.4%, and insignificant inhibition in Flacara and Mary Gratefully - 5.8 and 10.2%, respectively. Under the influence of *A. alternata* CF significant inhibition was found in the varieties Florina (44.9%), Pontina (29.1%), Măriuca (25.0%) and L 66 lines (23.2%), L 11 (20.1%). An inhibition of less than 15% in all evaluated CFs was recorded in the Flacara, Mary Gratefully and L 10B varieties, thus showing interest in breeding programs as possible sources of resistance to these fungal pathogens.

It was found that in the case of the root, the genotypes showed quite high sensitivity to CF (Figure 2). Thus, the above-mentioned culture filtrates inhibited root growth within the limits of 35.0 … 82.0%. The evaluated genotypes were most strongly influenced by *F. solani* and *A. alternata*, the average values relative to the control varying within the limits -46.9 ... -80.3% and -47.1 ... -82.0%, respectively. There were strong repressions at L 71, Florina, L 66, Flacara variant with *F. oxysporum* CF. In 8 of the 30 cases there was an inhibition of the growth of the embryonic root within the limits 35.0 ... 47.9%. Therefore, no genotypes with low sensitivity were attested, but the lowest sensitivity to the CF studied was recorded at L 66.

In the case of stem length, a wider amplitude of variability was identified in response to the CF fungus (Figure 3). Stem inhibition in relation to the control ranged within the limits -48.3 … -71.1% in *F. oxysporum*, -55.0.2… -84.7% - *F. solani*, -61.2… -85,7% - *A. alternata*. As in the case of the root, the genotypes were most strongly influenced by *F. solani* and *A. alternata*. For example, in the variant with *F. solani* CF, inhibition of more than 60.0% was observed in all genotypes, except for the L 66 line, where the growth of the strain was suppressed by 55.0%.

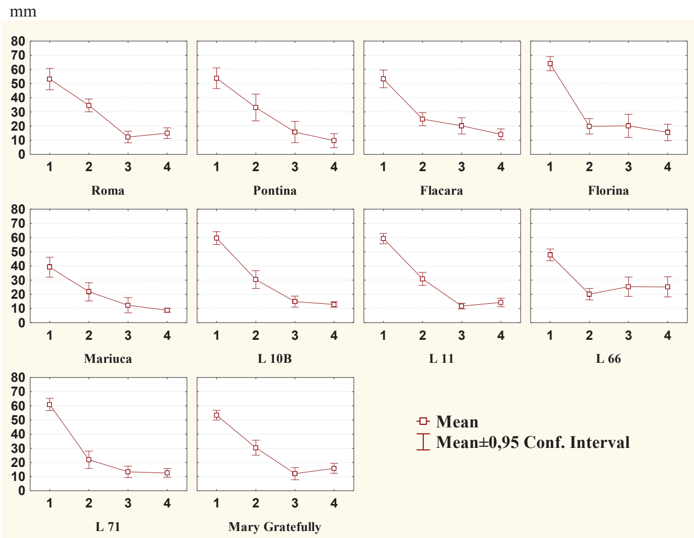

Figure 2. Influence of *F. oxysporum, F. solani, A. alternata* culture filtrates on root growth in tomato seedlings
Vertical: the root length
Horizontal: H_2O (control); 2. *F. oxysporum*; 3. *F. solani*; 4. *A. alternata*

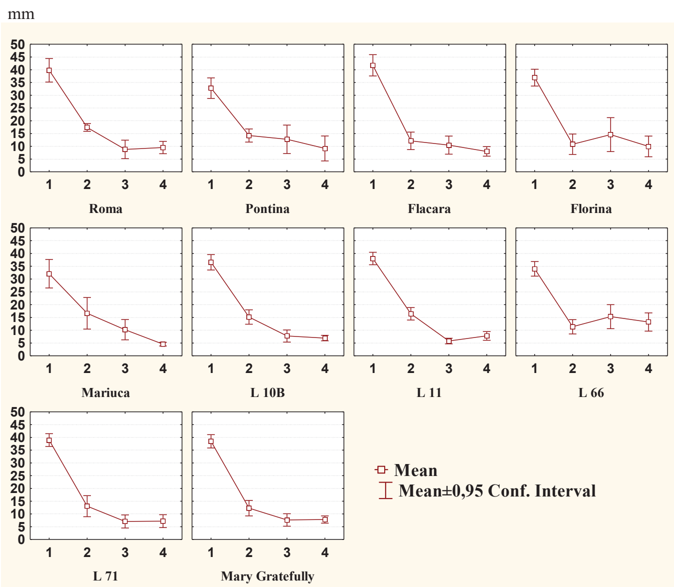

Figure 3. Influence of *F. oxysporum, F. solani, A. alternata* culture filtrates on stem growth in tomato seedlings
Vertical: stem length, mm
Horizontal: 1. H_2O (control); 2. *F. oxysporum*; 3. *F. solani*; 4. *A. alternata*

Based on the analysis of distribution dendrograms of varieties and lines of tomatoes, we found similarities and differences of distributions regarding the reaction of the embryonic root, stem and germination to the fungal metabolites (Figure 4). The highest similarity in the case of the embryonic root was found for L 10 B and L 11, of the stem - L 71 and Mary Gratefully, and of the germination – Pontina and L 66, Flacăra and L 11, which formed the smallest clusters. The other genotypes differed both from the control and from each other.

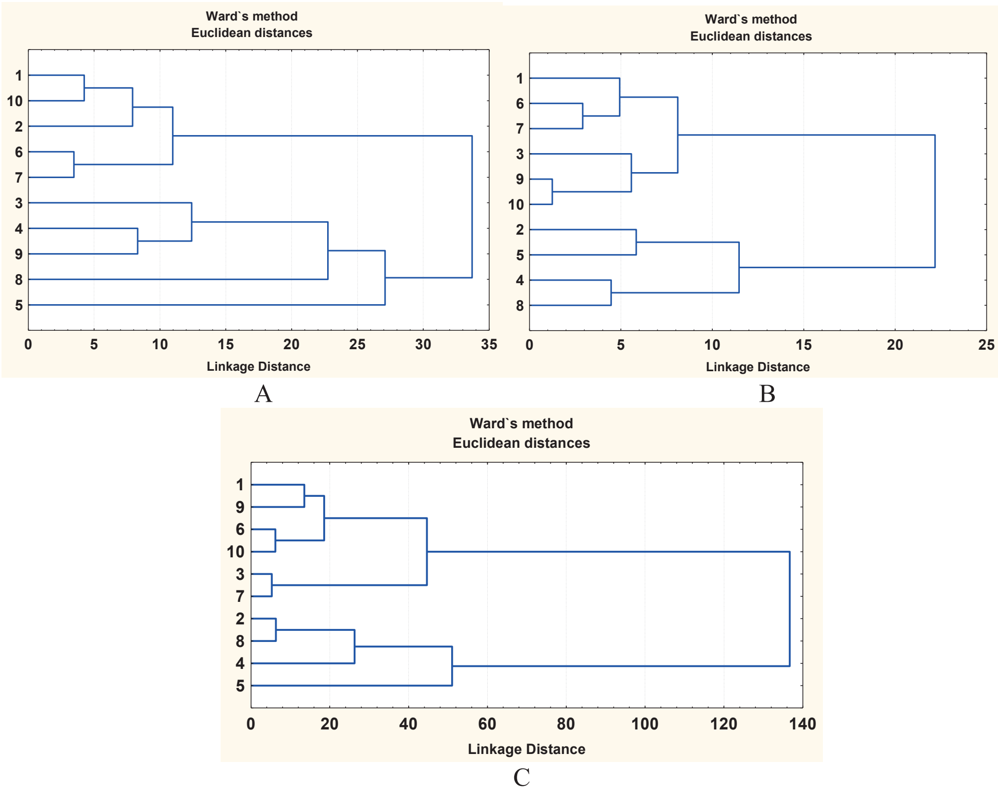

Figure 4. Dendogram of tomato varieties and lines distribution based on the reaction of the embryonic root (A), the stem (B) and the germination (C) to the fungi pathogens *F. oxysporum, F. solani, A. alternata*
1. Roma; 2. Pontina; 3. Flacara; 4. Florina; 5. Mariuca; 6. L 10 B; 7. L 11;
8. L 66; 9. L 71; 10. Mary Gratefully

Cluster analysis by *k*-means method showed that for all 3 studied characters, in the control variant the interclusterian variance was much higher than the intraclusterian one, which denotes that the 10 genotypes studied showed pronounced differences (Table 1).

Of great importance for the successful separation in clusters is the ability to discriminate characters and factors taken as cases - germination, root length, stem length. We can see that in general, the interclusterian and intraclusterian variance were much higher for germination, then for root length and lastly – for stem length.

In the case of germination, the separation of genotypes into clusters was successful for the all 4 variants - control, *F. oxysporum* CF, *F. solani* CF and *A. alternata* CF. It was observed that *A. alternata* CF was a factor with low genotypic differentiation power based on root length, and *F. oxysporum* CF - based on stem length, which indicates that the respective characters of the tomato genotypes studied did not specifically interact with these pathogens.

Table 1. Analysis of the inter- and intraclusterian variance in the interaction of tomato genotypes with some fungal pathogens

Variant	Interclusterian variance	Df	Intraclusterian variance	df	F	p
Germination						
Control (H_2O)	1079.601	2	180.468	7	20.94	0.00
F. oxysporum CF	1555.205	2	435.800	7	12.49	0.01
F. solani CF	761.029	2	517.560	7	5.15	0.04
A. alternata CF	1575.296	2	381.188	7	14.46	0.00
Root length						
Control (H_2O)	267.589	2	202.735	7	4.62	0.05
F. oxysporum CF	176.494	2	111.702	7	5.53	0.04
F. solani CF	160.621	2	31.820	7	17.67	0.00
A. alternata CF	85.309	2	96.880	7	3.08	0.11
Stem length						
Control (H_2O)	68.203	2	20.398	7	11.702	0.01
F. oxysporum CF	21.757	2	28.088	7	2.71	0.13
F. solani CF	79.243	2	16.118	7	17.207	0.00
A. alternata CF	26.683	2	19.773	7	4.72	0.05

By classifying the genotypes based on the 3 characters, it was found that in the control variant, cluster 1 had 6 genotypes - Rome, Florina, L 10B, L11, L71 and Mary Gratefully, with the highest values of the analyzed characters: germination - 96,38 %; root length - 58.43 mm and stem length - 38.1 mm. In the variant with FC, 4 of the mentioned genotypes - Rome, L 10B, L71 and Mary Gratefully formed cluster 3, with the highest germination values - 85.59%, the root and stem length being practically equal in the 3 clusters, which indicates that germination, compared to other 2 characters, was a factor with higher discriminant capacity (Table 2).

Table 2. Descriptive analysis of clusters

Cluster	Character	Control		Cultural filtrate	
		x	Genotype	x	Genotype
1	Germination, %	96.38	1 - Roma, 4 - Florina, 6 - L 10B, 7 - L 11, 9 - L 71,10 - Mary Gratefully	60.88	4 - Florina, 5 - Măriuca, 8 - L 66
	Root length, mm	58.43		18.80	
	Stem length, mm	38.1		11.86	
2	Germination, %	60.0	5 - Măriuca	76.64	2 - Pontina, 3 - Flacăra, 7 - L 11
	Root length, mm	39.1		19.42	
	Stem length, mm	32.1		10.74	
3	Germination, %	85.60	2 - Pontina, 3 - Flacăra, 8 - L 66	85.59	1 - Roma, 6 - L 10B, 9 - L 71, 10 - Mary Gratefully
	Root length, mm	51.63		18.87	
	Stem length, mm	36.20		10.05	

The evaluation of the data obtained regarding the crop structure (Table 3) demonstrated essential differences both after the general harvest and the commodity harvest that depended on the genotype and the weather conditions.

The climatic conditions of the year 2019 were extremely unfavorable for the growth of the plants, which favored the development of the virotic diseases that led to a considerable decrease of the productivity and the quality of the fruits. According to the data obtained in 2018, the general harvest ranged from 22.0 t/ha (Luci) to 78.6 t/ha (Măriuca), and the freight harvest from 40.1 to 69.6 t/ha, respectively.

Increased productivity in 2018 was recorded in the varieties Mariuca, Mary Gratefully, Flacara, L 66, whose productivity was 78.6, 73.0, 63.4 t/ha, 61.4 t/ha, respectively.

Table 3. The influence of the year conditions on the productivity characteristics of tomatoes

Variete, line	Harvest, t/ha						Share of fruit freight, %	
	General		% in comparison with 2018	Freight		% in comparison with 2018		
	2018	2019		2018	2019		2018	2019
Mary Gratefully	73.0	31.5	-56.9	59.6	28.2	-52.7	81.6	89.5
Roma	39.7*	29.0	-27.0	33.1*	23.7*	-28.8	83.4	81.7
Luci	22.0*	25.3*	+15.0	16.4*	20.9*	+27.4	76.8	82.6
Alex	41.4*	24.6*	-40.6	21.4*	19.9*	-7.1	51.7	80.9
Pontina	55.7*	29,3	-47.4	35.1*	25.5*	-27.4	63.0	87.0
Flacara	63.4*	25.7*	-59.5	44.6*	19.2*	-57.0	70.3	74.7
Kristina	32.3*	26.5*	-18.0	28.3*	22.5*	-20.5	87.6	84.9
Mariuca	78.6*	21.9*	-72.1	54.6*	19.5*	-64.3	69.5	89.0
Florina	59.7*	24.4*	-59.1	35.7*	22.2*	-40.8	59.8	91.0
Darsirius	56.0*	23.6	-57.9	50.6*	20.6*	-59.3	90.4	87.3
L 10B	55.4*	28.7	-48.2	48.6*	23.4*	-51.9	87.7	81.5
L 11	59.4*	17.0*	-71.4	52.3*	14.7*	-71.9	88.0	86.5
L 66	61.4*	28.2	-54.1	54.3*	25.1*	-53.8	88.4	88.6
L 71	50.6*	32.2	-36.4	39.1*	27.7	-29.2	77.3	86.0

*veridical difference at the $p < 0.05$ level of the control.

The climatic conditions of the 2019 year have significantly influenced both the general and the freight harvest. Significant decrease of the general harvest compared to 2018 year was attested to all the evaluated forms, falling within the limits of 18.1 ... 71.4%, except for the variety Luci at which the general harvest in 2018 year was of 22.0 t/ha, and in 2019 year - 25.3 t/ha. The highest productivity in 2019 year was found in the varieties Mary Gratefully, Pontina, Roma and lines L 10, L 66, L 71. Commodity harvest compared to 2018 years decreased by 7.1… 71.9%. The share of fruit freight in the samples taken in the study proved to be quite high and ranged from 51.7% (Alex) to 90.4% (Darsirius) in 2018 year and from 74.7% (Flacara) to 91.0% (Florina) in 2019.

CONCLUSIONS

It was found that the reaction of tomato plants (germination, root and stem growth) to *F. oxysporum, F. solani* and *A. alternata* CF's under controlled conditions was different and depended on the genotype growth organ, and the fungal species.

Cluster analysis by the *k*-means method found that all fungal species showed a higher discriminative capacity of tomato clusters for seeds germination, and for the length of the stem and root - *F. oxysporum* and *F. solani*, respectively, which reveals the specificity of more pronounced interaction with these pathogens.

By *k*-mean cluster analysis it was found that in the variant with fungal culture filtrates, 4 of the tested genotypes - Rome, L 10B, L71 and Mary Gratefully formed the cluster with the highest values of germination, root and stem length, which denotes their less pronounced sensitivity.

The highest productivity in the 2019 year showed the Mary Gratefully (31.5 t/ha) Rome (30.3 t/ha), Pontina (29.3 t/ha) and L 71 lines (32.2 t/ha), L 10 B (28.7 t/ha), L 66 (28.2 t/ha) varieties. The Mary Gratefully variety and the L 71 line can be successfully used in breeding for creating of new varieties with high adaptability to the pedoclimatic conditions of the Republic of Moldova.

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GENOTYPE RESPONSE OF DIFFERENT PEPPER VARIETIES TO THE ACCELERATED AGING TEST OF THE SEEDS

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Abstract

The main goal of the present study was to identify the genotypic response of different pepper varieties to the accelerated seed aging test and to optimize the regimen for this test in pepper. The experiments were carried out with eight varieties of pepper with a different origin. The seeds were subjected to accelerated aging according to the International Seed Testing Association (ISTA) rules. The temperature of 35^{0}C and 40^{0}C and duration of aging from 24, 48 and 72 hours were investigated. The seeds after treatment were subjected to a standard germination test. Initial seed moisture, germination energy, germination, mean germination time and uniformity of germination, fresh and dry weight of one sprout, length of embryo root and hypocotyls were determined. Severe germination suppression was observed at 35^{0}C for 72 hours and at 40^{0}C for 24 hours depending on the variety. Speed and camaraderie have almost doubled at 40^{0}C in 24 hours. The results were compared with non-aging seeds. The best physiological sensitivity was established at 40^{0}C for 24 hours.

Key words: *germination, viability, vigour, sprouts, mean germination time.*

INTRODUCTION

The high quality of seeds is of particular importance for the sustainable development and cultivation of vegetable crops (Peñaloza, 2005). During the storage of seeds, several of physiological and biochemical processes take place in them, which cause their vitality to be reduced (Kaewnaree et al., 2011).
TeKrony (1995) and Torres and Marcos-Filho (2003) emphasize that accelerated aging test is one of the most commonly used method for assessing the physiological potential and seed quality of the seeds.
Information obtained through accelerated seed aging supplement the results of other assessment methods, such as the standard germination test, while at the same time also showing seed performance during storage and field conditions (Almeida et al., 2014). According to Marcos-Filho (1999) and Freitas and Nascimento (2006) the principle of this test is to estimate the intensity and speed of deterioration of the sowing qualities by placing the seeds under stress conditions of high temperature and air humidity, and in the case of lower seeds quality this deterioration occurs much faster. Ferguson-Spears (1995) points out that, after cold test, the accelerated seed aging are the most commonly used method for assessing their vigour.
Demir and Mavi (2008) consider that, among other methods, accelerated seed aging is very suitable for evaluating sowing qualities and longevity in several of vegetable crops. Dutra et al. (2006), who also report that accelerated seed aging is particularly common in evaluating the quality of vegetable seeds. In small-seeded vegetables the water content of the seeds and faster absorption of the water and reaching its maximum content after a much shorter period than filed crops are the main problems which are the cause for the more limited studies of this test.
At the same time, it is a very good alternative for a more complete assessment of the seed (Jianhua and McDonald, 1996). In this sense, Marcos-Filho (1998) also reported that in small seeded crops, further studies are needed to improve the methods of determination vigour, which can be successfully achieved through accelerated aging and its advantages are easy and a quick way for qualification of the seeds.
To classify pepper seeds from individual lots, Panobianco and Marcos-Filho (1998) recommend the application of the accelerated

aging test. Gagliardi and Filho (2011) point out that this test is very suitable for assessing the physiological potential of pepper seeds. One of the most commonly studied factors in the application of the accelerated aging test for pepper seeds is the influence of exposure time at high temperatures (TeKrony, 1995).

The main goal of the present study was to identify the genotypic response of different pepper varieties to the accelerated seed aging test and to optimize the regimen for the test in this crop.

MATERIALS AND METHODS

The experiments were carried out in the experimental field of the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria with the following varieties, with the corresponding humidity indicated in the brackets Jalapeno (8.8%), Dzhulunska shipka 1021 (8.6%), Wulkan (9.2%), Picante de Cayene (10.4%), Romanian Giallo (10.1%), Padron (9.8%), Pyramid (10.6%) and Beros (9.4%). The seeds were produced in the field of the Horticulture Department from original sowing material provided by the seed companies.

The standard Bulgarian technology for middle early field production was applied, with sowing on March 15 and planting on May 20 according to the 60 x 15 cm scheme. In the complete botanical maturity phase, the fruits were harvested and the seeds were extracted and dried to the moisture that cover the requirements of ISTA (2013), as it was above mentioned.

The initial moisture content of the seeds was pre-determined using the method described in ISTA Rules (2013). The seeds of all varieties were placed for accelerated aging according to the method described by ISTA (2013). Temperatures of 35^0C and 40^0C and duration of 24, 48 and 72 hours were tested. The seeds by 4.0 g of each variety were placed in a lid-closed box of the following dimensions 11 × 11 × 3.5 cm for length, width and depth, respectively. Distilled water from 40.0 ml was added to the bottom of the box. The seeds were placed on a pre-assembled plastic mesh with measures 10 x 10 cm at a height of 3 cm from the bottom. The boxes thus prepared were kept in a water-jacketed ageing chamber at the temperatures and processing times indicated above.

Immediately after accelerated aging was completed within one hour, the seeds were subjected to a standard germination test in four replicates according to the method of ISTA (2013). First count and final count were determined in 4 replicates using the method described in ISTA (2013). Mean germination time by method of Battle and Whittington and uniformity of germination by method of Strona, both described in Panayotov (2015)
were established.

At the time of germination account, the length of the embryo root and hypocotyls were measured on ten seedlings from each replicates as well as the fresh and dry weight of a seedling. The seedlings dry weight was determined by the method described in Georgiev et al. (1980). The disperssion analysis of data (ANOVA) were processed (Foel and Cohen, 1992)

RESULTS AND DISCUSSIONS

The most of the tested varieties there were relatively good initial germination energy, from 35.3% to 87.3%, for Romanian Giallo and Jalapeno respectively (Figure 1). High values were also account for Dzhulunska shipka 1021 (85.3%) and Padron (83.3%). Silva and Vieira (2006) consider that germinating energy data provide information on seed vigour, adding that the deterioration of seeds may first be detected much earlier from changes in germination energy than in germination.

Accelerated seed aging causes significant changes in this indicator yet in the first treatment period of 24 hours at 35^0C. The highest decrease in comparison with control was observed for the seeds of the Pyramid variety - with 49.02%, followed by those of Romanian Giallo - with 37.68%. Both varieties have low initial germination energy. According to Torres and Marcos-Filho (2003), low-quality seeds deteriorate much stronger and faster than higher-quality one.Very close to the initial, remains the germination energy at Jalapeno and Beros, as well as for Dzhulunska shipka 1021. The deterioration is also significant at 48 and 72 hours, especially for the Wulkan and Romanian Giallo. A temperature of 40^0C leads to an even greater reduction in germination energy, as early as 48 hours it is zero for the seeds of Jalapeno and Pyramid, and at the next 72 hours exposure no germinating seeds were found in almost all the varieties tested. At this temperature for 24 hours, high values still show Dzhulunska shipka 1021, Padron and Jalapeno, i.e., seeds with high initial germination energy. Similar are the observations of TeKrony's (2003), according to him the initial seed status determines the strength and intensity of the accelerated aging effect.

All varieties included in the experiment there were very high germination, except for Romanian Giallo, as the values ranging from 81.6% for Picante de Cayene to 94.7% for Jalapeno and Padron. Compared to these data, as a result of accelerated aging at 35^0C for 24 hours the Wulkan deteriorated by 26.7%, followed by Picante de Cayane by 16.9% and Beros by 15.3%. In the next two exposures, as well as for 24 hours at 40^0C, only the Dzhulunska shipka 1021 and Padron retain good germination. The deterioration at 24 hours/40^0C is very strong, especially for Pyramid and Jalapeno, with 77.0% and 67.4% respectively of the initial germination. At this temperature for 72 hours of aging, only the seeds of Dzhulunska shipka 1021 retained acceptable germination, although not by the standard, with a reduction of 36.6% than non-aging seeds. This indicates that these seeds are with good storability and longevity, which is possibly related also with their higher vigour.

In most of the varieties there is also a complete loss of germination and lethality of the seeds. The sharp deterioration, but with a very well pronounced difference between the separate varieties and the ability to clearly highlight the samples with high vitality and vigour, is established at 40^0C for 48 hours. This gives reason for recommending a temperature of 40^0C with duration of 48 hours as an appropriate regime, with good physiological sensitivity for conducting the accelerated aging test of pepper seeds. This regime indicates very well the high seed potential of the Dzhulunska shipka 1021, Padron, Wulkan and Beros varieties.

In the previous exposure, severe deterioration was also observed, but the variability between the variants was lower and at the highest duration of 72 hours lethality was observed in many samples. Similar parameters for a suitable regimen for accelerated aging of pepper seeds have been reported by other researchers, such as Bhering et al. (2006), Gagliardi and Filho (2011) and Silva da et al. (2018). Serious deterioration of pepper seeds during accelerated aging was also observed by Kaewnaree et al. (2011), emphasizing that this is primarily due to lipid peroxidation and damage to cell membranes, which is associated with increased electrolyte leakage and inability to protein transport.

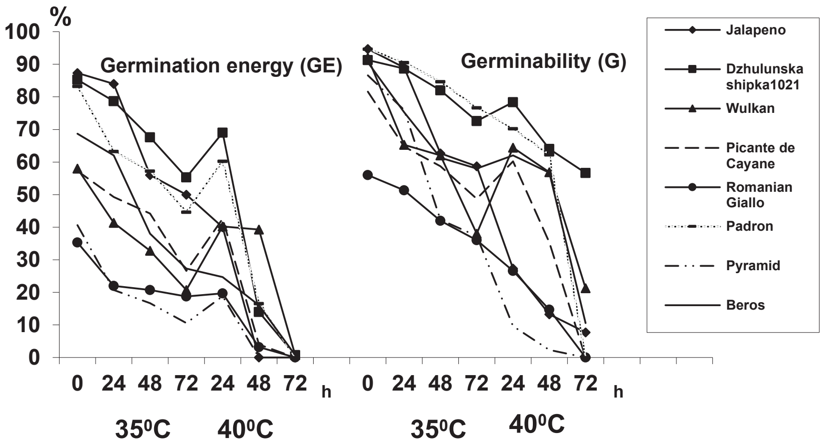
Zhang et al. (1999) and Tang and Song (1999) also point out as one of the main reasons for the deterioration of the seed quality in accelerated aging are the lipid membrane peroxidation and damage of biomembrane integrity. Statistical significance of the established results except for the variants for 74 hours at 40^0C has been established.

Kapoor et al. (2010) and Rastegar et al. (2011) reported that accelerated aging causes an increase in mean germination time. The initially mean time for seed germination of the tested varieties is between 3.0 (Jalapeno) days to 5.93 days (Romanian Giallo) (Figure 2). After 24 hours at 35^0C, the required germination time increases by approximately one day. The slightest changes are in the Romanian Giallo and Padron varieties, and the strongest one are in the Picante de Cayene.

With increasing temperature and treatment time mean germination time increases steadily, although at a slower rate to reach an increase at 40^0C/72 hours between 3 and 4 days while for Jalapeno variety even more - 7.7 days compared to untreated seeds.

The uniformity of germination as a result of stressful conditions in accelerated aging decreases. The control values of the Dzhulunska shipka 1021 (16.7%) and Jalapeno and Padron varieties with 14.6% and 13.4%, respectively are characterised with the highest values.

After their placement at 35^0C for 24 hours, this indicator is most strongly affected about Picante de Cayene. Relatively high values at 40^0C/72 hours are maintained at Dzhulunska shipka 1021. A strong decrease is observed at 40^0C/48 hours, but in this regime, both mean germination time and uniformity also have the best physiological sensitivity. Tores and Marco-Filho (2003) emphasize that accelerated aging is of particular importance for the correct evaluation of individual lots. According to Tomes et al. (1988) the effect of temperature compared to aging time has a stronger suppressive influence. The data for these two indicators are with statistical significance except for those for the uniformity of a variety Beros at 40^0C in 72 hours.



LSD p=5%

GE	35^0C			40^0C			G	35^0C			40^0C		
0	24	48	72	24	48	72	0	24	48	72	14	48	72
4.4	3.8	4.1	3.5	5.2	4.8	2.2	3.1	2.6	3.0	3.2	4.3	3.6	5.1

Figure 1. Germination behaviors of pepper seeds after accelerated aging

In addition to vitality, the accelerated aging strongly influences the morphological development of the sprouts. One of the most important indicators related to vigour is the fresh weight of a sprout (Copeland and McDonlads, 2003).

The highest initial fresh weight (Figure 3) was developed by the Jalapeno varieties (82.1 mg) and the lowest one by the Wulkan variety (47.9 mg). With the lightest aging regime of 35^0C/24 hours, the changes are relatively small. A greater decrease was observed only in the Pyramid variety - by 13.0 mg.

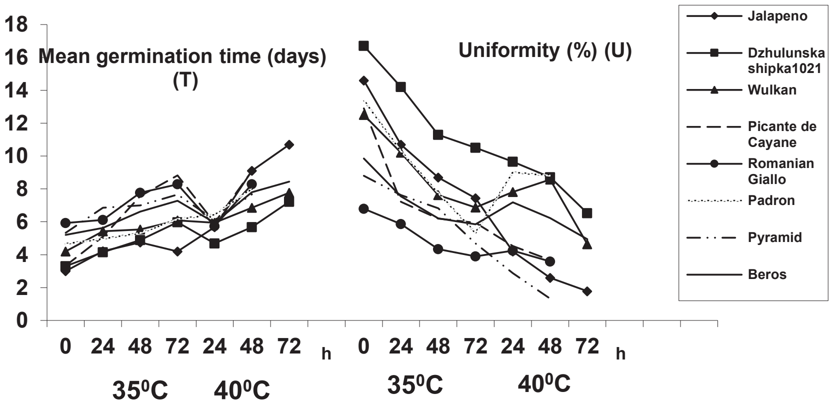
With an increase in aging time of 48 hours, the deterioration is more significant. At 72 hours, fresh weight decreased from 22.87% for the Beros variety to 56.1% for Pyramid compared to the control. This characteristic is much lowers at a temperature of 40^0C at 48 hours and as significantly larger stands the weight of sprouts of Jalapeno variety, while the values for the other genotypes are much smaller. This trend is also maintained for 72 hours.

The effect of accelerated aging is very clear also on the accumulated dry weight of sprouts. Deterioration was detected even at 35^0C/24 hours, the highest for the Jalapeno variety- with 39.0%, compared to the control and the lowest one for Romanian Giallo and Pyramid, by 17.2% and 19.1%, respectively. With increasing the temperature and aging time, the deterioration processes increase steadily and by 72 hours at 40^0C the dry weight is more than 50% lower than the control.

The differences for the fresh and dry weight with the controls are mathematically proven, except those of the Beros variety for the 72/40^0C and the Dzhulunska shipka 1021 variety for the dry weight under the same regime.

The effects of stress conditions from accelerated aging are also found on the length of the embryo root (Figure 4). A decrease is reported at both tested temperatures over a 24-hour period, although differences between controls and between varieties are not well demonstrated. It is stronger for the next 48 hours, especially at 40^0C. In this regime, as well as at 35^0C for 72 hours, the physiological sensitivity to accelerated aging, expressed in more significant varieties differences, is much clearer and well noticeable. At 40^0C/72 hours the values are insignificant.

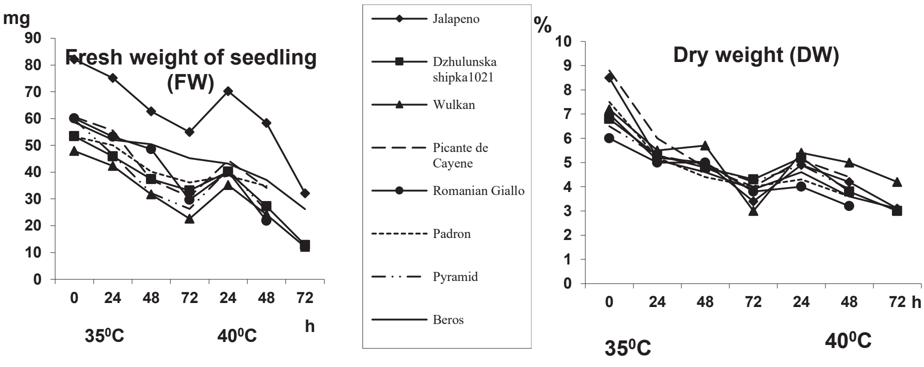
The tendency for the length of the hypocotyls is similar, as the degree of deterioration being stronger. A greater reduction at 35^0C/24 hours is established for the Jalapeno variety. Varietal differentiation is more significant at 35^0C/72 hours and especially at 40^0C/48 hours. This once again indicates that the 40^0C/48 hour regime is suitable to be applied to evaluate the potential, vigour and the storability of pepper seed lots.



LSD p=5%

T	35⁰C			40⁰C			U	35⁰C			40⁰C		
0	24	48	72	24	48	72	0	24	48	72	14	48	72
1.1	1.6	2.0	1.8	1.0	0.7	1.6	1.9	1.6	1.4	2.2	1.9	2.3	2.9

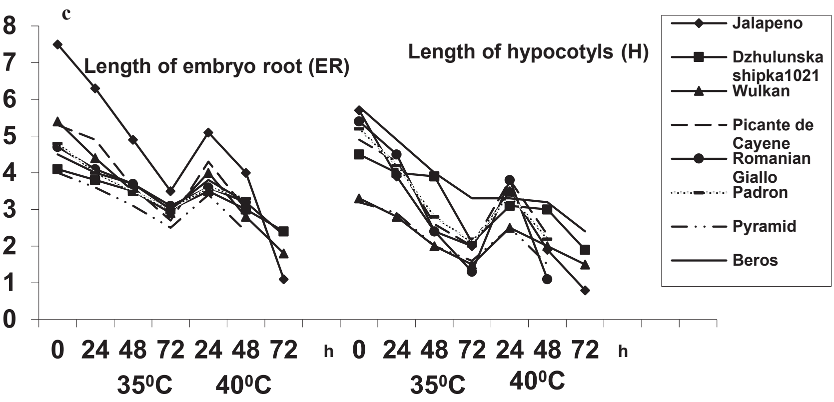
Figure 2. Sowing characteristics of pepper seeds after accelerated aging



LSD p=5%

FW	35⁰C			40⁰C			DW	35⁰C			40⁰C		
0	24	48	72	24	48	72	0	24	48	72	14	48	72
6.4	5.1	4.8	7.2	8.9	5.4	8.8	1.2	0.9	1.6	0.4	0.6	0.5	0.9

Figure 3. Development of pepper seedling after accelerated aging test of the seeds



LSD p=5%

ER	35^0C			40^0C			H	35^0C			40^0C		
0	24	48	72	24	48	72	0	24	48	72	14	48	72
0.9	1.3	1.2	0.8	1.5	1.0	1.5	1.1	2.0	1.4	1.9	0.9	1.6	1.1

Figure 4.Morphological characteristics of pepper seedling after accelerated aging of the seeds

CONCLUSIONS

Using of the accelerated seed aging test, accurate assessments of the vital status and the storability of individuals lots of pepper seeds can be successfully carried out and classified in descending order.

The highest viability potential and storability respectively, after application of the accelerated aging test, demonstrated the seeds of the varieties Dzhulunska shipka 1021 and Padron, followed by those of Wulkan and Beros.

Depending on the applied regime - temperature and time of impact, the sowing qualities of pepper significantly reduced, but a strong deterioration is also observed on the morphological development of the sprouts.

The most accurate estimation of the potential, vigour and storability of pepper seeds and a high physiological sensitivity in the same time are achieved by applying accelerated aging regime form 48-hours at 40^0C.

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PRODUCTIVITY AND ELEMENTS OF THE YIELD OF CARROT SEEDS IN THE APPLICATION OF DIFFERENT REGIMES OF FERTILIZATION

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Abstract

The main aim of this study was to determine the effect of different levels of mineral fertilizers and fertilization methods on the productivity of carrot seeds. The experiments were conducted with the Tushon variety. The seed plants were grown using standard technology through stecklings. Three levels of NPK were tested, as follows $N_{0,50,70,90}$ kg/ha, P_2O_5 $_{0,90,140,190}$ kg/ha and K_2O $_{0,100,150,200}$ kg/ha, applied once and twice. The number of umbels per order, number of umbellate per umbels, diameter of umbels, weight of seeds per umbel and percentage of normally developed seeds as well as yield and part of different orders for yield formation for central umbel (king umbel) and umbels from I, II and III orders were studied. The highest yields were found to occur with a single application of $N_{90}P_{90}K_{200}$ and double fertilization with $N_{90}P_{90}K_{100}$. Seeds obtained from the first and second orders are characterized with the highest ration in yield formation, the average for tested variants is 50.61% and 30.57% respectively. The strongest positive correlation between yield and number of umbels and umbellate, umbel diameter and weight of seeds per umbel were established.

***Key words**: order, central umbel, yield ratio, Apiaceae, seed production.*

INTRODUCTION

Seed production of many vegetable crops, including carrot seeds, is significantly suppressed in the absence of an optimal nutrient regime. Particularly important for normal seed production is the full satisfaction of the needs of plants with N, P, K and Ca (Harrington, 1990). The author emphasizes that nitrogen and phosphorus deficiency reduces the percentage of normally grown seeds.

According to Amjad et al. (2005) carrot seed yield depends essentially on the proper combination of nitrogen and potassium fertilization. They obtained the highest seed productivity when fertilizing with N 75 kg.ha^{-1} and K 90 kg.ha^{-1}, and most yield is low when N 225 kg.ha^{-1} and K 50 kg.ha^{-1} are applied. Similar are the conclusions formulated by Satyaveer et al. (1994), Singh and Singh (1996) and Rao and Maurya (1998).

Black et al. (2008) and George (1999) describe the carrot seed plant, pointing out that the central or king umbel is developed firstly and subsequently the umbels of the first, second and so on order, which take place over a long period, and this affects both the seed setting and formation, and their yield and quality.

According to Gray et al. (1983) the morphology of the carrot seed plant determines to a large extent the formation of the total yield, as the part of seeds obtained from first-order umbels occupying between 25% and 62%. Gray (1981) also estimates that the first-order umbels take a 60% part in total seed productivity of carrots.

Gill et al. (1981) emphasized that the total productivity of carrots seeds is determined extremely by the number of umbels of the different orders.

Mengistu and Yamoah (2010) report that the average seed yield between first, second and third-order branches are differs significantly. They found strong correlation relationships between yields with: number of branches per plant, plant height, umbel diameter, number of umbels per plant, number of umbellate per umbel and seed weight per umbel, with the strongest influence being the number of umbellate per umbel and umbel diameter. Similar trends of seed formation of this crop have been reported by El-Adgham et al. (1995).

The main aim of this study was to determine the effect of different levels and ways of application of mineral fertilizers and on the productivity of carrot seeds.

MATERIALS AND METHODS

The experiments were carried out during 2017-2019 in the Experimental field of the Department of Horticulture at the Agricultural University - Plovdiv, Bulgaria with carrot Tushon variety. The standard, well-established technology for seed production of carrots in Bulgaria by steklings was applied. The seeds were sown at the end of June, the roots were harvested in the middle of November and stored in a pit. In mid-March, the steklings were planted in an 80 x 30 cm scheme. The experiments were arranged at four replicates, with a plot size of 7 m^2 and a reported area of 6 m^2. The soil was prepared by deep plowing in the autumn and profiling the furrows in the spring.

Two regimes of fertilization were investigated:

1. By applying phosphorus and potassium fertilizers once in the autumn before deep plowing and nitrogen during planting;
2. Twice fertilization - half of the phosphorus and potassium fertilizers were applied before the autumn deep plowing, the other half in spring in planting, and the nitrogen fertilizer - half during planting and the other part during the growing season at the beginning of flowering.

The following variants with different fertilizer levels were studied:

- once fertilization: 1. $N_0P_0K_0$ - control; 2. $N_{70}P_{140}K_{150}$ (recommended); 3. $N_{50}P_{90}K_{100}$; 4. $N_{50}P_{90}K_{200}$; 5. $N_{50}P_{190}K_{100}$; 6. $N_{50}P_{190}K_{200}$; 7. $N_{90}P_{90}K_{100}$; 8. $N_{90}P_{90}K_{200}$; 9. $N_{90}P_{190}K_{100}$; 10. $N_{90}P_{190}K_{200}$;
- twice fertilization: 11. $N_{50}P_{90}K_{100}$; 12. $N_{50}P_{90}K_{200}$; 13. $N_{50}P_{190}K_{100}$; 14. $N_{50}P_{190}K_{200}$; 15. $N_{90}P_{90}K_{100}$; 16. $N_{90}P_{90}K_{200}$; 17. $N_{90}P_{190}K_{100}$; 18. $N_{90}P_{190}K_{200}$.

The different fertilization levels were determined based on the recommended fertilization for carrots seed production in Bulgaria - $N_{70}P_{140}K_{150}$ (Murtazov et al., 1984).

The following fertilizers were used: triple superphosphate (P_2O_5 46%), potassium sulfate (K_2O 50%) and ammonium nitrate (N 34%). During the growing season, all agro-cultural practices about the normal development of seed plants were applied in the technology. Seeds were harvested in 60-70% of the ripe seeds and others in waxy maturity. Post-harvest maturation was implemented over ten days.

The number of umbels, the number of umbellate in the umbel, the diameter of the umbels and the weight of seeds from one umbel were determined. The percentage of normally developed seeds for each order of branches in an average sample of 1.0 g was investigated, by as enumeration of fully developed seed and not well-developed, and the percentage of normal seed development was calculated. These studies were performed separately for the central umbels and the umbels of I, II and III order on five plants of each replicate. Seed extraction was performed individually for the umbels of the different orders and the total yield was determined.

The data were subjected to dispersion and of correlation using the methods described by Fohel and Cohen (1992).

Because the trends of the data during the three separate vegetation seasons are unidirectional presented results are average three-year values.

RESULTS AND DISCUSSIONS

The number of umbels per carrot seed plant (Table 1) varies both between the different branches and the fertilization applied. After a once fertilization with $N_{50}P_{90}K_{100}$ and $N_{90}P_{90}K_{100}$, the first-order umbels increase the most, compared to the control by 26.79% and 25.86%, respectively. The second-order umbels are the most - 19.8 and 18.94, respectively for $N_{90}P_{90}K_{200}$ and $N_{90}P_{190}K_{200}$, i. e. for variants with the highest tested levels of nitrogen and potassium.

Singh and Singh (1996) emphasize that nitrogen fertilization contributes to the formation of a greater number of umbels in the carrot seed plant. Again, the $N_{90}P_{90}K_{200}$ combination also causes the formation of the most tertiary order umbels, followed by $N_{90}P_{90}K_{100}$. The total number umbels of different orders in once fertilizer is the highest - 45 for $N_{90}P_{90}K_{200}$ variant.

In the twice application of fertilizers, the first umbels reach the highest value for $N_{90}P_{90}K_{100}$ - 13.97, followed by $N_{90}P_{90}K_{200}$ - 11.7 numbers. As with once fertilization, the numbers of the second-order umbels are the highest, ranging from 16.24 for variant $N_{50}P_{190}K_{200}$ to 24.72 for $N_{50}P_{190}K_{100}$, followed by $N_{90}P_{90}K_{100}$ with 23.52 pcs, with differences to the control are with statistically significance. In the tertiary order, the values are between 10.02 ($N_{90}P_{190}K_{100}$) to 14.59 ($N_{50}P_{190}K_{100}$). In total, the umbels in this method of fertilization reach a maximum number of 50.64 when applying $N_{90}P_{90}K_{100}$, and they are at least 33.38 pieces for $N_{50}P_{90}K_{200}$. Mengistu and Yamoah (2010) point out that the numbers of umbels per carrot seeded plant, especially those of the first and second-order, are an essential element in carrot seed production.

Table 1. Morphological characteristic of seed stalk and umbels

№ order	Number of umbels			Number of umbellate				Diameter of umbels (cm)			
	I	II	III	C	I	II	III	C	I	II	III
Once fertilization											
1	9.63	14.35	11.58	84.38	74.66	51.10	30.05	10.27	8.10	6.05	4.05
2	10.31	18.31	12.78	95.44	79.66	55.44	28.22	10.49	8.66	6.44	4.38
3	12.21	16.56	11.39	83.38	71.55	61.22	33.00	10.97	9.36	6.74	4.41
4	10.51	15.18	11.16	101.55	92.33	61.00	32.22	10.66	9.66	6.72	4.83
5	11.04	17.74	10.87	87.83	86.05	59.94	32.77	10.47	8.97	6.36	4.41
6	11.38	16.59	11.70	87.99	76.22	57.55	32.66	10.05	8.61	6.16	4.33
7	12.12	16.29	13.07	96.44	78.44	62.33	34.00	10.88	9.72	6.38	4.55
8	11.41	19.80	13.79	112.88	97.77	64.77	36.33	11.30	9.38	7.00	4.49
9	9.38	16.52	11.32	95.88	82.33	57.77	31.66	10.33	8.88	6.61	4.22
10	7.45	18.94	11.75	108.44	84.55	59.22	35.38	10.99	9.05	6.60	4.60
Twice fertilization											
11	11.29	17.86	11.83	100.72	88.16	59.05	33.49	11.41	9.99	6.99	5.24
12	5.90	16.28	11.20	99.50	84.83	65.44	33.72	11.80	9.88	7.16	4.94
13	10.98	24.72	14.59	98.22	89.44	61.16	38.38	11.63	9.47	6.88	4.55
14	10.99	16.24	12.12	104.05	88.22	66.16	39.22	11.77	10.05	7.55	5.05
15	13.97	23.52	13.15	115.44	91.11	67.44	36.83	12.11	10.13	7.11	4.88
16	11.70	20.24	12.63	93.05	90.77	65.44	37.16	11.63	9.24	6.97	4.83
17	11.59	17.87	10.02	103.55	83.99	61.27	35.83	11.27	10.05	7.22	4.85
18	8.83	16.68	12.77	100.22	89.88	63.50	38.55	11.72	9.11	7.02	4.52
LSD p=5%	2.82	5.93	4.99					1.41	1.01	0.83	0.70
r*	0.81	0.76	0.64	0. 71	0.69	0.66	0.40	0.68	0.76	0.69	0.58
r* with diameter				0.80	0.67	0.65	0.58				
r **.	0.78			0.66				0.68			

r* - correlation coefficient with yield of the respective umbel;
r** - correlation coefficient with wild of the whole plants.

In addition to the number of umbels for carrot seed productivity, the number of umbellate in one umbel is also important. For the central umbel in both fertilization methods, it is the highest, respectively, reaching in once to 112.8 at $N_{90}P_{90}K_{200}$, and at twice to 111.44 at $N_{90}P_{90}K_{100}$. In most of the twice fertilization variants except $N_{50}P_{90}K_{200}$, $N_{90}P_{90}K_{200}$ and $N_{90}P_{190}K_{200}$ it is higher than the respective variants in the once.

A similar trend is observed in the number of umbellate in first and second-order umbels. Again, the $N_{90}P_{90}K_{200}$ once fertilization variant and the $N_{90}P_{90}K_{100}$ with twice one there was the highest number of umbellate, with an increase of 30.95% and 22.03%, respectively, for the non-fertilized plants. The number of umbellate decreases as the order of the branches increases. At least number is developed the tertiary order umbels, almost two times less than the umbellate of the first order umbels. In once fertilization, the most umbellate was also reported for $N_{90}P_{90}K_{200}$, with 31.97% more than the non-fertilized control. Otherwise, these values are between 33.49 for $N_{50}P_{90}K_{100}$ to 39.22 for $N_{50}P_{190}K_{200}$.

Higher numbers of umbellate in the umbels of the first, second and tertiary order are also reported in twice fertilization, except for $N_{50}P_{90}K_{200}$ and $N_{90}P_{90}K_{200}$ for the first order and $N_{50}P_{90}K_{100}$ for the second one. It can be argued that at higher fertilization rates, the values of this index are increasing.

According to Ahmed and Tanki (1989), the combination of different levels of nitrogen, phosphorus, and potassium fertilizers have a strong influence on the formation of umbels in a carrot seed plant. The number of umbellate depends essentially on the diameter of the umbel. This can be seen very clearly from the strong positive correlations found between these two signs. The highest correlation coefficient r = + 0.80 is the dependence for the central umbels followed by that for the first order umbels with r = + 67.

An important feature of carrot seed production is the diameter of the umbel. Significant differences are observed between the different orders, with the largest size characterized by the central umbel. A similar point of view that the umbels from different orders differ in their size reported also Mengistu and Yamoah (2010).

However, the differences between the umbels within the frame of one of the same order are smaller. At the same time for this sign, the twice fertilization causes a significantly stronger effect and the values are higher than the reciprocal variants of once fertilization. In most cases, a higher level contributed to the development of the larger umbel diameter.

According to Ravinder and Kanwar (2002), the development of carrot seed plants including the umbels once and twice fertilization, there is a strong influence. In all variants, the diameter of the umbel is larger than that of the control.

The diameter of the central umbels is the highest for once fertilization with $N_{90}P_{90}K_{200}$ and twice for $N_{90}P_{90}K_{100}$, with an increase of 10.02% and 17.91% respectively. In once fertilization, the largest umbel of the first order is found for variant $N_{90}P_{90}K_{100}$, in the second it is observed for $N_{90}P_{90}K_{200}$, and in the tertiary one for $N_{50}P_{90}K_{200}$.

The twice application contributes than the non-fertilizing variant, to the largest increase in the diameter of the first order umbels in $N_{90}P_{90}K_{100}$, by 25.06%, for the second by 24.79% at $N_{50}P_{190}K_{200}$, and for the tertiary of the lower levels, $N_{50}P_{90}K_{100}$, by 29.38%. The statistical significance of the differences between these variants in comparison with the control has been established.

The weight of the seeds from the individual umbels is one of the main elements in the formation of carrot seed yield (Anjum and Amjad, 2002). The weight of seeds from the central umbels and those from the first and second orders after a single application of $N_{90}P_{90}K_{200}$ was the largest (Table 2), with 6.04%, 13.65% and 9.77% more than the control, respectively. In variant $N_{50}P_{90}K_{200}$, the weight of the umbels of tertiary branches it almost doubled that of the non-fertilized plants.

More significant differences between the variants are observed in the tertiary order, which is possibly due to the uneven development of these umbels, associated with their occurrence at a later stage of the vegetation or the lack of sufficient time for their full formation. Their weight in comparison with the others is characterized by the lowest values. The strongest increase of twice fertilization in the first, second and tertiary order was found at $N_{90}P_{90}K_{100}$, while for the central umbels it was established from fertilization with $N_{90}P_{190}K_{200}$.

The quantity of fully developed seeds is essential for successful seed production as well as for the quality of the obtained seeds. The differences between the separate variants are relatively small, and they are more significant depending on the sequence of the orders.

The highest percentage of fully developed seeds is reported for those of central umbels. Sadhu (1993) also reported that most of the seeds in the central umbel, unlike the others, are very well and fully formed. In the cases of the once fertilization, the values are between 78.65% for the control to 84.31% for the $N_{90}P_{90}K_{200}$, this difference are statistically significant. In this variant, this percentage for the seeds from first, second and tertiary order is also the highest, although the differences are smaller.

In most of the variants, the twice application of fertilizers helped to better seed development from the central umbel, with maximum values reported for $N_{90}P_{190}K_{200}$ and $N_{90}P_{190}K_{100}$. Although weak, compared to other variants in this way of fertilization with $N_{90}P_{90}K_{200}$, the highest amount of normally formed seeds from first and second order was reported, while for tertiary this was observed in the $N_{50}P_{90}K_{200}$.

Table 2. Specific behaviors of umbels development

№	Weight of seeds in umbels (g)				Normal developed seeds (%)			
order	C	I	II	III	C	I	II	III
	Once fertilization							
1	2.67	5.59	2.89	0.61	78.65	71.39	67.95	61.96
2	3.08	8.38	2.92	0.62	79.12	77.39	69.12	62.15
3	3.04	6.59	2.42	0.87	80.06	77.49	72.98	63.01
4	2.76	7.91	3.75	1.40	80.65	76.97	72.62	63.39
5	2.68	8.44	2.79	0.55	79.75	74.96	73.95	64.78
6	3.31	8.86	4.50	0.63	83.19	76.39	71.31	64.77
7	2.89	7.86	3.04	0.71	81.34	77.09	72.59	66.56
8	3.51	10.07	4.94	1.16	84.31	77.88	75.79	68.70
9	3.44	9.25	3.82	0.65	82.75	75.53	73.12	64.71
10	2.87	7.63	2.25	0.92	80.75	75.39	69.92	65.30
	Twice fertilization							
11	2.66	5.64	2.22	0.66	81.21	76.12	73.21	64.86
12	3.37	9.61	3.54	1.22	82.00	74.55	70.96	67.50
13	3.53	7.89	2.78	0.89	81.36	76.13	70.47	65.98
14	2.52	5.14	3.07	0.53	79.92	71.76	67.64	64.72
15	2.82	12.05	3.75	1.32	82.44	75.36	71.12	66.10
16	3.30	6.66	3.08	0.99	83.27	76.67	71.62	65.24
17	2.92	7.59	2.33	0.84	83.45	75.48	71.15	65.64
18	3.59	7.69	2.73	0.65	83.48	73.94	69.27	63.63
LSD p=5%					3.65	3.43	2.65	3.97
r*	0.72	0.78	0.67	0.67	0.68	0.72	0.52	0.44
r **	0.71				0.61			

r* - correlation coefficient with yield of the respective umbel;
r** - correlation coefficient with wild of the whole plants.

Seed yield (Table 3) is significantly influenced by the different levels and types of fertilization. In all variants, it is higher than the non-fertilized control. At the same time, twice fertilization improves productivity compared to reciprocal combinations of once fertilizer applications. An exception is observed when higher levels are applied, i. e. for $N_{90}P_{90}K_{200}$ and $N_{90}P_{190}K_{100}$, the increase being the highest for combinations $N_{50}P_{90}K_{100}$ and $N_{90}P_{90}K_{100}$, respectively by 13.49% and 22.26%, compared to the same levels, but on a once application.

In the first method of application of mineral fertilizers, the highest yield was reported in variant $N_{90}P_{90}K_{200}$ - 746.46 kg.ha^{-1} or by 29.68% above the control. Next are $N_{90}P_{190}K_{100}$ and $N_{50}P_{190}K_{100}$ with 19% over the seeds, obtained from the non-fertilized plants. These three variants also account for a relatively higher number of umbels, especially from first-order, as well as a high umbellate number.

The correlation between yield and these two traits is strongly positive with coefficient r = 0.78 and r = 0.66 for the whole plant. The correlation coefficients for the separate orders are also high, except for the umbellate number for the tertiary order, as it is the strongest and positive correlation with the number of umbels in the first and second orders with r = 0.81 and r = 0.76, respectively, followed by this for the umbellate counted in the central umbels (r = 0.71) and first-order (r = 0.69). The correlation of productivity with the weight of seeds per umbel (r = 0.71) is high and positive and it is the highest for the umbels of the first order (r = 0.78) and the central umbels (r = 0.72). Similar strong correlations for carrot seed productivity have been found by Mengistu and Yamoah (2010). Elballa and Cantliffe (1997) also point out that seed yield varies greatly depending on the sequence of umbels development on the mother plant and emphasized that the yields mainly formed by the seeds of first and second-order umbels. Similar are the conclusions of Amjad et al. (2005), as they reported that seed yields for carrots are determined very much by the number of first-order umbels.

Table 3. Productivity ($kg.ha^{-1}$) and contribution of different orders in yield formation (%)

№	Productivity	Part of different orders in yield formation			
order		Central	I	II	III
Once fertilization					
1	575.60	10.69	49.37	33.39	6.10
2	618.41	10.48	52.09	30.78	6.58
3	648.30	11.48	53.39	25.83	7.49
4	666.08	16.24	45.26	30.63	7.85
5	684.26	10.20	52.12	31.26	6.40
6	673.98	9.59	44.53	37.30	8.55
7	643.03	12.59	53.47	26.43	7.47
8	746.46	13.09	48.47	31.68	6.97
9	686.12	13.42	51.31	29.21	6.03
10	653.86	11.84	49.22	28.06	8.37
Twice fertilization					
11	735.81	8.27	52.61	31.68	5.98
12	662.60	15.67	40.50	33.86	9.95
13	728.42	8.28	52.39	33.00	5.19
14	688.16	11.08	52.89	27.42	8.58
15	786.19	9.43	56.77	28.44	5.33
16	710.26	10.20	47.71	34.51	7.56
17	677.58	11.14	59.10	23.73	6.02
18	702.85	10.33	49.81	33.13	5.21
LSD p=5%	58.3	6.45	12.9	7.90	4.61

The maximum yield of twice fertilization was established after the application of $N_{90}P_{90}K_{100}$ - 786.19 $kg.ha^{-1}$, with 36.58% above the control. This is the highest productivity among all the tested variants. In this case of fertilization, the numbers of umbels and the individual constituent parts, as well as the diameter, are the highest. High productivity of this type of fertilization also was observed in $N_{50}P_{90}K_{100}$ and $N_{50}P_{190}K_{100}$. Statistical proof of differences was found in all variants except $N_{70}P_{140}K_{150}$.

The specific morphology of the carrot seed plant also determines the different involvement of the individual umbels in the formation of the yield. On average for all tested variants, the proportion of the first order is 50.61%, followed by second-order with 30.57%. This is due to the larger number of umbels that they develop.

The tertiary umbels and the central umbels are with the least involved. Mengistu and Yamoah (2010) point out that, although the central umbel is the largest and with the highest weight of seeds, first-order umbels have a major proportion in the yield, which is related to their large number. The participation of the central umbel in productivity is the highest both once and twice fertilization in $N_{50}P_{90}K_{200}$ combination of 16.24% and 15.67%, respectively.

The proportion of first umbels increases the most after application of $N_{90}P_{90}K_{100}$ (once) and $N_{90}P_{190}K_{100}$ (twice). The second and third umbels have the strongest involvement in the yield formation in once fertilization with $N_{50}P_{190}K_{200}$ and twice - with $N_{90}P_{90}K_{200}$ and $N_{50}P_{90}K_{200}$, respectively.

CONCLUSIONS

The way of fertilizer application significantly influenced the formation of the carrot seed plant and stronger development of the main attributes responsible for productivity is established by twice fertilization of mineral fertilizers.

The highest number of umbels is developed in the first and second-order branches, while about the number of umbellate this is in the central umbel and in those of the first order. The values of these signs are most strongly affected by once fertilization with $N_{90}P_{90}K_{200}$ and twice one with $N_{90}P_{90}K_{100}$. A similar trend is observed for the diameter of the umbels and the weight of seeds per umbel.

The percentage of normally developed seed is the highest for central umbels at variant $N_{90}P_{90}K_{200}$ for once fertilization and at variant $N_{90}P_{190}K_{100}$ for twice one.

The highest productivity of carrot seeds is established in once fertilization with $N_{90}P_{90}K_{200}$ - 746.46 kg.ha^{-1}, and in twice fertilization with $N_{90}P_{90}K_{100}$ - 786.19 kg.ha^{-1}. The yield is determinated mainly from the seeds obtained from the first order and their largest participation is after fertilization with $N_{90}P_{90}K_{100}$, and at twice it is for $N_{90}P_{190}K_{100}$, followed by the quota of the second-order umbels. Twice fertilization of mineral fertilizers contributes to higher yields than the corresponding levels but in once fertilization.

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THE INFLUENCE OF COVER CROPS AND ANTAGONISTIC FUNGI ON THE HEALTHINESS OF CARROT (*DAUCUS CAROTA* L.)

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Abstract

Cover crops and antagonistic fungi have a very positive effect on the soil environment. Such cover crops as oats, rye, common vetch, tansy phacelia, white mustard, sunflower and fodder radish can be used in the cultivation of different species of vegetables. They increase the biological activity of the soil by stimulating the growth and development of microorganisms antagonistic and can protect cultivated plants from soil-borne phytopathogens. The purpose of the field and laboratory studies was to determine the antagonistic activity of selected fungi species occurring in the soil under carrot cultivated with the use of oats, tansy phacelia and spring vetch as cover crops. The healthiness of the roots of this vegetable was also investigated. Cover crops contributed to the increase of the population of antagonistic fungi (Trichoderma spp., Clonostachys spp., Myrothecium spp. and Penicillium spp.) in the soil. Regardless of the experimental treatment, those antagonistic fungi were most effective in limiting the growth of Sclerotinia sclerotiorum, Alternaria dauci and A. radicina. The effect of those fungi was a little smaller towards Fusarium oxysporum and Rhizoctonia solani. Oats and spring vetch were most effective in limiting the occurrence of soil-borne fungi. Cover crops had a positive effect on the healthiness of carrot roots. Alternaria dauci, A. alternata, A. radicina, Fusarium oxysporum, Globisporangium irregulare, Neocosmospora solani, Phytophthora sp., Rhizoctonia solani and Sclerotinia sclerotiorum proved to be the most harmful towards the studied underground parts of carrot. Oats proved to be the most effective in inhibiting the occurrence of the pathogenic fungi for Daucus carota L.

Key words*: Daucus carota L., cover crops, soil-borne fungi, phytopathogens, healthiness of plants.*

INTRODUCTION

Cover crops and antagonistic fungi have a very positive effect on the soil environment. Such cover crops as oats, rye, common vetch, tansy phacelia, white mustard, sunflower and fodder radish can be used in the cultivation of different species of vegetables. They increase the biological activity of the soil by stimulating the growth and development of microorganisms antagonistic and can protect cultivated plants from soil-borne phytopathogens (Hallama et al., 2019; Schmidt et al., 2018).

Cover crops can increase the number antagonistic microorganisms in the soil and improve the quality of the plants yield (Himmelstein et al., 2016; Oliveira et al., 2016).

MATERIALS AND METHODS

The field experiment was conducted in district of Lublin (22º56'E, 51º23'N, Central Eastern Poland, 200 m a.s.l.), on Haplic Luvisol formed from silty medium loams. The object of the studies was the soil taken from the field where carrot cv. 'Flakkee 2' was cultivated.

The experiment took into consideration cover crops such as oats (*Avena sativa* L.), spring vetch (*Vicia sativa* L.), tansy phacelia (*Phacelia tanacetifolia* Benth.) and one system of soil tillage, i.e.: tillage before winter (ploughing) and spring tillage (a combined cultivator). The conventional cultivation, i.e. without any cover crops, was the control.

Microbiological analysis of the soil was made according to the method described by Patkowska and Błażewicz-Woźniak (2014). All fungi isolates from the genera of *Clonostachys, Myrothecium, Penicillium* and *Trichoderma*, obtained from particular experimental treatments, were used to establish their antagonistic effect towards such fungi as *Altenaria dauci, Alternaria radicina, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Moreover, the healthiness of carrot roots were determined.

The mycological analysis was conducted according to the method described by Patkowska and Krawiec (2016) for pea. This analysis made it possible to determine the quantitative and qualitative composition of fungi infecting the underground organs of carrot.

RESULTS AND DISCUSSIONS

Cover crops contributed to the increase of the population of antagonistic fungi (*Trichoderma* spp., *Clonostachys* spp., *Myrothecium* spp. and *Penicillium* spp.) in the soil. Regardless of the experimental treatment, those antagonistic fungi were most effective in limiting the growth of *Sclerotinia sclerotiorum* (Figure 1), *Alternaria dauci* and *A. radicina.* The effect of those fungi was a little smaller towards *Fusarium oxysporum* and *Rhizoctonia solani. Trichoderma* spp. and *Clonostachys* spp. can limit the occurrence of soil pathogens and they improve the plants' healthiness (Sarma et al., 2014; Smitha et al., 2014).

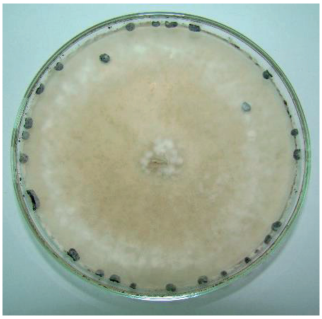

Figure 1. The 10-days colony of *Sclerotinia sclerotiorum* on the malt medium (photo by E. Patkowska)

Oats and spring vetch were most effective in limiting the occurrence of soil-borne fungi. A similar effect of the studied fungi antagonistic towards different species of pathogenic fungi was shown by Patkowska and Konopiński (2014) in the cultivation of scorzonera where cover crops were used. Banaay et al. (2012), Krauss et al. (2013) and Teshome et al. (2013) report that the ability of *Trichoderma* spp., *Clonostachys* spp. and *Penicillium* spp. to inhibit the growth and development of plant pathogens is based on antibiosis, competition and parasitism.

Cover crops had a positive effect on the healthiness of carrot roots. After the harvest of carrot, among fungi considered to be pathogenic, species from the genera of *Alternaria, Fusarium*, *Phytophthora, Rhizoctonia* and *Sclerotinia* were isolated from diseased roots (Table 1). A little more the enumerated fungi were isolated from the infected roots of carrot (Figure 2) cultivated conventionally or after tansy phacelia as a cover crops, while the least after oats.

Figure 2. Sclerotia and mycelium of *Sclerotinia sclerotiorum* on the carrot roots (photo by E. Patkowska)

Table 1. Fungi isolated from diseased roots of carrot after harvest

Fungus species	Experimental treatment/Number of isolates										
	Oats		Spring vetch		Tansy phacelia		Control		Total		Total (%)
	a*	b	a	b	a	b	a	b	a	b	
Acremonium murorum (Corda) W. Gams	-	-	-	-	3	-	4	2	8	2	10 (1.5)
Alternaria alternata (Fr.) Keissl.	1	-	3	-	5	2	16	6	25	8	33 (4.8)
Alternaria chartarum Preuss	-	-	-	-	3	-	9	3	12	3	15 (2.2)
Alternaria dauci (J.G. Kühn) J.W. Groves & Skolko	1	-	1	-	4	2	11	6	17	8	25 (3.6)
Alternaria radicina Meier, Drechsler & E.D. Eddy	1	-	1	-	6	4	17	14	25	18	43 (6.3)
Cladosporium cladosporioides (Fresen.) G.A.de Vries	-	-	-	2	2	3	6	3	8	8	16 (2.3)
Clonostachys rosea (Link)	11	9	9	7	6	3	-	-	26	19	45 (6.6)

Schroers, Samuels, Seifert & W. Gams											
Cylindrocarpon didymum (Harting) Wollenw.	-	-	-	-	3	2	8	4	11	7	18 (2.6)
Epicoccum nigrum Link	-	-	1	-	2	2	7	3	10	5	15 (2.2)
Fusarium avenaceum (Fr.) Sacc.	-	-	-	-	2	2	6	4	8	6	14 (2.0)
Fusarium oxysporum Schltdl.	4	2	6	4	8	4	10	6	28	16	44 (6.4)
Neocosmospora solani (Mart.) L. Lombard Crous	-	-	2	2	3	5	8	3	13	10	23 (3.3)
Penicillium aurantiogriseum Dierckx	2	1	3	1	4	2	8	4	17	8	25 (3.6)
Penicillium chrysogenum Thom	-	-	-	1	2	1	5	3	7	5	12 (1.7)
Penicillium meleagrinum Biourge	-	-	-	-	3	2	9	5	12	7	19 (2.8)
Penicillium simplicissimum (Oudem.) Thom	-	-	-	-	1	-	6	4	7	4	11 (1.6)
Phytophthora sp.	2	-	2	1	4	2	6	5	16	8	24 (3.4)
Rhizoctonia solani J.G. Kühn	5	3	7	4	8	6	12	10	32	23	55 (8.0)
Rhizopus stolonifer (Ehrenb.) Vuill.	2	3	3	2	8	3	16	8	29	16	45 (6.5)
Sclerotinia sclerotiorum (Lib.) de Bary	2	2	5	3	7	5	14	12	28	22	50 (7.3)
Trichoderma harzianum Rifai	7	5	6	4	5	3	2	-	20	12	32 (4.6)
Trichoderma koningii Oudem.	14	12	12	10	10	9	3	3	39	34	73 (10.6)
Trichoderma viride Pers.	10	7	8	5	6	4	2	-	26	16	42 (6.1)
Total	62	44	69	46	105	66	186	108	422	264	686 (100.0)
Total	106		115		171		294		686		

a* - root; b - head of root

Studies by Koike et al. (2017) and Zafar et al. (2017) also pointed to considerable harmfulness of these fungi towards carrot plants. According to Rogers and Stevenson (2010), Tülek and Dolar (2015) and Zafar et al. (2017), fungi, especially *Alternaria dauci*, *A. radicina*, attacked carrots at all stages, causing damping-off, and rotting of roots, crowns, seedlings, petioles, leaves and crowns of maturing carrots.

Studies conducted by Patkowska et al. (2016) and Dawadi et al. (2019) showed that cover plants (rye, oats, white mustard) significantly reduce the population of pathogenic fungi in the soil environment, thus positively affecting the healthiness of the cultivated plants.

CONCLUSIONS

The present studies confirmed the positive effect of cover crops on the growth and healthiness of *Daucus carota.*

Oats, tansy phacelia and spring vetch inhibited the occurrence and development of soil-borne fungi and - consequently - improved the healthiness of the examined plant.

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MACROELEMENTS STATUS IN LETTUCE AFFECTED BY DIFFERENT FORMS OF PHOSPHORUS FERTILIZATION

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Abstract

Lettuce (Lactuca sativa L.) is annual vegetable,belonging to the family of Asteraceae. Soils in Croatia are generally poor in phosphorus, so phosphorus fertilization is of great significance. The aim of this research was to determine the effect of fertilization by different phosphorus fertilizers on the status of macroelements in lettuce. The field fertilization experiment was set up with lettuce according to the Latin square method with three fertilization treatments: T1 (control, without fertilization), T2 (phosphorus in polyphosphate form) and T3 (phosphorus in orthophosphate form). Nitrogen was determined by the Modified Kjeldahl method, phosphorus spectrophotometricaly, potassium flamephotometricaly, calcium, and magnesium atomic absorption spectrometricaly. The highest content in 100 g of fresh lettuce of phosphorus (28.24 mg P/100 g) and magnesium (48.54 mg Mg/100 g) were determined in the T1 treatment, while the highest content in 100 g of fresh lettuce of nitrogen (204.34 mg N/100 g), potassium (189.63 mg K/100 g) and calcium (122.85 mg Ca/100 g) were determined in the T3 treatment.

***Key words**: Lactuca sativa L., magnesium, minerals, nitrogen, potassium.*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is annual herbaceous vegetable belonging to the Asteraceae family. It originates from West Asia and East Africa; for instance in Egypt it has been used in the diet for 2500 years (Lešić et al., 2016).
Lettuce is a vegetable crop that has high nutritional and health value and is best consumed fresh. Fresh lettuce leaves contain about 91-96% water, 0.8-2.3% protein, 0.1-0.4% fat, 0.1% sugar, 0.54-1.5%fiber, 1.4% mineral substances. Leaves are also rich in vitamins C, B1, B2 and carotene, as well as in fiber. Energy rate (fat, protein and carbohydrates) is low, which is why nutritionists have recommended it as a staple meal for anyone who has to take care of being overweight (Matotan, 2004; Parađiković, 2009; Lešić et al., 2016).
During the growing season for quality growth and development of lettuce, all the biogenic macronutrients, that the plant uptake through the roots from the soil solution, are needed. That is why it is important that biogenic macronutrients are in an available or physiologically active form for the plant in optimal concentrations.
Nitrogen is an integral part of proteins, participates in the formation of amino groups in amino acids, participates in the structure of nucleotides where it is located in purine and pyrimidine bases, and plays an important role in the human body too. It is essential in the biochemistry of many non-protein components such as coenzymes, photosynthetic pigments, secondary metabolites and polyamines. Nitrogen is an integral part of the enzyme nitrate reductase and nitrite reductase, whose main function is the conversion of the nitrate form of nitrogen to ammonia (Maathius, 2009).
The plant uptakes nitrogen in its mineral forms as NO_3^- and NH_4^+ ions (Marschner, 1995). The ammonia ion can bind to soil colloids while the nitrate form has great mobility in the soil and can be leached, as it cannot be bind to soil colloids due to its negative charge. The plant

will uptakesoil nitrogen that is not immobilized (Haynes, 1986). Plants growing on acidic soils and soils of poorer mechanical composition uptake better the NH_4^+ ion, compared to plants that grow on alkaline soils and soils of suitable water-air relationships that better uptake NO_3^- ion (Maathius, 2009).
Nitrogen content in lettuce leaves, according to literature data range from 1.13 to 5.02 % N in dry weight (Table 1).

Table 1. Nitrogen content in lettuce leaves (% N in dry weight)

Source	% N in dry weight
Tosic et al. (2019)	3.69-5.02
Wojciechowska et al. (2019)	2.56-3.32
Yarsi et al. (2019)	2.45-3.43
Solaimanet al. (2019)	3.80-4.10
Zandvakili et al. (2019)	1.13-4.08
Broadley et al. (2003)	4.2

According to Landis and Sreenis (2009) phosphorus is a macronutrient that is of great importance for plant growth and development, as well as it is part of every cell of all living organisms.
In acidic soils, which occupy about 40% of the total arable land, phosphorus is bound to aluminum and iron into insoluble compounds and as such is unavailable to plants (Vance, 2001). In alkaline soils, which occupy about 25% of the total arable land, phosphorus is bound to calcium into low soluble calcium phosphates. Therefore, phosphorus deficiency is one of the major limiting factors for successful agricultural production (Lynch and Brown, 2001).
Plants uptake phosphorus in anionic forms as $H_2PO_4^-$ and HPO_4^{2-}, and their concentration depends on soil pH (Kastori, 1983). Phosphorus in plant has a function in storing and releasing energy by storing all the solar energy transformed by the photosynthesis process into the chemical bonds between the phosphorus atoms in the ATP molecule. It is an integral part of the phospholipids that is the cell membrane consist of (Landis and Sreenis, 2009).
According to different authors lettuce phosphorus content ranged from 21 to 68 mg P/100 g of fresh weight (Table 2).
According to Landis (2009) potassium is uptaken by the plant root as the K^+ cation. Potassium is mobile in plants and its concentration decreases by the time. Potassium plays a key role in photosynthesis, phloem assimilate transport, nitrogen metabolism and storage processes of reserve compounds (Vukadinović & Vukadinović, 2011).
The potassium plant needs in large quantities. It plays an important role in the regulation of water in plants, effects the opening and closing of stomata, the synthesis of ATP and the activation of more than 60 enzymes, and therefore is also important for the synthesis of proteins, sugars and fats (Bergmann, 1992).
When potassium deficiency occurs, a decrease in stomata activity occurs too, which affects three important physiological processes: the inability to absorb CO_2 in photosynthesis, decrease of transpiration and turgor, and impedes the absorption of oxygen (Landis, 2009).
Potassium content in lettuce leaves ranged from 133 to 530 mg K/100 g fresh weight (Table 3).

Table 2. Phosphorus content in lettuce leaves (mg P/100 g fresh weight)

Source	mg P/100 g fresh weight
Mou (2009)	32
USDA (2018)	29
Botanical – online (2018)	23
Anilakumar et al. (2017)	30
Lešić et al. (2016)	21-68

Table 3. Potassium content in lettuce leaves (mg K/100 g fresh weight)

Source	mg K/100 g fresh weight
Mou (2009)	198
USDA (2018)	194
Botanical – online (2018)	257
Anilakumar et al. (2017)	247
Lešić et al. (2016)	133-530

Calcium is uptaken by the plant root in the Ca^{2+} ionic form (Vukadinović & Lončarić, 1998) and it has a significant importance in the process of respiration and photosynthesis (Maathius, 2009). Calcium plays a role in both increasing and decreasing the availability of other nutrients, for example increasing the availability of manganese in soil and reducing the availability of phosphorus (Maathius, 2009) due to soil pH or antagonism. It participates in the repair of soil structure by neutralizing soluble humic acids in insoluble calcium humates, which contributes to stable soil

structure (Znaor, 1996). It has a beneficial effect on the processes of ammonification, nitrifycation, biological nitrogen fixation and sulfur oxidation in soil (Vukadinović & Vukadinović, 2011).
Calcium has also an important role in plant growth. It participates in the composition of calcium pectinate, phytin salts, the composition of crystalline bodies, oxalates and calcite, and calcium phosphate buffer (Jug, 2016). It strengthens cell walls and stabilizes biomembranes, increases disease resistance and protects against toxins (Lešić et al., 2016).
Different authors quote calcium content in lettuce leaves from 13 to 60 mg Ca/100 g fresh weight (Table 4).

Table 4. Calcium content in lettuce leaves (mg Ca/100 g fresh weight)

Source	mg Ca/100 g fresh weight
Mou (2009)	40
USDA (2018)	36
Botanical - online (2018)	32
Anilakumar et al. (2017)	33
Lešić i sur. (2016)	13-60

Magnesium is an essential element that plants need for normal growth and development, and plays an important role in the plant's defense mechanism during abiotic stress. Plants uptake it in the Mg^{2+} ion form (Senbayram et al., 2015).
Magnesium affects the metabolism of carbohydrates, fats and proteins and activates a large number of enzymes (Vukadinović & Vukadinović, 2011). Magnesium is present in the chlorophyll molecule from 25 to 30%, while the remaining percentage is present in more mobile forms. Due to its good mobility in plants, magnesium can be easily translocated from the older parts of the plant and root to the younger parts, where it plays a role in the formation of the chlorophyll molecule (Senbayram et al., 2015). Therefore, the magnesium deficiency occurs in old leaves.
Magensium content in lettuce leaves ranged from 7 to 23 mg Mg/100 g fresh weight (Table 5).
Croatian soils are generally poor in phosphorus, so fertilization with phosphorus is of great importance. Fertilizers with phosphorus in the form of orthophosphates are most common used, while phosphorus in the form of polyphosphates are also available on the market.
Therefore, the aim of this research was to determine the effect of fertilization by different phosphorus fertilizers on the status of macroelements in lettuce.

Table 5. Magnesium content in lettuce leaves (mg Mg/100 g fresh weight)

Source	mg Mg/100 g fresh weight
Mou (2009)	7
USDA (2018)	13
Botanical - online (2018)	13
Anilakumar et al. (2017)	14
Lešić et al. (2016)	7-23

MATERIALS AND METHODS

Field fertilization experiment was set up in Velika Kosnica in Zagreb County (Croatia) with lettuce (*Lactuca sativa* L.), cultivar 'Aquarel' (Bejo). The experiment was set up using the Latin square method with three fertilization treatments: T1 - control, without fertilization; T2 - 500 kg/ha YaraMila Complex (55 kg/ha P_2O_5; Producer Yara, NPK 12-11-18 + 3MgO + 8S + B, S, Fe, Mn, Zn; phosphorus in form of polyphosphates); T3 - 370 kg/ha NPK 15-15-15 (55 kg/ha P_2O_5; Producer Petrokemija; phosphorus in form of orthophosphates).
Planting of lettuce seedlings was conducted on 18 June 2018. Rows spacing was 39 cm and 27 cm in rows. Standard agricultural technology was used, plowing was carried out in the spring. Application and incorporation of fertilizers was performed manually. Harvesting of lettuce was carried out at once on 6 August 2018 and average lettuce head samples were taken for chemical analysis.
Prior to setting up the fertilization experiment, a chemical soil analysis was performed (Table 6) which showed that the soil was alkaline, with poorly humus content, well supplied with nitrogen, poorly supplied with physiologically active phosphorus and rich in physiologically active potassium.
Average head lettuce samples were delivered to the Analytical Laboratory of the Department of Plant Nutrition, Faculty of Agriculture, University of Zagreb, where chemical analyses were performed. The lettuce leaves were cut into small pieces, dried at 105°C, after which

they were ground and homogenized. To determine the macronutrients content, the ground samples were digested with concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a microwave oven, after which phosphorus was determined spectrophotometrically, potassium by flame photometer, and calcium and magnesium by atomic absorption spectrometry AOAC (2015). Nitrogen was determined by the Adjusted Kjedahl Method (HRN ISO 11261: 2004). The dry matter was determined gravimetrically by drying to a constant mass.

Table 6. Soil chemical properties prior to field experiment set up

pH	H_2O	8.16
	nKCl	7.35
%	Humus	2.68
	N	0.17
AL-mg/100 g	P_2O_5	9.5
	K_2O	26
%	$CaCO_3$	3.8
	CaO	-

Statistical data analyses were performed using the SAS System for Win program ver 9.1 (SAS Institute Inc.)(SAS, 2002-2003). Analysis of variance (ANOVA) was performed following a Latin square experimental design. A Tukey's multiple comparison test (Tukey's HSD) was applied to identify differences among treatments.

RESULTS AND DISCUSSIONS

Dry matter content in lettuce leaves

Graph 1 shows the dry matter (DW) content in the analyzed lettuce leaves samples. No statistically significant differences were found in the dry matter content according to the fertilization treatments T1, T2 and T3, which ranged from 8.22 to 8.85%. The highest dry matter content was determined in the T3 treatment where phosphorus was applied in the form of orthophosphate.

According to Koudela and Petříková (2008) lettuce dry matter values range from 7 to 10%, and according to Anilakumaret al. (2017) this value is 5.4%. So, literature values are similar to the values obtained in this research.

Macroelemets content in dry weight of lettuce leaves

Nitrogen content in lettuce dry matter in the three fertilization treatments ranged from 2.29 to 2.38% N DW (Graph 2). The highest nitrogen content was determined in T2 treatment where phosphorus was applied in the form of polyphosphate (2.38% N DW), and the lowest nitrogen content was determined in T1 treatment without fertilization (2.29% N DW). Values obtained in this research are mostly lower compared to the literature data (Table 1), although Zandvakili et al. (2019) reported also even lower values that were obtained in this research.

Phosphorus content in lettuce dry matter in the three fertilization treatments ranged from 0.31 to 0.34% P DW (Graph 2). The highest phosphorus content was determined in T1 treatment where was no fertilization (0.34% P DW), while the lowest phosphorus content was determined in T2 and T3 treatment where phosphorus was applied in the form of polyphosphate and orthophosphate (0.31% P DW). Phosphorus data determined in this research is lower than the literature data, of 0.50% P DW (Broadley et al., 2003).

Potassium content in lettuce dry weight in the three fertilization treatments ranged from 2.15 to 2.24% K DW (Graph 2). The highest potassium content was determined in T1 and T2 treatment (2.24% K DW). The lowest potassium content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (2.15% K DW). The obtained values are almost twice lower than reported by Broadley et al. (2003) who reported 4.5% K DW in lettuce.

Calcium content in lettuce dry weight in fertilization treatments ranged from 1.35 to 1.42% Ca DW (Graph 2). The highest calcium content was found in T1 treatment where there was no fertilization and was 1.42% Ca DW, while the lowest was found in T2 treatment where phosphorus was applied in the form of polyphosphate (1.35% Ca DW). The average value of calcium in lettuce dry matter was 1.39% Ca DW, and is higher than reported in the literature, of 0.50% Ca DW (Vukadinović & Vukadinović, 2011).

Magnesium content in lettuce dry matter in all fertilization treatments ranged from 0.48 to 0.57% Mg DW (Graph 2).

The highest magnesium content was determined in the T1 treatment with no fertilization (0.57% Mg DW), and the lowest magnesium content was determined in the T2 treatment where phosphorus was applied in the form of polyphosphate (0.48% Mg DW).

The average magnesium content in lettuce dry matter was 0.52% Mg DW, and value obtained in this research is higher than reported,of 0.15 to 0.35% Mg DW (Vukadinović & Vukadinović, 2011).

Microelemets content in fresh weight of lettuce leaves

Nitrogen content in fresh weight (FW) of lettuce in the three fertilization treatments ranged from 192 to 204 mg N/100 g FW (Graph 3). The highest nitrogen content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (204 mg N/100 g FW).

The lowest nitrogen content was determined in T1 treatment where there no fertilization was applied (192 mg N/100 g FW).

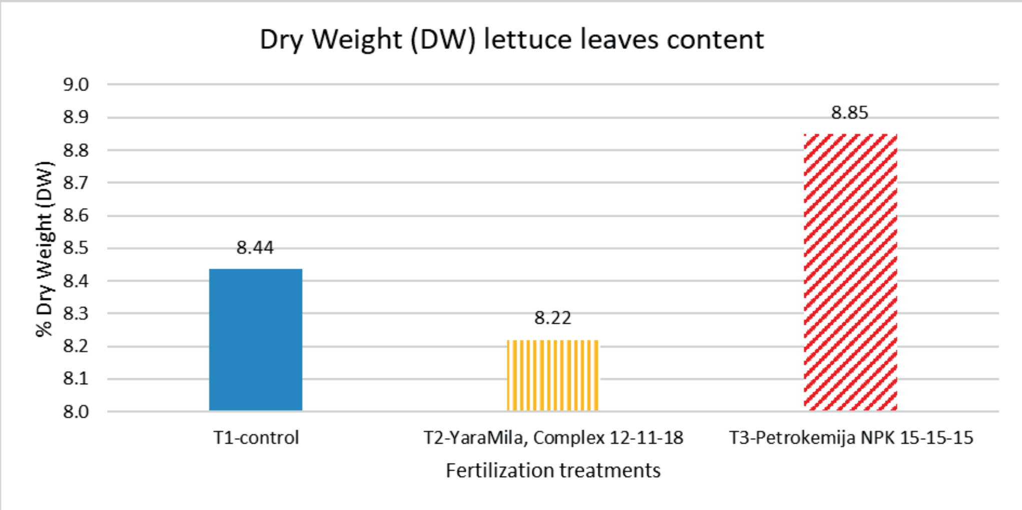
Phosphorus content in lettuce fresh weight regardless fertilization treatments ranged from 25 to 28 mg P/100 g FW (Graph 3).

The highest phosphorus content was determined in T1 treatment with no fertilization applied (28 mg P/100 g FW), while the lowest content was determined in T2 treatment where phosphorus was applied in the form of polyphosphate (25 mg P/100 g FW). The values obtained in this research are lower than the values from literature (29 and 30 mg P/100 g FW (USDA, 2018 and Anilakumar et al., 2017, respectively)).

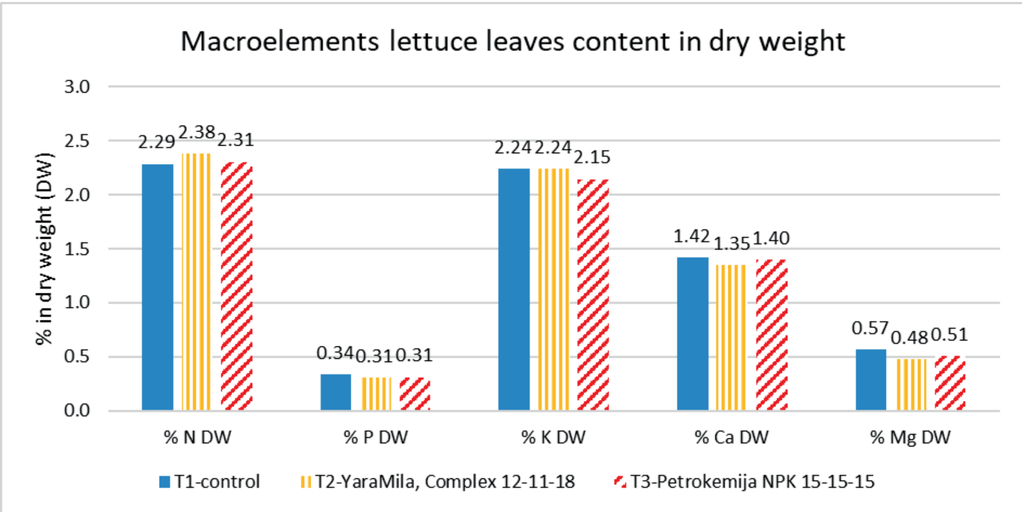
Potassium content in lettuce fresh weight in all fertilization treatments ranged from 184 to 190 mg K/100 g FW (Graph 3). The highest potassium content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (190 mg K/100 g FW), and the lowest content was found in T2 treatment where phosphorus was applied in the form of polyphosphate (184 mg K/100 g FW). The values obtained in this research are similar to those of Mou (2009) and USDA (2018) and lower than the values reported in Botanical – online (2018).

Calcium content in lettuce fresh weight regardless fertilization treatments ranged from 111 to 123 mg Ca/100 g FW (Graph 3). The highest calcium content was determined in T3 treatment where phosphorus was applied in the form of orthophosphate (123 mg Ca/100 g FW), and the lowest content was determined in T2 treatment where phosphorus was applied in the form of polyphosphate (111 mg Ca/100 g FW). The average value obtained in this research of 118 mg Ca/100 g FW is much higher than those reported in the literature (13 and 40 to mg Ca/100 g FW (Lešić et al., 2016 and Mou, 2009, respectively).

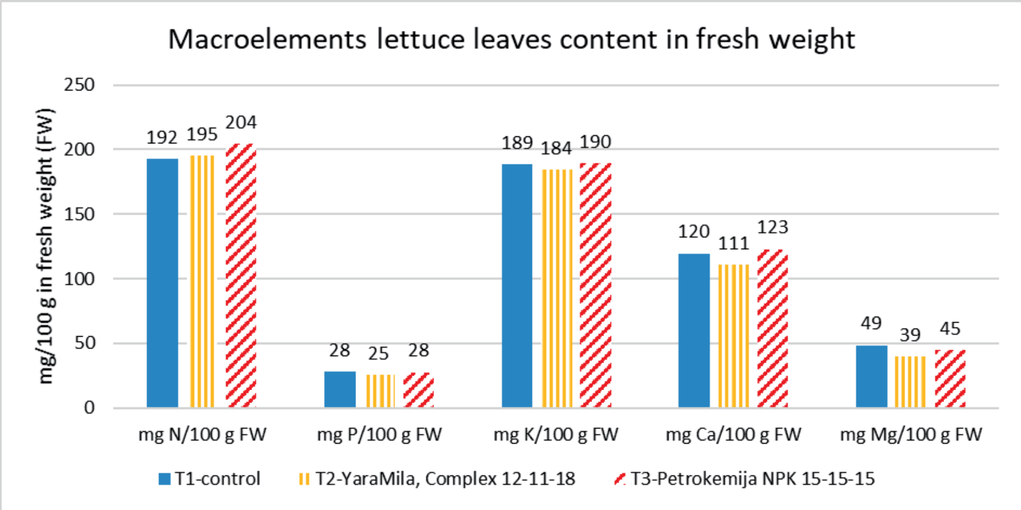
Magnesium content of fresh lettuce regardless fertilization treatments ranged from 39 to 49 mg Mg/100 g FW (Graph 3). The highest magnesium content was determined in the T1 treatment without fertilization (49 mg Mg/100 g FW), and the lowest content was determined in the T2 treatment where phosphorus was applied in the form of polyphosphate (39 mg Mg/100 g FW). The average value obtained in this research of 44 mg Mg/100 g FW are much higher than literature data (7-23 and 14 mg Mg/100 g FW (Lešić et al., 2016 and Anilakumar et al., 2017, respectively).



Graph 1. Dry Weight (DW) lettuce leaves content (% DW) according to different fertilization



Graph 2. Macroelements lettuce leaves content in dry weight(%in DW) according to different fertilization



Graph 3. Macroelements lettuce leaves content in fresh weight (mg/100 g in FW) according to different fertilization

CONCLUSIONS

This research has determined the status of macroelements in fertilized lettuce with different forms of phosphorus. The research was conducted in VelikaKosnica, Zagreb County (Croatia), using the Latin square method with three fertilization treatments.
The highest total dry matter (DM) content in lettuce leaves was determined in the T3 treatment (8.85% DM) where phosphorus was applied as orthophosphate.
Treatment T1 resulted with the highest content of minerals in dry weight (DW) of lettuce as follows: phosphorus 0.34% P DW, potassium 2.24% K DW, calcium 1.42% Ca DW and magnesium 0.57% Mg DW, while T2 treatment, where phosphorus in the form of polyphosphate was applied, resulted with the highest content of nitrogen (2.38% N DW).
In the fresh weight (FW) of lettuce in the T3 fertilization treatment, where phosphorus was applied in the form of orthophosphate, the highest content of nitrogen (204 mg N/100 g FW), potassium (190 mg K/100 g FW) and calcium (123 mg Ca/100 g FW) were determined, while in T1 treatment the highest content of phosphorus (28 mg/100 g FW) and magnesium (49 mg Mg/100 g FW) were determined.

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IMPACT OF BIOLOGICAL FERTILIZER ON THE ANATOMICAL STRUCTURES OF SHEET FROM LEAF LETTUCE (*LACTUCA SATIVA*)

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Abstract

The different growing conditions and in particular fertilization, affects the physiology, morphology and anatomy of the plants and provides the necessary information to compare their biological plasticity. The aim of the present study was to trace the effect of optimized nutrition in at organic cultivation of greenhouse lettuce using different formulations of organic fertilizers, on the anatomical structure of the leaves in order to increase plant resistance to the abiotic stress. The experimental production of seedlings was conducted at the base of Agricultural University - Plovdiv, Bulgaria. Four bio fertilizers were used in six variants of biological plant cultivation. Morpho-anatomical analyzes of the leaf blate, epidermis and stomata were performed using the methods of comparative anatomy. In all variants of lettuce with organic fertilization applied, a positive effect on the anatomical parameters of the leaves was observed, which was a prerequisite for increasing the biological resistance of the plants.

***Key words**: anatomy, biological fertilization, leaf, lettuce.*

INTRODUCTION

Greenhouse vegetable production is associated with the use of significant amounts of fertilizers and pesticides, which increases the risk of soil, groundwater and production of hazardous substances (Chen et al., 2014; Zhang et al., 2017). Today, technologies in agricultural production are closely linked to consumer needs and the demand for products that meet the requirements of food quality and safety (Gheorghiu et al., 2013; Terziev & Arabska, 2015; Day et al., 2008)

Biological analysis of plants is the best method for measuring the effect of the application of various chemical and organic substances in the soil (Basta et al., 2005). A number of studies point to varying degrees of phytochemical availability of organic and inorganic elements in soil (Bell et al., 1991; Brown et al., 1998; Cunningham et al., 1975; Mahler et al., 1987). Factors such as variety, agro-technical practices, climatic conditions, degree of ripeness, harvest time and storage conditions are of particular importance. The change in the individual elements of the technology is an important tool for obtaining healthy and higher biological value plant products with increased amount of bioactive substances. This is a potential for increasing commercial production of leafy vegetables (Dumas et al., 2003; Barba et al., 2016; Ebert, 2014).

Of the leafy vegetables group, lettuce is grown on all continents, but the largest consumers and producers are the United States (91,000 hectares) and Europe (a total EU area of 80 000 ha) (Pink & Keane, 1993). In Europe, it is the most widely used vegetable product for fresh consumption. (Serna et al., 2012). In Bulgaria, salad is a widely grown field and greenhouse culture (Retrieved from http://www.sunoilbg.org/language/en/uploads/files/documents0/document_6638d0d4b9d0a517e62b8bd4aef20ced.pdf)

The objectives of modern lettuce selection are divided into three main areas: (1) resistance to diseases and pests, (2) increased yield and uniformity, and (3) improvement of horticultural characteristics, such as quality and sustainability (Pink & Keane, 1993).

When the nitrate content of lettuce leaves exceeds the daily allowance, consumption of this crop poses a health hazard (Lorenz, 1978; Sanchez et al., 2005). On the other hand, increased nitrate uptake generates more vegetative growth, improves leaf morphology (e.g. length and width), but leaf

thickness can significantly reduce (Soundy & Smith, 1992). High nitrogen content in lettuce usually leads to storage difficulties and the ability to quickly rot after harvest. Decay in this crop generates significant financial losses for producers (Hoque et al., 2010; Fageria, 2009).
The quality of the salad can be enhanced by organic farming. The use of low nitrogen organic fertilizers is cost-effective and provides the necessary nutrients for this crop. For the purposes of industrial production, it is important to determine the appropriate organic fertilizers ensuring high yield and quality according to the production line and technology used (Fontes et al., 1997; Heckman et al., 2003).
There are a number of characteristics that make lettuce suitable for biological research. It has a relatively short growing season, forms a large amount of leaf mass with a large leaf area and accumulates the most nitrates in the leaves compared to other leafy vegetables (Pink & Keane, 1993).
Studies by Falla et al. (2000) and Honor et al. (2009) indicate that plant species reveal the quality of the environment in which they develop by altering their leaf anatomy and physiology, and these changes in leaf indices may be used in the reasonable and accurate assessment of habitat quality.
The epidermis, as the outermost layer of the assimilating organs, performing a protective and regulatory function, provides information on the degree of response of the plant species (Aneli, 1975). Changes in the size and number of major epidermal cells are one of the main indicators of the response of a plant organism to favorable or unfavorable changes in the environment, and in particular soil. (Ninova & Dushkova, 1977; Koev et al., 2001; Gostin & Ivanescu, 2007).
As the main structures of the epidermis, carrying out gas exchange and transpiration, the morphology and number of the stomata are also the main object of anatomic-morpholytic analysis for the plant response to abiotic stress (Dimitrova, 2000; Gostin & Ivanescu, 2007).
The purpose of this study is to investigate the effect of optimized nutrition on organic greenhouse salad cultivation with organic fertilizers of different composition on the anatomical structure of the leaves in order to increase the resistance of plants to abiotic stress.

MATERIALS AND METHODS

The initial phase of the experimental activity was carried out in 2019 by planting a greenhouse experience in the first ten days of month X 2018 and harvested II-III months of 2019 in economic maturity - 200-300 g. A leaf salad-type Batavia variety "Maritima" was used for analysis. The plants were planted in a polyethylene greenhouse at the experimental base of Agricultural University - Plovdiv, Bulgaria.

Seedling production

For the purpose of the experiment, bio-seeds of the above variety were purchased. Seeds for seedling production were sown on October 10. 1000 plants were grown and container technology was applied using 150-ounce stereo boards. An organic seedling mixture was used - 80% Perlite: 20% Lumbricompost developed by the authors (Kostadinov & Filipov, 2013) for bioproduction of seedlings.

Planting

The plants were planted in phase of 4-5 leaf on November 8 in the polyethylene greenhouses of AU-Plovdiv. After the plow was milled and milled, the test surface was profiled on a high flat bed. A 4-row 70 + 30 + 30 + 30/30 cm 30 row was used. The experiment was based on a block method of four replicates of 28 plants per replicate, with a plot size of 3.36 m^2. Internal guards were 8 units/rep. The front and rear guards were 12 each plants. The reported plots included 20 pieces each plants in repetition.

Paid options

The greenhouse experience was laid out with 6 variants with a total area of 450 m^2, of which 375 m^2 with organic fertilization. Irrigation was carried out with a drip system. The following options were used to study the effect of selected biotors on the anatomical arrangement of lettuce leaves under conditions of transition to organic production:

1. Control-MT (mineral fertilization - NPK)
2. Control - not fertilized
3. Italpolina
4. Arkobaleno
5. Lumbricompost
6. Ecoprop NX

The Italpolina and Lumbrikompost granular organic fertilizers have been applied in organic vegetable production, while the Arkobaleno (granular) and Ekoprop NX (liquid) organic fertilizers are less known but promising.
The granulated fertilizers were imported according to a pre-developed scheme as basic fertilization with pre-seed tillage in the following norms: N - 12.5 kg/da, P2O5 - 1.25 kg/da, + K2O - 4.75 kg/da, Italpolina - 25 kg, Arkobaleno - 100 kg/da, and Lumbrikompost - 400 l/da. The organic fertilizer Ekoprop NX dissolved in water was introduced by

four times pouring at a dose of 100g / da- from phase 5 leaf at 14-day intervals.

Characteristics of biotores

Italpolina (4N - $4P_2O_5$ - $4K_2O$) - is a dried poultry manure. Due to the high content of organic and active ingredients, in a short time leads to an increase in the microbiological, physical (structure, water retention) and chemical (buffering) properties of the soil. All these benefits help to reduce the loss of nitrogen, phosphorus and trace elements. Italpolina is rich in: organic ingredients, trace elements and beneficial microflora. COMPOSITION: N - 4%, P_20_5 - 4%, K_20 - 4%, M_2O - 0.5%, Water-soluble Fe - 0.8%, Water-soluble B - 0.2%, Organic C - 41%, Organic matter 70, 7%, Humic acids 5%, Fulvo acids 12%, Humidity 12%, pH 7.

Arkobaleno - simultaneously supplies nitrogen, phosphorus, potassium and organic matter (high fraction). Its completely organic form is not subject to washing phenomena. Its organic content is guaranteed as its components are from farms controlled. There is a slow transfer, therefore transmits nutrients to the plant throughout the growing season and allows for a healthy and balanced development. Improves the quality and storage capacity of plants. Allows gradual restoration of soil fertility and humus. Does not contain solid urban waste. Contains: Organic N - 45% - 3.5%; K20 - 3.5%; CaO - 5-8%; MgO - 0.8-1%; Organic C of biological origin - 30%; Organic matter (CX1.724) - 55-60%; Extractable organic matter (% of organic matter) - 30-35%; Humified organic matter (% of organic matter) - 12-14%; Humified organic matter (% of recoverable material) - 38-40%; Humification rate (HR) - 10-13%; Degree of Humification (DM) - 40-42%; Humification Index (Hi) - 1.3-1.4%; Fe - 3100-3200 ppm; B - 40-50 ppm; Cu - 190-200 ppm; Mn - 850-900 ppm; Zn - 550-560 ppm; Humidity - 13-15%; pH (H2O) 6-8.

Lumbrikompost (Organic fertilizer). An eco-friendly bioproduct resulting from the nutrition of organic red California worms. It is homogeneous, odorless. Contains dry matter 44.2%, moisture 55.8%, organic matter 47.24%, organic carbon 27.4%, pH (H2O) 7.85, salts 3.89 mS, total N (abs. Dry) - 1.71%, total P_2O_5 (abs. Dry) - 3.49 %, total K_2O 1.71 (abs. dry), total CaO (abs. dry) 6.25, total MgO (abs. dry) 2.14, Water-soluble (natural moisture) N-NH_4 - 1.75 mg/kg, Water-soluble (natural moisture) N- NO_3 - 804 mg/kg, Water soluble (natural moisture) P_2O_5 - 432.5 mg/kg, Water soluble (natural moisture) K_2O - 3282 mg/kg, Water soluble (natural moisture) CaO - 491 mg/kg, Water soluble (natural moisture) MgO - 353 mg/kg, Water-soluble (natural moisture) Cl - 555 mg/kg, Water-soluble (eu natural moisture) Cl - 555 mg/kg, Water soluble (natural moisture) SO4 - 547 mg/kg, Water soluble (natural moisture) Na - 229 mg/kg, Cd <0.3 mg/kg, Cr 46.8 mg/kg, Cu 124 mg/kg, Ni 24.0, Pb 17.1 mg/kg, Hg <0.05 mg/kg, Zn 295 mg/kg, B 50.8 mg/kg, Fe 8546 mg/kg, Mn 531 mg/kg.

Ekoprop NX is a microbiological fertilizer developed specifically for depleted soils. Its composition improves the efficiency of plant nutrition and the efficiency of water absorption. The number of young roots is greatly increasing, which in turn increases the better absorption of nutrients. Fertilizer contains 1% Glumos spp Applied to vegetables at a dose of 100 g / da. After planting, fertilize immediately using a large amount of water. It is applied 3-4 times during the growing season with an interval of 12-15 days. In thinner soils and sensitive crops, the dose is up to 150 g/da. It can also be applied with the last treatment, spreading superficially with subsequent incorporation of 10-15 cm and subsequent treatment after 7-15 days.

Plant material for anatomical studies

The plant material was collected when the plants reached maturity at the end of February - early March 2019. 3 fully developed middle leaves of 10 plants were used, which were fixed in 70% ethyl alcohol. Semi-permanent microscopic preparations were prepared from the middle of the leaf blade. Anatomic-morphological analyzes were performed using the methods of comparative anatomy (Metcalfe & Chalk, 1950) by examining the following indicators:

• Thickness leaf layers µm - TLL;
• Thickness of the upper covering tissue µm - TUE;
• Thickness of the lower covering tissue µm - TLE;
• Number of stomata upper epidermis in 1 mm^2 - NSUE;
• Length of stomata of the upper epidermis µm - LSUE;
• Width of stomata upper epidermis µm - WSUE;
• Number of stomata lower epidermis in 1 mm^2 - NSLE;
• Length of stomata lower epidermis µm - LSLE;
• Width of stomata lower epidermis µm - WSLE;
• Number of epidermal cells of the upper epidermis in 1 mm^2 - NCUE;
• Number of epidermal cells lower epidermis in 1 mm^2. - NCLE.

For each indicator 30 measurements were made.

The results were processed using the Descriptive statistic method, with minimum and maximum values, arithmetic mean, variation coefficient (VC %), mean arithmetic mean error (Sx %) determined. Using the SPSS 20 statistical package, using the Dunkan test, the degree of difference in the

arithmetic mean of the eleven indicators for the six variants examined was determined.
Using a Hierarchical Cluster Analysis, a cluster was created to compare the six Lactuca variants according to the degree of similarity of the measured indicators to compare the link between the groups (link groups) using the Squared Euclidian distance method.
The anatomical analyzes and photographs of each of the variants examined were made using a Magnum T microscope equipped with a Si5000 photographic documentation system at magnifications of x100 to x1000 in the Department of Botany and Methodology of Biology Education at the “Paisii Hilendarski” University of Plovdiv.

RESULTS AND DISCUSSIONS

Leaf lamina

Leaf mesophyll in *Lactuca sativa* is not divided into a palisade and spongy parenhyma (Bolhar-Nordenkampfand and Draxler, 1993), which determined the measurement of the total thickness of the leaf lamina (Figures 1, 2, 3).

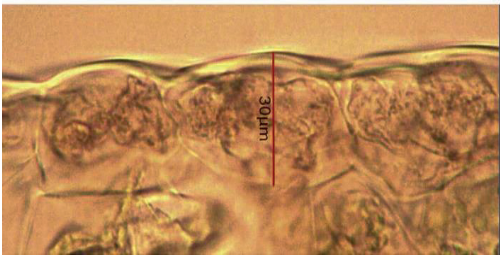

Figure 1. Upper covering tissue

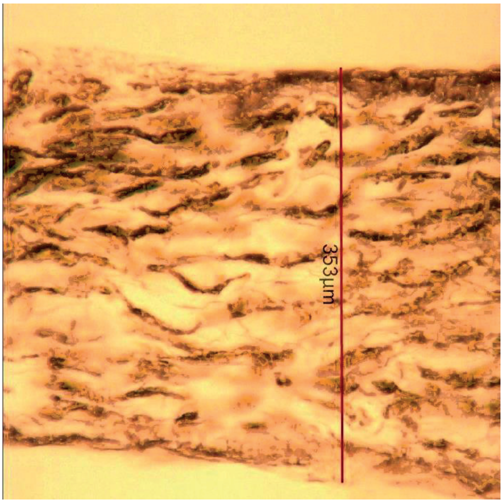

Figure 2. Leaf lamina

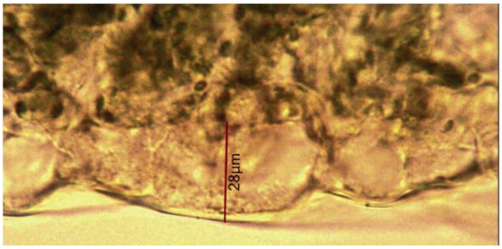

Figure 3. Lower cover tissue

The maximum value for leaf lamina thickness was reported for variant 3 (522.5 µm) and the minimum value for 1 (151.8 µm) (Table 1). In the mean values of this indicator, differences between the two variants are significant and statistically proven (Table 5). For the other variants, a statistically proven difference in the mean thickness of the leaf lamina was reported for variant 5 (234.9 µm), while differences in values for variants 2, 4, and 6 were not significant (Table 5).
For the thickness of the upper covering tissue, a maximum value was reported for variant 2 (38.5 µm), followed by variant 6 (35.2 µm) (Table 1). Minima were reported at 3 (19.8 µm) and 1 (20.9 µm) (Table 1). The highest mean values of this indicator were reported for variants 2, 6 and 4, with the statistical difference between the three variants being insignificant (Table 5). A minimum mean value for the thickness of the upper covering tissue was reported for variant 5, while the difference with the other variants statistically significant (Table 5).
The thickness of the lower covering tissue was also highest in variant 2 (35.2 µm), followed by variant 4 (31.9 µm) (Table 1). Minima were reported for 1 (14.3 µm) and 6 (15.4 µm) (Table 1). Maximum averages for this indicator were reported for variant 4 and 2 (Table 5). The statistical difference between the two variants is insignificant but statistically significant compared to the other four variants (Table 5). A minimum mean with a proven difference was reported for variant 1 (Table 5).
A number of studies have noted a decrease in the size of assimilating organs in plants in adverse environmental conditions. Dineva (2004) reported a nearly double reduction in leaf lamina size for *Fraxinus americana* L. and *Platanus acerifolia* Willd. exposed to road pollution. According to Ilkun (1978), Ninova (1970), Ninova Dushkova (1981), Dineva (2004), Radoukova (2009) unfavorable environmental conditions lead to a significant reduction in the overall thickness of the leaves, and especially that of the cover tissues.
In our study, the reported average of four times the mean leaf lamina at variant 3 (463.2 µm) compared to variant 1 (172.0 µm) is an indicator of the positive effect of Italpolina organic fertilizer.

With respect to the thickness of the upper and lower covering tissues, a clearly visible positive effect is considered in variant 4 where Arcobaleno is used.

The epidermis

Form of major epidermal cells

The degree of folding of the anticlinal cell walls of the major epidermal cells is one of the diagnostic features in examining the response of plants to environmental stressors. Stronger flexion of the anticline walls is an indication of greater sensitivity, i.e. for adverse effects (Ninova & Dushkova, 1978).

The shape and degree of folding of the anticline walls of the major epidermal cells of the upper and lower epidermis in the six variants examined are similar, i.e. are not affected by the nutrition. The major epidermal cells of the two epidermis in the six variants are characterized by a strong folding of the anticline walls. According to the classification of Aneli (1975), they refer to a curvilinear clan with zigzag folded cell walls. (Figures 4, 5), and according to the classification of Sveshtnikov (1970) are defined as curved to strongly curved.

Number of major epidermal cells in 1 mm²

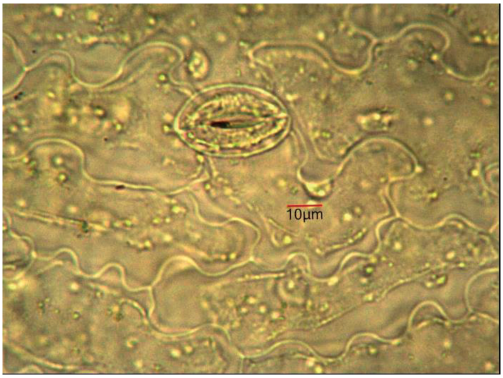

Figure 4. Upper epidermis

Figure 5. Lower epidermis

The smaller size of the major epidermal cells, as well as the increase in their number, are some of the main indicators of the response of the plant organism to adverse environmental changes. (Ninova & Dushkova, 1977; 1978; Koev et al., 2001; Gostin & Ivanescu, 2007; Radoukova et al., 2018; Dospatliev et al., 2018). According to Ninova and Dushkova (1981), low temperatures, excessive lighting, poor nutrition, and environmental pollution are among the factors causing reduce of the cell size.

Maximum values with respect to the number of epidermal cells of the upper epidermis are shown in variant 1 (487.8) and with respect to the lower variant 6 (560.9) (Table 2). The lowest values for the indicator were reported in variant 2 (195.1) for the upper epidermis and variant 1 and 2 for the lower (219.5) (Table 2). The statistically proven highest mean for the number of epidermal cells of the upper epidermis was reported for variant 2 (443.9) and the lowest for 2 (Table 5). In the lower epidermis, the averages of the six variants are statistically proven different. Variant 6 (491) is the maximum, and variant 2 (232) is the minimum (Table 5).

The imported organic fertilizers tend to increase the number of epidermal cells, especially the lower epidermis, with Arkobalenf and Ekoprop NX having the strongest impact.

Number of stomata in 1 mm²

Increasing the number of stomata and reducing their size is a major response of plant species to adverse changes in environmental conditions. Similar epidermal syndromes are reported in comparative anatomical studies of the same species under conditions of environmental stressors. (Ninova & Dushkova 1970; 1977; 1978; Dimitrova 2000; Radoukova, 2009)

Lactuca sativa is characterized by amphistomatic leaves and anomocytic type of stomata (Esau, 1977) (Figures 4, 5).

The number of stomata on the upper epidermis is maximal in variant 2 (95.1). In some of the test specimens of variants 1, 3, 4 and 5, visual acuity on the upper epidermis was not reported on the visual field (Table 3). At the mean values of the number of stomata in the upper epidermis, statistically proven maxima are reported at 2 and 6 (Table 5).

In the lower epidermis, maximal number of stomata was reported for variants 1 (243.9) and 2 (195.1). The minimum is reported in variant 5 (24.4) (Table 4). The mean values of this indicator are also highest for variants 1 and 2 (196.7 and 157.7 respectively) and lowest for variant 5 (52.8), with differences statistically significant (Table 5).

Variant 5 reports the maximum length of the stomata on the upper epidermis (10.1 µm), while in terms of width the maximum value of the variant 1 (7.6 µm) is shown (Table 3).

The smaller number of stomata on the lower epidermis reported for variant 5 corresponds to their larger size. The average values for the length and width of the stomata for this variant are highest (Table 5). This determines the use of Lumbrikompost as particularly favorable in terms of the number and size of the stomata. Although less pronounced positive effect is observed in the other 3 organic fertilizers. In both control variants, the reported maximum values in terms of number and minimum in terms of stomata size indicate the presence of environmental stressors, most likely due to a lack of organic matter in the soil.
Cluster analysis shows a clear clustering in standalone groups of variants 3 and 5, as well as 4 and 6. Variant 2 joins the group of 4 and 6, while the strongest degree of difference is shown in variant 1 (Figure 6).

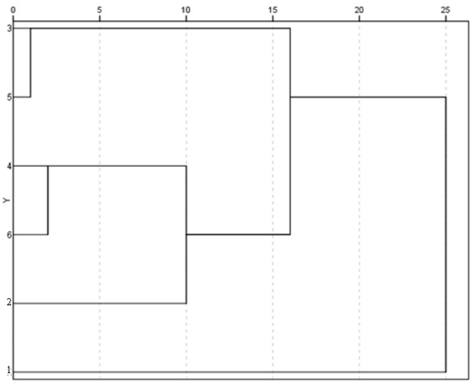

Figure. 6. Hierarchical cluster analysis to compare the relationship between indicators using the square Euclidean distance method

Table 1. Reported values for leaf lamina thickness and cover tissue for the six variants tested (μm)

Thickness of leaf lamina					
Variant	min	x ± Sx	max	VC%	Sx%
1	151.8	172.0±4.9	192.5	8.1	2.8
2	244.2	350±18.4	394	15.8	5.2
3	418	463.2±13.5	522.5	8.2	2.9
4	221.1	382.2±22.7	436.7	17.8	5.9
5	191.4	234.9±11.5	276.1	12.9	4.9
6	303.6	316.9±4.9	343.2	4	1.5
Thick upper cover tissue					
1	20.9	23.6±0.3	25.3	5.8	1.1
2	29.7	31.6±0.44	38.5	7.7	1.3
3	19.8	24.6±0.7	30.8	15.7	2.8
4	27.5	31.4±0.2	33	4.1	0.7
5	22	23.2±0.2	25.3	5.6	1
6	27.5	31.5±0.5	35.2	8	1.4
Thick lower cover tissue					
1	14.3	19.5±0.4	24.2	11.1	2
2	19.8	29.4±0.9	35.2	16.7	3.1
3	18.7	24.2±0.6	29.7	13.9	2.5
4	24.2	29.4±0.4	31.9	6.9	1.2
5	20.9	22.4±0.2	24.2	5.2	0.9
6	15.4	21.8±0.5	24.2	12.1	2.2

Table 2. Reported values for the number of epidermal cells of the upper and lower epidermis in the six variants examined

Number of epidermal cells upper epidermis					
Variant	min	x ± Sx	max	VC %	Sx %
1	356.9	443.9±8.3	487.8	10.3	1.9
2	195.1	211.4±3.4	268.3	8.7	1.6
3	219.5	265.0±5.6	317.1	11.5	2.1
4	317.1	380.5±5.3	414.6	7.6	1.3
5	317.1	334.9±3.1	365.9	5	0.9
6	292.7	338.2±4.6	365.9	7.5	1.3
Number of epidermal cells lower epidermis					
1	219.5	273.9±6.9	341.5	13.9	2.5
2	219.5	232.5±2.3	243.9	5.3	0.9
3	341.5	425.2±7.3	487.8	9.3	1.7
4	365.9	386.2±3.7	414.6	5.2	0.9
5	292.7	323.8±3.2	341.5	5.2	0.9
6	414.6	491.1±7.6	560.9	8.4	1.5

Table 3. Reported values for number and size of stomata (µm) of the upper epidermis in the six variants examined

Variant	min	x ± Sx	max	VC%	Sx%
Number of stomata upper epidermis					
1	0	30.9±3.3	73.2	8.3	1
2	121.9	156.9±2.8	195.1	9.7	1.8
3	0	26.0±3.7	48.8	7.6	1.1
4	0	30.9±3.5	73.2	6.9	1.2
5	0	21.9±2.4	48.8	6.9	1.1
6	24.4	47.9±3.2	73.2	6.5	1.6
Length of stomata upper epidermis					
1	6.2	7.0±0.09	7.8		
2	6.7	7.9±0.1	8.5	7.2	1.3
3	8.3	8.8±0.05	9.2	2.9	0.5
4	7.4	7.7±0.04	8.1	3.1	0.5
5	8.7	9.3±0.08	10.1	4.6	0.7
6	7.8	8.1±0.04	8.51	2.7	0.4
Width of stomata upper epidermis					
1	4.8	5.3±0.05	7.6	5.2	0.9
2	5.1	5.6±0.04	5.9	4.2	0.7
3	4.6	5.5±0.06	5.8	5.5	0.9
4	5.5	5.7±0.03	5.9	2.8	0.5
5	5.5	5.8±0.05	6.2	4.8	0.8
6	5.3	5.6±0.03	5.9	3.5	0.5

Table 4. Reported values for the number and size of the stomata (µm) of the lower epidermis in the six variants examined

Number of stomata lower epidermis					
Variant	min	x±Sx	max	VC%	Sx%
1	170.7	196.7±3.3	243.9	9.1	1.6
2	97.6	157.7±4.3	195.1	15	2.7
3	48.8	81.3±2.9	97.6	19.8	3.6
4	73.2	86.9±2.2	97.6	14.1	2.5
5	24.4	52.8±3.1	73.2	32.2	5.8
6	97.6	125.2±3.6	146.3	15.9	2.9
Length of stomata lower epidermis					
1	7.4	8.5±0.09	9.2	5.8	1.1
2	7.6	8.3±0.06	8.9	4.2	0.7
3	7.1	7.9±0.06	8.3	3.9	0.6
4	8.1	8.4±0.05	8.7	3.3	0.5
5	8.1	8.5±0.03	8.7	2.2	0.3
6	6.9	7.6±0.05	7.8	3.9	0.6
Width of stomata lower epidermis					
1	5.1	5.4±0.03	5.5	2.9	0.6
2	5.1	5.7±0.06	6.2	5.4	0.8
3	5.5	5.9±0.05	6.4	4.7	0.8
4	4.8	5.4±0.07	5.8	7	1.2
5	5.5	6.2±0.06	6.44	5.6	0.9
6	5.1	5.4±0.06	5.8	5.3	0.9

Table 5. Duncan test for similarity between the arithmetic value of the studied parameters for the six variants p 0.05

Variant	TLL	TUE	TLE	NSUE	LSUE	WSUE	NSLE	LSLE	WSLE	NCUE	NCLE
1	172.0^{d}	$23.6^{b,c}$	19.5^{d}	30.9^{c}	7.0^{f}	5.3^{c}	196.7^{a}	8.5^{a}	5.4^{c}	443.9^{a}	273.9^{e}
2	$350.3^{b,c}$	31.6^{a}	29.4^{a}	156.9^{a}	7.9^{d}	5.6^{b}	157.7^{b}	8.3^{b}	1.9^{d}	211.4^{e}	232.5^{f}
3	463.2^{a}	24.6^{b}	24.2^{b}	26.0^{c}	8.8^{b}	5.5^{b}	81.3^{d}	7.9^{c}	5.9^{b}	265.0^{d}	425.2^{b}
4	382.2^{b}	31.4^{a}	29.4^{a}	30.9^{c}	7.7^{e}	5.7^{a}	86.9^{d}	$8.4^{a,b}$	5.4^{c}	380.5^{b}	386.2^{c}
5	243.9^{d}	23.2^{c}	22.4^{c}	21.9^{c}	9.3^{a}	5.8^{a}	52.8^{e}	8.5^{a}	6.2^{a}	334.9^{c}	324.4^{d}
6	316.9^{c}	31.5^{a}	21.8^{c}	47.9^{b}	8.1^{c}	5.6^{b}	125.2^{c}	7.6^{d}	5.4^{c}	338.2^{c}	491.1^{a}

Average with identical letters have no statistically proven differences

CONCLUSIONS

The lowest overall thickness of the leaf lamina, the upper and lower covering tissues in variant 1 reported a negative reaction to the growing conditions for this variant. The high average values for these indicators reported for variants 3 and 4 determine their response to soil-borne organic fertilizers (Italpolina and Arkobaleno) as favorable.

With respect to the number of epidermal cells, the minimum values obtained for both epidermis in variant 2 are indicative of a positive response to environmental conditions, which contrasts with the values reported for the same variant in terms of both leaf thickness and number. stomata on both epidermis. The maximum number of stomata reported for both epidermis in variant 2 determines the presence of a negative reaction to growing conditions.

The results obtained for variant 5, which shows the smallest number and largest size of stomata for both epidermis, determined the plant response to fertilization with Lumbrikompost as particularly positive. A similar tendency with respect to the stomata is observed in other variants with the application of organic fertilizers, in contrast to the two controls.

Grouping of variants 3 and 5 and 4 and 6 into separate cluster groups in terms of anatomical parameters is an indicator of a positive response to growing conditions.

The application of organic fertilizers can be defined as favorable to the anatomical structures of the leaves during greenhouse lettuce cultivation.

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STUDY OF THE VEGETATIVE BEHAVIOUR AND PRODUCTIVITY OF SOME GENOTYPES OF FABA BEAN (*VICIA FABA* L.)

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Abstract

The aim of the study was a comparative analysis of several genotypes of faba bean (Vicia faba L.). The sowing of the seeds was carried out in November. The percentage of overwintering plants was determined. The vegetative behavior of the plants in dynamic as plant height, numbers and length of branches and numbers of leaves were investigated. The generative features of the plants and the yield of the green pods were registered. The results indicate that the plants of the studied genotypes were successfully survived during winter and in the spring, they developed a plant-like shrub with 3 or 4 branches. Differences in generative characteristics of plants were founded. The B1E238 genotype began flowering earlier and have the largest numbers of flowers. This genotype is distinguished also the highest yield of green fruits. The investigated genotypes of faba bean do not have significant differences by morphological features of the fruits and seeds.

***Key words**: faba beans, genotypes, productivity, vegetative behaviour.*

INTRODUCTION

A faba bean (*Vicia faba*) is an annual plant of Fabaceae. The genetic variability of the species is quite large, and most researchers refer tofour botanical varieties: *V. faba paucijuga*, *V. faba mayor*, *V. faba equina* and *V. faba minor*. Differences between subspecies are mainly based on differences in seeds weight, shape and size (Metayer N., 2004; L´opez-Bellido et al., 2005). In Bulgaria the varieties of large-seeded of faba bean are mainly distributed. From the small-seeded and the middle-seeded mainly local forms occur (Kartalov et al., 1999). Faba bean is the third most important leguminous vegetable crop after beans and peas. In addition to human food, faba bean is also used as food for certain ruminants (Singh and Bhat, 2012a). Many researchers (Singh et al., 2009; Singh et al., 2010; Singh et al., 2013a) have indicated that the cultivation of faba bean affects soil fertility. Although the cultivation of faba bean in Bulgaria is limited, this doesn't define the importance of culture as unimportant. According to many authors, the importance of the faba bean will become ever greater. The reasons for this are social and environmentally sound (Hoffmann et al., 2007; Singh et al., 2010; 2013b). In the period 2010-2012, S. Angelova et al., visit different areas of the country for the purpose of collecting old varieties, populations and forms of faba bean (*Vicia faba*), which are grown and maintained by the local people. More than 70 specimens have been collected that differ significantly in plant habitat, duration of the growing season, size and color of seeds and crude protein content. According to the authors, the loss of this culture as a tradition in many places in the country is due to the aging of the population and depopulation of villages, as well the diminishing interest of the crop due to the wide variety of leguminous crops on the market.

MATERIALS AND METHODS

For a two years of research 4 type of genotype of faba bean were investigated. The experiment was carried out on experimental field of Agricultural University Plovdiv. Three of the investigation genotypes are local forms - B1E217, B1E235 and B1E238 (Figure 1), provided by the Institute of Plant Genetic Resources - Sadovo. The seeds are part of collection of old varieties, populations and forms of faba bean. Super Simonia variety was studied also (Figure 2). The sowing of seeds was carrying out in November. The experiment was carried out in 3 replications. The field germination of seeds and the percentage of overwintering plants were determined. The biometric measurements of the plants were performed during the beginning of flowering and mass fruiting. The vegetative behaviour of the plants as plant height, numbers and length of branches and numbers of leaves were investigated. Some generative features of the plants and the yield of green pod were registered. Green fruits were harvested at technological maturity before seed growth in them, when they reached ¾ of the normal size for the variety and their consistency is juicy and fragile, without a parchment layer (Cholakov, 2009). Some morphological features of green pods and seeds were investigated.

Figure 1. Genotype - B1E238

Figure 2. Super Simonia variety

RESULTS AND DISCUSSIONS

Seed germination is one of the most important determinants of seed quality. The investigated genotypes have a high percentage of field germination (Table 1) - between 90% in genotype B1E235 and 98% in Super Simonia variety.

An essential indicator for the winter sowing of vegetables is the percentage of wintering plants (%). This indicator, as well as germination, is essential in determining the sowing rate. In two variants, the percentage of overwintering plants coincides with field germination, while in Super Simonia variety and B1E217 genotype it decreases by 3% to 5.5%. Nevertheless, the percentage of overwintering plants is highest in Super Simonia. In conclusion, it can be noted that the plants of the test variants successfully overwinter despite the decrease in temperatures during the winter months.

During the flowering period (Table 2), the plants do not have a significantly differences by the morphological characteristics. The height of the central stem is from 22 cm (B1E238) to 29 cm (Super Simonia). As for the number of branches per plant, two of the variants are characterized by a lower degree of branching- the Super Simonia variety and the local form B1E235 (var. 3). The number of reported branches in these variants is three, while in the other two variants it is four. The most intense branching growth is in Super Simonia variety (var. 1), where the length of all branches exceeds in value the other variants. The leaves number on the central stem is from 7.4 to 9.2. In all variants, the number of leaves on the branches is highest in the first forms, and then decreases.

During the period of mass fruiting phase (Table 3), the maximum stem length was reported at genotype 3 (62.2 cm). For the period between the two reports, the growth of the stem in this variant was most intense, 30 cm. The stem is lowest at variant 2, respectively 42 cm, which is stands with the slowest rate of growth of the stem, 17 cm for the period. Although for variants 1 and 4 the difference in the values of this indicator is 8 cm, we can say that the growth rate of the stem between the two reporting periods is similar by 26 cm for variant 4 and by 26.8 cm for variant one.

The number of branches does not change, three or four per plant. The average length of the branches depending on the genotype is between 37 cm and 55.3 cm. In variants with a higher central stem, the number of branches is smaller, an average of three plants. Variants with a lower central stem have a larger number of branches, but shorter than the others.

Table1. Field germination (%) and percentage of overwintering plants

Variant №	Field germination, %			Overwintering plants, %		
	2016	2017	average for the period	2016	2017	average for the period
1.Super Simonia	98	98	98	96	94	95.0
2. B1E217	93	91	92	88	85	86.5
3. B1E235	90	90	90	90	90	90.0
4. B1E238	92	90	91	92	90	91.0

Table 2. Biometric indicators of the plants in the beginning of flowering, average for the period 2016-2017

Variant	Branch number per plant	Length of:					Numbers of leaves on:				
		central stem	1st branch	2nd branch	3th branch	4th branch	central stem	1st branch	2nd branch	3th branch	4th branch
1.Super Simonia	3	29.1	25.4	25.0	21.1	-	9.2	8	7	5	-
2. B1E217	4	24.2	21.0	19.2	17.4	17.2	7.4	7	7	5	4
3. B1E235	3	27.5	21.7	17.4	13.1	-	9.2	8	5	3	-
4. B1E238	4	22.0	21.0	16.	12.2	10.2	8.4	7	6	5	5

Table 3. Biometric indicators of the plants during mass fruiting period, average for the period 2016-2017

Variant №	Length of central stem	Number of branches	Branch lengths on:					Numbers of leaves on:	
			1st branch	2nd branch	3th branch	4th branch	average length of branch	central stem	branch
1. Super Simonia	56.1	3	51.1	50.5	49.3	-	48	20	16
2.B1E217	42.9	4	40.5	37.5	36.4	35.0	37	18	14
3.B1E235	62.2	3	59.0	56.1	48.1	-	55.3	21	16
4.B1E238	48.7	4	46.3	43.4	37.4	35.2	41	17	13

The generative organs of the faba bean are embedded in the leaf petiole. Therefore, the number of leaves per plant is essential. Although the number of leaves is from 17 to 21 and relative to the length of the stem, differences between the studied genotypes appear in the length of the internodes. This, as well as differences in the degree of branching, show differences in the habitus of the plants. The average number of leaves on the branches at the time of reporting is between 13 and 16. The lowest is in the variants with a lower stem and larger number of branches.

The location of the generative organs is important to determining maturity. Tested genotypes had differed on first flowers on the central stem (Table 4). The earliest betting on the first flowers was reported in genotype B1E238 - in second leaf. In plants of the other genotypes, this occurs after the 3rd - 5th leaf.

This trend for the early generation of the first flowers in genotype B1E238 is observed in all branches.

For the other genotypes, emergence of the first flowers on branches begins after 2 - 4 leaves.

Betting more flowers, as well as providing optimal conditions for the pollination and fertilization processes, are an important prerequisite for greater plant productivity. Depending on the genotype, the number of flowers per plant is between 38 and 46. The smallest is for genotype B1E235 and the largest is for B1E238.

The total standard yield of green pods (Table 5) is from 1036.4 kg/da - B1E217 (variant 2) to 1212 kg/da at B1E238 (variant 4).

Table 4. Some generative plant manifestations average for 2016-2017

Variant №	Betting first flower and number of flowers on:					Average number of flowers per 1 plant
	central stem	1st branch	2nd Branch	3rd branch	4th branch	
1. Super Simonia	$2^{(4)}$	$3^{(4)}$	$4^{(4)}$	$3^{(4)}$	-	45
2. B1E217	$3^{(3)}$	$2^{(4)}$	$2^{(2)}$	$3^{(2)}$	$2^{(3)}$	42
3. B1E235	$3^{(5)}$	$2^{(4)}$	$2^{(3)}$	$2^{(3)}$	-	38
4. B1E238	$3^{(2)}$	$2^{(2)}$	$3^{(1)}$	$4^{(1)}$	$3^{(2)}$	46

*The index in parentheses () shows in which leaves the first flowers are laid

Table 5. Yield (kg/da) and morphological characteristics of the green pods average for the period 2016-2017

Variant №	Yield, kg/da	Pod weight, g	Pod length, cm	Pod width, cm	Pod thickness, cm
1.Super Simonia	1153.5	7.8	7.63	1.20	0.94
2. B1E217	1036.4	6.3	6.81	1.08	0.81
3. B1E235	1064.0	6.6	6.84	1.10	0.84
4. B1E238	1212.0	7.6	7.83	1.13	0.87

The higher yield is due to the greater number of fruits per plant and the higher average weight of the pods. Good results were obtained for the Super Simonia variety. The reported yield exceeds variant 2 and variant 3, by an average of 103.3 kg/da.

Although the plants of variants 2 and variant 3 have some differences in habitus, the difference between the reported total yield is small - 26.7 kg. There are no differences between these genotypes of faba bean on the morphological features of the fruits.

Average for the period, green pods weigh is from 6.3 to 7.8 g, the length of the pods is 6.81-7.83 cm, the width of 1.08-1.20 cm, and the thickness of 0.81 to 0.94 cm. The higher values are for the Super Simonia variety, which is inferior to the B1E238 genotype only at the pod length indicator.

The values of the indicators are very similar in both variants.

With the passage of the pods into botanical maturity, their length slightly increases (Table 6). Depending on the variant, the length of the pod is from 7.6 (at variant 2) to 11.3 cm (at variant 4). The number of seeds per pod depending on the variant is from 3 to 4. The largest seeds are in the variant B1E238 (var. 4), where the weight per 100 seeds is 133.82 g., and the smallest is in B1E217 (var. 2), 90.56 g.

Table 6.Some morphological characteristics of maturity pod and seeds, average for the period 2016-2017

Variant №	Pod length,cm	Number of seed per pod	Mass of 100 seeds, g
1.Super Simonia	10.7	4	122.6
2. B1E217	7.6	3	90.56
3. B1E235	8.9	3	105.32
4. B1E238	11.3	4	133.82

CONCLUSIONS

The investigated genotypes successfully overwinter, which make them suitable for early production with winter sowing in region of Plovdiv. The Super Simonia variety is characterized by the highest percentage of overwintering plants.

The genotypes have a shrubby habitus with 3 or 4 branches and length of stem at the phase of mass fruiting from 42 cm to 62.2 cm. Plants with a lower central stem have a greater number of branches, and shorter internodes.

The genotypic differences are observed in the betting of the first generative organs. The earliest betting is on genotype B1E238, which is characterized by the better generative development due to the betting of the largest number of flowers. The reported yield in this genotype is by 5.1 to 13.9% higher than others.

The investigated genotypes don't have differed in the morphological characteristics of the pods and seed number, but seed masses are difference.

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CLIMATE ANALYSIS AND EFFECTS OF ABIOTIC STRESS ON SALAD GROUNDED IN UNDERGROUND GREENHOUSE AND OUTDOOR AND EFFECTS OF ORGANIC FERTILIZERS IN THE FIGHT WITH STRESS FACTORS

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Abstract

In the study was determined the impact of organic fertilizers as anti-stress agents and their impact on the particular species. Also was carried out an evaluation of the plant anti-stress response, by applying different methods of reporting and looking for ways to overcome it. Climate change and coming with them abiotic stressors, a consequence of extreme weather conditions, especially affect on salad grown outdoors. What are the climate fluctuations in hydrothermal conditions and how they can affect the growth and development conditions? This question is very relevant at the moment. Therefore a general hydrothermal characterization of the climate in the Plovdiv region for the period 1990-2019 has been made. In the study were made analyses of the basic conditions during the period investigated, the presence of stressors, as well as an assessment of the impact of major meteorological factors on salad crop productivity, on three different varieties, using four different formulations of organic fertilizers, and also control variants with mineral fertilizer and without fertilizing. The goal was to increase plant resistance to abiotic stress and to guarantee the yield by optimizing nutrition.

***Key words**: Lettuce (Lactuca sativa), organic fertilization, climate change, Plovdiv region.*

INTRODUCTION

In the face of climate change, organic production is among the leading modern strategies for adapting green systems to changing conditions of growth and development. Agricultural production, including leaf and head lettuce, fed with organic fertilizers, guarantees the safety and purity of food while at the same time having the function of preserving and enriching soil biodiversity. The high interest in the use of organic fertilizers of different concentrations is justified due to the fact that their use leads both to the conservation of soil resources and to an increase in soil fertility. Organic fertilizers combined with soil temperature increase the usability of nutrients. The rhizosphere is the area around the roots of the plants where maximum microbial activity occurs. Bacteria that improve plant growth and health are known as rhizobacteria that stimulate plant growth (PGPR). On the combinations and mode of action of plant growth-promoting rhizobacteria (PGPR) are studies by Vessey, 2003. The use of PGPR in agriculture is definitely a priority, as synthetic chemicals pose a serious threat to agro-ecosystems (Verma et al., 2019).

The agricultural production is mainly performed outdoors, and the importance of the basic meteorological elements - temperature and precipitation - is well known. Despite the existing mechanisms for sustainability and environmental plasticity, the cultivation of early spring crops, including lettuce, is determined by the climatic conditions of the individual territories. The salad is grown in our country, both in unheated greenhouses and in the open air, with any change in hydrothermal conditions affecting the economic efficiency in the first case and directly on the productivity in the second. The analysis of the multiannual data of the major meteorological elements over the last century in Bulgaria shows a tendency to increase temperatures and decrease or change the distribution of rainfall by seasons (Kazandjiev, 2008; Koleva & Alexandrov, 2008). There has been a change in humidity through the index I_{DM}DeMartonne (1926) from moderately moist to moderately dry, with a proven decrease in the productivity of non-irrigated crops (Kazandjiev et al., 2009). There is a change in the duration of the potential growing season (Slavov et al., 2006) to a faster accumulation of the required temperature sums and shortening of the interphase periods, which adversely affects the crop yield. Moisture conditions are directly related to the amount of precipitation. Trends in declining annual precipitation in the Danube Plain and the Thracian Plain, as well as an increase in the frequency of dry years over the last century, have been identified by Koleva & Alexandrov, 2008; Alexandrov et al., 2011. In the first 15 years of this century, some weather stations have experienced an increase in rainfall during the autumn season and moisture accumulation of up to 10% (Georgieva et al., 2017). In recent years, the frequency of extreme weather events has caused damage to varying degrees and loss of agricultural production, both worldwide and in our country. More recent climate studies in Bulgaria show a clear trend of warming after the mid-1980s. In the period 1988-2016, the average annual air temperature in areas up to 800 m above sea level increased by an average of 0.8°C compared to the climatic norm for the reference period 1961-1990, and the trend for precipitation amounts is inversely determined by the previous climatic period. Quantities have increased and heavy rainfall has caused severe flooding harmful to various socio-economic sectors, including outdoor agriculture (Marinova et al., 2017). On the other hand, the process of water distribution and irrigation presupposes rationality and balance in the guaranteed human right to have water and protect ecosystems in the face of climate change (Kolcheva, 2019).

The salads are grown for fresh consumption and have a great variety, color and taste. They are characterized by a relatively short growing season, reaching economic maturity 50-60 days after planting.The optimum temperature for growth and development is 23°C during the day and 7.0°C at night. At high temperatures, the plants bloom, deform and become bitter. Freezing causes damage to varying degrees, especially on the wrapping sheets and reduces their resistance to storage and transport. The recommendations for their cultivation, the timing of planting in the different regions of the country, nutrition and drifts, depend on the climatic conditions. As leafy plants, they are susceptible to nitrate accumulation, which makes them organically grown extremely important and promising. With this publication, the authors aim to make a hydrothermal analysis of the Plovdiv area for the last 30 years. For a period of 3 years an attempt has been started to test the behavior of three varieties of salad in conditions of transition to organic fertilization. Data are currently being collected, but the experiment has not been completed and some preliminaryresults will be analyzed here. In the course of the study the influence of biotors as anti-stress agents and their impact on the specific species was determined. The anti-stress response of the plants has also been evaluated by applying different methods of reading it and looking for ways to overcome it. One of the main goals is to optimize nutrition and increase the resistance of salads to abiotic stress.

MATERIALS AND METHODS

Daily air temperature data, rainfall (mm), air humidity (%), etc. were used from the weather stations in Plovdivaccording to the recommendations of WMO and the methodological guides of NIMH (Stanev, 1969). For the analysis and calculation of the climatic norm for the reference period of the De Martonne Index (1926), daily values of air temperature of 2m above the earth's surface and 24 hours of precipitation from the NIMH database available at the AU Plovdiv were used. Annual and monthly indices are calculated that are appropriate for both planetary and regional analysis using the formulas (Mitkov & Topliyski, 2018):

$$J_{DM} = \Sigma R / T + 10),$$

where ΣR is sum of the rainfall in mm and T is the average temperature °C; J (mm/°C) index (De Martonne, 1926) by months.

$$J_{DM} = 12 * \Sigma R / T + 10),$$

where ΣR is the monthly sum of the rainfall in mm and T is the average monthly temperature

°C using the index and rating scale appropriate for the index.

A professional agrometeorological station was installed for the purpose of the experiment and immediately to the observed plantsMeteobot® Pro (https://meteobot.com/meteostancii/), with a set of sensors: for rainfall, air temperature, soil temperature at planting depth, etc. Attempts have been made to plant plastic greenhouses with planting respectively - before winter and outdoors - in the spring of 2018-2019. The plants were planted in phenophase 4-5 leaves in November 8 in the polyethylene greenhouses of AU-Plovdiv in 4 rows according to the scheme 70+30+30+30/30 cm with a profile of the soil surface a high level bed (100+60 cm.) The experiment was based on the block method in four repetitions in 28 plants per repetition, with a plot size of 3.36 m^2. Organic seeds were provided for seedling production using container technology using 150-hole Styrofoam boards.

Organic seedlings are used - 80%, Perlite - 20%, Lumbricompost, developed by us for bio-production of seedlings(Kostadinov & Filipov, 2013). The following fertilization options are being studied: 1. Control-non-fertilization; 2. Control-MT (mineral fertilization-NPK); 3. Italpollina; 4. Arkobaleno; 5. Lumbricompost; 6. Ekoprop NX. Granular fertilizers were introduced as basic fertilization, with pre-transplantation of soil, into the following norms: N- 12.5 kg/da, P_2O_5 - 1.25 kg/da, + K_2O - 4.75 kg/da, Italpollina- 25 kg, Arkobaleno - 100 kg/da, Lumbricompost - 400 l/da. Ekoprop NX is applied by double treatment at a dose of 100g/dka, the first in the seedling phase, the second after planting and planting.

The salads are three varieties of Batavia variety ‘Maritima’, Leaf lettuce-type Lolo rosa variety ‘Tuska’, Head lettuce variety ‘Winter Oil Head’, 3000 plants (1000 plants of each variety). The greenhouse experience is based on 6 variants with a total area of 450m^2, of which 375m^2 with organic fertilization. A drip system was used for watering in the greenhouse and outdoors.

Biometric studies include three-fold measurement of phenophase commercial maturity every 7-10 days for each variety and variant of: Fresh whole plant mass (g); Socket - D - diameter (mm) and mass (g); Head - arrangement, shape, coloring of the rosette leaves (superficial and internal); Leaf count and fresh mass (g); Stem mass (g), diameter and length. The total yield, quality, appearance, texture, organoleptic evaluation, was reported by repetition.

Statistical Package for the Social Sciences (SPSS) (SPSS Inc., 2007) and Microsoft® Office products were used for statistical data analysis. The visual evaluation of the smoothed curves is subjective, so the existence of a trend is investigated using the nonparametric test of Mann-Kendall (WMO, 1990).

RESULTS AND DISCUSSIONS

What are the current climate studies in Plovdiv, what are the climate fluctuations in hydro-thermal conditions and how they can affect the growth and development conditions?

Given the climate fluctuations discussed, a general hydrothermal characterization of the climate in the Plovdiv region has been made.

The air temperature, precipitation and soil temperature were studied, as well as the De Martonne (1926) index, which gives an estimate of the humidity conditions.

For the period 1990-2019. a positive, statistically significant trend (Test Z 3.55; Signific. ***) in annual air temperature was observed in the study area (Figure 1). The deviation from the reference period (1961-1990) in degrees is significant +0.9°C. It should be noted that the highest values occur after 2000, with the two warmest years being 2000 and 2019, with values of 14.6°C and 14.2°C, respectively, with an average value for the whole period of 12.9°C (Figure 1).

In this sense, it is necessary to conclude that there is a change in temperature conditions, i.e. a statistically significant trend, an increase in the new period, and that the increase is most significant at the beginning of our century.

The deviation from the period 1990-2019 is between + 1.6°C and -1.2°C, but after 2010 is only positive (Figure 2). For January the deviation from the reference period is positive + 0.7°C.

For February the deviation for the period 1990-2019, compared to the reference period is positive + 0.5°C.

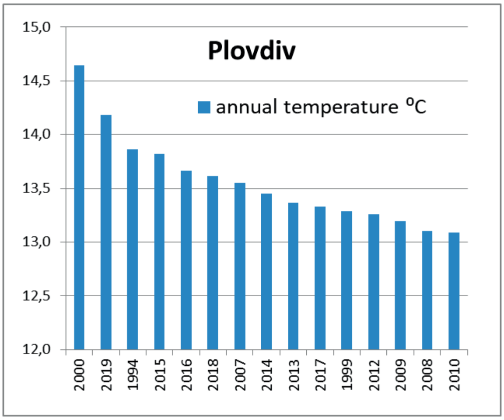

Figure 1. Highest annual temperature °C since 2000

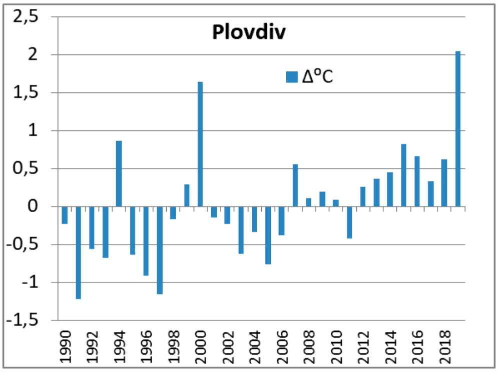

Figure 2. Deviations from 1990-2019

A positive trend (Z 1.89 +) was observed at the average monthly temperature for March, and the deviation of the period 1991-2019 from the reference 1961-1990 was 0.9°C.

A positive trend was observed with a level of significance (Z 1.74 +) and a deviation for April 1991-2019, compared to the reference 1961-1990 of 0.5°C. The trend in May is significant (Z 1.71 +) and the deviation from the reference period is 0.7°C.

In June, the increase over the reference period was 1.2°C, in July 1.4°C, August 1.9°C. For September, the increase compared to the reference period is 0.7°C. Mann-Kendall tests report a positive trend (Z 2.82**) for the period 1990-2019.

For October the deviation is 0.6°C. November deviation from the reference period is 0.5°C positive trend with significance level (Z 2.36*). The only month with a slight, negative deviation of less than one-tenth is December (-0.1°C) (Figure 3). Previous surveys of the area, but for the period 1986-2015 they give a negative course of two winter months and it with a greater deviation (Georgieva et al., 2017).

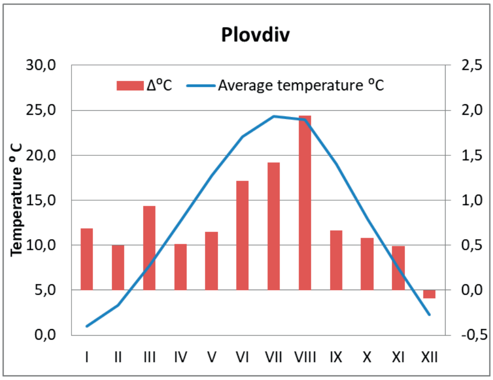

Figure 3. Average temperature by months and deviations from 1961-1990

The annual rainfall shows a significant increase over the period 1990-2019 (Z 2.50*), with a deviation from the reference period 1961-1990 of 34 mm.

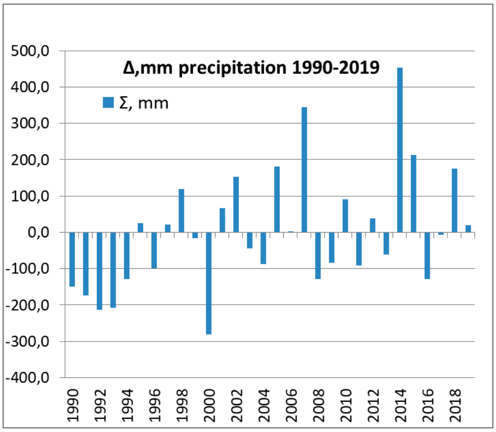

Figure 4. The annual rainfall deviations (Σ mm) 1990-2019

The highest amounts are predominantly after 2000, the two largest being in 2014 and 2007, respectively, 993.9 mm and 884.1 mm (Figure 5). Also, the highest negative deviation (annual ∑ mm) is during the same period, which shows an increase in the frequency of extreme events (Figure 4).

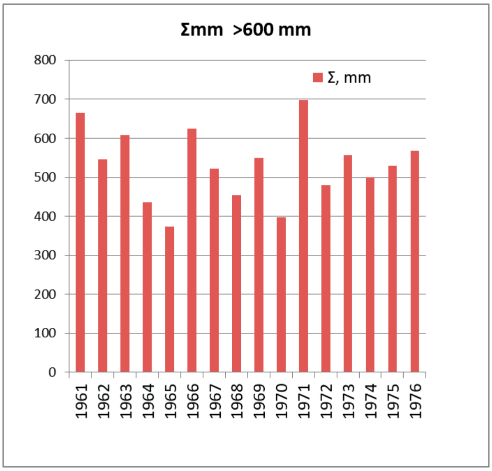

Figure 5. The highest annual rainfall (Σ mm) 1961-2019

The monthly analysis shows negative deviations in the spring - April, May and November and positive during the other seasons, with the highest values in September and October, about 30%, respectively 12.2 mm and 13.3 mm (Figure 6). Here, Georgieva et al., 2017, found a 5-10% increase in rainfall outside active vegetation over the period 1986-2015, compared to the reference 1961-1990. There is a statistically significant trend for the period 1990-2019 (Z 1.96*) and a deviation of 3.9 mm in June, compared to the value for the same month during the reference period.

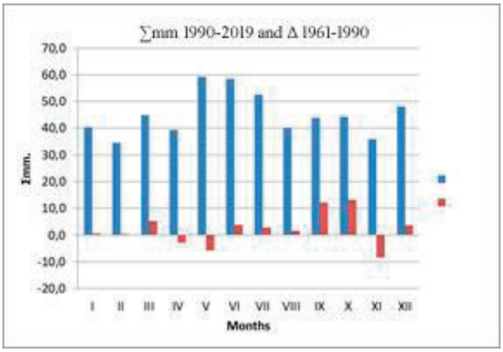

Figure 6. Monthly rainfall and deviations (Σ mm) 1990-2019 from the reference period

Soil temperature is no less important element in the development of salads. An analysis of the period 1990-2019 has been made (Figure 7). Here, the values of 20 cm are investigated here, because at this depth the planting is carried out and the basic root system of the observed species develops. The great importance of PGPR in the rhizosphere has already been mentioned. In addition to their activity, soil temperature also affects the speed of biochemical processes in the growth and development of plants and through the absorption of biological fertilizers. The soil temperature by months shows a positive trend throughout the period, including the winter months. The most pronounced and of the highest statistical significance are summer and autumn - July, August, September and October (March 20 cm (Z 1.76 +); April 20 cm (Z 2.26*); May 20 cm (Z 3.25**); June 20 cm (Z 2.36*); July 20 cm (Z 3,55***); August 20 cm (Z 3,40***); September 20 cm Z 4 , 04 S***); October 20 cm (W 4.04***); November 20 cm (Z 2.80**); December 20 cm (Z 2.16*). Only three are months with a temperature of 20 cm <5.0°C - December, January and February.

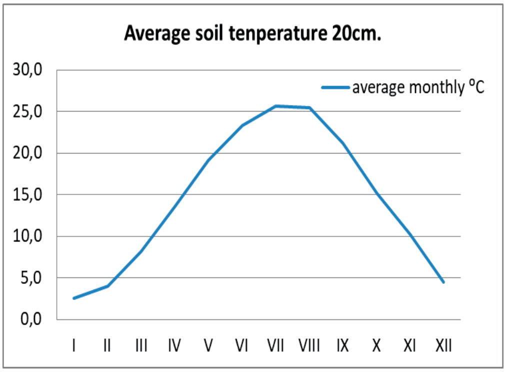

Figure 7. Average monthly soil temperature (1990-2019) at a depth of 20 cm in °C

The humidity conditions were estimated using the De Martonne Index (1926). The calculations show a difference of 1.7 compared to the period 1961-1990 and a positive trend (Test Z 3.46; Signific. * * *) For the period 1990-2019. The values for the two periods are respectively 23.2 and 24.9, which on the respective scale (Figure 8) characterizes the area as Semiarid, with the second period very close to the beginning of moderately arid.
All highest values were found in the period after 2000, and the highest values were in 2018, 2014, 2017 and 2019 as follows: 52, 42, 40, and 39 and characterize the years as Very humid, Humid and Moderately humid (Figure 9).

J(mm/ºC)	Climate classification
<10	Arid
10-25	Semiarid
25-30	Moderately arid
30-35	Slightly humid
35-40	Moderately humid
40-50	Humid
50-60	Very humid
60-187	Excessively humid

Figure 8. The classifications by DeMartonne,1926

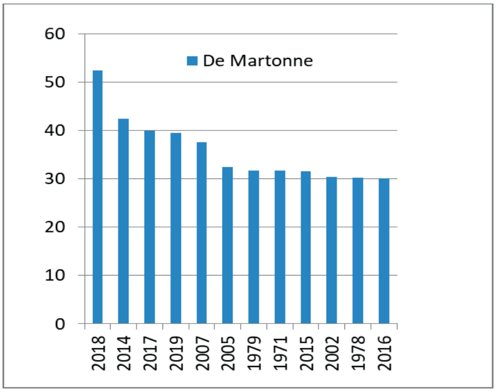

Figure 9. Highest De Martonne Values 1961-2019

An analysis of the De Martonne Index (1926) noted an improvement in the humidity conditions during the period 1990-2019 in the Plovdiv area. At the same time, extreme phenomena such as intense droughts and intense rainfall are observed (Figure 10).

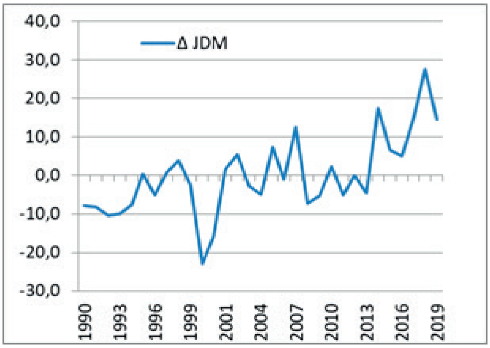

Figure 10. De Martonne indexes (1990-2019 deviations)

Greenhouse experience

Biometric Characteristics of Harvesting Plants –'Winter Oil Head' Variety - 2019. More significant differences are reported in the biometric parameters of the vegetative organs. In the first harvesting date (March 13th) With a higher average mass of the whole plant than the control - mineral fertilization is only option 6 (Ekoprop NX) with an excess of 12.7%. On the second date (March 20th), the trend from the first is preserved. Similar values to NPK control values were reported in Option 5. At the last harvest, Option 3 and Option 5 had similar values to those of the mineral fertilization control, with Option 6 exceeding 6.2%. Although most organic fertilizer options are inferior to mineral fertilization control, they meet the requirements of the standard (300-400 g) for individual harvests. The number of leaves showed more significant differences in the variants studied. They are more pronounced in the second and third harvest. The excess of 7 leaves is reported for option 5 and option 6 for the second date and from 4-5 sheets for the last harvest. The average results on this indicator give an advantage to the studied variants over the control - mineral fertilization. The organic fertilizers used stimulated the formation of 2 to 4 leaves more per plant. The average values of the stem indices do not differ significantly between the variants except for variant 6. It exceeds the control (variant 1) by 4.4% in diameter and by 16.7% by mass. Fertilizing had the least impact on the rosette diameter. The differences between the variants are insignificant in the harvests and in the average data. They are within 2-3 cm. In conclusion, it can be noted that fertilization had the most significant effect on the number and size (mass) of the leaves. A smaller effect is recorded with the size of the leaf rosette (diameter) and the size of the stem. Given that the salad consumption part is the leaves, a positive effect is observed in all organic fertilizer variants tested.

Morphology and biometrics of the 'Tuska' variety B. The biometric indices of the individual organs were less affected by the fertilization applied. The average mass of the whole plant in the variants with biofertilization for the 1st and 2nd harvest is inferior to the control - mineral fertilization. At the 3rd harvest date, Option 6 exceeds it. All the variants tested have better values than the control - not rough. Other leaf, stem and rosette indices show a similar trend to that of the control over the mean mass of the whole plant.

The values of the individual indices for the 1st and 2nd harvest are the highest for the control - mineral fertilization. At the 3rd harvest date, Option 6 slightly outperforms it. All biofertilizer variants have slightly better values than the nonfertilized rosette variants. However, the excess is insignificant within 1-2 cm.

Morphology and biometrics of grade A 'Maritima'. The effect of applied biofertilization on the morphological characteristics of plants follows the trends, as in the other two varieties. The highest values for the indices of the individual organs are reported in the mineral fertilization control. The highest number of harvested leaves and average for the growing season shows option 1 - control. The variants tested had lower values, with only Option 6 approaching the Option 1 control. Stem diameter values, averaged and harvested, show an advantage of the mineral fertilization control over most of the variants tested. This tendency also persists with the mass of the stem. A better stimulating effect of the biotors is accounted for by the diameter of the leaf rosette. The difference between the control and the individual variants is small within 1.0-1.5 cm. An important indicator affecting the quality of the salad and the consumer rating is the average mass of the whole plant. With the highest average harvest weight and average during the harvesting period, the mineral fertilizer version is about 800g. With close weight Option 6 about 775g. The other variants tested, with the use of different biotors, are lagging between 136g - 165g. Despite the lower weight, the plants cover and exceed the requirements of the standard (300-400 g).

Outdoor experiment

Biometric measurements. In order to determine the influence of different biotrains on the growth and development of lettuce plants, the indicators characterizing vegetative growth were monitored. Two observations were made and measurements were made on the plants after planting. The impact of individual biotores on the growth and development of salads was determined. Fertilization (with mineral and organic fertilizers) has an extremely strong impact on overall biology, growth and development. This effect is especially pronounced in plants with a consumptive part of the vegetative organs, such as salad. Fertilizers have a complex effect, greatly stimulating the productivity of the salad. In order to assess this impact, two readings of the basic morphological parameters were conducted twice during the growing season in accordance with the salad standards. The first reading took place when entering maturity. The following is contacted - as the vegetation progresses, the positive effect of the fertilizers tested on the consumption part increases, and on the non-consumption part, this effect weakens. These indicators are reported at anomalous in terms of humidification year conditions and higher than usual average temperatures. In all the variants examined, plants meeting the requirements of the lettuce harvesting standards developed. The field experiment was planted on April 17thand harvested on June 24th. Due to the extreme meteorological conditions (floods), the C-head lettuce entered premature phenophase full flowering 50% on May 24th.

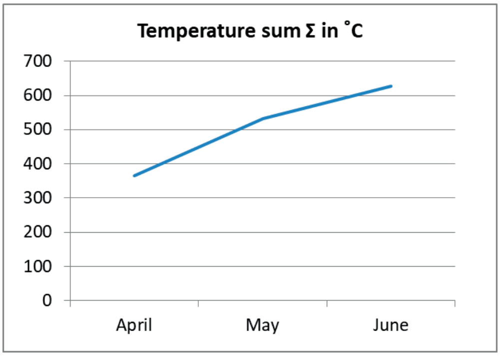

Figure 11. Accumulated temperature sums during thefield experiment

A very high correlation was found in all three varieties to the temperature sum by months (Figure 11), with the correlation coefficient R=0.97 and the determination coefficient R2=94.1%, respectively, indicating that 94% of the changes in the resulting variable were the result of the changes of the factor variable. The number of leaves in the different varieties is in direct proportion to the accumulated temperature sums per month for the trial period (Figure 12).

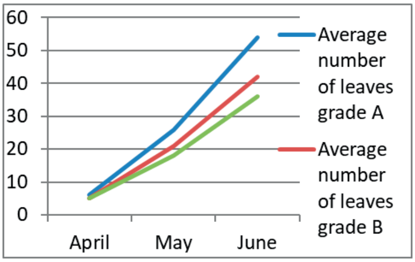

Figure 12. Average number of leaves at the beginning is the end of the trial for the different varieties

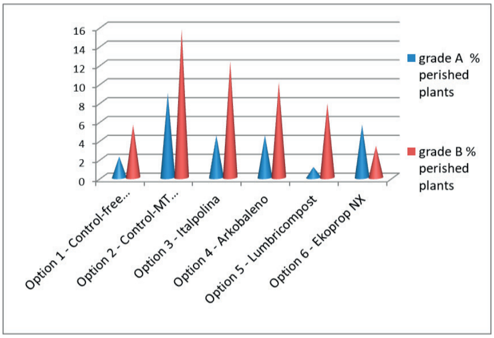

Figure 13.Average number of leaves at the beginning is the end of the trial for the different varieties

Different survival rates are observed for the different fertilization options (Figure 13). It should be emphasized that in some of the variants with biofertilization, the survival of plants subjected to extreme weather conditions is significantly higher. In the case of variety A Option 5 - Lumbricompost gives the lowest percentage of dead plants only 1.1%, in variety B the best result with Option 6 - Ekoprop NX only 3.4% of dead plants. These percentages are lower than in the non-fertilized control variants, which are 2.2% for cultivar A and 5.6% for cultivar B. It is crucial to note, however, that the dead plants in the mineral fertilizer variants in both the varieties are 8.7% higher for variety A and 15.7% higher for variety B, respectively, which is a statistically significant difference.

CONCLUSIONS

When comparing the values of the air temperature for the period 1990-2019 to the reference 1961-1990, the average deviation in the annual value is 0.9°C. Checking the trend from 1990-2019 with the Mann-Kendall test shows a statistically significant increase (Z 3.55; Signific. ***). The highest value 14.6°C was established in 2000, the second highest in the period and above 14°C in 2019. The variations during the summer are the highest - August, July and June with values of 1.9°C, respectively 1.4°C and 1.2°C. Negative deviation in air temperature is observed in winter - in December. In the remaining months, the distribution is relatively uniform, between 0.5°C and 0.7°C. Compared to previous surveys for the period 1986-2015, which found negative deviations during the two winter months - November and December, in the period 1990-2019 the negative value remained only in December, but was significantly mitigated.

The trend in the amount of precipitation is opposite to that observed during the 1971-2000 period. There is a positive trend (Z 2.50; Signific. *) and an increase of 34 mm (annual Σ mm) according to the period 1961-1990. Extreme events have also intensified - intense droughts, intense torrential rainfall, with both highest amounts since 2000.

At a soil temperature of 20 cm, which is a factor in the development of lettuce, the activity of rhizobacteria and the absorption of organic fertilizers, statistically significant trends are observed, with the most pronounced positive increase in the summer and autumn months. Statistical significance was not taken into account only in the two winter months - January and February.

An analysis of the De Martonne (1926) Index noted an improvement in the humidity conditions over the period 1990-2019. At the same time, it can be seen that extreme phenomena are observed, with intense droughts and intense rainfall.

Fertilizers tested for outdoor salad cultivation show a good overall stimulating effect, combined with rapid initial effects, under conditions of temperature and water stress per year, with extreme rainfall and temperature variations between 2.0°C and 4.0°C. The study shows the importance and benefits of biofertilization, both for the ecology and for the survival of plants exposed to abiotic stress. An adverse effect mitigation effect has been identified, which implies that the study should be continued outdoors. The experience goes on and these are initial results that are very good and give us hope for the future. The results obtained show the advantage of organic fertilizers and warrant being tested in a longer study covering different meteorological conditions.

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PATHOGENICITY AND VIRULENCE OF SOME STRAINS OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS*, FROM ROMANIA

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Abstract

The harmful organism that produces the "bacterial canker of tomato" (Clavibacter michiganensis subsp. michiganensis) produces a hypersensitivity reaction by infiltration a bacterial suspension in the leaves of Nicotiana tabacum plants and leads to the appearance of characteristic symptoms of the disease by inoculation a bacterial suspension in stems of Solanum lycopersicum seedlings. Analysis of eight isolates of Clavibacter michiganensis subsp. michiganensis, from five counties of the country, in 2019, established the existence of strains with different pathogenicity and virulence.

***Key words**: bacteria, hypersensitivity, patogenicity, vascular, strain.*

INTRODUCTION

Clavibacter michiganensis is the only recognized species of the genus *Clavibacter* and it contains nine host-specific species, namely: *Clavibacter michiganensis* subsp. *michiganensis* ("bacterial canker of tomato"), *Clavibacter michiganensis* subsp. *insidiosus* ("bacterial wilt of alfalfa"), *Clavibacter michiganensis* subsp. *sepedonicus* ("ring rot of potato"), *Clavibacter michiganensis* subsp. *nebraskensis* ("wilt of maize"), *Clavibacter michiganensis* subsp. *tessellarius* (infects wheat), *Clavibacter michiganensis* subsp. *phaseoli* (infects bean), *Clavibacter michiganensis* subsp. *capsici* (infects pepper), *Clavibacter michiganensis* subsp. *californiensis* and *Clavibacter michiganensis* subsp. *chilensis* (infects tomato) (Yasuhara-Bell & Alvarez, 2015).

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is a monophyletic species, being clearly separated from other pathogenic and non-pathogenic *Clavibacter* species, with which it can share the same habitat (Jacques et al., 2012). It is one of the pathogenic bacteria that causes significant damage to tomato crops. It attacks not only tomato plants but also others *Solanaceae* plants. Occasionally, it attacks non-solanaceous plants (pepper, corn, peas, beans and onions) (https://gd.eppo.int/taxon/CORBMI/hosts; https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3721; Calle-Bellido et al., 2012). It is an aerobic bacteria, Gram-positive, usually non-motile, curved rod shape and encapsulated (Severin & Iliescu, 2006).

It was the first isolated and describe on the tomato plants, in 1910, by Smith, in North America (Singh & Bharat, 2017; Fatmi et al., 2017; Valenzuela et al., 2018). In Romania, it was reported, in Ilfov county, by Elena Bucur, in 1955 (Marinescu et al., 1986; Rădulescu et al., 1970).

Throughout time artificial inoculations with this bacteria have been carried out at different host or non-host plants, for example: *Avena sativa*, *Citrullus lanatus*, *Cucumis sativus*, *Helianthus annuum*, *Hordeum vulgare*, *Secale cereale*, *Triticum aestivum*, *Cyphomandra betacea*, *Lycopersicum pimpinelifolium* (*Solanum racemigerum*), *Nicotiana glutinosa*, *Solanum humboldii*, *Solanum muricatum*, *Solanum nigrum* var. *guineese*, *Solanum pruniforme*, *Solanum tuberosum*, *Solanum racemiflorum*, *Solanum melongena*, *Capsicum annuum* and *Phaseolus vulgaris* (https://gd.eppo.int/taxon/CORBMI/hosts; Severin & Iliescu, 2006).

Behavior and resistance of tomato plants to the attack of *Clavibacter michiganensis* subsp. *michiganensis* were highlighted by such artificial inoculations, obtaining different results: irregular results after root inoculation and uniform results after inoculation of bacterial suspensions in the stems of fourteen-year-old plants. For differentiating sensitive plants from resistant plants, the most suitable method is the method of cutting the petiole, which gives a partial resistance, due to bacterial inhibition. And in order to determine the optimal period of attack with this bacteria, the method of inoculation by splashing or injury in the cotyledon stage is the most recommended (Severin & Iliescu, 2006).

Temperature required for development of *Clavibacter michiganensis* subsp. *michiganensis* is between 20 and 30^{0}C, with an optimal growth at 25^{0}C and a maximum survival at 50^{0}C. The pH also influences the growth and development of this bacteria. A pH of 7-8 is more favorable for the development of the disease than a pH of 5 (Sen et al., 2015).

The pedoclimatic conditions of the environment influence the manifestation of the symptoms of the disease, obtaining:

- a vascular attack at high temperatures (more than 23^{0}C) and low rainfall;
- a fruit attack at low temperatures (less than 21^{0}C) and heavy rainfall;
- a stronger attack if the soil temperature is higher (above the optimum growth temperature) and a slower attack if the soil temperature is lower (below the optimal growth temperature);
- an external infection of plants at medium temperatures and high humidity (Rădulescu et al., 1970).

To determine whether a strain is pathogenic or not in plants, **hypersensitivity reaction** can be performed on certain sensitive plants. The hypersensitivity reaction occurs when the tissues of a sensitive plant are invaded by some phytopathogenic agents, which cause necrosis on the inoculated leaves. If a young bacterial suspension of *Clavibacter michiganensis* subsp. *michiganensis* infiltrates into the intracellular spaces of the leaves of ***Mirabilis jalapa*** or ***Nicotiana tabacum***, under certain temperature conditions (18-43^{0}C), an inoculated plant response (collapse or internervurian necrosis) is obtained, after 24 hours on the leaves of *Nicotiana tabacum* and after 48-72 hours on the leaves of *Mirabilis jalapa* (Gitaitis, 1990; Montesinos, 2000; Burokiené et al., 2005; Shaker, 2014).

Studies show that for a better understanding of the mechanism of disease production and evolution, nineteen bioluminescent strains of *Clavibacter michiganensis* subsp. *michiganensis* were isolated. All mutant strains induced hypersensitivity reaction in *Mirabilis jalapa* and caused wilting symptoms in tomato plants (Xu et al., 2010).

For establishing the virulence of the different strains of *Clavibacter michiganensis* subsp. *michiganensis* **pathogenicity tests** can be performed. The pathogenicity test is performed in the greenhouse and allows the artificial reproduction of the disease, under certain conditions controlled by temperature and humidity. The EPPO protocol "PM 7/42 (3) *Clavibacter michiganensis* subsp. *michiganensis*", recommends two methods of inoculation: **the test in tomato plantlets** and **the cotyledon test**. The second method is performed, in particular, when the tomato isolates did not produce wilting through the seedling test. The presence of symptoms of wilting of the infected plants or the appearance of wounds on the stems (in the case of the seedling test) and the appearance of white blisters or craters on the surface of the cotyledons (in the case of cotyledon test) indicate the presence of infection with *Clavibacter michiganensis* subsp. *michiganensis*. The absence of any symptoms suggests a negative reaction, which means that the disease has not reproduced and the inoculated suspensions are not contaminated with *Clavibacter michiganensis* subsp. *michiganensis*.

Pathogenicity tests performed on tomato seedlings allowed different strains of *Clavibacter michiganensis* subsp. *michiganensis* to be grouped into strains with high, medium and low virulence (Burokiené et al., 2005).

In this case we aim to analyze and establish the pathogenicity and virulence of some strains of *Clavibacter michiganensis* subsp. *michiganensis*, in Romania, by the hypersensitivity test and the pathogenicity test.

MATERIALS AND METHODS

For this experiment were used two reference strains of *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm* PD 223; *Cmm* NCPPB 2979) and eight isolates of *Clavibacter michiganensis* subsp. *michiganensis,* from *Lycopersicon lycopersicum*, from five county of Romania: Argeș (cv. Colibri - *Cmm* 19-3857 and *Cmm* 19-3861; cv. Paris - *Cmm* 19-3858), Dolj (cv. Prekos - *Cmm* 19-4088), Suceava (cv. Colibri - *Cmm* 19-4326; cv. Kyveli - *Cmm* 19-4327), Hunedoara (cv. Kingset - *Cmm* 19-4735), Satu-Marte (cv. “Inimă de bou” - *Cmm* 19-4819).

The contaminated plants were taken by the phytosanitary inspectors from the Phytosanitary Offices and sent to the National Phytosanitary Laboratory, within the National Phytosanitary Authority, Romania. After conducting laboratory analyses, they were declared contaminated with *Clavibacter michiganensis* subsp. *michiganensis*.

The strains used were kept in the freezer at -80^{0}C, on PROTECT^{TM} beads. To obtain bacterial colonies, from each strain we used two beads contaminated with *Clavibacter michiganensis* subsp. *michiganensis*. They were placed on the surface of a common culture medium YPGA (Difco yeast extract 5 g, Difco Bacto peptone 5 g, D(+) glucose 10 g, Difco Bacto agar 15 g). Petri dishes inoculated with the strains of interest were incubated at 27^{0}C, for 5 days. Colonies with typical morphology were collected and used to make of bacterial suspensions in one ml sterile water. The suspensions had slight turbidity and were first tested by indirect immunofluorescence to determine bacterial concentration. Bacterial suspensions with an approximate concentration of 10^7-10^8 ufc/ml were used as such, while suspensions with a concentration greater than 10^8 ufc/ml were diluted to the desired concentration. The bacterial suspensions thus obtained were used to induce the hypersensitivity reaction and to performe the pathogenicity test.

For the hypersensitivity reaction, the bacterial suspensions were infiltrated, with a hypodermic syringe, in intercellular spaces of completely developed leaf by *Nicotiana tabacum* cv. White Barley. Plants are inoculated and incubated at 27-30^{0}C and followed for 1-4 days, until symptoms appear.

For the pathogenicity test, there were used ten plants of *Solanum lycopersicum* cv. Moneymaker and nine plants of *Solanum melongena* cv. Black Beauty. The plants used for inoculation were young plants in the stage of two-three true leaves. These were inoculated with a bacterial suspension of *Clavibacter michiganensis* subsp. *michiganensis*, in five points of the stems, by injection. The plants thus inoculated were incubated in the quarantine greenhouse, at a temperature of 26-28^{0}C, humidity 60-72%, alternation day/night of 15/9 hours and they were observed for 25 days.

After 25 days, the stems of the eggplants and tomato plants inoculated were cut, with a scalpel, at the soil surface. The leaves were removed and the remaining material was cut and then milxed using a Homex. To these material, a sufficient amount of sterile water was added and then stirred for 20-30 min. The extract obtained was tested by indirect immunofluorescence, according to the EPPO protocol "PM 7/98 Indirect Immunofluorescence test for plant pathogenic bacteria" and were observed under the fluorescence microscope.

RESULTS AND DISCUSSIONS

Hypersensitivity reaction. The leaves of the plants of *Nicotiana tabacum* cv. White Barley inoculates were evaluated after 24, 48, 72 and 96 hours. In the areas where the bacterial suspension was infiltrated, different reactions were obtained, such as: yellowing, necrosis and collapse (Table 1).

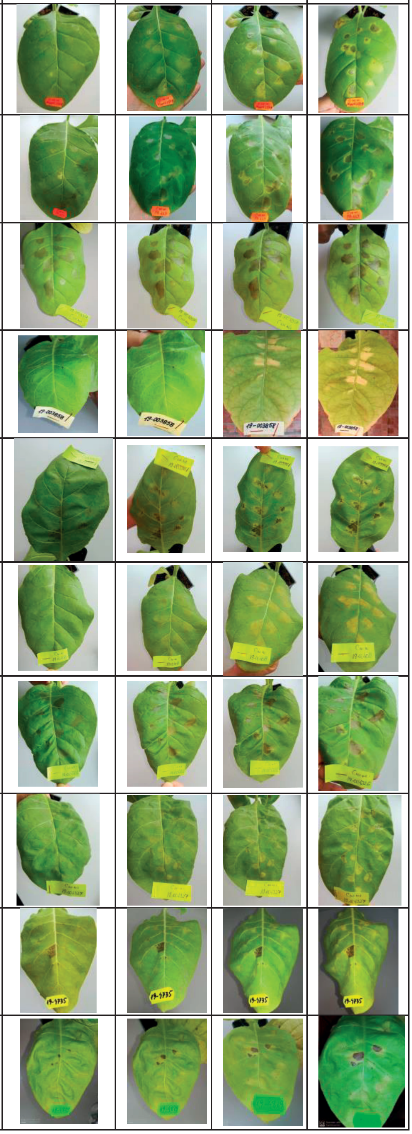
By observing the reaction of the inoculated tobacco leaves it was found:

- the strains of *Cmm* PD 223, *Cmm* 19-3857 and *Cmm* 19-4326 are highly pathogenic strains, because an internervurian collapse was obtained after 24 hours; after 96 hours, the internervurian collapse was intensified and browned, with or without the yellowing of the areas around it;
- the strains of *Cmm* NCPPB 2979, *Cmm* 19-3861, *Cmm* 19-4735 and *Cmm* 19-4819 are medium pathogenic strains because initially, after 24 hours, a slight collapse or necrosis of

the inoculated areas occurred, followed by an intensification of the necrosis areas in the following hours and by the yellowing of the areas adjacent to them;

Table 1. Hypersensitivity reaction
- the evolution of symptoms

Strains of *Cmm*	Leaves of *Nicotiana tabacum* cv. White Barley			
	24 hours	48 hours	72 hours	96 hours
Positive control *Cmm* NCPPB2979				
Positive control *Cmm* PD223				
Cmm 19-3857				
Cmm 19-3858				
Cmm 19-3861				
Cmm 19-4088				
Cmm 19-4326				
Cmm 19-4327				
Cmm 19-4735				
Cmm 19-4819				



- the strain of *Cmm* 19-3858 is a low pathogenic strain whereas, 24 hours, a necrosis of the inoculation points appeared, followed after 72 hours by the yellowing of the inoculated areas;
- the strains of *Cmm* 19-4088 and *Cmm* 19-4327 are very low pathogenic strains as they did not produce any reaction within 24 hours; the yellowing of the inoculated areas starting after 48 hours;
- the negative control did not show any reaction.

Pathogenicity test. Bacterial suspensions of *Clavibacter michiganensis* subsp. *michiganensis* used produced in the seedlings of *Solanum lycopersicum* cv. Moneymaker in which they were inoculated, characteristic symptoms of "Bacterial canker of tomato".
At 7 days after inoculation, in some plants, the margins of the leaflets turned upwards, the entire leaflet bent down, along the median rib, the petioles of the affected leaves curved downwards, leading to the bending of the whole leaves. The stems of plants were deformed and the plants with symptoms remained smaller compared to those where the symptoms have not manifested yet (Figure 1).

Figure 1. Foliar symptoms of *Clavibacter mchiganensis* subsp. *michiganensis* on *Solanum lycopersicum*

After 10-15 days, initially, at the inoculation points of the stems appeared small, lenticular cankers, which extended along the stem causing longitudinal cracks, in advaced stages (Figure 2).

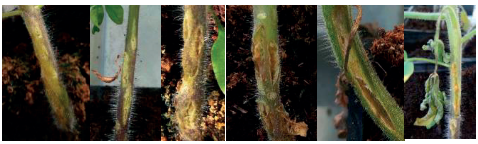


Figure 2. Symptoms of *Clavibacter mchiganensis* subsp. *michiganensis* on stem plants of *Solanum lycopersicum*

After 20-25 days, some plants died and at others plants the leaves were completely wilted and they remained attached to the plants.
Therefore, at 7 days after inoculation, the procent of plants with symptoms was:

- 90% affected plants - at plants infected with strains of *Cmm* PD 223, *Cmm* 19-3857 and *Cmm* 19-4326;
- 70% affected plants - at plants contaminated with strains of *Cmm* NCPPB 2979, *Cmm* 19-3861 and *Cmm* 4819;
- 30% affected plants - at plants inoculated with the strains of *Cmm* 19-3858 and *Cmm* 19-4327;
- 10% affected plants - at plants infected with the strains of *Cmm* 19-4088 and *Cmm* 19-4735;
- no symptoms plants at negative control.

We considered that a strain is more virulent than other if the number of plants showing symptoms is higher and the number of days after inoculation until appearance of symptoms is smaller. And we considered that a strain is less virulent if the symptoms appear in a longer period of time than those on other plants.

Therefore, the studied strains can be grouped as follows:

- with high virulence - *Cmm* PD 223, *Cmm* 19-3857 and *Cmm* 19-4326;
- with medium virulence - *Cmm* NCPPB 2979, *Cmm* 19-3861 and *Cmm* 19-4819;
- with low virulence - *Cmm* 19-3858 and *Cmm* 19-4327;
- with very low virulence - *Cmm* 19-4088 and *Cmm* 19-4735.

In the case of artificial infection with *Clavibacter michiganensis* subsp. *michiganensis* of the seedlings of *Solanum melongena* cv. Black Beauty, the foliar symptoms were almost completely absent. Only a few leaves showed symptoms of wilting and interveinal chlorosis (Figure 3).

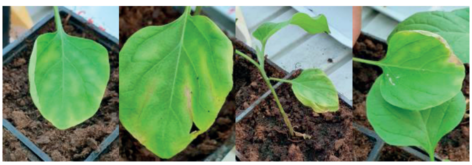


Figure 3. Foliar symptoms of *Clavibacter mchiganensis* subsp. *michiganensis* on *Solanum melongena*

Also, the simptom of deformation of the stem of the inoculated eggplant was almost absent. Only one or two inoculated plants showed such a symptom. In contrast, all plants showed lenticular lesions on the stems at the place of inoculation, lesions that expanded and led to the appearance of longitudinal cracks (Figure 4).

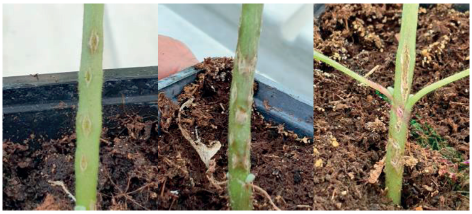


Figure 4. Symptoms of *Clavibacter mchiganensis* subsp. *michiganensis* on stem plants of *Solanum melongena*

Regarding the analysis of tomato and eggplant extracts obtained after the grinding of stems of these inoculated plants and after performing the immunofluorescence test, the presence of the target bacteria was found in all the analysed extracts and their absence in the negative control. It was obvious that the number of bacteria in tomato extracts was much higher than in eggplant extracts. Therefore, the bacterial concentration was much higher in *Solanum lycopersicum* plants, compared to those of *Solanum melongena*.

CONCLUSIONS

Through the **hypersensitivity reaction** it was established that all the strains analyzed were pathogenic. The reactions on the leaves of *Nicotiana tabacum* cv. White Barley were variable, as follows: the pathogenic strains caused a faster reaction whereas the less pathogenic strains caused a later reaction. The stronger pathogenic strains caused, after 24 hours, collapse and necrosis in the areas where the bacterial suspension was infiltrated, and the low pathogenic strains caused, after 48-72 hours, only a yellowing of the inoculated areas.
Pathogenicity test allowed the establishment the virulence of the studied strain, depending on the speed of the onset of symptoms. Any studied strain was not devoid of virulence. Low virulent strains of *Clavibacter michiganensis* subsp. *michiganensis* caused a slower reaction and a delay in the onset of symptoms on test plants of *Solanum lycopersicum*, while more virulent strains caused a faster onset of symptoms. As established Burokiené'study, in 2005, the strains of *Clavibacter michiganensis* subsp. *michiganensis* has different degrees of

virulence. We can conclude that, the strains analyzed also showed different degree of virulence, namely: high, medium, low and very low virulence.

Solanum melongena plants showed very low foliar symptoms, lacking in intensity and aggressivity. They appeared later compared with the foliar symptoms of *Solanum lycopersicum* plants. The wounds on the stems are similar to the two types of test plants used for pathogenicity. We can say that the intensity of the symptoms on the stem was much lower in *Solanum melongena* plants, compared to those on *Solanum lycopersicum* plants. The indirect immunofluorescence analysis revealed the existence of a larg number of bacteria in tomato plants compared to eggplant.

Regarding the reproduction of the disease on test plants, the EPPO protocol "PM 7/42 (3)" recommends the use of tomato plants. However, we can conclude that although the eggplants have an apparent resistance to the attack of the bacterium *Clavibacter michiganensis* subsp. *michiganensis*, they can be used as test plants when tomato test plants are not available.

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THE EFFECTS OF BIOCHAR ON THE GROWTH AND YIELD OF ZUCCHINI (*CUCURBITA PEPO* VAR. *GIRAUMONTIA* FILOV)

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Abstract

The aim of the study was to determine the influence of biochar, incorporated as a soil meliorant on the growth and yield of zucchini. The experiment was carried out in the experimental field on University of Forestry - Sofia (42° 7' N, 23° 43' E). The soil type on the experimental field is fluvisols. Six variants have been developed: 1) V1 - pure soil; 2) V2 - with manure (4 t/ha^{-1}); 3) V3 - with biochar (500 kg/ha^{-1}); 4) V4 - manure (4 t/ha^{-1}) + biochar (250 kg/ha^{-1}); 5) V5 - manure (4 t/ha^{-1}) + biochar (500 kg/ha^{-1}); 6) V6 - manure (4 t/ha^{-1}) + biochar (750 kg/ha^{-1}). Within the experiment, the average weight of one fruit, dry matter content, water content, was determined. The dynamics of yield between different harvests were monitored. Nine harvests were implemented, with the highest yield of all variants reported at the fourth harvest. The highest yield was reported for variant 6 - 3219 kg/ha^{-1}, followed by variant 2 - 3168 kg/ha^{-1}.

Key words:*:biochar, manure, yield, zucchini.*

INTRODUCTION

Biochar is rich in C product, which is produced by pyrolysis of various organic materials.

Depending on the type of material used and the conditions of pyrolysis, biochar shows various physical and chemical characteristics. This makes it suitable for various uses: for remediation of contaminated soils, for reducing greenhouse gas emissions, for controlling soil erosion, etc. (Ippolito et al., 2012; Oldfield et al., 2018; El-Naggar et al., 2019; Oni et al., 2019).

Biochar shows good results in improving soil properties and plant growth, alleviating stress from drought and salinity, interacting with heavy metals and organic pollutants, and more. (Nigussie et al., 2012; Kavitha et al., 2018; El-Naggar et al., 2019; Eissa, 2019; Guo et al., 2020).

As regards the physical properties of the soil, the application of biochar increases the stability of the soil aggregates and the water retention capacity, by improving the characteristics of the soil pores and water retention. (Kavitha et al., 2018).

There are a number of studies worldwide focused on the effect of biochar and its influence on the yields of different crops grown on different soil types, under different climatic conditions. (Zhang et al., 2010; Sovu et al., 2012).

Studies on the beneficial effects of biochar have been conducted with a number of vegetable crops (Blackwell et al., 2010; Zhang et al., 2011; Nigussie et al., 2012; Ty et al., 2013; William and Qureshi, 2015; Ni et al., 2017), arable crops (Kimetu et al., 2008; Husk and Major, 2011) and vineyards (Sovu et al., 2012).

Experimental application of biochar for crops of Cucurbitaceae family, in different soil types, in most cases shows an increase in yields.

In some studies, researchers have found that the application of biochar increases the yield of zucchini compared to the control variant (Van Zwieten et al., 2009).

Amin и Eissa (2017) find that application of biochar changes the pH of calcareous sandy soil from 7.9 to 6.65, increases soil organic matter and N utilization efficiency which increases the yield of zucchini (Yu et al., 2019).

Although the majority of experiments and analyzes show the positive aspects of the application of bio-carbon in agriculture, there are also data showing various problems and negative results in its application. (Kavitha et al., 2018; El-Naggar et al., 2019)

Such data were obtained in eksterimenst with zucchini. For example Gartler et al. (2013) have found that treatment with biochar (alone or in combination with biosolids) does not lead to significant changes in the biomass of a number of vegetable crops with an aboveground product, including and zucchini, while significantly affecting the root vegetables.

There are also data that report a decrease in zucchini yield compared to control variants in a one-year experiment (Gaskin G. et al., 2010).

Spokas et al. (2012) in their review they summarize that the increase in crop yields is reported mainly in degraded or weathered soils, while the negative or neutral effects of biochar on yields are in fertile soils. (El-Naggar et al, 2019).

To the similar conclusion are reached and Ippolito et al. (2012), pointing out that due to their low retention capacity for nutrients or water, it would be much more beneficial to use biochar in degraded and sandy soils than in high-yield soils.

Vista and Khadka (2017) found that biochar has a great effect on changes in soil properties such as: pH, organic carbon, phosphorus, but is highly effective at increasing the potassium content. However, it has been found that nitrogen content has been reduced to a great extent, and that an overdose of biochar is not beneficial for vegetable crops.

The aim of this study was to investigate the effect of incorporated low rates carbonized plant residues as ameliorant on the growth on fluvisols. In this context, the article presents data on the impact of biochar on phenological development and yield of zucchini.

MATERIALS AND METHODS

The experiment was conducted on the experimental field of the Faculty of Agronomy at the University of Forestry - Sofia (42° 7′ N, 23° 43′ E). The experiment is conducted on fluvisol, an area of 150 m^2.

For the purpose of the experiment, two ameliorants were used - biochar and manure. Six variants have been developed as follows:

1) control (C) - no ameliorants;
2) manure control (M) - 4 t/ha^{-1};
3) biochar control (BC) - 500 kg/ha^{-1};
4) combination with manure - 4 t/ha^{-1} and biochar - 250 kg/ha^{-1} ($M+BC_{250}$);
5) combination with manure - 4 t/ha^{-1} and biochar - 500 kg/ha^{-1} ($M+BC_{500}$);
6) combination with manure - 4 t/ha^{-1} and biochar - 750 kg/ha^{-1} ($M+BC_{750}$).

The experiment was carried out by randomized complete block design with four replications and protection zones.

In combination variants the manure is as a basic background - with the same optimum amount and biochar with reduced, optimal and increased rate. The ameliorants were incurporated one month before the sowing of the zucchini (at the end of March), by spreading them over the surface, followed by rotary cultivation to a depth of 15-20 cm.

For the field experiment was carry out the zucchini variety Izobilna, which is the standard variety in the country. Three beds are formed and the sowing of zucchini was by a standard two-row cultivation scheme (100 cm+60 cm x 50 cm).

The following phenological phases from the development of the zucchini were monitored: - beginning of germination; - beginning of formation of the first leaf; - the beginning of flowering of female flowers; - the first harvest.

The total yield was calculated in kilograms per $hectare^{-1}$, by variants, and for each variant the dynamics of harvests was monitored. Data were analyzed statistically by Anova.

RESULTS AND DISCUSSIONS

Meteorological conditions

During the experimental period (from the last ten days of April to the first ten days of July), the meteorological conditions were favourable for the growth and development of the zucchini. Only on the day of sowing, the average daily temperature was below 10°C (7.4°C) and till the end of April, on average for the period, daytime temperature was around 15°C (Figure 1).

The first ten days of May are relatively cool - with an average temperature of 14°C. In the following period, the temperature rises, with several cooler days again combined with rainfall at the end of May. Average daily temperatures in June range from 17°C to 24.4°C, with a rise in average daily temperatures above 25-27.1°C and 26.5°C at the end of the month. There is a uniform distribution of rainfall during the zucchini vegetation, ranging from 0.2 mm to 21.9 mm.

The total rainfall is 63.1, with more than two-thirds falling in the first half of the month (Figure 1).

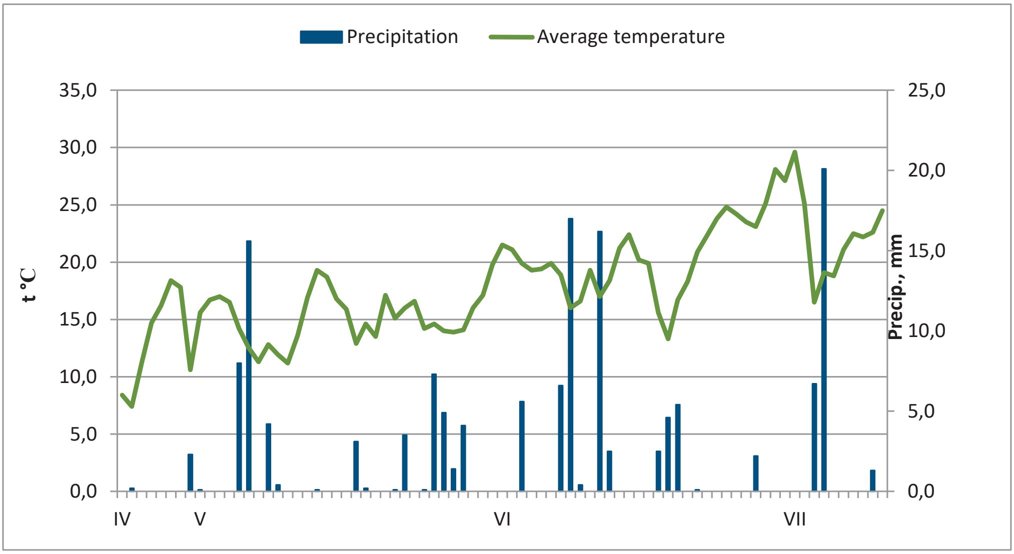

Figure 1. Average daily temperature and precipitation during the experimental period of the zucchini

Phenological monitoring

The sowing was carried out on 24 of April, with the average daily temperatures below 15°C for the first three days.

This affected the germination of plants and up to 5-day such was not registered.

On day 9, the beginning of the germination was reported in all six variants (Figure 2).

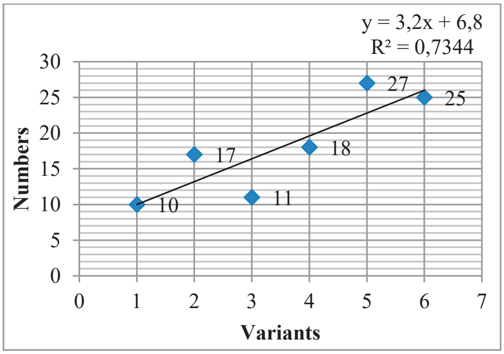

Figure 2. Number of plants in the cotyledon phase by variants (beginning of germination)

In two of the variants - control (1) and treatment only with biochar (3) the germination is less. In all variants with included manure, the number of germination plants was higher as compared with these two variants. After another 9 days (on the 18th day) the next phase was reported - the first leaf (Figure 3).

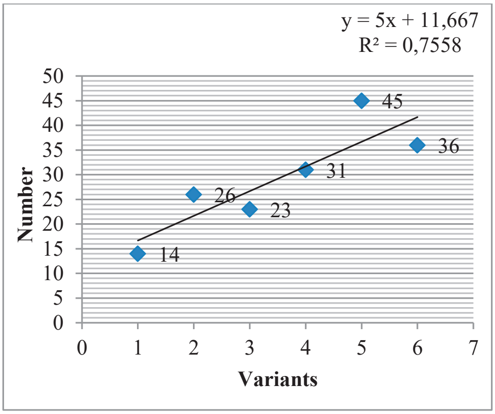

Figure 3. Number of plants in the formation phase of the first true leaf of variants

The trend persists - again the combined variants are with more plants with the first formed leaf. In the biochar-only variant (3), the number of plants in this phase exceeds that of the manure-only variant (2).

The beginning of flowering of female flowers in the individual variants, was reported between the 46th and the 48th day after sowing, with minor differences between different variants (Figure 4).

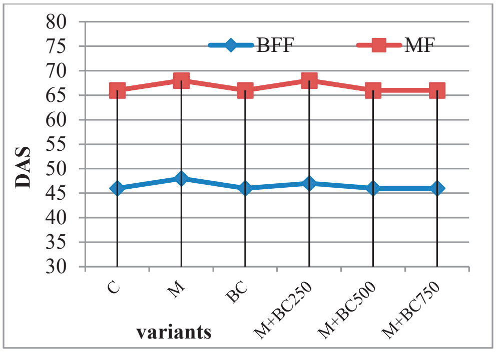

Figure 4. Number of days after sowing (DAS) to the beginning of flowering and mass flowering of female flowers in variants

No effect of the application of ameliorants (alone or in combination) was observed on the beginning of flowering of the female zucchini flowers.

This means that by this stage of development, the plants of the control variant have reached the rate of development of the plants of the other variants.

Nine harvests were carried out, according to variants and replications, which form the total yield (Figure 5).

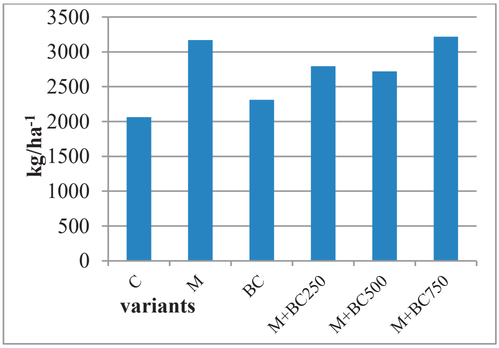

Figure 5. Average zucchini yield obtained by variants

There remains a tendency that the variants involving manure (M, M+BC$_{250}$, M+BC$_{500}$, M+BC$_{750}$) to be better than those without manure (C and BC).

It was found that the variant with individual use of biochar (BC), also has a higher yield than the control variant (C). This result shows that adding only biochar to the soil as a soil ameliorant (although it does not contain nutrients) leads to an increase in yield compared to the control, which confirms the conclusions of Zwieten et al. (2009).

The highest yield was obtained at two variants – M+BC$_{750}$ and M (Figure 5).

The second soil ameliorant - manure, incorporated on its own, leaded to higher yields. Compared to the control (no ameliorants and no fertilizers) and compared to incorporated biochar alone, the total yield of this variant (among the tree control variants) was highest, because the manure contains nutrients and can be used both as a soil improver and as a fertilizer (Figure 5).

For the other three variants (with combined incorporation of the two ameliorants), the obtained yields were also higher than the two controls (without ameliorants - C and with the incorporation of biochar - BC), which is in agreement with the findings of other researchers (Spokas et al., 2012; Ippolito et al., 2012; El-Naggar et al., 2019) that the use of biochar in light and sandy soils leads to improved yields.

Among the three combined variants (M+BC$_{250}$, M+BC$_{500}$ and M+BC$_{750}$) with the highest obtained yield was the variant with highest applied rate of biochar (M+BC$_{750}$).

Nine harvests during which realized yields of zucchini were grouped into three harvesting periods (Figure 6).

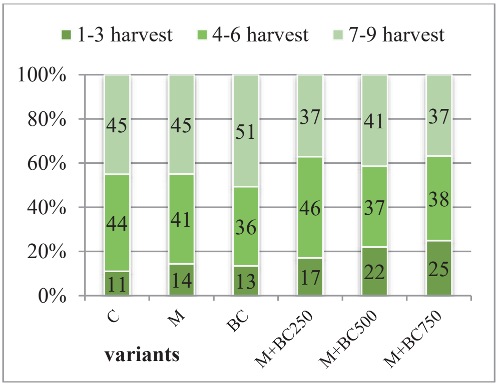

Figure 6. Percentage distribution of the yield obtained in the three harvesting period for each variant

The first of these included the beginning of the harvest (on the 59th day after sowing), as harvests were obtained only from variants with a combination of ameliorants.

This is clearly seen in the percentage of the first harvest of the individual variants, with the other harvests (Figure 6).

In the three combined variants, the yield of the first three harvests was higher due to an earlier first harvest.

In the biocarbon-only variants (BC), yield from different harvests groups increased gradually and was highest during the last harvesting period, covering the 7-9th harvest, which was in early July.

Probably the impact of biochar was initially to retain nutrients and then to gradually mineralize and release the plants in the later stages of their development. This effect is not noticeable in the other three combined variants because manure is also fertilizer and feeds the plants throughout all stages of their development.

In variant M+BC_{750}, the final yield is distributed almost evenly between the three harvest periods and is the highest yield achieved.

CONCLUSIONS

Of the two ameliorants, with a more pronounced influence on the growth, development and yields of zucchini was manure, regardless of the way of its application - alone or in combination with biochar.

Self-applied biochar has no effect on growth and yield of zucchini.

The combination of biochar (750 kg/ha^{-1}) with manure, applied to light soils, resulted in the highest yield obtained from zucchini.

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SPREAD OF STOLBUR IN SOME TOMATO VARIETIES AND INDICATORS OF THEIR PRODUCTIVITY

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Abstract

The aim of this study was to identify the distribution of 'Ca. P. solani' in the four Moldavian tomato varieties during growing season and to evaluate their productivity and fruit quality. Molecular diagnosis of phytoplasma was used. The most significant difference in the percentage of infected tomato plants between the studied varieties was recorded at the stage of mass ripening of fruits. It has been found that the varieties Elvira and Desteptarea were more susceptible to 'Ca. P. solani' compared with Cerasus and Mary Gratefully. At the end of the period of vegetation all analyzed varieties were significantly loaded with phytoplasma infection. Differences in the indicators of productivity and quality of fruits in the studied tomato varieties were determined. In general, varieties that were more resistant to phytoplasma infection, respectively, had a higher yield and better fruit quality. Thus, we can conclude that, in addition to other plant diseases, climatic conditions of the year, some features of the genotype, etc., stolbur negatively affected productivity and, especially, reduced the quality of tomato fruits.

Key words: *phytoplasma, molecular diagnosis, tomato varieties, productivity, fruit quality.*

INTRODUCTION

'*Candidatus* Phytoplasma solani' is a pathogen that affects a wide range of crop plants including tomatoes (EFSA, 2014). This phytopathogen colonizes the phloem of plants and is transmitted by insects vector of the order Hemiptera, families *Psyllidae*, *Cicadellidae* and *Cixiidae* (Bertaccini & Duduc, 2009; Weintraub & Beanland, 2006).

Phytoplasmas belong to the Mollicutes class. The genus Candidatus Phytoplasma comprises more than 43 candidate species that fall into ribosomal groups and subgroups. Molecular tools have been developed that allow more precise differentiation of phytoplasma. In particular, non-conservative single-copy non-ribosomal genes, such as ribosomal protein (rp), secY, secA, rpoB, tuff, groEL (chaperonin) have been widely used to differentiate most phytoplasmas (Bertaccini, 2019).

Phytoplasmosis causes considerable losses in the quality and productivity of agricultural production (Bertaccini & Duduk, 2009). This disease (stolbur) is a widespread in tomato in Moldova. Its distribution in tomato fields is controlled by the climatic conditions of the year, being more or less abundant in different growing seasons. However, stolbur is presented in Moldavian tomato fields every year and has a negative effect on the fruits production. Disease control is possible. Modern agricultural techniques, biological control, use of resistant varieties, chemical treatments are considered as the basic components of disease management Chemical control is usually effective, but it has some negative consequences such as the reduction of amount of many beneficial organisms, as well as a toxic impact on the human health and the environment (Gavrilescu & Chisti, 2005). The use of tomato varieties resistant to pathogens for the control of phytoplasma infection has a number of economic and ecological advantages. The degree of damage, as well as the percentage of infected plants, vary from one variety to another.

Analyzing the sensitivity of some varieties of tomato to phytoplasma infection, it is necessary to take into account the combination of the environmental factors of the current year. From the other hand, these factors directly affect the productivity and quality of tomatoes.

The aim of this study was to detect the spread of '*Ca*. P. solani' in the four Moldavian tomato varieties and to evaluate their productivity and fruit quality during the growing season of 2018.

MATERIALS AND METHODS

The phytoplasma infection spread was studied on the Moldavian tomato varieties Elvira, Cerasus, Desteptarea and Mary Gratefully created in the Institute of Genetics, Physiology and Plant Protection (Chisinau, Moldova). The phytoplasma presence was identified by molecular analysis in individual tomato plants (20 plants in each variety) collected at the period of mass fruit ripening (August) and the end of the season of vegetation (September) of 2018.

DNA for the molecular identification was isolated from the fruit peduncle of each plant by express alkaline boiling method (Guo et al., 2003). This method consists of few fast consequent steps: thin sections of fruit peduncles are boiled in 10 µl 0.3 N NaOH for 5 minutes; this mix is neutralized by adding 10 µl 0.3 N HCl; obtained mix is centrifuged for 3 minutes at 10000 rpm. A 1 µl aliquot of obtained solution is used as a template DNA in the first round of nested-PCR. Two pairs of specific primers designed on the base of nucleotide sequence of '*Ca*. P. solani' chaperonin gene were used in both rounds of nested-PCR: cpn421F / cpn421R (round I) and cpn200 F / cpn200R (round II) (Zamorzaeva, 2015). The following programs for amplification were used: I - 94°C 5 minutes; II - 94°C 30 seconds, 60°C 30 seconds, 72°C 30 seconds × 30 cycles (round I) or × 35 cycles (round II); III - 72°C 10 minutes; IV - 4°C ∞. The products of round II of PCR were registered in UV light after the electrophoresis on 1.5% agarose gel in TBE×1 stained by ethidium bromide.

The productivity (t/ha) of the four tomato varieties were evaluated in the growing season of 2018. The yield of marketable fruits was additionally registered in each studied variety. The percentage of marketable fruits was calculated as an important indicator of fruits quality of analyzed tomato varieties.

A statistical processing the data obtained by molecular methods and quantitative measurements were carried out. Fisher's criterion was applied to qualitative traits in limited random sampling.

RESULTS AND DISCUSSIONS

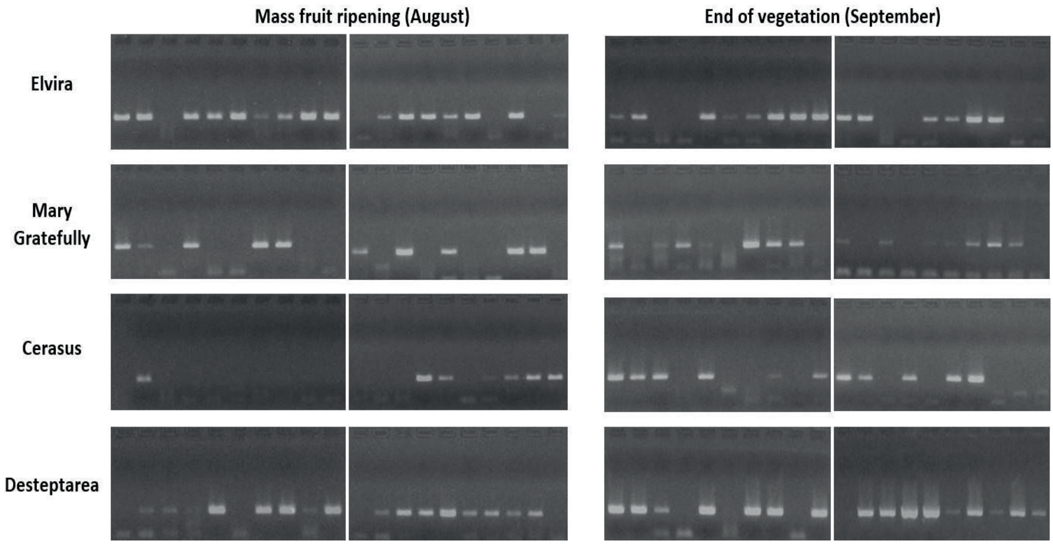

Figure 1. Results of the molecular diagnosis of '*Ca*. P. solani' presence in plants of the four tomato varieties at the period of mass fruit ripening and at the end of the season of vegetation: corresponding fragment 200 b.p. obtained by the electrophoresis of products of nested-PCR (round II) was registered

The evaluation and comparative analysis of the four tomato varieties demonstrated the difference in the phytoplasma infection spread between these varieties. Results of the molecular diagnosis of '*Ca*. P. solani' presence in tomato plants are summarized in Table 1 and presented in Figure 1.

Table 1. Number of infected plants in the four analyzed tomato varieties (20 plants were analyzed in each variety)

Variety	Number of infected plants	
	Mass fruit ripening (August)	End of vegetation (September)
Elvira	17	17
Mary Gratefully	10	14
Cerasus	7	11
Desteptarea	16	16

It was found that the most significant difference in the number of infected tomato plants between studied varieties was recorded at the stage of mass fruit ripening. The spread of phytoplasma infection significantly increased in the tomato field to the end of the season of vegetation and the difference in the percentage of infected plants in studied varieties was less pronounced at this period (Figure 2).

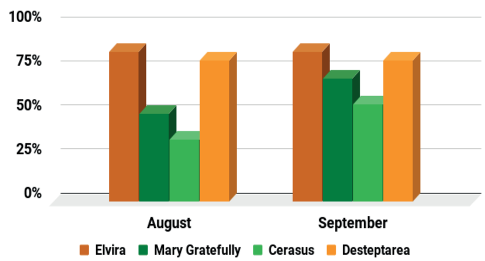

Figure 2. Distribution of '*Ca*. P. solani' in the four tomato varieties

Analyzing the spread of phytoplasma infection in the tomato field at the stage of mass fruit ripening it was found that the varieties Elvira and Desteptarea were more susceptible to '*Ca*. P. solani' (85% and 80% of infected plants, respectively) compared with Cerasus (35% of infected plants) and Mary Gratefully (50% of infected plants). Comparing pairs of the contrast in the susceptibility to '*Ca*. P. solani' varieties one can see that the difference in the percentage of infected plants remained significant for all analyzed pairs with $P \leq 0.01$ (pairs Cerasus-Elvira, Cerasus-Desteptarea) or $P \leq 0.05$ (pairs Mary Gtatefully-Elvira, Mary Gtatefully-Desteptarea).

The comparison of '*Ca*. P. solani' distribution in August and September allowed to see that the level of the infection in varieties Elvira and Desteptarea was no changed, it consisted of 80-85% of infected plants in September. The percentage of infected with phytoplasma plants significantly increased in variety Mary Gratefully having reached of 70%. Variety Cerasus manifested the highest resistance to '*Ca*. P. solani' infection till the end of the season of vegetation: only about a half of plants (55%) were infected in September. Thus, the difference in the level of phytoplasma infection in studied varieties was not considerably pronounced in September: significant difference ($P \leq 0.05$) was recorded only in comparison of the best (Cerasus) and worst (Elvira) varieties.

Some variability of the indicators of productivity and fruit quality was established in studied tomato varieties in climatic conditions of 2018 in the Republic of Moldova. This vegetative season was in whole favorable for the tomato growing and ripening (warm and wet June, hot and wet July, hot and drought August). At the same time, these climatic conditions were advantageous for the phytoplasma infection spread in the tomato field. It is known that hot temperature increases the activity of insect vectors as well as the reproduction of the phytoplasmas in their bodies (Murral et al., 1996). In our study, 20% from 35 insects of families *Cixiidae* and *Psyllidae* collected in July-August of 2018 near the tomato field, were infected with '*Ca*. P. solani' (unpublished data). These insects may be potential vectors of phytoplasma to tomato plants, increasing the level of infection in the field in August-September. It has to be emphasized that the incubation period of phytoplasmosis can be relatively long, consisting of one month or more (Blancard, 2012). Increasing the infected insects' activity in July-August impacted on the phytoplasmosis spread in August-September.

So, the productivity and the yield of marketable fruits (important characteristic of fruits quality) were evaluated in the four tomato varieties (Table 2).

Table 2. Indicators of productivity and fruit quality of tomatoes in 2018

Variety	Productivity (t/ha)	
	total	marketable fruits
Elvira	58.0	40.1
Mary Gratefully	73.0	59.5
Cerasus	74.4	60.9
Desteptarea	67.8	50.9

In general, varieties that were more resistant to phytoplasma infection, respectively, had a higher yield. It has to be emphasized that the productivity is, firstly, a characteristic of the genotype. Secondly, it is under the influence of the climatic conditions of the year (changes in temperature, humidity, number of sunny or cloudy days during the growing season, *etc.*). Diseases of tomato plants other than stolbur can also affect the productivity and fruit quality. On the other hand, it is known that ‘*Ca*. P. solani’ infection negatively influences on productivity and, especially, reduces the quality of tomato fruits (Bertaccini & Duduk, 2009).

Our results demonstrated that the highest productivity was registered in varieties Cerasus and Mary Gratefully (see Table 2, Figure 3). These varieties were less damaged by phytoplasmosis at the period of mass fruit ripening (August), when tomato fruits were mainly harvested. Worst productivity (58 t/ha) was recorded in the most susceptible to ‘*Ca*. P. solani’ variety Elvira.

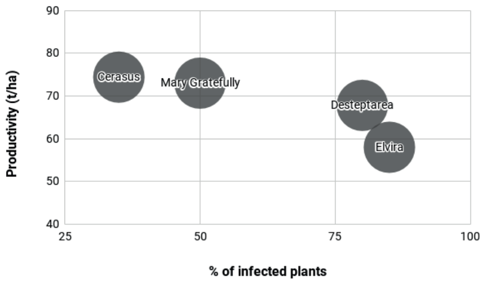

Figure 3. Correlation between the phytoplasma spread in the four tomato varieties in August and their productivity in 2018

The correlation of the level of phytoplasma spread in the four studied tomato varieties and yield of marketable fruits was additionally analyzed. It was established that the percentage of the marketable fruits varied in different varieties (Figure 4).

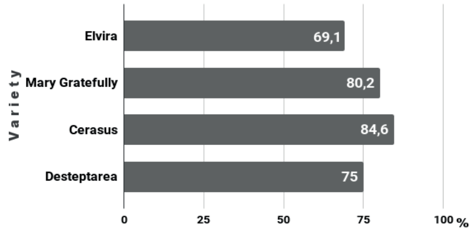

Figure 4. Percentage of the marketable fruits collected from the four tomato varieties in 2018

The correlation between the degree of phytoplasma infection in a variety and the yield of marketable fruits was perceptible in August (see Figures 4, 5). Namely, the percentage of marketable fruits in the varieties Cerasus and Mary Gratefully, which were more resistant to phytoplasma, was above 80%. The best indicator was registered in Cerasus (84.6% of marketable fruits from all harvested fruits). This indicator in the more susceptible to phytoplasma variety Desteptarea was 75%; the most susceptible to phytoplasma infection variety Elvira showed the worst yield of marketable fruits, it was less than 70%.

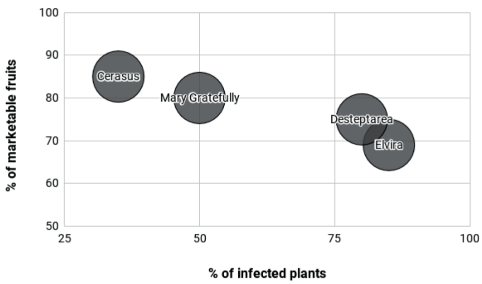

Figure 5. Correlation between the phytoplasma spread in the four tomato varieties in August and the yield of marketable fruits

In fact, analyzing the correlations between the phytoplasma spread in the tomato field in August of 2018 and the total productivity or the yield of marketable fruits, the four studied tomato varieties may be divided into two groups: (1) more resistant to ‘*Ca*. P. solani’ Cerasus and Mary Gratefully which had better indicators of fruits quantity and quality; (2) more susceptible to ‘*Ca*. P. solani’ Elvira and Desteptarea which had worse indicators of these traits (compare Figures 3 and 5).

The correlation between the degree of the phytoplasma presence in tomato field in September, at the end of the growing season, and the yield of marketable fruits in the variety had other pattern of distribution (Figure 6). It has to be mentioned that the infection distribution at the later stages of plants development, after a period of mass fruit ripening, does not affect the quantity and quality of fruits. Significant spread of '*Ca*. P. solani' in a tomato field in September is not important in terms of productivity of the variety and the yield of marketable fruits. This indicator may be useful for the assessing the degree of resistance of tomato varieties to '*Ca*. P. solani'.

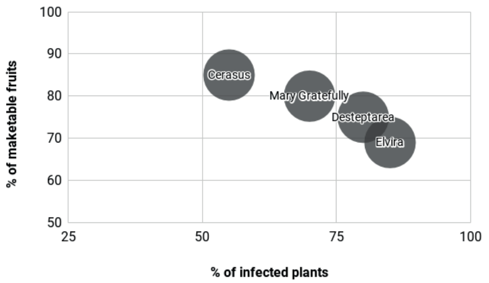

Figure 6. Correlation between the phytoplasma spread in the four tomato varieties in September and the yield of marketable fruits

Nevertheless, analyzing this correlation one can see that the best variety was Cerasus and the worst was Elvira. Varieties Mary Gratefully and Desteptarea were intermediate in terms of fruit quality and plants loading by phytoplasma infection in September.

CONCLUSIONS

The difference in the '*Ca*. P. solani' infection spread between the four tomato varieties was established in the growing season of 2018. This difference was more pronounced at the stage of mass fruit ripening, in August. At the end of the period of vegetation, in September, all analyzed tomato varieties were significantly loaded with phytoplasma. Nevertheless, the difference between the best (Cerasus) and worst (Elvira) varieties remained significant (P≤0.05) in September.

The difference in productivity and yield of marketable fruits was found comparing the four tomato varieties. In general, varieties that were more resistant to phytoplasma infection, respectively, had a higher yield.

Having in mind that stolbur was only one of the factors (along with other plants diseases, genotype features, adverse climatic conditions of the year, *etc.*), which negatively affected the economically important indicators of productivity and quality of tomato fruits, the correlations between these indicators and the level of phytoplasma spread in the tomato field provided an additional information for characterizing the four tomato varieties.

Analysis of correlations between the phytoplasma spread in the tomato field in August and total productivity or yield of marketable fruits allowed to divide the four studied tomato varieties into two groups: first group consisted of more resistant to '*Ca*. P. solani' Cerasus and Mary Gratefully with better indicators of fruits quantity and quality; second group consisted of more susceptible to '*Ca*. P. solani' Elvira and Desteptarea with worse indicators of productivity and yield of marketable fruits. These results have to be considered in the tomato breeding process. Additionally, summing up comprehensive study of the four tomato varieties in 2018 we may conclude that Cerasus was the best variety and Elvira was the worst.

ACKNOWLEDGEMENTS

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ROLES OF SPRAYING AMINO ACIDS AND CHELATED MAGNESIUM ON GROWTH, FLOWERING AND PRODUCTION OF CORMS OF *FRESSIA HYBRIDA*

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Abstract

An experiment was conducted in the nursery of the Faculty of Agriculture, University of Kufa during agricultural seasons 2018-2019 to study the effect of spraying amino acid and chelated magnesium of growth, flowering and production of corms of Fressia. Experiment was adopted in Randomized Completed Block Design (R.C.B.D) with three replicates in two factors. First spraying nutrition solution (Taravert Amifol) at a three concentration (0, 2 and 4 ml. L^{-1}). Second spraying three concentration of chelated magnesium (EDTA) (0, 100 and 200 mg. L^{-1}).
The results show that spraying amino acid at a concentration of 4ml. L^{-1} and chelated magnesium at a concentration 100 mg.L^{-1} increased significantly: number of leaves, shoot dry weight, leaves content of total chlorophyll and total soluble carbohydrates, number of inflorescence, number of floret, floret diameter, inflorescence stalk length and vase life (6.67 leave.plant^{-1}, 2.74 g, 39.40 mg.100 g fresh weight, 9.10 mg. g^{-1}dry weight, 8.00 inflorescence. plant^{-1}, 12.95 floret. inflorescence, 8.16 cm, 39.63 cm and 9.33 days) compared to control treatment which gave the lowest values (3.66 leave.plant^{-1}, 1.49 g, 33.39 mg.100 g fresh weight, 6.12 mg. g^{-1} dry weight, 3.33 inflorescence. plant^{-1}, 5.00 floret. inflorescence, 3.63 cm, 29.77 cm and 4.66 days), respectively.

***Key words**: floriculture bulbs, growth stimulated, minerals nutrient.*

INTRODUCTION

Fressia plant (*Fressia hybrida*) belongs to the Iridaceae family which includes many important floriculture plant. This family includes more than 50 plant genera of annual winter which live under Iraqi climatic conditions. It is considered as a one of the important plants in the world because its floral inflorescences are suitable for picking, and for its pleasant aromatic smell when opened, and for the multiplicity of its colors and long visa life (Al-Batal, 2010). Its flowers are ranked sixth in the world among the cut flowers (Armitage & Luashman, 2003).
Amino acids, which are the primary plant units for protein synthesis (Ria, 2002) are one of the stimuli for plant cell growth as they have many physiological effects in plants, especially the free amino acids that are involved in building the enzymes responsible for photosynthesis, as it is a source of carbon and chelating materials for microelements (Goss, 1973). It also stimulates the process of photosynthesis, builds carbohydrates, and increases the effectiveness and activity of anti-oxidant enzymes (Tantawy et al., 2009). It is also characterized by its ease of penetration and absorption, as it affected the permeability of plant membranes and consequently the ease of transporting nutrients within the plant, which ultimately improves plant growth and development (Koksal et al., 1999). Khattab et al. (2016) show that spraying *Gladiolus grandiflorus* L. with amino acids (Glycine and Methionine) resulted in an increased plant height, number of leaves, shoot dry weight, number of inflorescences, floret diameter, its dry weight, number of corms per plant and its dry weight.
Magnesium is also an important and necessary element for plant growth and nutrition, as many carbohydrate enzymes need magnesium as a catalyst for its action, as well as it enters into building the chlorophyll molecule and activates almost all enzymes that participate in the processes of photo phosphorylation (Taiz & Zeiger, 2006). Abdallateef et al. (2017) mentioned that spraying the element magnesium on the plant *Matthiola incana* at a concentration of 6 g^{-1} resulted in a significant

increase in the height of the plant, number of leaves and flower inflorescences, length and diameter of inflorescences' stem.

The fact that bulbs of freesia are suitable for commercial picking in many producing countries for cut flowers (Imanishi, 1993) conducted this research with the aim of studying the effect of spraying the nutrient solution Taravert Amifol and chelated magnesium in several concentrations in order to improve the growth and flowers parameters and flowering visa life of these bulbs suitable for commercial cut flowers. Reducing the time required to flowering of these commercially important bulbs is also studied.

MATERIALS AND METHODS

An experiment was conducted in the nursery of the Faculty of Agriculture, University of Kufa, in a house covered with green saran during the 2018-2019 agricultural season, to study the effect of spraying nutrient solution "Taravert Amifol" and chelated magnesium on the growth, flower and production of freesia corms. The corms planted on 01/10/2018 in plastic pots with a diameter of 20 cm in which they consisted sand and peatmoos in a ratio of 1: 3. Table 1 shows the analysis of the soil of the pots done in the Graduate Studies Laboratory of the Faculty of Agriculture - University of Kufa.

Table 1. The physical and chemical properties of potting soils

pH	EC. ds.m⁻	OM %	N g 100 g^{-1}	P mg. L^{-1}	K mg L^{-1}	Mg^{++} mg. L^{-1}	clay %	Silt %	Sand %	Soil Texture
7.21	.211	0.92	0.62	2.14	14.1	1.50	3.10	5.3	91.6	sandy

A factorial experiment was carried out in Randomized Complete Block Design (R.C.B.D) with two factors. The first one, three concentrations (0, 2 and 4) ml.L^{-1} of nutrient solution "Taravert Amifol" which consists of: total nitrogen 6.5%, organic matter 15%, total amino acids 20% and free amino acids 11% were used. The second factor which consists in spraying chelated magnesium (Mg-EDTA) in three concentrations (0, 100 and 200) mg. L^{-1} was applied twice: the first time after the emergence of 3 real leaves and the second after 21 days from the first spray. Means were compared according to the Least Significant Difference (L.S.D) at a 5% probability (SAS, 2000).

All operations like watering and weeding were done whenever the plant needed it. On 2/2/2019, the following growth parameters were measured: Number of leaves per plant; Shoot dry weight; Leaf content of total chlorophyll (mg.100 g^{-1} fresh weight) measured by acetone in UV-Visible Spectrophotometer and wavelength 663 and 645 nm according to Goodwin (1976); Leaf content of total soluble carbohydrates (mg.g^{-1}dry weight) measured by UV-Visible Spectrophotometer in wavelength 490 nm according to Duboies et al. (1956); Number of corms per plant; Corms diameter; Number of days required for opening the first floret calculated from planting seeds to the appearance of first floret; Number of inflorescences per plant; Number of floret per inflorescences; Floret diameter (cm); Inflorescence stalk length (cm); Vase life (day): inflorescences were harvested early in the morning with a sharp blade when 2-4 florets opened on the inflorescence, then transferred directly to the laboratory and then placed in a one-liter glass bottle containing one liter of water, aspirin tablet and 2 g of sugar, than number of days was calculated until wilting inflorescence (Singh, 2006).

RESULTS

The results in Table 2 showed that spraying nutrient or chelated significantly increased growth parameters. Also spraying nutrient solution at a concentration of 4 ml.L^{-1} and chelated magnesium at 100 mg. L^{-1} significantly increased of: Leaves per plant, Shoot dry weight, Leaf content of total chlorophyll and total soluble carbohydrates, Number of corms per plant and corms diameters (6.67 leaves, 2.74 g, 39.40 mg.100 g^{-1} and 9.16 mg.g^{-1}, 3.66 corm and 1.86 cm) compared to control treatment which gave the lowest values.

Table 2. Effect of spraying nutrient and chelated magnesium on growth parameters

Treatments			Number of Leaves (leaf.plant^{-1})	Shoot dry weight (g)	Leaves content of Total Chlorophyll (mg.100g^{-1})	Leaves content of total soluble carbohydrats (mg. g^{-1})	Number of corms per plant	Corm diameter (cm)
Nutrient Solution		0	4.00	1.60	33.70	6.34	1.22	1.18
		2	5.11	2.02	35.69	7.44	2.44	1.48
		4	6.33	2.53	38.77	8.31	3.22	1.73
L.S.D. 0.05			0.34	0.08	0.42	0.43	0.30	0.08
chelated magnesium (mg.L^{-1})		0	4.77	1.90	35.34	7.13	2.00	1.53
		100	5.22	2.13	36.13	7.56	2.33	1.48
		200	5.44	2.11	36.69	7.39	2.55	1.56
L.S.D. 0.05			0.34	0.08	0.42	0.435	0.30	0.08
Nutrient Solution (mg.L^{-1}) × chelated magnesium (mg.L^{-1})	0	0	3.66	1.49	33.39	6.12	1.00	1.10
	2	100	4.00	1.62	33.62	6.21	1.00	1.20
	4	200	4.33	1.69	34.10	6.71	1.66	1.26
	0	0	4.66	1.87	34.88	7.14	2.00	1.43
	2	100	5.00	2.04	35.63	7.33	2.33	1.46
	4	200	5.66	2.17	36.57	7.84	3.00	1.56
	0	0	6.00	2.36	37.76	8.14	3.00	1.53
	2	100	6.67	2.74	39.40	9.16	3.66	1.86
	4	200	6.33	2.49	39.14	7.64	3.00	1.80
L.S.D. 0.05			0.60	0.15	0.73	0.75	0.52	0.13

Spraying nutrient or chelated significantly increased flowering parameters. Also spraying nutrient solution at a concentration 4 ml.L^{-1} and chelated magnesium at 100 mg. L^{-1} significantly decreased the number of days required for opening the first floret and increased the number of inflorescences per plant, number of floret per inflorescences, floret diameter, inflorescence stalk length and vase life to (129.33, 8.00 inflorescences, 12.95 floret, 8.16 cm, 39.63 cm and 9.33 days) compared to control treatment which gave the lowest values (Table 3).

Table 3. Effect of spraying nutrient and chelated magnesium on growth parameters

Treatments			number of days required for opening the first floret (day)	number of inflorescences per plant	number of floret per inflorescences	floret diameter (cm)	inflorescence stalk length	vase life (day)
Nutrient Solution		0	138.89	3.88	6.00	3.84	30.32	5.22
		2	134.22	5.55	8.66	5.08	34.40	6.88
		4	130.22	7.33	10.00	7.10	38.87	8.00
L.S.D. 0.05			0.73	0.46	0.48	7.10	0.67	0.41
chelated magnesium (mg.L^{-1})		0	135.67	5.00	7.55	0.23	33.38	6.33
		100	134.11	5.77	8.77	4.76	34.51	7.11
		200	133.56	6.00	9.33	5.31	35.70	7.66
L.S.D. 0.05			0.73	0.46	0.48	5.95	0.67	0.41
Nutrient Solution (mg.L^{-1}) × chelated magnesium (mg.L^{-1})	0	0	140.33	3.33	5.00	0.23	29.77	4.66
	2	100	138.67	4.00	6.00	3.63	30.10	5.00
	4	200	137.67	4.33	7.00	3.86	31.10	6.00
	0	0	136.33	5.00	7.00	4.03	32.33	6.00
	2	100	134.33	5.33	7.66	4.56	33.80	7.00
	4	200	132.00	6.33	8.33	5.03	37.07	7.66
	0	0	130.33	6.66	10.26	5.66	38.03	8.33
	2	100	129.33	8.00	12.95	6.10	39.63	9.33
	4	200	131.00	7.33	12.00	8.16	38.93	9.12
L.S.D. 0.05			1.274	0.80	0.84	7.03	1.16	0.72

DISCUSSIONS

The results of Tables 2 and 3 show significant increasing when spraying nutrient solution in the growth and flowering parameters. That may be due to the role of nutrient solution which contain many nutrients necessary for plant growth such as nitrogen and direct or indirect for amino acids that are important in many physiological processes, as well as its role in stimulated photosynthesis, which leads to improvement plant growth by increasing the manufacture of carbohydrates and proteins, finally that lead to the development plant growth parameters (Thomas et al., 2009). Also, amino acids are an important source of nitrogen which stimulate proteins synthesis, formation of nucleic acids (RNA and DNA), essential amino acids especially tryptophan which is the initial starters product of IAA that it necessary to the division and elongation of plant cells and has role in promoting apical dominance (Wona, et al., 2011). This activates the efficacy of the photosynthesis, enzymes and CO_2 assimilation in plant which increase carbohydrates manufacture and soluble amino acids transportation from the sources to corms (Calvo et al., 2014). In addition to that, increasing number of leaves and leaf content in total chlorophyll (Table 2) stimulates photosynthesis and increases the manufacture of carbohydrates (Table 2) which leads to an excess of sugars that are ready and available to enhancement the flowering growth and give the highest number of inflorescences and improvement flowering growth parameters (Al-Said & Kamal, 2008).

Also, it is observed from the results of Tables 2 and 3 a significant increase when spraying magnesium in the growth characteristics, and that may be due to the main role of magnesium is activation of Ribulose 1-5 Bisphosphosphate carboxylase enzyme which is necessary for the stabilize of carbon dioxide in the Calvin Cycle in the dark reactions of photosynthesis, which has a role in increasing nitrogen and it is involved in protein formation (Taiz & Zeiger, 2006), also has an important role in manufacturing chlorophyll molecule (Al-Sahaf, 1989). Finally stimulates the process of photosynthesis and increases the amount of carbohydrates (Table 2), also flowering parameters had increased significantly as a result of spraying magnesium (Table 3), and that may be due to an increases in the available of the elements ready for absorption by the plant, including magnesium, which may lead to an increase in the efficiency of photosynthesis and the manufactured of soluble carbohydrates in the leaves. In addition to that magnesium has a role in the representation of proteins (Al-Sahaf, 1989) and finally ultimately has improved flowering parameters (Mastalers, 1984).

CONCLUSIONS

Through the research results we conclude that spraying nutrient solution “Taravert Amifol” at 4 mg. L^{-1} and chelated magnesium at 100 mg. L^{-1} have improved growth and flowering parameters especially long vase life for cut flower.

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MORPHOLOGICAL AND PHYSIOLOGICAL PARTICULARITIES OF HOSTA LEAVES VARIETIES CULTIVATED IN ROMANIA

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Abstract

The paper presents the results of the morphological characteristics of Hosta leaves (leaves area, the perimeter, the length of the leaves, width of leaf, the surface of the dominant colour) and physiological studies (photosynthesis, respiration, transpiration and chlorophyll) got from 'T. Rex', 'American halo', 'White feather' and 'Christmas island' varieties of Hosta cultivated in "I. Todor" Botanical Garden within the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The intensity assessment of the photosynthesis, respiration and transpiration was determined with the LCPro+ device, directly into the experimental field. The intensity of photosynthesis varied between 2.33 µmol CO_2 m-^{2} s^{-1} ('American halo') and 22.08 µmol CO_2 m^{-2} s^{-1} ('Christmas island'), intensity of transpiration 2.54 mmol H_2O $m^{-2}sec^{-1}$('American halo') and 6.12 mmol H_2O $m^{-2}sec^{-1}$ ('Christmas island'), intensity respiration 2.81 µmol CO_2 m^{-2} s^{-1} ('White feather') and 6.55 µmol CO_2 m^{-2} s^{-1} ('Christmas island'). The intensity of physiological processes varied depending on the light intensity, temperature and species. The photosynthetic pigments content was determined using 80% acetone extraction and by spectophotometry at wavelengths of 470 nm, 663 nm and 646 nm.

Key words*: chlorophyll, spectophotometry, photosynthesis, respiration, transpiration.*

INTRODUCTION

Hosta, also known as "Autumn lily", originates from Japan, China and Korea and it was first introduced in Europe in the late 1700s and in the US in the mid-1800s (Greenfell & Shadrack, 2004). Dr. Engelbert Kaempfer was the first man ever to see, draw and describe a Hosta, later identified as *Hosta lancifolia*. Hosta has become one of the best-selling herbaceous flowers in the world (Greenfell, 1996).

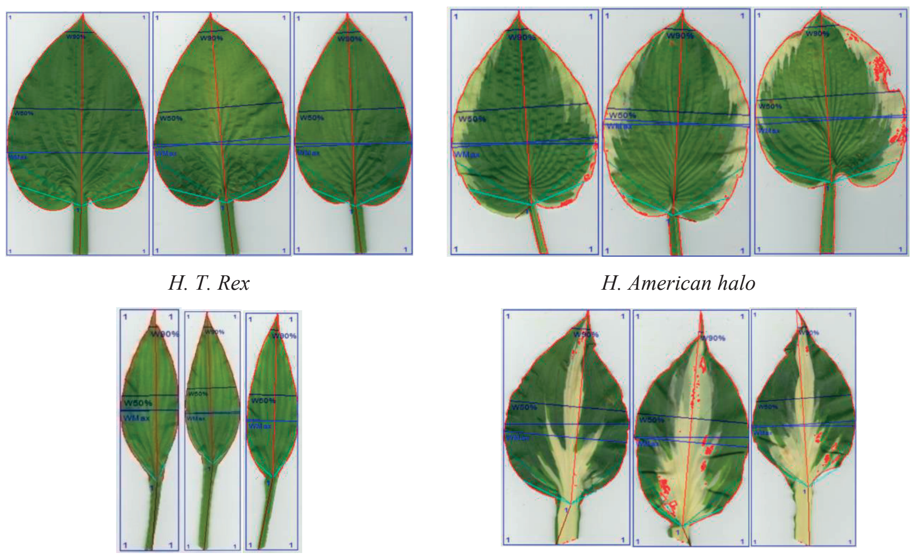
The number of currently known species is about 43 and of cultivars over 2500 (Șelaru, 2007). The most widespread species are: *Hosta plantaginea* Aschers. (syn. *Funkya subcordata* Spreng.*), Hosta sieboldiana* Engl. (syn. *Hosta glauca* Stearn.*), Hosta fortunei* (Hort.) Engl., *Hosta undulata* (Hort.), *Hosta lancifolia* Engl. (syn. *Funkia japonica* Voss.) și *Hosta albo-marginata* Hook. (syn. *Hosta sieboldii* Aschers.) (Toma, 2009). All these species are decorative outside the flowering season as well, due to the general aspect of the plant and to the characters of the leaves (Toma, 2009). Hosta is part of the *Asparagaceae* family and is a perennial, herbaceous plant, having different sizes, from miniature species to giant species. It is considered a decorative plant mainly for its leaves in various sizes, shapes and colours, but also for its delicate flowers. The leaves may be green, blue, yellow, golden or white and may be one colour or variegated. Most varieties of Hosta show colours of flowers from purple to white.

They are the best-selling perennials in the USA and are popular in Europe and Japan, and therefore have considerable economic importance (Zonneveld & Pollock, 2012). Yu et al. (2016) indicate that nitrogen metabolism regulation, photosynthesis and energy supply, chloroplast development, and chloroplast protein import/processing play crucial roles in leaf colour changes in variegated leaves. The results provide novel insights into understanding the mechanisms of leaf colour regulation in variegate leaves. Yoshioka et al.

in 2009 found that the rate of photosynthesis (on an area basis) of the variegated leaves increased almost linearly according to the increase in the proportion of green area to total leaf area. In contrast, dark respiration rate was nearly constant irrespective of the extent of leaf variegation.

MATERIALS AND METHODS

The plants taken into study were cultivated in the "I. Todor" Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The morphological and physiological characteristics were analysed in 4 varieties: Hosta cv. *T. Rex*, with green leaves, Hosta cv. *American halo*, with green-blue leaves having streaked, irregular, ivory-white edges, Hosta cv. *White feather*, with ivory-white leaves that turn to green in June due to the bright sunlight, and Hosta cv. *Christmas island*, with leaves having white color in the middle and dark green edges.
In order to determine the morphological characteristics of the leaves, 3 leaves were harvested from each of the 4 cultivars and varieties of Hosta, in July 2019. The leaves were washed with water, dried and scanned using a scanner Epson Expression 11000XL and after analysed with the WinFolia Software. The determinations done are the leaf area (cm^2), the perimeter (cm), the length (cm) and the width (cm) of the leaves (Figure 1) and the percentage of the dominant and secondary colours of the leaves. The variants were noted with V_1 - Hosta cv. *T. Rex*, V_2 - Hosta cv. *American halo*, V_3 - Hosta cv. *White feather*, V_4 - Hosta cv. *Christmas island.*
The determination of the intensity of photosynthesis, transpiration and respiration was performed directly on the green part of leaves, in the field of experience, using the LCPro+ equipment, according to Lascu et al. (2019). Triplicates of independent determinations were reported.
The quantitative analysis of the assimilating pigments was performed through the Arnon spectrophotometric method, which is based on the extraction of pigments in an organic solvent (80% acetone) and measuring the absorbance of the extract, by reading the sample extinction at a spectrophotometer at three different wavelengths: 470 nm, 646 nm and 663 nm, according to Asănică et al. (2017). In the case of the varieties with variegated leaves, the determinations were done both on the whole leaf and separately, on the green and white parts.



H. T. Rex *H. American halo*

H. White feather *H. Christmas island*

Figure 1. Morphological characteristics of the plant leaves for *Hosta T.Rex, American halo, White feather* and *Cristmas island*

The samples (consisting in the green part of the leaf, the white part of the leaf and the whole leaf) are marked with S_1 - S_8, as follows:
S_1 - *T. Rex,* the whole leaf
S_2 - *American halo,* the white part of the leaf
S_3 - *American halo,* the green part of the leaf
S_4 - *American halo,* the whole leaf
S_5 - *White feather,* the whole leaf
S_6 - *Christmas island,* the white part of the leaf
S_7 - *Christmas island,* the green part of the leaf
S_8 - *Christmas island,* the whole leaf.
Independent extract solutions were analysed in triplicate. Statistical data processing was done by Microsoft Office Excel 2013.

RESULTS AND DISCUSSIONS

From the data regarding the biometric measurements presented in Table 1 and Figure 2 it can be observed that the average leaf area has varied from 40.87 cm (V_3 - *White feather*) to 183.19 cm (V_1 - *T. Rex*).

Table 1. Biometrical measurements of the leaves

Var.	Leaf Area (cm^2)	Perimeter (cm)	Length (cm)	Width (cm)
V1	183.19±24.08	52.87±2.77	24.50±1.45	13.19±1.33
V2	181.82±31.23	64.08±2.01	22.91±2.08	13.79±1.60
V3	40.87±9.19	31.51±3.48	20.12±2.40	4.59±0.70
V4	83.29±4.39	57.44±20.0	18.40±2.43	8.87±0.49

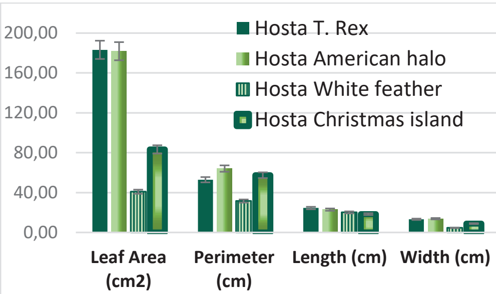


Figure 2. Biometrical measurements of the leaves (cm^2/cm)

As for the perimeter, the variant V_2 had the highest value (64.08 cm) and V_3 had the lowest value (31.51 cm), mainly correlated with leaf width.
The length of the leaves was between 24.50 cm for V_1 and 18.40 cm for V_4.
The width of the leaves was between 13.79 cm for V_2 variant and 4.59 cm for V_3.
The perimeter and the width showed the highest values in the V_2 variant and the smallest in the V_3 variant.
The determinations for the leaves of Hosta plants were made with the LCPro+ analyzer in a relatively small range of variation of the intensity of photosynthetic active radiation of 1518-1586 µmol m^{-2} s^{-1}, at temperature 36-38.6ºC (Table 2). In these conditions, it was registered different degrees of stomatal opening (between 0.06 - V2 and 0.22 - V3) that influenced physiological parameters.

Table 2. Intensity of photosynthesis, transpiration and respiration of Hosta leaves

Variant	Light intensity on leaf (Q leaf) µmol $m^{-2}s^{-1}$	Leaf Temp °C	Gs Degree of stomatal opening	Photosynthesis µmol CO_2 m^{-2} s^{-1}	Transpiration mmol H_2O m^{-2} sec^{-1}	Respiration µmol CO_2 m^{-2} s^{-1}
V_1	1586	36.5	0.15	19.85	4.53	3.17
V_2	1528	36.0	0.06	2.33	2.54	6.16
V_3	1518	38.3	0.12	21.43	4.51	2.81
V_4	1579	38.6	0.22	22.08	6.12	6.55

Q leaf - intensity of light incident on leaf, T°C - leaf temperature

The intensity of the photosynthesis process ranged from 2.33 µmol CO_2 m^{-2} s^{-1} for *American halo* (with green inside leaves) to 22.08 µmol CO_2 m^{-2} s^{-1} for *Christmas island* (with dark green on the edges). According to Zhang et al. (2018), the intensity of photosynthesis is higher in the green areas of the leaves compared to the yellow areas for the species *Aucuba japonica*.
The leaf surface and the position of the leaves against the solar radiation are important factors that condition the intensity of the photosynthesis process (Burzo et al., 2004). The results obtained by Toshoji et al. (2012), in the study over 12 ornamental plants, among which *Hosta* sp. *Reversed,* demonstrated that gross photosynthetic rates were lower in white sectors than in green sectors. Loss or reduction of green colour in the white sectors of the variegate leaves did not result from an optical effect (such as random reflection of light), simply was an effect of functional chloroplasts decrease.

Regarding the intensity of the transpiration process according to the data presented in Table 2, the variation limits were between 2.54 mmol H_2O m^{-2} sec^{-1} for *American halo* and 6.12 mmol H_2O m^{-2} sec^{-1} for *Christmas island*. The intensity of transpiration varies with the species, the age of the plant and the environmental conditions (Toma & Jităreanu, 2007).

A significant correlation between intensity of the photosynthesis and transpiration was recorded ($R^2 = 0.79$, $y = 5.73\ x - 8.94$, where y = intensity of photosynthesis and x = intensity of transpiration), probably according to the degree of stomatal opening.

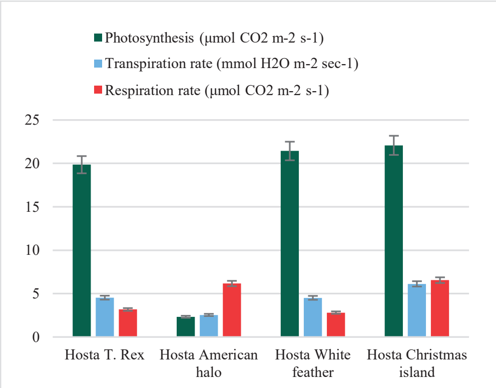


Figure 3. Intensity of the physiological processes

Regarding the intensity of the respiration process (Figure 3) it can be appreciate that the lowest value of the intensity of the respiration was recorded for V_3 (*White feather*), 2.81 µmol CO_2 m^{-2} s^{-1}, and the highest value was recorded for V_4 (*Christmas island*), 6.55 µmol CO_2 m^{-2} s^{-1}. According to Zhang et al. (2018), the respiration intensity in the yellow areas of the *Aucuba japonica* species is higher than photosynthesis. White sectors tended to exhibit dark respiration rates lower than those in green sectors (Toshoji et al., 2012).

Determinations regarding the content of chlorophyll and carotenoid pigments in the species of the *Hosta* genus led to the results presented in Table 3. They highlighted the modification of the content of assimilating pigments according to the species, as follows: the chlorophyll a content was higher in the case of *Christmas island*, the green sample (S_7), at a value of 19.82 mg/100 g, and the smallest quantity was recorded for the S_6 variant, *Christmas island*, the white sample, at the value of 0.4 mg/100 g, according to Figure 4.

The quantity of chlorophyll b ranged from 0.16 in sample S_6 (*Christmas island* white) to 14.56 in sample S_7 (*Christmas island* green).

It should be noted the increased content of chlorophyll and carotenoid pigments in S_7 (*Christmas island* green) in positive correlation with the photosynthesis rate.

Tabel 3. Assimilatory pigments content

Sample	Chl. a (mg/100 g fw)	Chl. b (mg/100 g fw)	Total chlorophyll (mg/100 g fw)	Carotenoids (mg/100 g fw)	Cl a/Cl b ratio
S1	18.93	7.74	26.67	5.41	2.45
S2	3.26	0.92	4.18	1.60	3.99
S3	18.61	7.27	25.88	5.67	2.56
S4	16.28	4.93	21.21	5.45	3.30
S5	17.08	5.73	22.81	5.95	2.98
S6	0.40	0.16	0.56	0.30	2.50
S7	19.82	14.6	34.38	6.24	1.36
S8	19.09	8.24	27.33	5.71	2.32

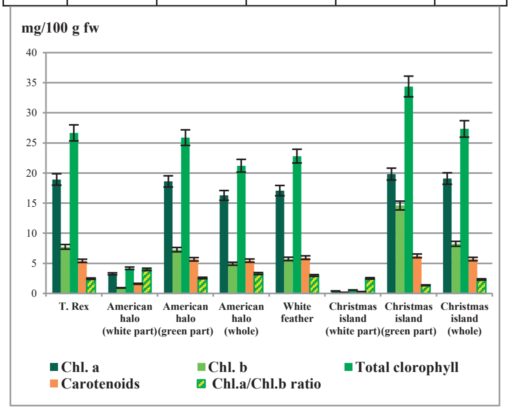


Figure 4. Content of chlorophyll and carotenoid pigments (mg/100g)

Following the analysis of the obtained results regarding the carotene content of the leaves, a smaller quantity of it was noticed in samples S_2 (1.6 mg/100 g) and S_6 (0.3 mg/100 g) compared to the other samples, where the quantity was over 5 mg/100 g.

The amount of chlorophyll a, chlorophyll b, total chlorophyll and carotene is higher in the

green areas of the leaves compared to the white areas.
The results are similar to those obtained by Zhang et al. (2018) for the *Aucuba japonica* species. In *Hosta "Gold Standard"* leaves, total Chls, Chl a, and Chl b in golden regions were obviously lower than in green regions of variegated leaves, and the Chl b accumulation was more reduced than Chl a (Yu et al., 2016).
The ratio between chlorophyll pigments and carotenoid pigments in the leaves may provide indications for leaf colour changes.
The content of chlorophyll pigments is correlated in the case of numerous plants with the intensity of the photosynthesis process and respectively with the accumulation of nutrients in the plants.
As for the dominant colour of the leaves determined with Winfolia software (Table 4, Figure 5), the colour recorded for the variant *Hosta T. Rex* was 100% green, the *Hosta American halo* variant had the dominant colour percentage of 78.93%, *Hosta White feather* of 92.95%, and in *Hosta Christmas island* the percentage of green from the total leaf area was 62.19%.

Tabel 4. Percentage of green color in the leaves

Sample	Dominant colour Green (%)	Secondary colour White (%)
Hosta *T. rex*	100.00	0.00
Hosta *American halo*	78.93	21.07
Hosta *White feather*	92.95	7.05
Hosta *Christmas island*	62.19	37.81

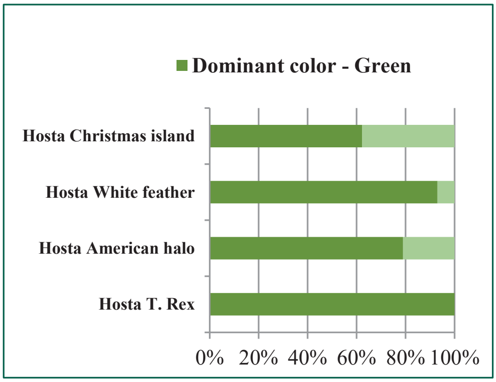


Figure 5. The dominant colour of the leaves

Leaf variegation is seen in many ornamental plants and is often caused by a cell-lineage type formation of white sectors lacking functional chloroplasts (Sakamoto et al., 2009).

CONCLUSIONS

The ornamental plants taken into study are useful resources for further research regarding the morphological aspects correlated with physiological processes that are carried out at the variegate leaf level. The results obtained varied according to the cultivars and the assimilatory pigments distribution in leaf, which positively influenced both the physiological processes in leaves and their dominant colour.

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MORPHOMETRIC AND MORPHOLOGICAL ANALYSES OF *ANACAMPTIS* × *TIMBALI* NOTHOSUBSPECIES *REINHARDII* A NEW ORCHID HYBRID POPULATION TO ROMANIA

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Abstract

This is the first confirmed occurrence in Romania of a significant (notho)population of seven hybrids, Anacamptis × timbali nothosubspecies reinhardii (Ugr. ex E.G. Camus) H. Kretzschmar, Eccarius & H. Dietr., 2007, hybrids between two highly divergent species, Anacamptis coriophora (from Anacamptis coriophora group) and Anacamptis palustris subsp. elegans (from Anacamptis palustris group). The seven hybrids, very likely F1 generation plants representing a single interspecific/intrageneric pollination event, were first studied at Grădiştea Muncelului-Cioclovina Natural Park, Hunedoara County, Romania. The hybrids were phenotypically intermediate between their parental species in most of the 25 morphometric and 41 morphological characters scored, but significantly, they closely resembled Anacamptis palustris subsp. elegans parent. Additionally, pollination studies were performed. Since the parental species occurred in near proximity (at less than 1 meter distance), we suggest that the production of this hybrid required a minimum travel distance of ca 1-10 meters, by the pollinators and frequent exchange of pollen between the parental species was very likely. The parental species A. coriophora and the hybrid, which display a considerable synchronicity in their flowering time, were proved to overlap in pollinator community, very successfully sharing the solitary bees belonging to genus Lasioglossum. The presence of fruits in almost all the hybrids is another proof that they were successfully cross-pollinated. It is clear that even contrasting pollination syndromes such as generalized food deception (in the nectarless Anacamptis palustris subsp. elegans) and generalised food foraging behaviour (in the nectar-producing Anacamptis coriophora) mechanisms are insufficient to fully stop the gene flow between the two species. We set this hybrid population discovery in the context of the recent, expanding evidence of the occurrence of wild species of orchids in Grădiştea Muncelului-Cioclovina Natural Park.

Key words: *anacamptis, hybrid, orchid, timbali, speciation, Orchidaceae.*

INTRODUCTION

This intrageneric/interspecific hybrid is a combination between two highly divergent and taxonomically controversial species *Anacamptis coriophora* (L.) R.M. Bateman, Pridgeon & M.W. Chase, 1997 (from Section *Coriophorae* (Parl.) H. Kretzschmar, Eccarius & H. Dietr., 2007) and *Anacamptis palustris* (L.) R.M. Bateman, Pridgeon & M.W. Chase subsp. *elegans* (Heuff.) R.M. Bateman, Pridgeon & M.W. Chase, 1997 (from Section *Laxiflorae* (Soó & Keller) H. Kretzschmar, Eccarius & H. Dietr., 2007). They are now members of the newly supplemented genus *Anacamptis* Rich., 1817 (previously, these two parental species were members of the genus *Orchis* Tourn. ex L. 1753; following extensive molecular analyses, recently, they have been moved into *Anacamptis* genus).

The generic name, *Anacamptis*, originates in the Ancient Greek word anákamptein, which ad litteram means bent-backward, a reference to the bent-backward (reflexed) tips of the pollinia (the sacs of pollen), a characteristic of this genus. The hybrid (nothospecies) epithet, *timbali*, was given in honour of Édouard-Pierre-Marguerite Timbal-Lagrave (1819-1888), a French pharmacist and botanist who specialized in the flora of southwestern France, including the Pyrénées and Corbières mountains, hence its vernacular name, Timbal's Anacamptis.

The hybrid (nothosubspecies) epithet, *reinhardii*, was given in honour of Reinhard Gustav Paul Knuth (1874-1957), a German taxonomist and botanist hence its other possible vernacular name, Reinhard's Anacamptis Hybrid. Taxa with the specific or nothosubspecific epithet, *reinhardii*, commemorate his name. The accepted scientific name was established in 2007, as *Anacamptis* × *timbali* nothosubspecies *reinhardii* (Ugr. ex E.G. Camus) H. Kretzschmar, Eccarius & H. Dietr., Orchid Gen. Anacamptis, Orchis & Neotinea, ed. 2: 428 (2007). Its basionim (the first name ever given to a taxon) is *Orchis* × *reinhardii* Ugr. ex E.G. Camus, Monogr. Orchid.: 230 (1908). This nothosubspecies belongs to nothospecies *Anacamptis* × *timbali* (Velen.) H. Kretzschmar, Eccarius & H. Dietr., 2007, a member of genus *Anacamptis* Rich., 1817, subtribe Orchidinae Dressler & Dodson, 1960/Verm., 1955, tribe Orchideae Dressler & Dodson, 1960/Verm. 1977, subfamily Orchidoideae Lindl., 1826, family Orchidaceae Juss., 1789. Since we found at least *6 different individuals*, the hybrids have been given the *nothospecies status*. This is the first nothopopulation of *Anacamptis* × *timbali* nothosubspecies *reinhardii* ever mentioned in Romania. Consequently, we strongly propose this hybrid as a new addition to/candidate for the Romanian flora. Moreover, we believe is imperious to put the entire area under strict protection.

MATERIALS AND METHODS

Location description

The extreme rarity of this crossing is due to the fact that both parents are rather rare and to find them together is rather exceptional. The preferred habitat is a seasonal, full sun, grassy, alkaline marsh, periodically prone to flooding. The soil composition may be a mixture of calcareous alluvium that provides a substrate for the entire marsh, sand, clay, etc., derived from the adjacent slightly well-drained areas and dunes. The surface of the soil was moist but not water logged (marshy meadow). The sample sites for all three taxa were located within a few metres of the ecotone between the marsh and back landward. To our surprise, the area was already being developed for housing, whereas the adjacent marsh has retained much of its original flora. The vegetation of this wetland reserve is dominated by herbaceous swampy species. No other orchid species were noticed to occur in the area. The researched area covered approximately 1 square kilometre. It was reported that it is periodically grazed by cattle and three weeks after this study was performed, it was almost completely mowed. *Anacamptis palustris* subsp. *elegans* occurred in significant numbers in the marshy parts of the area, reaching approximately 150-200 individuals. The foetid-smelling *Anacamptis coriophora* grew immediately adjacent to the hybrids, in the ecotone and the drier parts of the swamp. Its numbers were significantly higher, probably encountering several thousand plants (2,000-3,000). All 6 hybrids were growing in the close vicinity of *Anacamptis coriophora*, within the drier parts of the swamp. The distances between the hybrids and *Anacamptis coriophora* parent were very short, in some cases measuring only 5-10 centimetres, in other cases slightly higher, just over 1 meter. The distances between the hybrids and *Anacamptis palustris* subsp. *elegans* were significantly longer, from 1-2 metres up to 20-30 metres or more, if we take in consideration the distance to the edges of the swamp, where scarce *Anacamptis palustris* subsp. *elegans* groups were found.

Flowering time

The flowering times of all three species (parents and hybrid) overlapped almost entirely. *Anacamptis coriophora* parent was the first to flower. Approximately one week later, *Anacamptis palustris* subsp. *elegans* parent came into bloom. The hybrids flowering time seemed to be intermediate between parents, although, at the time the studies were performed, all three species were at the peak of anthesis. In some parts of the swamp, some *Anacamptis coriophora* individuals were slightly off the peak of anthesis.

Parental species description

Anacamptis coriophora (L.) R.M. Bateman, Pridgeon & M.W. Chase, 1997

The specific epithet, *coriophora*, originates in the Ancient Greek words khórion (crust, insect) and phór(os) (to carry, to transport,), ad litteram meaning smelling like a bug, a reference to the unpleasant, strong scent that its flowers usually emit, which resemble that of some species of bugs, hence its vernacular name, the Bug Orchid. The flowers can display considerable variation, particularly in the shape and colour of the labellum and the helmet. The commonest variant has a dark-reddish or brownish-purple helmet and a pale-coloured labellum, but the helmet can also be green flecked with dark brown, greenish-white, or reddish purple. The colour of the labellum can vary from whitish with extremely faint spots to intense purple-brown, with strong dark-purple spots. The leaves are linear, linear-lanceolate or lanceolate, numerous, standing straight up, most at the base. Inflorescence are prolonged-cylindrical. The bracts are linear-lanceolate (Kuhn et al*.,* 2019). The flowers are small, coloured in greenish-red to brown-purple hues. The petals are pointed, gathered in a solid beak-shaped helmet. The labellum is bent down and backwards, trilobed, olive green colour, at the base whitish with purple spots. The spur is tapering, bend downwards, shorter than the ovary. The flowers are rewarding, abundant in nectar and usually many are pollinated (Claessens & Kleynen, 2011). Diploid chromosome number: *2n = 36, 3*

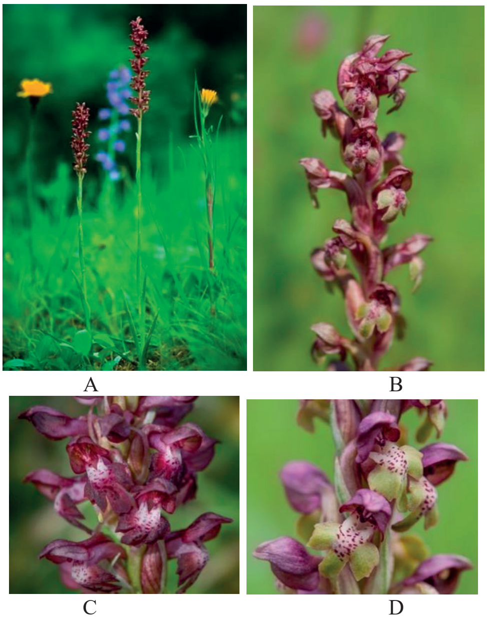


Figure 1. *Anacamptis coriophora.* Entire plant (A) and details of several inflorescences with different chromatic variations (B-D) Photos A-D © N. Anghelescu originals

Anacamptis palustris **(L.) R.M.Bateman, Pridgeon & M.W.Chase subsp. *elegans* (Heuff.) R.M.Bateman, Pridgeon & M.W.Chase, 1997**

The infraspecific epithet (subspecies epithet), *elegans*, originates in the Old Latin word ēlegāns (tasteful, select), ad litteram meaning delicate, refined, a reference to the elegant, tall inflorescences of this subspecies, which bears beautiful, large flowers, hence its (potential) vernacular names, the Elegant Anacamptis, the Elegant Swamp Orchid or the Elegant Marsh Orchid. It is a tall, beautiful and imposing plant that may reach 50-100 centimetres in height. The lanceolate leaves are larger and longer, the flower bracts are longer than the ovary/receptacle (Delforge, 2006). Similar to *Anacamptis coriophora, Anacamptis palustris* subsp. *elegans* is a wintergreen species. They emerge in autumn and wither after anthesis. The flowers are conspicuously and more or less uniformly purple and show little variation. Most of the plants can reach 60-90 centimetres, but oscillates most often between 10 and 25 centimetres. Its lanceolate erect leaves are located at the base of the plant and others smaller, not very visible, are fixed on the stem (stem-leaves). The dense inflorescence forms a pyramidal spike of tight flowers. The deep pink-purple flowers, whose colour can vary from light pink to purple, are very rarely white (Presser, 2002). The labellum, clearly trilobed, provided with two protruding ridges at the base, forms towards the back, a filiform spur. The plant does not have nectar, the attraction of the butterflies for the latter is therefore a decoy. As it is a question of ensuring the fertilization, the morphology of the flowers is well adapted to the proboscides of the Lepidoptera, which may be diurnal or nocturnal (Pridgeon et al., 2001).

Diploid chromosome number: *2n = 36, 42*

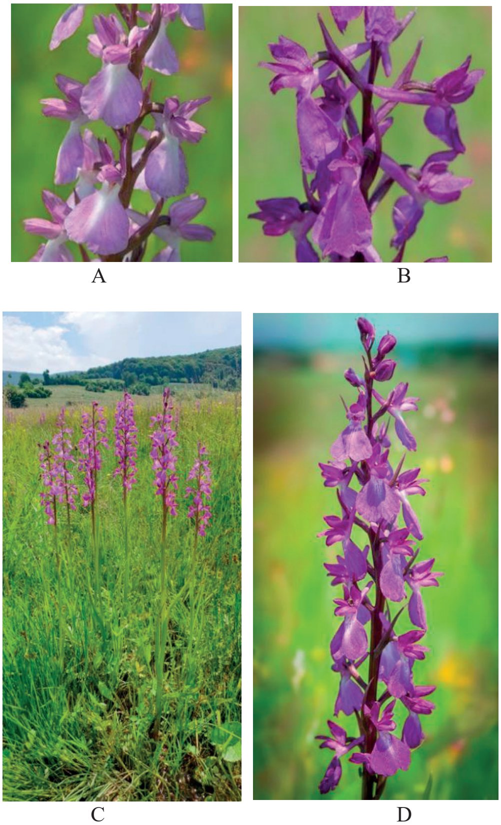


Figure 2. *Anacamptis* palustris subsp. *elegans.* Details of hypo- and hyperchromatic chromatic inflorescences (A-B), entire plant (C) and detail of a full inflorescence (D). Photos A-D © N. Anghelescu originals

General descriptions of the hybrids

The six hybrids closely resembled each other morphologically in size and shape, suggesting that they might have the same parental origin. Primary hybrids (F_1 generation) are mainly much scarcer than their parents and, in general, appear phenotypically intermediate between the parental species.

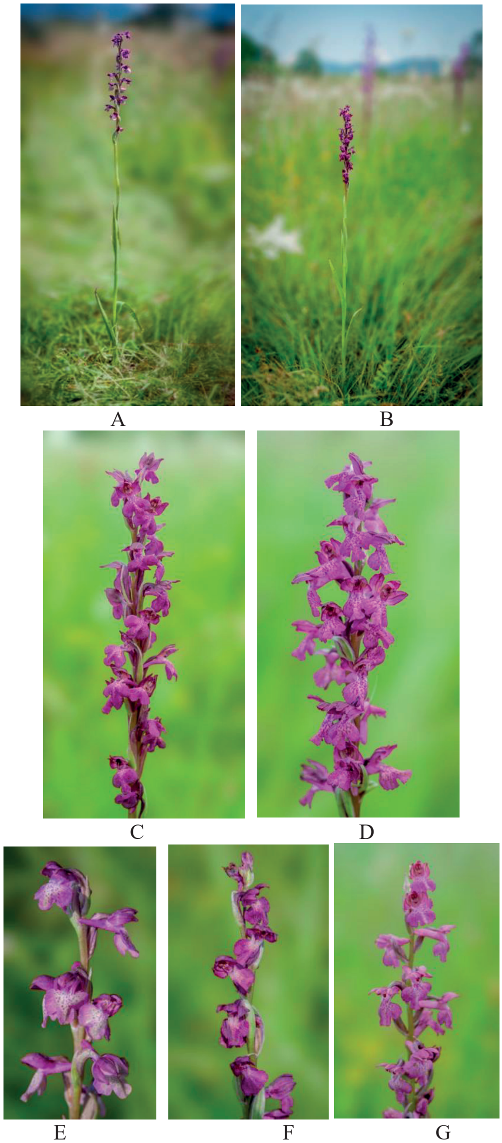
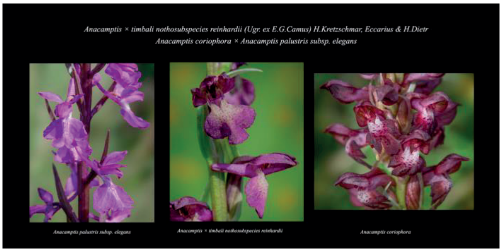
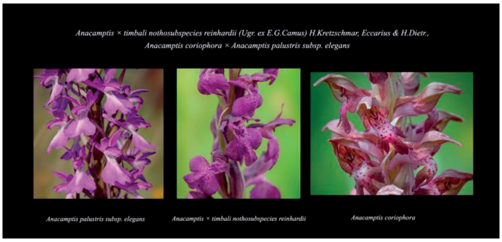


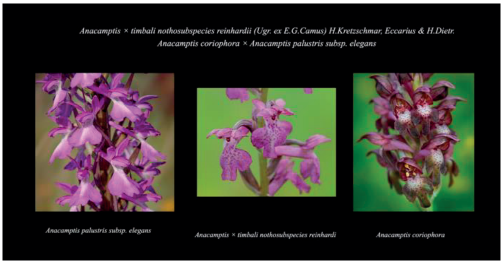
Figure 3. *Anacamptis* × *timbali* nothosubspecies *reinhardii*. Entire plants (A-B) and details of several inflorescences that present different polymorphic (shape, size, colour) variations (C-G). The hybrids are very tall, a feature inherited from their *Anacamptis palustris* subsp. *elegans* parent. The inflorescences are laxer with smaller flowers, resembling more those of *Anacamptis coriophora* parent. Photos A-G © N. Anghelescu originals



A



B



C

D

Figure 4. Morphological comparisons of *Anacamptis* × *timbali* nothosubspecies *reinhardii* and parents (A-D). The illustrations show the intermediate characters of the hybrids, compared to those of its parents. Photos A-D © N. Anghelescu originals

RESULTS AND DISCUSSIONS

Morphometric/biometric methods:
Given that the parental species differ considerably in morphology, the identification of any hybrids between them, appear relatively straight-forward (the images are very explicit).
In most cases, the phenotypical characters/traits of the hybrid appear intermediate between the parental species in the majority of morphological characters.
It should be emphasised that a positive determination of hybrids implies a deep knowledge of the variation of the parental species and a deep characterisation of the biotope in which they are found.
Therefore, for the correct identification of a hybrid, it is imperious that at least one unequivocal character of each parental partner is demonstrable and cannot come from the other supposed partner (Bateman & Hollingsworth, 2004).
In order to describe the plants as comprehensive as possible, a wide range of characters are taken in consideration and biometrically/morphometrically analysed in millimetres, unless otherwise stated (Jacquemyn *et al*., 2012).
The quantitative measurements encompass all organs except the tubers and gynostemium.
Measurements are examples of several parental plants and of hybrid no. 4 & 5 (in all individuals measured, it has to be mentioned that older flowers found at the bases of inflorescences are better developed than the younger flowers found in the upper half).

Table 1. Morphometric comparison of the parental species and potential hybrids. The quantitative measurements (in mm unless otherwise stated) encompass all organs except the tubers and gynostemium

Vegetative & Floral Organs	Characters / Features (milimetres)	*Anacamptis palustris* subsp. *elegans*	*Anacamptis* × *timbali* nothosubspecies *reinhardii*	*Anacamptis coriophora*
1. Stem & Inflorescence				
	Overall height	560/600	550	200
	Stem diameter	6.3	5.5	3.4
	Stem anthocyanins	moderate/strong	moderate/strong	low/absent
	Inflorescence length	180/250	180	70/90
	Number of flowers	24/30	21	48/53
2. Leaves				
	Distribution of sheathing leaves on stem	even	basal/even	basal
	Longest leaf posture	erect	slightly recurved/erect	slightly recurved/erect
	No. of sheathing leaves	3	3	4/8
	No. of leaves non-sheathing	3	3	2/5
	Length of longest leaf	200/250	150/180	22/72
	Width of longest leaf	30/38	20/30	18/20
	Outline shape of longest leaf	linear-lanceolate	linear-lanceolate	linear-lanceolate
	Leaf conduplicate	strong	moderate	moderate
	Apex hooding	strong	moderate	moderate
	Leaf colour	vivid/light green	deep green	deep green
	Leaf dorsal side	light green	light green	darker green
	Leaf ventral side	green	green	greyish green, veined
	Leaf margins	entire	entire	entire
	Leaf markings	unmarked	unmarked	unmarked
	Upper leaves	bract-like	cauline	bract-like
3. Bracts & Ovary				
	Length of basal bracts	33/36	39/45	9/12
	Width of basal bracts	5/8	7/8	3/3.2
	Length of floral bracts	20/22	22/23	2.2/2.3
	Width of floral bracts	3.2/3.6	3.8/4.2	2.1
	Texture of bracts	robust	robust	membranous
	Bract anthocyanins	strong	absent/moderate	Moderate/strong
	Marginal wall thickness	thick	thick	thin
	Ovary length	16/20	12/15	5/8
	Ovary diameter	2.3/4	2.5/3	2.3
	Ovary anthocyanins	absent/moderate	moderate	strong
4. Sepals & Lateral Petals				
	Lateral sepals position	near-erect	near-erect	erect
	Lateral sepals connivent	no	yes	yes
	Sepal fusion from base (%)	40	60	70/80
	Sepal apex	Oval/blunt	acute	acute
	Sepal iridescent green pigment	absent	present	present
	Median sepal length	11.1/12.8	9.2/9.9	8.1/8.7
	Median sepal width	3.2/5	2.9/3.2	1.3/1.6
	Lateral sepals width	3.2/4.6	2.3/2.9	1.4/1.6
	Lateral sepals length	12.2/12.6	7.9/8.1	8.3
	Lateral petals width	3.3/4.3	2.8/3	1.4/2
	Lateral petals length	7.9/11.2	7.3/7.8	5.3/6.2
5. Labellum				
	Outline shape	elongated-oval, nearly flat	circular/heart shaped, nearly flat	longitudinal-oval, lobesfolded backwards
	Lobes	nearly entire	trilobed	deeply trilobed
	Median lobe length	12.4/13.4	7.8/8.9	7.1/8.9
	Lateral lobes length	11.8/12.1	7/7.8	5.6/6

Vegetative & Floral Organs	Characters / Features (milimetres)	*Anacamptis palustris* subsp. *elegans*	*Anacamptis* × *timbali* nothosubspecies *reinhardii*	*Anacamptis coriophora*
	Sinuses separating the three lobes	shallow	medium/deep	very deep
	Width	16.5/17.8	9.3/11	7.3/8
	Lateral lobe reflexion	absent	absent/moderate	strong
	Central lobe apex	shallow, bilobed, flat	prominent, entire, flat	prominent, strongly recurved
	Central lobe width	4.2/5.3	4.2/4.6	1.6/2.1
	Base colour of labellum	whitish, purple-dotted	pink to light purple, purple-dotted	whitish, grooved, red-dotted
	Centre colour	whitish, elongated streak, purple-dotted	whitish -yellowish-pinkish, convex, slightly to strongly uniformly dotted	whitish, grooved, convex, dark-red dotted
	Margins colour	deep-purple, uniform	purple, brownish to very dark-purple	dark-red, green, brown
	Markings type	purple stripes	dense purple spots	dark red or brown spots
	Surface markings papillate	no	moderately	strong
	Markings distribution in centre	linear-vertical	circular	circular
	Markings contrast	weak	strong (weak at times)	strong
	Lateral lobe indentation	entire, scalloped	entire/moderate	prominent
6. Spur				
	Spur length	15.2/17	8.2/8.9	7.5/8
	Spur shape	cylindrical thin, ascendant	cylindrical, thick, horizontal	conical, thick, slightly descendant
	Spur width entrance	3.2/3.6	4.6/4.9	4.8/5/4
	Spur width halfway	1.2/1.5	2.6/2.9	2.1/3.2
	Spur down-curvature	none	none/slightly down-curved	down-curved
7. Nectar				
	Presence	no	?	yes
	Amount	-	?	abundant
8. Smell				
	Presence	vaguely fruity	vaguely bug-like	Strong, bug-like, carrion

Morphometric comparisons

Habitus: The hybrids are very tall and sturdy, reaching up to 60 centimetres, a feature inherited from *Anacamptis palustris* subsp. *elegans*.

Stem: Is moderately to strongly purple washed. The presence of anthocyanins is also a feature inherited from *Anacamptis palustris* subsp. *elegans*.

Leaves: Two out of the 6 hybrids show larger cauline leaves, erect, lanceolate, sheathing the stem, a feature inherited from *Anacamptis palustris* subsp. *elegans*. The other 4 hybrids resemble more *Anacamptis coriophora*, by presenting 2-3 basally concentrated leaves and 1-2 cauline leaves, a lot smaller, sheathing the stem. Also, the colour of the leaves is intermediate between the parents, the hybrid presenting slightly darker leaves than *Anacamptis palustris* subsp. *elegans* which has vividly to light green leaves.

Flower: The flower sizes of the hybrids lie between that of both parents. They show a variety of intermediate sizes (they differ very slightly) and range mostly in the median range of the parental sizes (length x width).

Bracts: The bracts are very variable in size. They are shorter to almost equal to the ovaries like in *Anacamptis coriophora*. In hybrid no. 3, the bracts are very long, almost twice as long as in other hybrids, and longer than in both parents. This is a feature which shows a hybrid enhanced character.

Lateral Sepals & Petals: The sizes are again, intermediary between the parental species.

Helmet/hood: the lateral petals and sepals that construct the helmet are not linked and the helmet opens slightly at the crown, in intermediary condition between the parental species.

Labellum: The morphology of the labellum is particularly interesting as the parents differ considerably in the labellum size and shape. The labella of the hybrids are all different in shapes, lobe depth and colour. One of the hybrids (hybrid no. 4) resembles more *Anacamptis palustris* subsp. *elegans* (has very shallow lobe separation and the lobes are equal, almost undefined), presenting a shallower labellum indentation. Hybrid no. 6 lies at the other extreme: it presents a deeply trilobed labellum resembling more *Anacamptis coriophora* (has a narrow, prominent central lobe and allows all three lobes to reflex). The rest of the hybrids are intermediary between these two. In all 6 hybrids, the lobes are not reflexed and the labellum appears almost flat.

Consequently, we speculate that a prospective pollinating insect will perceive a flatter and proportionally wider landing stage in the hybrid than in the parent. Moreover, the purple labellum colour of the hybrid, represents a combination of anthocyanin pigments, which is intermediate between the dark-red of *Anacamptis coriophora* and the vivid purple of *Anacamptis palustris* subsp. *elegans*.

A particularly interesting phenomenon of overexpression of pigmentation was observed in 4 hybrids (especially in hybrid no. 4). In all cases, the flowers become dark, deep-purple, different from either parent. The high density of floral anthocyanins is evident only in the hybrids, relative to either of the parents. This interesting example of a hybrid enhanced character reinforces the supposition that the over-expression of pigmentation, which will result in lower reflectivity of the flowers is more commonly observed in hybrids between distantly related species.

The increased hybrid vigour in some particular features, which became superior to both parents, is called the ‘heterosis effect’ (Kretzschmar *et al.,* 2007). These character shifts are capable of modifying pollinator specificity, indicating a potential evolutionary future for the hybrid. These data will show and explore whether specific patterns of inheritance of specific characters suites and determine whether particular novel combinations of character states (or novel states) in such hybrids, impair, neutralise or enhance with respect to functionality. On the other extreme, lies hybrid no. 2, which has a lot less anthocyanin on the labellum, shifting more towards *Anacamptis coriophora* in this respect.

Labellar markings: In all hybrids, the markings of the labella present a heart-shaped pattern of distribution (more circular), strikingly resembling the distribution in *Anacamptis coriophora*. None of the hybrids inherit the longitudinal whitish streak of *Anacamptis palustris* subsp. *elegans*. Also, the texture of the central area of the labellum is strongly papillose, reminding of the brownish-red papillose spots of *Anacamptis coriophora* feature.

Spur: The spur is generally long (but shorter than the ovary), cylindrical, thick and horizontal, in most of the cases. In one case, hybrid no. 5 is conical, with a pointed tip, resembling *Anacamptis palustris* subsp. *elegans*. The others are rather thick showing a strong *Anacamptis coriophora* influence. Only one of the hybrids, hybrid no. 1 has a slightly downward pointing spur.

Hybridisation within *Anacamptis* genus

According to Goulet & Hopkins (2017), the term *hybridisation* is rather controversial and needs a new, updated definition. Often, hybridisation is only considered between species, but ‘*from a genetic point of view, interspecific hybridisation is only a special case of a much more widespread phenomenon*’ (Stebbins, 1950). Therefore, especially when referring to orchidology, it would be useful to redefine the phenomenon of hybridisation independent of species strict definitions, which stated that ‘*the species is the basic category of biological classification, composed of related individuals that resemble one another and who are able to breed only among themselves’*.

In this article, we will adopt its broader definition given by Harrison (1990), in which ‘*hybridisation is a cross between individuals from separate populations that differ in one or more heritable traits’*. Defining hybridisation independent of the species distinction, elegantly circumvents the problem of species definition. Over millions of years of evolution, hybridization had a major role in shaping the history of life on earth. The evolutionary history of a population is reflected in the genetic variation of its genomes. In natural populations, hybridization can act as an evolutionary engine by overcoming the reproductive barriers between populations.

In 1786, in his treaty Disquisitio de sexu plantarum (A Dissertation on the Sexes of Plants), Carl Linnaeus (1707-1778) first suggested that new species arose [mainly] by hybridization, thus rejecting the notion of species 'immutability' or the incapacity of species to change through time (Coyne & Orr, 2004). A new hybrid lineage is formed through parental genome mixing. Hybridization is widespread, but the generation of a unique, natural hybrid lineage to occur is likely very rare. New hybrid lineages must establish reproductive isolation and a unique ecological niche in order to overcome genetic mixing and competition from parental species (Mayr, 1942). As a result, hybridisation was shown to have a significant role in speciation, generating new species with better genetic, adaptive variation (Arduino et al., 1996).

By definition, hybridisation is the crossing of two different genotypic parents, parent generations $P_1 \times P_2$. The genes from P_1 & P_2 exist in the first subsequent generation, named F_1 (Soltis & Soltis, 2009). They will be present in the hybrid genotype and can be dominant, recessive or intermediate (Ramsey & Schemske, 1998). The totality of all successful hybrid types that originate of the crossing of two parental taxa (natural species, not of hybrids) is called a nothotaxon.

Scarcity of orchid hybrids: In the wild, the maintenance of species integrity has major importance. In the case of sexually compatible sympatric populations, species integrity depends upon several reproductive barriers that secure the reproductive isolation between species. They are classified in:

- pre-mating barriers - spatial segregation, phenology (scientific study of cyclical biological events, such as flowering periods, breeding, seed production, in relation to climatic conditions) and pollinators;
- post-mating barriers - significant differences in their haploid chromosome number (n), fruit abortion, seed unviability, hybrid unviability and hybrid sterility (Bateman & Hollingsworth, 2004).

Because of the sequential action of these isolating mechanisms, it is generally assumed that pre-mating isolation barriers are more important to reproductive isolation than post-mating barriers, although conclusive evidence for this is still largely lacking (Scopece et al., 2008).

Within *Anacamptis* genus, hybridisation among various sympatric species was shown to be quite successful, as the pre-mating barriers barriers against hybridisation are otherwise low. Between some species, hybrid swarms often appear, e.g. between *Anacamptis morio* x *Anacamptis papilionacea*.

Space segregation: In the case of *Anacamptis palustris* subsp. *elegans* and *Anacamptis coriophora,* the space segregation was extremely low, very often measuring less than 5 centimetres. Also, on this particular location, the two sympatric orchid species occurred in high density, in very large numbers - dozens of dozens of *Anacamptis palustris* subsp. *elegans* were surrounded by a high density of hundreds (or even thousands) of *Anacamptis coriophora*.

Phenology: They also display considerable overlap in flowering time: whereas flowering in *Anacamptis coriophora* starts at the beginning of May and lasts until the beginning of June (the latest), *Anacamptis palustris* subsp. *elegans* usually starts flowering two weeks later (mid-May) and flowering lasts until mid-June. On the date the hybrids were found, on 5th-9th of June, both parents were in full flower, although *Anacamptis coriophora* was slightly over the peak of anthesis, reaching the late stage of flowering. The majority of individuals had the lower flowers of the inflorescences already withered, with thick ovaries developing into fruits. *Anacamptis palustris* subsp. *elegans* and *Anacamptis* × *timbali* nothosubspecies *reinhardii* were both approaching the peak of anthesis. Is needs to be determined whether the hybrid actually flowers 1-7 days before or after its parents.

Chromosome number: These findings also comply with the fact that the parental species have the same chromosome number, $2n = 36$, allowing them to cross relatively easily.

Pollination: All species of *Anacamptis* genus are nectar-deceit orchids. The spurs of those species do not produce any nectar, and as a consequence, they do not reward their pollinators with any food bodies /substances.

They are known as food-deceptive orchids and this particular pollination mechanism is classified as generalized food deception mechanism (Jersáková et al., 2006).
In order to attract insects and successfully accomplish their pollination, they usually grow among rewarding plant species, which they very often mimic in one or more floral traits such as, inflorescence shape, flower colour, floral scents (that mimic the presence of food - nectar), nectar guides, spurs and pollen-like papillae. Little (1983) termed this type as ‘mimicry based on naïveté’.
But there is one exception within this non-rewarding genus: the foetid *Anacamptis coriophora*, which is a rewarding species that produces abundant amounts of nectar in its spur and recompenses its pollinators with this very nutritious food. This pollination mechanism is known as generalised food foraging behaviour mechanism (Galizia et al., 2005). It also attracts a large variety of visitors with its poignant and heavy smell that usually reminds of carrion (dead flesh) or bug-odour (hence its vernacular name, the Bug Orchid).
All food-deceptive orchids exploit the preexisting plant-pollinator relationships, especially the food foraging behaviour and achieve their pollination by deception. They are also generalist pollinators, usually pollinated by bees, bumble-bees, beetles, butterflies, flies, and share most of their pollinators with all the neighbouring nectariferous, rewarding plant species (Claessens & Kleynen, 2011).
As mentioned, the parental species occurred in the very near proximity of each other, sometimes at a distance measuring less than a few centimetres. This implies that the pollinating insects required a minimum travel distance between the parents, in order to generate the hybrids. Since *Anacamptis coriophora* and *Anacamptis palustris* subsp. *elegans* may, at least partially, overlap in their pollinator community and display a considerable synchronicity in their flowering time, frequent exchange of pollen between the parental species was very likely.


A B

Figure 5. *Anacamptis coriophora* pollinated by solitary bees belonging to genus *Lasioglossum* Curtis, 1833, Family Halictidae Thomson, 1869 (A-B). Photos A-B © N. Anghelescu originals. Insect ID: Prof. Bogdan Tomozei


A B

Figure 6. *Anacamptis* × *timbali* nothosubspecies *reinhardii* pollinated by the solitary bees belonging to genus *Lasioglossum* Curtis, 1833, Family Halictidae Thomson, 1869 (A-B). Photos A-B © N. Anghelescu originals. Insect ID: Prof. Bogdan Tomozei

This is one important example of strong overlap in pollinator community between *Anacamptis coriophora* & *Anacamptis* × *timbali* nothosubspecies *reinhardii*. It shows that the hybrid may, very successfully share at least on very efficient pollinator, the solitary bees belonging to genus *Lasioglossum* (see image), with one of its parents. The presence of fruits in almost all the hybrids is another proof that they were successfully pollinated.
Fruit & Seed: In some of the hybrids fruit was apparently already forming. Fruit set and seed formation are two of the most important post-mating reproductive barriers. The fact that the fruit was already developing may demonstrated that the hybrids are fertile (allogamous or, very scarcely, facultatively autogamous). Whether the seeds are able to mature and successfully germinate (develop beyond the protocorm stages), remains to be clarified. Seed formation will be of primary interest in our further studies. The dust-like seeds travel via air currents even further, thus conquering new territories and establishing new populations. It

is well known that biology dictates that the pollinia are the viable means of achieving gene flow within populations and the seeds are the viable means responsible of establishing new populations. Nevertheless, distribution of pollen is limited to the pollen viability and endurance of the pollinating insects (Bateman & Hollingsworth, 2004).

Mycorrhizal Associations: According to our observations, all the hybrids were found to grow in the near proximity of *Anacamptis coriophora* plants, which occurred in high densities and surrounded them. Rather often, the distances between the hybrids and *Anacamptis coriophora* were only less than a few cm. On the other hand, the distances between the hybrids and *Anacamptis palustris* subsp. *elegans*, ranged from 3-5 metres up to 30-50 metres. Therefore, the hybrids were intimately admixed with one parent but were separated from the other. This may be in favour of *Anacamptis coriophora* of being the mother or the seed carrier, as it is well-known that the seeds (in this case the hybrid seeds) fall within the close vicinity of the maternal parent and, by making use of the mycorhizal fungi available, they successfully germinate.

Maternity-Paternity Testing: As many other hybrid studies show, within a hybrid population, some first-generation hybrids (F_1 hybrids or direct hybrids), may have 'inversed' same parents.

In our small hybrid population, this phenomenon of 'inverse parenting' can be translated as follows:

- some hybrids may have *Anacamptis coriophora* as mothers (*Anacamptis coriophora*♀ - ovule donors and seed carriers),
- others may have *Anacamptis coriophora* as fathers (*Anacamptis coriophora*♂ - pollen donors). Once emasculated, the *Anacamptis coriophora*♂ fathers may become *Anacamptis coriophora*♀ mothers, if pollinia of an *Anacamptis palustris* subsp. *elegans* plants successfully lands on their stigma, thus generating hybrid seeds. The same is valid for the *Anacamptis palustris* subsp. *elegans* reproductive partners. This suggests considerable mobility of the pollinia, seeds or both across the ecotone, rendering the potential results of 'maternity/paternity testing' especially intriguing.

CONCLUSIONS

Hybridisation evidence supports the recent expansion of the genus *Anacamptis*. The new hybrid described in this report represents a cross between an exceptionally small-flowered species producing nectar and one of the largest-flowered orchid species in the genus *Anacamptis*, which is completely devoid of nectar.

It is clear that even these contrasting pollination syndromes (generalized food deception & generalised food foraging behaviour mechanisms) are insufficient to fully stop the gene flow between the two species. Together, the hybrids formed within the genus *Anacamptis*, provide further evidence of the genetic cohesion of this genus.

We set this hybrid discovery in the context of the expansion of the newly occurring evidence of the occurrence of wild species of orchids in Gradistea Muncelului-Cioclovina Natural Park.

Future research will imply extensive:

- molecular analyses to confirm the identity of the plants as hybrids;
- molecular analyses to distinguish between maternal and parental species for each hybrid (*Anacamptis coriophora*♀ × *Anacamptis palustris* subsp. *elegans*♀ or *Anacamptis coriophora*♂ × *Anacamptis palustris* subsp. *elegans*♂).

The value of more careful morphological and molecular investigations will reveal the amount and direction of gene flow in orchids:

- analyses of specific enhanced characters (heterotic characters) in the hybrids relative the parental species;
- analyses of the potentiality of seed germination and determine if the hybridisation may continue beyond the first generation, F_1;
- the clarification of whether the area is either private property or may fall under the protection of the park.

Observation: the whole area was mowed probably before the seeds were able to fully mature.

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PARTIAL RESULTS OBTAINED ON PROPAGATING HERBACEOUS PEONY FROM GREEN SHOOTS CUTTINGS

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Abstract

The research work conducted is of great importance both to professional growers and peonies lovers who like to admire them in gardens and parks, because of the method of herbaceous peony propagation that it presents and because of the measurements, observations and determinations made during the research. It is especially important because its outcomes. This experience brings new information regarding peony propagation by different methods. It contributes to the scientific enrichment regarding the culture technology and the propagation of the herbaceous peony through the studies conducted on this method of propagation. It brings in the contributions and benefits of the herbaceous peony cultivators regarding the propagation rate, percentage of rooting, rooting substrate, use of different solutions and rooting substances but also on the peony cultivars that can influence the rooting percentage. The research conducted is of topical importance, especially since the peony flower has a significant and high ornamental value regarding the culture and marketing of peony flowers which is growing on the flower production market, bringing information and solutions to the problems encountered in the culture. and peony marketing. The present research presents some results obtained: the propagation rate is high between 10 seedlings/plant in Kansas cultivar and 26 seedlings/plant in Dorren cultivar; although in this experience the rooting rate was below 30%, this method of propagation cannot be neglected but improved in the future; the method can be recommended with a high propagation rate; from the partial data obtained so far, this method can be recommended in order to propagate the herbaceous peony.

***Key words**: cuttings, peony, rooting, shoots.*

INTRODUCTION

The research work conducted is of great importance both to professional growers and peonies lovers who like to admire them in gardens and parks, because of the method of herbaceous peony propagation that it presents and because of the measurements, observations and determinations made during the research. It is especially important because its outcomes. The propagation rate by this method is higher than the propagation rate obtained by applying other methods. For example, from one plant, one can obtain up to 30 cuttings that can be rooted, after which 30 plants can be obtained if the rooting percentage is 100%.

This method does not disturb the root of the plants, the cuttings being made from green shoots.

This experience brings new information regarding peony propagation by different methods. It contributes to the scientific enrichment regarding the culture technology and the propagation of the herb peony through the studies conducted on this method of propagation. It brings in the contributions and benefits of the herbaceous peony cultivators in terms of propagation rate, percentage of rooting, rooting substrate, use of different solutions and rooting substances but also on the peony cultivars that can influence the rooting percentage.

The research conducted is of topical importance, especially since peonies have significant and high ornamental value given that their growing and marketing are nowadays increasing on the flowers production market. This research brings information and solutions to the problems faced in peonies' growing and marketing.

Peonies are propagated by the division of the shrub, layering, cuttings, grafting, by seeds and in vitro.

Herbaceous peony species are propagated by the division of the shrub, an operation carried out on mature plants, 4-5 years old. After partly removing the soil from the roots, the mother plants are cut into pieces with a very sharp knife or divided by bare hand in 3-6 parts, depending on the size of the mother plant (Toma Florin, 2005).

Division of peony shrubes may be annually made in intensive flowering plants (*Aster, Chrysanthemum*), once 2-3 years (*Delphinium*) and once 5-6 years in slow growing or sensitive to separation plants (*Paeonia*) (Cantor Maria, 2009).

In temperate climate areas in the Northern hemisphere, peonies flower between May and July. Modern propagation researches conducted in the last years allowed growers to obtain some cultivars with various flowers and forms (Cantor Maria, 2016).

MATERIALS AND METHODS

The research was conducted in 2018 at USAMV Bucharest, in the experimental field of the department of floriculture, in the University's botanical garden, in the greenhouse, and in the bower. The greenhouse and the experimental field of the department of floriculture were arranged for conducting the experiment.

The research material used in this experiment consists of herbaceous peony cultivars from both private garden and the university's collection.

This research is bifactorial and has two components: factor A and factor B.

Factor A includes the following herbaceous peony cultivars: Festiva Maxima, Dorren, Pink Giant and Kansas.

Factor B is represented by the following solutions used for rooting: Rootip Basic, Rootip Mix, Kerafol Evo, Fighter Phos and Atonik. The 4 variants presented in Table 1 resulted from the combination of the two factors. Between May and April, cuttings were taken and planted in alveolar pallets. The substrate used for planting the cuttings is a mixture made of 60% peat and 40% perlite with 4 mm granulation.

Throughout the research period, biometric measurements, and visual determinations and observations were used as research methods.

Biometric measurements were done by means of the following indicators:

- Length of cuttings;
- Diameter of cuttings;
- Diameter of the passing area located between root and base of the stem;
- Diameter of earth bale around the roots;
- Length of formed roots.

Visual observations based on the following indicators:

- Propagation rate;
- Rooting percentage;
- Percentage of young/lignified roots.

Table 1. Experimental variants, herbaceous peony cultivars and rooting solutions

Variant	Cultivar	Rooting solution
V1	Festiva Maxima, Dorren, Pink Giant, Kansas	Rootip Basic
V2	Festiva Maxima, Dorren, Pink Giant, Kansas	Rootip Mix
V3	Festiva Maxima, Dorren, Pink Giant, Kansas	Rootip Basic 50 ml+ Kerafol Evo 25 ml + 10 ml Fighter Phos la 10 l apa
V4	Festiva Maxima, Dorren, Pink Giant, Kansas	Atonik

RESULTS AND DISCUSSIONS

By the research studies conducted, this experiment brings partial outcomes regarding peonies' propagation by cuttings green shoots highlighting the variants experienced in terms of cultivars and solutions that have been used, but also the rate of propagation, the percentage of rooting and the characteristics of the roots formed.

By analysing the data in Table 2 we observe a minimum propagation rate of 6 cuttings/ plant related to the Kansas cultivar and a maximum rate of propagation of 26 cuttings/plant related to the Dorren cultivar. Regarding the dimensions of the cuttings, we have obtained cuttings with length between 4 and 13 cm, and diameter between 2 and 5 mm.

Table 2. Propagation rate and characteristics of green shoots cuttings of some herbaceous peonies cultivars, 2018

Cultivar l	**Propagation rate Number of cuttings per plant**		**Length of cuttings (cm)**		**Diameter of cuttings (mm)**	
	min	**max**	**min**	**max**	**min**	**max**
Festiva Maxima	12	22	5	11	2	5
Dorren	14	26	6	13	2	4
Pink Giant	8	12	4	9	2	3
Kansas	6	10	5	10	2	4


Figure 1. Types of green shoots cuttings


Figure 2. Cuttings before being planted

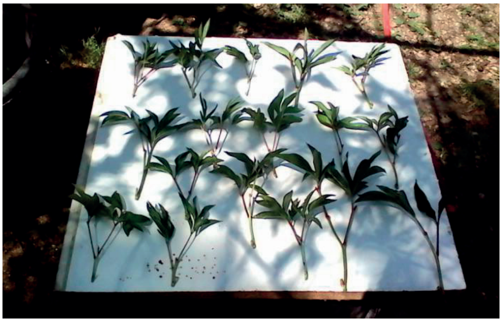
Figure 3. Trimming of cuttings before root

Figure 4. Bathing of cuttings in solutions for rooting before planting

Figure 5. Putting of cuttings in various solutions for rooting before planting


Figure 6. Planting of cuttings in the rooting substrate


Figure 7. Placing pallets with cuttings under arches covered with canvas in the greenhouse


Figure 8. Putting of cuttings in the greenhouse and their covering with a canvas

Figure 9. Cuttings during the rooting phase

Figure 10. Cuttings during the rooting phase

Table 3. Characteristics of rooted cuttings of some herbaceous peonies cultivars

Variant	Cultivar	Maximum length of the root (cm)	Diameter of the passing area between the root and the stem base (mm)	Diameter of the earth bale around the root (cm)	Percentage of roots	
					Young	Lignified
V1	Festiva maxima	9 cm	8 mm	2.5 cm	70%	30%
V2	Kansas	11 cm	15 mm	4 cm	40%	60%
V3	Dorren	8 cm	10 mm	4.5 cm	80%	20%
	Kansas	17 cm	14 mm	4.5 cm	90%	10%
	Pink Giant	4.7 cm	2 mm	1 cm	100%	-

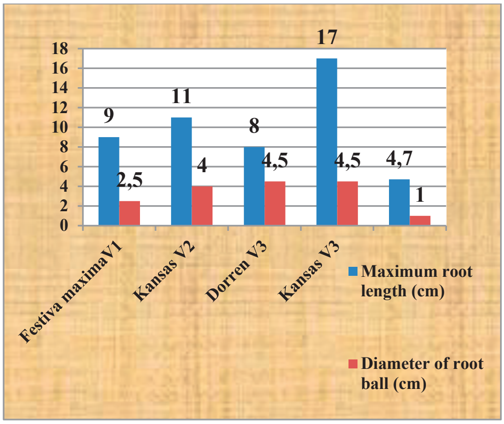

Figure 11. Dimensions of cuttings' roots of some herbaceous peony cultivars

The determinations regarding the characteristics of the rooted cuttings are presented both in Table 3 and Figure 11. Thus, the maximum root length was between 8 cm in the Dorren cultivar in the V3 variant and 17 cm in the Kansas cultivar in the V3 variant. The diameter of the passing area between the root and the base of the stem was between 2 mm in the Pink Giant cultivar in the V3 variant and 15 mm in the Kansas cultivar in the V2 variant. The earth bale around the root diameter was between 1 cm in the Pink Giant cultivar in the V3 variant and 4.5 cm in the Dorren and Kansas cultivars in the V3 variant.

The percentage of young roots was between 40% in the Kansas cultivar in the V2 variant and 100% in the Pink Giant cultivar in the V3 variant, and the percentage of lignified roots was between 10% in the Kansas cultivar in the V3 variant and 60% in the Kansas cultivar in the variant V2.

Rooting percentage in all cultivars was below 25%.

Figure 12. Cuttings during rooting phase, Dorren cultivar

Figure 13. Cuttings during rooting phase, Pink Giant cultivar

Figure 14. Cuttings during rooting phase, Festiva maxima cultivar

Figure 15. Cuttings during rooting phase by variants

Figure 16. Cuttings during rooting phase by variants, 2018

Figure 17. Cutting during rooting phase

Figure 18. Appearance and characteristics of rooted cuttings, 2018

Figure 19. Root appearance, cultivar, Kansas, V3, 2018

Figure 20. Cutting and root, Kansas cultivar, V2, 2018

Figure 21. Rooted cutting, cultivar, Festiva maxima, V1, 2018

Figure 22. Rooted cutting, cultivar Dorren, V3, 2018

Figure 23. Rooted cutting cultivar, Festiva maxima, V1, 2018

Figure 24. Rooted cutting, cultivar Kansas, V3, 2018

Figure 25. Cutting and root, Pink Giant cultivar, V3, 2018

Figure 26. Planting of rooted cuttings in pots, 2018

CONCLUSIONS

Through the observations, determinations and measurements made this research led to the following conclusions:

- the propagation rate is high between 10 cuttings/plant in Kansas cultivar and 26 cuttings/plant in Dorren cultivar;
- the rooting solution in V3 variant, Rootip Basic 50 ml + Kerafol Evo 25 ml + 10 ml Fighter Phos per 10 l water was the most effective regarding the rooting of peonies cuttings green shoots;
- albeit during the first experiment the rooting percentage was below 30%, this method of propagation cannot be neglected but improved in the future; the method can be recommended because of its high propagation rate;
- the maximum length of cuttings roots was obtained in V3 variant;
- the maximum diameter of the passing area located between the root and the stem base, and of the earth bale around the formed roots was obtained in variant V3, too;
- the best percentage in terms of young roots was obtained V3 variant, too;
- based on the partial data obtained so far, this method may be recommended for propagating herbaceous peonies.

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PHYSIOLOGICAL PARAMETERS CHANGES IN TULIP BULBS DURING COLD STORAGE

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Abstract

During the cold season, it is known that the tulip bulbs (Tulipa gesneriana L.), which are specialized underground organ, go through a series of physiological (respiration and transpiration) and biochemical changes (mass loss and total soluble solids). The main purpose of this study is to compare the physiological parameters changes during storage in cold room conditions of ten tulip cultivars (Allegretto, Margarita, Menton Exotic, Lion King, Sensual Touch, Jaap Groot, Marilyn, Indiana, Atlantis, Washington), before and after storage period. The samples were stored in conditions like: T: 1°C and RH: 90%, in the Research Center for Studies of Food Quality and Agricultural Products, of the USAMV Bucharest. From obtained results it was observed decreases with 10% of the mass loss between the initial and the final moment of analyses for Allegretto, Menton Exotic, Sensual Touch cultivars. For respiration rate were observed increases with 25% (for Lion King cv.) up to 6.6 times more (for Menton Exotic cv.) between the initial and final moments.

Key words: *Tulipa, respiration rate, transpiration rate, TSS, glucose*

INTRODUCTION

Over the years numerous studies have had as main directions the optimal conditions for storing different horticultural products. The main factors that differentiate the vegetative buds into flowering buds are the temperature and the duration of storage (Delian, 2013; Toma, 2009). It is known that the tulip bulbs (*Tulipa gesneriana* L.) go through their dormancy period in summer (Burzo, 2016). During their storage, the bulbs go through a series of physiological and biochemical changes, because the low temperatures cause the end of the dormant state and the growth of the flowering buds (Burzo, 2016).

According to Toma (2009) and Marasek-Ciolakowska et al. (2018) tulips can be classified according to shapes (dwarf and tall), depending on the flowering period (from mid-spring to mid-summer), and not least by the appearance of the flower (with single or double petals, with shape of lily or with fringed petals). The bulbs are microblasts with spare materials in the fleshy leaves and are protected on the outside by cataphylls (dry leaves, thin). The stem itself is shaped like a disc, it has adventitious roots in the lower part, in the center 1-2 buds, from which develop fleshy leaves, called tunics, which have the role of storage. At the base of the fleshy leaves, the terminal and lateral buds form (Toma, 2009). In some cases the buds are tuberized and they turn into bulbs.

The main purpose of this study is to compare the changes in the physiological parameters of ten tulip cultivars, before and after the long term storage period, in cold room conditions.

MATERIALS AND METHODS

The variety groups of tulips studied are: Double late, Double fringed, Darwin, Lily flower, Fringed, Simple late and Triumph. The tulip bulbs, in uniform size for each cultivar, were stored in perforated paper bag. The tulips cultivars studied were: 'Allegretto', 'Margarita', ‘Menton Exotic’, ‘Lion King’, ‘Sensual Touch’, ‘Jaap Groot’, ‘Marilyn’, ‘Indiana’, ‘Atlantis’, and ‘Washington’ (Figure 1; Annex 1); and were stored and monitored in cold room, under following conditions: temperature (T): 1°C and relative humidity (RH): 90%, for 160 days (Burzo, 2005; Burzo, 2017), in Postharvest Technologies Laboratory of the Research Center for Studies of Food Quality and Agricultural Products - UASMV Bucharest.

The physiological parameters like: respiration and transpiration rates and the correlations with the biochemical changes like: mass loss, total soluble solids (TSS), and the concentration of glucose and fructose, were the main purpose of this study, so with T0 was noted the initial moment and with T1 the final moment, after 160 days.

Figure 1. Tulip cultivars bulbs used for physiological measurements

Respiration rate was determined with a static, closed system, in containers with hermetic closure with a volume of 280 ml (for smaller bulbs) and 1180 ml (for the bigger bulbs). With the Lambda T NDIR Monitor, ADC BioScientific LTd., the respiration rate was measured and the results were expressed in mg CO_2/kg/hour (Popa et al., 2019; Fonseca et al., 2002). By gravimetric analysis (Fante, 2014), the transpiration rate was measured and the results were expressed in g water/100 g f.w./hour.

By using the Memmert UN110 oven for drying, for 24 hours at 105°C, the water content of the samples was determined, method also used by Delian (2011). The content of total soluble solids, glucose and fructose were determined from 3 bulbs for each sample, with refractive device Kruss DR301-95 (% Brix), refractive device Milwaukee MA873 (%) for glucose and refractive device Milwaukee MA872 (%) fructose.

Using Excel, statistical analyses were performed, like: mean, standard deviation, ANOVA single factor, T Test and correlations (Pomohaci, 2017).

RESULTS AND DISCUSSIONS

For tulip bulbs, the respiration rate (Figure 2) during storage registered increases with 25% (for Lion King cv.) up to 6.6 times more (for Menton Exotic cv.) between the initial and final moments, Kanneworff W. (1994) suggesting that this behavior is an adaptation to low temperatures, being high energy users, due to number increased of mitochondria according to Khodorova (2013).

Koksal (2010) suggest that for long-term storage is not convenient due to high mass loss (Table 1).

All studied tulip cultivars registered a significant increase of respiration rate ($P<0.05$), between T0 and T1, with some particularities. Allegretto, Lion King, Marilyn and Indiana cvs. started the long term cold period storage with respiration rates up to 20 mg CO_2/kg/h, while the others registered, for the initial moment, lower values of this physiological indicator (Figure 2).

For Allegretto bulbs, between respiration rate (Figure 2) and TSS (Table 1) a very significant positive correlations $R^2 = 0.8521$, with linear regression equation $y = 0.286x + 2.9835$ and between transpiration rate (Figure 2) and water content (Table 1), a very strong significant negative correlations $R^2 = 0.9485$, with linear regression equation $y = -84.572x + 60.43$. At respiration rate, for Margarita, significant differences ($P<0.05$) were registered, for the initial moment, with Jaap Groot cv., and for the final moment with Lion King, Jaap Groot, and Indiana cvs.

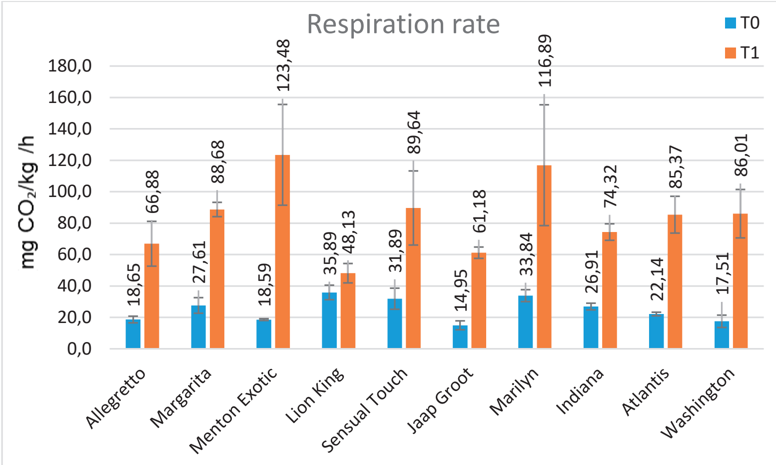

Figure 2. Respiration rate during storage in cold room

For Margarita bulbs, between respiration rate (Figure 2) and TSS (Table 1) a very strong significant positive correlations $R^2 = 0.9233$, with linear regression equation $y = 0.2399x + 2.426$ and between transpiration rate (Figure 2) and water content (Table 1), a negative correlations $R^2 = 0.6399$, with linear regression equation $y = - 48.885x + 53.061$.

At respiration rate, for Menton Exotic, significant differences ($P<0.05$) were registered, for the initial moment, with Lion King, Marilyn, Indiana, and Atlantis cvs.

For Menton Exotic bulbs, between respiration rate (Figure 2) and TSS (Table 1) a strong significant positive correlations $R^2 = 0.8178$, with linear regression equation $y = 0.0989x + 13.034$ and between transpiration rate (Figure 3) and water content (Table 1), a negative correlations $R^2 = 0.5334$, with linear regression equation $y = - 114.24x + 60.089$.

At respiration rate, for Lion King, significant differences ($P<0.05$) were registered, for the initial moment, with Jaap Groot, Atlantis, and Washington cvs., and for the final moment with Indiana, Atlantis, and Washington cvs.

For Lion King bulbs, between respiration rate (Figure 2) and TSS (Table 1) a positive correlations $R^2 = 0.5064$, with linear regression equation $y = 0.5372x - 4.5628$ and between transpiration rate (Figure 3) and water content (Table 1), a significant positive correlations $R^2 = 0.8321$, with linear regression equation $y = 100.66x + 55.588$.

At respiration rate, for Sensual Touch, significant differences ($P<0.05$) were registered, for the initial moment, with Jaap Groot cv. For Sensual Touch bulbs was determined between respiration rate (Figure 2) and TSS (Table 1) a positive correlations $R^2 = 0.7639$, with linear regression equation $y = 0.2111x + 7.0618$ and between transpiration rate (Figure 3) and water content (Table 1), a negative correlations $R^2 = 0.5135$, with linear regression equation $y = - 54.826x + 56.116$.

At respiration rate, for Jaap Groot, significant differences ($P<0.05$) were registered, for the initial moment, with Marilyn, Indiana, and Atlantis cvs. and for the final moment with Indiana cv.

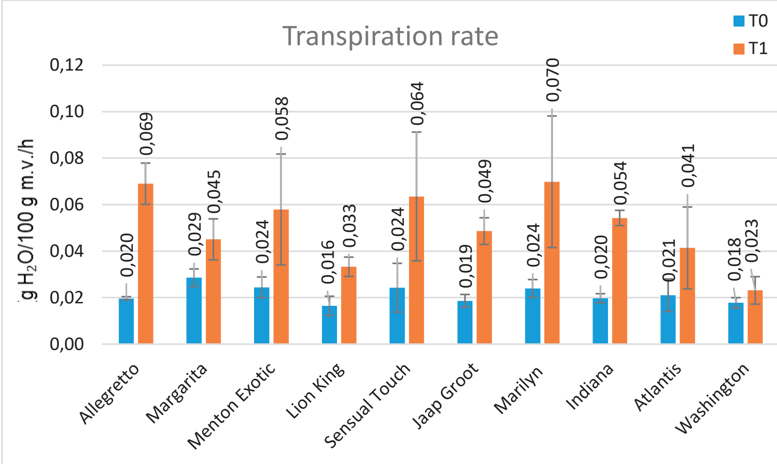

Figure 3. Transpiration rate during storage in cold room

For Jaap Groot bulbs, between transpiration rate (Figure 3) and water content (Table 1), a very significant positive correlations $R^2 = 0.9302$, with linear regression equation y = 54.504x + 56.74. At respiration rate, for Marilyn, significant differences ($P<0.05$) were registered, for the initial moment, with Atlantis and Washington cvs. For Marilyn bulbs, between respiration rate (Figure 2) and TSS (Table 1) a positive correlations $R^2 = 0.5868$, with linear regression equation y = 0.0868x + 17.725 and between transpiration rate (Figure 3) and water content (Table 1), a positive correlations $R^2 = 0.608$, with linear regression equation y = 6.4281x + 50.022.

At respiration rate, for Indiana, significant differences ($P<0.05$) were registered, for the initial moment, with Washington cv.

For Indiana bulbs, between respiration rate (Figure 2) and TSS (Table 1) was determined a positive correlations $R^2 = 0.6115$, with linear regression equation y = 0.1334x + 16.908 and between transpiration rate (Figure 3) and water content (Table 1), a very strong significant negative correlations $R^2 = 0.98$, with linear regression equation y = -9.5894x + 51.342.

Table 1. Variation of water content and TSS(%), glucose(%) and fructose(%) during storage in cold room

Samples	Water content (%)		TSS (%)				Glucose (%)				Fructose (%)			
	T0	T1	T0		T1		T0		T1		T0		T1	
			Average	Std	Average	Std	Average	Std	Average	Std	Average	Std	Average	Std
Allegretto	58.9	54.5	7.3	0.7	23.1	1.7	7.5	0.8	23.7	1.9	7.6	1.0	24.1	1.8
Margarita	51.9	50.6	8.9	0.1	23.9	2.8	8.6	0.5	24.7	2.2	9.7	1.1	23.9	1.5
Menton Exotic	59.0	51.8	14.1	1.9	26.1	0.9	14.1	2.1	27.0	0.7	14.0	1.7	27.6	0.5
Lion King	57.1	59.1	12.6	2.7	23.4	2.4	12.6	2.6	24.6	2.3	12.6	2.6	25.1	3.0
Sensual Touch	55.8	51.6	12.1	0.7	27.7	2.1	12.8	1.2	29.5	2.3	11.9	1.3	30.3	2.6
Jaap Groot	57.7	59.5	20.5	2.8	20.4	3.4	20.6	2.8	21.5	3.2	20.9	2.9	21.6	3.2
Marilyn	50.1	50.6	19.2	3.2	29.3	0.8	19.8	3.0	30.8	0.9	20.2	3.2	31.2	1.5
Indiana	51.2	50.8	20.4	2.7	26.9	2.9	21.2	2.3	27.8	3.1	21.2	2.5	30.4	1.3
Atlantis	54.2	55.5	15.9	1.8	23.3	0.8	16.1	1.8	24.3	0.8	15.9	1.4	25.0	0.7
Washington	53.5	53.4	15.6	1.3	23.6	1.2	15.9	1.4	25.0	1.5	15.4	1.7	25.5	1.8

For Atlantis bulbs, between respiration rate (Figure 2) and TSS (Table 1) a strong significant positive correlations $R^2 = 0.8424$, with linear regression equation y = 0.1113x + 13.629 and between transpiration rate (Figure 3) and water content (Table 1) was a positive correlations $R^2 = 0.4079$, with linear regression equation y = 27.33x + 54.005.

For Washington bulbs, between respiration rate (Figure 2) and TSS (Table 1) a strong significant positive correlations $R^2 = 0.8456$, with linear regression equation y = 0.1061x +

14.11 and between transpiration rate (Figure 3) and water content (Table 1), a negative correlations $R^2 = 0.2991$, with linear regression equation $y = -4.5106x + 53.501$.

The transpiration rate for tulip bulbs, (Figure 3) during long term cold storage registered great variation between cultivars, most of them due their genetics characteristics, correlated with their humidity. According to Atanasova (2020) the bulbs quality could influence the profitability ratio due to their flowering capacity and price costs.

The transpiration rate registered low variations during long term cold storage for Atlantis and Washington cvs., and significant differences ($P<0.05$) were registered for Allegretto, Jaap Groot or Indiana cvs.

The tulip bulbs water content varied according to the cultivar. The most important water loss was registered at Menton exotic bulbs, probably with the transpiration rate increase, despite the high relative humidity (RH 90%) storage conditions. Significant differences ($P<0.05$) were registered between Jaap Groot and Washington transpiration rates at final moment, as well as between Indiana and Washington cv.

The Allegreto bulbs metabolism registered in that experimental conditions, a high, increased, intensified metabolism, while the physiological processes took place at the lowest intensities at variety Lion King.

According to Burzo (2005) and Koksal (2010), exposure of the bulbs to low temperatures determine increased glucose, fructose and sugar levels that could increase the respiratory quotient and respiratory rate, too. For Allegreto and Margarita the glucose, fructose and TSS concentrations increased greatly during long term cold storage (Table 1), beeing correlated with increased respiration rates (3-4 times in 160 storage days).

CONCLUSIONS

Allegretto, Margarita, Menton Exotic, Atlantis and Washigton cultivars presented significant positive correlations between respiration rate and TSS content during storage period. Allegretto and Indiana presented significant negative correlations between transpiration rate and water content. Lion King and Jaap Groot presented significant positive correlations between transpiration rate and water content.

However, future research is needed for a better understanding of the influence of temperature on physiological and biochemical processes in tulip bulbs during long term cold storage.

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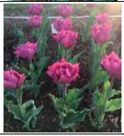
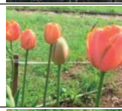
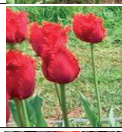
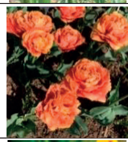
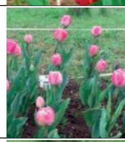
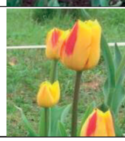
Annex 1. Characteristics of studied tulips cultivars

Variety	Group	Description	
Double late	Allegretto	the height of the flowers reaches up to 35 cm with red flowers in the lower part and yellow in the upper part	
	Margarita	the height of the flowers reaches up to 30 cm with a cyclamen flower, fragrant	
	Menton Exotic	the height of the flowers reaches up to 45-50 cm with a pink flower	
Double fringed	Lion King	the size of the flowers reaches up to 30-35 cm with a red flower beaten with a serrated edge	
	Sensual Touch	the height of the flowers reaches up to 40 cm with orange blossom beaten with a serrated edge	
Darwin	Jaap Groot	talia reaches up to 50-60 cm with yellow flower with flames and white margin, with medium flowering	
Lily flower	Marilyn	the height of the flowers reaches up to 50 cm with a white flower with red flames	
Fringed	Indiana	the height of the flowers reaches up to 50 cm, a red flower with a serrated edge	
Simple of late	Atlantis	the height of the flowers reaches up to 40 cm, the flower is purple with a yellow-white edge	
Triumph	Washington	the height of the flowers reaches up to 60-70 cm with a yellow flower with red flames, with medium flowering	

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SOME METHODS OF IDENTIFYING SURFACES WITH COMPACT SOILS FROM RESIDENTIAL AREAS

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Abstract

Healthy soils are good for sustainable landscape and for the environment protection. Its protect wildlife, allow water infiltration, reduces soil erosion, allow carbon sequestration from the atmosphere, ensure the growth of trees. These functions are disturbed if the soil drainage is not ensured due to the strong compaction or to the presence of artefacts (bricks, concrete, plastic and others). Frequently, the soils in the individual gardens are highly compact in the underlying layer at about 20 cm deep. Top soil is usually loose because the setting up of the gardens in the residential buildings occurs by levelling of land surface and cover it with fertile soil brought from other locations. After carrying out field investigations we found that the fertile soil material used to cover the land surface is not sufficient to ensure growth of all species in individual gardens. The results obtained during the investigations made it possible to develop a useful diagram for the identification of the owners of the dwellings and the gardens of the areas where the soil properties are deficient and some measures necessary to reduce the restrictions for plant growth.

***Key words**: residential area, diagram, compacted soils, identification.*

INTRODUCTION

The development and improvement of the urban horticulture is one of the current demands of residents and communities from residential area. The benefits of urban gardens and green spaces are also included control urban temperatures (i), offers favourable conditions for wildlife (ii); improve human health (iii) etc.
Achieving sustainable landscapes in individual gardens could be done only if the soil is kept loose, healthy (without pathogens), well supplied with nutrients and without pathogens contaminants. Soil in individual gardens must also ensure organic matter recycling, rain or irrigation water retention for continuously supply of plants, vigorous root system development, water infiltration and, last but not least, plant protection against pathogens.
For communities interested in gardening on a site, it is usefully to know the characteristic of the soil.
For residential area it is very important to test soil characteristics such as pH and nutrient availability, state of compactness etc. In order to create green spaces in residential areas it is also required to take into consideration the site's land use history and test the soil accordingly for potential contamination.
Soil compaction in the residential areas occurs during construction cutting and filling operations, general grading works, and other processes of running heavy equipment over the soil. After construction, soil compaction can occur with site activities such as walking, sports, and even parking heavy vehicles on grassed areas.
The strong compaction of soil causes a considerable decrease in the rate of infiltration of water into the soil. It should be noted that the root systems of plants are not symmetrical and are not a "mirror image" with the aerial part of the trees. In compacted soil a "pancake" type root system will be evident. Trees with a "pancake" type root system have not roots in a well-known shaped root ball, root growth. In compacted soil roots spread up to more than three times the diameter of the tree crown. Compaction also appears to decrease tree establishment, as roots are unable to penetrate dense soils encountered beyond the planting hole. This dramatically also reduces shoot growth (Malerechera et al., 1991; George et al., 2014; Filipov et al., 2019).

Frequently, the soils in the individual gardens are highly compact in the underlying layer at about 20 cm deep. Top soil is usually loose because the settings up of the gardens in the residential buildings occurs by levelling of land surface and cover it with fertile soil brought from other locations. After carrying out field investigations we found that the fertile soil material used to cover the land surface is not sufficient to ensure growth of all species in individual gardens. Our results obtained during the investigations made it possible to develop a useful diagram for the identification of the owners of the dwellings and the gardens of the areas where the soil properties are deficient and some measures necessary to reduce the restrictions for plant growth.

MATERIALS AND METHODS

The studied sites are located in the residential area of Iasi city (North East of Romania).

We studied several locations in the urban area of Iasi. In each location we studied topography and realized several soil profiles.

The representative soil for the studied area is Haplic Chernozems (Figure 1).

Figure 1. Haplic Chernozems with high biological activity

The choice of the location of the soil profiles was made according to the following criteria: uniformity of soil surface color (i); soil crusting susceptibility (ii); uniformity of the wetting strip after drip irrigation (iii); the growing stagnation of plants (iv); and the uniformity of root distribution (vi). The presence of the artefacts was also taken into account. In determining the locations we also took into account the instructions within Soil Survey Methodology (Florea et al., 1987).

The studied soils have been diagnosed according to the Romanian System of Soil Taxonomy (Florea et al., 2012) and World Reference Base for Soil Resource (WRB, 2014). Characterization of soil profiles was done following the instructions from guidelines for soil and land descriptions (Munteanu, 2009; Guidelines for soil description FAO, 2006).

Soil samples were taken from each pedogenetic horizon in order to conduct laboratory analyses: according to the current methodology (Dumitru et al., 2009; Lacatusu et al., 2017).

Following the processing and analysis of the data obtained in the field and laboratory, we develop a useful diagram for the identification of the owners of the dwellings and the gardens of the areas where the soil properties are deficient and some measures necessary to reduce the restrictions for plant growth.

RESULTS AND DISCUSSIONS

Our studies regarding on the establishment of some criteria for identifying surfaces with compact soils from residential areas were made in residential areas of Iasi.

The representative soil of the studied region is a Cambic Cernoziom Chernozem (after Soil Taxonomy System of Romanian Soils - 2012) or a Haplic Chernozems (after World References Base for Soil Resources-WRB, 2014).

In most cases, in the studied residential area there are several types of soil even on small areas around buildings. Within the studied location was also frequently identified Urbic Technosols, Ekranic Technosols, Humic Technosols etc.

The soil survey of disturbed soil in urban area has certain peculiarities compared to soil survey of undisturbed soil.

Disturbance of urban soil properties occurs through cutting, filling (Figure 2), compaction (Figure 2) insignificant residential areas.

Figure 2. Covering the surface of the land with subsoils material followed by strong compaction

In our studies we frequently find high proportion of artefacts (Figure 3) such as bricks, concrete, bricks, concrete, pieces of iron, nails.

Figure 3. Strong compacted soil with artefacts and stones

In frequent cases, after finishing the buildings, the soil is levelled and covered with humiferous soil material. Input of good soils does not remove plant restrictions of soil covered with a fertile layer of soil. In the first stage the plants can develop in the imported soil, but later the growing stalling or even dried. In the first stages the plants can grow in the imported soil, but later the growth is slowed down or even the plants dry out.

After construction, soil compaction can also occur with site activities such as walking, sports, and even parking heavy vehicles on grassed areas.

Identifying areas with poor soil for plants and establishing limiting properties for growing plants are useful for a successful landscaping.

After carrying out field investigations we found that the fertile soil material used to cover the land surface is not sufficient to ensure growth of all species in individual gardens. The results obtained during the investigations made it possible to develop a useful diagram (Figure 4) for the identification by the owners of the dwellings and the gardens of the areas where the soil properties are deficient and some measures necessary to reduce the restrictions for plant growth.

The required input data in order to identify area with where the soil properties are deficient for plant growing are uniformity of soil surface color, soil crusting susceptibility, uniformity of the wetting strip after drip irrigation, the growing stagnation of plants, uniformity of root distribution etc.

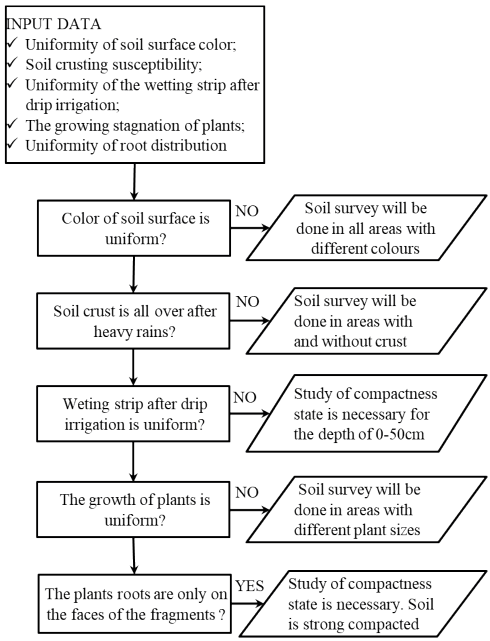

Figure 4. Flowchart for the identification area with possible restriction for plants growth

If the areas delimited according to the criteria in the diagram (Figure 4) are common for

several restrictions, then a larger study is made corresponding to all the limiting factors for plant growth.
In the cases where after the application of drip irrigation, the width of the wet band from the soil surface extends in some places, then we consider that the soil is compact and prevents water infiltration.
The restoration of compacted soils on the depth of 0-50cm could be done by the spading works and incorporation of the plants stems fragments (Figure 5).

Figure 5. Reclamation works of the strong compacted Urbic Technosols by soil loosening and incorporation into the soil of the plant stems for providing vertical drainage

The incorporation of plant stems fragments in the soil layer on the depths 20-50 cm will gradually improve the internal drainage of the macro pores that will result after decomposition of the organic matter.
The stages of improvement of the highly compacted consist of the loosening and removing of the soil of a strip of 1m and depth of 0-20cm (i), the loosening of the soil on the depth of 20-50 cm and the incorporation in the vertical or slightly oblique position of the fragments of plant stems (ii), covering the loose soil layer with the soil that was initially removed from the depth 0-20cm (iii), covering with a fertile soil layer of a thickness of 10-15cm.

CONCLUSIONS

After carrying out field investigations we found that the fertile soil material used to cover the land surface is not sufficient to ensure growth of all plants in individual gardens.
We develop a useful diagram for the identification of the owners of the gardens of the areas where the soil properties are deficient and some measures necessary to reduce the restrictions for plant growth.
If the areas delimited according to the criteria in the diagram are common for several restrictions, then a larger study is made made corresponding to all the limiting factors for plant growth.
In the cases where after the application of drip irrigation, the width of the wet band from the soil surface extends in some places, then we consider that the soil is compact and prevents water infiltration.

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ANALYSIS OF THE EXISTING RESEARCH REGARDING THE USE OF THE SPECIES *ROSA CANINA* L.

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Abstract

Rosa canina L. is a shrub found in the wild or cultivated flora, from which mature, fresh or dried pseudofruits (Cynosbati fructus) are used. This species has a key position in the sustainable culture strategy, can offer solutions to social, economic and environmental problems. Numerous scientific studies mention various uses of the seeds, petals, flowers and fruits of the wild rose in different stages of maturity as well as their biochemical content and antioxidant properties. In this regard, a comprehensive literature review was conducted to promote the usefulness of this species in several areas: nutrition, pharmacology, improvement and environmental protection. Developing knowledge about the importance of the Rosa canina species can take full advantage of a small fraction of the genetic diversity of nature.

Key words: *rosehip, food, pharmacology, breeding, environmental protection.*

INTRODUCTION

Rose hip (*Rosa canina* L.) is a species of *Rosaceae* present in Europe, Western Asia and North Africa. It is a perennial shrub of spontaneous flora of 2-3 m high, with elongated stems and branches, with solitary pink, red or white flowers (Figure 1a).

The fruit has a red-brick colour, to a deep red with a pleasant sour taste and inside there are numerous small and hairy achenes (Figure 1b).

The rose hip is commonly used as a food or medicinal plant, in the form of natural pharmaceutical products. This species has a key position in the sustainable culture strategy and can offer solutions to social, economic and environmental problems (Roșu et al., 2011).

Rose hip fruits can be used as organic products either from spontaneous flora, where treatments with synthetic products are not applied or from organic plantations.

Rosa canina hip has been used for foods, making marmalades and soups or drinks wine or tea.

Sadigh-Eteghad et al. (2011) suggest that the *Rosa canina* extract used in traditional medicine might have immunomodulatory effects.

Numerous studies indicate *Rosa canina* L. fruits with a high content of ascorbic acid, phenols, flavonoids and antioxidants (Soare et al., 2015a; Roman et al., 2013).

The highest content of the vitamin C was identified in Turkey in fresh rosehip pulp (fruit flesh of rose hip) by Kazaz et al. (2009) of 2,200 mg/100 g and by Demir & Özcan (2001), respectively 2,712 mg/100 g.

Figure 1a. Shrubs with pink flowers of *Rosa canina*

Figure 1b. Shrubs with fruits of *Rosa canina*

Other scientific studies mention different uses of seeds, petals, flowers, as well as comparisons between biochemical components and their antioxidant properties. Seeds and oil from *Rosa canina* seeds can be good sources of phytonutrients. Ilyasoglu (2014) found that rose hip seeds contain phenolic compounds (2,554 pg/g), carotenoids (2.92 pg/g) and ascorbic acid (1,798 pg/g) and also seed oil was rich in acids, polyunsaturated fats, linoleic acid (54.05%), linolenic acid (19.37%), and phytosterols, mainly β-sitosterol (82.1%).
The aim of the study was to present a review of the literature to summarize the usefulness of this species in several areas: nutrition, pharmacology, improvement and environmental protection.

MATERIALS AND METHODS

The current study concerns the actual research regarding the use of the species *Rosa canina* L., benefiting from a small fraction of the genetic diversity of nature. In this respect, the following aspects were pursued:
- use of the species in nutrition;
- pharmacological importance;
- use in breeding of the *Rosa* genus;
- importance in agri-environment measures on combating wind deflation.

This paper includes articles published in journals and books written by specialists.

RESULTS AND DISCUSSIONS

Food importance

Numerous scientific studies mention different uses of seeds, petals, flowers and rose hip fruits in different stages of maturity, as well as comparisons between biochemical components and their antioxidant properties.
As food, rose hip fruits can be used as a raw material in making tea, pasta, jam, marmalade, dessert soup, wine and juices and other simple or combined preparations with other fruits (apples, seafood, tomatoes, etc.), contributing to the diversification of nutrition. Roșculete et al. (2013) studied different processing times of rose hip fruits in combination with tomatoes, in different ratios and concluded that the time of exposure with heat treatment has influenced the properties of the final product.
Recently studies have been conducted regarding the use of rosehips in obtaining preparations in combination with various vegetables, fruits and dairy products with high nutritional properties. Mocanu et al. (2009) conducted a study on the combination of rosehip extract and milk (sample B). Sensory evaluation of descriptors: the appearance and texture, colour, smell, taste, made in comparison with the control sample, consisting of milk plus probiotics (sample A) and sample C (milk plus licorice- sweet wood extract). The best qualities were recorded in sample B.
In Turkey, projects were started to get completely new products such as rosehip ice cream (Duman et al., 2005).
In Hungary, rose hip fruits are used in the manufacture of Palinka, in Denmark powder is available from the dried fruit and in Sweden and Scandinavia, fruits of rose hip are mainly used for the production of commercial soups (Gaik, 2011).
Polyphenols extracted from plants can reduce oxidative processes, namely oxidation of myoglobin and oxidation of unsaturated fatty acids, processes that change the colour and odour of meat during refrigeration. In order to determine the antioxidant effect of polyphenols extracted from rose hip fruits (*Rosa canina*), on chilled beef, Papuc et al. (2013), found that their antioxidant activity is dependent on their concentration, maintaining the colour and aroma of this meat.

Rose hip fruits stimulate digestion and are slightly diuretic and are recommended as a decoction.

Rosa canina tea is a source of supply with natural antioxidants. Tumbas et al. (2012), following the investigation of the effect of phytochemicals in the tea of masses, showed that vitamin C and flavonoids are responsible for the antioxidant activity of the rose hip tea, while the polyphenols contribute to its anti-proliferative activity on the tumor cells (anti-proliferative activity).

Pharmacological importance

The results of many studies have shown the potential for the use of the *Rosa canina* species from a therapeutic (medicinal) point of view also. The fruits of rose hip have shown their effectiveness against arthritis, inflammation, diabetes, heart ailments, pathogens, gastric ulcers.

Numerous studies indicate that *Rosa canina* L. fruits with a high content of ascorbic acid, phenols and flavonoids, with antioxidant and antimutagenic effects. Thus, the results obtained by Kilicgun and Altiner (2010) following studies on the correlations between the content of phenolic compounds and the antioxidant mechanisms/prooxidant effects, suggest that *Rosa canina* extracts may act not only as an antioxidant, but also as a prooxidant with different effects in depending on their concentrations.

The antioxidant properties of polyphenols from fruits have been confirmed in many scientific studies, with effects against many diseases, including anti-tumor. Human erythrocytes are oxygen transporters that can be exposed to oxidative damage. Several micronutrients can protect red blood cells from oxidative stress. Widen et al. (2012), after investigating the protective effect of fruits on erythrocytes, found that they contain many compounds with antioxidant protection.

Ozturk and Ercilsi (2011), following the study of antibacterial and antioxidant activities of four taxa (*Rosa pisiformis, Rosa canina, Rosa villosa* and *Rosa dumalis subsp. Antalyensis*), found that the most effective antibacterial agent was found in *Rosa canina* which inhibited the growth of most of the bacteria tested.

The antioxidant and antibacterial characteristics of the various extracts of *Rosa canina* fruit harvested from Iran were studied *in vitro* by Montazeri et al. (2011) and their data suggest a possible use of methanol extract of *Rosa canina* as a source of natural antioxidant and antimicrobial agents.

Celik (2011) found that infusion time is a factor that has an effect on antioxidant activity. Thus, a maximum antioxidant effect was observed in the infused tea for six minutes, and a minimum antioxidant effect was observed in the infused tea for 60 minutes. Regarding the optimal dose of antioxidant, another important factor of the antioxidant activity, Kilicgun and Dehen (2009), studied five different concentrations of tea (1%, 2%, 3%, 4% and 8%), suggesting that *Rosa canina* has the potential to be used as an antioxidant at a concentration of 3%.

The *in vitro* and *in vivo* anti-inflammatory effects of *Rosa canina* extracts are still under investigation. Lattanzio et al. (2011), following the study of the anti-inflammatory and gastroprotective effect of a crude hydroalcoholic extract of *Rosa canina* in rats, found that the extract has an effect similar to that of indomethacin, suggesting its use as an adjunctive therapeutic tool for the treatment of inflammatory diseases.

The possibility of the therapeutic potential of the fruits of *Rosa canina* L. in the prevention of functional renal disorders has been studied by numerous researchers. Gholampour et al. (2012), following studies in this direction, found that *Rosa canina* appears to be useful as a preventive agent against renal damage induced by ischemic injury in rats. Similar results were obtained by Gholampour and Owji (2013), who investigated the effect of *Rosa canina* extract on liver dysfunction induced by renal ischemia in rats, and found that *Rosa canina* has a hepatoameliorative effect.

Gallic acid is another phenol present in rose hip fruits, which is appreciated for its antioxidant properties, but also for cytotoxicity against human cancer cells (Fiuza et al., 2004).

In recent years, it has been cultivated to obtain pharmacological preparations, based on rose hip, to be used in the treatment of certain diseases. Many recommendations are for osteoarthritis, rheumatoid arthritis and low back pain. However, conclusive research is needed to ascertain the therapeutic effects of mass-based products.

Several clinical studies have investigated the effects of rose hip powder on arthritis and pain caused by it. A major feature of arthritis, especially osteoarthritis, is the degradation and erosion of the extracellular-extracellular matrix (ECM) in cartilage (Schwager et al., 2011). There are few relevant studies that indicate that *Rosa canina* is a source of natural compounds (flavonoids, polyphenols, etc.), which may alter the symptoms and evolution of osteoarthritis, inducing patients an effect similar to that of analgesic drugs, steroids or anti-arthritis drugs.

Rose hip extracts have also been shown to be effective in rheumatoid arthritis, which is a chronic, inflammatory and autoimmune disease. Willich et al. (2010), following a randomized, double-blind, placebo-controlled, parallel trial with 5g/days of rose hip powder for 6 months in patients with this disease, found a significant improvement in pain, similar to that of analgesics, steroids and anti-rheumatic drugs.

Extracts obtained from the fruits of *Rosa canina* are capable of reducing the cell proliferation of cancer cells. In this respect Cagle et al. (2012) and Idassi et al. (2012), following the investigation of the effectiveness of rose hip extracts in the prevention of cell proliferation of three human glioblastoma cell lines A-172, U-251 and U -1242 MG (tumors that develop in the brain) have shown that rose hip extracts may serve as an alternative or supplement to current chemotherapy regimens for glioblastomas.

Use in breeding of the *Rosa*

• Breeding for rootstock production

Rose hip presents importance in breeding of the *Rosa* genus, especially as rootstocks for ornamental roses (Shirdel et al., 2013; Nețoiu et al., 2008). Roses are traditionally propagated by cuttings and grafting. Choosing the right type of rootstock is the key to success for *Rosaceae* family vigour, productivity and flower quality.

Farzad et al. (2009) following the study of some parameters of plant growth and flower quality in four cultivars of roses, grown on their own roots and on rootstocks of *Rosa canina*, indicated that, the parameters studied were higher in the case of cultivars obtained on the rootstocks.

Khosh-Khui and Zargarian (2010), following the study of three rose varieties grafted on four different rootstocks of Rosa (*Rosa banksiae* Ait., *Rosa canina* L., *Rosa chinensis* Jacq. *"Masquerade"* and *Rosa multiflora* Thunb), it was concluded that *Rosa canina* was the best rootstock. As the grafting is more expensive and takes a long time, lately, there has been an increasing use of "tissue culture" for rootstock regeneration, being an alternative method of rapid multiplication (Horn et al., 1998). Thus, Moallem et al. (2013) managed the induction of callus and regeneration of *Rosa canina* from leaf explants on a medium containing 1 mg l IAA. Tian et al. (2008) reported a new protocol for the regeneration of leaves of *Rosa canina*.

• Breeding for fruits

Güne (2010) in Turkey, following the study on the pomological and phenological characteristics in 11 genotypes of *Rosa canina*, selected five genotypes, respectively MR - 12, MR - 15, MR - 26, MR - 84 and YL - 04, recommending them for cultivation and certification.

Dog roses are grown in Croatia on a very small scale and most of the rose hip used in the domestic processing industry is imported (Tomljenovic et al., 2019). Also, in their study, they stated that through selection and hybridization of perspective genotypes it would be possible to create clonally propagated varieties suitable for commercial cultivation. *Rosa canina* is widespread in different plant communities. This plant community is divided into the following groups: forestry, shrublands, grasslands and ruderal (Niculescu et al., 2015).

In Romania, the natural biodiversity of this species is high, even in small habitats, where valuable genotypes can be identified for inclusion in breeding programs (Soare et al., 2014a; Soare et al., 2015b). Roman et al. (2013) studied the chemical composition of eight *Rosa canina* biotypes from the spontaneous flora of Romania (Transylvania) regarding ascorbic acid, total polyphenols, total flavonoid content and their antioxidant activity. Based on these results, two perspective biotypes were selected for breeding these species.

The cultivation of this *Rosa canina* species raises the problem of promoting valuable forms (productive and high quality fruits), or of the forms with less prickles on the stems and branches. The most effective solution in this case is to identify and take from the spontaneous flora some perspective genotypes and to include them in a breeding program. Thus, in Romania, following a study of the variability of some fruit parameters of *Rosa canina* L. in 36 genotypes, belonging to 25 indigenous populations from Bacău, Neamț and Vrancea, Ghiorghiță et al. (2012) identified valuable genotypes in terms of fruit/plant density, lower spike density, fresh biomass/fruit quantity and high vitamin C content, recommending them as gene sources in breeding this species. Also, Soare et al. (2014b) identified 20 valuable genotypes in Vâlcea county that showed a great variability of morphological characters, probably caused by altitude and phytosociological associations.

Roșu et al. (2011) identified in the Northeast area of Romania, genotypes of *Rosa* spp. with a high content in bioactive principles.

Importance in agri-environment measures

Due to reduced environmental requirements, especially those from the ground, shrubs of *Rosa* genre can be used in the establishment of plantations on land consolidation role in the establishment of shelterbelts role in combating deflation wind and the establishment of biological barriers against destructive action role the snow (Nețoiu et al., 2008; Soare and Paniță, 2009).

CONCLUSIONS

The fruits of *Rosa canina* contain a higher concentration of bioactive components which have a positive impact on health. Those are a source of vitamins and antioxidants which can be used in the food industry as functional food. But, the biochemical accumulation of useful compounds is influenced by the pedo-climatic conditions and the harvesting area.

Plenty of studies reveal the potential of rose hips uses in complementary and alternative medicine for their ameliorative effects.

By harvesting rose hip fruits and implicitly by seeds, plantations can be set up to provide biological material for grafting of the rose.

The specie *Rosa canina* should be introduced in stable cultures for rich biochemical composition and pharmacological importance and value nutritional.

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INFLUENCE OF WEATHER AND MINERAL FERTILIZERS ON GROWTH, FLOWERING AND FLOWER QUALITY OF CHINA ASTER IN THE NORTHEASTERN FOREST-STEPPE OF UKRAINE

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Abstract

This study investigates the influence of weather conditions and rates (3.0 g m^{-2}, 6.0 g m^{-2}, 9.0 g m^{-2}) of complex mineral fertilizer (Nitroamophos - $N_{16}P_{16}K_{16}$) on growth, flowering, plant height, and number and diameter of inflorescences of five China aster (Callistephus chinensis L. Nees) cultivars ('Olenka', 'Leleka', Litnia Nich', 'Tsarivna' and 'Yabluneva') during a three-year (2015-2017) study in the Northeastern Forest-steppe of Ukraine. Differences in plant height, number and diameter of inflorescences of cultivars were influenced by variations in weather conditions of the research area, cultivar characteristics, as well as the rate of mineral fertilizer. The increase in the amount of precipitation during the growing season contributed to the growth of the vegetative mass, but negatively affected the formation of the number of inflorescences. The largest inflorescence diameter was formed in the wet condition of 2016 for most cultivars. Unlike weather conditions, mineral fertilizer had considerable effect on the diameter of inflorescence. The most valuable morphological and decorative features were formed for the application of 6.0 g m^{-2} complex mineral fertilizer. Further increase in rate of fertilizer was not appropriate.

Key words: *Callistephus chinensis, cultivar, flower quality, mineral fertilizer, weather.*

INTRODUCTION

Callistephus chinensis belongs to the family *Asteraceae* (*Compositae*) of the genus callistephus (*Callistephus*). The name of the genus comes from two Greek words, *Kalistos* - "the most beautiful'' and *Stephus* - "crown", which refers to inflorescence of the flower and thus reflects the high decorative qualities of the plant. The Callisthephus genus includes only the single species *Callistephus chinensis* (L.) Ness, which has become widespread in the flower garden of Europe and other countries of the world, and also called annual aster since 1728 (Kumar & Chandary, 2018).

The natural range of *Callistephus chinensis* is northeastern China, South of the Far East of Russia, and the northern part of the Korean Peninsula. Until now, in these regions, the species is found on the rocks and clay-stony asphalts of the southern slopes of the mountains. In the wild, the plant is characterized by a fairly large habitus, non-terry inflorescences of blue color, and less decorative (Sowmya & Prasad, 2017). The first cultivars of *C. chinensis* were also unattractive, although Chinese gardens have cultivated for a long time (Chawdhuri et al., 2016).

With the development of modern floral business, *C. chinensis* is among the top three most popular crops, second only to chrysanthemums and marigolds in commercial cultivation (Kumar & Chandary, 2018; Wani et al., 2018; Chawdhuri et al., 2016). It is one of the most widespread seasonal ornamental annual flower plants worldwide, which is easily cultivated in open field, pots and bouquets (Maheta et al., 2016).

Scientists have recently and considerably increased interest in the study of this plant. Most of the works published in the last decade were devoted to investigating the growing and agricultural practices of *C. chinensis* under different conditions (Khanna et al., 2016; Kirar et al., 2014; Levandovska et al., 2017; Kumari et al., 2018). Regarding the growth and development of *C. chinensis*, the level of productivity of various cultivars is very specific to their response to weather and climatic conditions of the particular region of cultivation. Thus, *C. chinensis* cultivars do not always maintain their decorative qualities when the region of cultivation is changed. In addition, under different natural and climatic conditions, the variability of plant morphological features and the level of seed productivity are observed (Levandovska 2015; Levandovska et al., 2017).

Special interest of scientists is also focused on the influence of mineral and organic fertilizers on the morphological parameters of the plant, and as a consequence, its decorative qualities. Most of the results indicate that, the recommended rates of mineral and organic fertilizers increased the plant height, number of leaves and flowers, particularly studies by Munikrishnappa and Chandrashekar (2014). Khanna et al. (2016) also emphasize the use of manure and forest litter to produce high-quality floral products for commercial cultivation of *C. chinensis*. Additionally, the influence of fertilizers on the profusion of flowering and duration of seed maturity have been reported to accelerate seed maturity; and the specificity of action of phosphorus fertilizers and bacterial agents respectively accelerated and delayed flowering (Singh et al., 2017). Also, maximum levels of development of the vegetative and reproductive organs of *C. chinensis* were noted for the application of the recommended rates of Nitrogen and Phosphorus and combinations of NPK + Vermi-compost + Azotobactor + PSB (Maheta et al., 2016; Kirar et al., 2014). Similarly, there was an increase in vegetative growth of *C. chinensis* when micronutrients were applied (Verma et al., 2018).

In spite of these well documented positive effects, global environmental changes suggest that, mankind uses excessive amounts of mineral fertilizers (Kumari et al., 2018; Vijayakumar, 2017), which leads to negative effects on air, water and soil, and particularly soil fertility is disturbed (Barman et al., 2017). Alternatively, it is appropriate to combine mineral and organic fertilizers and apply scientifically-based doses (rates) of fertilizers for each specific plant and soil-climatic conditions (Bose et al., 2018; Sharma et al., 2017).

The present study therefore investigates the influence of different rates of mineral fertilizers (Nitroamophos - $N_{16}P_{16}K_{16}$) on growth, flowering and morphometric parameters of *C. chinensis* under the climatic conditions of the Northeastern Forest-steppe of Ukraine. For this region, such studies are very relevant and of great importance for the effective breeding of cultivars, gene pool creation and the expansion of the range of *C. chinensis* in modern landscaping.

MATERIALS AND METHODS

The research was carried out in accordance with the methodological recommendations of Dospekhov (1985). The three-year (2015-2017) research was conducted at the Educational Research and Production Center of Sumy National Agrarian University (ERPC SNAU) in the Northeastern Forest-steppe of Ukraine (N 50° 52.9; E 34° 46.2), to ascertain the effect of three different rates (3.0 g m^{-2}, 6.0 g m^{-2}, 9.0 g m^{-2}) of mineral fertilizers (Nitroamophos – $N_{16}P_{16}K_{16}$) on a set of qualitative and quantitative characteristics of the vegetative and reproductive organs of five cultivars of *C. chinensis* ('Olenka', 'Leleka', Litnia Nich', 'Tsarivna' and 'Yabluneva'). The chernozem soil of the experimental site is coarse-medium on loess loam. The arable layer has a humus content of 4.0%, nitrogen nitrate – 2.2-3.3 mg, ammonia nitrogen – 10.6-11.2 mg, mobile phosphorus forms – 137-158 mg, exchangeable potassium – 35-70 mg g^{-1} of soil. The reaction of the soil solution is close to neutral (pH 6.0).

The influence of various rates of mineral fertilizers on the growth and flowering of *C. chinensis* was studied in small-field experiments and the placement of plots was randomized. Seedlings were grown in a controlled environment of the greenhouse of the ERPC SNAU. Sowing of seeds in boxes was undertaken in the third decade of March of the studied years (2015, 2016, and 2017) respectively, on March 21, 25 and 24. In open soil, seedlings were transplanted in rows manually on areas of 3 m^2 with three replications on May 28 (2015, 2017), and May 25 (2016). Fertilizers were applied directly during the planting process in a row. Plots with no fertilizer served as control. All cultural practices including pest and disease control were performed.

Phenological observations were conducted throughout the vegetation period of plants. The phenological phases were established according to the method described by Beideman (1974) and the methods of phenological observations in the botanical gardens of the Union of Soviet Socialist Republic Academy of Sciences (USSR AS, 1979). A comparative assessment of the morphological and economic characteristics of *C. chinensis* cultivars was carried out during the flowering period in accordance with the methodology of Bilov (1978). Observations and measurements were based on the following parameters: plant height, number of inflorescence and diameter of inflorescence. Data were subjected to analysis of variance (ANOVA) using the Statistica 8 software and Duncan Multiple Range Test (DMRT) was used to separate the means at 5% level of probability.

RESULTS AND DISCUSSION

An important economic and valuable feature of *C. chinensis* is the beginning and the duration of flowering. Using the conventionality described (Levandovska, 2017) in the grouping of cultivars for the flowering period, the following are distinguished: *early-flowering* (flowering at the end of July, the duration of the period “shoots – the beginning of flowering” is 120-130 days); *intermediate-flowering* (flowering in the first half of August, the duration of the period “shoots – the beginning of flowering” is 131-145 days); and *late-flowering* (flowering in the second half of August and later, the duration of the period “shoots – the beginning of flowering” is 146-160 days). The interphase period from emergence of seedlings (germination) to the onset of seed formation for different cultivars of *C. chinensis* lasts 150-190 days. As stated (Iskrenko et al., 2015), the duration of ontogenesis and the flowering period is a genetically endemic feature that strongly depends on the varietal characteristics and favorable weather conditions during the growing season. The influence of the latter factor (weather) can vary the duration of flowering from 40 to 60 days. Under favorable conditions in the northern regions of Ukraine, the end of flowering can occur only through strong autumn frosts, which lead to the death of plants. For the southern regions, the time limit for flowering is the end of October, after which the plants dry out.

The researched varieties of *C. chinensis* ('Olenka', 'Leleka', 'Litnia Nich', 'Tsarivna' and 'Yabluneva'), according to the State Register of Plant Varieties suitable for distribution in Ukraine (SRPV, 2017), fall into three groups based on the terms of flowering, namely: (1) *Early-flowering*: (a) 'Olenka' (the plant is strong, forms a compact plant height of 28-30 cm and a diameter of 25-30 cm. The early flowering begins at the end of July and lasts until the beginning of September and at the same time blooms 8-10 inflorescences with 15-18 inflorescences in total); (b) 'Yabluneva' (compact, colon-shaped, branched plant with a height of 65-70 cm and a plant diameter of 30-35 cm. Seven (7) branches of the first-order, during mass flowering on the plant, there are 6-9 concurrently exposed inflorescences, and only forms up to 30 pieces. It begins to bloom in late July and bloom until the second half of September); (2) *Intermediate- flowering*: (c) 'Litnia Nich' (a plant with a height of 45 cm, a width of 30 cm, compact, strong, rounded form, and is very branched out). During massive flowering, there are 7-10 inflorescences that are opened simultaneously, 19-25 in total, and the flowering begins in the first decade of August. (3) *Late-flowering*: (d) 'Leleka' (broad-leaved plant, 55-60 cm tall and 50 cm wide, slightly branched, blooming at the end of August and bloom for a month). (e) 'Tsarivna' (plant height 45 cm, width 32 cm, semi-stalks, very strong. Flowering begins at the end of the second decade of August. During mass flowering, 3-4 inflorescences sprout in the plant simultaneously, with total of 6-10 pieces).

To determine the influence of weather conditions on the growth and development of *C. chinensis* cultivars, the main hydrothermal indicators provided by the North-East branch of the Institute of Agriculture of the National Academy of Sciences of Ukraine were analyzed by years of research (Table 1).

Table 1. The sum of active temperatures, amount of precipitation and hydrothermal coefficient for the years of research in the conditions of the ERPC SNAU (2015-2017 years)

Year	The sum of active temperatures (°C)	Amount of precipitation for the period of active temperatures (mm)	Hydrothermal coefficient	Moisture condition
2015	2696.1	279.9	1.04	Normal
2016	2793.0	445.8	1.60	Wet
2017	2491.0	148.0	0.59	Dry
Average long-term	2568.0	294.0	1.21	Normal

The 2015 conditions were characterized by a vegetation period close to normal. Compared to the average perennial rainfall, April was 4.1 mm, which was 35.9 mm less than normal. Respectively, in May and June, rainfall was lesser by 41.2 and 58.3 mm, whiles in July and August by 70.8 and 54.6 mm. The air temperature for all months of the growing season in 2015 exceeded the average annual figures. Specifically, in April and May by 0.4°C and 1.2°C, in June and July by 2.3°C and 1.7°C, and greatest in August by 2.5°C. During the vegetation period (April-August), the amount of active temperatures was 2696.1°C, and the precipitation was 279.9 mm.

The growing season of 2016 was characterized by high temperatures and excessive precipitation over certain months. The rainfall in April, May and August were 58.0 mm, 153.1 mm and 124.8 mm respectively, which were 18.1 mm, 99.1 mm and 67.8 mm higher than the average perennials. In June and July, rainfall fell below the norm by 3.4 mm and 13.8 mm. The air temperature during all the months of the vegetation period exceeded the average annual figures. Particularly, in April and July, the highest was by 3.0°C and in June and August by 2.0°C and 2.3°C, respectively. During the vegetation period (April-August), the amount of active temperatures was 2793.0°C, and the precipitation was 445.8 mm.

Weather conditions for the growing season in 2017 were largely characterized by high temperatures and insufficient rainfall compared with the average long-term data. In April and May the amount of precipitation was 13.4 and 31.4 mm, which were lesser by 26.6 and 22.6 mm. The least precipitation compared to the long-term data fell in June (33.2 mm) and August (15.1 mm), which were below the long-term data by 33.8 and 41.9 mm. In July, precipitation was 77.7 mm, exceeding the long-term figures by 1.7 mm. The air temperature in May was lower than the average long-terml values by 0.6°C. In all other months of the vegetation period, the temperature was higher than the norm, in particular the highest in August by 3.9°C, while in April by 0.3°C and in June and July by 0.8°C. During the growing season (April-August), the amount of active temperatures was 2491.0°C, and the precipitation was 148.0 mm.

Thus, the analysis of weather conditions, in particular the hydrothermal coefficient (HTC) as described (Selyaninov, 1937) revealed that the vegetative period of 2015 was normal (HTC = 1.04), 2016 was wet (HTC = 1.60) and 2017 was dry (HTC = 0.59). HTC were computed by the formula: HTC = $\Sigma p \times 10 / \Sigma t$, where Σp is the amount of precipitation or rainfall (mm), for a period with an average daily air temperature above 10°C; Σt is the sum of temperatures (°C), for the period with average daily air temperature beyond 10°C.

Over the years, the cultivars studied passed all stages of ontogenesis and formed seeds. The beginning of the flowering of most cultivars occurred at the end of July to the first half of August. The duration from "shoots - the beginning of flowering" varied between 120-145 days. The results on the phenology of the cultivars allowed establishing certain differences in the varietal characteristics given in the State Register of Plant Varieties suitable for distribution in Ukraine (SRPV, 2017) and the obtained three-year data shown (Table 2).

Table 2. The duration of "shoots - the beginning of flowering" and "flowering" of cultivars of *C. chinensis* (2015-2017)

Phenophase	Cultivar														
	Olenka			Yabluneva			Litnia Nich			Leleka			Tsarivna		
Year	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
Appearance of shoots	25.03	29.03	30.03	25.03	29.03	30.03	25.03	29.03	30.03	25.03	29.03	30.03	25.03	29.03	30.03
Beginning of flowering	30.07	26.07	03.08	12.08	02.08	09.08	29.07	09.08	12.08	04.08	09.08	11.08	16.08	05.08	11.08
Duration of interphase (days)	128	120	127	141	127	133	127	134	138	133	134	138	145	130	137
Duration of flowering (days)	41	49	37	30	41	37	52	39	41	55	42	41	39	44	40

Thus, according to the observations among the studied cultivars, only one cultivar ('Olenka'), which had the average period for the three years from the "shoots – the beginning of flowering" occurring within 125 days can be classified under the group of early-flowering in the conditions of the ERPC SNAU. The other cultivars have shown to be of intermediate-flowering with the duration from the "shoots – the beginning of flowering" ranging between 133-137 days. Suprisingly, the 'Yabluneva' cultivar which was declared early-flowering, began to flower on average 10 days later than the norm. Also, in contrast, the late group cultivars 'Leleka' and 'Tsarivna' reduced the onset of flowering by 18 and 16 days, respectively.

Assessing the influence of weather conditions of the year on the beginning and duration of flowering, it is revealed that, for most of the studied cultivars, the conditions of 2016, defined as wet, was the best. However, for the 'Litnia Nich' and 'Leleka' cultivars, optimum conditions were in 2015 which had a normal moisture condition. Under arid (dry) conditions in 2017, all cultivars began flowering late and had a shorter flowering period as a whole.

The use of fertilizers in the process of cultivating decorative (ornamental) plants allows for highly decorative plants with abundant number of flowers and inflorescences, a bright coloration of flowers and leaves, as well as more pronounced varietal signs. Therefore, a very important step in the cultivation of flower crops, both in closed and open soil is the rational use of fertilizers, which depends on the environment and biological characteristics of plants (Maheta et al., 2016).

The issue of applying fertilizers to ornamental herbaceous plants in the open field has been worked out rather weakly. In literature, contradictory data on the mineral nutrition of individual annual and perennial flower crops are presented or there are no such recommendations at all.

Based on the current growth in demand for aesthetic annual flowering plants, for both private and public landscaping, determining the optimal rate of fertilizer and the method of its application is an urgent necessity. During the three growing seasons, the influence of different application rates of complex mineral fertilizers on the main economic and valuable features of *C. chinensis* was investigated. Since for iliac plants, the determining factor for decoration is the duration and abundance of flowering, these parameters were subjected to analysis. Duration of flowering of plants according to cultivars for different rates of mineral fertilizers is presented (Table 3).

Table 3. Beginning and duration of the phenophase (flowering) of *Callistephus chinensis* cultivars (2015-2017)

Rates of fertilizer	Phase and date of flowering									Duration of flowering (days)			
	Beginning			Massive			End						
	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	Average
Early-flowering													
'Olenka'													
Control	30.07	26.07	3.08	6.08	29.07	8.08	10.09	13.09	10.09	41	49	37	42.3
3.0 g m^{-2}	30.07	26.07	1.08	5.08	29.07	5.08	10.09	13.09	10.09	41	49	39	43
6.0 g m^{-2}	30.07	25.07	30.07	5.08	28.07	3.08	10.09	13.09	10.09	41	50	41	44
9.0 g m^{-2}	30.07	25.07	1.08	5.08	28.07	5.08	10.09	13.09	10.09	41	50	39	43.3
'Yabluneva'													
Control	12.08	9.08	9.08	14.08	11.08	13.08	12.09	19.09	16.09	30	41	37	36
3.0 g m^{-2}	10.08	5.08	7.08	12.08	8.08	10.08	12.09	18.09	16.09	32	44	39	38.3
6.0 g m^{-2}	8.08	4.08	9.08	10.08	7.08	13.08	12.09	17.09	16.09	34	44	37	38.3
9.0 g m^{-2}	3.08	4.08	7.08	7.08	7.08	10.08	12.09	17.09	16.09	39	44	39	40.7
Intermediate-flowering													
'Litnia Nich'													
Control	29.07	9.08	12.08	5.08	10.08	16.08	19.09	17.09	23.09	52	39	41	44
3.0 g m^{-2}	29.07	2.08	12.08	5.08	5.08	16.08	19.09	16.09	23.09	52	45	41	46
6.0 g m^{-2}	29.07	1.08	11.08	5.08	4.08	15.08	19.09	15.09	23.09	52	45	42	46.3
9.0 g m^{-2}	29.07	1.08	11.08	5.08	4.08	15.08	19.09	15.09	23.09	52	45	42	46.3
Late-flowering													
'Leleka'													
Control	4.08	9.08	11.08	5.08	12.08	15.08	22.09	19.09	15.09	49	42	34	41.7
3.0 g m^{-2}	4.08	8.08	10.08	5.08	11.08	14.08	22.09	18.09	15.09	49	41	35	41.7
6.0 g m^{-2}	29.07	5.08	2.08	5.08	8.08	5.08	22.09	16.09	15.09	55	42	43	46.7
9.0 g m^{-2}	29.07	5.08	4.08	5.08	8.08	7.08	22.09	16.09	15.09	55	42	41	46
'Tsarivna'													
Control	16.08	5.08	11.08	23.08	9.08	15.08	25.09	18.09	21.09	39	44	40	41
3.0 g m^{-2}	18.08	4.08	11.08	21.08	7.08	15.08	25.09	18.09	21.09	37	45	40	40.7
6.0 g m^{-2}	6.08	4.08	10.08	13.08	7.08	14.08	25.09	17.09	21.09	49	44	41	44.7
9.0 g m^{-2}	13.08	4.08	10.08	15.08	7.08	14.08	25.09	17.09	21.09	42	44	41	42.3

The abundance of flowering depends on the biological characteristics of the cultivar, and its duration may vary depending on the soil-climatic conditions of cultivation. Also, optimal conditions for light, heat, and moisture in certain periods of plant development can help accelerate the flowering of *C. chinensis* for 5-10 days (Masaye & Rangwala, 2009;

Munikrishnappa & Chandrashekar, 2014). Thus, the analysis of the average flowering time for 2015-2017 indicated that, the minimum index (36 days) occurred in the control of 'Yabluneva' and the maximum (46.7 days) in the 'Leleka' cultivar for the application of 6 g m^{-2} mineral fertilizer. There was no significant dependence for the duration of flowering on the different rates of mineral fertilizer. However, it is proved that the duration of flowering depends on the characteristics of the cultivar and weather conditions of the year.

The plant height, according to literature, depends largely on the biological characteristics of the cultivar and is stable. It provides the strength of the plant and is one of the most important indicators that determine the general characteristics of habitus. For flowering plants, the size and shape of the plant is quite important, since it regulates the direction of its use. With the use of *C. chinensis* as a flower border, where the height variation is unacceptable, the positive response of plant height to mineral fertilizers is negative. At the same time, when cultivating varieties by cut, increasing the height increases their commercial grade.

The height of *C. chinensis* plants for application of various rates of mineral fertilizers varied within the options (Table 4). According to the description of cultivars, the samples can be divided into three groups: short (plant height 20-30 cm), medium (plant height 30-50 cm) and tall (plant height above 50 cm). Also, according to the general morphological characteristics, the 'Olenka' cultivar is short; the cultivars 'Tsarivna' and 'Litnia Nich' belong to the medium-height group, while 'Leleka' and 'Yabluneva' are very tall. However, comparing the values of measurements made in the present study at the experimental sites of the ERPC SNAU and the results of tests of the originators of the cultivars, there was a considerable decrease in plant height of the cultivars 'Leleka', 'Litnia Nich', 'Tsarivna' and 'Yabluneva' except for the early-flowering 'Olenka' cultivar.

The application of mineral fertilizers during the cultivation of *C. chinensis* had a positive effect on plant height (Table 4). The average plant height for the application of the three rates of fertilizer on 'Olenka' cultivar exceeded the control by 2-8.7%. The rest were as follows: 'Leleka' (2.4-5.6%); 'Litnia Nich' (5.0-12.7%); 'Tsarivna' (10.5-15.9%); 'Yabluneva' (8-13.9%). There was a significantly ($P<0.05$) taller plant for all five cultivars for the application of 6 g m^{-2} or 9 g m^{-2} fertilizer compared with control. For all cultivars, when compared with the maximum rate of fertilizer (9 g m^{-2}), application of 6 g m^{-2} fertilizer resulted in taller plants and the difference was as well significant for two cultivars ('Olenka' and 'Yabluneva'). Additionally, application of the minimum rate of fertilizer (3 g m^{-2}) generated significantly ($P<0.05$) taller plants in only two cultivars ('Yabluneva' and 'Tsarivna') compared to their controls.

The analysis of the influence of weather conditions on plant height showed that, the year 2017 was the most favorable for the formation of tall plants for all cultivars of *C. chinensis*, except 'Tsarivna' (Table 4). The climatic conditions of 2016 negatively affected the height of the cultivars 'Leleka' and 'Litnia Nich'. At the same time, 'Olenka' and 'Yabluneva' cultivars had a stable value for this morphoparameter for the three years. Thus, it can be concluded that, plant height was affected by the cultivar, weather conditions and mineral fertilizer.

It is important to note that, the application of mineral fertilizers during the cultivation of the cultivars 'Leleka', 'Litnia Nich', 'Tsarivna' and 'Yabluneva' did not affect the formation of sufficiently tall plants with characteristic values for these cultivars. Thus, the given cultivars for specific soil-climatic conditions tend to reduce the size, particularly, the heights and diameter of the plant, regardless of the weather conditions of the year of cultivation and supply of nutrients.

Table 4. Influence of rates of mineral fertilizers on plant height of *C. chinensis* (2015-2017)

Rates of fertilizer	Plant height (cm)			
	2015	2016	2017	Average*
′Olenka′				
Control	25.3	24.5	26.2	25.3 c
3.0 g m^{-2}	25.8	25.3	26.2	25.8 c
6.0 g m^{-2}	27.3	27.4	27.7	27.5 a
9.0 g m^{-2}	26.8	26.8	26.6	26.7 b
′Leleka′				
Control	35.7	36.3	40.4	37.5 a
3.0 g m^{-2}	39.1	35.1	41.0	38.4 a
6.0 g m^{-2}	40.1	37.4	41.4	39.6 a
9.0 g m^{-2}	39.9	36.8	41.3	39.3 a
′Litnia Nich′				
Control	37.4	36.3	35.3	36.3 c
3.0 g m^{-2}	40.9	36.5	36.8	38.1 bc
6.0 g m^{-2}	41.7	39.4	41.5	40.9 a
9.0 g m^{-2}	41.0	37.6	41.4	40.0 ab
′Tsarivna′				
Control	26.6	28.9	27.3	27.6 b
3.0 g m^{-2}	32.0	31.4	28.1	30.5 a
6.0 g m^{-2}	34.8	32.3	28.8	32.0 a
9.0 g m^{-2}	32.1	31.4	28.7	30.7 a
′Yabluneva′				
Control	36.5	38.7	37.4	37.5 c
3.0 g m^{-2}	40.2	41.3	39.9	40.5 b
6.0 g m^{-2}	42.2	42.9	42.9	42.7 a
9.0 g m^{-2}	41.9	41.7	40.6	41.4 b

*In each column for each cultivar, means with same letter (s) are not significantly different at $P<0.05$

Table 5. Influence of different rates of mineral fertilizers on the number and diameter of inflorescences of *Callistephus chinensis* ('Olenka', 'Leleka' and 'Litnia Nich' cultivars)

Rates of fertilizer	Number of inflorescences (pcs.)				Diameter of inflorescences (cm)			
	2015	2016	2017	Average	2015	2016	2017	Average*
'Olenka'								
Control	3.7	3.1	5.2	4.0 a	6.0	5.9	6.2	6.0 c
3.0 g m^{-2}	5.0	4.8	5.8	5.2 a	6.8	7.0	6.5	6.8 b
6.0 g m^{-2}	5.7	5.0	6.4	5.7 a	7.0	7.3	6.9	7.1 a
9.0 g m^{-2}	5.3	4.6	6.3	5.4 a	7.0	7.1	6.8	7.0 ab
'Leleka'								
Control	9.6	5.0	9.5	8.0 a	7.8	7.6	8.7	8.0 b
3.0 g m^{-2}	13.0	5.4	10.9	9.8 a	8.0	7.9	8.7	8.2 ab
6.0 g m^{-2}	14.0	6.6	13.0	11.2 a	8.5	8.7	8.9	8.7 a
9.0 g m^{-2}	13.3	5.7	11.3	10.1 a	8.2	8.2	8.8	8.4 ab
'Litnia Nich'								
Control	9.0	6.3	10.4	8.6 a	6.0	6.5	5.8	6.1 c
3.0 g m^{-2}	9.3	7.6	11.2	9.4 a	6.5	6.8	5.9	6.4 bc
6.0 g m^{-2}	10.3	8.2	15.3	11.3 a	7.0	7.5	6.5	7.0 a
9.0 g m^{-2}	10.3	8.0	12.9	10.4 a	6.8	7.3	6.5	6.9 ab

*In each column for each cultivar, means with same letter (s) are not significantly different at P<0.05

One of the most important indices of ornamental plants is the number and diameter of inflorescences (Levandovska, 2015). They are important because they determine the level of decoration and affect performance. The effect of different rates of mineral fertilizers on number and diameter of inflorescences of *C. chinensis* cultivars are given in Table 5 and Table 6.

The average number of inflorescences formed on plants of the 'Olenka' cultivar in the three variants exceeded the control by 30 to 42.5%. The others were as follows: 'Leleka' (22.5-40%); "Litnia Nich" (9.3-31.4%); 'Tsarivna' (16.3-48.8%); 'Yabluneva' (15.4-40.4%). Hence, the application of mineral fertilizers positively influenced the number of inflorescences for all *C. chinensis* cultivars.

However, among the treatments, application of 6 g m^{-2} fertilizer generated the highest number of inflorescences in all cultivars, but compared to control, it was only significantly ($P<0.05$) higher for just two cultivars ('Tsarivna' and 'Yabluneva').

Table 6. Influence of different rates of mineral fertilizers on the number and diameter of inflorescences of *Callistephus chinensis* ('Tsarivna' and 'Yabluneva' cultivars)

Rates of fertilizer	Number of inflorescences (pcs.)				Diameter of inflorescences (cm)			
	2015	2016	2017	Average	2015	2016	2017	Average
'Tsarivna'								
Control	5.0	3.6	4.2	4.3 b	9.0	9.3	9.0	9.1 b
3.0 g m^{-2}	6.0	3.7	5.3	5.0 ab	9.0	9.4	9.3	9.2 b
6.0 g m^{-2}	8.0	4.9	6.3	6.4 a	9.5	10.1	9.9	9.8 a
9.0 g m^{-2}	6.6	3.9	5.9	5.5 ab	9.2	9.8	9.7	9.6 a
'Yabluneva'								
Control	5.3	4.2	6.1	5.2 b	7.9	7.6	8.1	7.9 b
3.0 g m^{-2}	6.0	5.1	6.8	6.0 ab	8.0	7.9	8.7	8.2 b
6.0 g m^{-2}	9.0	6.0	6.9	7.3 a	8.7	8.5	9.2	8.8 a
9.0 g m^{-2}	8.5	5.3	6.5	6.8 ab	8.5	7.9	8.8	8.4 ab

*In each column for each cultivar, means with same letter (s) are not significantly different at P<0.05

Weather conditions of vegetation had an impact on the number of inflorescences. The wet conditions of the year 2016 proved to be the least favorable for the implementation of the reproductive potential of all studied cultivars (Table 5; Table 6). For the cultivars 'Leleka', 'Tsarivna' and 'Yabluneva', the greatest number of inflorescences was formed in 2015 (normal moisture conditions), whiles for 'Olenka' and 'Litnia Nich', it was in 2017 (dry). According to the research findings, there was a certain correlation between the weather conditions of the year and the number of inflorescences. So, the increase in the amount of precipitation during the growing season contributes to the development of the vegetative mass, but also negatively affects the formation of the number of inflorescences in *C. chinensis*. For most cultivars, the optimal conditions for the development of the reproductive phase occurred in the year (2015) that had normal conditions for moisture, but for the 'Litnia Nich' cultivar, it was the dry condition of the year 2017.

The inflorescence diameter like other indicators, tended to increase for the application of different rates of mineral fertilizers in all studied cultivars (Table 5; Table 6). For the 'Olenka' cultivar, the increase in the average diameter of inflorescences due to fertilizing was 13.3-18.3% compared to control. The others were as follows: 'Leleka' (2.5-8.8%); 'Litnia Nich' (4.9-14.8%); 'Tsarivna' (1.1-7.7%); 'Yabluneva' (3.8-11.4%). Consequently, the diameter of inflorescence responded positively to the application of mineral fertilizers. Nonetheless, for all treatments, application of 6 g m^{-2} fertilizer resulted in a significantly ($P<0.05$) larger average diameter of inflorescences except for the maximum fertilizer applications (9 g m^{-2}) and also application of 3 g m^{-2} fertilizer for the 'Leleka' cultivar.

Based on the weather conditions of the research years, variations in the diameter of inflorescence for the five cultivars are shown (Table 5; Table 6). It was established that the largest average inflorescence diameter were formed in 2016 for three cultivars 'Olenka' (6.8 cm), 'Litnia Nich' (7.0 cm) and 'Tsarivna' (9.7 cm), and in 2017 for 'Leleka' (8.8 cm) and 'Yabluneva' (8.7 cm). For most cultivars in 2015, the average diameters of inflorescences were average, with values ranging between that of the other research years (2016 and 2017), except for 'Tsarivna' which recorded the least (9.2 cm). Unlike mineral fertilizer, weather condition did not have a considerable effect on the diameter of inflorescence.

CONCLUSIONS

During the growing season, differences in plant height, number and diameter of inflorescences of *C. chinensis* cultivars ('Olenka', 'Leleka', 'Litnia Nich', 'Tsarivna' and 'Yabluneva') were influenced by variations in the weather conditions of the research area, cultivar characteristics, as well as the rate of mineral fertilizer. The largest increase in height when fertilizing, compared to control was observed in the cultivar 'Tsarivna' (15.9%), while the smallest was in 'Olenka' (2.0%). It was established that, increases in the amount of precipitation during the growing season contributes to the growth of the vegetative mass, but negatively affects the formation of the number of inflorescences of *C. chinensis*. For most cultivars, the optimal conditions for the growth of the reproductive phase occurred in the year (2015) that had normal moisture, but dry condition (2017) for the 'Litnia Nich' cultivar. The largest inflorescence diameter was formed in the wet condition of the year 2016 for most cultivars ('Olenka', 'Litnia Nich' and 'Tsarivna') and in 2017 for 'Leleka' and 'Yabluneva'. Largely, cultivars in 2015 had average inflorescence diameter. Unlike weather conditions, mineral fertilizer had considerable effect on the diameter of inflorescence. Thus, in comparison with control, the inflorescence diameter was the largest for the cultivar 'Olenka' (18.3%) and the least for 'Tsarivna' (1.1%). The most valuable morphological and decorative features were formed for the application of 6.0 g m^{-2} complex mineral fertilizer (Nitroamophos – $N_{16}P_{16}K_{16}$). Further increase in rate of fertilizer was not appropriate.

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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF WATER EXTRACTS FROM *ROSA DAMASCENA* PETALS GROWN IN KAZANLAK VALLEY, BULGARIA

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Abstract

The current study aimed to evaluate the phenolic content, total flavonoid content, as well as the antioxidant activity in water extracts obtained from Rosa damascena petals grown in Kazanlak valley, Stara Zagora region, Bulgaria. The rose samples were collected during May 2019 from the plantations in Skobelevo, Asen, Iasenovo, and Koprinka villages grown under different conditions. The contents of total polyphenols and flavonoids in the water extracts from rose petals were determined. Additionally, the antioxidant activity of rose water extracts was evaluated by four reliable methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays. The highest values of total phenols and flavonoids were found in the rosewater extract from organically grown plantation: 47.09±2.89 mg GAE/g dry weight and 6.87±3.00 mg QE/g dry weight, respectively. The highest radical scavenging activity demonstrated the extracts from organic plantations, while the metal-reducing assays showed higher antioxidant potentials in the extracts from conventionally grown roses. The results demonstrated the potential application of these edible flowers rose extracts as ingredients or additives for food and cosmetic purposes with the potential antioxidative properties.

Key words*: antioxidant activity, petals, phenolic content, Rosa damascena, water extracts.*

INTRODUCTION

Rosa genus, belonging to the *Rosaceae* family, includes 200 species and more than 18.000 cultivars. One of the most important Rosa species is *Rosa damascene* Mill (Patil et al., 2015), which is the hybrid between *R. gallica* and *R. phoenicia* Mahboubi (2016). This plant is called damask rose because it was originally brought to Europe from Damascus (Patil et al., 2015). Nowadays, Bulgaria and Turkey are the main producers of *R. damascena* essential oil in the world and the Bulgarian *R. damascena* oil is the known best ones (Mahboubi, 2016).
The oil-bearing rose (*Rosa damascena* Mill. f. Trigintipetala Dieck) is an emblematic plant species for Bulgaria. The industrial rose cultivation in Bulgaria involves exclusively the species *R. damascena* Mill f. Trigintipetala Dieck (Kazanlashka roza), due to its higher rose oil content and superior essential oil quality (Kovacheva et al., 2010). Recently, there has been a growing interest in antiviral, antibacterial, anticancer, and antioxidant activities on *R. damascena* products (Mahboubi, 2016). For instance, Baydar and Baydar (2013) reported that fresh and spent flowers extract obtained from *R. damascena* could be a good natural antioxidant source.
In recent years, industrial production of organic oil-bearing roses has become one of the fastest developing sectors of organic agriculture in Bulgaria. Today, all rose plantations and rose flower processing facilities are private property. Organic producers are concentrated in southern Bulgaria. Essential oil crops including roses are produced mainly in the Sub-Balkan Valley as well as in the Thracian Valley, which is considered the most appropriate for oil rose and lavender production (Chalova et al., 2017).
The interest in organic products is growing worldwide principally because consumers are concerned about the amounts of agrochemicals, pesticides, synthetic growth stimulants, and antibiotics in foods, as well as in genetic

modifications (Torjusen et al., 2001). The application of an organic source of nutrients is rapidly attracting attention. Organic maturing has beneficial impacts on soil properties and produces safe plants. It is reported in the literature that some vegetables of organic origin present a higher content of flavonoids when compared to the same products coming from conventional cultivation, as in the work of Chassy et al. (2006), where authors compared organic with conventional tomatoes. Ren et al. (2001) determined the content of polyphenols in five commonly consumed in Japan, produced by organic and conventional cultivation. The contents of flavonoid (quercetin) and caffeic acid in organically grown plants were 1.3-10.4 times higher than those found in conventional plants. The effects of organic and conventional cultures on anthocyanins, total phenolics, and antioxidant activity in blueberry fruit were studied (Wang et al., 2008), and the results showed that fruit produced following organic procedure showed a higher content of some concerning conventionally grown plants. Many investigators obtained the best results by using organic compost for several medicinal and aromatic plants (Taie et al., 2010). Besides, Ginova et al. (2013) evaluated total phenols and radical scavenging activity of rose samples collected from seven industrial-scale plantations in Bulgaria during 2009-2010 (Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo and Gurkovo). But unfortunately, there are not so many studies connected with the influence of different agriculture systems on the antioxidant activity of *Rosa damascena.*

The aim of the current study was to evaluate the phenolic content, total flavonoid content, as well as the antioxidant activity in water extracts obtained from *Rosa damascena* petals grown in Kazanlak valley, Stara Zagora region, Bulgaria.

MATERIALS AND METHODS

Plant material

The samples were collected from arable areas in Southern Bulgaria, a region called Rose (Kazanlak) Valley. The Rose Valley is situated in the middle of the country between the Stara Planina Mountain (North) and Sredna Gora Mountain (South). The valley is around 90 km long and around 10 km wide and the average altitude 350 m. For the experimental study, six oil rose plantations were selected (Table 1). In three of them have been applied to an organic agriculture system and in the rest of the farms, a conventional agriculture system was used. The google maps coordinates, altitude, soil type, and geographical area are presented in Table 1. Conventional agricultural practices utilized nitrogen fertilizers and pesticides, drip irrigation, and mechanization. Organic crops cannot be genetically engineered, flowers were collected manually, fields were not irradiated, or not fertilized. Additionally, farmland used to grow organic crops is prohibited from being treated with synthetic pesticides and herbicides for at least 3 years before harvest. Rose flowers of *R. damascena* were collected in May 2019 in the morning (6-8 a.m.), in 3, 4, and 5 phases as described by Staikov and Zolotovich (1954). Plant material was air-dried in shade at room temperature and grounded in a mechanical grinder (final powder size less than 400 µm). The samples are stored in a dark and cool room at 16-18°C before the analysis.

Table 1. Locations of the sampling plantations in Kazanlak valley, Bulgaria

Farm	Area	Google maps coordinators Latitude (N) Longitude (E)		Altitude	Soil type
	Conventional agriculture system				
1	Damascena 1, Skobelevo	42.670050	25.196783	520 m	Fluvisols
2	Damascena 2, Skobelevo	42.668950	25.198150	520 m	Fluvisols
3	Koprinka	42.633433	25.329483	400 m	Luvisols
	Organic agriculture system				
4	Skobelevo	42.672067	25.177383	522 m	Fluvisols
5	Asen	42.643567	25.175150	483 m	Fluvisols
6	Yasenovo	42.693767	25.277983	510 m	Fluvisols

Extracts preparation

Rose petals were extracted with distilled H_2O in solid to liquid ratio 1:10 (w/v). The extraction procedure was performed in an ultrasonic bath (SIEL, Gabrovo, Bulgaria, 35 kHz, and 300 W) for 20 minutes, at 65 °C. The obtained extracts were filtered, and the residues were extracted once again under the above-mentioned conditions. The combined extracts were used for further analysis.

Total phenolic contents

Total phenolic content was measured using a Folin-Ciocalteu reagent. Briefly, 1 mL Folin-Ciocalteu reagent (diluted five times) was mixed with 0.2 mL rose petals extracts and 0.8 mL 7.5% Na_2CO_3. The reaction was performed for 20 minutes at room temperature in darkness. The absorbance was measured at 765 nm against the blank. The results were expressed as mg equivalent of gallic acid (GAE) per g dried weight (dw), according to Ivanov et al. (2014).

The total flavonoids content

The total flavonoids content was analyzed using $Al(NO_3)_3$ reagent as previously described (Kivrak et al., 2009). After 40 minutes the absorbance was measured at 415 nm against the blank. The results were presented as mg equivalents quercetin (QE) per g dry weight (dw) according to the calibration curve linear in the range of 10-100 μg/mL quercetin (Ivanov et al., 2014).

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Rose petals water extract (0.15 mL) was mixed with 2.85 mL 0.1 mM solution of DPPH in 100% methanol. The sample was incubated for 15 minutes at 37 °C. The reduction of absorbance was measured at 517 nm in the comparison to the blank containing methanol and % inhibition was calculated (Ivanov et al., 2014).

2,2′-azino-bi's-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay

ABTS radical cation ($ABTS^+$) was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Then 0.15 ml rose petal extract was mixed with 2.85 ml of the ABTS solution previously diluted with methanol (1:30; v/v). After 15 min at 37 ºC in darkness, the absorbance of the formed complex was measured spectrophotometrically at 734 nm (Ivanov et al., 2014).

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain (1996) with slight modification. The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6- tripyridyl-striazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3 \times 6H_2O$ in distilled H_2O. FRAP (3.0 ml) reagent was mixed with 0.1 mL rose petal extract. After 10 mins at 37 °C in darkness, the absorbance was measured at 593 nm against blank prepared with water (Ivanov et al., 2014).

Cupric reducing antioxidant capacity (CuPRAC) assay

Rose petal extract (0.1 mL) were mixed with 1 mL $CuCl_2 \cdot 2H_2O$, 1 mL Neocuproine (7.5 mL in methanol), 1 mL 0.1 M ammonium acetate buffer and 1 mL distilled water. The solution was incubated at 50 °C for 20 minutes in darkness and the absorbance was measured at 450 nm (Ivanov et al., 2014).

All the results from the determination of anti-oxidant activity were performed in triplicates and expressed as mM Trolox equivalents (mM TE) by dry weight. All the data were expressed as mean ± standard deviation (SD).

Data analysis

The data were expressed as mean ± standard deviation (SD) from three replicates for each sample. To determine the significant differences between values, and used to perform ANOVA statistical analysis. Statistical analysis with all data was also done with Unscrambler (Camo, Norway) software packages.

RESULTS AND DISCUSSIONS

These results for antioxidant activity and phenolic content from the water extract of rose were summarized in Table 2.

The highest values of total phenols and flavonoids were found in the rosewater extract from organically grown plantation from farm 3 Asen area: 47.09±2.89 mg GAE/g dry weight and 6.87±3.00 mg QE/g dry weight, respectively. In general, total phenol content in rose petals decreased in the following order of the collecting area: Asen> Skobelevo> Yasenovo (organic agriculture system)>

Damascena 1, Skobelevo> Damascena 2, Skobelevo> Koprinka (conventional agriculture system). In rose farms with conventional agriculture systems, the level of total phenols ranged from 19.64 to 13.97 mg GAE/g dw (Table 2). The reported in our study values for total phenols were higher than the reported values for rose petals from seven industrial-scale Bulgarian plantations (Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo, and Gurkovo) (from 5.85 to 11.80 mg GAE/g dw). Damask rose gave the highest total phenolic content (31.9 mg GAE g-1 sample) (Sommano et al., 2018), which was following our results for organic rose petals. According to Sengul et al., (2017) the total phenolic content of Turkish rose petals (*Rosa damascena* Mill.) was 481.54 µg GAE/mg sample, while Ge and Ma (2013) found that total phenolic content in petals of Yunnan edible roses was 2087.43 ±17.37 mg GAE/100 g fresh weight. The range of total phenols content in rose teas was 50.7 to 119.5 mg gallic acid equivalents (GAE) per gram of dry matter (Vinokur et al., 2006). Our values for total phenols in organically grown roses were also comparable with reported values for phenolic content in Turkish rose petals syrup 64.94 µg GAE/mg sample (Sengul et al., 2017).
The values of total flavonoids were the highest in the organic agriculture system (farm 4 to 6), where the content varied between 4.63 to 6.87 mg QE/g dw. In comparison to the roses from the conventional agriculture system, the content of total flavonoids was twice higher (Table 2).
It was observed that the levels of total phenols and flavonoids in rose petals from an organically grown area demonstrated approximately 45-50 % higher values in comparison with conventionally grown roses (Figure 1).

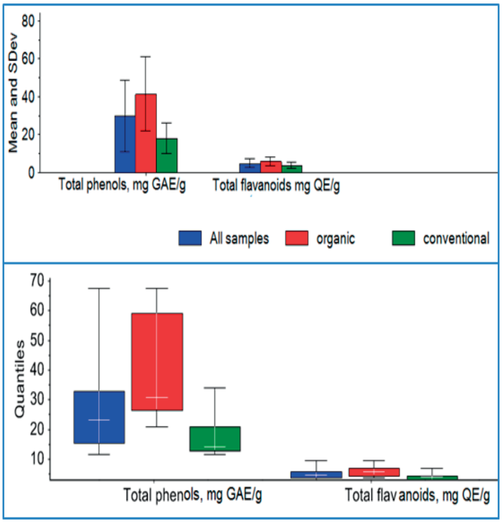

Figure 1. Statistical data of total phenols and total flavonoids of water extracts from *Rosa damascena* petals

Table 2. Total phenols, total flavonoids and antioxidant activity in water extracts from conventionally and organically grown roses

Farm	Area	Total phenols, mg GAE/g dw	Total flavonoids, mg QE/g dw	Antioxidant activity, mM TE/g			
				DPPH	ABTS	FRAP	CUPRAC
Conventional agriculture system							
1	Damascena 1, Skobelevo	19.64±0.08	4.58±1.88	439.38±11.98	379.77±4.81	811.62±91.08	541.59±20.90
2	Damascena 2, Skobelevo	17.62±0.24	3.05±1.71	316.38±2.45	371.11±16.24	803.34±2.70	560.81±19.36
3	Koprinka	13.97±0.13	2.58±0.15	298.68±10.74	320.08±11.04	945.94±12.36	925.85±27.88
Organic agriculture system							
4	Skobelevo	39.01±0.21	4.63±0.99	311.20±5.94	240.97±6.52	237.39±7.58	486.34±13.10
5	Asen	47.09±2.89	6.87±3.00	347.20±8.76	546.13±22.84	322.75±4.25	848.13±5.92
6	Yasenovo	30.50±0.04	5.32±0.27	583.83±13.18	638.44±18.48	1875.80±11.05	563.90±5.04

It was reported previously that quercetin and kaempferol are antimicrobial compounds synthesized by plants in response to pathogen attack (Dixon and Paiva, 1995). Because organically and sustainably grown products were produced by cultural methods utilizing no or very little pesticides, pathogenic pressures may explain the higher total phenolic levels found in the organically and sustainably grown samples (Asami et al., 2003).
The antioxidant activity of rose petals extracts was evaluated by four methods based on the

different mechanisms (DPPH, ABTS, FRAP, and CUPRAC). It was found that organically grown samples (Table 1) demonstrated better radical scavenging activity evaluated by methods base on hydrogen transfer (DPPH and ABTS assays). However, conventionally grown roses showed higher results for antioxidant activity evaluated by methods based on electron transfer as FRAP and CUPRAC (Table 1 and Figure 2). In general, the roses collected from farm 6, Yasenovo area showed the highest result for antioxidant activity evaluated by DPPH, ABTS and FRAP methods from 583.83 to 1875.8 mM TE/g dw. Organic rose petals from Asen and Yasenovo areas demonstrated the highest antioxidant activity evaluated by ABTS method. Vinokur et al. (2006) also reported that rose petal teas from different cultivars exhibited scavenging capacity toward 2,2′-azino-bis-(3-ethylbenzothiazoline)-6sulfonate cation radical (ABTS+) ranging between 712.7 and 1770.7 μM Trolox equivalents (TE) per gram of dry petals. However, reported in the current study results for antioxidant activity of *Rosa damascena* were higher than these of Vinokur et al. (2006) and comparable with reported for rose petals from seven industrial-scale Bulgarian plantations in Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo and Gurkovo (Ginova et al., 2013).

All these results suggested that the contents of polyphenolic and antioxidants in rose petals are strongly influenced by environmental, e.g. geographical and edaphic, factors as previously described by Ginova et al. (2013). Moreover, the current study demonstrated that agriculture practices also strongly influence the content of total phenols and flavonoids in rose petals (Table 1). A similar trend of the increase in phenolic compounds in organically grown samples, as fruits and vegetables were reported by some authors (Ren et al., 2001; Asami et al., 2003; Borguini et al., 2013).

Vickery and Vickery (1981) mentioned that many secondary metabolites as phenolic compounds, for example, act as fungicides and antibiotics to protect the plants from fungi and bacteria. Therefore, higher content of phenolic compounds in organic food occurs because of the possible incidence of pests and pathogens in the organic cultivation method, in which pesticides are not used. An increase in phenolic compounds production is connected with the natural defences of plants (Borguini et al., 2013).

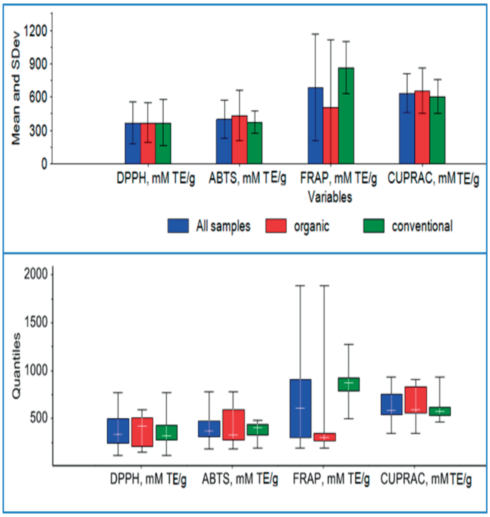

Figure 2. Statistical data of antioxidant activity of water extracts from *Rosa damascena* petals among the different methods

Table 2 presents the correlation coefficient (r) values established based on linear correlations between phenolic content and antioxidant activity, evaluated by four methods. It was established a high correlation between total phenols and total flavonoids in rose flower water extracts, r = 0.78 (Figures 3). The content of total flavonoids exhibited a significant correlation with antioxidant activities in the DPPH and ABTS, with r = 0.58 and 0.69, respectively. That correlation is graphically illustrated in Figure 4. Correlation analysis showed that total antioxidant activity, evaluated by DPPH and ABTS assay, is highly related to the total phenols and total flavonoids (Table 2 and Figure 4). Amongst the methods used for quantifying antioxidant activities, the correlation between ABTS and CUPRAC was 0.53. Büyüktuncel el al. (2014) reported a high correlation between FRAP and CUPRAC; FRAP and ABTS with 0.886 and 0.870, respectively for wine samples. Probably, the low correlation in our study among the rest of the methods could be explained by the use of the water extracts, not organic solvents. According to many authors (Vinokur et al., 2006; Hou et al., 2014; Patil et al., 2015), the antioxidant activity of *R. damascena* is not related to anthocyanin level but is correlated to total phenolic, flavonol contents of *R. damascena*. According to Vinokur et al., 2006, the antioxidant capacity of rose petals is correlated well (r = 0.78-0.88) with the contents of total polyphenols. In our case, total flavonoids expressed as quercetin equivalent is correlated well with radical scavenging activity of water extracts (Figure 4).

Table 2. Pearson's correlation coefficients (r) between total phenols, total flavonoids content and antioxidant activities of water extracts from *Rosa damscena*

	Total phenols, mg GAE/g	Total flavonoids, mg QE/g	DPPH, mM TE/g	ABTS, mM TE/g	FRAP, mM TE/g	CUPRAC, mM TE/g
Total phenols, mg GAE/g	1.00					
Total flavanoids, mg QE/g	**0.78**	1.00				
DPPH, mM TE/g	**0.51**	**0.58**	1.00			
ABTS, mM TE/g	0.39	**0.69**	0.43	1.00		
FRAP, mM TE/g	-0.38	-0.18	0.33	0.37	1.00	
CUPRAC, mM TE/g	0.39	0.42	0.14	**0.53**	0.05	1.00

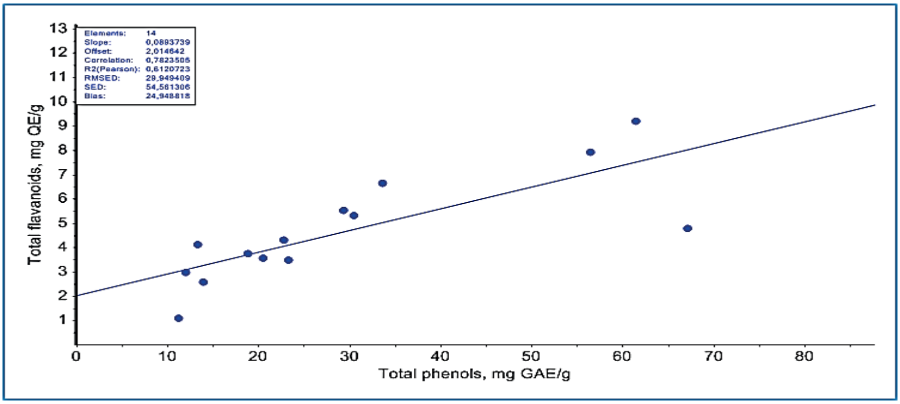

Figure 3. Scatter plots of correlation between total phenols and total flavonoids

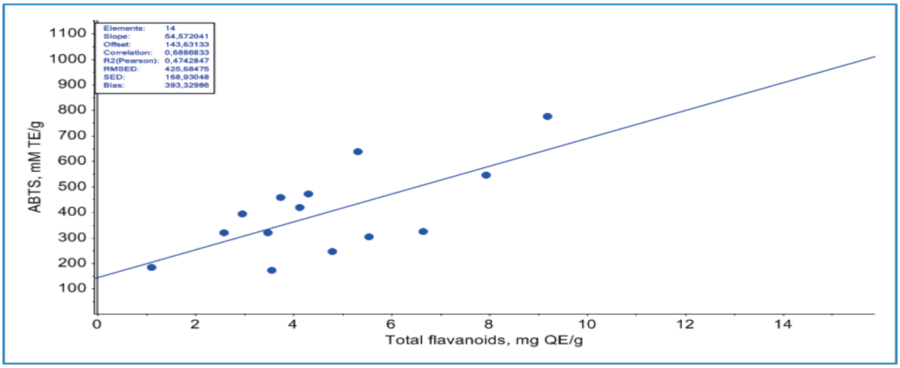

Figure 4. Scatter plots of correlation between total flavonoids and ABTS assay

CONCLUSIONS

In conclusion, the current results demonstrated that antioxidant properties and phenolic compounds of oil-bearing rose petals grown at conventional and organic agriculture systems. A significant variation was found in antioxidant activity and phenolic compounds between two agriculture practices. The radical-scavenging activity in rose petals was mostly due to the high levels of total flavonoids, followed by total phenols. Although the amount of these compounds varied between samples, organically grown samples exhibited high levels of radical scavenging activity. The metal-reducing assays showed higher antioxidant potentials in the extracts from conventionally grown roses. The results demonstrated the potential application of these rose water extracts as antioxidative ingredients or additives for food and cosmetic purposes.

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SARMIZEGETUSA REGIA BETWEEN AN ARCHAEOLOGICAL SITE AND A NATURAL PROTECTED AREA. CULTURAL LANDSCAPE AS THE MISSING LINK

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Abstract

The present paper, based on the first extensive landscape and biodiversity study lead in Romania on an archaeological site, tackles the main problems generated by the double protection of the site of Sarmizegetusa Regia in Orăștie Mountains, the most iconic archaeological site of the Dacian period. Both part of the chain of Dacian fortresses enlisted on the UNESCO World Heritage List and part of the Natural Park Grădiştea Muncelului - Cioclovina (IUCN category V), the site of Sarmizegetusa Regia is facing serious management issues due to this dual strict protections systems. The paper will present a detailed analysis concerning the problems generated by the lack of harmonisation between the two management plans issued from two different types of protection, but also by the lack of a specific category of heritage and its specific protection instruments: the cultural landscape. The article aims to find, based on the site analysis, a series of landscape-based solutions for a coherent management plan, based on landscape values and methods, in order to respond to the present and urgent problems that the Dacian site is facing today.

Key words: *Sarmizegetusa, UNESCO archaeological site, Natural Park, cultural landscape, protection systems.*

INTRODUCTION

Sarmizegetusa Regia represents one of the most important archaeological sites of Romania, being registered in the National Archaeological Repertory with the RAN code: 90397.01 and classified as a historical monument - LMI code HD-I-s-A-03190. Its importance also led to the inclusion in the World Heritage List - UNESCO - 906/1999/C, Dacian fortresses in the Orăștiei Mountains, being declared by OMCC 2483/2006 area of priority archaeological interest. The statute of historical monument also led to the establishment of the protection area of the actual archaeological site (18.3 ha), this occupying 66 ha. An additional protection area, which includes the part of the Dacian fortress that was not thoroughly explored and properly valorised, occupies 281.2 ha (Figure 1).
Simultaneously, Sarmizegetusa Regia is an integral part of the Grădiștea Muncelului-Cioclovina Natural Park (PNGM-C), established at the county level in 1979 by Decision no. 452 of the Executive Committee of the People's Council of Hunedoara County and reconfirmed in 1997 by the Decision of the County Council no. 13.

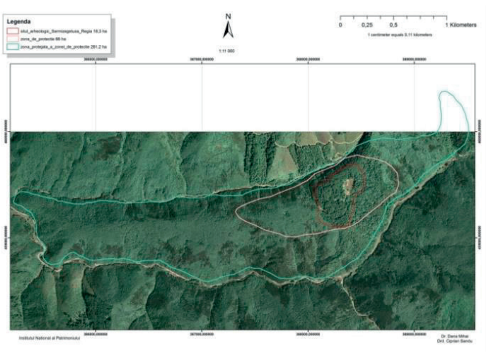

Figure 1. The limits of the Sarmizegetusa Regia protection area. Source: INP - Heritage National Institute

By Law no. 5/2000, PNGM-C it is declared a natural protected area of national interest, being classified, according to OUG (Government Emergency Ordinance) no. 57/2007, approved with modifications and addenda by Law no. 49/2011, with the subsequent modifications and addenda, in the category of natural parks, which corresponds to category V IUCN – "*Protected landscape: protected area managed mainly for landscape conservation and recreation*". The purpose of the establishment of this natural park is to preserve a series of landscape ensembles where the interaction of human activities with nature has created over time a distinctive area, with significant landscape and / or cultural value, often with a great biological diversity. Grădiștea Muncelului-Cioclovina Natural Park covers an area of 39818 ha, its limits being established by HG (Government Decision) no. 230/2003.

In addition, the PINMATRA/2001/018 project, finalized in 2002, integrated the area of Sarmizegetusa Regia within the surfaces with virgin and quasi-virgin forests. With a high degree of naturalness, these forests were included in the areas of integral protection. In such forests, their protection status being equivalent to the IUCN I categories - *areas intended for the protection of "wilderness"*, where no active human intervention is allowed.

In this context of double protection - as a historical monument included in the list of UNESCO world heritage (cultural heritage) and as an integral part of PNGM-C (natural heritage), it would have been expected that Sarmizegetusa Regia would enjoy not only upon a special attention from the public and local and central administration but also on a number of suitable management and protection measures. However, the onsite reality is far from an ideal one.

The incorporation of Sarmizegetusa Regia, as well as a whole series of Dacian fortresses within PNGM-C as well as within the virgin and quasi-virgin forests (Dacian beech forests) led to a series of conflicts between the policies for natural heritage protection and those of protection and valorisation of the cultural heritage. Thus, the Management Plan of PNGM-C initially included Sarmizegetusa Regia in the areas with complete protection, which subsequently led to the impossibility of interventions to manage the arboreal vegetation within the archaeological site. This fact led in time to the degradation of the historical monument, degradation caused, among others, by the collapse of the trees over the ruins of the Dacian fortress. Only in 2016, with the revision of the management plan of PNGM-C, the archaeological site, with an area of 18.3 ha, was included in the areas of sustainable management, which generated the initiation of projects and intervention plans for the protection and the valorisation of the Dacian fortress. On the other hand, the rest of the archaeological site remains in the area of integral management, which keeps on the major difficulties regarding the protection and the valorisation of the archaeological site. This recent registration of the archaeological site within the sustainable management area finally allows tree maintenance and management interventions as well as conservation works and forestry treatments that promote the natural regeneration of the forest: the treatment of transformation cuts toward gardening, the treatment of gardening and quasi-gardening cuts.

MATERIALS AND METHODS

Sarmizegetusa Regia archaeological site and its relation with the landscape

The first aim of the study was to understand the landscape in a past-present relation both at large and detailed scale. Thus, our understanding of the landscape was based on historical and archaeological data and on *in situ* reading of the ancient landscape. An important aspect was to understand the relation with the natural landscape within the Dacian culture. Looking at the larger scale we have to understand Sarmizegetusa Regia as part of a (military) system that generated a specific landscape (Olteanu, 2007). The fortifications in Orăștie area are built using stone walls: Costești, Blidaru, Vârful lui Hulpe, Bănița, Luncani-Piatra Roșie and the linear fortification at Cioclovina-Ponorici. There are also discovered a series of civil settlements in Costești, Fața Cetei, Fețele Albe, Sarmizegetusa Regia or scattered households in Rudele, Tâmpu and Meleia (the latter being apparently related to the iron exploitation). The development of the Dacian settlements in this area occupied by forests involved the massive deforestation of the territory, both to allow land shaping (the setup of the terraces on which it was being built) and in order to get the wood that represented the main building material and that was also used for the exploitation of the iron ore (Neamțu et al., 2016; Oltean, 2007). A number of researchers, following C. Daicoviciu, have advanced the hypothesis that these fortifications had a system of communication with each other (using signal fires) but this seems unlikely given the structure of the relief that prevents a visual connection between them. However, conform to our topographical analysis made in AutoCad, such communication was possible through a possible "relay" placed on Muncel or Godeanu Peaks that could have visual communication with the cities in the area (Figure 2). In this respect, the presence of today's forests is cancelling these potential visual relations that are difficult to understand in the current landscape.

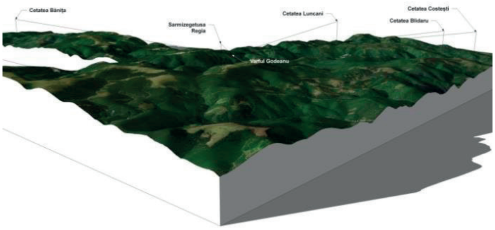

Figure 2. The visual-territorial relations between the settlements within Orăștie Mountains area. Source: Tudora, 2018, p. 37

From the excavations carried out at Sarmizegetusa Regia it results that the households grouping was made on a series of flat terraces, forming true neighbourhoods located to the west and east of the fortification and the sacred area (Glodariu, 1983; Oleanu, 2007). The civil settlement of Sarmizegetusa is not accessible to the public and archaeological research has revealed only a part of the habitable terraces. The terraces occupied by the housing within the ensemble are difficult to read in the landscape due to the presence of the dense forests (Figure 3). So far, the households groping model and their relation with the urban structure (public space?) has not been analysed. At Sarmizegetusa Regia the largest households as well as the ones with the most important artefact inventory are clustered near the fortress, this fact determining renowned historian and archaeologist C. Daicoviciu to name this area "the aristocratic neighbourhood".

Other annex constructions discovered in the residential areas are the workshops, in Dacian antiquity the production of the objects and utensils necessary for the daily activity being made either within the household (pottery, weaving, leather processing) or by craftsmen who worked in specialized workspaces (Neamțu et al., 2016)

Figure 3. Area archaeologically researched (2017) within the space of the western neighbourhoods of Sarmizegetusa. Source: personal archives (2018)

A settlement of such dimensions as it was Sarmizegetusa Regia, or better said the surface where discoveries have already been made, extends over 6 km, most of these sites not being valorised and being actually covered by forests- also presumes the development of specific infrastructures with traces legible in the landscape.

The roads from Dacia were, for the most part, natural, following without special setups the water courses or the ridges. Often the route of the ancient roads is perfectly visible on long sections to this day, for example in Căpâlna, Costeşti and Sarmizegetusa. Some of these roads, described by C. Daicoviciu and H. Daicoviciu (1960), are no longer visible due to their coverage with vegetal layers and dry leaves. There were also paved roads such as the one from Sarmizegetusa Regia between the western civil neighbourhood, the fortification and the sacred area (Glodariu, 1983). Within the current visit route, the present arboreal vegetation has a positive effect on the perception of the ancient road due to the filtering of the images of the lower terraces, which unfold as a surprise during the site's visit, and create a vegetal arch along the route (Figures 4 and 5).

The most extensive works to adapt at the landscape were represented by the setup of the terraces, these representing the basic infrastructures on which the buildings were erected. These were usually bricked up on the lateral sides and towards the hill, being open to the valley (usually toward south) but there are also simple terraces, without supporting walls, including at Sarmizegetusa. The width of the terraces ranged from 20-30 m to hundreds of meters. Ground levelling in variable proportions is found everywhere in the settlements located on slopes (Glodariu, 1983). Even now, part of the households in the area are organized on landscaped terraces, whereas terraces developed in the past being still legible in the landscape.

Figure 4. The axial perspective along the paved road made more dynamic by the rhythm of the trees

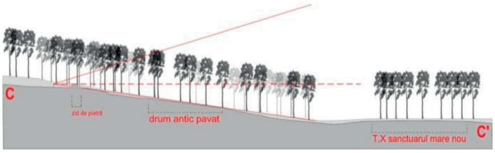

Figure 5. Section through the ancient paved road. Source: Personal archives/Tudora, 2018

However, most of the terraces are still unreadable within the forests that cover them (Figure 3). The site currently open to the public was also covered by forests at the time of discovery, but then massive deforestation was allowed in order to support archaeological research and to valorise the site thus unveiled (Figure 6).

The fortifications built of stone *(murus dacicus)* in the area of Orăștie Mountains were built from the 1stcentury B.C. In the fortified areas there were usually discovered two or three more valuable buildings, traces of barracks and other household setups. The fortification from Sarmizegetusa Regia is built in several stages that have not been clearly identified. An attempt to discern the successive stages of construction and the techniques used was performed by Oltean and Hanson (2017) based on LiDAR technology (Figure 7).

Figure 6. a. The construction on the terrace II - appearance during the excavations. b. The andesite plinths on the terrace X - appearance at their discovery. Source: C. Daicoviciu apud Neamțu *et al.,* 2016

Figure 7. The present contour of the fortification mapped with LiDAR technology and the paths of the stone road (west) and the paved road (east). Source: I.A. Oltean and W.S. Hanson, 2017

The forests covering of the fortification area make impossible its overall perception or its relation with the topography and the territory (Figure 8), the perception of the general landscape being impossible on terrace I, the highest one within the site (Figure 9).

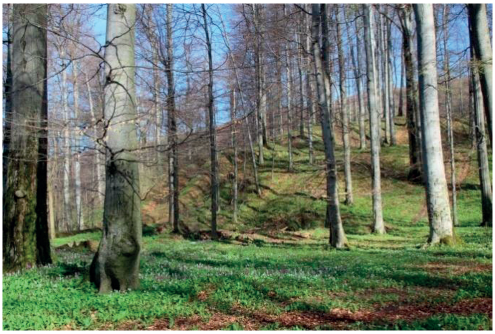

Figure 8. Crossing path and closed perspective to the top with terrace I. Source: personal archives (2018)

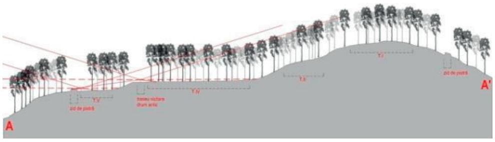

Figure 9. Close perspectives within the fortification and the lack of valorisation for the topography (generator of the fortress ensemble) within the perspectives. Source: Tudora, 2018

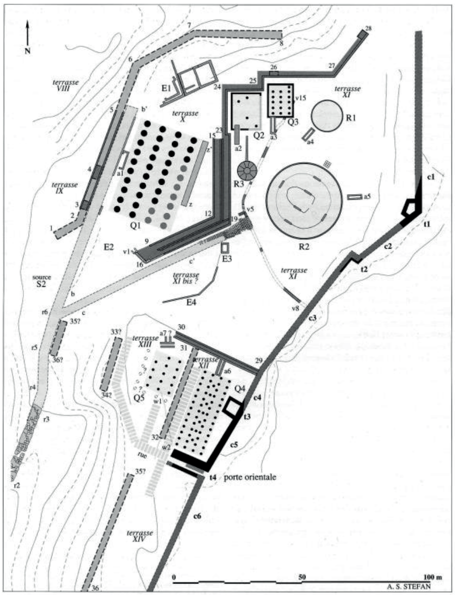

Figure 10. Number designation for the terraces from Sarmizegetusa Regia. Source: Ștefan, 2001

Clearly, the major interest within the Sarmizegetusa Regia is represented by the sacred area that unfolds on terraces VIII-XIII (Figure 10). From these terraces the arboreal vegetation was almost totally removed to allow a clear perception of the site and its structures. The presence of the two sprouts *(Picea abies)* generates a separation between terrace XI - the largest, which includes the circular sanctuaries - and the terraces XII and XIII, located at a small difference in level but having totally different structures (Figure 11). The terraces within the sacred ensemble are delineated by dense forests toward the edges of the area opened to the public. The whole sacred area is cut out like a clearing in the surrounding forest (Figures 12 and 13).

Figure 11. The presence of the two spruces on the terrace XI. Source: personal archive (2018)

Figure 12. General aerial view on Sarmizegetusa Regia. Source: Oltean, 2007.

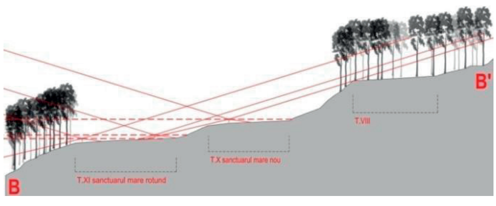

Figure 13. Section with the succession of the main terraces within the sacred area, Source: Tudora, 2018

On the other hand, in this area, a number of problems caused by the presence or absence of arboreal vegetation become much more visible. Thus, some of the slopes from the terraces show stability problems, the roots of the trees located on the cornices leading to the fragmentation and the collapse of the slopes (Figure 14). The most serious such situation is represented by the pentagonal tower, partially destroyed by the push generated from the trees that collapsed over time (Figure 15).

These matters send to another part of the research regarding the state of the arboreal vegetation from this site, an issue that will be analysed in the next subchapter.

Beyond the terraces of the sacred area, the eastern and western civil neighbourhoods, otherwise clearly marked on the information panels at the entrance to the site, as well as the Roman baths, are practically inaccessible and unreadable due to the vegetation from the site (Figures 16-17).

Figure 14. Terraces IX and VIII seen from the terrace XI – the collapse of some slopes between terrace IX and VIII can be observed here, in the background it is also noticeable the mound within the fortification. Source: Culescu, 2018

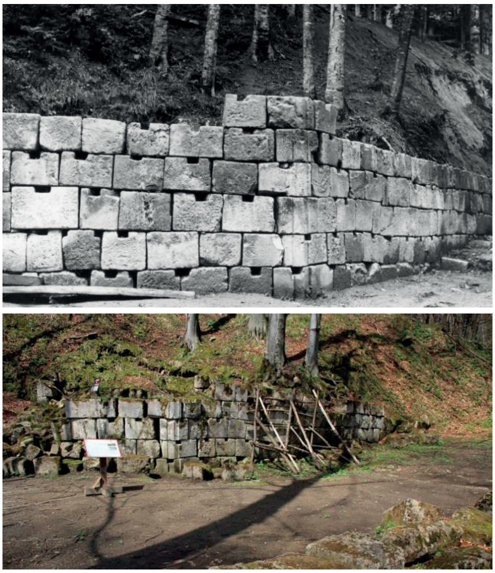

Figure 15. a. The pentagonal tower: photograph from the University Babeș-Bolyai archives (1960s) b. Current image: pressure created by trees on the wall and the destabilization of the terrain edge under the trees' weight are both visible. Source: personal archives (2018)

Figure 16. The pathway within the western civil neighbourhood. Source: personal archive (2018)

Figure 17. The Roman Bath. Source: personal archives (2018)

The state of the arboreal vegetation and the relation with the archaeological site

The assessment of the vegetation in the Sarmizegetusa Regia site was done conforming to the CODIT method described and developed by Alex Shigo (1998) and VTA method (visual tree assessment) that was and Claus Mattheck (1994, 2007). For a better legibility of the images used in the article the trees' injuries, crack, open wounds, or other problems assessed *in situ* are highlighted in red, using Adobe Photoshop CS3.

Regarding the vegetation, the largest share in the forest mix is represented by the beech *(Fagus sylvatica)* and the hornbeam *(Carpinus betulus)*. Within the protection zone and, respectively, the protected area, compact groups of spruce *(Picea abies)* and pine *(Pinus sylvestris)* can be found sporadically. At this vegetal level it is also important to mention the presence of two species introduced by man: the black locust *(Robinia pseudoacacia)* and the plum tree *(Prunus sp.)*. Thus, at the beginning of the access road to the archaeological site (in the DJ705A parking area) a group of black locust trees (invasive species) can be observed. Inside the site, south of the large round Temple, a plum tree is inserted, which still exhibits characteristics specific to the planting material developed for production, as well as indicators regarding its planting on this site, thus excluding the hypothesis of spontaneous occurrence.

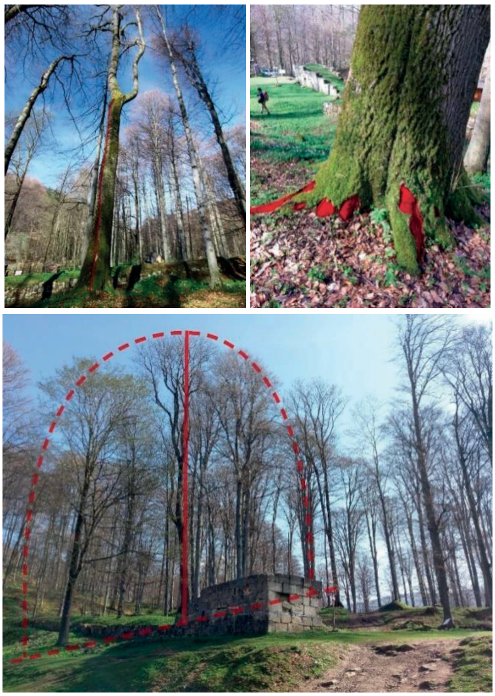

Figure 18. Cracks extended throughout the entire height of the trunk; injuries to the base of the trunk/the potential fall space, which intersects the fortress walls. Source: Culescu, 2018

These interventions are narrow, but they are extremely important due to the implications they bring in an area that has already been, for a significant period of time, under national and international protection regarding the conservation of the habitats form this space.

From the health point of view, broadly speaking, the general state of the tree vegetation within the site is currently relatively precarious. Many of the trees placed in the vicinity of the areas used by visitors or near the archaeological components exhibit a series of problems (open wounds, cracks, etc.) or deficiencies in the general architecture of the plant (broken branches, forks, stumps, etc.), some of them presenting the risk of collapsing and destroying the archaeological remains, as is the case with the ash *(Fraxinus excelsior)* located at the western gate of the fortress (Figure 18). This is not the only tree with the risk of collapse, such situations being found throughout the entire perimeter of the site (Figure 19).

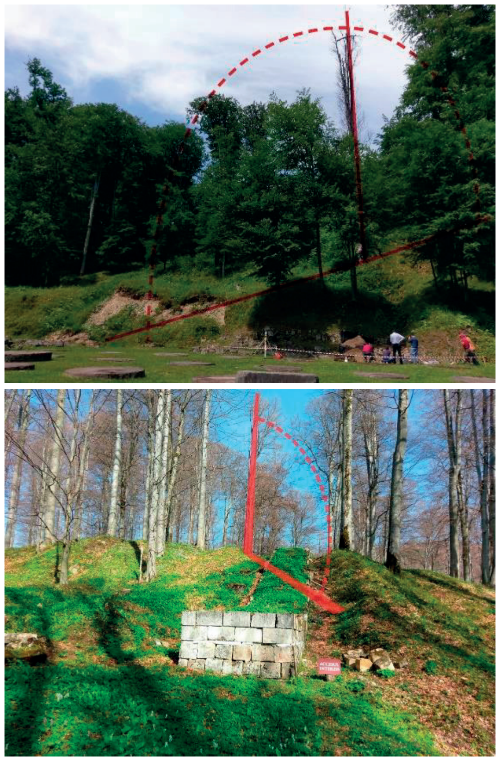

Figure 19. The potential fall space for other trees with problems within the site that intersects other elements of the archaeological ensemble. Source: Culescu, 2018

The trees located on the cornices of the terraces generate the danger of their collapse, thus leading to the destruction of the component structures of the Dacian fortress. These destructions, from the point of view of cultural heritage, are practically irreparable and irrecoverable (Figure 20).

Figure 20. Landslides and other problems associated to them. Source: Culescu, 2018

If the trees from the site can have somewhat negative impact on the general status and safety of the archaeological remains, as well as the safety of the visitors, in turn the visit of the site, in the absence of properly marked routs, leads to the alteration of the arboreal vegetation. Of the effects of improper site setup or lack of it, we mention here only two aspects. The first is related to the visiting routes that are not properly made or maintained. This results in the exposure of the roots and can, in time, determine the destabilization of the affected specimens. Injuring or cutting the roots opens new gates for diseases and pests, thus leading in time to the debilitation of the trees. Moreover, because those problems cannot be detected visually, it is difficult to know where and how to intervene, in a timely manner, in order to stop or to slow down the debilitation (Figure 21).

Figure 21. Tree roots of exposed due to the usage patterns. Source: Culescu, 2018

Another aspect related to the site management is the attachment of information panels or “site management tools” to the trees, which leads, in addition to the derisory image of the site, to injuring the trees and, subsequently, to generating other health problems for the affected trees (Figure 22).

Figure 22. Panels fixed on trees. Source: personal archives (2018)

Regarding the state of the arboreal vegetation, two problems are recurrent, being encountered on this site for a large number of trees. One of these is the presence of the wounds at the base of the trunk (Figure 23) - a defect that, especially in the absence of an adequate maintenance process, questions the future integrity of the affected specimens.

Figure 23. Trees with wounds at the base of the trunk. Source: Culescu, 2018, p. 47

The second problem is the presence of cracks in the trunk (Figure 24) - an aspect that, in combination with the wind, can in time lead to the disintegration of the plants. The defective crown architecture contributes already very visible with trunk pressure forces. Also, the elimination of other trees can change the microclimate specific to each individual and, by exposing them to different wind directions and forces, it can potentiate the existing defects, ultimately leading to the loss of the affected plants.
Within the site, there can be encountered several other problems, such as: the occurrence of open wounds in the trunk caused by the fall of other trees, tumours, branches with defective growth or insertion caused by the friction of the elements. Although these problems have a lower recurrence, they cannot be neglected, constituting additional factors in destabilizing the trees from this site. Last but not least, the presence of wood in a more or less advanced process of decomposition (improvised furniture, pavement, etc.) is also affecting the general condition of the trees, often speeding up their weakening and, finally, leading to their disappearance.

Figure 24. Trees with cracks along the trunk.Source: Culescu, 2018

RESULTS AND DISCUSSIONS

As the *in situ* landscape analyse and vegetation assessment that we lead in 2018 are the first of this kind made on an archaeological site in Romania comparison with similar situation are difficult to be made. We can only imagine that similar situation are to be found also in other Dacian fortresses sites of Orăștie Mountins as all of them are part of the Natural Park of Grădiștea Muncelului – Cioclovina and thus exposed to the same conflictual situation generated by the two protection systems.
As the 2018 study was more of a preliminary one, for a more clear and precise image on the situation in Sarmizegetusa Regia a more detailed analyse has to be foretaken. Such a detailed study should be led by INP together with its collaborators, with specialists of Romsilva that are in charge with the management of the forests in the area, and with specialists in charge with the management of the Natural Park.
Our partial result concerning the landscape in general and the trees in particular are only pointing to the otherwise chronical problems of the Sarmizegetusa Regia archaeological site that is, for the most part, not valorised and inaccessible.

The degradation of the vegetation and its poor maintenance lead to the destruction of this archaeological site of world importance. Within its perimeter, the interventions on the trees are allowed only at the request of the specialized bodies (to read forestry bodies), in order to carry out the works of repair, current maintenance, archaeological research, restoration, consolidation and conservation of the historical monument. The real problem is that these interventions cannot be performed at the request of the site administration or the archaeologists who work on the site and are prone to react only to the danger of destroying the components of the historic ensemble. In addition, when these interventions are carried out, the protection measures are unsuitable or lack completely from the execution process, and this has already generated additional damage. The way of organizing the tree cuts, the lack of protection measures and the approach of the site only as a part of the forest generates irreparable damage (Figure 25).

Figure 25 a. Trees collapsed due to natural causes (2012). Source: http://www.anchetadehunedoara.ro/sarmizegetua-regia-o-istorie-furtunilor-care-au-devastat-monumentul-unesco-ii// b. Trees collapsed following cuts. Source: personal archives (2018)

What can be easily observed is the fact that we are facing a lack of correlation of the mechanisms and instruments of protection for the natural and cultural values within the PNGM-C, although it is appointed, according to Law no. 5/2000, in the category of natural parks, corresponding to the category I IUCN - “Protected landscape: protected area administered mainly for landscape conservation and recreation.” Thus, in accordance with Annex 1 of OUG no. 57/2007 -The purpose and the management regime for the categories of protected natural areas - it is stipulated in letter e): *Natural parks are those protected natural areas whose aims are the protection and conservation of some landscape ensembles where the interaction of human activities with the nature has created over time a distinct area, with significant landscape and / or cultural value, often with a great biological diversity. The management of the natural parks aims to maintain the harmonious interaction of man with nature by protecting the diversity of habitats and landscape, promoting the preservation of traditional land uses, encouraging and consolidating the activities, practices and traditional culture of the local population. Likewise, recreational and tourism opportunities are offered to the public and scientific and educational activities are encouraged.* However, the PNGM-C Management Plan is limited to a number of measures aimed at protecting natural habitats but does not provide sustainable development resources for communities.

Even though the PNGM-C Management Plan established the reassignment of the surface of the archaeological site (only the part accessible to visitors) within the sustainable management category, within the Forest Management Plan this space is still embedded in the integral management category. As a result of the spring 2018 cuts, Grădiște Forestry Department has requested the reassignment within the Forest Management Plan for the area related to the archaeological site from the integrated protection category to the one designated for sustainable management (the classification made at the time of the development of the Forest Management Plan is not complying with the PNGM-C Management Plan) but, however, this measure does not guarantee a change of attitude for the workers on field. In this regard, beyond the necessary changes to be made to the Forest Management Plan (where the situation was not resolved during the last 6 months), it is also necessary to draw up a vegetation management plan for the site, accompanied by a plan for organizing the execution works to ensure avoiding any subsequent damages caused by the condition of the trees or by the works for their removal.

Actually, in the current legislative system the landscape remains a topic reduced only to natural and environmental aspects although the international legal framework emphasizes the cultural dimension of the landscape. The landscape is defined by IUCN as an area "where the interaction of people and nature over time has produced an area of distinct character with significant ecological, biological, cultural and scenic value: and where safeguarding the integrity of this interaction is vital to protecting and sustaining the area and its associated nature conservation and other values". Yet, the landscape values within PNGM-C are reduced to biodiversity issues, without taking into account the cultural values of the area or the scenic features that could enhance both the natural and cultural heritage.

Further analysis on Sarmizegetusa Regia and on other Dacian fortresses in Orăștie Mountains can help to a better understanding of values and site management for these unique UNESCO recognised archaeological sites. For the moment, our preliminary conclusions are related only to this first landscape study.

CONCLUSIONS

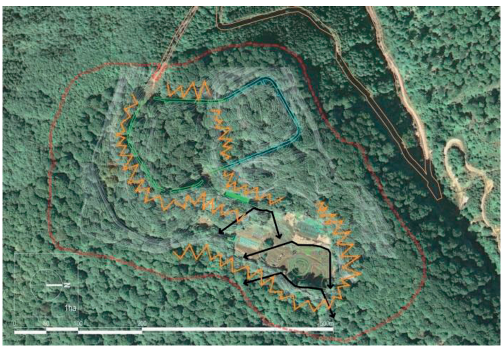

Figure 26. Perception of arboreal vegetation as visual screens around and within the site. Source: Tudora, 2018

From the cultural landscape point of view, the spatial and visual relation of Sarmizegetusa Regia with the territory and the landscape, the relation that led to the development of the settlement on these places, is today non-existent (Figure 26) due to vegetal screens (in orange) that block the major downward perspective towards point of interest (in black). Although the forest has long been a factor of protection of the site and, to a large extent, it represents one even today, there are a number of obvious problems generated by the need to valorise the archaeological site in the context of retaining the forest. On the other hand, dead or decaying trees are a real danger for the site. In addition, obviously, the fact that the forest has invaded the former Dacian settlement makes detailed archaeological research difficult, which also leads to misapprehending the importance and the territorial dimension of this settlement by the general public.

For now, it is very obvious the inability to mediate the two protection systems - natural and cultural - established within the space where the archaeological site is located. In addition, turning the site into a tourist attraction and opening it to visitors brings additional pressure for the ensemble. It is important to note that, although the area benefits from the presence of valuable habitats, they are not unique. On the other hand, the interaction of visitors with these habitats should be done safely, but this is not possible considering especially the degradation state of the arboreal vegetation. This degradation has so far led to the destruction of unique archaeological artefacts and, most likely, will continue to cause damage in the absence of a proper management.

Beyond solving the problems that result from the inter-institutional non-corroboration of the protection measures for cultural and natural values, the following interventions or projects for valorising the site appear as necessary starting from landscape architecture principles:

- Development (with an interdisciplinary team) of a project for valorising, restore and conserve the Sarmizegetusa Regia entire site as a whole, not only for the 18.3 ha area;
- Modernization and adequacy of the infrastructure for visiting and comprehend the site based on its valorising project;

- Development, together with the PNGM-C, of mountain routes for tourist that include the archaeological sites - on the old Dacian and Roman roads and pathways, marked as such, allowing pedestrian access to the area;
- Clear on field marking of the protection perimeters for the archaeological sites;
- The immediate recovery and protection of the materials dislocated from the walls of the fortress for future restoration;
- Valorisation, restoration and preservation of the monuments within the site and from the extended protection area, based on the aforementioned project, to ensure protection when the number of visitors increases;
- Performing interventions at landscape level to valorise the site and its protection area based on a landscape design plan.

As short-term emergencies, it stands out, beyond the safety of the ensemble components, the need to reorganize and setup the visiting routes, an intervention that is directly related to the safety. Route setup must be done with non-invasive and minimal means and should consistently aim to limit the interaction between visitors and valuable elements of the site and the continuous degradation of the land. In this respect, traditional methods of modelling the terrain or making paths, steps or pathways are recommended. The reversibility of these setups is a criterion of outmost importance. Another requirement is to maintain the naturalness of the site from the point of view of the image provided by the site, this being one of its major qualities at this moment. The *mise-en valeur* of the site can be done with soft installations and instruments specific to landscape architecture and land art.

To ensure the success of the maintenance of the arboreal vegetation it is recommended to appoint a so-called gardener of the site, respectively a professional - preferably with experience in the field of arboriculture - who will come to know in detail the condition of the trees that form the vegetal ensemble. Thus, the interventions carried out on each tree will take into account its vulnerabilities, its strengths, etc. and to ensure continuity and consistency in decision-making regarding tree interventions.

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USE OF VEGETABLE FUNCTIONAL INGREDIENTS TO ACHIEVE HYPOGLYCEMIC BREAD WITH ANTIOXIDANT POTENTIAL, FOR DIABETICS

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Abstract

For diabetics, diet is a major therapeutic tool and a special nutrition form, in which food is adapted to metabolic disorders of disease. Bread is a key ingredient of the human diet and is consumed by billions of people worldwide. In this paper are presented results of the performed research to achieve a hypoglycemic bread assortment, with antioxidant potential, for diabetics. In the composition of bread, nutritionally valuable functional ingredients were used, but also with antioxidant potential: Jerusalem artichoke flour, hemp flour, apple waste flour, hemp husked seed and oat bran. The biphasic process was applied to obtain bread, and when the dough was fermented, the natural sourdough enriched with phenolic compounds and inulin was used. Bread for diabetics has superior sensory quality, high nutritional value and antioxidant potential. It has a low content of available carbohydrates (38.56-39.49%) and is noted for its content in protein (8.85-9.12%), total fiber (3.15-3.70%) and total polyphenols (100.85-115.12 mg GAE/100 g). At the same time, this product has an antioxidant capacity (117.45-125.15 mg TE/100 g). Shelf-life of this bread is 4 days.

Key words: *antioxidant, bread, diabetics, hypoglycemic, potential.*

INTRODUCTION

Diabetes is a metabolic disease, with an increased incidence both internationally and nationally. According to the International Diabetes Federation, in 2017, there were worldwide, 425 million people living with diabetes (largest age group: 40-59 years) and 352 and 352 million pre-stage of diabetes, called Impaired Glucose Tolerance (IGT). Also, by 2045, the number of people with diabetes is expected to rise by 48% worldwide (International Diabetes Federation, 2017).
Jerusalem artichoke tubers (*Helianthus tuberosus*) are characterized by their content in proteins, minerals potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and inulin. Inulin can be used in the diet of diabetics as a sugar substitute without having an impact on glycaemia (Meyer & Blaauwhoed, 2009; Long et al., 2016). Jerusalem artichoke tuber (*Helianthus tuberosus* L.) is considered a functional food (Radovanovic et al., 2015). Catană et al. (2018) achieved a functional ingredient (powder) with high nutritional value and antioxidant potential by processing of Jerusalem artichoke tubers (Red Jerusalem artichoke and White Jerusalem artichoke varieties). This powders achieved from Jerusalem artichoke tubers are important sources of minerals (K, Fe, Mg, Ca, phosphor - P) inulin and bioactive compounds. Thus, Jerusalem artichoke powders are characterized by a total polyphenol content of 18.51-44.03 mg GAE/g. Also, these powders have antioxidant potential being beneficial in a healthy diet for prevention of diseases caused by free radicals. On the other hand, powders achieved in this study are characterized by high inulin-type fructans content (51.60-57.45%), being beneficial for achieving of bakery and pastry products for diabetics. Hemp flour is a valuable component for enriching physicochemical and antioxidant properties of

wheat bread (Mikulec et al., 2019). Flours from hemp are also a rich source of bioactive compounds from the polyphenols group, which have an anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-viral, anticancer and cardioprotective effects (Callaway, 2004; Manach et al., 2005). Bread with hemp flour was characterized by significantly higher protein content (13.38–19.29 g/100 g DW), in comparison to wheat bread (11.02 g/100 g DW). The share of hemp flour influenced the polyphenols content by increasing from 256.43 (standard bread) to 673.59 mg GAE/kg (50% of the additive) (Mikulec et al., 2019). Hemp flour has been used for the production of gluten-free bread (Korus et al., 2017). Catană et al. (2018) achieved a functional ingredient from apple wastes resulting from the apple juice industry. Powders achieved from apple pomace are important sources of minerals (K: 450.12-508.45 mg/100 g; Fe 2.31-2.73 mg/100 g; Mg: 41.15-55.65 mg/100 g); Ca: 76.32-92.44 mg/100 g; Zn: 1.54-1.96 mg/100 g), dietary fibres (60.62-64.75%), polyphenols (17.83-38.83 mg GAE/g) and have antioxidant capacity (1.77-5.12 mg TroloxEquivalents/g). Due to the complex biochemical composition, the powders achieved from apple pomace can be used to obtain hypoglycemic bread with antioxidant potential, for diabetics. Also, for the achievement of hypoglycemic bread with antioxidant potential, for diabetics, an important role has sourdough. Lappi et al. (2010) showed that sourdough fermentation of wholemeal wheat bread increases solubility of arabinoxylan and protein and decreases postprandial glucose and insulin responses. Sourdough is considered the gold standard for bread-making. The biochemical changes deriving from the action of microbial enzymes along with indigenous flour ones greatly influence sourdough characteristics and ultimately the quality of bread. Effects of dough fermentation on texture, aroma, shelf life and nutritional value of the bakery products have been elucidated and highlight its positive role on bread production (Siepmann et al., 2018). Burnete et al. (2019) obtained sourdough enriched in phenolic compounds and inulin, using flour from Jerusalem artichoke tubers (*Helianthus tuberosus*).

In this paper are presented results of the performed research to achieve a hypoglycemic bread assortment, with antioxidant potential, for diabetics. Composition of the products and the proposed technological solutions considered the decrease of glycemic index, on the one hand and the increase of antioxidant capacity, on the other hand.

MATERIALS AND METHODS

Materials

In order to obtain the product “Hypoglycemic bread with antioxidant potential” the following raw materials and auxiliary materials were used: white wheat flour type 650, whole wheat flour, hemp flour, Jerusalem artichoke flour, apple waste flour, hemp husked seeds, flax seeds, oat bran and sea salt. For the fermentation and final proofing processes, natural sourdough enriched in phenolic compounds was used, using Jerusalem artichoke flour.

Bread-making

In order to achieve the product “Hypoglycemic bread with antioxidant potential”, there were performed two experimental variants (PDV1 and PDV2), togheter with the control sample (C:“White bread with natural sourdough”) (Figure 1).

Figure 1. Product “Hypoglycemic bread with antioxidant potential” (PDV1 and PDV2) and control sample (C)

Reduction of the glycemic index was achieved by introducing in the composition of products of some natural ingredients, in well defined proportions, scientifically based and by applying the biphasic technology, which allows the preservation, in the highest concentrations, of the bioactive compounds from these.

Methods

Sensory analysis

Sensory evaluation of the product “Hypoglycemic bread with antioxidant

potential" was performed 12 hours afterbaking, using descriptive method and *"Comparison method with unitary score scales"*. Sensory quality of the product was established based on the total average score by comparison with a scale from 0 to 20 points (18.1 ... 20 - qualyfing "very good"; 15.1 ... 18 - qualyfing "good"; 11.1 ... 15 - "satisfactory"; 7.1 ... 11 - "unsatisfactory"; 0 ... 7 - "inadequate").
Measurement of the colour parameters of samples was performed at room temperature, using a CM-5 colorimeter (Konica Minolta, Japan), equipped with SpectraMagic NX software, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), *L**, *a** and *b**: *L** - colour luminance (0 = black, 100 = white); *a** - red-green coordinate (-a = green, +a = red); *b** - yellow-blue coordinate (-b = blue, +b = yellow).
The texture properties of the product were measured through a compression test using an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA).

Physic-chemical analysis
The moisture content was determined according to the AACC 44-15A method. Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005). Fat content was determined according toAOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005).Total dietary fiber (TDF) was determined by enzymatic method using the assay kits: KTDFR "Total dietary fiber" (AOAC Method 991.43).
Calorie contents were calculated using the following conversion factors: 9 for fat, 4 for carbohydrates, 4 for protein and 2 for fibre, according to the Commission Regulation no. 1169/2011 (European Commission, 2011). Joule contents were calculated using thefollowing conversion factors: 37 for fat, 17 for carbohydrates, 17 for protein and 8 for fibre,according to the Commission Regulation no. 1169/2011 (European Commission, 2011).

Total polyphenol content
Total polyphenol content was conducted according to Horszwald & Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in methanol: water = 50:50 and the absorbance of the extracts were determined at a wavelenght λ = 755 nm. Results were expressed as mg of Gallic Acid Equivalents(GAE) per g product.

Antioxidant capacity
The DPPH scavenging radical assay was conducted according to Horszwald & Andlauer (2011) with some modifications (concerning extract volumes of the used sample
and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g product.

Microbiological analysis
Yeasts and molds were determined by the method SR ISO 21527-1:2009. *Enterobacteriaceae* were determined according to the SR EN ISO 21528-1:2017 method.

RESULTS AND DISCUSSIONS

Sensory analysis
A complex mixture of dietary fibers, slow carbohydrates (mainly sourced from whole wheat flour, Jerusalem artichoke flour, hemp flour, apple waste flour, oat bran) and vegetable proteins (derived from hemp flour and hemp husked seeds) and dough fermentation, using natural sourdough, enriched in phenolic compounds and inulin, lead to the production of some hypoglycemic products and will decrease the inulin resistance (and, consequently, the inulin secretion), when there are consumed.
Sensory analysis plays an important role in characterizing the quality of food products.
As a result of the sensory analysis it was found that the product „Hypoglycemic bread with antioxidant potential", achieved in two experimental variants (PDV1 and PDV2) is

well developed, presents an elastic, dense core, with uniform pores and pleasant taste and aroma, specific to the product. So, the analyzed products were tested by an expert panel receiving qualifying „very good",with next scores: Control Sample - 19.76; PDV1-19.44; PDV2-19.76 (Figure 2).

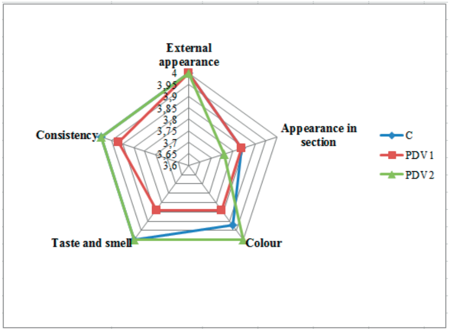

Figure 2. Sensory evaluation of the product “Hypoglycemic bread with antioxidant potential” (PDV1 and PDV2) and of the control sample (C)

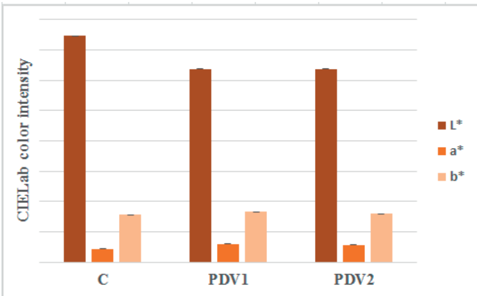

Figure 3. Color parameters of the product “Hypoglycemic bread with antioxidant potential” (PDV1 and PDV2) and of the control sample (C)

As a result of the ingredients used to decrease the glycemic index and to increase the antioxidant potential, the product “Hypoglycemic bread with antioxidant potential”, intended for diabetics, has a darker color, compared to the control sample, recording lower values of luminance (PDV1 - L^* = 63.68; PDV2 - L^* = 63.69) and higher values of the parameters a^* and b^* (Figure 3).
Also, the texture of the product “Hypoglycemic bread with antioxidant potential” was analyzed, compared to the control bread sample (C), for a period of 6 days (Table 1). The products were packed in polypropylene foil (oxygen permeability 1336.47 $cm^3/m^2 \cdot 24$ h·bar; water vapor permeability 1.284 $g/m^2 \cdot 24$ h·bar). Using the Instron texture analyzer (model 5944), the following texture parameters were calculated: firmness (hardness), elasticity, cohesiveness, and guminess. The test parameters were: compression speed: 100 mm/min; deformation of the sample: at a distance of 10 mm in height; load cell: 50 N. Figures 4-7 show the compression curves obtained for the product “Hypoglycemic bread with antioxidant potential” (experimental variants PDV1 and PDV2) on day 1 and day 3, respectively, on the date of manufacture.

Table 1. The textural properties of the product “Hypoglycemic bread with antioxidant potential”, compared to the control sample C

Product	Perioad (days)	Firmness (N)	Elasticity	Cohesiveness	Gumminess (N)
C	1	7.23 ± 0.26	1.00 ± 0.00	0.22 ± 0.18	2.11 ± 1.33
	2	9.74 ± 0.66	0.98 ± 0.00	0.30 ± 0.08	2.86 ± 0.61
	3	11.62 ± 1.87	1.04 ± 0.09	0.22 ± 0.11	2.70 ± 1.48
	6	14.57 ± 2.59	0.98 ± 0.00	0.28 ± 0.09	4.19 ± 1.95
PDV1	1	7.18±1.73	0.99±0.01	0.33±0.03	2.33±0.66
	2	8.71±0.51	0.99±0.00	0.35±0.03	2.98±0.09
	3	8.98±2.01	0.98±0.00	0.44±0.02	3.82±0.73
	6	11.94±3.79	0.98±0.01	0.28± 0.02	3.32±1.23
	7	13.67±3.74	1.14±0.19	0.23±0.02	3.62±1.33
PDV2	1	5.46±1.47	0.98±0.00	0.31±0.07	1.71±0.69
	2	5.78±1.12	0.98±0.00	0.32±0.09	1.82±0.49
	3	7.16±1.72	0.98±0.00	0.46±0.00	3.26±0.77
	6	8.97±2.90	1.03±0.04	0.36±0.04	3.35±1.11
	7	9.98±2.38	1.01±0.03	0.27±0.11	2.88±1.56

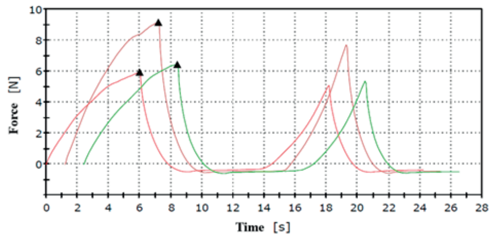

Figure 4. Compression curves for the product “Hypoglycemic bread with antioxidant potential” (PDV1) - day 1

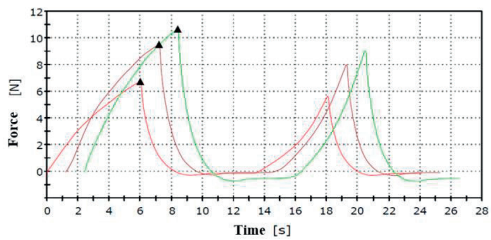

Figure 5. Compression curves for the product “Hypoglycemic bread with antioxidant potential” (PDV1) - day 3

According to the obtained results, during the 7 days from the date of manufacture, the firmness of the product "Hypoglycemic bread with antioxidant potential", achieved in two experimental variants PDV1 and PDV2, varied in the range 5.46 N-13.67 N (the minimum value was registered in the case of the experimental variant PDV2, one day from the date of manufacture, and the maximum one, in the case of the experimental variant PDV1, 7 days from the date of manufacture).

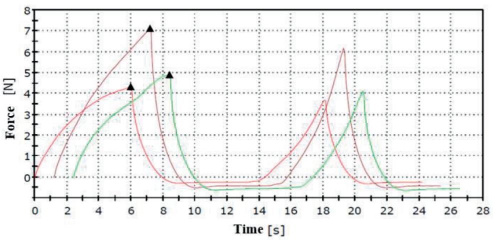

Figure 6. Compression curves for the product "Hypoglycemic bread with antioxidant potential" (PDV2) - day 1

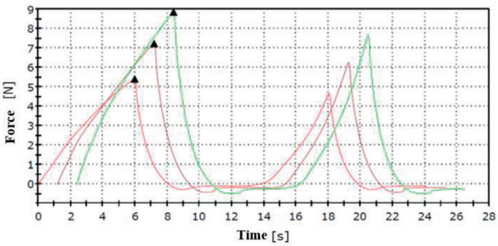

Figure 7. Compression curves for the product "Hypoglycemic bread with antioxidant potential" (PDV2) - day 3

It is noteworthy that in the case of the experimental variant PDV2, there were registered lower values of firmness, during the 7 days from the date of manufacture, compared with the experimental variant PDV1 and the control sample (C). Thus, the bread corresponding to the experimental variant PDV2 has a less dense, less hard core, compared to the experimental variant PDV1 and the control sample (C).

The elasticity of the product "Hypoglycemic bread with antioxidant potential" (experimental variants PDV1 and PDV2) and of the control sample (C) recorded close values, during the 7 days from the date of manufacture.

The product "Hypoglycemic bread with antioxidant potential" (experimental variants PDV1 and PDV2) had higher values of cohesiveness compared to the product "White bread with natural sourdough" (control sample - C). According to the values recorded for this parameter of texture, the product "Hypoglycemic bread with antioxidant potential" has a higher compressive strength compared to the control bread sample (C), which leads us to the conclusion that it has stronger internal bonds, which maintain its structure. The highest value of cohesiveness was recorded after 3 days from the date of manufacture, in the case of the experimental variant PDV2 (0.46).

The product "Hypoglycemic bread with antioxidant potential" (experimental variants PDV1 and PDV2) had lower of guminess, compared to the product "White bread with natural sourdough" (control sample - C).

Physic-chemical analysis

As a result of the physic-chemical analysis it was found that the product "Hypoglycemic bread with antioxidant potential", achieved in two experimental variants (PDV1 and PDV2) for diabetics, has a low carbohydrate content and is noted for its protein, ash and total fiber content (Table 2).

Table 2. Physic-chemical analysis of the product "Hypoglycemic bread with antioxidant potential" and of the control sample C

Component	C	PDV1	PDV2
Nominal mass (kg)	0.404	0.412	0.416
Volume (cm³/100 g)	272	233	236
Porosity (%)	71.30	69.53	69.04
Elasticity (%)	95	95	95
Acidity (degrees)	3.2	4.2	4.4
Moisture (%)	41.25	45.68	45.41
Ash (%)	0.50	1.12	1.27
Protein (%)	7.58	8.85	9.12
Fat (%)	0.88	1.71	1.94
Carbohydrates (%)	49.79	42.64	42.26
Available carbohydrates (%)	49.21	39.49	38.56
Total dietary fiber (%)	0.58	3.15	3.70
Energy value (kcal/100g)	236.24	215.05	215.58
Energy value (kJ/100g)	1002.63	910.25	911.94

Product "Hypoglycemic bread with antioxidant potential", achieved in two experimental variants (PDV1 and PDV2) has higher moisture compared to that of bread with *Aronia*, achieved by Catană et al. (2018), but also the content in protein, lipids, ash and total fiber, lower than that of this bread. Chemical characteristics of the bread achieved within this study determine a fast and lasting satiety when consumed and a low glycemic impact, being

beneficial in the diet of diabetics and obese people. Due to the ingredients used to decrease the glycemic index and to increase the antioxidant potential, the product "Hypoglycemic bread with antioxidant potential" has a smaller volume (PDV1 - V = 233 cm^3/100 g; PDV2 - V = 236 cm^3/100 g), compared to the control sample (C - V = 272 cm^3/100 g). Similar results were obtained by Šporin et al. (2017) in the case of the bread fortified with grape pomace flour, levels of fortification of 6, 10 and 15%. Reduction of the volume of the bread fortified with grape pomace flour could be explained by the activities of enzymes and yeasts. Amylases in the enriched dough could be inhibited by the phenolic compounds of the grape pomace flour, resulting inadequate concentrations of maltose. At the same time, in the case of bread fortified with grape pomace flour, a smaller quantity of gas is produced by the activity of yeasts, which results in a smaller volume of bread and a relatively compact texture.
Elasticity of the product "Hypoglycemic bread with antioxidant potential" (experimental variants PDV1 and PDV2) is similar to the control sample one, instead, the porosity recorded slightly lower values (C - 71.03%, PDV1 - 69.53% and PDV2 - 69.04%). Both the porosity and the elasticity are expressed as a percentage, the higher their value, the more bakery products are considered to be better qualitative and more appreciated by consumers.

Total polyphenol content
Total polyphenol content of the product "Hypoglycemic bread with antioxidant potential" recorded the following values: 100.85 mg GAE/100 g experimental variant PDV1 and 115.12 mg GAE/100 g experimental variant PDV2 (Figure 8).
Due to the valuable ingredients used in the composition (Jerusalem artichoke flour, apple waste flour, hemp flour, hemp husked seeds, flax seeds, oat bran etc.), the product "Hypoglycemic bread with antioxidant potential" has the total polyphenol content 1.53-1.75 times higher compared to the control sample, "White bread with natural sourdough".

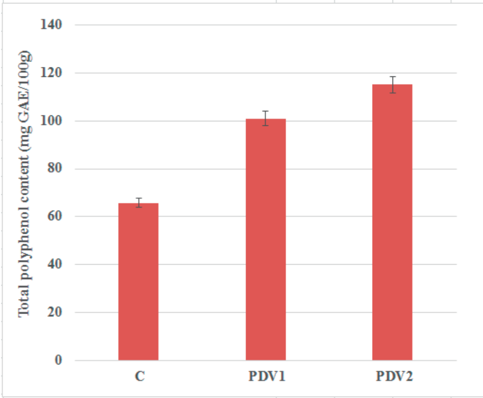

Figure 8.Total polyphenol content of the product "Hypoglycemic bread with antioxidant potential" (PDV1 and PDV2) and of the control sample (C)

Total polyphenol content of product "Hypoglycemic bread with antioxidant potential", is superior to those recorded in case of bread prepared with 10% of grape pomace powder (89.43 mg GAE/100 g; Hayta et al., 2014).
According to the research performed at international level (Lutz et al., 2019), phenolic compounds can be considered as natural inhibitors of platelet aggregation, helping to reduce the risk of cardiovascular diseases, caused by thrombosis. Also, performed studies have shown that a diet rich in phenolic compounds is associated with anti-inflammatory effects in the human body (Cassidy et al., 2015).

Antioxidant capacity
Due to its content in phenolic compounds, the product "Hypoglycemic bread with antioxidant potential" has antioxidant capacity (Figure 9). The highest values of antioxidant capacity were registered in the case of experimental variant PDV2: 125.15 mg Trolox Equivalents/100 g. Due to the ingredients with antioxidant potential, used in the composition of product "Hypoglycemic bread with antioxidant potential" for diabetics, it has an antioxidant capacity of 1.7, respectively 1.8 times higher, compared to the control sample (C).

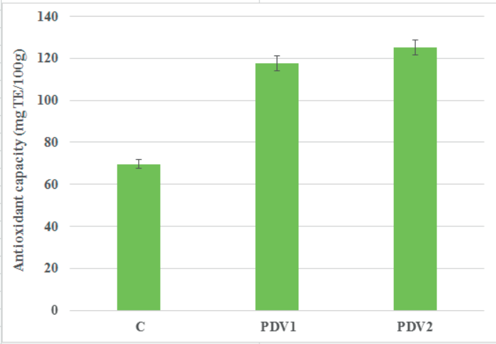

Figure 9. Antioxidant capacity of the product "Hypoglycemic bread with antioxidant potential" (PDV1 and PDV2) and of the control sample (C)

Corroborating the results of the sensory analysis with those of the physico-chemical analysis, respectively with those obtained for the content in polyphenols and for the antioxidant capacity, for the product „Hypoglycemic bread with antioxidant potential", the experimental variant PDV2 was selected as the optimal variant.

Microbiological analysis

Following the microbiological analysis of the products "Hypoglycemic bread with antioxidant potential" and "White bread with natural sourdough" (Control) packed in polypropylene bags, it was found that they comply with the provisions of the legislation into force and at 7 days from the date of manufacture (Table 3).

Table 3. Microbiological analysis of the product "Hypoglycemic bread with antioxidant potential" and of the control sample (C)

Product	Microbiological indicator					
	Yeasts and molds (CFU/g)			*Enterobacteriaceae* (CFU/g)		
	24h	5 days	7 days	24h	5 days	7 days
C	< 10	< 10	< 10	< 10	< 10	< 10
PDV1	< 10	< 10	< 10	< 10	< 10	< 10
PDV2	< 10	< 10	< 10	< 10	< 10	< 10

Corroborating the results of the microbiological analysis with those of the sensory analysis, the minimum durability of the product "Hypoglycemic bread with antioxidant potential", corresponding to the experimental variant PDV2, was established at 4 days. Shelf-life relative high of the product "Hypoglycemic bread with antioxidant potential" can be explained mainly by antioxidant and antibacterial potential of Jerusalem artichoke flour, hemp flour, apple waste flour, used in the composition of the product, due to their total polyphenol content. Flours from hemp are a rich source of polyphenols, which have an anti-microbial and anti-viral effects (Callaway, 2004; Manach et al., 2005, Mikulec et al., 2019). Also, Zhang et al. (2016) demonstrated antioxidant and antibacterial activities of phenolics from *Golden Delicious* apple pomace (phloridzin and phloretin are the most important phenolic compounds that have antioxidant and antibacterial activities).

However, lactic acid bacteria from sourdough enriched in phenolic compounds, used for fermentation and final proofing, act as a natural antibiotic, thereby increasing the shelf-life of product "Hypoglycemic bread with antioxidant potential" (Catană et al., 2018).

CONCLUSIONS

Bread for diabetics has superior sensory quality, high nutritional value and antioxidant potential. It has a low content of available carbohydrates (38.56-39.49%) and is noted for its content in protein (8.85-9.12%), total fiber (3.15-3.70%) and total polyphenols (100.85-115.12 mg GAE/100 g). At the same time, this product has an antioxidant capacity (117.45-125.15 mg TE/100 g). Shelf-life of this bread is 4 days.

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CONTENT IN BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF FLOURS OBTAINED FROM WINEMAKING BY-PRODUCTS

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Abstract

Grape pomace is particularly noted for its content of fiber, phenolic compounds, unsaturated lipids and sterols, vitamins and antioxidants. In this paper are presented results of the performed research for determination of vitamin C, total polyphenol content and antioxidant capacity in case of flours (grape pomace flour, grape seed flour, grape skin flour) obtained from winemaking by-products. There were taken into the study, winemaking by-products from three grape varieties: Blauer Zweigelt, Burgund Mare and Fetească Regală. Vitamin C content of flours obtained from winemaking by-products varied in the range of 18.25-25.75 mg/100 g FW and total polyphenol content varied in the range of 57.45-258.75 mg gallic acid equivalent (GAE)/g FW. Due to the content in bioactive compounds, the obtained flours have an antioxidant capacity (9.98-46.12 mg trolox equivalent (TE)/g FW). The highest value of the antioxidant capacity was recorded in the case of grape seed flour, this one being followed by the grape pomace flour, on the last place being the grape peel flour. Due to the complex biochemical composition, the flours obtained from winemaking by-products, can be considered as functional ingredients.

Key words*: winemaking by-products, polyphenols, vitamin C, antioxidant capacity.*

INTRODUCTION

Vinification is an important agro-industrial activity, in countries such as Italy, Spain and France, but also in Romania. Following the winemaking process, significant quantities of winemaking by-products (grape stalk, grape skins, grape seeds, traces of pulp) are produced. It is estimated that the production of 100 L of white wine results in about 30 kg of winemaking by-products (Mendes et al., 2013). Grape pomace represents a mixture of skins, seeds and traces of grape pulp, resulted after the wine is obtained. At the international level, there is a great interest for the increasing of the added value of industrial cultures, both for economic reasons and for the protection of the environment, associated with the tendency to obtain new food products and ingredients with nutritional and functional properties (García-Lomillo et al., 2017; Gil-Sánchez et al., 2018).
Grape pomace is particularly noted for its content in fiber, phenolic compounds, unsaturated lipids and sterols, vitamins and antioxidants (Kalli et al., 2018). According to the international performed studies (Brenes et al., 2016), the content of bioactive compounds of grape pomace depends on the variety of grapes, the area where the culture is located, the conditions of fertilisation, the soil and the period of grape harvest. Also, research conducted by Cappa et al. (2015), showed that more than 70% of the phenolic compounds in grapes remain in the grape pomace. Therefore,

it is very important their recovery from this food matrix and use them in the composition of food products, as preservatives, antioxidants, colorants or fortifying agents. Sousa et al. (2014) evaluated the biochemical composition of the flour obtained from grape pomace (*Vitis vinifera* L.), *Benitaka* variety, grown in the semiarid region of Northeast Brazil. The results showed that this flour obtained from these residues had below neutral pH (3.82), moisture (3.33 g/100 g), acidity (0.64 g of citric acid/100 g), noting the content in ash (4.65%), total dietary fiber (46.17%) carbohydrate (29.2%), protein (8.49%), lipids (8.16%), vitamin C (26.25 mg/100 g), and anthocyanins (131 mg/100 g). Also, these authors mention that the minerals iron (Fe), potassium (K), zinc (Zn), manganese (Mn), and calcium (Ca) were present in higher concentrations. Flours achieved from winemaking by-products (black seed flour, black pomace flour) by Catană et al. (2017) are noted by their content in ash (2.80-6.61%), protein (10.53-10.85%), fat (8.49-15.36%), total fibre (58.86-66.06%), K (1102.35-3406.67 mg/100 g), Ca (476.62-988.45 mg/100 g), Mg (146.55-223.75 mg/100 g), Fe (3.97-8.67 mg/100 g), total polyphenols (200.15-322.75 mg/100 g) and antioxidant capacity (40.75-51.25 mg Trolox Equivalents/g).

In this paper are presented results of the performed research for determination of vitamin C, total polyphenol content and antioxidant capacity in case of flours (grape pomace flour, grape seed flour, grape skin flour) obtained from winemaking by-products.

MATERIALS AND METHODS

Samples

The winemaking by-products from three grape varieties *(Blauer Zweigelt, Burgund Mare* and *Fetească Regală),* were provided by the National Research & Development Institute for Biotechnologies in Horticulture Ştefăneşti Argeş. Experiments were performed within the Pilot Experiments Plant for Fruits and Vegetables Processing from the National Research & Development Institute for Food Bioresources. Till processing, winemaking by-products were stored under refrigeration (3°C). The winemaking byproducts were subjected to dehydration process in a convection dryer at temperature of 50ºC to a moisture which allows their milling and conversion into flours and, at the same time, their stability in terms of quality. Milling of dried semi-finished products was performed by using Retsch mill. The achieved functional ingredients (flours) were packed in glass containers, hermetically sealed, protected by aluminum foil against light and stored in dry and cool areas (temperature of maximum 20ºC), till to the biochemical analysis. Figures 1-3 show flours obtained from grape pomace, resulted from the three studied grape varieties: *Blauer Zweigelt, Burgund Mare* and *Fetească Regală*.

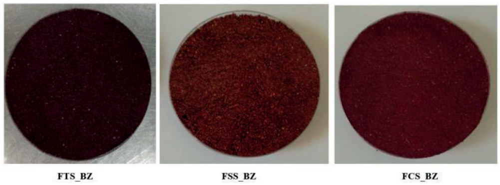

Figure 1. Flours achieved from grape pomace, *Blauer Zweigelt* variety (FTS_BZ - grape pomace flour; FSS_BZ - grape seed flour; FCS_BZ - grape skin flour)

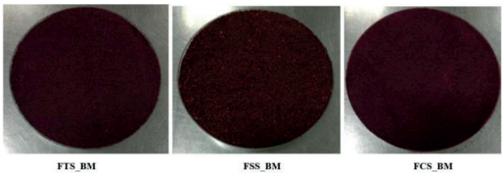

Figure 2. Flours achieved from grape pomace, *Burgund Mare* variety (FTS_BM - grape pomace flour; FSS_BM - grape seed flour; FCS_BM - grape skin flour)

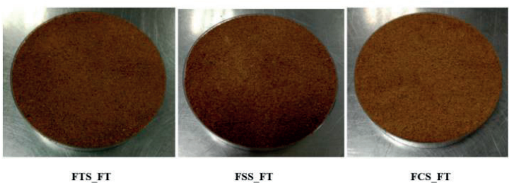

Figure 3. Flours achieved from grape pomace, *Fetească Regală* variety (FTS_FT- grape pomace flour; FSS_FT - grape seed flour; FCS_FT - grape skin flour)

Methods

Vitamin C content

Determination of vitamin C was performed by high performance liquid chromatography (HPLC) (Accela, Thermo Scientific) coupled with high resolution mass spectrometry (HRMS) (LTQ OrbitrapXL Hybrid Ion Trap-

Orbitrap MassSpectrometer, Thermo Scientific) using hippuric acid as internal standard.
LC conditions: Column (Hypersil GOLD aQ, 150 x 2.1 mm, 3 µm); Column temperature: 40°C; Sample temperature: 4°C; Mobile phase A: 990 mL water: 10 mL 1M ammonium formate (aq): 1 mL formic acid; Mobile phase B: 990 mL methanol: 10 mL 1M ammonium formate (aq): 1 mL formic acid; Flow rate: 0.400 mL/min; Injection volume: 25 µL;
MS conditions: Analyzer: Fourier Transform Mass Spectrometry (FTMS); Resolution: 60000; Ionization mode: Electrospray ionization in negative ion mode (ESI-); Specific ions were: m/z = 175.02438 (for vitamin C) and m/z = 178.05051 (for hippuric acid). In Figure 4 is presented the calibration curve of vitamin C, achieved in the concentration range 1500-10000 µg/L.

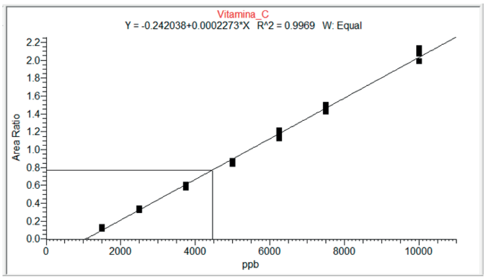

Figure 4. Calibration curve of vitamin C

Total polyphenol content
Total polyphenol content was conducted according to Horszwald & Andlauer (2011), with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range of 0-0.20 mg/mL (Figure 5).

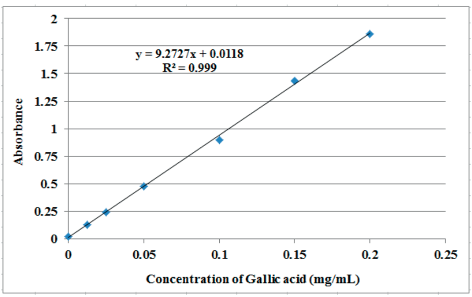

Figure 5. Calibration curve of gallic acid

The extraction of phenolic compounds was performed in methanol: water 50:50 (v:v), and the absorbance of the extracts was determined at a wavelength λ = 755 nm. Results were expressed as mg of gallic acid equivalents (GAE) per g flour.

Antioxidant capacity
The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging radical assay was conducted according to Horszwald & Andlauer (2011), with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range of 0-0.4375 mmol/L (Figure 6). Results were expressed as mg Trolox Equivalents per g flour.

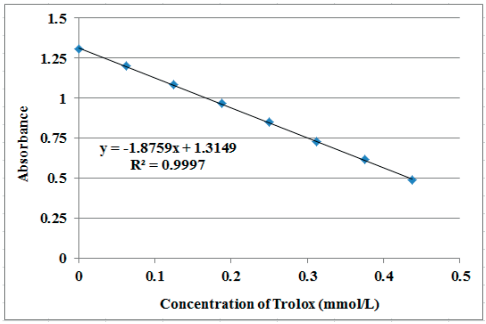

Figure 6. Calibration curve of Trolox

RESULTS AND DISCUSSIONS

Vitamin C content
Vitamin C content of the flours obtained from winemaking by-products varied in the range of 18.25-25.75 mg/100 g (the minimum value was registered in case of grape pomace flour *Burgund Mare* variety, and the maximum one in case of grape skin flour *Fetească Regală* variety) (Figure 7).Vitamin C content of these powders is comparable with that reported by Sousa et al. (2014) for grape pomace flour (26.25 mg/100 g) and higher than that reported by Nayak et al. (2018), in case of *Cabernet* grape pomace (22.8 mg/100 g). Concerning the type of flour obtained from winemaking by-

products, the highest value of the vitamin C content was registered in the case of grape seed flour, this one being followed by grape pomace flour and, on the last place being grape skin flour.

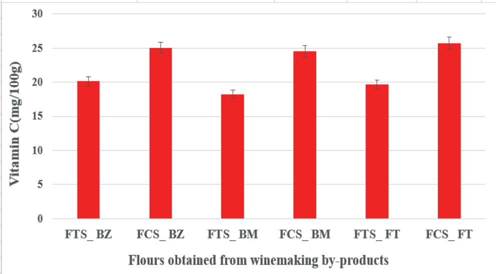

Figure 7. Vitamin C content of the flours obtained from winemaking by-products

Vitamin C plays a role in many processes as a cofactor for enzymes involved in processes and effects important for cancer transformation: antioxidant defence, transcription, and epigenetic regulation of gene expression (Granger & Eck, 2018). Vitamin C is an antioxidant that may scavenge reactive oxygen species preventing DNA damage and other effects important in cancer transformation (Pawlowska et al., 2019). Vitamin C is also reported to exert beneficial effects in the immune system and inflammation, which is crucial in fighting precancerous and cancer cells by the host (Ang et al., 2018). It is important to mention that vitamin C is needed for the repair of tissues in all parts of the body (Devaki et al., 2017).

Total polyphenol content

The flours achieved from winemaking by-productsare also noted by total polyphenol content (Figure 8).Total polyphenol content of flours obtained from the winemaking by-products taken into study varied in the range of 57.45-258.75 mg GAE/g (the minimum value was registered in the case of grape skin flour *Fetească Regală* variety*,* and the maximum one, in the case of grape seed flour, *Burgund Mare,* variety). Concerning the type of flour obtained from winemaking by-products, the highest total polyphenol content was registered in the case of seed flour, this one being followed by grape pomace flour, on the last place being skin flour. Results are according to those obtained by Muncaciu et al. (2017), who studied two grape varieties *Fetească neagră* (variety for red wine) and *Italian Riesling* (variety for white wine).

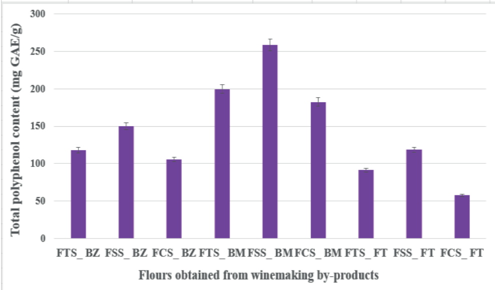

Figure 8. Total polyphenol content of the flours obtained from winemaking by-products

In results obtained by Muncaciu et al. (2017), grape seed flour presented the highest total polyphenol content (103.62 mg epicatechin/g DW in he case of grape seed flour, *Italian Riesling* variety and, respectively, 94.40 mg epicatechin/g DW in the case of grape seed flour, *Fetească neagră* variety).

Concerning the grape variety, in the case of experiments carried out, powders obtained from winemaking by-products belonging to the *Burgund Mare* variety, presented the highest values of the total polyphenol content: 199.87 mg GAE/g in the case of grape pomace flour; 258.75 mg GAE/g in the case of seed flour; 182.55 mg GAE/g in the case of skin flour.

It is also worth noting that the total polyphenol content of grape pomace flour, obtained from winemaking by-products belonging to the three grape varieties taken into study (*Blauer Zweigelt, Burgund Mare, Fetească Regală)* is higher than that reported by other authors: 3.64 mg GAE/g (*Barbera* variety) and 16.06 mg GAE/g (*Chardonnay* variety) (Marchiani et al., 2016); 55.80 mg GAE/g (*Quebranta* variety) and 49.67 mg GAE/g (*Torontel* variety) (Solari-Godiño et al., 2017); 41.14 mg GAE/g (*Cabernet Sauvignon* variety) (Urquiaga et al., 2015).

Polyphenols are an important class of compounds that have antioxidant, anti-inflammatory (Chedea et al., 2018), anti-aging (Kostyuk et al., 2018) and anti-cancer (Cipolletti et al., 2018) effects, as well as the prevention of different diseases (Figueira et al., 2017; Kujawska et al., 2018).

Costabile et al. (2019) evaluated the acute effects of the consumption of a drink rich in polyphenols from red grape pomace on glucose/insulin and triglyceride responses to a standard meal in healthy individuals, and the relationship between plasma levels of phenolic metabolites and metabolic parameters. These authors have shown that red grape pomace consumption acutely reduced postprandial insulin levels and improved insulin sensitivity. This effect could be likely related to the increase in gallic acid levels. The drink rich in polyphenols from red grape pomace added to the regular diet, it could contribute to the increasing of the daily intake of polyphenols, with potential health benefits. Lu et al. (2019) have shown that grape polyphenol extracts have a great influence on the recovery of gut microbiota after antibiotics and high-fat diet treatment.

Antioxidant capacity

Due to their content in phenolic compounds, flours achieved from grape pomace (grape pomace flour, grape seed flour, grape skin flour) have antioxidant capacity (Figure 9).

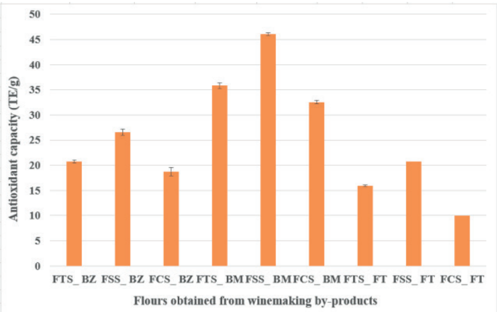

Figure 9. Antioxidant capacityof the flours obtained from winemaking by-products

Antioxidant capacity of the flours obtained from winemaking by-products taken into study, varied in the range of 9.98-46.12 mg TE/g (the minimum value was registered in the case of grape skin flour, *Fetească Regală* variety, and the maximum one, in the case of seed flour, *Burgund Mare* variety). Concerning the type of flour obtained from winemaking by-products, the highest value of the antioxidant capacity was registered in the case of seed flour, this one being followed by grape pomace flour, on the last place being grape skin flour. As regards as the grape variety, in the case of experiments carried out, powders obtained from winemaking by-products belonging to the *Burgund Mare* variety, presented the highest values of the antioxidant capacity: 35.17 mg TE/g in the case of grape promace flour; 45.60 mg TE/g in the case of grape seed flour; 32.10 mg GAE/g in the case of grape skin flour. Between the total polyphenol content and antioxidant capacity of the powders obtained from winemaking by-products, there is a linear correlation ($y = 5.5381x + 2.5929$, $R^2 = 0.9999$) (Figure 10).

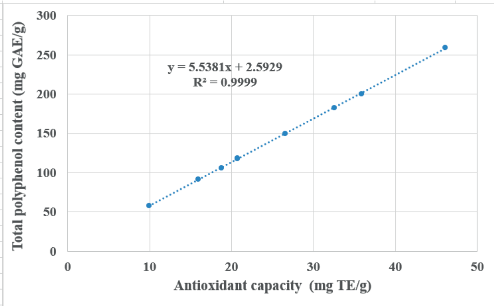

Figure 10. Correlation between the total polyphenol content and antioxidant capacity in case of flours obtained from winemaking by-products

The results presented are consistent with those reported by Catană et al. (2017), which also obtained a linear correlation between the total polyphenol content and the antioxidant capacity values, in case of the flours achieved from tomato waste and winemaking by-products.

CONCLUSIONS

Flours (grape pomace flour, grape seed flour, grape skin flour) obtained from winemaking by-products are noted for their vitamin C content (18.25-25.75 mg/100 g) and total polyphenol content (57.45-258.75 mg GAE/g). Also, due to their content in bioactive compounds, these powders have antioxidant capacity: 9.98-46.12 mg TE/g.

Flours obtained from winemaking by-products can be regarded as functional ingredients and can be used to fortify food products (bakery and pastry products, especially) in order to increase the nutritional value and their antioxidant capacity.

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THE MAHALEB CHERRY (*PRUNUS MAHALEB* L.) - A SPECIES SPECIFIC TO DOBROGEA'S PLATEAU

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Abstract

The present article has taken into account data from forest management plans realized during 1993-2007 for forests that belong to Forest Districts located in Dobrogea. All the data regarding the stand elements of Prunus Mahaleb L. were extracted from these plans for the management parcels in which this species is present. As such, the purpose of this paper was to analyze the main qualitative characteristics of stands (age), their structural characteristics (consistency, composition, average diameter and average height) as well as their site characteristics (altitude, slope, soil type). The total surface occupied by this species is of 1103.1 ha, with significant percentages in Constanța, Hârşova, Casimcea, Babadag and Măcin Forest Districts. The altitude at which Prunus mahaleb L. appears in Dobrogea Plateau ranges between 15 m at Cernavodă and 410 m at Măcin. The field's configuration is mainly undulated where Mahaleb species are present and covers a surface of 574 ha. The soils on which this species vegetates are: chernozem (218 ha), cambic chernozem (152 ha), lithic rendzic leptosol (60 ha), cambic phaeozem (43 ha), and rendzic leptosol (33 ha). The forest types in which Prunus mahaleb L. is present are: Soft oak from Dobrogea's silvosteppe with superficial soil (102 ha), Silvosteppe soft oak with oriental hornbeam (102 ha) and Dobrogea's Silvosteppe Turkey oak mixed hardwood stand (96 ha).
Mahaleb cherry has an important ecologic purpose, being used in improving degraded fields.

***Key words**: altitude, mahaleb cherry, site conditions, soils, stands.*

INTRODUCTION

Dobrogea Plateau is bordered by the Danube Meadow and Delta at West and North, which overlap on evident tectonic dislocations. On the East, the Black Sea has underneath strong Dobrogean structures that continue through the seashore platform.

Dobrogea is a plateau region where fragmentation has caused the development of forms such as hill summits, large valley breeches and basins. They cover approximately 10 400 km^2, namely 4.3% from Romania's territory. From a morphologic point of view, the area is characterized by low altitudes (only some North-West peaks exceed 400 m; 89% are situated under 200 m), relief energy frequent under 100 m, values of 0.5-1 km/km^2 for horizontal fragmentation with 42-47 semi-permanent leakage and two evident slope categories (0-5º - predominantly on plateaus, glacis, meadows - and over 30º on structural, petrographic slants and sea fronts) (Popescu, 2003). Even though Dobrogea is situated in a dry climate, the surface of degraded lands is sufficiently large (Mănescu, 2000).

Prunus mahaleb L. is present in Central and South Europe and West Asia. In our country, the species can be found in thinned forests from Dobrogea's silvosteppe ("shibliacs"), Moldova de Sud, and Banat. The species is thermophile, resistant to drought and requires a lot of summer heath. It vegetates well on skeletal and chalky dry soils and sometimes even on meadow ones (Clinovschi, 2005).

It is considered a very resistant tree to drought, sturdy and resistant to diseases. Consequently, the tree was used as parent stock for the horticulture production of cherries cultivated in the majority of Mediterranean countries

(Berrin, 2012; Al-Absi, 2010; Rankova, Z., 2006).

The plants' parts were used as traditional medicine for diabetes, gastro-intestinal problems and as tonic for curing different affections in the traditional Turkish medicine, having also an antibacterial activity (Seyyednejad et al., 2008). Furthermore, the resin obtained from the wood's exterior surface was used in treating gastritis. The oil extracted from seeds was used for fabricating liqueur and special wines due to its aromatic taste. The seeds were also used in treating diarrhea for Sudanese children. The fruits were used in pastries and bakeries (Berrin et al., 2012).

Concerning the genetic diversity, cluster analysis using UPGMA method and Dice's coefficient grouped the Mahaleb cherry into two main clusters with similarity coefficient ranging from 0.16 to 0.93 (Abedian et al., 2012).

The adaptability of Mahaleb cherry to continental climate, tolerance to drought, hot summer, poor soils, high pH and lime tolerance are valuable rootstock traits considering the forecasted environmental conditions due to the climate changes (Hrotko, 2016). Because seeds of the Mahaleb cherry, used as a rootstock in cherry production, germinate and emerge poorly due to seed dormancy, it is recommended that GA_3 should be used in addition to cold stratification for improving germination percentage and speed of Mahaleb cherry seeds (Al-Absi, 2010).

In Romania the species is one of the most frequent cherry species used in the reconstruction of forest fields, being used for controlling soil erosion of degraded fields (Enescu, 2015), together with sea buckthorn (Constandache et al., 2016), black locust (Murariu et al., 2018), or pines (Silvestru-Grigore, 2018).

The species is useful on coarse soils with a high content of calcium carbonate, in field's marginal and post-marginal rows and in highway protection belts (Costandache et al., 2006). In addition, it is well adapted to climatic changes (Vizitiu et al., 2018).

MATERIALS AND METHODS

The present article was created based on data from forest management plans realized during 1993-2007 for forests that belong to forest districts located in Dobrogea (10 forest management plans realized during 1993-2007 – ***Forest management plans).

Data regarding the Mahaleb cherry stand elements were extracted from these plans.

They contain a description of all environmental and stand characteristics. Firstly, the stand elements represented by Mahaleb cherry were extracted with the help of Excel (853 elements), followed by the data for each element.

The following stand and environment characteristics were analysed, specific to areas where Mahaleb cherry species are present: distribution, altitude, relief forms, soils, forest type, mixture, pruning, stand structure, current growth and production class.

RESULTS

1. Distribution of Mahaleb cherry in Dobrogea Plateau

The investigations have shown that Mahaleb cherry is present in all 10 forest districts from the studied area, occupying a total surface of 1103.1 ha. If we consider the total surface of forests from Dobrogea Plateau, Mahaleb cherry occupies only 1%.

The forest districts with a higher presence for this species are: Constanța (342.4 ha), Hârșova (117.6 ha), Casimcea (106.1 ha), Babadag (97.8 ha) and Măcin (88.3 ha). They are followed by a group of forest districts where the species has an average distribution: Ciucurova (71.9 ha), Cerna (82.6 ha) and Băneasa (76.6 ha). Mahaleb cherry has a reduced presence in Niculițel (52.2 ha) and Cernavodă forest districts (68.7 ha) (Figure 1).

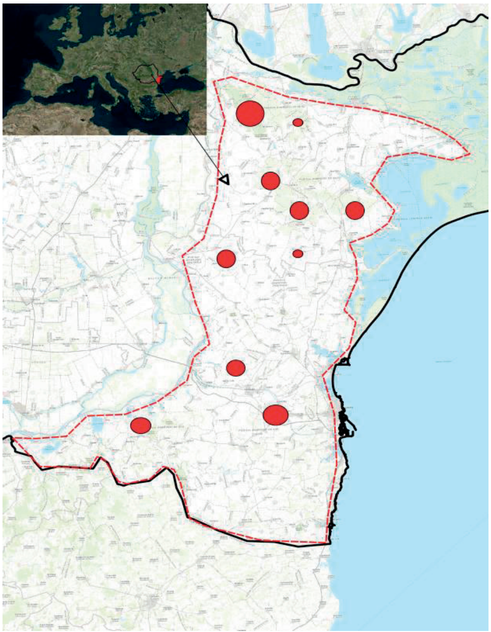

Figure 1. Distribution of *Prunus mahaleb* stands from Dobrogea Plateau

2. Site characteristics specific to Mahaleb cherry stands from Dobrogea Plateau

The main site characteristics of these stands are the following: the relief form, the field's configuration and the altitude.

The slope is **the relief form** characteristic for these stands with the plateau occupying 31% of the stands' total surface while low plain and average plain have values of 1% and 3% (Figure 2).

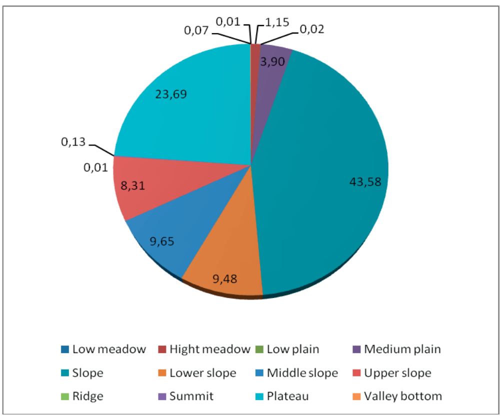

Figure 2. Relief forms characteristic for *Prunus mahaleb* stands from Dobrogea Plateau

The field's configuration is levelled and covers 566 ha, followed by the sinuous one (510 ha) and the fragmented one (26 ha).

The relief forms cause changes in the climatic and edaphic regimes, influencing indirectly the forest vegetation.

The altitude at which Mahaleb cherry appears in Dobrogea Plateau ranges between 15 m at Cernavodă and 410 m at Măcin.

Generally speaking, the altitude characteristic for this species is situated between 101-150 m. By calculating the average altitude of all parcels in which this species is present in this area, we reach a value of 122 m (Figure 3).

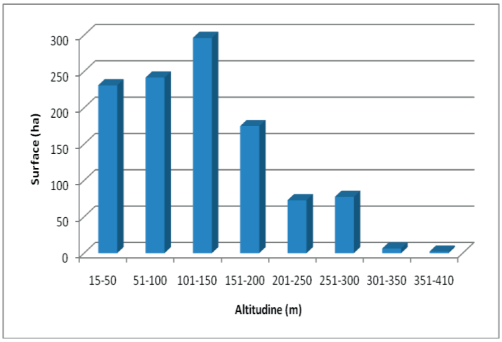

Figure 3. Altitude of *Prunus mahaleb* stands from Dobrogea Plateau

The field's slope is situated between 6^g and 50^g, with most fields characterised by reduced slopes of 0^g-20^g (Figure 4).

The slope is strongly correlated with the exposition and altitude and influences environment conditions.

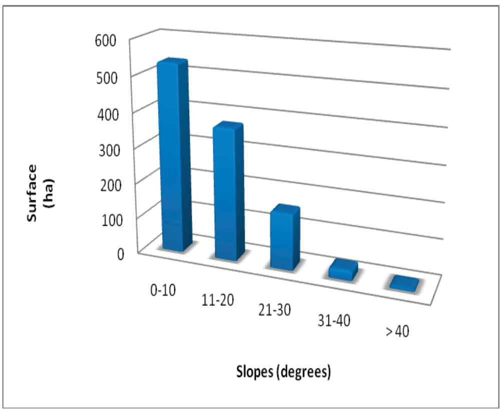

Figure 4. Field slope for *Prunus mahaleb* stands from Dobrogea Plateau

The exposition of the fields characteristic for these stands is mainly South-East, as well as North (Figure 5).

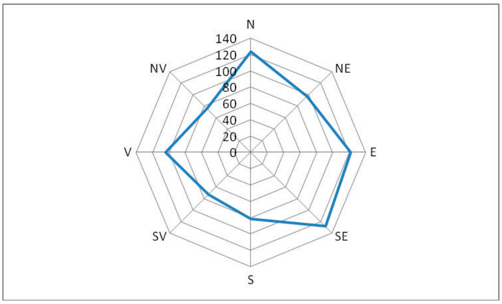

Figure 5. Field exposition for *Prunus mahaleb* stands from Dobrogea Plateau

The soils on which the Mahaleb cherry vegetates are: chernozem (218 ha), cambic chernozem (152 ha), lithic rendzic leptosol (60 ha), cambic phaeozem (43 ha), and rendzic leptosol (33 ha).

These soils have an intense microbiologic activity (Onet et al., 2019) and proper chemical properties (Crisan et al., 2020), but suffer from a lack of water supply in certain periods of the year (Dinca et al., 2020).

3. The characteristics of Mahaleb cherry stands from Dobrogea Plateau

The forest types in which the Mahaleb cherry is present are: Soft oak from Dobrogea's silvosteppe with superficial soil (102 ha), Silvosteppe soft oak with oriental hornbeam (102 ha) and Turkey oak mixed hardwood stand from Dobrogea's silvosteppe (96 ha).

As it can be seen, almost all forest types are specific to Dobrogea and situations in which a certain type of forest has in its name a well-defined geographic area are extremely rare in the Romanian forest typology.

These forests ensure numerous ecosystem services, besides the wood that they provide to their owners, such as game species (Dincă et al., 2018) or numerous non-wood forest products (Dincă et al., 2020).

The stand's age is a quantitative structural characteristic with direct implications in the qualitative structural aspects. The Mahaleb cherry stands age is young, ranging between 1 and 100 years (Figure 6).

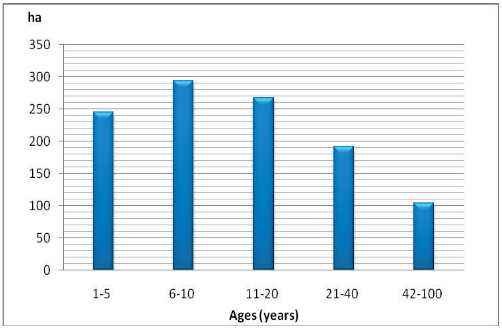

Figure 6. Age of *Prunus mahaleb* stands from Dobrogea Plateau

The specie's participation percentage in the stand's composition is of 100% - pure stands or lower - mixed stands.

The mixture is intimate (244 ha), in stripes (241.1 ha) or mixt (intimate + groups = 220.7 ha) (Figure 7).

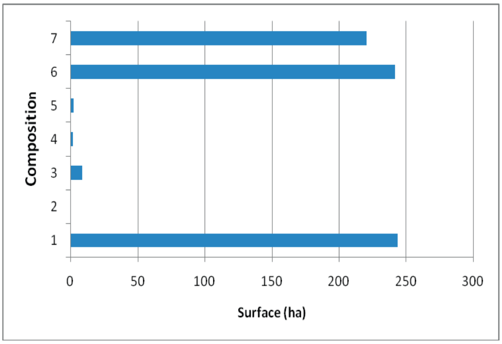

Figure 7. Composition of *Prunus mahaleb* stands from Dobrogea Plateau

The current growth of Mahaleb cherry stands from this area rangs between 0.1 m^3/year/ha and 2.1 m^3/year/ha.

The stand's production class is inferior (4^{th} class = 419.9 ha; 5^{th} class = 393.5 ha), and at best average (3^{d} class = 5.9 ha).

CONCLUSIONS

The realized study has led to the identification of 853 stands from Dobrogea Plateau in whose composition we can encounter Mahaleb cherry species (from pure stands to stands in which the specie's presence is of 10%).

The total surface occupied by this species is of 947 ha, with a higher percentage in Constanța, Casimcea, Hârșova, and Babadag Forest Districts.

The local conditions are represented by sinuous slopes situated at average altitudes of 101-150 m, with an average slope of 12^g, mainly north and southeast expositions and a typical chernozem soil type.

The main characteristics of these stands are the following: **forest types** - Soft oak from Dobrogea's silvosteppe with superficial soil (102 ha), Silvosteppe soft oak with oriental hornbeam (102 ha) and Turkey oak mixed hardwood stand from Dobrogea's silvosteppe (96 ha); **young stands**, aged 6-20; **inferior production classes** (classes 4 and 5) and at best average; **pure stands or mixed** with other broad-leaved species (in this case, the mixtures are intimate, in stripes or mixed); **current growth** ranging between 0.1 m^3/year/ha and 2.1 m^3/year/ha.

Mahaleb cherry has an extremely important ecologic purpose, being used in improving degraded lands. This species is one of the few broad-leaved species that can vegetate on sunny slopes and on arid fields.

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PRELIMINARY STUDY REGARDING THE USE OF MEDICINAL AND DECORATIVE PLANTS IN THE CONCEPT OF PERI-URBAN GARDENS WITH ROLE ON ENVIRONMENTAL PROTECTION

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Abstract

Urban gardening is becoming more and more popular in our day to day lives. The desire for such activity arose due to the massive urban growth, the daily stress and an increasing desire to have fresh fruits and vegetables for personal use. Although the presence of a vegetable garden in a private urban or peri-urban area offers many advantages – a source of fresh vegetables and aromatic plants, as well as a recreational area – it faces a major disadvantage – pollution. In the city there are countless sources of pollution, the most common being the one created by traffic. Having this in mind, the purpose of this paper is to present an overview on selecting medicinal and decorative plants in order to provide protection for vegetables against urban pollution. The results gathered were used to create a list of plants including vegetable, medicinal and floricultural species, which helped to design a planting plan for a vegetable and decorative garden.

Key words: *urban garden, edible landscape, vegetable garden, pollution*.

INTRODUCTION

Since ancient times, cultivating plants has been an integral part of human history. From the human need to feed, the garden appeared as a source of safe and fast food. Initially, the role of a garden was primarily functional, the production of food and medicinal plants being its main purpose, while later on it developed also an additional aesthetic role (Hangan *et al.,* 2018).

In many cities around the world, air pollution is a major problem. When designing a garden, an important factor that must be taken into account is the plants pollution absorption degree.

Urban horticulture is becoming an essential element of urban planning in many cities of the world (Vittori Antisari *et al.,* 2015).

Compared to traditional farming, growing food in the urban area depends on different conditions. In the cities, according to the accessible space, the horticultural gardens are distributed differently (for example, close to main roads), instead of following rational and agronomical aspects (such as potential pollution sources, access to light) (Alloway, 2004).

The concentration of various trace elements from the atmosphere are considerably affected by human actions, and their quantification in atmospheric accumulation can be useful to attribute to different sources of pollution (Vittori Antisari *et al.,* 2013).

Contaminants that are stored in soil, water, and air can affect the product quality and healthiness of the plants (Leake *et al.,* 2009), but not only. These accumulations threaten the health of people too by entering in human bodies through stomach, lungs, and contact with the skin (Timofeev *et al.,* 2019).

The presence, in urban soils, of heavy metals originating from intense human activities, especially road traffic, represent the main risk (Khan *et al.,* 2008; Salvagio Manta *et al.,* 2003). Although, these elements can be absorbed by plants, their accumulation among plant organs and plant species may vary (Säumel *et al.,* 2012). Urban areas contain multiple sources of pollutants with different degrees of intensity. A garden situated on a rooftop or in a courtyard is more protected than a garden located near a railway. Furthermore, researches on heavy metal absorption have established that distance from the road and pollution are generally inversely associated (Gherardi *et al.,* 2009).

In order to improve the quality of air and soil in a small edible garden, some small decorative and medicinal plants were studied.

MATERIALS AND METHODS

The study presented in this research was done based on the existing studies and information in literature. The main research methods used are the case study regarding the degree of air pollution absorbed by plants and a study based on designing a small vegetable garden replica representing the ideal combination between vegetable plants, decorative and medicinal plants.

The plants that will be studied are the ones commonly used in a private garden.

All the information gathered during this study, will result in a small design simulation of a vegetable garden which will be situated in the experimental field of the Horticulture Faculty in Iasi, "V. Adamachi" farm. The design programs used are AutoCAD 2015 and Photoshop 6.

The area and site for the design will be evaluated and natural factors such as water, soil, light and local fauna will be taken into consideration. The area to be designed has 37 m^2 applied on three modules.

Current methods of assessing tolerance thresholds and risks for agricultural soils are focused on the types of metals (Murray *et al.,* 2009). However, two species of plants grown in the same soil do not present the same risk to human consumers. Different plant species have different abilities to absorb and accumulate metals, both in general and in different types of tissue (Alexander *et al.,* 2006). Spinach leaves (*Spinacia oleracea* L.), for example, contain higher concentrations of Mn than its roots, while in radish *(Raphanus sativus* L.) the highest concentrations of Mn are found in roots. The leaves of plants have a higher concentrations of metal than the stems, so leafy vegetable species grown on contaminated soil may be at a greater risk (Qadir *et al.,* 2000; Harrison, 2001). According to Ramos *et al.* (2002) lettuce (*Lactuca sativa* L.) is considered as potential hyperaccumulator of heavy metals. The concentrations of heavy metals in lettuce shoots and roots rises by increasing the exposure duration (Khan *et al.,* 2015). According to Garate *et al.* (1993), lettuce has higher capacity to accumulate heavy metal in different tissues.

The samples collected by Säumel *et al.* (2012) inside the city have a much higher metal content than the supermarket samples (for example, basil 4.4, nasturtium 1.7, thyme 3.5 and parsley 1.9 times more Cr).

The amount of particulate matter on the leaves of different plant species alongside a road vary according to traffic density, particle type and species. Species with dispersed haired or glabrous leaves captures less particles than plants with densely haired leaves. Plants higher than 15 cm collect more particulate matter than plants lower than 15 cm (Weber *et al.,* 2013).

A small edible garden does not have enough space for large plants to grow thus, some small decorative and medicinal plants that improve the quality of air and soil were studied.

Studies conducted in the literature enumerate as species that can be used the following: yarrow, aster, wild geranium, common ivy, lavender and sage.

Achillea millefolium L., commonly known as yarrow, is one of the species of plants that can be used in the urban environment and can cover a quite large area providing support for the deposition of polluting particles. Due to the hairy and rough appearance of the leaves (Figure 1), the plant can capture a large amount of particles (Weber *et al.,* 2013).

The plant is cultivated as an ornamental plant for gardens and natural landscaping. Even if the plant can grow in less ideal conditions, it prefers a well-drained soil and full sun.

Figure 1. *Achillea millefolium* L. plant detail (https://iransaffronhouse.com/product/achillea-millefolium/)

Aster spp., also known as aster, is one of the types of plants that absorbs heavy metals from the soil such as cadmium, lead and selenium. Also, due to the hairy appearance and the small leaves, *Aster* can capture particles thus preventing the dispersion of this pollutant (Figure 2) (Gabrys, 2018).
Aster is a genus of perennial flowering plants from the family Asteraceae.
The plant has large clusters of flowers in white, pink, lavender, purple and red colours. Although it can tolerate dryness and poor soil it will bloom poorly (Gilman, 1999). Asters blooms forms late summer and autumn.

Figure 2. *Aster* spp. plant detail (https://www.forestryimages.org/browse/detail.cfm?imgnum=5110040)

Aster is sensitive to ozone thus, indicating the high or low presence of this pollutant on the basis of appearance and health of the plant (Gabrys, 2018).
It can be used in borders, rock gardens, or wildflower gardens. It attracts butterflies and bees, providing the pollinators with a late-season supply of nectar Gilman, 1999).
Geranium maculatum L., or wild geranium, contributes to urban biodiversity by attracting pollinators due to flowers. The palmately lobed leaves situated at the base are large and appear in spring. The lower leaf surface has rough white hairs while the upper surface is covered with fine white hairs that helps capturing the particles (Figure 3) (Gabrys, 2018).
It is a perennial plant known as wild geranium or spotted geranium. It is an herbaceous plant growing up to 60 cm in high and flowers in spring to early summer. The flowers are rose-purple, pale or violet-purple.

Figure 3. *Geranium maculatum* L. underside leaf detail (https://wimastergardener.org/article/wild-geranium-geranium-maculatum/)

The plant is used in herbal medicine as well as an ornamental plant in gardens.
Hedera helix L., commonly known as common ivy, is one of the few species ideal for air purification due to the resistance of urban pollution. This is a climbing plant that is very common in landscaping. Although it has a moderate ability to capture particles, by its volume it can capture more particles than any other small plant (Figure 4) (Sternberg *et al.,* 2010).

Figure 4. *Hedera helix* L. ground covering detail (https://www.dapplelandscapedesign.com.au/climbers/climbers/Hedera-helix-English-Ivy.htm)

The plant is a rampant, evergreen vine, commonly known as English ivy, European ivy, common ivy. It is a flowering plant species from the Araliaceae family.
Ivy grows up to 20-30 m in high where climbing surfaces, like trees, walls, pergolas, are available, but it also grows as a groundcover.

Common ivy is cultivated as an ornamental plant. There are multiple cultivars with different ornamental characteristics, such as variegated cultivars with yellow or white, or deeply lobed leaves, dwarf growth or with purple stems.
Although it is a species often used in landscaping, it is considered an invasive species, so more attention should be paid to maintenance.
Lavandula spp., or lavender, is an odoriferous plant that offers a fragrance to the environment. Its small leaves help to capture the particles from the air and reduce their dispersion (Figure 5) (Gabrys, 2018).

Figure 5. *Lavandula* spp. leaf details (https://garden.org/plants/photo/334136/)

Lavander is a species of flowering plant in the family Laminaceae (Șelaru, 2007).

Figure 6. *Salvia officinalis* L. leaves details (https://theoriginalgarden.com/p/seeds/aromatics_medicinals/aromatics_medicinals/seeds-salvia-officinalis-common-sage#gallery-4)

Salvia officinalis L., commonly named sage, is a bioaccumulative plant absorbing heavy metals such as zinc. Hairy leaves help to capture particles (Figure 6), and the flowers attract pollinators (Gabrys, 2018).
Other ideal plants for capturing particle matter with the help of leaves or the way of growing are *Cosmos bipinnatus* cvs. (garden cosmos), *Stachys byzantina* K. (lamb's-ear), *Cineraria maritima* L. (silver ragwort) and *Phalaris arundinacea* L. (reed canary grass).
Some tree and shrub species may also be used to improve air quality and absorb heavy metals.

Figure 7. Protective hedge and trees (http://gardendrum.com/2013/07/15/red-cow-farm-garden/

These may be used in plant compositions or to create protective walls (Gabrys, 2018) as seen in Figure 7. *Taxus baccata* L (yew) and *Pinus mugo* Turra (creeping pine), for example, help to capture the particles by having small leaves (Tatiana, 2009) and high resistance to dust, smoke and gas, while *Betula pendula* Roth. (silver birch), besides capturing particles and having a high resistance to smoke and gas (Tatiana, 2009), is also a bioaccumulator of heavy metals thus improving the quality of the soil (Beckett, 2000).

RESULTS AND DISCUSSIONS

Plants are living organisms that constantly feel and change our environment. There are plants that are much more efficient in taking pollutants, either by attracting heavy metals through their roots, or by channelling and depositing particles in the leaves or by absorbing gaseous pollutants through stomata (Gabrys, 2018).

Studies conducted by Imperial College (Shackleton *et al.,* 2010) have shown that vegetation hedges made from shrubs or perennials near pollution sources have reduced particle levels and contributed to the absorption of gases such as nitrogen dioxide.

Another study (Tremper *et al.,* 2015) shows how a protective curtain made of ivy reduced the level of pollution near a playground.

The study carried out by Säumel *et al.* (2012) indicated that high global traffic increases the content of heavy metals while the presence of barriers between the place of cultivation and roads decreases their content. The main protective curtains of urban crops are buildings and vegetation curtains (Säumel *et al.,* 2012).

Leake *et al.,* (2009) notes that although vegetable plants were grown on urban sites with high contamination, no evidence was found regarding the negative health effects when compared to typical urban sites. Thus, the study offers additional assurance that sites classified with high degree of contamination are not a major risk.

Growing one's own food in urban spaces has a possible health benefit thus improving the mental and psychological health of applicants (Leake *et al.,* 2009).

The most common pollutants in urban areas are anthropogenic (Vittori Antisare *et al.,* 2015). These are caused by the emissions of road traffic, incinerator, industrial deposits and the industrial history of the site (Chen *et al.,* 2005; Vittori Antisare *et al.,* 2015).

Intense human activity produces the main pollutant of the soil - heavy metals (Khan *et al.,* 2015). Studies on heavy metals have shown that the distance from contaminated roads or areas plays an important role in reducing the degree of contamination of plants (Gherardi *et al.,* 2009).

The place in the city where vegetables are grown influences the concentration of heavy metals in them. As an example, plants grown 10 m from the road have a higher concentration of heavy metals compared to plants grown at a distance of 60 m from the road (Vittori Antisari *et al.,* 2015).

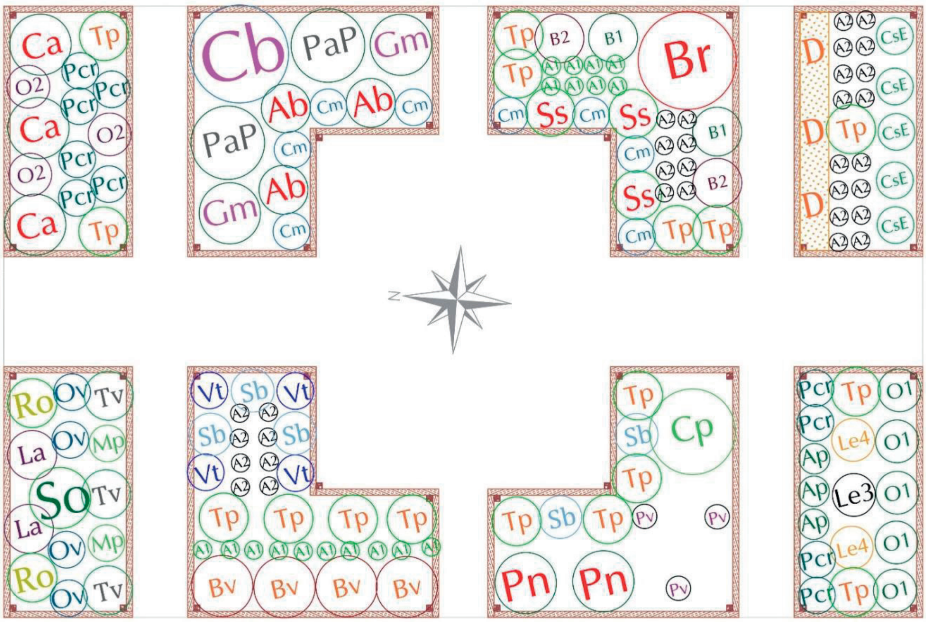

Figure 8. Example of planting plan

Plants that can reduce pollution or can be used as bioindicator of air pollution in urban areas are usually plants that have a surface where particulate matter can be trapped on (plants with hairy leaves) - *Alchemilla mollis* Rothm. (garden lady's mantle), *Euonymus japonicas* Thunb. (evergreen spindle), *Forsythia x intermedia* Sab. (border sorythia), *Hydrangea arborescens* L. (smooth hydrangea), *Parthenocissus quinquefolia* Planch. (Virginia creeper), *Parthenocissus tricuspidata* Planch. (Boston ivy), *Spiraea* spp. (spirea), *Salvia* spp. (sage), *Stachys byzantine* K. (lamb's-ear) etc. - or plants that are sensitive to some pollutants like ozone - *Phaseolus vulgaris* L. (common bean), *Artemisia* spp. (mugwort), *Festuca rubra* L. (red fescue), *Rhus typhina* L. (staghorn sumac), *Solanum tuberosum* L. (potato). etc (Gabrys, 2018).

As a result of this study, the following vegetable garden design was created, which can be seen in Figure 8.

The list of plants (Table 1) has been carefully made according to the ecological requirements of the plants (light, water, soil), their way of association and succession, the decorative elements (height, form, colour and texture of the leaves, flowers and fruits) and the possibility to decorate for a longer period of time.

Table 1. List of plants used in the planting plan

Abbreviation	Scientific name and cultivar	Common name
A1	*Allium sativum* L.	garlic
A2	*Allium schoenoprasum* L. cv. Chinese Chives	chives
Ab	*Aster novi-belgii* L. cv. Marie Ballard	New York aster Marie Ballard
Ap	*Apium graveolens* L. var. rapaceum	celery
B1	*Brassica oleracea* L. convar. acephala cv. Kadet	curly kale green Kadet
B2	*Brassica oleracea* L. convar. acephala cv. Scarlet	curly kale Scarlet
Br	*Brassica oleracea* L. var. capitata f. rubra	red cabbage
Bv	*Beta vulgaris* var. cicla cv. Chard Bright Lights	leaf beet Chard Bright Lights
Ca	*Capsicum annuum* L. cv. Brilliant	pepper Brilliant
Cb	*Cosmos bipinnatus* Cav. Cv. Sensation	garden cosmos Sensation
Cm	*Cineraria maritima* L.	silver ragwort
Cp	*Cucurbita pepo* L. cv. Óvári Fehér	Patison pumpkin
CsE	*Cucumis sativus* L. cv. Ekol	cucumber Ekol
D	*Daucus carota* L. cv. Rondo	carrot Rondo
Gm	*Geranium maculatum* L. cv. Splish Splash	wild geranium Splish Splash
La	*Lavandula x intermedia* L. cv. Grosso	lavender Grosso
Le3	*Lycopersicon esculentum* Mill.	tomato
Le4	*Lycopersicon esculentum* Mill. cv. Yellow Pearshaped	cherry tomato Yellow Pearshaped
Mp	*Mentha x piperita* L.	peppermint
O1	*Ocimum basilicum* L. cv. Italiano classico Genovese	basil Italiano classico
O2	*Ocimum basilicum* L. cv. Serafim	basil Serafim
Ov	*Origanum vulgare* L.	oregano
PaP	*Phalaris arundinacea* Picta	reed canary grass
Pcr	*Petroselinum crispum* Mill.	parsley
Pn	*Phaseolus vulgaris* conv. nanus L. cv. Lingua di Fuoco Nano	dwarf bean Lingua di Fuoco Nano
Pv	*Phaseolus vulgaris* L. cv. A Cosse Violette	bean A Cosse Violette
Sb	*Stachys byzantina* K.	lamb's-ear
So	*Salvia officinalis* L.	sage
Ss	*Salvia splendens* Raf. cv. Scarlet	scarlet sage
Tp	*Tagetes patula* L.	French marigold
Tv	*Thymus vulgaris* L.	common thyme
Vt	*Viola tricolor* L.	Johnny Jump up

CONCLUSIONS

The main pollution sources in urban and peri-urban areas are anthropogenic. These are caused by incinerators, industrial deposits, industrial history of the site and mainly, from the emissions of road traffic. The contaminants that are stored in water, soil and air can affect the product quality and healthiness of the plants but can also threaten the health of the people.

Plants have the ability to change our environment. There are plants that have a high resistance to urban pollution and are more efficient in capturing pollutants throughout their roots, stomata or depositing particles on the leaves.

In order to create an environmentally friendly vegetable garden some aspects were taken into consideration. It is best to have a location far away from a high traffic main road but this aspect is not always met. The main protective barrier of a private garden consists of a green hedge. To enhance the ability of capturing more particle matter it is recommended to plant trees that can have also the ability to capture air and soil pollutants (for example silver birch).

Different plant species have different abilities to absorb and accumulate metals thus, two species of plants grown in the same soil do not present the same risk to human consumers.

Another way to reduce pollution in a small vegetable garden is to take advantage of companion planting and integrate species of plants that are capable of attracting particle matter with their leaves (e.g. *Stachys byzantina* K.*, Cineraria maritima* L.) or have the ability to absorb heavy metals from the soil (e.g. *Salvia officinalis* L., *Aster* spp.).

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THE USE OF THERMOPHILIC MICROMYCETES IN THE PREPARATION OF SUBSTRATES FOR THE CULTIVATION OF EDIBLE MUSHROOM BICUSPID

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Abstract

The paper describes the results of research in using the thermophilic mashroom Thielavia terrestris (Apinis) Malloch et Cain in the practice of preparing compost substrate for cultivation of Agaricus bisporus (J.E. Lange) Imbach. Industrial production of poultry produces a large amount of waste - litter and litter materials, potentially suitable for use in champignon composts. However, the litter mass contains a large number of ligno-cellulose inclusions that adversely affect the development of champignon mycelium and are poorly amenable to bioconversion processes. The solution to the problem may be in preliminary implementation of thermophilic mushroom with a rich spectrum of ligno- and cellulolytic enzymes. The use of Thielavia terrestris accelerated compost ripening by 3-5 days, increased the content of sugars available for champignon mycelium in compost by 12-18%, improved the compost structure and increased champignon yield on the converted substrate by 20-22%. The methods proposed in the current article provide the possibility of bioconversion of significant waste amounts in the form of litter and litter mass into valuable mushroom protein.

Key words: *biotechnology, thermophilic mushroom, compost substrate, bioconversion processes.*

INTRODUCTION

Cultivated edible mushrooms are of great consumer importance. It is a source of valuable protein, vitamins, trace elements, and biologically active substances (Ramos et al., 2019). Investigations are being actively conducted in the world on optimizing the cultivation of champignon in a culture and increasing its nutritional value (Rzymski et al., 2016; McGee, 2018).

In recent years, researchers have been paying their attention to reducing of the biological hazard in the resulting product, as well as the problems of the interaction of microorganisms living in compost (Singh et al., 2012; Šantrić et al., 2018; Qiu et al., 2019).

The scientific articles related to the study of the compost microorganisms role in the development of mushrooms are of great interest for our research. It has been shown that *Mycothermus thermophilus* and a number of other thermophilic microorganisms can stimulate the development of champignon (Kertesz et al., 2018).

Studies on cellulose destruction and substrate adaptation for the cultivation of an edible fungus using a complex of microorganisms are noteworthy for our research (Zhang et al., 2014; Zhang et al., 2019). A number of researchers point to the fact that most of the compost carbohydrates remain unused in the process of growing mushrooms, noting the feasibility of searching for *A. bisporus* strains that can utilize components that are poorly used by commercial strains (Pontes et al., 2018). However, the high productivity of commercial strains remains an important factor.

In this context, studies relevant to the investigation the thermophilic microorganisms role that have cellulolytic potential and are able to provide preliminary degradation of materials intended for the production of compost are relevant.

MATERIALS AND METHODS

The current research was conducted on the basis of the biotechnology laboratory in the institution of higher education - Penza State Agrarian University (Russia). In the experiments we used the commercial strain *Agaricus bisporus* 512, which is widespread in Russian mushroom farms, as well as the culture

of the *Thielavia terrestris* mushroom strain F144, obtained from the VKPM collection. The cultivation of the strains was carried out according to standard methods.
As a substrate for composting the utilized underlay materials were used such as waste products generated during the industrial rearing of turkeys. These materials contain a significant amount of cellulose, a material difficult to assimilate champignon mycelium.
The biological efficiency of growing basidiomas was determined as the ratio of the fresh mass of mushrooms to the dry mass of the substrate. The conversion coefficient was calculated as the ratio of the dry mass of mushrooms to the dry mass of the substrate. All experiments were performed in triplicate. Statistical processing was carried out using the program for processing and analysis of data "Statistica 10.0".

RESULTS AND DISCUSSIONS

The following compost options were prepared for the research: option 1 - based on broiler underlay (control); option 2 - based on underlay material from laying hens; 3 - based on turkey underlay material.
The prepared composts were placed in the 6 liter plastic crates, inoculated with champignon mycelium (*Agaricus bisporus* 512 strain) and incubated in laboratory conditions to obtain growing mushrooms. Compost based on broiler droppings was mastered first of all on 12 ± 2 days. The emergence of the rudiments of growing mushrooms was noted on 7 ± 2 days after applying the integumentary earth. Compost based on dropping materials from laying hens was mastered more slowly, although the differences were within the statistical error. The third version of compost, based on the litter material of the turkey was mastered by mycelium at a longer time by 18 ± 3 days, the emergence of the rudiments of growing mushrooms was weakly expressed. They were formed singly.
The first wave of uprising had been lasting for 4-8 days in different versions of the experiment. The maximum yield in the first uprising wave was 6.9 ± 0.7 kg/m^2 and was obtained on the control compost. In the second and third variants, using litter and laying materials of laying hens and turkeys, 5.7 ± 0.7 kg/m^2 and 4.4 ± 0.1 kg/m^2 of biomass of growing champignons, relatively, were obtained in the first uprising wave.
During the second wave of uprising, the yield was proportionally lower. The total yield for two waves of growing is presented in Table. 1.

Table 1. The yield of mushrooms on different composts, kg/m^2 ($p < 0.05$)

Variants	The first wave of uprising	Total yield
Option 1 (control)	6,9±0,7	9.8±0.2
Option 2	5.7±0.7	7.9±0.2
Option 3	4.4±0.1	6.7±0.1

Thus, from the studied compositions, compost with turkey litter materials was the least effective.
The conversion of substances of various composts is ambiguous. The conversion rate of compost prepared on the basis of broiler litter was 8.9%; based on litter materials from laying hens 5.7%; and compost based on turkey litter materials was only 2.4% (Figure 1).
Such results can be explained by a relatively higher dry matter content, as well as low yield of champignon on compost with turkey litter.
In this regard, it becomes necessary to optimize this material in order to change its structure and properties. To do this, the turkey-added substrate was fermented using the thermophilic fungus *Thielavia terrestris* strain F 144, which biodegradable sawdust contributed to a qualitative change in the compost composition.

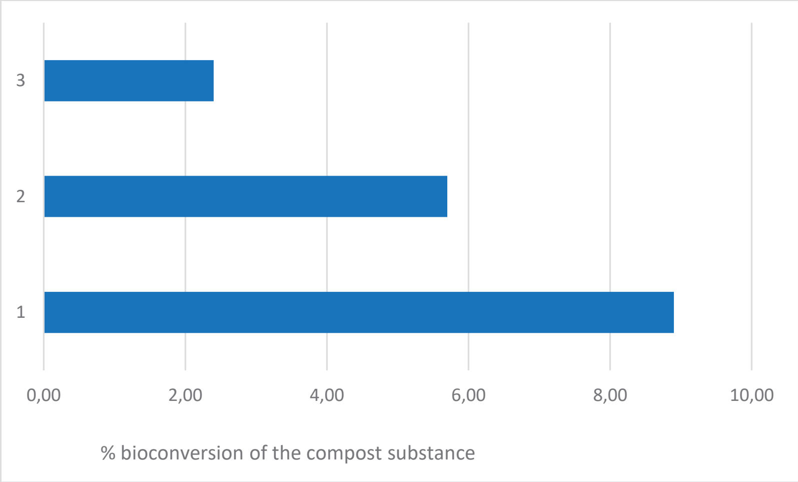

Figure 1. Conversion factors of substances with mycelium on different composts: 1 - compost based on broiler litter; 2 - compost based on litter material from laying hens; 3 - compost based on turkey litter materials ($p < 0.05$)

The mushrooms grown in deep conditions was added to the compost at the stage of layering according to the scheme "layer by layer" in the amount of 10.0 g/kg of compost.
The control material was the compost prepared in the usual way (litter-bedding mixture and wheat straw). Fermentation was carried out for two weeks. Accounting for yield on compost preliminarily fermented with thermophilic mycelial fungus *Thielavia terrestris* showed that the result significantly exceeded the control indicators (Table 2) and amounted to 9.4 ± 0.3 kg/m^2, which is comparable to the yield of champignon on compost using broiler litter.

Table 2. Productivity of champignons on composts based on litter material of turkeys, kg/m^2 ($p < 0.05$)

Compost Features	**1-wave yield**	**Total yield**
Compost without pre-fermentation	4.4±0.1	5.8±0.3
Thielavia terrestris fermented compost	6.7±0.1	9.4±0.3

This is probably due to the fact that the fungal enzymes *Thielavia terrestris* contributed to the conversion of lignocellulosic components of the litter into sugar, which is accessible for assimilation by mycelium.
This is also indicated by the sharply increased coefficient of substrate nutrient conversion, which is 3 times higher than in the control (without using *Thielavia terrestris*) (Figure 2).
It should be noted that the obtained conversion rate of 6.3% was higher than in the variant with the use of litter-laying materials of laying hens, although slightly lower than compost based on broiler litter.

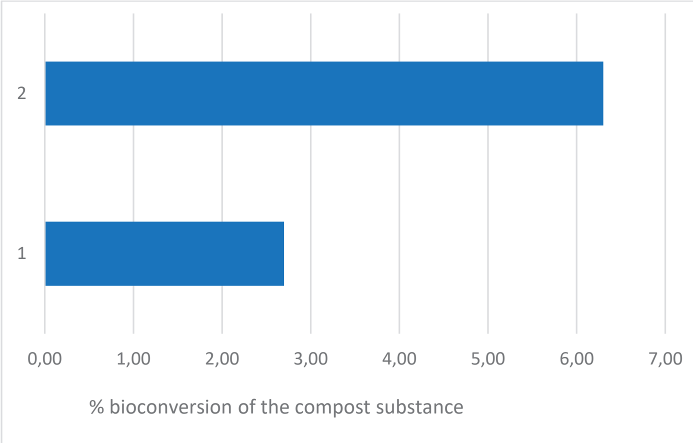

Figure 2. The conversion factors of substances with mycelium on different composts: 1 - compost based on litter materials of turkeys without fermentation *Thielavia terrestris*; 2 - compost based on material previously fermented with *Thielavia terrestris* ($p < 0.05$)

Reliably noted increase in the efficiency of substrate nutrient conversion and an increase in the yield of champignons with the addition of the compost, originally rich in lignocellulosic components, but fermented by the thermophilic cellulosolytic fungus *Thielavia terrestris*. Using the pre-fermentation process with cellulolytic enzymes makes it possible to utilize waste materials that are massively produced in turkey growing – litter and bedding materials containing a significant amount of straw and sawdust as nutrient substrates. Thus, in laboratory conditions, the positive effect of using preliminary fermentation of a substrate rich in cellulose and lignin sources for the subsequent utilization of nutrients by the mycelium of edible champignon fungus and increasing yield was established (Figure 3).

Figure 3. Growing of *Agaricus bisporus*: on the left - compost without preliminary fermentation, on the right - compost pre-fermented with *Thielavia terrestris*

CONCLUSIONS

Thus, in laboratory conditions, the positive effect of using preliminary fermentation of a substrate (turkey litter and bedding materials) rich in cellulose and lignin sources, mycelium of the thermophilic fungus *Thielavia terrestris* for subsequent utilization of nutrients by the mycelium of edible champignon mushroom and increase its productivity was established.

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VALORISATION OF *ARONIA MELANOCARPA* POMACE FOR DEVELOPMENT OF FUNCTIONAL INGREDIENTS WITH HIGH NUTRITIONAL VALUE AND ANTIOXIDANT CAPACITY

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Abstract

At the industrial level, the fruits of Aronia melanocarpa are processed mainly in the form of juice, resulting significant quantities of Aronia pomace, which is noted by the content of polyphenols, antocyanins, dietary fibers, pectic substances, minerals and vitamins. In this paper are presented the results of the research performed to achieve a functional ingredient (powder) from Aronia waste resulting in fruit juice industry. For this purpose, Aronia pomace (from the conventional and organic culture of our country) was subjected to a convective drying process at 50°C or lyophilisation at -55°C to protect the bioactive compounds to a moisture content to allow their milling and turning them into powder and their stability in terms of quality. Powder achieved from Aronia pomace is characterized by total dietary fiber content (55.42-58.67%), minerals (1.97-2.73%), total sugar (12.72-15.89%), vitamin C (9.05-26.08 mg/100 g), and total polyphenols (50.26-96.92 mg GAE/g). At the same time, powder achieved from Aronia pomace has antioxidant capacity. Due to its complex biochemical composition and antioxidant potential, the functional ingredient achieved from Aronia pomace can be used to fortify bakery and pastry products.

Key words: *Aronia, pomace, powder, total polyphenols, dietary fiber.*

INTRODUCTION

Aronia melanocarpa fruits are some of the richest sources of bioactive compounds, having high biological and nutritional values (Horszwald et al., 2013; Janković et al., 2016; Chrubasik et al., 2010). At the same time, these fruits are a good source of polyphenols, which have high antioxidant activity. The main group of polyphenols found in this plant are anthocyanins, which are responsible for astringent taste and intense coloring (Caruso et al., 2016; Kulling and Rawel, 2008). Many recent studies have demonstrated the positive multidirectional effects of these compounds on health. Anthocyanins positively influence the circulatory system and the functioning of the heart. They stimulate insulin secretion and improve retinal functioning (Bermúdez-Soto et al., 2007; Lala et al., 2006; Li et al., 2017). Jurikova et al. (2017) mention that the fruits of *Aronia melanocarpa* are an important source of antioxidants, especially polyphenols, such as phenolic acids (neochlorogenic and chlorogenic acids) and flavonoids (anthocyanins, proanthocyanidins, flavanols and flavonols), especially, cyanidine-3-galactoside and cyanidine-3-arabinoside, as well as units of (-) epicatechin. Due to the high content of these bioactive compounds, the fruits of *Aronia melanocarpa* have a wide range of positive effects, such as a strong antioxidant activity and potential medicinal and therapeutic benefits (gastroprotective, hepatoprotective, antiproliferative or anti-inflammatory effects). These also could contribute to the the prevention of chronic diseases, including metabolic disorders, diabetes and cardiovascular diseases, due to the impact on lipid profile, of plasmatic glucose à *jeun* and blood pressure. Popularity of *Aronia melanocarpa* fruits is increasing due to its association with potential health benefits, such as: antioxidant, antibacterial, antiviral, antimutagenic, anticancer, cardioprotective, hepatoprotective, gastroprotective, antidiabetic and anti-inflammatory properties. Benefiacial effects of *Aronia melanocarpa* fruits have been demonstrated by *in vitro* and *in vivo* studies (Cvetanović et al., 2018; Kokotkiewicz, Jaremicz, & Luczkiewicz, 2010). Due to the astringent taste, to be accepted by consumers, *Aronia melanocarpa* fruits are dehydrated and then are ground and transformed into powders and used to obtain teas, instant juices, fruit yogurt, desserts and food supplements (Sadowska et al., 2019).

After obtaining the *Aronia* juice, the juice of and the juice of *Aronia and* elderberry (*Sambucus nigra* L.) result important quantities of waste (*Aronia* pomace, elderberry pomace), which is noted for its content in polyphenols, anthocyanins, food fibers, pectic substances, minerals (K, Ca, Mg, Fe) and vitamins (Grunovaite et al., 2016; Rodrigues et al., 2018). Due to the high content of polyphenols, waste *Aronia* fruits and elderberry (*Sambucus nigra* L.) fruits, they have *antioxidant potential* and have beneficial effects on the human body, to prevent diseases caused by oxidative steess, such as cardiovascular diseases, neurodegenerative diseases, cancer, diabetes and osteoporosis (Dufour et al., 2018).

In this paper are presented the results of the research performed to achieve a functional ingredient (powder) from *Aronia* waste resulting in fruit juice industry.

MATERIALS AND METHODS

Samples

Aronia melanocarpa pomace resulted by *Aronia melanocarpa* fruits processing into juice within the Pilot Experiments Plant for Fruits and Vegetables Processing in IBA Bucharest, using a juicer extractor (Philips). Within experiments were used *Aronia melanocarpa* fruits, purchased from Romanian farmers. Within experiments were used *Aronia melanocarpa* fruits from conventional (II) and organic (I and III) culture. Till processing, *Aronia melanocarpa* pomace was stored under refrigeration (3°C). *Aronia melanocarpa* pomace was subjected to dehydration by two procedures: convective dehydration with hot air at 50°C and lyophilisation at -55°C. Dehydration process of *Aronia melanocarpa* pomace, was achieved to a moisture (3.20-6.18%) which allows their milling and conversion into powder and, at the same time, their stability in terms of quality. Milling of dried semi-finished products was performed by using Retsch mill. The achieved functional ingredients (powders) were packed in glass containers, hermetically sealed, protected by aluminum foil against light and stored in dry and cool areas (temperature of maximum 20ºC), till to the biochemical analysis. Figure 1 shows *Aronia melanocarpa* pomace powders obtained by dehydration at 50°C, and Figure 2 those obtained by lyophilisation.

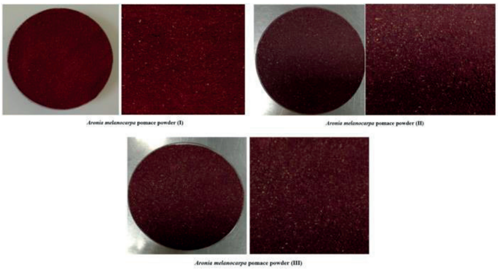

Figure 1. *Aronia melanocarpa* pomace powder (dehydration at 50°C)

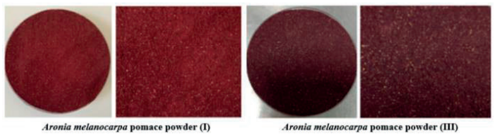

Figure 2. *Aronia melanocarpa* pomace powder (lyophilisation at -55°C)

Methods

Sensory analysis

Sensory analysis (appearance, taste and smell) was performed by descriptive method.

Physico-chemical analysis

Measurement of the colour parameters of samples was performed at room temperature, using a CM-5 colorimeter (Konica Minolta, Japan), equipped with SpectraMagic NX software, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), L^*, a^* and b^*: L^* - colour luminance (0 = black, 100 = white); a^* - red-green coordinate (-a = green, +a = red); b^* - yellow-blue coordinate (-b = blue, +b = yellow).

Moisture determination was performed with Ohaus Moisture Analyzer MB45 at temperature 105°C.
Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005). Fat content was determined according to AOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005).
In order to determine minerals samples were mineralized by calcination, with the addition of hydrochloric acid and hydrogen peroxide. The minerals sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and zinc (Zn) were determined by Atomic Absorption Spectrophotometer (type *AAnalyst* 400, Perkin–Elmer). Iron (Fe) was determined by Graphite Furnace Atomic Absorption Spectrophotometer (type *AAnalyst* 600, Perkin–Elmer).
Total sugar content was determined according to Schoorl method.
Total dietary fibre (TDF) was determined by enzymatic method using the assay kits: K-TDFR ''Total dietary fibre'' (AOAC Method 991.43). Determination of pectic substances (expressed as calcium pectate) was achieved by gravimetric method.
Determination of vitamin C content was performed by high performance liquid chromatography (Accela, Thermo Scientific) coupled with high resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) using hippuric acid as internal standard (Catană et al., 2017).
Determination of vitamin E (α-tocopherol) content was performed by high-performance liquid chromatography (HPLC-DAD) (Popović et al., 2015).

Total polyphenol content
Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extraction media, time and mode of extraction, extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in methanol:water 50:50, and the absorbance of the extracts was determined at a wavelenght λ = 755 nm. Results were expressed as mg of Gallic Acid Equivalents (GAE) per g *Aronia melanocarpa* pomace powder.

Antioxidant capacity
The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f(Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g *Aronia melanocarpa* pomace powder.

Microbiological analysis
The water activity (*A*w) was determined by an instrument Aquaspector AQS-2-TC, Nagy. The measurements were performed at 25ºC. Yeasts and molds were determined by the method SR ISO 21527-1:2009. *Enterobacteriaceae* were determined according to the SR EN ISO 21528-1:2017 method and *Escherichia coli* by SR ISO 16649-2:2007 method. *Salmonella* was determined by the method SR EN ISO 6579-1:2017.

RESULTS AND DISCUSSIONS

Sensory analysis
As a result of the sensory analysis, it was found that the powders achieved from *Aronia melanocarpa* pomace, have colours from cherry to dark cherry and, respectively, brown, and have pleasant taste and smell, characteristic of these fruits.
As a result of the instrumental colour analysis, it was found that the powders obtained from *Aronia melanocarpa* pomace, by dehydration with hot air, at 50°C, are darker in colour compared to those obtained by lyophilisation, at -55°C, registering lower luminance values, respectively, L* = 38.55, in the case of sample II (Figures 3, 4).

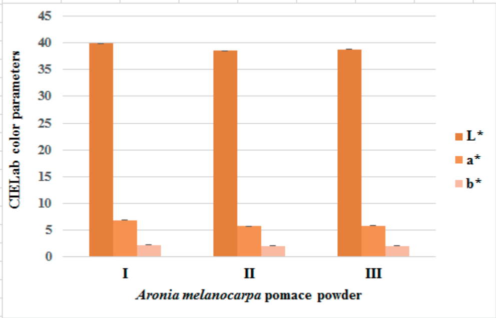

Figure 3. Colour parameters of the powders achieved from *Aronia melanocarpa* pomace (dehydration at 50°C)

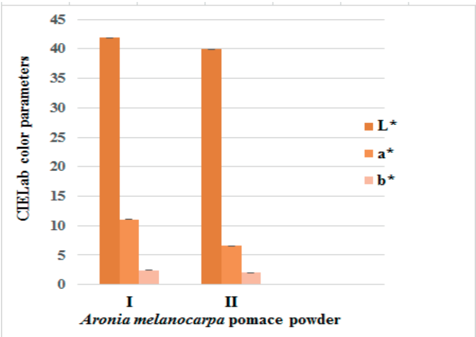

Figure 4. Colour parameters of the powders achieved from *Aronia melanocarpa* pomace (lyophilisation at -55°C)

Also, the minimum values of parameters a* (red colour coordinate) and b* (yellow colour coordinate) were also recorded for powders obtained from *Aronia melanocarpa* pomace, by dehydration with hot air, at 50°C (a* = 5.71 and b* = 1.98, values recorded in the case of sample II).

Physico-chemical analysis
Composition of the powders achieved from *Aronia melanocarpa* pomace is presented in Table 1.

Table 1. Physico-chemical composition of powders achieved from *Aronia melanocarpa* pomace

Functional ingredient	Water (%)	Ash (%)	Protein (%)	Fat (%)	Total sugar (%)	Total fibre (%)
Dehydration with hot air at 50°C						
Sample powder I	5.79±0.14	2.63±0.03	6.81±0.06	3.11±0.03	15.40±0.08	56.85±1.05
Sample powder II	6.18±0.15	1.97±0.02	6.38±0.06	3.30±0.04	12.72±0.06	55.42±1.03
Sample powder III	5.22±0.13	2.01±0.02	5.67±0.05	3.19±0.04	15.52±0.08	57.60±1.06
Lyophilization at -55°C						
Sample powder I	3.20±0.08	2.73±0.03	7.03±0.06	3.21±0.03	15.89±0.08	58.50±1.08
Sample powder III	3.85±0.10	2.04±0.02	5.80±0.05	3.27±0.04	15.80±0.08	58.67±1.09

Following the physic-chemical analysis, it was found that the powders achieved from *Aronia melanocarpa* pomace, are noted for their content in protein (5.67-7.03%), total ash (1.97-2.73%), total sugar (12.72-15.89%) and total fiber (55.42-58.67%), the obtained results being in accordance with those from specialty literature (Reiβner et al., 2019; Sidor and Michalowska, 2019).
Powders achieved from *Aronia melanocarpa* pomace (I) has the highest total ash content (2.63% in the case of powder achieved by dehydration with hot air, at 50°C and 2.73% in the case of that achieved by lyophilisation, at -55°C), protein (6.81% in the case of powder achieved by dehydration with hot air, at 50°C and 7.03% in the case of that achieved by lyophilisation, at -55°C), and the powder achieved from *Aronia melanocarpa* pomace (III) has the highest content of total sugar (15.52% in the case if powder achieved by dehydration with hot air, at 50°C and 15.80% in the case of that achieved by lyophilisation, at - 55°C) and total fiber (57.60% in the case of powder achieved by dehydration with hot air, at 50°C and 58.67% in the case of that achieved by lyophilisation, at -55°C). For *Aronia melanocarpa* powder, achieved by dehydration with hot air, at 60°C and grinding of *Aronia melanocarpa* pomace, resulted after the juice obtaining, Reiβner et al. (2018), have obtained similar values for the principal physicf-chemical indicators: moisture = 2.72%; lipids = 3.61% s.u.; protein = 5.97% s.u.; ash= 1.92%s.u.; total fiber = 59.50% s.u. Also, Sidor and Michalowska (2019), report for the powder achieved from *Aronia melanocarpa* pomace a protein content of 10.67%, lipid content of 5.15%, ash content of 1.95%.
Also, powders obtained from *Aronia melanocarpa* pomace, are noted for their content in pectic substances (6.23 – 7.75%), expressed as calcium pectate (Table 2).
Content in pectic substances of the powders achieved from *Aronia melanocarpa* pomace, obtained in this study, are in accordance with that reported by Sidor and Michalowska (7.52%).
The consumption of pectins generates an increase in beneficial microbial populations in the gastrointestinal tract, increasing production levels of short chain fatty acids and gases such

as methane, carbon dioxide and hydrogen, positively affecting health (Blaut, 2002; David et al., 2014). Also, researches in the field have shown a relationship between pectins consumption and maintenance of normal blood cholesterol levels or a reduction of post-prandial glycaemic responses (Ferretti et al., 2014).

Table 2. Content in pectic substances of powders achieved from *Aronia melanocarpa* pomace

Functional ingredient	Pectic substances (% calcium pectate)
Dehydration with hot air at 50°C	
Sample powder I	7.10±0.18
Sample powder II	6.23±0.16
Sample powder III	7.50±0.19
Lyophilization at -55°C	
Sample powder I	7.28±0.18
Sample powder III	7.75±0.19

Powders achieved from *Aronia melanocarpa* pomace, are noted for their mineral content (K, Ca, Mg, Fe, Zn). Figures 5 and 6 show the mineral content of the powder achieved from *Aronia melanocarpa* pomace by dehydration, at 50°C.

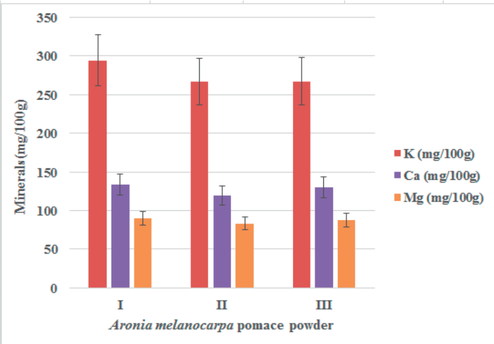

Figure 5. Mineral content (Na, K, Ca and Mg) of the powders achieved from *Aronia melanocarpa* pomace (dehydration at 50°C)

Powder obtained from *Aronia melanocarpa* pomace, the sample I, has the highest potassium content (294.04 mg/100 g). Calcium content of the powders achieved from *Aronia melanocarpa* pomace, varied in the range 119.94-133.29 mg/100 g, the maximum value being recorded in the case of powder achieved from the fruits sample III (organic culture). Magnesium content of the powders taken into study represents about 67-69.6% of the calcium content of them.

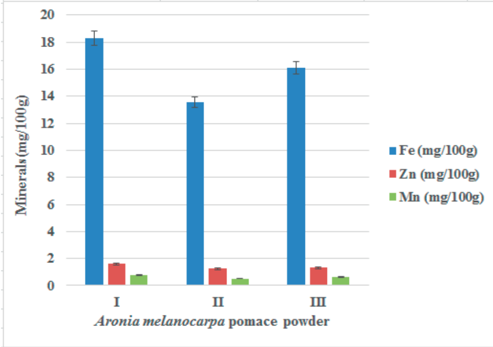

Figure 6. Mineral content (Fe, Zn and Cu) of the powders achieved from *Aronia melanocarpa* pomace (dehydration at 50°C)

Iron content of the powders achieved from *Aronia melanocarpa* pomace, varied in the range 13.55-18.30 mg/100 g, the maximum value being recorded in the case of powder achievde from *Aronia melanocarpa* pomace, sample I. Zinc content of the powders in this experimental study, varied in a narrow range: 1.24-1.59 mg/100 g (maximum value was also recorded for powder achieved from *Aronia melanocarpa* pomace, sample I. The lowest values were recorded for the manganese content (0.52-0.77 mg/100 g).

Content in K, Mg, Fe and Zn of powders achieved from *Aronia melanocarpa* pomace, by dehydration with hot air, at 50°C, is comparable to that reported by Sidor and Michalowska (2019), as being obtained by Pieszka et al. (2015), in the case of *Aronia melanocarpa* pomace, resulted after juice obtaining (K: 278 mg/100 g; Mg: 88 mg/100 g; Fe: 19.7 mg/100 g; Zn: 1.57 mg/100 g). In contrast, the Ca and Mn content of the powders taken into study is lower, compared to that obtained by Pieszka et al. (2015), in the case of *Aronia melanocarpa* pomace, resulted after the juice obtaining (Ca: 275 mg/100 g; Mn: 3.15 mg/100 g).

Between the mineral content of the powders achieved by dehydration at 50°C and, respectively, lyophilisation at - 55°C (in the case of *Aronia melanocarpa* I and III fruit samples), there are very small differences, caused by the difference of sample moisture (Table 3).

Table 3. Mineral content of the powders achieved from *Aronia melanocarpa* pomace (lyophilisation at -55°C)

Minerals	Powders achieved from *Aronia melanocarpa* pomace	
	Sample powder I	Sample powder IIII
K (mg/100g)	303.35 ±34.28	287.23±32.46
Ca (mg/100g)	136.95±14.12	131.56±13.56
Mg (mg/100g)	92.10±9.21	88.79±8.88
Fe (mg/100g)	18.80±0.56	16.33±0.49
Zn (mg/100g)	1.63±0.09	1.34±0.08
Mn (mg/100g)	0.79±0.02	0.64±0.02

Bioactive compounds content

Powders achieved from *Aronia melanocarpa* pomace are sources of bioactive compounds: total polyphenols, vitamin C and vitamin E (α-tocoferol) (Table 4).

Table 4. Bioactive compounds content of the powders achieved from *Aronia melanocarpa* pomace

Functional ingredient	Total polyphenols (mg GAE/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)
Dehydration with hot air at 50°C			
Sample powder I	6105.55±152.64	12.44±0.41	1.27±0.07
Sample powder II	5025.65±125.64	9.05±0.30	1.12±0.06
Sample powder III	6585.28±164.63	14.25±0.47	1.40±0.08
Lyophilization at -55°C			
Sample powder I	8566.85±214.17	20.53±0.68	2.16±0.12
Sample powder III	9692.32±234.63	26.08±0.86	2.45±0.13

Total polyphenol content of the powders achieved from *Aronia melanocarpa* pomace varied in the range 5025.65-9692.32 mg GAE/100 g, and vitamin C content in the range 9.05-26.08 mg/100 g. It is worth noting that the powders achieved from *Aronia melanocarpa* pomace, by lyophilisation, have a higher content of polyphenols, respectively, vitamin C, compared to those achieved by dehydration at 50°C (1.4 times, respectively, 1.47 times in the case of total polyphenols; 1.65 times, respectively, 1.83 times, in the case of vitamin C). Also, in the case of these powders, the content of vitamin E (α-tocopherol) varied in the range 1.12-2.45 mg/100 g (the minimum value was recorded in the case of powder achieved from *Aronia melanocarpa* pomace, by dehydration, at 50°C, and the maximum one in the case of that obtained by lyophilisation, at -55°C).

Antioxidant capacity

Due to their content in antioxidants (total polyphenols, vitamin C, vitamin E etc.), powders achieved from *Aronia melanocarpa* pomace, have antioxidant capacity (Figure 7).

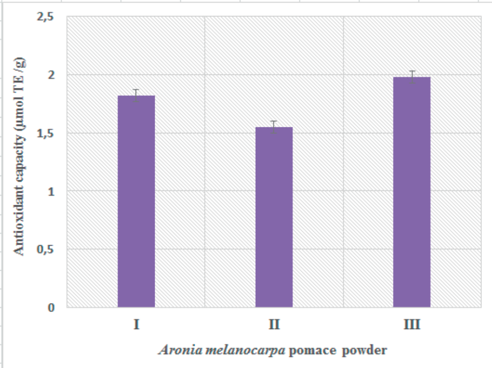

Figure 7. Antioxidant capacity of the powders achieved from *Aronia melanocarpa* pomace

In the case of the powders achieved from *Aronia melanocarpa* pomace, by dehydration, at 50°C, the antioxidant capacity varied in the range 1.55-1.98 µmol Trolox Equivalents/g (Figure 7). Powders achieved from *Aronia melanocarpa* pomace, by lyophilisation, at -55°C, recorded higher values of antioxidant capacity: 2.60 µmol Trolox Equivalents/g (sample I) and, respectively, 2.95 µmol Trolox Equivalents/g (sample III).

Microbiological analysis

Results of the microbiological analysis of the powders achieved from *Aronia melanocarpa* pomace are presented in the Table 5.

Table 5. Microbiological analysis of powders achieved from *Aronia melanocarpa* pomace

Functional ingredient	Yeast and mold (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Escherichia coli* (CFU/g)	*Salmonella* (in 25 g)	Water activity (Aw)
Dehydration with hot air at 50°C					
Sample powder I	< 10	< 10	< 10	absent	0.305
Sample powder II	< 10	< 10	< 10	absent	0.315
Sample powder III	< 10	< 10	< 10	absent	0.300
Lyophilization at -55°C					
Sample powder I	< 10	< 10	< 10	absent	0.285
Sample powder III	< 10	< 10	< 10	absent	0.290

Microbiological analysis shown that the achieved powders are in the frame of the provisions of the legislation into force. These powders show low values of water activity (0.285-0.315), which give them microbiological stability.

CONCLUSIONS

Powders achieved from *Aronia melanocarpa* pomace are important sources of minerals (K, Fe, Mg, Ca and Zn), dietary fibres and bioactive compounds.

The powders achieved in this study, are noted for their content in polyphenols (5025.65-9692.32 mg GAE/100 g) vitamin C (9.05-26.08 mg/100 g) and vitamin E (1.12-2.45 mg/100 g). Also these powders have antioxidant capacity (1.55-2.95 μmol Trolox Equivalents/g), being beneficial in a healthy diet for prevention of diseases caused by free radicals.

On the other hand, powders achieved from *Aronia melanocarpa* pomace are characterized by high dietary fibre (55.42-58.67%) and pectic substances (6.23-7.75%), content being important sources to increase the fibre and pectic substances content of foods (bakery products, pastry products, etc.). Increase of the fibre content in case of the sweet flour products is very important because it reduces their glycemic impact on the human body, thus preventing the development of diabetes mellitus and obesity. Also, dietary fibre have an important role in promoting feeling of satiety.

Powders achieved from *Aronia melanocarpa* pomace can be regarded as functional ingredients and can be used to fortify food products (bakery and pastry products, especially) in order to increase the nutritional value and their antioxidant capacity.

ACKNOWLEDGEMENTS

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JUSTIFICATION OF CONSTRUCTION OF INSTALLATION FOR THERMAL DECOMPOSITION OF BIOMASS

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Abstract

An important part of the overall technical base of the modern greenhouse is the heating system. Choosing the most efficient - both technical and cost-effective, both of the thermal energy source and the heat carrier, is one of the most important factors that a greenhouse can successfully fulfill its purpose. The heating system should be selected in such a way that, as soon as possible, the large investment needed to build large-scale greenhouse production is recovered. The article will propose a possible technical solution for greenhouse heating, focusing on modern tools and systems as well as basic approaches for increasing the energy efficiency of the production made in the facility

Key words: *greenhouse, heating, heating system for greenhouses.*

INTRODUCTION

For heated greenhouses production is close to industrial: high yields of good quality resulting from modern cultivation technologies; regular deliveries not dependent on the season of the year. This provides access to buyers with a significant share and lasting position on the market with fresh vegetables. Achieving these strategic goals requires high investment costs. By literary data (Ivanov et al., 2015) - 100 thousand euros per hectare of greenhouse area. For the conditions of Bulgaria, where greenhouse production is characterized by the existence of free - built but not working greenhouse complexes, a variant for reconstruction and modernization is possible. These costs can be recovered as quickly as possible only through the maximum possible production at competitive prices. In order to minimize the cost of the activity in order to reduce the cost of production, it is necessary to carry out thorough economic analyses of each item.

Despite the wide variety of existing greenhouse heating methods, every individual case of investment intention for greenhouse production needs to be approached strictly individually. Given the large share of the cost of maintaining the microclimate in the heated greenhouses - 55-60% according to (Ivanov et al., 2015) of the total costs, apart from the selection of the heating method, the energy carrier should be strictly observed. It should be at the lowest possible price and combined with the most efficient heating technology to lead to minimal costs for providing the microclimate. On the other hand, the energy carrier must also guarantee relative "autonomy" to the consumer, "Independence" from the prices of other existing energy carriers. The aim is, in the case of a sharp rise in fuel and electricity prices, that there is not a corresponding one in the cost and hence in the price of the greenhouse production produced.

The membership of our country in United Europe imposes restrictions on the use of solid fuels - wood and coal. Directive 2009/125 / EC of 21 October 2009 laying down requirements for the reduction of ambient air pollution by fine particles, carbon monoxide (CO) and nitrogen oxides (NOx) emitted during the combustion process. European Commission Regulation 2015/1185 of 24 April 2015 laying down the rules for the implementation of that Directive. According to these two European normative documents of 1 January 2022, a change in the requirements for the heating systems used and the ban on solid fuels is expected.

In Bulgaria, which is poor in natural fossil fuels, the question of the energy efficiency of each production is of utmost importance. Achieving "energy independence" allows for strategies to develop some economic activity without taking into account the growth of energy prices. This provision of energy at "acceptable" prices makes it possible to produce lower-priced products which guarantee their availability at competitive prices.

A great advantage in this respect is provided by renewable energy sources (RES). Biomass, as one of them, is a significant resource contributing to the overall energy consumption in Bulgaria.

Biomass is created from the living matter of Earth as a result of its vital activity and includes all organic matter.

The continuous generation of biomass on Earth characterizes it as RES. The conversion of biomass energy stored in plant biomass into fuel resembles its mode of formation. Expressed by chemical formulas, the process can generally be written: (Benev A., 2008, http://www.shtrakov.net/RET/Lect_09.pdf)

$$CO_2 + 2\,H_2O \underset{heat}{\overset{light}{<\!=\!=\!=\!=\!=\!=\!=\!=\!=\!=\!=\!=\!=\!=\!>}} [(CH_2O) + H_2O] + O_2$$

Expressed in this way - the right reaction expresses photosynthesis, in which biomass - [(CH2O) + H2O] is formed and oxygen (O2) is released.

Reverse - the oxidation of the biomass is accompanied by the release of heat. The exothermic nature of the interaction of biomass with oxygen (oxidation) characterizes it as a fuel. The heat generated during this process is the released solar energy that plant biomass generates during photosynthesis and stored in chemical form. The amount of carbon dioxide (CO2) released by combustion of biomass is as much as is necessary to obtain it through photosynthesis (Panzhava et al., 2002; Effective use of biomass, 2010). Therefore, its use as a fuel does not pollute the natural environment with additional damages and it is also called "green energy" (Nazmeev et al., 2001; Moses et al., 2006).

According to (Hayes, 1987; Benev et al, 1994; Benev, 2008) the net amount of energy is in the range of about 8 MJ/kg for green wood, about 20 MJ / kg for dried plant products, 40 MJ/kg for fats, natural oils and oilseed esters, 55 MJ/ kg for methane. For comparison, these fossil fuels are 27 MJ/kg and 46 MJ/kg, respectively for coal and oil. The energy derived from these fuels is also called "brown" because its use always leads to the release of additional polluting materials. Biomass is the resource that is most easy to use and whose resource is yet to develop (Zaharinov et al., 2014). More than 80 high-calorie technologies have been developed in the application of thermal, biological or chemical treatment, in some of the developments being fully equivalent to diesel oil and even better (Jhon et al, 2008; Balat, 2009).

The relative shares of the different biomass fuels are presented in Figure 1.

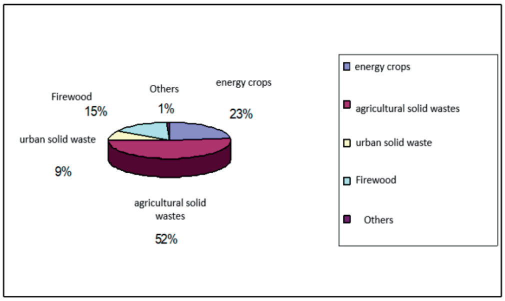

Figure 1. Relative percentages of the various fuels from biomass in Bulgaria
Source: Legal framework for the use of biomass in the countries of the European Union and Republic of Bulgaria, 2005

From the exhibition it is clear that there is a large amount of unused plant and forest residues in Bulgaria. Their use by developing techniques and technologies for harvesting, harvesting and storing them is a challenge for modern society. Using this energy resource will improve the energy independence of both the individual company and our entire country.

In 2020, with full absorption of the biomass energy potential presented in the program, its share will reach 8.5% in gross domestic consumption. About 38% of spent biomass in 2020 is expected to be used to generate electricity and heat, amounting to about 837,000 tons net equivalent. About 70% of the biomass and about 30% of electricity production will be used for this heat production. The share of biomass in final energy consumption will reach 10.7% in 2020, was 8% (National Long-Term

Program to Promote the Use of Biomass for 2008-2020, 2008).

MATERIALS AND METHODS

To explore autonomous technology for the processing of plant residues and low quality wood for heating greenhouses.

The subject of the study is a technology for the use of different types of biomass (plant residues and low-quality wood) as a raw material for heating energy when heating greenhouses.

The means of the study is an installation for the thermal decomposition of plant residues and low quality wood for the purpose of heating the heat carrier.

RESULTS AND DISCUSSIONS

1. Experimental thermal expansion process module.

In Figure 2 is a schematic diagram of an installation of industrial furnaces with periodic action for the thermal decomposition of solid biomass without access of air - retorts. The individual nodes and components of the installation are labelled in order to get an idea of their device - Figure 2.

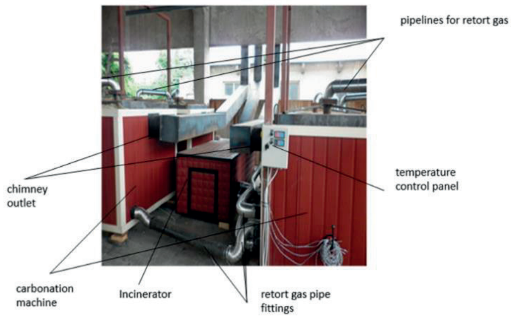

Figure 2. Thermal decomposition plant for biomass

2. Device

The installation consists of: two machines for carbonization (low temperature pyrolysis); insufflator; piping and flue system; control dashboard. Underneath each of the machines an electronic scale is installed. For the interaction between the individual elements of the installation - the thermal decomposition machines of solid biomass and the incinerator, a pipeline installation is used for circulating the thermal decomposition of the biomass gases (Figure 3). A pipe connection exists both between the two machines and between them and the injector. Exhaust gases are used to discharge into the atmosphere through the flue gas chimney. Manipulation, through a change in temperature, of the working processes occurring in low-temperature pyrolysis biomass of solid biomass is using a control panel. Each of the machines consists of a furnace, two chambers for decomposition of solid biomass - returns with a hermetic lid, a system of pipes for the transfer of the gases obtained during the process and for the remainder of their combustion - the smoke. The cameras can also be positioned sequentially depending on the work area.

In this type of equipment, solid biomass is placed in the rhetoric that are heated from outside by hot gases resulting from the thermal decomposition of solid biomass (pyrolysis gases). Retractors are metal containers in the shape of a parallelepiped. They are made of 2 mm thick sheet steel.

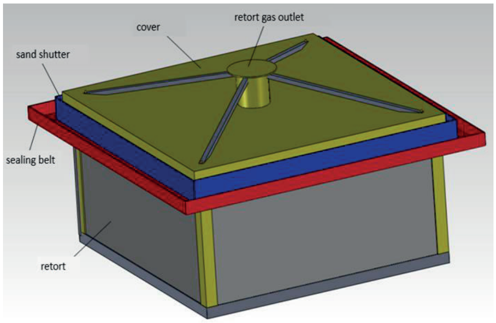

Figure 3. The heat from a thermal decomposition plant for biomass

The rattles are placed in a housing of fireproof insulation. For a tight fit between the machine body and the retort, a sealing belt is used. There is a pressurized biomass filling lid that connects to the body of the retort via a sand block. The steam-gas mixture formed in the thermal decomposition process of the solid biomass is passed through a hole located low at the back of the retort. Then this mixture through a three-way distributor is made: in its first position, to the chimney for discharge into the atmosphere; in the second - for heating the biomass in the next chamber; the third gas-gas mixture goes to the injector.

The incinerator burns the gases obtained from the machines as a result of the thermal decomposition of the solid biomass. The heat generated by this burning is used to heat water in the heat exchanger of the incinerator. From here, the heated water is directed to heating the greenhouse through a pipeline installation.

With the control panel, the temperature in the rhetoric is controlled during the process - the thermal airless decomposition (low temperature pyrolysis) of solid biomass. From the numerical value of the temperature depends the flow of the pyrolysis gases, which in turn determines the output required for the operation of the greenhouse heating system. The pipelines for the separated pyrolysis gases and for the residues from their combustion (smoke) make the connection between the individual elements of the system and that of the environment. To measure the amount of gas emitted during the process of pyrolysis of solid biomass, an electronic balance is used at the base of each machine. The plant is capable of operating in several technological modes, which correspond to the actual heating requirements for greenhouses. In addition, depending on the area of the greenhouse facility and the corresponding heating power, the installation may be composed of a corresponding number of modules.

3. Power management of the installation

The thermal power of the plant is controlled by two circuits:

1. Power of pyrolysis chambers. It is achieved by controlling the separated combustion gases from the retorts by the weighing method;
2. Heat exchanger power. This is achieved by measuring the temperature in the greenhouse or the difference in the temperatures of the outgoing and the water entering the heat exchanger.

For power management of pyrolysis chambers, a system for measuring the mass of exhaust gases is installed in the system.

Figure 4. System for monitoring and setting of modes management of the pyrolysis installation

The management of the separated mass upon biomass heating is carried out by controlling the temperature in the pyrolysis chambers. The temperature is measured with four thermocouples.

Figure 5. Temperature measurement system in the pyrolysis installation's rhetoric

CONCLUSIONS

A constructive concept of a biomass combustion plant is proposed.
An installation for greenhouse heating is synthesized by combustion.
The gasification plant under investigation is in compliance with the national energy efficiency programs and the use of renewable energy sources.
The proposed greenhouse heating technology, in addition to using "green energy", also uses the maximum capacity of biofuel - it has a significantly higher efficiency than its direct incineration.

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RESEARCH ON THE ENTOMOFAUNA STRUCTURE OF COLEOPTERS FROM APPLE TREE PLANTATIONS ACCORDING TO THE GROWING AREA AND IN THE CLIMATE CHANGE CONTEXT

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Abstract

The investigations were carried out in 2 statonaries, in 2 stages that took place during the years 2010 to 2011 and 2018 to 2019 in two apple treeorchards, belonging to SC Service SRL Delesti County of Vaslui, and the Didactic- Farm Vasile Adamachi of the Iasi country. The collection of the biological material has been carried out using soil traps Barber type, during the period May to August, seven harvests were carried out each year when the salt solution was completed or replaced, and the samples taken have been labeled and brought to the laboratory for the determination. The collected material has been cleaned of plant debris and then prepared for identification and of all specimens only the species of coleopterers which were then determined to the species level and then listed. From the analysis of the collected material it appears that the collected specimens belonging to the Hexapoda class, the Coleoptera order of which the larger ones are: Opatrum sabulosum., Omias rotundatus , Coccinella 7punctata , Brachysomus hirtus, Meligetes maurus, Otiorrhynchus pinastri, Polydrosus flavipes.

Key words: *entomofauna, Coleoptera, soil traps type Barber, climate change.*

INTRODUCTION

Culture of the fruit tree apple is the most widely known and widespread in temperate climate areas, and apples are the first in terms of both production volume, quality - food value and demand on the market (Talmaciu et al., 1996).

The apple culture is very old; pomological descriptions, as well as cultural practices, we meet in the writings of many scholars with about. 2000-3000 years before Christ (Saffo, Hippocratic, Teofrast), but also later (Cato, Varo, Columella, Pliniu The Old, etc.). The high ecological plastness of this species has allowed a wide geographical spread of the many varieties formed or created in the most diverse areas of the world.

However, the modern culture of the fruit tree apple, by default, has led to an increase in the use of pesticides, fertilizers and other active chemical substances, whose shortcomings we know well. In this context, environmental pollution by pesticides and fertilizers, fruit cultivation occupies one of the main places. (Talmaciu et al., 2006; 2007).

Global attitudes toward the environment and human health are becoming increasingly apparent, sustainable exploitation of natural resources and, in particular, agriculture as an essential factor in environmental change. (Varvara et al., 1981)

MATERIALS AND METHODS

Insects of the Coleoptera order are considered to be one of the main groups of insects which are recommended for the indication of soil type, vegetation and environmental chemism.

Local climatic conditions have a direct influence on their biology and ecology.

The sampling was carried out using Barber traps with a diameter of 10 cm and a height of 8 cm and a 25% salt solution (NaCl) was used as a fixer (Figure 1).

At the station under study, six traps were placed on a row of trees from the edge inwards in a straight line at a distance of 20 m from the edge and between traps 6 to 8 m in a row. The collection took place once a month during the growing season (june, july, august).

Figure 1. The Barber trap placed in the ecosystem

Samples of the biological material collected were labeled with: sample number, date of collection, and stationary. The samples thus labeled have been kept free from sunlight and transported to the laboratory for analysis and determination.

The soil trap type Barber method was used in the research orchards of apple on the Vasile Adamachi teaching farm in Iasi country and at the S.C. Loturi Service SRL, in the Delesti, Vaslui country, from june to September, to establish the structure and dynamics of the entomophasic epigee fauna, determined / identified using yhe books of determinations (Chatened du Gaetan, 1990; Panin I., 1951; Reitter E., 1908; Rogojanu V et al., 1979) and other sources on the internet.

RESULTS AND DISCUSSIONS

Due to their geographical position, the counties of Iasi and Vaslui have a rather pronounced continental climate integration into the land of climatical of high hills.

Apart from its geographical location and relief, the climate is also relevant to other factors, given the solar radiation and the general circulation of anticyclonal air masses that channel air currents.

The amount of solar radiation at the ground level is quite high having a particular influence on the air temperature, the average value being 8.3°C.

Due to the physical-geographical particularities the general nature of the climate in the Moldovian region represents differences characterized by high temperature jumps from summer to winter, but also daily allowances.

The main climatic characteristics of the year 2018-2019 recorded at Vasile Adamachi stationary

The characteristics of the climate for Iasi county registered at Vasile Adamachi meteorological station in the period 2010-2011 will be made by analyzing its basic elements: temperature, precipitation and relative humidity of the air. Thus, the annual average temperature in the area is 8.9°C with a peak in 2011, in August 21.5°C and in January 2010 the minimum of -4.0°C. Average annual rainfall is 500.5 mm in 2010 and 399.8 mm in 2011, but they are spread out unevenly and meet the water requirements of growing plants at the limit.

Table 1. The main climatic characteristics of 2010-2011 at the meteostation located at Vasile Adamachi, Iasi stationary

Month	Average temperature (C°)		Precipitaţion (mm)		Average relative humidity (%)	
	2010	2011	2010	2011	2010	2011
January	-4.0	-2.2	15.8	8.6	87	81
February	-2.7	-1.4	24.4	19.4	90	79
March	1.3	7.3	25.4	28.6	73	72
April	8.5	10.0	53.5	24.8	76	62
May	14.0	16.2	46.2	30.2	68	62
June	17.3	17.6	68.1	83.6	72	63
July	18.7	21.1	85.7	26.2	75	62
August	18.1	21.5	85.6	100.6	70	63
September	14.1	16.8	35.4	30.0	67	66
Octomber	8.6	8.7	20.1	10.4	83	73
November	3.2	2.2	9.3	1.4	84	78
December	-1.2	-0.1	30.7	36.0	82	82
Date /year	7.9	9.8	500.5	399.8	77.25	70.25

Over the period 2018-2019 from a thermal point of view, Monthly average values ranging from -3.7 to 25.41°C were recorded, which can render 2018 quite warm. The rainfall regime, throughout 2018, had a rainfall of 287.6 l/m^2, which shows a very large deficit.

Table 2. The main climatic characteristics of 2018-2019 at the station located at Vasile Adamachi, Iasi stationary

Month	Average temperature (C°)		Precipitaţion (mm)		Average relative humidity (%)	
	2018	2019	2018	2019	2018	2019
January	-1.0	-0.74	23.8	33.2	81.2	86.9
February	-3.7	5.12	9.4	41.2	79.0	49.84
March	1.7	5.34	11.4	1.0	72.1	65.25
April	9.53	12.05	15.9	20.6	77.53	64.9
May	13.0	14.62	9.2	3.5	62.9	74.23
June	19.8	19.92	62.1	21.1	73.15	78.38
July	19.17	21.75	38.7	26.2	69.53	65.92
August	25.41	20.16	38.6	43.2	65.47	69.2
September	17.1	20.26	16.4	13.0	56.1	17.62
Octomber	7.68	6.5	18.1	44.2	73.0	34.65
November	2.5	2.63	30.3	43	78.0	87.38
December	-2.21	-1.3	13.7	31.1	82.12	80.1
Date /year	9.08	10.52	287.6	321.3	72.5	64.53

In 2019 the annual average temperature in the area is 10.52°C with a maximum in July of 21.7°C and a minimum in January of -1.3°C. The average annual rainfall is 321.3 mm, but it is spread out unevenly throughout the year, which makes the crop of horticultural crops seriously affected.

Air humidity is expressed as the absolute maximum in November at 87.38% and the minimum in September at 17.62% and the average of the two years of research is 68.52%.

The main climatic characteristics of the year 2010-2011 recorded at Delesti stationary

In Vaslui county, in the summer months, the average temperatures recorded in the air were higher than normal, with the summer of 2010 being warm. The maximum temperature was recorded in August (monthly average of 22.8°C in air) and the minimum temperature recorded in January 2010 was -5°C.

Table 3. The main climatic characteristics of 2010-2011 in Delesti stationary

Month	Average temperature (C°)		Precipitation (mm)		Average relative humidity (%)	
	2010	2011	2010	2011	2010	2011
January	-5.0	-4.0	26.7	18.1	85	81
February	-2.1	-2.7	24.9	22.7	81	81
March	3.4	1.3	29.2	64.0	73	73
April	8.7	8.5	46.6	78.4	68	61
May	16.5	14.0	61.4	47.8	66	60
June	20.4	17.3	82.5	49.0	70	59
July	21.8	18.6	83.8	67.6	71	62
August	22.8	19.1	62.7	24.0	70	56
September	16.5	14.1	61.1	26.6	74	62
Octomber	9.2	8.6	38.9	64.2	79	68
November	5.2	3.2	30.8	37.0	83	83
December	1.9	-1.2	31.0	47.2	86	82
Date /year	9.94	8.06	579.5	546.6	76	69

September was warmer than normal, with an average air temperature of 16.5°C in 2010 and 14.1°C in 2011.

The annual average temperature was 9.94°C, in 2010 1.8°C higher than the annual average in 2011 when the temperature was 8.06°C.

The rainfall in the period 2010-2011 was unevenly spread, thus being months when very small quantities were recorded, well below the normal values, as they were in january, february and november.

Under these conditions, relative air humidity values were much lower than multi-annual averages, between 56 and 86%.

The main climatic characteristics of 2018-2019 at the Delesti stationary

The average annual temperature recorded in the area between 2018-2019 was 8.9°C in 2018 and 9.6°C in 2019 with a peak in July of 21°C and a minimum in January of -5.9°C. The average annual rainfall is 399.8 mm but they are distributed unevenly, in other areas reaching an average of 511.6 mm, satisfying the water needs of the crop plants.

Air humidity is expressed as the absolute maximum in February at 90% and the minimum in September at 67%.

Table 4. The main climatic characteristics of 2018-2019 in Delesti stationary

Month	Average temperature (C°)		Precipitation (mm)		Average relative humidity (%)	
	2018	2019	2018	2019	2018	2019
January	-5.9	-2.2	65.2	8.6	87	61
February	-1.3	-1.4	51.6	19.4	90	89
March	1.8	7.3	100.9	28.6	73	76
April	10.9	10.0	46.6	24.8	76	68
May	15.7	16.2	53.5	30.2	68	53
June	17.9	17.1	44.0	83.6	72	79
July	18.7	21.0	66.9	26.2	75	57
August	18.9	20.5	26.2	100.6	70	81
September	13.5	16.8	63.2	30.0	67	55
Octomber	8.9	8.7	11.2	10.4	63	26
November	6.3	2.2	17.9	36.0	54	44
December	0.5	-0.1	66.1	1.4	82	39
Date /year	8.9	9.6	613.3	399.8	73.08	60.66

Analyzing the climate, we note that the territory is in a continental climate with the highest air temperature range of 26.9°C. The annual amount of precipitation varying between 400-600 mm.

Results with regard to the structure, abundance and type of the Coleopters fauna collected from the two stationary in the research period.

In the stationary under study, were placed six traps on a row of trees from the edge inwards in a straight line at a distance of 20 m from the edge and between traps 6 to 8 m in a row.

The plastic pots have been used to do this, with a volume of 500 ml, with 10 cm in diameter and 9-10 cm in height, and it was used as a fixer a solution of salt with 25% concentration.

The situation of the collections in the period **2010-2011, located at Vasile Adamachi, Iasi stationary,** is presented in Table 5.

Thus, after identification of the 507 specimens, it were registered 20 species, belonging to several families of the choleopters.

The species with the highest number of specimens collected were: *Harpalus distinguendus* with 146 specimens, *Otiorrhynchus raucus* with 71 specimens, *Calathus fuscipes* with 48 specimens, *Opatrum sabulosum* with 32 specimens, *Omias rotundatus* with 17 specimens, *Coccinella 7punctata* with 9 specimens, *Brachysomus hirtus* with 9 specimens, *Meligetes maurus* with 7 specimens, and *Polydrosus flavipes* with 4 specimens. A number of 38 identified species had between 1 and 3 specimens collected.

After diet, 18 species have predatory behavior (Pd), which falls into the group of useful species (U) and 27 species are phytopiophage, thus being considered as harmful species (D). Also, of the total specimens collected, 100 specimens (73%) belong to the harmful entomofauna and only 37 (27%) specimens belong to the useful entomofauna.

Table 5. Structure, dynamics, abundance and type of the coleopter fauna collected from the Iasi stationary in 2010-2011

No	Name of species	Dates/ No. of samples						Total	Type of fauna
		2010			2011				
		V	VI	VII	V	VI	VII		
1.	Amara crenata Dejean	-	-	2	-	2	1	5	Pd
2.	Amara familiaris Duft.	11	-	5	9	1	2	28	Pd
3.	Amara similata Gyll.	2	-	-	-	3	-	5	Pd
4.	Anisodactylus signatus Paz	1	-	16	1	7	7	32	Pd
5.	Attagemus unicolor	-	-	5	1	2	2	10	D
6.	Brachynus crepitans L.	15	-	4	-	4	7	30	D
7.	Brachysomus hirtus	-	-	-	-	8	11	19	D
8.	Calathus fuscipes Goeze	1	-	12	1	13	21	48	Pd
9.	Carabus besseri Fischer	4	-	3	1	-	1	9	Pd
10.	Carabus cancellatus Illyg	1	1	2	1	1	1	7	Pd
11.	Carabus coriaceus L	2	-	5	1	2	2	12	Pd
12.	Coccinella 7punctata	-	-	2	-	11	1	14	Pd
13.	Dermestes laniarius	-	7	2	-	2		11	Pd
14.	Harpalus distinguendus Duft	35	27	31	3	28	22	146	Pd
15.	Harpalus tardus Panz.	-	3	3	-	1	1	8	Pd
16.	Microlestes maurus Strm	7		2	2		1	12	D
17.	Otiorrhynchus raucus	-	13	22	9	20	7	71	D
18.	Pterostichus niger Schall	2	-	-	-	3	8	13	Pd
19.	Notiophilus palustris Duft.	-	4	5	-	1	9	19	D
20.	Zabrus tenebrioides Goeze.	-	2	-	4	2	-	8	D
Number of species/Total		81	57	121	33	111	104	507	159D=31,36% 348U=68,64%

Pd- predator species D- harmful species U- useful entomofauna

The situation of the collections in the period **2018-2019, located at Vasile Adamachi, Iasi stationary**, is presented in Table 6.

Table 6. Structure, dynamics, abundance and type of the Coleopter fauna collected from the Vasile Adamachi, Iasi stationary in 2018-2019

No	Name of species	Dates/ No. of samples						Total	Type of fauna
		2018			2019				
		V	VI	VII	V	VI	VII		
1.	Abax carinatus Duft	1	2	-	-	3	1	7	Pd
2.	Amara crenata Dejean	-	-	2	-	-	1	3	Pd
3.	Amara similata Gyll.	2	-	-	-	-	-	2	Pd
4.	Amara familiaris Duft.	28	2	5	7	1	1	44	Pd
5.	Anisodactylus signatus Paz	1	-	16	1	7	7	32	Pd
6.	Brachynus crepitans L.	18	-	7	-	4	14	43	D
7.	Calathus ambiguus Payk.	-	-	2	-	12	1	15	Pd
8.	Calathus fuscipes Goeze	1	-	-	10	39	29	79	Pd
9.	Carabus besseri Fischer	4	-	3	1	-	-	8	Pd
10.	Carabus cancellatus Illyg	1	1	2	-	-	-	4	Pd
11.	Carabus coriaceus L	14	1	5	2	22	24	68	Pd
12.	Carabus scabrisculus Ol	-	-	-	-	8	13	21	Pd
13.	Dolichus chalensis Schal.	-	2	-	2	-	-	4	Pd
14.	Harpalus calceatus Duft	-	-	-	-	1	1	2	Pd
15.	Harpalus distinguendus Duft	33	31	46	-	25	10	145	Pd
16.	Harpalus tardus Panz.	-	3	-	-	1	1	5	Pd
17.	Leistus ferrugineus L	-	-	-	1	-	-	1	Pd
18.	Nebria picicornis F	7	-	2	-	2	-	11	D
19.	Otiorrhynchus raucus	7	8	2	-	-	2	19	D
20.	Poecilus cupreus L	1	-	1	-	-	1	3	D
21.	Pseudophonus griseus Panz.	-	13	22	46	20	0	101	Pd
22.	Pseudophonus rufipes Müll	18	4	50	13	41	29	155	Pd
23.	Pterostichus cylindrichus Hr.	-	-	5	1	2	9	17	Pd
24.	Pterostichus niger Schall	2	-	-	-	3	8	13	Pd
25.	Zabrus tenebrioides Goeze.	-	-	2	-	4	2	8	D
Total specimens		138	59	232	84	195	212	810	84D=10,37% 726 U=89,63%

Pd- predator species D- harmful species U- useful entomofauna

Thus, after identification of the 810 specimens, it were registered 25 species, belonging to several families of the coleopters.

The species with the highest number of specimens collected were: *Pseudophonus rufipes* with 205 specimens, *Harpalus distinguendus* with 145 specimens, *Pseudophonus griseus* with 111 specimens, *Calathus fuscipes* with 97 specimens, *Carabus coriaceus* with 68 specimens, *Amara familiaris* with 44 specimens, *Brachysomus crepitans* with 43 specimens, *Carabus scabriusculus* with

21 specimens and *Otiorrhynchus raucus* with 19 specimens. A number of 13 identified species had between 1 and 10 specimens collected.

After diet, 20 species have predatory behavior (Pd), which falls into the group of useful species (U) and 5 species are phytopiophage, thus being considered as harmful species (D). Also, of all the specimens collected, 84 (10.37%) of the specimens belong to the harmful entomofauna and 726 (89.63%) specimens belong to the useful entomofauna.

The situation of the collections in the period **2010-2011, at Delesti, Vaslui stationary** is presented in Table 7.

Thus, after identification of the 161 specimens, it were registered 28 species, belonging to several families of the coleopters.

The species with the highest number of specimens collected were: *Omias rotundatus* with 17 specimens, *Opatrum sabulosum* with 16 specimens, *Coccinella 7punctata* with 12 specimens, *Brachysomus hirtus* with 11 specimens, *Otiorrhynchus pinastri* with 10 specimens, *Mordella aculeata* with 9 specimens, Harpalus tardus with 7 specimens, *Cantharis fusca* and *Phyllotreta vittula* with 6 specimens. A number of 20 identified species had between 1 and 5 specimens collected.

After diet, 11 species have predatory behavior (Pd), which falls into the group of useful species (U), and 18 species are phytopiophage thus being considered as harmful species (D). Also, of the total number of specimens collected, 107 (66.46%) belong to the harmful species and only 48 (33.54%) specimens belong to the useful entomofauna.

The situation of the collections in the **period 2018-2019, at Delesti, Vaslui stationary** is presented in Table 8.

Thus, after identification of the 168 specimens, it were registered 45 species, belonging to several families of the coleopters.

The species with the highest number of specimens collected were: *Opatrum sabulosum* with 31 specimens, *Omias rotundatus* with 20 specimens, *Brachysomus hirtus* with 9 specimens, *Coccinella 7punctata* with 8 specimens, *Meligetes maurus* with 6 specimens, *Dermestes laniarius* with 5 specimens, *Sitona crinitus* with 5 specimens, *Harpalus tardus, Mordellla aculeata, Mordella fasciata*, *Otiorrhynchus raucus* and *Polydrosus flavipes* with 4 specimens. A number of 33 identified species had between 1 and 3 specimens collected.

Table 7. Structure, dynamics, abundance and type of the Coleopter fauna collected from Delesti, Vaslui stationary in 2010-2011

No	Name of species	Dates/ No. of samples						Total	Type of fauna
		2010			2011				
		17.05	21.06	23.07	28.05	21.06	25.08		
1.	Coccinella 7punctata	3	2	4	2	1	-	12	Pd
2.	Dermestes laniarius	-	1	-	-	-	3	4	Pd
3.	Harpalus distinguendus	1	-	1	1	-	1	4	Pd
4.	Harpalus pubescens	-	1	-	-	1	-	2	Pd
5.	Harpalus tardus	1	2	2	-	-	2	7	Pd
6.	Carabus violaceus	-	1	-	-	2	-	3	Pd
7.	Licinus cassideus	1	3	-	-	-	-	4	Pd
8.	Amara crenata	2	-	-	1	-	-	3	Pd
9.	Amara eurynota	1	-	2	2	-	-	5	Pd
10.	Pterostichus niger	2	-	1	-	-	1	4	Pd
11.	Apion automarium	-	-	1	-	1	-	2	D
12.	Attagemus unicolor	1	1	-	-	-	2	4	D
13.	Brachysomus hirtus	9	-	-	2	-	-	11	D
14.	Longitarsus anchusae	1	-	-	-	-	-	1	D
15.	Meligetes maurus	-	5	-	-	1	-	6	D
16.	Mordela aculeata L.	4	-	-	1	1	3	9	D
17.	Mordella fasciatta	-	1	-	-	1	2	4	D
18.	Omias rotundatus	-	13	-	-	3	2	18	D
19.	Opatrum sabulosum	5	6	-	1	4	-	16	D
20.	Orchestes lonicerae	1	2	-	-	1	-	4	D
21.	Otiorrhynchus pinastri	1	3	1	2	3	-	10	D
22.	Otiorrhynchus raucus	-	1	-	2	-	2	5	D
23.	Otiorrynchus ovatus	1	1	1	-	-	1	4	D
24.	Otiorrynchus obsidianus	1	-	-	1	-	-	2	D
25.	Phyllotreta nemorum	-	-	-	1	-	1	2	D
26.	Phyllotreta vittula	-	1	2	-	3	-	6	D
27.	Scymnus nigrinus	-	1	-	-	1	-	2	D
28.	Tychius 5 punctatus	1	2	-	-	-	-	3	D
TOTAL		37	48	15	16	27	20	161	107D=66,46 % 48 U=33,54%

Pd- predator species D- harmful species U- useful entomofauna

After diet, 18 species have predatory behavior (Pd), which falls into the group of useful species (U) and 27 species are phytopiophage, thus being considered as harmful species (D). Also, of the total specimens collected, 119 specimens (72%) belong to the harmful entomofauna and only 47 (28%) specimens belong to the useful entomofauna.

Table 8. Structure, dynamics, abundance and type of the Coleopter fauna collected from Delesti, Vaslui stationary in 2018-2019

	Name of species	Dates/ No. of samples						Total	Type of fauna
		2018			2019				
		23.05	17.06	18.07	02.06	02.07	05.08		
1.	Acrolocha sulcula	-	1	-	-	-	1	2	Pd
2.	Amara crenata	-	-	-	1	-	-	1	Pd
3.	Amara eurynota	1	-	-	-	-	-	1	Pd
4.	Apion automarium	-	-	-	-	1	1	2	D
5.	Attagemus unicolor	1	-	-	-	-	2	3	D
6.	Baryplithes araneiformis	-	-	2	-	-	1	3	D
7.	Brachysomus hirtus	9	-	-	-	-	-	9	D
8.	Cantharis fusca	1	-	-	-	-	2	3	Pd
9.	Carabus violaceus	-	-	-	-	2	-	2	Pd
10.	Chaetocnema hortensis	-	-	1	-	-	2	3	D
11.	Coccinella 7punctata	1	1	4	2	-	-	8	Pd
12.	Cymindis vaporariorum	1	-	-	-	-	1	2	Pd
13.	Dermestes haemorrhoidalis	-	1	-	-	-	-	1	Pd
14.	Dermestes laniarius	-	2	-	-	-	3	5	Pd
15.	Harpalus calceatus	1	1	-	-	-	1	3	Pd
16.	Harpalus distinguendus	-	-	1	1	-	1	3	Pd
17.	Harpalus pubescens	-	-	-	-	1	-	1	Pd
18.	Harpalus tardus	1	2	-	-	-	1	4	Pd
19.	Leistus ferrugineus	2	-	-	-	-	-	2	Pd
20.	Leptura maculicornis	-	-	-	1	-	-	1	D
21.	Leucoparyphus fullo	-	2	-	-	-	-	2	Pd
22.	Licinus cassideus	-	3	-	-	-	-	3	Pd
23.	Longitarsus anchusae	1	-	-	-	-	-	1	D
24.	Meligetes maurus	-	5	-	-	-	1	6	D
25.	Mordela aculeata L.	-	-	-	1	-	3	4	D
26.	Mordella fasciatta	-	1	-	-	1	2	4	D
27.	Mordellistena abdominalis	1	-	-	-	-	1	2	D
28.	Omias rotundatus	-	17	-	-	-	3	20	D
29.	Opatrum sabulosum	5	27	-	-	-	-	31	D
30.	Orchestes lonicerae	-	1	-	-	-	-	1	D
31.	Otiorrhynchus tristis	-	-	-	1	-	-	1	D
32.	Otiorrhynchus fullo	-	2	-	-	-	-	2	D
33.	Otiorrhynchus pinastri	1	-	-	-	-	-	1	D
34.	Otiorrhynchus raucus	-	-	-	3	-	1	4	D
35.	Otiorrynchus ovatus	-	2	-	-	-	1	3	D
36.	Otiorrynchus obsidianus	-	1	-	-	-	-	1	D
37.	Phyllotreta nemorum	-	-	-	1	-	2	3	D
38.	Phyllotreta vittula	-	-	1	-	-	-	1	D
39.	Polydrosus flavipes	4	-	-	-	-	-	4	D
40.	Pterostichus niger	-	-	1	-	-	1	2	Pd
41.	Scymnus nigrinus	-	-	-	1	-	-	1	D
42.	Sitona crinitus	-	3	-	-	-	2	5	D
43.	Telmatiphilus typhae	-	-	-	1	-	1	2	D
44.	Tillis elongatus	-	-	-	1	-	1	2	Pd
45.	Tychius 5 punctatus	-	1	-	-	-	-	1	D
TOTAL		30	77	10	14	5	37	168	119D=72 % 47U=28 %

Following the three harvests carried out each year in the study in the stationary for collecting the coleopterans species using soil traps type Barber, the results shall be presented:

In 2010-2011, at the Vasile Adamachi stationary from Iasi country, were collected 20 species, with a total of 507 specimens.

In 2080-2019, at the Vasile Adamachi stationary from Iasi country, were collected 25 species, with a total of 810 specimens.

In 2010-2011, at the Delesti stationary from Vaslui country, were collected 28 species, with a total of 161 specimens.

In 2018-2019, at the Delesti stationary from Vaslui country, were collected 45 species, with a total of 168 specimens.

CONCLUSIONS

Due to the physical-geographical particularities the general nature of the climate in the Moldovoan region presents differences which allow at least two conclusions:

- The valey microclimate , located on the lower terraces of the rivers that transvert the two localities where the experimental stationary were located, the mcroclimat of valley was characterized by high temperature jumps from summer to winter, but also diurns. To mention the frequencies of thermal inversions, the fog, the brums, the relatively heavy moisture.

- The microclimate of terrace and the one on sunny lyrics implies annual average temperatures of around 8.5-9°C, where the insolation is more pronounced in summer and a lower reative moisture.
- Following the application of the soil trap type Barber collection method, in the two stationary, we selected only specimens of coleopters belonging to both the species category of species that make up the useful fauna and the harmful fauna.
- It is to be noticed that in Vasile Adamachi stationary in Iasi county was collected with a significantly higher percentage of the species of coleopters belonging to useful fauna, whereas in the Delesti stationary in Vaslui county the dominant species belong to the harmful fauna to the *Coleoptera* order.

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