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INFLUENCE OF COPPER SPRAYING ON PHOTOSYNTHETIC PERFORMANCE, GRAPE RIPENING, WOOD MATURATION AND FROST RESISTANCE IN GRAPEVINE

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Abstract

The research was carried out in a family vineyard from Arad County, Buteni village, during 2018-2019, on Cardinal, Victoria, and Merlot, varieties. In the experimental versions, 1 or 2 copper treatments were added in the normal scheme of phytosanitary treatments for diseases and pest control. Copper is essential in grapevine growing, for grapevine downy mildew (Plasmopara viticola) control. The research plots were superior to the control concerning the percentage of annual wood maturation, the canes content in carbohydrates and the buds viability. In the V_7 variant were obtained the best results, sprayed with 2.42 kg/ha copper hydroxide ($Cu(OH)_2$) applied three times: before flowering, berry development, respectively after veraison. The second was V_5 variant in which was applied 1.61 kg/ha of copper hydroxide ($Cu(OH)_2$) two times: at berry development, and after veraison. In the last place as concern the percentage of wood maturation, the content of the cane in carbohydrates and the buds viability, was the control variant V_1 on which was applied a single treatment with copper, respectively 0.807 kg/ha of copper hydroxide ($Cu(OH)_2$) after veraison.

Key words*: copper, carbohydrates, wood maturation, sensitive varieties, frost resistance.*

INTRODUCTION

Copper is very important for plants, with unique fungistatic and bactericidal activity due to the copper ions (Cu ++) released in the water (Yruela, 2005). Copper ions are passively absorbed by fungi and bacteria during growth and accumulate until the concentration becomes lethal to the cells (Sommers, 2008). Copper is component of enzymes and enzyme activator and acts as a catalyst (Festa and Thiele, 2011). Copper is vital to all plants and is involved in the chlorophyll biosynthesis, seed germination, in increasing drought resistance and in water supply (Yamasaki et al., 2008). Copper is involved in respiration and protein synthesis, in the nutrients and water assimilation, in the lignin synthesis, in the stiffening of the cell wall and in increasing of plant resistance to pathogens (Sundin et al., 2016).

Copper-based fungicides used in agriculture took place for the first time in the seventeenth century, when farmers treated the wheat seeds for sowing with copper sulphate (blue stone) against corn cockle (*Agrostemma githago*) (Morton and Staub, 2008). In 1882, the French scientist Millardet discovered the properties of copper as a fungicide, using copper sulphate as "Bordeaux Mixture" (*Bouille Bordelaise*) to control the grapevine downy mildew (*Plasmopara viticola*), and in 1956 the first copper-based pesticide was approved.

Nowadays copper has become essential for the grapevine growing, due both to the grapevine downy mildew (*Plasmopara viticola*) control as well as for several side effects (Andras-Sauca et al., 2018a; Borca et al., 2018).

When is applied in the late growing season in vineyards, the copper decreased the powdery mildew (*Erysiphe necator*) infection due to effect on cleistothecia and mycelium, decrease the noble rot (*Botrytis cinerea*) infection by thickening the berries skin, delays the leaves falling, which helps to ripen the shoots tissues and increase the resistance to winter low temperatures (Gruau et al., 2016; Blanco-Ulate et al., 2015).

Copper-based treatments are currently included in all grapevine integrated disease and pest

control management, which finally increase the production costs (Gadoury et al., 2012).

High amount of nitrogen and phosphorus can lead to copper deficiency (Brunetto et al., 2015). For example, measureless amounts of nitrogen increase the abundant development of canopy, the sequestration of total copper and the decrease of the grapevine photosynthesis rate (Hendrickson et al., 2004). Copper deficiency, correlated with climate variability, decreases the vines frost resistance, with negative financial impact on vineyards management (Dobrei et al., 2010).

However, the copper use must be moderate, because the copper excess can cause chlorosis and burns on the leaves, it can negatively affect the quality of grapes, must and wine (Lamichhane et al., 2018). Achievement of high quality wine by-products is essential to withstand an increasingly demanding market with more and more efficient competitors (Andraş-Sauca et al., 2018b).

MATERIALS AND METHODS

The research was carried out during 2018-2019 in a private vineyard from Buteni village, Arad County, during the full maturity growing stage. Two table grapes varieties (Victoria and Cardinal) and Merlot wine grapes variety were investigated, known as being more sensitive to the lower temperatures in winter, therefore with issues concerning the maturation of the one year old canes and the buds viability (Dobrei et al., 2018; Nistor et al., 2018).

In the experimental plots were add one or two copper treatments, in the current scheme of phytosanitary treatments for diseases and pests control (usually with only one copper treatment applied after veraison). Copper-base treatment applied was Kocide which has as active substance copper hydroxide ($Cu(OH)_2$) 53.8% concentration; the treatment dose was 1.5 kg/ ha commercial product.

The experimental plots were: V_1 - treatment with copper (0.807 kg/ha $Cu(OH)_2$) applied after early veraison stage, considered as control; V_2 - treatment with copper (0.807 kg/ ha $Cu(OH)_2$) applied before flowering stage; V_3 - treatment with copper (0.807 kg/ha $Cu(OH)_2$) applied in lag phase; V_4 - two treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied before flowering stage + (0.807 kg/ha $Cu(OH)_2$) applied at lag phase; V_5 - two treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied in lag phase + (0.807 kg/ha $Cu(OH)_2$) applied after early veraison stage; V_6 - two treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied before flowering stage + (0.807 kg/ha $Cu(OH)_2$) applied after starting veraison stage; V_7 - three treatments with copper (0.807 kg/ha $Cu(OH)_2$) applied before flowering stage + (0.807 kg/ha $Cu(OH)_2$) applied to lag phase + (0.807 kg/ha $Cu(OH)_2$) applied after veraison stage starting.

During the research, minimum temperatures of -17ºC were recorded in three nights, respectively -18ºC in one night.

Observations and determinations were made regarding several indicators: sugar concentration in grape must (g/l), was determined with Bellingham + Stanley OPTi digital hand held refractometer; the amount of sugar per hectare was calculated, respectively the canopy area necessary for the accumulation of one kg of sugar (or photosynthetic efficiency); the total annual growth (expressed in m/vine and calculated in m/ha were determined); following the first frosts the measurements were redone, after the wood the fragments affected by the frost were removed, resulting the one year old matured wood (measures were in m/vine, m/ha and as a percentage of total annual wood growth; the amount of carbohydrates in canes was determined in two different stages: before early autumn frosts (15th November), respectively after the late frosts in spring (1th March). Measurements were done by the antrona reagent method by which the soluble sugar and starch were determined, and their amount was evaluated as the total carbohydrates; the rate of viable eyes was determined after the late frosts in spring and before the bleeding start (1th March). The research aimed to study the influence of copper treatments on the grape yield and quality, photosynthesis efficiency, the well-matured canes wood, the carbohydrate amount in canes and the buds viability.

Statistics were done by GraphPad InStat version 5.04 version for Windows (GraphPad Software, Inc. 5755 Oberlin Drive #110 San Diego, USA) and data analysed using statistical ANOVA and Tukey's test ($p \leq 0.05$) for quantitative variables.

RESULTS AND DISCUSSIONS

The quality of grapes or wine is essential for successful products on the market. Quality cannot be achieved without a performant growing technology, which provides high quality grapes for winemaking with economic efficiency (Nan et al., 2018).

Table 1. Influence of copper applied during phytosanitary treatments on the grapes quality and photosynthetic efficiency

Treatment variants	Variety	Sugar g/l	Sugar kg/ha	m^2 canopy/kg sugar	Difference to control (kg sugar/ha)	Significance
V_1 -control	Victoria	148	1538	12.7	-	-
	Cardinal	127	1201	20.43	-	-
	Merlot	194	1675	9.09	-	-
V_2	Victoria	144	1461	12.75	-77	0
	Cardinal	124	1170	20.2	-40	0
	Merlot	190	1640	8.87	-35	0
V_3	Victoria	151	1532	12.76	-6	-
	Cardinal	131	1239	19.81	38	*
	Merlot	196	1693	8.99	18	-
V_4	Victoria	153	1591	11.71	53	*
	Cardinal	135	1275	18.54	74	**
	Merlot	198	1709	8.51	34	-
V_5	Victoria	161	1676	11.66	171	***
	Cardinal	144	1364	17.99	163	***
	Merlot	208	1798	8.51	123	***
V_6	Victoria	157	1632	11.42	94	**
	Cardinal	138	1304	18.12	103	**
	Merlot	202	1745	8.33	70	*
V_7	Victoria	159	1654	11.26	167	***
	Cardinal	141	1333	17.73	132	***
	Merlot	204	1763	8.25	88	**
DL - Victoria			5% - 37.1		1% - 67.4	0.1% - 117.9
DL - Cardinal			5% - 34.9		1% - 62.5	0.1% - 109.9
DL - Merlot			5% - 42.3		1% - 71.1	0.1% - 122.6

In Table 1 is presented the data regarding the influence of copper applied in the mixture of phytosanitary treatments on the sugar concentration and the photosynthetic efficiency. Regardless of whether were grapes for wine or fresh consumption, varieties from experimental plots reaction was positive to the copper doses applied. In all three varieties the sugar concentration was influenced both by the amount of copper applied and by the growing stage of treatment application.

The highest concentrations of sugar resulted in V_5 plot: 161 g/l for the Victoria, 144 g/l for the Cardinal and respectively 208 g/l for the Merlot variety. In V_5 plot was applied 1.61 kg/ha copper twice: in lag phase and in the first stage of veraison.

In the second place was ranked the V_7 plot on which was applied 2.42 kg/ha copper in three stages: before flowering, lag phase and veraison. The V_2 was the only experimental plot that recorded values lower than the control, with 144 g/l sugars in the Victoria variety, 124 g/l sugars in the Cardinal variety, respectively 190 g/l sugars in the Merlot variety. Compared to the control, in V_2 was applied the same copper dose (0.807 kg/ha), but much earlier - before flowering.

The largest concentration of sugars was reported in the V_5 experimental plot: 1676 kg sugars/ha in the Victoria variety, 1364 kg/ha in the Cardinal variety, respectively 1798 kg sugars/ha in the Merlot variety.

Statistically the V_5 experimental plot was the highest statistical significant variant; the differences between V_5 variant and the control variant, were for all three varieties very significant.

The photosynthetic efficiency recorded higher values in V_7 due to the lower canopy area per kilogram of sugar amount, in which was applied 2.42 kg/ha copper.

In varieties more sensitive to low winter temperatures, a proper maturation of the one year old canes is essential for an adequate resistance in winter time. The maturation of the shoots tissues begins in the summer and can be positively influenced by appropriate technology, so that the possible early frosts which are quite frequent during the month of October to affect less the canes maturation. Therefore, the introduction of copper into the phytosanitary treatments is beneficial from all points of view. Copper is efficient in downy mildew (*Plasmopara viticola*), does not charge the cost of treatments due to the reasonable prices of copper fungicides, it is also accepted in organic viticulture and in addition it is very efficient in wood maturation.

Research should establish precisely both the optimum amount of copper per vine and for each vineyard, as well as the most appropriate growing stage for application.

The results regarding the influence of the copper treatments on the total annual growths and one year old wood matured are presented in the Table 2.

As concern the total annual cane wood increases, it is noted that the high doses of copper (V_7 variant) and especially the early application of copper - before flowering (variant V_2), has led in all three varieties to a slight slowing of growth. In all three varieties, V_2 recorded the lowest values of the total growth of shoots. Therefore, the variant with the best results regarding the total one year old cane wood increases was the V_5 variant, in which were applied two treatments with copper: one applied in lag phase and the second in the first stage of veraison.

The one year old cane wood matured is very important indicator, especially in varieties with lower resistance to winter frosts (Dobrei et al., 2015). Therefore copper influence is higher on one year old canes wood than on the well-matured canes wood.

Table 2. The influence of the copper treatments on the one year old canes and well-matured canes

Treatment variants	Variety	One year old canes		Well-matured canes			Difference from control (% from total)	Significance
		m/vine	m/ha	m/vine	m/ha	% from total		
V_1 - control	Victoria	18.8	85465	13.3	60680	71	-	
	Cardinal	24.1	109534	13.9	63189	58	-	
	Merlot	13.6	61812	9.8	44451	72	-	
V_2	Victoria	16.7	75901	12.2	55461	73	2	-
	Cardinal	20.9	94990	12.3	55916	59	1	-
	Merlot	12.4	56358	9.3	42278	75	3	-
V_3	Victoria	17.4	79083	13.4	60916	77	6	*
	Cardinal	22.8	103626	13.7	62280	60	2	-
	Merlot	13.2	59994	10.3	46824	78	6	*
V_4	Victoria	16.9	76810	13.7	62280	81	10	**
	Cardinal	21.6	98172	14	63644	65	7	**
	Merlot	13.1	59539	10.7	48642	82	10	**
V_5	Victoria	18.9	85900	15.7	71372	83	12	**
	Cardinal	24.5	111352	16.6	75464	68	10	**
	Merlot	13.8	62721	11.7	53188	85	13	***
V_6	Victoria	16.8	76356	13.1	59553	78	7	*
	Cardinal	21.5	97739	13.5	61371	63	5	*
	Merlot	12.7	57734	10	45460	79	11	**
V_7	Victoria	16.7	75918	14.4	65462	86	15	***
	Cardinal	21.3	96830	15.3	69554	72	14	***
	Merlot	12.5	56825	11	50006	88	16	***
DL - Victoria		5% - 4.21		1% - 7.11			0.1% - 12.38	
DL - Cardinal		5% - 3.87		1% - 6.45			0.1% - 11.32	
DL - Merlot		5% - 4.72		1% - 7.78			0.1% - 12.92	

Regarding the well matured canes ratio from the total one year old wood, the best results were recorded in V_7 variant, in which was applied the highest amount of copper. In V_7, the well-matured canes represent 88% of the total one year old canes wood in the Merlot variety, 86% in the Victoria variety, respectively 72% in the Cardinal variety. The differences recorded between the V_7 and the control, were 15% in the Victoria variety, 14% in the Cardinal variety, respectively 16% in the Merlot variety, and were very highly significant.

The experimental variant that recorded the highest values of well-matured canes wood/ha was V_5, on which were applied two copper treatments (the first in lag phase and the second in the first decade of veraison stage).

In the varieties sensitive to the low temperatures during the winter, it is very important the sugars and starch concentration in one year old canes, which influence the time for wood maturation and the buds viability. In Victoria variety the carbohydrate amount in canes was influenced both by the number of copper treatments applied and by the growing stage of treatment application.

At the beginning of winter, the highest concentration of carbohydrates in canes was recorded in the V_7 variant, the only one in which were applied three copper treatments. On the second and third rank the variants V_5 and V_4, in which were applied two copper treatments. The same ranking of variants was noted for the carbohydrates in canes in the early spring; all the experimental variants recorded higher significant limits compared with the control.

In the Cardinal variety, the canes concentration in carbohydrate was lower compared to the Victoria variety, but the experimental variants ranking was similar. In Cardinal variety the higher differences between the variants and the control showed the higher influence of the copper treatments on the cane carbohydrates concentration (Table 4).

In Merlot variety was recorded the highest carbohydrates concentration in canes compared with other two varieties, both at the beginning of winter and spring. The differences between the control and the experimental variants were also smaller. In Merlot variety the best results were recorded in the variant with three copper treatments (Table 5).

Table 3. Carbohydrates concentration in Victoria variety canes

Variant	Carbohydrates concentration (g %)		Carbohydrates concentration (%)		Difference compared to control (%)		Significance	
	15 XI	1 III	15 XI	1 III	15 XI	1 III	15 XI	1 III
V_1 (Control)	10.2	7.7	100	100	-	-	-	-
V_2	10.6	8.3	103.9	107.79	3.9	7.79	-	*
V_3	10.9	8.5	106.9	110.38	6.9	10.38	*	**
V_4	12.7	10.6	124.5	137.66	24.5	37.66	***	***
V_5	13	11.1	127.4	144.15	27.4	44.15	***	***
V_6	12.1	9.9	118.6	127.27	18.6	27.27	**	***
V_7	13.3	11.5	130.4	149.35	30.4	49.35	***	***

DL - 15XI 5% - 5.92 1% - 10.71 0.1% - 19.48
DL - 1 III 5% - 5.21 1% - 9.33 0.1% - 17.28

Table 4. Carbohydrates concentration in Cardinal variety canes

Variant	Carbohydrates concentration (g%)		Carbohydrates concentration (%)		Difference compared to control (%)		Significance	
	15 XI	1 III	15 XI	1 III	15 XI	1 III	15 XI	1 III
V_1 (Control)	8.7	5.7	100	100	-	-	-	-
V_2	9.2	6.1	105.74	107.01	5.74	7.01	-	*
V_3	9.4	6.4	108.04	112.28	8.04	12.28	*	**
V_4	10.9	8	125.28	140.35	25.28	40.35	***	***
V_5	11.2	8.4	128.73	147.36	28.73	47.36	***	***
V_6	10.4	7.5	119.54	131.57	19.54	31.57	**	***
V_7	11.9	9	136.78	157.89	36.78	57.89	***	***

DL - 15XI 5% - 6.53 1% - 11.98 0.1% - 21.42
DL - 1 III 5% - 6.21 1% - 11.16 0.1% - 20.82

Zufferey et al. (2012) found in Chasselas variety the lowest level of carbohydrates concentration in the two-year-old cane wood around flowering growing stage.
In Chardonnay variety, Vaillant-Gaveau et al. (2014), found that grapevine is able to correlate the inflorescences with the available carbohydrate amount from perennial wood.

The buds are some of the most sensitive organs of vines at frost. The level of frost damage on grapevine buds is directly related to the grape yield in the current year and of the next year.
The varieties chosen for the research are well-known as low buds viability, especially Cardinal and Victoria. Merlot variety is ranking among red wine varieties frequently influenced by climate change and variability (Table 6).

Table 5. Carbohydrates concentration in Merlot variety canes

Variant	Carbohydrates concentration (g %)		Carbohydrates concentration (%)		Difference compared to control (%)		Significance	
	15 XI	1 III	15 XI	1 III	15 XI	1 III	15 XI	1 III
V_1 (MT)	12.7	10.1	100	100	-	-	-	-
V_2	13.2	10.4	103.9	102.97	3.9	2.97	-	-
V_3	13.6	10.6	107.08	104.95	7.08	4.95	*	**
V_4	14.6	12	114.9	118.81	14.9	18.81	**	***
V_5	14.9	12.1	117.32	119.8	17.32	19.8	***	***
V_6	13.9	11.8	109.44	116.83	9.44	16.83	**	***
V_7	15.1	12.3	118.89	121.78	18.89	21.78	***	***

DL - 15XI 5% - 5.31 1% - 9.12 0.1% - 16.21
DL - 1 III 5% - 4.91 1% - 7.98 0.1% - 13.78

Table 6. Buds viability

Variant	Victoria			Cardinal			Merlot		
	Buds viability			Buds viability			Buds viability		
	%	Difference to control	Significance	%	Difference to control	Significance	%	Difference to control	Significance
V_1 (MT)	63	-	-	41	-	-	83	-	-
V_2	66	3	-	46	5	*	85	2	-
V_3	67	4	*	48	7	*	86	3	*
V_4	72	9	**	58	17	***	90	7	**
V_5	76	13	***	61	20	***	92	9	**
V_6	70	7	**	52	11	**	88	5	**
V_7	78	15	***	62	21	***	94	11	***

DL - Victoria 5% - 3.85 1% - 6.12 0.1%- 12.03
DL - Cardinal 5% - 4.37 1% - 7.33 0.1%-14.12
DL - Merlot 5% - 2.72 1% - 4.98 0.1%-9.75

Although the temperatures during research years were high, in the Cardinal variety, the buds viability ratio was relatively low, with limits between 41 and 62% depending on the variant.
In the Victoria variety, the buds viability ratio was higher than in the Cardinal variety, ranging between 63 and 78%, while in the Merlot variety was recorded the highest buds viability ratio, ranging from 83 to 94%. In all three varieties, the highest buds viability was recorded in the V_7 variant, in which was applied the highest amount of copper, followed by the V_5 and V_4 variants, in which was applied 1.61 kg of copper/ha. In the variants V_1, V_2 and V_3, in which were applied the smallest amounts of copper, was recorded the lowest ratio of buds viability.
In Figures 1, 2 and 3 are presented the correlation between cane carbohydrates concentration and buds variability in all three varieties. The highest correlation is recorded in Victoria variety while in Cardinal and Merlot varieties cane carbohydrates concentration influence on buds viability is moderate. Buds viability in experimental variants V_4 and V_6 are the most influenced by the cane carbohydrates concentration recorded in the first decade of

March during the research. On contrary, in V_1 (control), V_2 and V_3 was recorded the less influence of cane carbohydrates concentration on buds viability. Calugar et al. (2010) reported significant correlations between canes carbohydrates concentration and buds viability in grape varieties from Blaj vineyards.

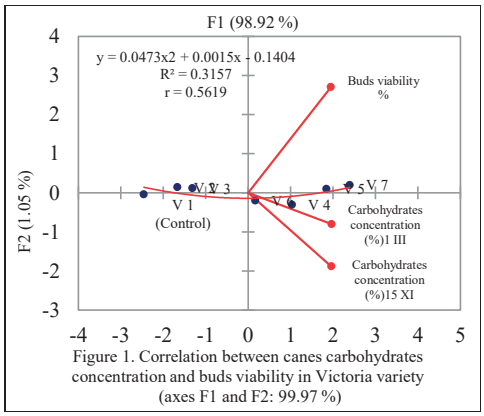

Figure 1. Correlation between canes carbohydrates concentration and buds viability in Victoria variety (axes F1 and F2: 99.97 %)

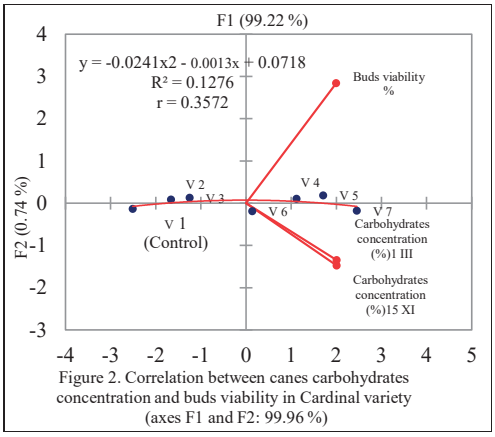

Figure 2. Correlation between canes carbohydrates concentration and buds viability in Cardinal variety (axes F1 and F2: 99.96 %)

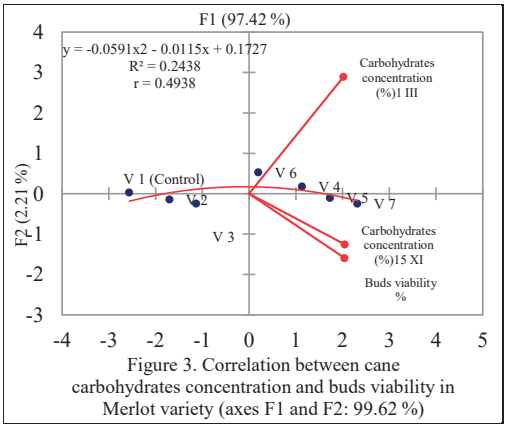

Figure 3. Correlation between cane carbohydrates concentration and buds viability in Merlot variety (axes F1 and F2: 99.62 %)

CONCLUSIONS

The must concentration in sugars was influenced both by the direct amount of copper applied during the phytosanitary treatments, as well as by the growing stage of the treatment application.

Too early copper application in V_2 variant (before flowering), led to the poorest results because the copper applied during the intense growth of the stems, decrease for short time the rate of photosynthesis.

The application of higher doses of copper maintains the canopy area in appropriate phytosanitary status in all three varieties and therefore the photosynthetic efficiency increased proportionally with the amount of copper applied. V_7, in which was applied 2.42 kg copper/per hectare, recorded the lowest canopy area necessary to synthesize one kilogram of sugars.

Copper treatments were favourable for canes carbohydrate concentration in all three grape varieties. The concentration carbohydrates in canes increased proportionally with the amount of copper applied; the highest values were recorded in V_7 variant, for which was applied 2.42 kg copper/ha.

The carbohydrate concentration in canes was influenced not only by the copper doses but also by the growing stage when copper treatment was applied. The control variant to which copper was applied only ones - in late ripening growing stage, recorded for all three varieties, the lowest concentrations of carbohydrates in canes. In the V_2 and V_3 variants were recorded higher concentrations of carbohydrates in canes compared to the control, for copper applied in a single treatment, but much earlier, before flowering and in lag phase respectively.

Copper treatments also had a favourable influence on the buds viability; number of viable buds increased gradually with the amount of copper applied. Besides the copper amount of copper applied, the buds viability was also influenced by the growing stage when treatments was applied; the lowest buds viability was registered when copper treatments were applied later in the ripening stage.

The late copper application does not have the expected effect on the one year old wood

maturation and buds viability due to the short time available until the first hoar frost. Since the maturation of the cane wood starts in the summer, it is very important for the vines to benefit from copper in moderate doses at least from the lag phase stage.

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