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THE IMPACT OF COPPER APPLICATION TIMING AND THE FREQUENCY OF TREATMENTS ON PHYSIOLOGICAL, BIOCHEMICAL, AND PRODUCTIVE PARAMETERS IN A VINEYARD

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Abstract

The research carried out between 2021 and 2023 was primarily focused on managing the timing and frequency of copper application - an essential treatment in the control of diseases and pests. The grape varieties (Victoria, Muscat de Hamburg, Merlot, Cabernet Sauvignon, Fetească neagră) involved in the research were for table grapes and wine, with peculiar tolerance to frost, diseases, and pests. Experimental plots were organized based on several copper treatments correlated with disease and pest management. Several grape yield parameters, as well as berry composition and canes (berry sugar and vitamin content; cane carbohydrate content), were tracked for each of the six experimental plots within each grape variety. Simultaneously, an examination of how the vines survived during the winter as regards of wood maturity and bud viability was carried out, taking into account the major influence of copper on these parameters. The results showed that the grape varieties behaved differently from year to year, depending on the individual qualities of each variety.

Key words*: viticulture, copper, cane maturation, grapes, quality.*

INTRODUCTION

Grapevine is one of the most vulnerable cultivated plants to disease and pest attacks (Hnatiuc et al., 2023). According to the majority of winegrowers, the most widespread and dangerous disease is downy mildew, which is caused by the fungus *Plasmopara viticola* (Peng et al., 2024). Copper-based treatments were previously the most widely utilised and effective products for downy mildew control (Dobrei et al., 2020). Copper was initially used as a fungicide in agriculture in the 17th century to treat wheat seed against downy mildew before sowing (Beckerman et al., 2023). Copper was applied for downy mildew control in vine treatments in 1882, in France, by Millardet, who applied copper as a fungicide in the Bordeaux mixture (Morton & Staub, 2008). Much later, in 1956, the first copper-based insecticide for grapevine mildew control was approved (Rajwade et al., 2024). Since then, the number of copper-based treatments approved for downy mildew control on vines has expanded substantially, making it nearly impossible to develop an efficient strategy to control diseases and pests on vines that should not be found in copper-based products (Puelles et al., 2024). Copper serves various roles in vine metabolism beyond its conventional use as a pesticide (Moine et al., 2023; Betancur-Agudelo et al., 2023). It contributes to essential processes such as protein and lignin synthesis, chlorophyll production, respiration, absorption, and winter resistance (Oyebamiji et al., 2024). As the concept of organic viticulture is more spread and vine-cultivated areas expand under organic systems, the significance of copper has increased (Jez et al., 2023; Vršič et al., 2023). Copper-based products are among the few chemical treatments approved for use in organic viticulture, underscoring their importance in sustainable grape cultivation practices (Döring et al., 2015). The proper application of copper in viticulture represents a critical and extensively investigated aspect in recent years (Volkov et al., 2023). Research findings have highlighted its multifaceted role beyond solely downy mildew control (Moine et al., 2023; Maddalena et al., 2023). Copper exhibits beneficial effects and unintended yet effective side effects, including mitigating powdery mildew incidence by impacting cleistotheca and mycelium, decreasing grey rot (*Botrytis cinerea*) susceptibility through thickening the skin of the berries, postponing leaf fall to promote shoot tissue maturation, and enhancing winter resilience (Widmer & Norgrove, 2023; Bleyer et al., 2023). Optimal utilization of copper in viticulture necessitates an integrated approach, involving the rotation of different copper formulations or its alternation with other products. This strategy is crucial to prevent the development of resistance in target pathogens (Trentin et al., 2023). Moreover, the timing of copper application holds significant importance and must be carefully synchronized with climate variability, grape variety, growing stage, and the purpose of application (Moine et al., 2023).

The objective of the paper was to investigate the management of timing and frequency of copper application in grapevine, particularly focusing on its influence on grape yield parameters, vine survival during winter, disease and pest control.

MATERIALS AND METHODS

The study was conducted in the Recaș vineyard area over the growing seasons of 2021 to 2023, with the main objective of improving copper treatment timing, amount, and frequency. Experimental plots were established to investigate various copper application protocols, which included different numbers of treatments and scheduling strategies, as part of overall disease and pest management strategies (V_1 - one treatment with copper (0.76 kg/ha Cu,) applied after veraison (Control); V_2 - one treatment with copper (0.76 kg/ha Cu) applied before flowering; V_3 - one treatment with copper (0.76 kg/ha Cu) applied at berry leg; V_4 - two treatments with copper (0.76 kg/ha Cu) applied at berry leg + (0.76 kg/ha Cu) applied after veraison; V_5 - two treatments with copper (0.76 kg/ha Cu) applied before flowering + (0.76 kg/ha Cu) applied after veraison; V_6 - three treatments with copper: (0.76 kg/ha Cu) applied before flowering + (0.76 kg/ha Cu) applied at berry leg + (0.76 kg/ha Cu) applied after veraison.

Cuproxat Flowable product was used (190 g/litre Cu derived from tribasic copper sulphate) in the copper treatments. The varieties chosen for research (for wine and fresh consumption), have variable resilience to frost, diseases and pests.

Observations were made and determined on varieties and experimental plots regarding: the percentage of matured annual wood, the fertile buds percentage, the carbohydrates composition of annual canes (at the onset of dormancy and at the end of physiological dormancy), the grape production, the sugar concentration in grape juice, the amount of assimilated sugar per hectare and the berry composition in vitamins. The latter was assessed in accordance with specific standards endorsed by the Association of Analytical Chemists (AOAC) and subsequently expressed per 100 grams of sample. The statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software for Windows version V22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables. Normality of data distribution was assessed using the Shapiro-Wilk test. For inferential statistics, a two-tailed t-test was performed to compare the means of two independent groups, with significance set at $p < 0.05$. The t-value was calculated for the difference between the means. The p-value was calculated for the probability of obtaining the observed difference between groups. A p-value less than 0.05 was considered statistically significant. The statistical analysis was conducted using XLSTAT 2018 software (Addinsoft, Paris, France). Principal Component Analysis (PCA) was performed to analyze the qualitative characteristics of the data.

RESULTS AND DISCUSSIONS

The majority of viticultural practices involve 1-2 copper treatments as part of disease and pest management strategies, which are often applied after veraison (Jez et al., 2023). During the three-year study period, climate conditions varied significantly throughout the growing seasons, ranging from very favourable in 2021 to moderately favourable in 2023 (Table 1). Therefore, the average results attained during these years can be considered consistent and reliable.

Table 1. Influence of treatments on one year old wood maturation in table grape varieties
(Mean 2021-2023 growing seasons)

Experimental plot	Cu (kg/ha)	No of days till the first hoar-frost			Percentage of one year old wood maturated (%)	Difference to the control (%)
		Treat. 1	Treat. 2	Treat. 3		
Victoria						
V_1(Control)	0.76	97	-	-	69	-
V_2	0.76	156	-	-	74	5^{NS}
V_3	0.76	121	-	-	76	7*
V_4	1.52	121	97	-	83	14^{**}
V_5	1.52	156	97		85	16^{**}
V_6	2.28	156	121	97	88	19^{**}
Muscat Hamburg						
V_1(Control)	0.76	69	-	-	60	-
V_2	0.76	156	-	-	69	9^{*}
V_3	0.76	94	-	-	71	11^{*}
V_4	1.52	94	69	-	76	16^{**}
V_5	1.52	156	69		78	18^{**}
V_6	2.28	156	94	69	82	22^{***}

The percentage of annual matured wood serves as a significant indicator profoundly impacting vine winter resilience and bud viability (Dobrei et al., 2021). Despite minimum temperatures across all three years remaining above the frost resistance threshold for vines, the percentages of one year old matured wood, particularly among table grape varieties, ranged between 60 and 85%. Bondok et al. (2012) found in the Roumi Ahmar table grape, that old wood have a significant role in grape yield increasing, bunch weight and berry quality. In both table grape varieties, the ranking of experimental plots was near similar, differing primarily in the percentage of matured one-year-old wood. The control plots recorded less matured wood, while plots receiving higher copper doses and extended copper application durations showcased elevated percentages of ripened wood. These differences compared to the control were statistically significant.

Table 2. Influence of treatments on one year old wood maturation in wine grape varieties
(Mean 2021-2023 growing seasons)

Experimental plot	Cu (kg/ha)	No of days till the first hoar-frost			Percentage of one year old wood maturated (%)	Difference to the control (%)
		Treat. 1	Treat. 2	Treat. 3		
Fetească neagră						
V_1 (MT)	0.76	68	-	-	73	-
V_2	0.76	156	-	-	75	2^{NS}
V_3	0.76	95	-	-	79	6^{NS}
V_4	1.52	95	68	-	84	11^{*}
V_5	1.52	156	68		85	12^{*}
V_6	2.28	156	95	68	91	18^{**}
Merlot						
V_1 (MT)	0.76	64	-	-	74	
V_2	0.76	156	-	-	77	3^{NS}
V_3	0.76	84	-	-	80	6^{NS}
V_4	1.52	84	64	-	85	11^{*}
V_5	1.52	156	64		86	12^{*}
V_6	2.28	156	84	64	90	16^{**}
Cabernet Sauvignon						
V_1 (MT)	0.76	61	-	-	83	-
V_2	0.76	156	-	-	83	-
V_3	0.76	79	-	-	84	1^{NS}
V_4	1.52	79	61	-	90	7^{*}
V_5	1.52	156	61		91	8^{*}
V_6	2.28	156	79	61	96	13^{*}

In Muscat Hamburg variety, renowned for its frost sensitivity and wood maturation challenges, the beneficial influence of copper surplus compared to the control was notably evident.

Despite the inherent good properties of wine grape varieties concerning the maturation of one-year-old wood and winter resilience, certain experimental plots exhibited lower percentages of wood maturation (Table 2). Nonetheless, across all plots, the wood demonstrated satisfactory maturation and remained undamaged by winter's low temperatures. Comparatively, the differences observed between experimental plots for wine grape varieties were less significant than those observed in varieties for table grapes. In plots with higher doses of copper treatments application, had increased percentages of matured wood. The timing of the copper application was also important: applications made too early, prior to flowering, or too late, at the onset of veraison, demonstrated lesser efficacy than applications made during the berry leg stage. In all three varieties, the most efficient treatment as indicated by wood maturation was in the V_6 plot, which received the highest copper dosage, applied in all three critical stages: before flowering, during berry leg development and at the onset of veraison. Among the wine grape varieties, the smallest differences between treatments were recorded in the Cabernet Sauvignon variety, known for its favourable properties regarding wood maturation and winter cold resistance. After two copper treatments application in Riesling variety (during and after flowering) for *Plasmopara viticola* control, Meissner et al. (2019) discovered significantly higher copper content in both leaves and vines wood, which may inhibit or even delay the maturation of cane wood.

The carbohydrate content of the canes during dormancy and at the winter dormancy was an indicator directly influenced by both the doses and timing of copper application. It should be noted that the varieties considered being less resistant to frost recorded lower carbohydrate concentrations in the canes compared to the varieties for wine, which are typically more resilient to frost. In all varieties, the influence of the treatment from the experimental plot on the carbohydrate content of the canes was similar, with all experimental plots recording values superior to the control. This indicator is significantly influenced by both the quantity of copper applied and the timing of application.

The highest carbohydrate content in the canes was observed in plot V_6, where the highest copper dose was applied in three rounds. The percentage of fertile buds was also obviously influenced by copper, with all varieties following a similar pattern of experimental plot scaling. Although the recorded minimum temperatures during the three winter seasons did not drop below the resistance limit of the varieties as outlined in specialized literature and practiced in viticulture significant percentages of buds affected by frost were noted in certain experimental plots (Nistor et al., 2023) (Table 3).

Table 3. Treatments influence on grape production and fertile buds percentage in table grape varieties (Mean 2021-2023 growing seasons)

Experimental plot	Grape production (kg/ha)	Sugar (g/l)	Sugar (kg/ha)	Canes carbohydrates g %		Fertile buds percentage	Difference to Control (%)
				15.XI	1.III		
Victoria							
V_1 (Control)	16220	150	1703.1	10.6	8.1	61	-
V_2	15750	147	1620.6	10.7	8.2	69	8^{*}
V_3	16953	157	1863.1	10.9	8.5	71	10^{*}
V_4	18230	161	2054.5	12.8	10.6	76	15^{**}
V_5	17570	159	2066.8	12.9	10.8	79	18^{**}
V_6	18810	163	2146.2	13.4	11.4	83	22^{***}
Muscat Hamburg							
V_1 (Control)	12530	165	1447	11.9	8.0	52	-
V_2	11254	160	1260	12.4	8.3	62	10^{*}
V_3	13457	171	1611	12.9	8.4	65	13^{*}
V_4	14838	174	1807	14.8	9.9	71	19^{**}
V_5	14325	172	1724	15.1	10.2	75	23^{***}
V_6	15740	180	1983	16.2	11.1	80	28^{***}

Table 4. Treatments influence on grape production and fertile buds percentage in wine grape varieties
(Mean 2021-2023 growing seasons)

Experimental plot	Grape production (kg/ha)	Sugar (g/l)	Sugar (kg/ha)	Canes carbohydrates g %		Fertile buds percentage	Difference to Control (%)
				15.XI	1.III		
Fetească neagră							
V_1 (Control)	9875	194	1341	13.6	11.2	74	-
V_2	9611	189	1271	13.7	11.6	75	1^{NS}
V_3	10276	198	1424	14.0	12.1	79	5^{NS}
V_4	10916	207	1581	15.7	12.5	83	9^{*}
V_5	10518	203	1495	15.9	12.7	84	10^{*}
V_6	11410	212	1693	17.1	13.2	89	15^{**}
Merlot							
V_1 (Control)	10125	186	1318	12.9	9.7	73	-
V_2	9836	178	1226	13.3	10.1	76	3^{NS}
V_3	10823	193	1462	13.7	10.3	78	5^{NS}
V_4	11510	201	1619	15.3	11.8	84	11^{*}
V_5	11798	196	1618	15.6	11.9	86	13^{*}
V_6	12216	206	1761	16.7	12.5	91	18^{**}
Cabernet Sauvignon							
V_1 (Control)	9751	196	1338	14.1	12.2	82	-
V_2	9598	188	1263	14.3	12.4	83	1^{NS}
V_3	9996	199	1382	14.5	12.7	85	3^{NS}
V_4	10217	205	1466	16.2	13.3	90	8^{*}
V_5	10098	202	1428	16.3	13.4	91	9^{*}
V_6	10832	210	1592	17.9	14.3	94	12^{*}

Muscat Hamburg was the most sensitive variety, followed by the Victoria variety. In these cultivars, the method of copper application played a significantly more important role compared to wine varieties known for their resilience. As the amount of copper applied increased, the percentages of fertile buds also increased, with the most effective treatments observed in plots V_6 and V_5 followed by V_4.

The observed differences among experimental treatments, although minor, were also evident when applied to wine grape varieties, demonstrating the importance of effectively managing copper use, even for varieties that typically have high bud fertility percentages in Romania's winter climate conditions (Table 4). Low winter temperatures, grape output levels, growing practices, and grape harvest timing all had an impact on wood maturity and bud fertility. Excessive grape production, particularly in conjunction with less favorable climatic conditions, resulted in delayed grapes technological maturity, with consequent negative impacts on wood maturation. Therefore, the importance of copper management in respect to both dosage and application time becomes very important (Facco et al., 2023); this ensures that plants can withstand winter with sufficient reserves of cane carbohydrates, thereby facilitating optimal wood maturation and corresponding frost resistance in buds.

In addition to the favorable influence on the wood maturation and the buds fertility, copper had an obvious influence on both the quantity and quality of grape production in all varieties. Upon evaluating the experimental plots based on production levels, a consistent pattern emerged across all five varieties. In experimental plot V_6, which received three copper treatments, was registered the highest grape production, followed by plots V_4 and V_5 with two copper treatments, and plots V_3, V_1, and V_2, to which was applied only one copper treatment each. Regarding the time of application, the lowest production was recorded at plot V_2, to which the copper treatment was applied in the first part of vine development, before flowering, which reduced the growth rate for a short time, and subsequent decrease in production. Delayed application of copper after veraison onset (V_1) is not indicated due to the too short interval for the copper to have its effect. Hence, the most optimal timing for copper application was during the berry leg stage, where a single treatment yielded the most favorable results.

When the treatment schemes were applied twice, the optimal time of application was during the berry leg stage and post-veraison. Evaluating grape quality based on sugar content proved that treatment applied to plot V_6, receiving three treatments, exhibited the highest sugar concentration. Too early application of copper (V_2) or too late (V_3) had the effect of reducing the concentration of sugars, compared to the application of copper during berry leg stage.

Table 5. Treatments influence o grape berry accumulation in vitamins for table grape varieties (Mean 2021-2023 growing seasons)

Experimental plot	Vitamin C, total ascorbic acid (mg)	B_1 -Thiamin (mg)	Vitamin B_6 (mg)	Vitamin A. (UI)	Vitamin E - alpha-tocopherol (mg)
Victoria					
V_1(Control)	11.739	0.046	0.441	27823	2.299
V_2	11.745	0.043	0.441	27821	2.322
V_3	11.747	0.049	0.453	27873	2.312
V_4	11.833	0.056	0.455	27881	2.306
V_5	11.827	0.062	0.462	27914	2.332
V_6	11.871	0.068	0.465	27914	2.324
Muscat Hamburg					
V_1(Control)	11.710	0.039	0.448	27723	2.323
V_2	11.731	0.041	0.448	27722	2.341
V_3	11.739	0.042	0.456	27779	2.349
V_4	11.812	0.053	0.458	27893	2.352
V_5	11.817	0.061	0.469	27952	2.356
V_6	11.859	0.065	0.472	27957	2.355

International unit (UI); 1 IU=0.3 micrograms; 1 IU=0.0003 mg; 1 IU of vitamin A: the biological equivalent of 0.3 μg retinol or 0.6 μg β-carotene; RAE mcg (retinol activity equivalent).

Table 6. Treatments influence o grape berry accumulation in vitamins for wine grape varieties (Mean 2021-2023 growing seasons)

Experimental plot	Vitamin C. total ascorbic acid (mg)	B_1 -Thiamin (mg)	Vitamin B_6 (mg)	Vitamin A. (UI)	Vitamin E - alpha-tocopherol (mg)
Fetească Neagră					
V_1(Control)	11.289	0.083	0.483	27691	2.412
V_2	11.290	0.082	0.482	27693	2.410
V_3	11.297	0.093	0.489	27723	2.423
V_4	11.321	0.097	0.494	27791	2.475
V_5	11.396	0.107	0.502	27823	2.498
V_6	11.342	0.103	0.498	27804	2.486
Merlot					
V_1(Control)	11.313	0.079	0.475	27832	2.391
V_2	11.312	0.078	0.476	27821	2.397
V_3	11.356	0.086	0.478	27843	2.399
V_4	11.443	0.091	0.487	27914	2.442
V_5	11.551	0.097	0.493	27923	2.447
V_6	11.543	0.098	0.495	27921	2.449
Cabernet Sauvignon					
V_1(Control)	11.402	0.083	0.463	27745	2.381
V_2	11.399	0.082	0.462	27751	2.380
V_3	11.421	0.091	0.469	27759	2.389
V_4	11.475	0.096	0.475	27843	2.393
V_5	11.574	0.099	0.478	27852	2.421
V_6	11.576	0.096	0.495	27854	2.420

The vitamin content, the differences among the experimental treatments were relatively low, in all varieties (Tables 5, 6). However, plots receiving copper supplementation, recorded slightly higher values due to improved tolerance to disease and pest and a better photosynthetic efficiency. These parameters were less influenced by the copper doses and application timing compared to the other parameters under consideration.

In similar topic, after two –years of copper treatments application on table grapes, results of Ma et al. (2019), showed vitamin C contents much higher in grape berries than those from the control or Bordeaux mixture treatments.

The PCA diagram was generated to visualize the relationships among the variables and to identify patterns or clusters within the data.

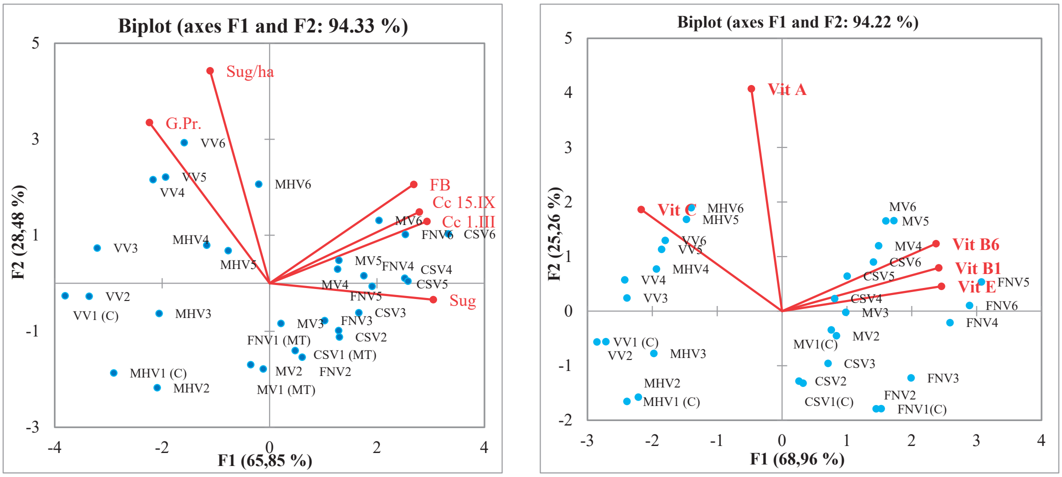

Figure 1. PCA diagram for copper treatments influence on berry quality in table grapes varieties and wine grape varieties (a); PCA diagram for vitamin concentration in table grapes varieties and wine grape varieties (2021-2023 growing seasons) (b) (V_1 – Control; V_2,V_3,V_4,V_5, V_6 – experimental plots; V – Victoria; MH – Muscat Hamburg; FN – Fetească neagră; M – Merlot; CS – Cabernet Sauvignon)

The statistical analysis was performed using Principal Component Analysis (PCA) to assess the influence of copper treatments on berry quality in table grape varieties and wine grape varieties. In the PCA diagram (Figure 1a) it was observed that the first principal component (F1) explained 65.85% of the variability in the dataset, indicating that F1 captured the majority of the variation in berry quality due to copper treatments. Grape production varied across different grape varieties and copper treatments. The Victoria variety exhibited the highest grape production overall, except for the control variant and V_2. However, Merlot, Fetească Neagră, and Cabernet Sauvignon had lower grape production but accumulated higher sugar in berries, particularly after copper application in V_6, V_5, and V_4. Furthermore, after copper application in V_6, V_5, and V_4, Merlot, Fetească Neagră, and Cabernet Sauvignon showed the highest percentage of fertile buds and the highest concentration of canes carbohydrates for both application times (15.XI and 1.III). Muscat Hamburg variety displayed the lowest sugar concentration, fertile buds percentage, and canes carbohydrates concentration overall. These findings suggest that copper treatments have varying effects on grape quality parameters across different grape varieties, with some varieties exhibiting improved berry quality metrics following copper application.

The PCA diagram (Figure 1b) revealed that the first principal component (F1) explained 68.96% of the variability in vitamin accumulation among table grapes. This suggests that F1 captured a significant proportion of the variance in the data related to vitamin accumulation.

Merlot, Cabernet Sauvignon, and Fetească Neagră varieties exhibited the highest accumulation of vitamins B_1, B_6, and E in plots treated with high amounts of copper (V_4, V_5, and V_6). This indicates that these grape varieties may respond positively to copper treatments in terms of vitamin accumulation.

Muscat Hamburg displayed the highest accumulation of vitamin A in plots V_5 and V_6, indicating a potential beneficial effect of copper treatments on this vitamin in this particular grape variety.

Furthermore, Muscat Hamburg also showed significant accumulation of vitamin C in plots V_5 and V_6, while Victoria variety was also rich in vitamin C. This suggests that these grape varieties may have a higher capacity to accumulate vitamin C compared to others under specific copper treatments.

Control experimental plot V_1 and V_2 plot, which received fewer copper treatments, exhibited lower levels of vitamins overall, indicating the importance of copper treatments in enhancing the accumulation of vitamins in grape varieties.

CONCLUSIONS

Copper plays an essential role in grapevine growing, serving both in disease control and various physiological and metabolic functions. The quantity and timing of copper application had significant influence on grape production, its quality, and the overall health of the vines. These factors impact several key aspects, including carbohydrate content of the canes, percentage of wood maturation, fertility of buds, and vine resilience during winter frost conditions. Research findings show a proportional increase in carbohydrate content of one-year-old canes, percentage of wood maturation, and bud fertility with higher levels of copper administered to the vines.

The moment of copper application is also important, to ensure that the treatment is the expected one. Early application before flowering or late application after veraison onset proved to be less effective compared to application at the berry leg stage. Among the monitored parameters, the most effective copper dose of 2.28 kg/hectare applied in three stages (before flowering, at the berry leg stage, and at veraison onset) yielded optimal results across all varieties. The differences between the experimental plots were much more evident in the Victoria and Muscat Hamburg varieties, which are more sensitive to winter frosts, and generally have a poorer annual wood maturation, particularly under conditions of high grape production or late harvesting.

Overall, the PCA analysis provided insights into the relationship between copper treatments and vitamin accumulation in different grape varieties, highlighting potential variations in response to copper treatments among grape varieties and the importance of copper in enhancing vitamin content in grapes.

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