THE IMPACT OF COPPER FUNGICIDES ON THE COPPER CONTENT IN ORGANS AND WINE FROM A 'SAUVIGNON BLANC' GRAPEVINE

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The framework of this project regarding organic viticulture was to determine the impact of copper fungicides on growth and yield of a 'Sauvignon blanc' grapevine and on the copper contents in the resulting grape juice and wine. Protection against diseases was carried out using copper in two forms: (a) 35 % Cu-oxychloride (Cuprablau Z) and (b) 8 % Cu-gluconate (Labicuper) in a dose of 6 kg Cu^{++}/ha/season (the maximum dose in organic viticulture in Slovenia), compared to a control (c) with copper-free fungicides. The experiment was designed as a randomised block system, with four replicates (260 plants in replicate; 650 m^2). At harvest time, five vines were selected randomly for each replicate to evaluate the yield (number and weight of clusters). The copper content was analysed within the grapevines' leaves, grape juices, and wines. The use of Labicuper reduced the yield per vine. The highest Cu content was detected in the leaves (2601 mg/kg) and in the juices (8.25 mg/l) of the Cu-oxychloride treated vines, whereas in the Labicuper treated vines it was in the berry skins (18.75 mg/kg). Lower Cu content was found in the juice obtained from macerated grapes. The Cu content in the bottled wines after all treatments was significantly below 0.25 mg/l (permitted limit 1 mg/l).
Keywords: grapevine, Cu fungicide, growth, wine, 'Sauvignon blanc'

Auswirkungen von Kupferfungiziden auf den Kupfergehalt in den Reborganen und im Wein von 'Sauvignon blanc'. Im Rahmen eines Projektes über Rebschutz im ökologischen Weinbau werden die Auswirkungen der Verwendung von Kupferfungiziden auf das Wachstum, den Ertrag und den Kupfergehalt im Traubenmost und Wein der Rebsorte 'Sauvignon blanc' bestimmt. Der Rebschutz mit Kupfer wurde in zwei Formen durchgeführt: (a) 35 % Cu-Oxychlorid (Cuprablau Z) und (b) 8 % Cu-Gluconat (Labicuper) in der Dosis von 6 kg Cu^{++}/ha/Saison (Höchstdosis im biologischen Weinbau in Slowenien) im Vergleich zur Kontrolle (c) mit kupferfreien Fungiziden. Der Versuch wurde als randomisiertes Blocksystem mit vier Wiederholungen (260 Pflanzen pro Wiederholung; 650 m^2) angelegt. Zur Zeit der Traubenreifung wurden fünf Rebstöcke in jeder Wiederholung zufällig ausgewählt, um den Ertrag (die Anzahl und das Gewicht der Trauben) zu bewerten. Der Kupfergehalt wurde in den Blättern, im Traubenmost und Wein analysiert. Der Ertrag pro Rebstock wurde durch die Verwendung von Labicuper reduziert. Der höchste Cu-Gehalt wurde bei den mit Cu-Oxychlorid behandelten Reben in den Blättern (2601 mg/kg) und im Saft (8,25 mg/l) nachgewiesen, während er bei den mit Labicuper behandelten Reben (18,75 mg/kg) in den Beerenschalen festgestellt wurde. Niedriger Cu-Gehalt wurde im Saft aus mazerierten Trauben festgestellt. Der Kupfergehalt in den abgefüllten Weinen aller Behandlungen lag deutlich unter 0,25 mg/l (zulässiger Grenzwert 1 mg/l).
Schlagwörter: Weinrebe, Cu-Fungizid, Wachstum, Wein, 'Sauvignon blanc'

The use of copper (Cu) in viticulture became widespread after the accidental discovery of a Bordeaux mixture at the end of the 19th century (BRUN et al., 1998; CHAIGNON et al., 2003). The intensive and long-term application of copper-based fungicides leads to an accumulation of Cu in vineyard soils and can potentially have adverse effects on the microbial functions and fertility of the soils (PELLEGRINI et al., 2010; STRUMPF and STRASSEMEYER, 2012; WIGHTWICK et al., 2013). Furthermore they cause proportional decreases in the photosynthetic efficiency (BAGLYAS and PÖLÖS, 2014a), and an increase of copper bioavailability and toxicity for plants (BAGLYAS and PÖLÖS, 2014b; MIOTTO et al., 2014; DA ROSA COUTO et al., 2015). The maximum limits of Cu-content in the soil are exceeded in many vineyards (PIETRZAK and MCPHAIL, 2004; MIRLEAN et al., 2007; KOVAČIČ et al., 2013). In the past, the annual Cu-inputs to vineyard environments were very high (30 kg/ha). In organic agriculture, a gradual reduction in Cu-input levels from 8 kg to 6 kg/ha is required (requirement of Commission Regulation EC No. 473/2002). The additions of spent mushroom substrates to vineyard soils increased the Cu-initial retention capacity (HERRERO-HERNANDEZ et al., 2011) and the growing of cover crops between vine rows also reduced Cu-concentrations in the soil (MACKIE et al., 2015). In vines treated with Cu-preparations the highest Cu-concentrations were usually found in the leaves, followed by wood and grapes (RUSJAN et al., 2007; BENI and ROSI, 2009; ALI et al., 2003). High levels of copper residues are especially problematic for table grapes (POULSEN et al., 2007).

One of the strategies for reducing the annual rates of Cu applied to vineyards is to develop new formulations of Cu-products, which can provide less wash-off due to rainfall (PEREZ-RODRIGUEZ et al., 2015) and a higher efficacy of disease control at significantly reduced rates of applied Cu (for example systemic acting Cu-gluconate), and consequently a reduction in soil Cu-residues can be achieved by their use (LA TORRE et al., 2011). A typical variety sensitive to aroma reduction due to the increased Cu-content in grapes and must is 'Sauvignon blanc' (DARRIET et al., 1995; ZUBLASING and PEDRI, 2011). Maceration usually decreases the copper content in the clarified juices (HATZIDIMITRIOU et al., 1996). The use of systemic acting Cu-preparations (e. g. Cu-gluconate, Cu-octanoate, Cu-peptidate, etc.) for the control of grape diseases has increased (DI MARCO et al., 2011; LA TORRE et al., 2011; KOVAČIČ et al., 2013). The effects of traditional Cu-preparations based on Cu-hydroxide, Cu-oxychloride, Cu-oxide, Cu-sulphate and similar on copper dynamics in grapes, must and wine are well-documented in scientific literature, but information on the effects of new systemic formulations is quite limited.

The aim of this study was to compare the effects of two different Cu-preparations (systemic acting Cu-gluconate vs. contact acting Cu-oxychloride) on the growth and yield of a 'Sauvignon blanc' grapevine and on Cu residues in the must and wine.

MATERIAL AND METHOD

In this research the impact of using copper fungicides on the growth and yield of a 'Sauvignon blanc' grapevine on 5BB rootstock, and the copper content in the grape juice and wine at all stages of vinification was studied. The experiment was conducted during 2011and 2012 in an 11-year-old vineyard (46º 39'N, 15º 56'E, 305 m.a.s.l.) owned by the company Radgonske gorice. The training system was double Guyot, and soil management was a permanent grass cover. Protection against downy mildew was carried out using copper in two forms: (a) contact acting traditionally used fungicide Cuprablau Z (35 % Cu^{++} in the form of Cu-oxychloride) and (b) systemic acting Labicuper (8 % Cu^{++} in the form of Cu-gluconate) in doses of 6 kg Cu^{++}/ha/season (maximum dose in organic viticulture in Slovenia), compared to variant (c) without the use of copper (control; plant protection was carried out according to integrated production by application of systemic and contact acting fungicides without any copper content). All preparations were applied in the form of 400 l spray per ha.

The incidences of downy mildew and other diseases during the two experimental years were low. All three spray programmes provided a very high level of disease control and therefore differences in yields of the three studied treatment variants were not related to diseases attack rate but to other factors (the inhibitory effects of copper on the development of the vine and the residues of copper in some vine organs).

The experiment was designed as a randomised block system, with three treatments and four replicates (260 plants per replicate; 650 m^2) and two rows as a buffer zone (untreated rows) between each replicate. At harvest time, five vines were randomly selected in each replicate to evaluate the yield (number and weight of clusters), and to analyse the chemical composition of the grape juices (soluble solids, total acidity and pH) 100 berries in each replicate were taken.

The copper content was analysed in the leaves, grape juices obtained from all vines in replicate (juice after pressing, decanted juice after 24 hours, and sediment), and in wine at all stages of vinification (wine after first decanting, lees, wine after filtration, and bottled wine) using the method of Kurnik et al. (2012), using an atomic absorption spectrophotometer Varian AA 240FS (Agilent Technologies (previously Varian BV), Middelburg, The Netherlands), by reading the absorbance at 324.8 nm. Samples of the leaves and berries (50 leaves and 100 berries in each replicate) were taken at the end of August (30. 8. 2011 and 29. 8. 2012), three weeks after the last treatment.

Vinification was carried out at the University Center for Viticulture and Enology Meranovo, Faculty of Agriculture and Life Sciences in 80 l stainless steel barrels. Half of the grapes were pressed immediately after the harvest whereas the other half were macerated for eight hours at a temperature of 15 °C.

Differences between treatments were detected using a one-way analysis of variance (ANOVA). Statistical evaluation of the data was performed by the SPSS 19.0 programme ($P \leq 0.05$).

RESULTS AND DISCUSSION

YIELD AND CHEMICAL COMPOSITIONS OF THE GRAPES

In 2011 and 2012, the use of copper fungicides influenced the yield and development of clusters and berries (Table 1). The weights of 100 berries and grapes per vine were significantly lower when Cu was used in the form of Cu-gluconate (Labicuper). Between the control (standard fungicides without Cu-content) and Cuprablau Z, the differences in the weight of 100 berries and their yield were insignificant, except in the yield per vine in 2012. Beni and Rosi (2009) also had not found differences in grape yields when comparing 'conventional' and 'organic' vineyards. In soluble solids regarding total acidity and juice pH there were no significant differences between treatments detected ($P \leq 0.05$).

The main reason for the ascertained significant differences in the weights of 100 berries and grapes per vine with Labicuper treated vines may lie in its phytotoxicity. In literature there is information available that systemic acting Cu-formulations may have higher phytotoxicity to grape organs than traditional contact acting formulations (Pertot et al., 2002 and 2006; Juang et al., 2012; Baglyas and Pölös, 2014).

COPPER CONTENTS IN GRAPES AND LEAVES

The treatments of vines with Cu-fungicides against downy mildew had an effect on the contents of the copper in the leaves (Table 2), and the highest content of Cu was detected in leaves treated with Cu-oxychloride (Cuprablau Z). Similar relationships of Cu-content were observed by Rusjan et al. (2007), and Beni and Rosi (2009). Leaves treated with systemic acting Cu-gluconate accumulated less Cu than leaves treated with Cu-oxychloride. We expected the opposite result. The main reason could be in its higher translocation rate from the leaves to other tissues of the grapevine. A much lower level of Cu was measured in berry skins and grape juice. In berry skins it was the highest when the vines were treated with

Table 1: Yield and chemical composition of grape juice regarding 'Sauvignon blanc' treated with different Cu-fungicides during the years 2011 and 2012

Treatment	Year	Number of clusters/vine	Yield (kg/vine)	100 berries-weight (g)	Soluble solids (°Oe)	Total acidity (g/l)	pH
Cuprablau	2011	38.45 ± 1.15 a	4.21 ± 0.16 a	163.75 ± 5.79 ab	70.25 ± 1.63 a	9.56 ± 0.11 a	2.92 ± 0.38 a
Control	2011	40.50 ± 1.26 a	4.39 ± 0.11 a	170.00 ± 4.32 a	71.00 ± 1.68 a	9.19 ± 0.24 a	2.97 ± 0.36 a
Labicuper	2011	38.35 ± 0.72 a	3.06 ± 0.12 b	150.25 ± 6.38 c	70.00 ± 0.96 a	9.56 ± 0.14 a	2.96 ± 0.10 a
Cuprablau	2012	34.10 ± 3.23 ab	2.88 ± 0.44 b	160.50 ± 10.41 a	82.00 ± 2.22 a	8.72 ± 0.56 a	3.01 ± 0.04 a
Control	2012	35.85 ± 3.01 a	3.46 ± 0.25 a	155.25 ± 8.99 ab	81.00 ± 3.30 a	8.44 ± 1.08 a	3.06 ± 0.02 a
Labicuper	2012	33.35 ± 3.53 a	2.08 ± 0.32 c	143.00 ± 10.42 b	82.00 ± 0.82 a	9.10 ± 1.03 a	3.01 ± 0.07 a

Values in each column followed by the same letters are insignificantly different according to LSD test ($P \leq 0.05$).

Labicuper, which confirms our assumption about the translocation of Cu-gluconate (Labicuper) from the leaves to the other tissues of a grapevine. La Torre et al. (2011) found that copper formulation characterised by a low metallic content (Labicuper) was effective in controlling downy mildew with a lower copper dosage than with the copper formulations used as a standard. Thus, the environmental impact of copper in organic viticulture could be minimised through the new copper formulations similar to Labicuper. Copper contents of leaves, berry skins and juices are also influenced by weather conditions (especially the summer season precipitation rate). In 2012, the growing season precipitation rate was higher by 25 % than in 2011 (Slovenian Environment Agency, 2015). In the first ten days of September (before the harvest) the amount of precipitation in 2012 (81.5 mm) was four times higher than in 2011 (19.4 mm). Consequently, the washing of copper deposit from grape surfaces was much higher and measured concentrations in grape organs were therefore lower.

grape juices from vines treated with copper were within the ranges also observed in other experiments (Hatzidimitriou et al., 1996).

Two methods of grape processing – immediate pressing (P) and skin contact (M) showed significant differences in the copper content in juices before fermentation. Using the skin contact method (maceration), in all fractions of the grape juices the copper contents decreased in comparison with immediate pressing. Reductions in the copper contents are within the ranges from 20 to 43 % in juices after pressing and from 32 to 49 % in decanted juices after 24 h (Table 3). That can be explained by the bonding of copper during skin contact with glutathione and other organic compounds that are present in 'Sauvignon blanc' grapes (Hatzidimitriou et al., 1996).

The impacts of different copper treatments of vines on copper residues in wine was also evaluated (Table 4). Generally, a big decrease in copper content in wine compared to grape juice can be observed due to the copper eliminating effect of lees. After filtration and bottling, also in wines from copper treated vines, the Cu-content was lower than the current legal limit (1 mg/l). The highest Cu-content was in wines from Cu-oxychloride-treated vines. In bottled wines the differences between (P) and (M) processed grapes were insignificant.

The intensive use of Cu-based fungicides to control downy mildew of vines has led to an accumulation of copper in plant organs. According to the commonly known fact that the translocation of copper from grape roots to above-ground organs is very limited (Juang et al., 2012), we assume that only a small amount of copper detected in grape leaves, juices and berry skins originated from translocation from roots, and that most of it relates to intake after application of Cu-fungicide formulations. Only a small portion of copper was detected in the juices and most was transferred to the wine. This means that

Table 2: Copper content in the leaves, juices, and berry skins of vines treated with different Cu-fungicides during 2011 and 2012

Treatment	Year	Leaves (mg/kg)	Juice (mg/l)	Berry skins (mg/kg)
Cuprablau	2011	2601.75 ± 303.52 a	8.25 ± 0.73 a	11.00 ± 0.91 b
Control	2011	150.20 ± 32.94 c	1.45 ± 0.12 b	5.75 ± 0.25 c
Labicuper	2011	1524.00 ± 129.74 b	6.90 ± 0.51 a	18.75 ± 1.65 a
Cuprablau	2012	732.00 ± 187.75 a	4.80 ± 0.85 a	5.21 ± 0.66 b
Control	2012	162.50 ± 21.99 b	3.20 ± 0.62 a	3.32 ± 0.36 c
Labicuper	2012	669.00 ± 53.12 a	5.25 ± 2.45 a	6.87 ± 1.11 a

Values in each column followed by the same letters are insignificantly different according to LSD test ($P \leq 0.05$).

COPPER RESIDUES IN WINE

In 2011 the copper content was also evaluated at different stages of micro-vinification. We compared different treatments on vines and the copper content in grape juice significantly increased in both treatments with copper (Cuprablau Z, Labicuper) when compared to untreated vines (control P/M; Table 3). In the juices after pressing, the copper content (3.23 to 6.16 mg/l) was significantly higher in comparison to the control (0.50 to 1.00 mg/l). In the decanted juice after 24 h, the copper content (2.83 to 5.90 mg/l) was again significantly higher in comparison to the control (0.47 to 0.80 mg/l). In the sediment the copper content increased from 10.93 to 14.86 mg/kg and was significantly higher in comparison to the control (3.46 to 3.53 mg/kg). The values for

Table 3: Copper content (mg/l) in juices of 'Sauvignon blanc' vines treated with different Cu-fungicides and different methods of grape processing in 2011; P = immediate pressing, M = maceration

Treatment	Juice after pressing	Decanted juice after 24 h	Sediment
Cuprablau P	6.16 ± 0.03 a	5.90 ± 0.06 a	14.16 ± 0.35 a
Cuprablau M	4.90 ± 0.26 c	4.00 ± 0.21 b	14.86 ± 0.38 a
Control P	1.00 ± 0.00 e	0.80 ± 0.00 d	3.53 ± 0.03 d
Control M	0.50 ± 0.00 f	0.47 ± 0.03 d	3.46 ± 0.03 d
Labicuper P	5.70 ± 0.06 b	5.47 ± 0.07 a	13.33 ± 0.33 b
Labicuper M	3.23 ± 0.03 d	2.83 ± 0.35 c	10.93 ± 0.12 c

Values in each column followed by the same letters are insignificantly different according to LSD test ($P \leq 0.05$).

the application of 6000 g of Cu^{++} per ha and season does not pose any threat for observing the legal limit of Cu-residue in wine. The only concern should be the potential phytotoxicity of systemic copper which according to our observations can retard grapevine growth and berry development. The environmental impact of copper in organic viticulture could be reduced through new copper formulations, and consequently reduce copper residues in soils and plants. After a whole season's use of new formulations similar to Cu-gluconate at a rate of 6 kg Cu^{++}/ha/season we do not expect increased values of Cu-residues in wine.

Table 4: Copper content (mg/l) in the wines of 'Sauvignon blanc' treated with different Cu-fungicides in 2011; P = immediate pressing, M = maceration

Treatment	Wine after first decanting	Lees	Wine after filtration	Bottled wine
Cuprablau P	1.63 ± 0.55 a	158.95 ± 117.950 a	0.10 ± 0.003 b	0.09 ± 0.015 ab
Cuprablau M	1.76 ± 0.29 a	48.43 ± 2.623 ab	0.36 ± 0.046 a	0.24 ± 0.800 a
Control P	0.23 ± 0.033 c	62.43 ± 25.120 ab	0.08 ± 0.015 b	0.08 ± 0.250 ab
Control M	0.16 ± 0.033 c	17.00 ± 0.929 b	0.06 ± 0.058 b	0.14 ± 0.750 ab
Labicuper P	1.30 ± 0.000 ab	111.86 ± 48.860 ab	0.06 ± 0.067 b	0.06 ± 0.050 b
Labicuper M	0.80 ± 0.000 bc	53.13 ± 22.338 ab	0.11 ± 0.033 b	0.09 ± 0.050 ab

Values in each column followed by the same letters are insignificantly different according to LSD test ($P \leq 0.05$).

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Received August, 4th, 2015