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Natural alternatives to copper and low-rate copper formulations to control grape downy mildew in organic farming

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Summary To control plant diseases in organic farming, growers can use preventive measures together with a few plant protection products. The control of downy mildew is based on the use of copper compounds, however copper can cause environmental problems due to its accumulation in the soil. In this study natural alternatives to copper and formulations with low rates of copper were evaluated in organic farming in order to control *Plasmopara viticola*. All of the products tested ensured effective control of grape downy mildew under the experimental conditions of the trial, characterized by moderate disease pressure. The best levels of protection were observed with copper products. Low-copper formulations (Glutex Cu 90 and Labicuper) with similar efficacy to the reference product offer benefits including lower quantities of copper. This paper recommends a cultural management of downy mildew in accordance with the risk of infection in order to reduce environmental copper input. We suggest that organic growers can minimize the use of copper in organic viticulture by using copper-alternatives when downy mildew infection is intermediate or low. On the other hand in years with high disease pressure new copper formulations can be used to reduce the amount of copper.

Additional keywords: copper, natural products, organic farming, plant protection, *Vitis vinifera* L.

Introduction

Downy mildew, caused by the oomycete *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni, is one of the world's most destructive grapevine diseases (2, 24, 12, 27, 23). In organic farming, the control of *P. viticola* is based on the use of copper, together with all preventive management measures necessary to minimize the development of disease. Copper is still a very important tool to manage the diseases in conventional agriculture and is actually indispensable in organic farming (24, 21).

With Commission Directive 2009/37/EC, copper compounds have been included in Annex I to Directive 91/414/EEC (concerning the placing of plant protection products on the market). "Therefore, it is necessary that Member States introduce monitoring plans in vulnerable areas, where the contamination of the soil compartment by copper is a matter of concern, in order to set, where appropriate, limitations as maximum applicable rates" (Commission Directive 2009/37/EC) (7). In organic farming, limitations such as maximum applicable copper rates have already been defined (Regulation EC No 889/2008) (6). The use of copper, in fact, may have long-term consequences due to its accumulation in the soil (4, 26, 28, 14, 9, 22) and this is incompatible with the principles of organic farming. In the near future the current legal amount of copper will probably be reduced, so that alternatives to copper compounds need to be developed. Several research programs have been carried out to find appropriate alternative solutions (24, 8, 16, 17, 31, 18, 32, 30, 5, 35).

In order to evaluate the possibility of replacing or reducing the use of copper, a field trial in an organic vineyard was carried out and the activity of natural products and

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low rate copper formulations was estimated against grape downy mildew.

Materials and Methods

Experimental trial

The experiment was conducted in 2010 in an organic vineyard near Rome (lat. 41.4°N, long. 12.3°E, 180 m a.s.l.). The grape variety tested was Malvasia di Candia, and the rootstock (44 years old) employed was Kober 5BB (*Vitis berlandieri* x *Vitis riparia*). The training system was "tendone", consisting of a continuous overhead canopy under which the bunches are disposed (25). Plots were prepared, each containing 12 plants and repeated four times in randomized blocks. Spacings between vines were 2.50 m x 2.50 m, with a buffer row between treatments. The test organism was *P. viticola*.

Products were sprayed until near run-off with a pulled sprayer (Martignani K.W.H. electrostatic sprayer system) at a pressure of 1.5 bar. The trial was carried out in accordance with the EPPO/OEPP PP1/31 (3) guidelines (11).

Environmental data

A weather station was placed at the trial site to record weather data such as precipitation, air temperature, soil moisture (at a depth of 20 cm and 40 cm), leaf wetness, solar radiation, relative humidity, soil temperature, and wind speed and direction. These data were acquired through the GSM modem that was on board the weather station for remote transmission to a WeatherLink software program.

Treatments

Table 1 reports commercial name of tested products, commercial manufacturers, composition, active ingredient, rate of appli-

Table 1. Commercial name, active ingredient, dosage and number of applications of the products tested against grape downy mildew.

Treatment	Commercial name	Manufacturer	Composition	Active ingredient (%)	Rate of application	Number of applications /treatment
RP[a]	Bentoram Cuproxat SDI	Dal Cin Gildo (IT) Nufarm Italia(IT)	Copper hydroxide Tribasic copper sulphate	10 15.2	2-3-4[b] 3[b]	13
1	Labicuper	Labin Italia (IT)	Copper gluconate	8	2-2.5[b]	10
2	Glutex Cu 90	Socoa Trading S.r.l. (IT)	Copper hydroxide	9	4-4.25[b]	11
3	Biplantol agrar Biplantol mycos V forte	Plantosan (CH) Plantosan (CH)	Homeopathic preparation Homeopathic preparation	- -	1[b] 1[b]	6 5
4	Sporatec	Brandt Consolidated (US)	Rosemary Oil - Clove Oil - Thyme Oil	18-10-10	1[c]	14
5	Myco-Sin VIN	Andermatt Biocontrol (CH)	Aluminium sulphate	75	0.5[c]	11
6	BM-608	Biomor (IL)	*Malaleuca alternifolia* essential oil	23.8	0.75[c]	13
7	Stimulase	Agronutrition (FR)	Purified enzymes extracted from *Trichoderma* sp.	-	1[b]	8

[a] Reference product (Standard)
[b] l/ha
[c] %

cation and number of applications per treatment. The products tested included new copper formulations with low metallic copper (Labicuper and Glutex CU 90) and natural substances (Biplantol, Sporatec, Myco-Sin VIN, BM-608, Stimulase). The commercial products were used according to the manufacturer's information. The products were compared with an untreated control and a reference product containing copper (standard).

Grape downy mildew assessment

The assessments were made at intervals of seven days starting from the first appearance of disease symptoms until harvest. Phenology was described according to the Biologische Bundesanstalt, Bundessortenamt and CHemical industry (BBCH) scale (3), in which grapevine phenological development stages are described by Lorenz *et al.* (1994) (19). Grapevine leaves and bunches were visually assessed, 100 leaves and 100 bunches were picked randomly from the central 10 vines of each plot. The percentage of leaves and bunches diseased out of a total number assessed (disease incidence) and the area of leaves and bunches showing symptoms of disease (disease severity), were estimated. Disease severity (infection degree, ID) was calculated using a scale of nine classes (0-8) using the Townsend-Heuberger formula (33):

$$\mathrm{ID}\ (\%) = \Sigma^{i}_{1}(n_i \times v_i) \,/\, N \times V$$

where v_i is the damage class, n_i is the number in one class, N is the total number, V is the highest class, i is the number of classes. The area under disease progress curves (AUDPC) based on disease severity was calculated for each treatment according to Shaner & Finney (1977) (29). The AUDPC was assessed with the formula:

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where y_i is the disease severity at the i^{th} observation, y_{i+1} is the disease severity at the i^{th} +1 observation, n is the total number of assessments and $t_{i+1} - t_i$ is the number of days between the two assessments.

AUDPC values were normalized by dividing each AUDPC value by the total area of the graph (= the number of days from the first to last assessment of the disease × 1.0) (13). The normalized AUDPC was referred to as the relative area under disease progress curve (RAUDPC).

The index effectiveness was calculated at harvest using Abbott's formula (1):

$$\%\ \text{effectiveness} = [(I_c - I_t)/I_c] \times 100$$

where I_c is the disease incidence of the untreated control, I_t is the disease incidence of the treatment.

Observations for the presence of phytotoxic effects of all tested products were made after each spray on the shoots, leaves, bunches and flowers.

Yields

The effect of the investigated products on annual production was recorded at harvest time. Grape yield was calculated as t/ha/treatment.

Statistical analysis

Data in percentages were transformed to arcsine square roots according to the formula $Y=\arcsin(\sqrt{x\%/100})$ to correct normality before analysis. The data obtained were subjected to statistical analysis using ANOVA, a parametric statistical method and Tukey's test (34) at 5% of probability. Duncan's multiple range test ($P \leq 0.05$) (10) was used to determine significant differences among treatments in terms of grape fruit yield. Statistics were performed with GraphPad InStat version 3.00 for Windows.

Results and discussion

Environmental data

In 2010 there was high spring rainfall,

concentrated mainly in May, when there was 99.8 mm of rain. In June and August rainfall was average for that period, while in July and September rainfall was low (Figure 1).

Fungicidal effect and amount of copper provided with the treatments

The spring weather conditions led to the appearance of symptoms of primary infection of downy mildew in the last week of May. The first oil-spots appeared on the 24th of May (BBCH 57-Inflorescences fully developed; flowers separating) due to the presumed infection by the rain of May 17th, which was the date of the first infection event. With regard to the fruits, the first symptoms of grapevine downy mildew were observed on June 23rd (BBCH 75-Berries pea-sized, bunches hang). The phytosanitary situation did not show a particularly critical framework. Continuous monitoring and prompt applications, as required by the organic production method, guaranteed that all of the products tested achieved an effective anti-downy mildew control.

Figure 2 reports the assessment of disease incidence on leaves and bunches at the harvest. The best protection was achieved with copper formulations used as reference product and with the Glutex Cu 90 formulation. The Labicuper formulation showed a lower control of *P. viticola* than the reference product and Glutex Cu 90 although there were no significant differences among these products. The experimental products that did not contain copper (Stimulase, Myco-Sin VIN, Sporatec, Biplantol and BM-608) provided a control of infection on leaves significantly different in comparison with the untreated control.

Disease severity measured using RAUDPC for both leaves and bunches is reported in Figures 3 and 4 respectively. RAUDPC values in plots treated with copper formulations were lower than the values in plots treated with alternatives to copper. The ANOVA showed that there was no significant differences between reference product, Glutex Cu 90 and Labicuper. Although protection based on the application of natural alternatives to copper did not guarantee the same level of control achieved with formulated copper compounds, it was nevertheless acceptable.

Figure 5 shows the relationship between the amount of metallic copper applied and the effectiveness of the treatments. Glutex Cu 90 formulation guaranteed similar results

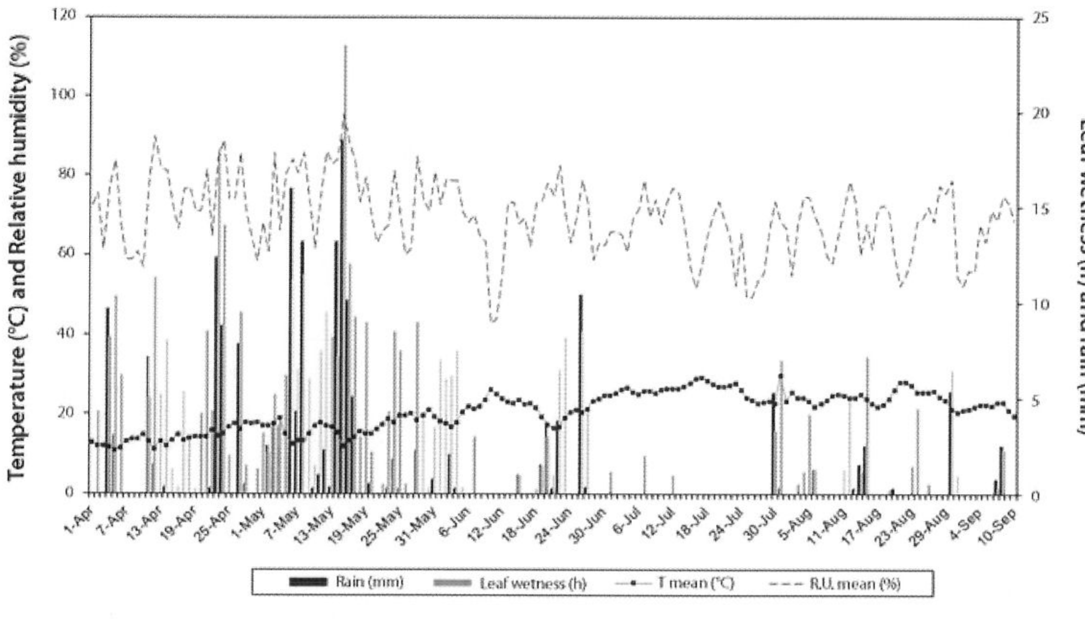

Figure 1. Climatic conditions during the experimental field trial .

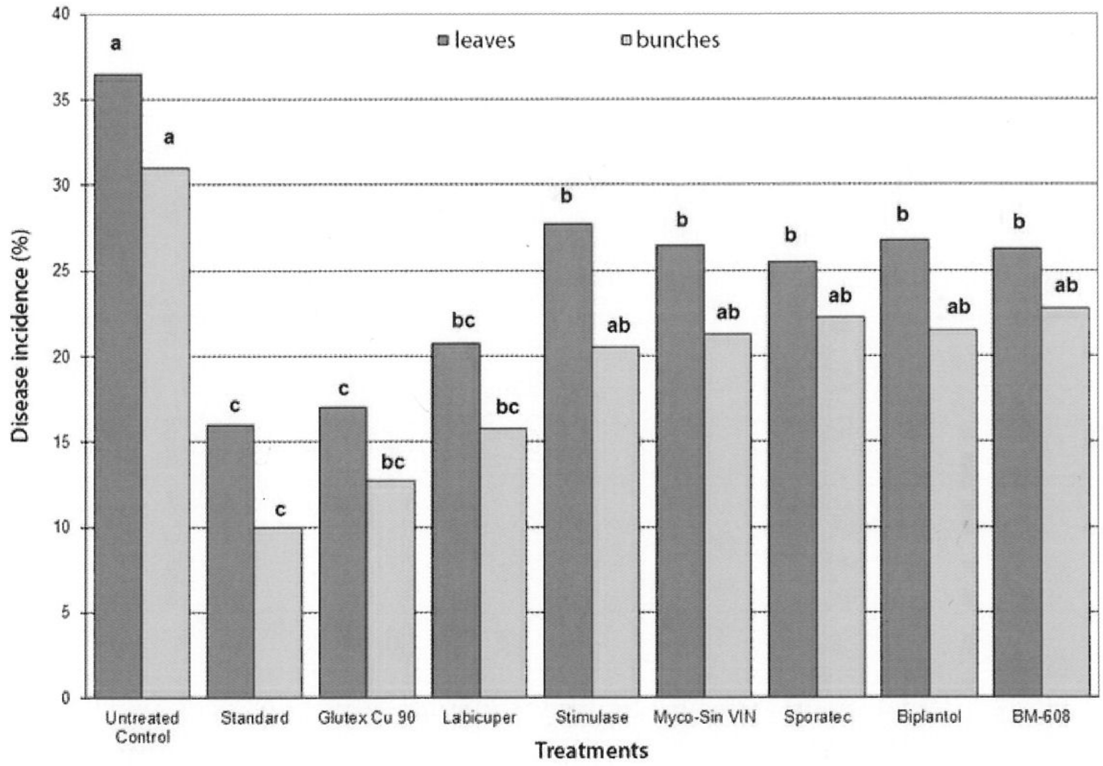

Figure 2. Disease incidence (expressed as % of infected leaves or bunches) at the harvest. Columns with the same letter are not significantly different according to Tukey's test ($P \leq 0.05$).

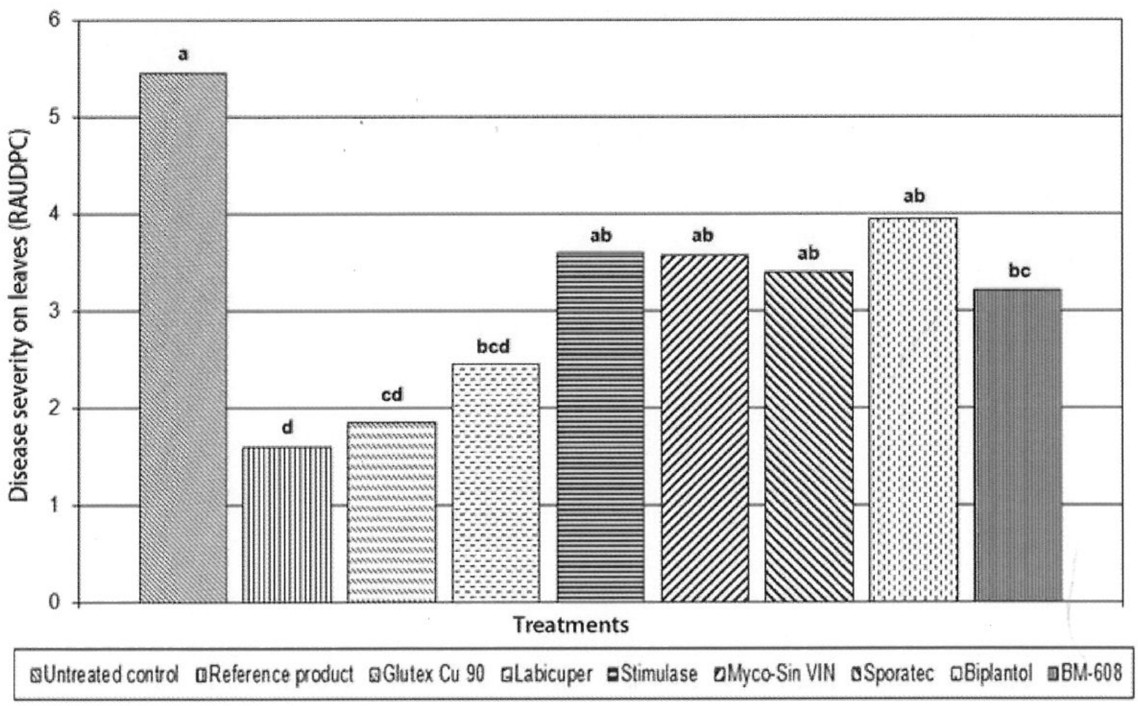

Figure 3. Downy mildew severity on leaves, expressed as relative area under the disease progress curve (RAUDPC). Columns with the same letter are not significantly different according to Tukey's test ($P \leq 0.05$).

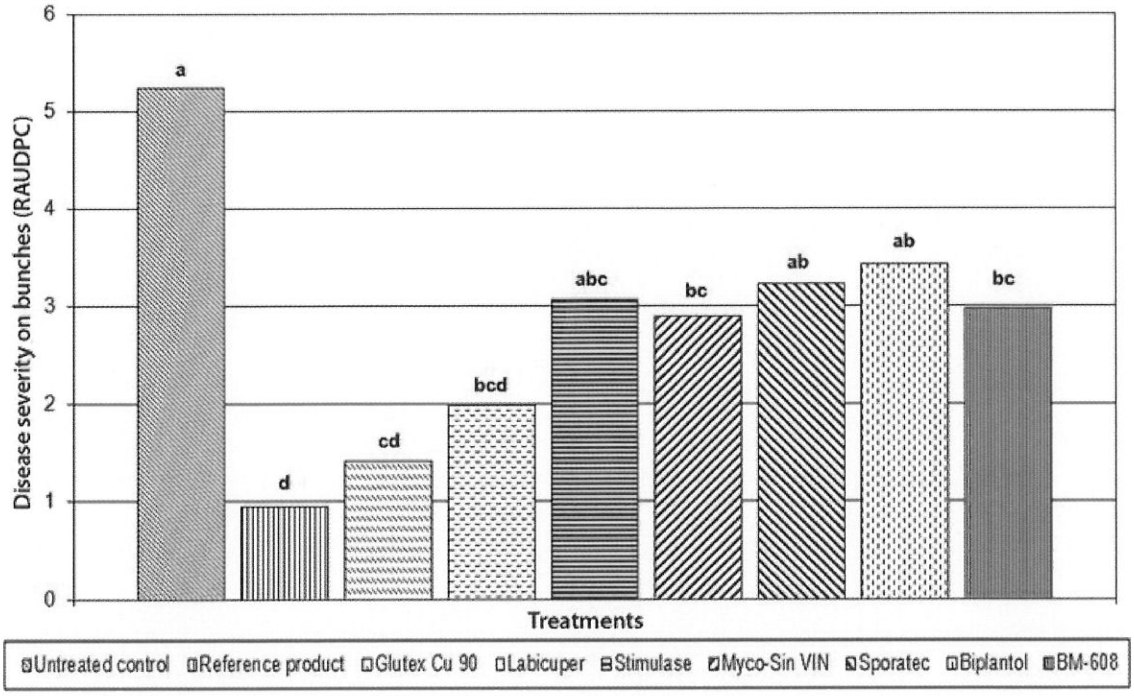

Figure 4. Downy mildew severity on bunches, expressed as relative area under the disease progress curve (RAUDPC). Columns with the same letter are not significantly different according to Tukey's test ($P \leq 0.05$).

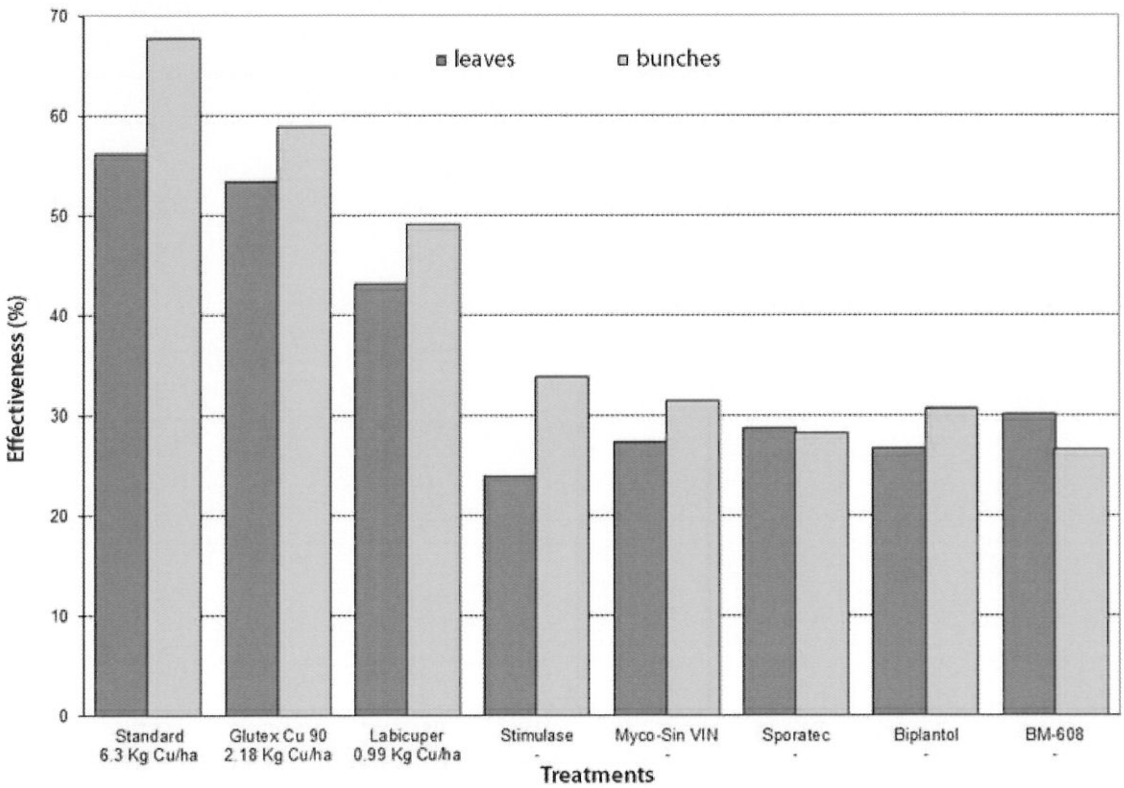

Figure 5. Effectiveness at the harvest of different products and total amount of copper applied with the treatments.

to the reference product but provided lower quantities of elemental copper (the total copper applied was about 2.2 kg/ha compared with approximately 6.3 kg/ha for the reference product). The total metallic copper applied with the Labicuper formulation was extremely low (0.99 kg/ha). Clearly, the quantities of elemental copper depend on the formulation used. Copper formulations used as reference product provided 482 g/ha of metallic copper per application, Glutex Cu 90 provided 200 g per ha per application, and Labicuper provided 99 g per ha per application. All non-copper based alternative products were essentially equally effective at harvest (Figure 5).

No visual signs of phytotoxicity were observed at any stage during this experiment.

Yield

Figure 6 shows the effect of different treatments on total yields (t/ha). The highest yields were obtained with reference product followed by the Glutex Cu 90 formulation. These two formulations were statistically different in comparison with the untreated control. There were also statistically different compared with the other tested products. Plants treated with Sporatec formulation BM-608 did not show significantly different results when compared with yields obtained with untreated control.

Conclusions

Under the experimental conditions of the trial, characterized by moderate disease pressure, all of the products tested gave an effective control of *P. viticola*. The best results were obtained with copper formulations, which were all very effective. The results showed that copper formulations (Glutex Cu 90 and Labicuper) were able to control grape downy mildew in the field using a third (Glutex Cu 90) or a sixth (Labicuper) of the amount of copper in comparison with the reference product (Figure 5). With the results obtained it was possible to identify alternatives to copper compounds that were less effective than copper but nevertheless showed an acceptable level of activity against *P. viticola* under medium disease pressure.

The overall conclusion from this study suggests that in order to minimize copper accumulation in the soil, flexible control

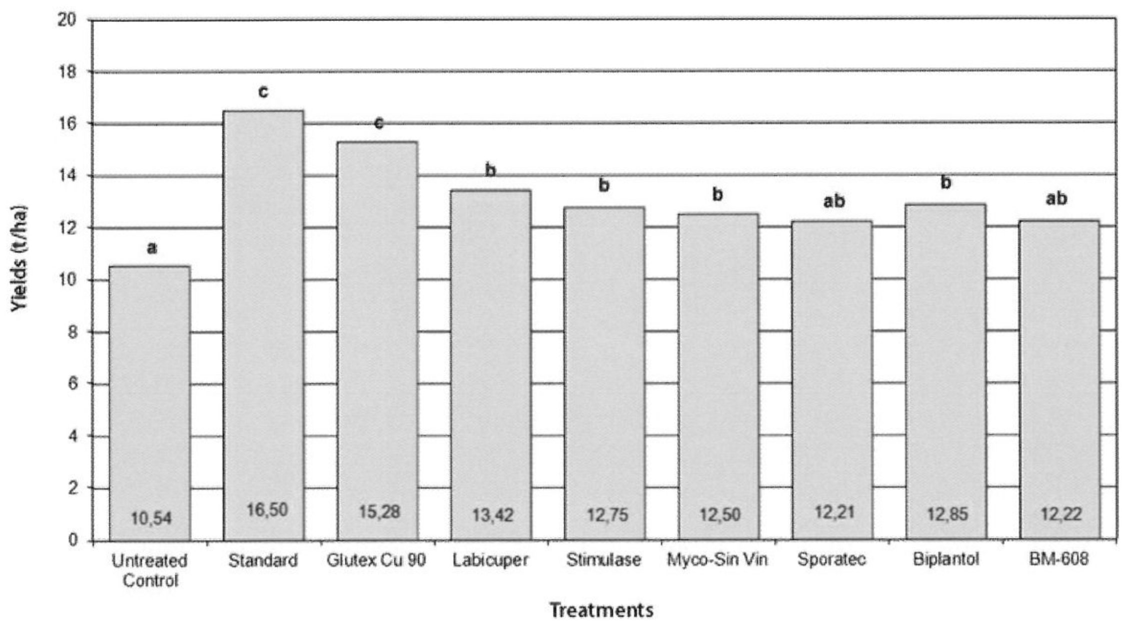

Figure 6. Effect of tested products on annual production: yields (t/ha). Values with the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

strategies should be adopted, which should be determined on the basis of the real risk of disease in the vineyard (20, 15). When downy mildew infection is low-intermediate, chemical control should include copper-alternatives and the use of copper compounds should be avoided. In this way the organic growers can economize on copper compounds under moderate infection pressure and to apply copper, using new improved copper formulations developed by agrochemical companies in order to help lower the total amount of copper, when *P. viticola* pressure is high. This approach has less of an environmental impact and is in accordance with EU restrictions on copper use.

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Χρήση φυσικών φυτοπροστατευτικών προϊόντων και σκευασμάτων με μειωμένη περιεκτικότητα σε χαλκό για καταπολέμηση του περονόσπορου σε βιολογικές καλλιέργειες αμπέλου

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Περίληψη Στα πλαίσια της φυτοπροστασίας, οι βιοκαλλιεργητές μπορούν να εφαρμόσουν μόνο προληπτικά καλλιεργητικά μέτρα σε συνδυασμό με χρήση μερικών εγκεκριμένων σκευασμάτων. Στη συγκεκριμένη μελέτη αξιολογήθηκαν σκευάσματα βιολογικής φυτοπροστασίας εναλλακτικά του χαλκού, που αποτελεί το κύριο μέσο καταπολέμησης του *Plasmopara viticola*, παθογόνου αιτίου του περονόσπορου της αμπέλου. Στο σύνολο των πειραμάτων τα χαλκούχα παρουσίασαν τα καλύτερα αποτελέσματα. Παρόλα αυτά, τα σκευάσματα ήταν αποτελεσματικά στο σύνολό τους στην περίπτωση ήπιων προσβολών και μάλιστα αυτά που περιείχαν μειωμένη περιεκτικότητα χαλκού σε σχέση με το σκεύασμα αναφοράς παρουσιάστηκαν το ίδιο δραστικά. Στη συγκεκριμένη ερευνητική μελέτη παρουσιάζουμε μια εναλλακτική μέθοδο φυτοπροστασίας- για τον περονόσπορο- προκειμένου να αντιμετωπιστεί το αυξανόμενο περιβαλλοντολογικό πρόβλημα της βιο-συσσώρευσης του χαλκού λόγω της συνεχής χρήσης του. Προτείνουμε τη χρήση σκευασμάτων εναλλακτικών του χαλκού σε περιπτώσεις ήπιας προσβολής και χρήση σκευασμάτων με χαμηλή περιεκτικότητα σε χαλκό όταν οι συνθήκες είναι ευνοϊκές για επιδημία.

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