PLURIENNAL TRIALS FOR THE CONTROL OF GRAPEVINE DOWNY MILDEW WITH NATURAL PRODUCTS

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Summary

Downy mildew, caused by *Plasmopara viticola*, is one of the most dangerous grapevine diseases. In organic vineyards, downy mildew is mainly controlled with copper compounds. However, due to its accumulation in soil, the use of copper could create problems of environmental impact. To this aim, the Commission Regulation (EC) N. 889/08 imposed severe standards to limit the use of metal copper per hectare per year. In order to minimize the use of copper or to find appropriate alternative solutions in line with the principles of organic farming, experimental trials have been conducted during five years of activity. In particular, this study has evaluated the anti-grape downy mildew activity of cupric products characterised by low metallic content (Glutex Cu 90 and Labicuper), natural products in association with low rate copper formulations (Brotomax and Biplantol), and natural products tested alone (Croplife, EcoMate Armicarb "0" and Biplantol). The experimental design adopted has been composed by randomized blocks with 4 replications and plots consisting of 12 plants. The assessments to evaluate the anti-grape downy mildew activity have been carried out every week on the 10 central plants of each plot. 100 leaves and 100 bunches per plot have been observed and it has been estimated the percentage of affected organs (disease incidence) and the percentage of the infected area (disease severity). Obtained data have been subjected to statistical analysis. These trials allowed to evaluate the effectiveness of different products against *P.viticola* under different conditions of epidemic pressure. In conditions of low and medium epidemic pressure, it is possible to achieve a satisfactory protection against *P.viticola* both with the use of copper compounds, or alternatives to copper-compounds; on the other hand, under a high pressure of disease, only copper compounds have been effective to control *P.viticola*. Finally, the outcomes of this study highlight the need to adopt flexible defensive strategies based on the seriousness of the disease.

INTRODUCTION

Grapevine downy mildew, caused by the fungus *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni, is one of the most potentially devastating diseases of *Vitis vinifera*. This disease can be extremely serious under humid climate conditions. In organic viticulture, the control of *P. viticola* is based on the use of copper compounds. Since the use of this heavy metal causes environmental problems, the European Commission fixed a limit of up to 6 kg per hectare per year of copper quantities in organic farming (Regulation EC N. 889/08). This ceiling is further reduced in Germany, Austria and the Czech Republic (Hofmann, 2002; Hofmann *et al.*, 2006; Keil *et al.*, 2008; www.organicrules.org) while the use of copper is not allowed for plant protection purposes in Denmark, Netherlands and Sweden (Speiser and Schmid, 2003; Trapman, 2010). European Commission will review copper authorisation for possible further restriction of allowable copper quantities in the light of new developments. In order to minimize the use of copper or to replace it with natural products, some research programs were carried out (Kühn and Friedrich., 2008; La Torre *et al.*, 2005; Sivčev *et al.*, 2010). This paper describes the field trials conducted in organic viticulture within the period 2006-2010.

MATERIAL AND METHODS

Field experiments have been performed in an organic vineyard located near Rome (central Italy). Data related to the farm are reported in Table 1.

Table 1. Trial Planning.

Years	2006-2007-2008-2009-2010
Location	Pavona (Rome, Italy)
Farm	Due Antichi Casali
Grapevine	Malvasia di Candia
Rootstock	Kober 5BB (*Vitis berlandieri* x *Vitis riparia*)
Year of planting	1966
Training system	Tendone
Plant distance (m)	2,5 x 2,5
Sperimental scheme	randomized blocks
Replicates	4
N. plants/plot	12
Spray machine	Electrostatic atomizer Martignani KWH
Year of conversion into organic	1989

The trials have been carried out according to the Guidelines EPPO/OEPP PP1/31 (3). In the experiments there were several treatments, including an untreated control and a farm reference product (Standard) that was managed totally by the owners following their usual approach, derived mostly by their experience. It has been evaluated the anti-grape downy mildew activity of cupric products characterised by low metallic content (Glutex Cu 90 and Labicuper), natural products in association with low rate copper formulations (Brotomax and Biplantol) and natural products tested alone (Croplife, EcoMate Armicarb "0" and Biplantol). Table 2 reports the characteristics of products tested during the 5 years of trials (2006-2010). Plots have been repeated 4 times in randomized blocks, where each plot contained 12 plants. The assessments to evaluate the anti-grape downy mildew activity have been performed on a weekly basis on the 10 central plants of each plot. 100 leaves and 100 bunches per plot have been observed. The percentage of affected organs (disease incidence) and the percentage of the infected area (disease severity) have been estimated. Disease severity (infection degree, ID) has been computed using a scale of nine classes

(0-8) with the Townsend-Heuberger formula (Towsend and Heuberger, 1943): ID (%) = Σ^{i}_{1} (ni x vi) / N x V (where vi is the damage class, ni is the number in one class, N is the total number, V is the highest class, i is the number of classes).

Data obtained have been subjected to statistical analysis using a parametric statistical method ANOVA and Tukey's test ($P \leq 0.05$) for quantitative variables (disease incidence).

For ordinal variables (disease severity), the individual antifungal activity differences of the products have been compared by using a Kruskal-Wallis test (a nonparametric test) with Dunn's post hoc test ($P \leq 0.05$).

The quantity of metallic copper applied with the different products during every year of activity has been calculated in kilogram per hectare.

Table 2. Characteristic of products tested during the 5 years of trials (2006-2007-2008-2009-2010).

Treatment		Composition	Year of activity
Standard	Cuprobenton DC blu	Copper oxychloride and copper sulphate	2006
	Cuproxat liquido Cuprobenton blu	Tribasic copper sulphate Copper oxychloride	2007
	Cuprobenton blu Zetaram 20 L	Copper oxychloride	2008
	Cuprobenton blu Cuproxat SDI	Copper oxychloride Tribasic copper sulphate	2009
	Cuproxat SDI Bentoram	Tribasic copper sulphate Copper hydroxide	2010
Labicuper		Copper gluconate	2009-2010
Glutex Cu 90		Copper hydroxide	2008-2009-2010
EcoMate Armicarb "0"		Potassium bicarbonate	2007-2008-2009
Brotomax + Copper	Brotomax Cupravit idro WG	Cu - Zn - Mn - N from urea Copper hydroxide	2006
	Brotomax Coprantol Ultramicron	Cu - Zn – Mn - N from urea Copper hydroxide	2007
	Brotomax Borboflow	Cu - Zn - Mn - N from urea Copper sulphate	2008
Croplife	Croplife	Citrofresh - Ethyl alcohol - Octanoic acid	2006-2007-2008
	Invigorator	N, P, P_2O_5, K, K_2O, Ca, Mg, Na, S, B, Fe, Mn, Cu^{++}, Zn, Mo, Co	
	Calcium carbonate	Calcium carbonate	
	Citofresh	Orange extract	2008
Biplantol + Copper	Biplantol agrar Biplantol mycos V forte	Minerals and microelements in homeopatic dynamic form (D6 – D200)	2007
	Cupravit idro WG	Copper hydroxide	
	Biplantol agrar Biplantol mycos V forte	Minerals and microelements in homeopatic dynamic form (D6 – D200)	2008
	Tepan 55 Cu	Copper sulphate pentahydrate	
Biplantol	Biplantol agrar Biplantol mycos V forte	Minerals and microelements in homeopatic dynamic form (D6 – D200)	2009-2010

RESULTS AND DISCUSSION

Results. The downy mildew attack has been influenced by the atmospheric conditions. The lowest disease pressure has been registered in 2006. During this year, weather conditions were not favourable for the development of *P. viticola.* Disease severity in the vineyard was very low and the symptoms were present on leaves but not on bunches. Figure 1 shows the results at the harvest. The best results have been obtained by the treatments that involved the use of Brotomax in association with copper hydroxide (Cupravit idro WG), though all tested products generated good results. Nevertheless, it is necessary to underline that the results are not very interesting because of the low infection pressure; therefore they don't permit to draw significant conclusions.

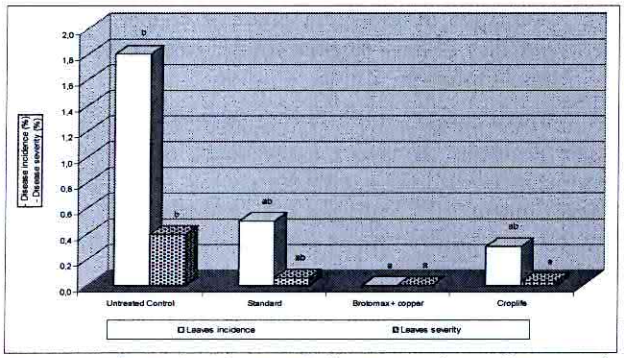

Figure 1. Disease incidence and disease severity on leaves at the harvest (2006).
Means with different letters on the top of each type of bar indicate significant differences between treatments according to Tukey test at $P \leq 0.05$ (disease incidence). Means with different letters on the top of each type of bar indicate significant differences between treatments according to Kruskal–Wallis test followed by Dunn's multiple comparison test at $P \leq 0.05$ (disease severity).

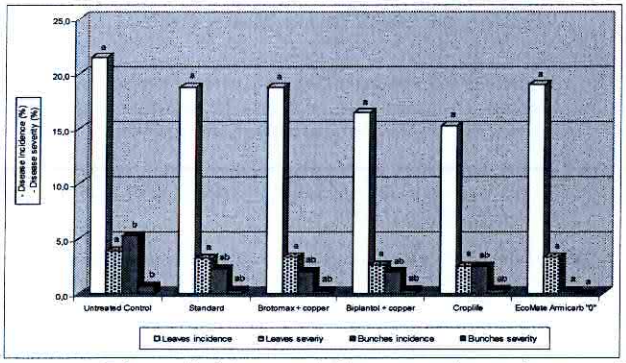

Figure 2. Disease incidence and disease severity on leaves and bunches at the harvest (2007).
Means with different letters on the top of each type of bar indicate significant differences between treatments according to Tukey test at $P \leq 0.05$ (disease incidence). Means with different letters on the top of each type of bar indicate significant differences between treatments according to Kruskal–Wallis test followed by Dunn's multiple comparison test at $P \leq 0.05$ (disease severity).

In 2007, the long rainy period of May, as well as the favourable temperatures for the development of *P. viticola,* caused the manifestation of the first symptoms at the end of the month on leaves. The symptoms occurred on bunches at the end of June. During the summer few rain events reduced the risk of infection. Figure 2 reports the results at the harvest. The best results on bunches have been obtained with potassium bicarbonate (EcoMate Armicarb "0") but all the products guaranteed a satisfactory anti-grape downy mildew difence.

The highest attack was in 2008 because of the specific weather conditions. Consequently, the disease pressure was extremely high. The best results have been obtained on standard farm reference. It is necessary to underline that not even copper formulations used as reference products guaranteed a good control of grape downy mildew. Unfortunately, it has been very difficult to carry out the treatments due to the unusually high number of rainy days and the intensity of the rain. The difficulty in applying the products at the right time (investigated products work preventively and are non-systemic) caused uncontrolled downy mildew development. Figure 3 reports the results at the harvest.

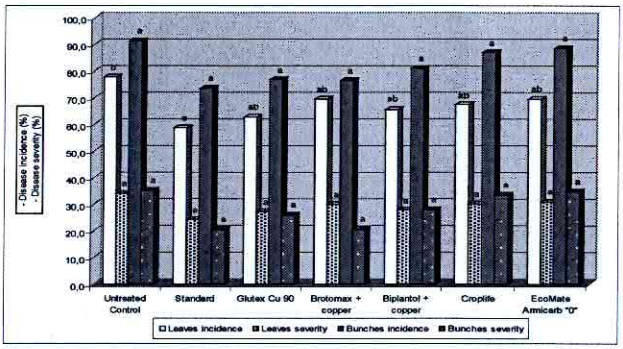

Figure 3. Disease incidence and disease severity on leaves and bunches at the harvest (2008).
Means with different letters on the top of each type of bar indicate significant differences between treatments according to Tukey test at $P \leq 0.05$ (disease incidence). Means with different letters on the top of each type of bar indicate significant differences between treatments according to Kruskal–Wallis test followed by Dunn's multiple comparison test at $P \leq 0.05$ (disease severity).

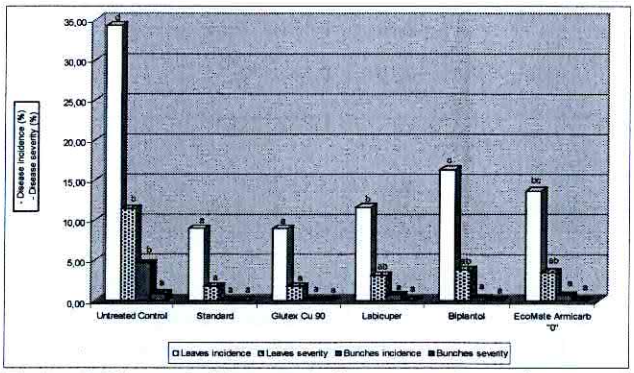

Figure 4. Disease incidence and disease severity on leaves and bunches at the harvest (2009).
Means with different letters on the top of each type of bar indicate significant differences between treatments according to Tukey test at $P \leq 0.05$ (disease incidence). Means with different letters on the top of each type of bar indicate significant differences between treatments according to Kruskal–Wallis test followed by Dunn's multiple comparison test at $P \leq 0.05$ (disease severity).

Years 2009 and 2010 were characterized by medium infection pressure. Figure 4 reports the data obtained in the 2009 at the harvest. The best results have been obtained

with copper formulations used as reference treatments (Cuprobenton blu and Cuproxat SDI) and with Glutex Cu 90 formulation. Nonetheless, all investigated products gave a good control of *P. viticola* and reduced the disease incidence and disease severity of grapevine downy mildew compared to the untreated control.

Figure 5 reports the results of the anti-downy mildew defence on leaves and bunches in the 2010. All investigated products gave a satisfactory protection against the disease and showed the statistically significant differences in comparison with the untreated control.

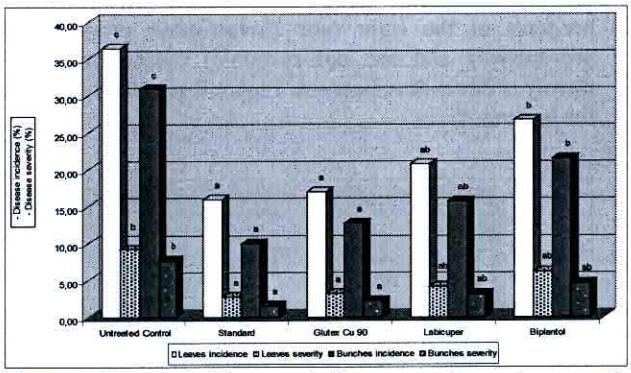

Figure 5. Disease incidence and disease severity on leaves and bunches at the harvest (2010).
Means with different letters on the top of each type of bar indicate significant differences between treatments according to Tukey test at $P \leq 0.05$ (disease incidence). Means with different letters on the top of each type of bar indicate significant differences between treatments according to Kruskal–Wallis test followed by Dunn's multiple comparison test at $P \leq 0.05$ (disease severity).

Table 3. Total amount of copper (kg ha^{-1} $year^{-1}$) provided by each treatment

Treatment	2006	2007	2008	2009	2010
Standard	3,35	5,53	5,88	4,53	6,27
Brotomax + Copper	2,19	2,42	2,58	-	-
Croplife	0,19	0,19	0,14	-	-
Biplantol + Copper	-	1,95	0,10	-	-
Labicuper	-	-	-	0,96	0,99
Glutex Cu 90	-	-	2,61	1,69	2,18

Table 3 reports the total amount of metallic copper (kg ha^{-1}) provided by each treatment during the 5 years of trials. The highest amounts of copper have been detected using reference products. Only during season 2010 (Table 3), reference treatments exceeded 6 kg per ha, that is the maximum amount of copper allowed in organic farming whitin the European Union. Nevertheless, Regulation EC N. 889/08 gives the possibility to apply this ceiling on an average basis over the 5 years for perennial crops. It is therefore possible to use a higher amount of copper in the years characterized by a high disease pressure and to reduce the copper when disease pressure is low or intermediate. No symptoms related to the presence of phytotoxic effects have been noted on leaves or bunches during the five years of trials.

Discussion. During the five years of field trials, it has been evaluated the activity against *P. viticola* of different products under high, medium and low disease pressure. The results showed that:

- Under high infection pressure of grape downy mildew, which was present in the 2008, none of the tested products gave a very good protection. The best results have been obtained with copper formulations used as reference products. Glutex Cu 90 performed slightly worse than the reference products while natural products gave an insufficient control of disease.
- Under medium and low infection pressure (2006-2007-2009-2010 field trials) all investigated products showed a good control of grape downy mildew. Therefore, when disease pressure is low or intermediate, organic vine-growers should use, other than the preventive measures, natural products alternative to copper in place of copper compounds, and they should reserve copper treatments when the risk of infection is high.

These considerations should guide organic growers in their decisions on disease management in order to reduce the copper when the conditions are not favourable for the development of *P. viticola* and to apply copper, also in excess of 6 kg per hectare per year, in situations with high infection pressure.

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