NATURAL PRODUCTS ALONE OR WITH COPPER VS. GRAPE DOWNY MILDEW: EFFICACY, COSTS, Cu IMPACT

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SUMMARY

In organic farming copper is one of the few fungicides that can be used and the only effective against downy mildew.
Due to the problems of environmental impact associated with the use of this heavy metal, the European Union decided to restrict its use (Annex II Regulation EC n. 889/2008). In order to assess the possibility of replacing the copper or reduce the quantities used, we tested natural products and low rate copper formulations in field trials.
The anti-downy mildew activity of these formulations has been studied in an organic vineyard. During the two years of activity we also estimated the economic cost of use of the products to assess their possible use in agricultural practice.

The examined products were:

- homeopathic products (Biplantol), tested either alone or in combination with a cupric formulation;
- potassium bicarbonate (Armicarb);
- copper hydroxide at low copper rate (Glutex).

The anti-downy mildew activity of the products tested was compared with an untreated control thesis, in order to follow the course of infection and a Standard farm reference thesis, where the treatments were carried out according to the usual farm procedures.
The results showed that under conditions of high risk of infection, only the cupric formulations provided a reasonable anti-downy mildew protection. However at medium epidemic pressure conditions even the substances of natural origin explicated a satisfactory anti-downy mildew defence, comparable with the one obtained by the use of copper formulations.
During the first year of activity the lowest economic cost at the end of the agricultural cycle was obtained with Glutex formulation treatments.
At the end of the second year of activity, cupric formulations used on the Standard thesis ensured the lower cost; little more expensive Armicarb and Biplantol theses.
Regarding the cost-effectiveness ratio in the two years of activity the cupric formulations used on the Standard thesis shown the best result. In the second year of testing, also the other formulations tested showed a good cost-effectiveness ratio.
Moreover we have carried out analytical determinations to evaluate the impacts caused by applied copper on soil, grapes and leaves. On the plant surface, persistence of copper contained in some different formulations has been studied, too.

INTRODUCTION

Few plant protection products are allowed in organic farming and they usually are less effective than chemical pesticides. Copper and sulphur are the most usable products and in particular, with regard to copper compounds, the European Community has establishedthe maximum level of metal copper that can be used per hectare per year. During the years 2008 and 2009, two field trials have been carried out in an organic vineyard in order to estimate the effectiveness, against *Plasmopara viticola* (Berk. *et* Curt) Berl. *et* De Toni, either of copper compounds at a low rate and alternative copper products. The costs of products have also been computed in order to evaluate their use in farming practice. Finally, chemical analyses have been carried out to determine the copper residues in the soil, leaves and grapes.

MATERIALS AND METHODS

The trials have beenperformed in an organic vineyard, near Rome, in central Italy. All data concerning the vineyard can be found in Table 1. Table 2 reports the characteristics of anty-downy mildew products tested in the trials. The trials have been carried out according to the European and Mediterranean Plant Protection Organization Standard PP1/31(3). The anti-downy mildew activity of the products has been compared either with an untreated control thesis, in order to follow the course of infection and with a Standard farm reference thesis, where the treatments were carried out according to the usual farm procedures.
We have constantly monitored the vineyards and the disease assessments, to evaluate the effectiveness of treatments on leaves and bunches.The assessments have been carried out periodically on 100 leaves and 100 bunches for each replicate, randomly identified on the central row of each plot. We computed the percentage of affected organs (diffusion), the infection percentage index (I.%I.) according to the Townsend-Heuberger formula, and the efficacy index according to Abbott's formula. The obtained data, after arc sin transformation, has been evaluated by the Tukey test. The amount of metallic copper applied per hectare with the different products has been calculated.

Table 1. Layout of the trial

Year of activity	2008-2009
Location	Pavona (Rome, Italy)
Farm	Due Antichi Casali
Grapevine	Malvasia di Candia
Year of planting	1966
Training system	Tendone
Rootstock	*Vitis berlandieri x riparia* Kober 5BB
Planting space (m)	2,5 x 2,5
Experimental design	Randomized blocks
N° of replicates	4
N° plants / plots	12
Sprinkling machine	Electrostatic Sprayer Martignani KWH
Year of conversion to organic farming	1989

Table 2. Characteristics of the anti-downy mildew products tested during the two-years trials

Theses	Active Ingredient	Product	Year of Activity	Dose of Formulation (ml-g/hl or l/ha)	Dose of Cu^{++} (g/hl)
Standard (Ref. prd.)	Copper (from copper oxychloride)	Cuprobenton blu	2008	400-500-600	56-70-84
	Copper (from tetracopper chloride hydroxide)	Zetaram 20L		300-350-400	90-105-120
	Copper (from copper oxychloride)	Cuprobenton Blu	2009	4000	560
	Copper (from tribasic copper sulphate)	Cuproxat SDI		2.5	487.5
Glutex	Copper (from copper hydroxide)	Glutex Cu 90	2008 2009	400-450 425-450	36-40.5 38.25-40.5
Biplantol	Complex of active agents in homeopathic dynamized form (D6 - D200)	Biplantol agrar	2008	1	0
			2009	1	-
	Minerals in homeopathic and dynamic form (D6 - D200)	Biplantol mycos V forte	2008	1	0
			2009	1	-
	Copper (from Pentahydrate copper sulphate)	Tepan 55 Cu	2008	75-100	4.5-6
Armicarb	Potassium bicarbonate	Eco Mate Armicarb "O"	2008 2009	600 500-600	0 0

Economic evaluations of products were made.
The chemical analyses have beenperformed to determine the copper residue in soil, leaves and grapes. The sampling, in numbers of four, has been carried out for every replicate of every thesis. The total copper has been determined in the soil, leaves and grapes by means of spectrophotometry in atomic absorption by acetylene-air flame (FAAS at λ = 3224.8) after mineralisation with microwave oven for the leaves; with regard to the soil, it was determined according to the “D.M.” of 13/ 09/ 99.

RESULTS

The results of the trials, conducted in the two-years activity, are reported in Table 3.
2008 has been an extremely favourable year towards a development of grape downy mildew. The reference product has guaranteed the best results. The defence realised in the Glutex thesis resulted to be slightly inferior to the reference product. The other tested products have highlighted a more modest protection.
The second year of activity (2009)has been characterized by a medium pressure of *P. viticola*.Reference product and Glutex showed the best results. The theses treated with Biplantol and Armicarb have shown good results too. All the theses showed significant statistically difference with the untreated control.
Table 4 reports the total number of carried out treatments, the costs of applications of tested products and cost/effectiveness ratio.
In the first year of activity (2008), copper compounds, used on Standard thesis, showed the less cost/treatment ratio but a high number of applications (12) has been done on this thesis; then, at the end of agricultural cycle, the cost was high (1422,06 €/ha). Glutex formulation realised the less overall cost.
In 2009Standard thesisshowedthe lesscostat the end of agricultural cycle (1.121 €/ha).
The best cost/effectiveness ratio was realized with copper compounds used on Standard thesis both in 2008 and in 2009.
Figure 1 and Figure 2 reportthe quantity of metal copper, expressed in kilogramme per hectare, distributed by the treatments during the first and the second years of activity.

With regard to the impact of treatments with cupric product on the soil of the vineyard, samplings of soil have been carried out in two successive periods in order to highlight the possible accumulation of metal within experimental annuity (2009's trial): the first one preceded the start of treatments of the second year of experimentation, while the second one has been made several months after the end of this experimentation; samplings have also been conducted on the soil of theses untreated with copper for control purposes. The Average results for the 3 replicates of each of 4 thesis plots are shown in Table 5.

The results of the total copper on leaves are shown in the Table 6.

In addition, for the Standard and Glutex, an average value (R_m) has been estimated over the all period (from first leaves collection to the third one). Moreover, the total amount of copper distributed(D_t) over relative thesishas been calculated for the same thesis; the values of the ratio R_m/D_thave also been calculated. The results of these evaluations are shown in Table 7.

The results of copper determinations on bunches are reported in the Table 8.

Table 3. Assessment at the harvest on leaves and bunches (I^{st} and II^{nd} year of activity)

Theses	I^{st}Year of Activity 22/09/08						Theses	II^{nd}Year of Activity 08/09/2009					
	LEAVES			BUNCHES				LEAVES			BUNCHES		
	Dif.%	I.%I.	Eff.%	Dif.%	I.%I.	Eff.%		Dif.%	I.%I.	Eff.%	Dif.%	I.%I.	Eff.%
Untreated Control	77.8b	34a	-	91.3b	35.3a	-	Untreated Control	34.3c	11.5c	-	4.7b	0.9b	-
Standard (Ref. prd.)	58.8a	24.3a	24.4	73.5a	20.5a	19.4	Standard (Ref. prd.)	9a	1.8a	73.8	0a	0a	100
Glutex	62.8ab	27.3a	19.3	76.8ab	26a	15.9	Glutex	9a	1.8a	73.8	0a	0a	100
Biplantol + copper	65.5ab	28a	15.8	81ab	28a	11.2	Biplantol	16.3b	3.9b	52.4	0.3a	0.1a	92.9
Armicarb	69.5ab	30.8a	10.6	88.5ab	34.8a	3	Armicarb	13.7b	3.5b	60.2	0.7a	0.1a	85.7

Different letters indicate significant different values by Tukey test for $P \leq 0.05$

Table 4. Treatments costs and ratio cost/effectiveness

Theses	Product	Year of Activity	N. of Treat-ments	Overall N. of Treatments	Cost to Treatments (€/ha)*	Overall Cost (€/ha)*	Ratio Cost/Eff. Leaves	Ratio Cost/Eff. Bunches
Standard	Cuprobenton blu Zetaram 20L	2008	9 3	12	118.51	1422.06	58.3	73.3
	Cuprobenton Blu Cuproxat SDI	2009	2 7	9	124.60	1121.41	15.2	11.2
Glutex	Glutex Cu 90	2008 2009	10 8	10 8	135.43 172.04	1354.30 1376.32	70.2 18.7	85.2 13.8
Biplantol + copper	Biplantol agrar Biplantol mycos V forte Tepan 55 Cu	2008	6 6 4	15	119.17	1787.49	113.1	159.6
	Biplantol agrar Biplantol mycos V forte	2009	6 6	12	111.79	1341.48	25.6	14.4
Armicarb	Eco Mate Armicarb "O"	2008 2009	10 8	10 8	249.37 165.58	1928.50 1324.64	235.3 22	831.2 15.5

*The costs shown derived from the sum of total costs per hectare (worker, tractor, maintenance fuel, plant protection product)

Note:

1. We considered that a vineyard both row crop that tendone to media intensity of planting requires a commitment of 1.3 hours/ha for performing fungicide treatments with mist-blower.
2. We considered a gross hourly cost for application of fungicide treatments (excluding the cost of products) of € 58.70 per hour (deducted from the regional tariff-agricultural mechanic working in force in Italy since 1 March 2007).
3. The cost of the anti-downy mildew products derived from the current retail prices.

Table 5. Increase in soil of distributed copper (before and afterIInd year of activity); residue expressed in mg/kg (Cu/dry weight 2mm sieved soil)

Theses	0 to 20 cm*		20 to 40 cm*	
	17.02.2009**	03.03.2010**	17.02.2009**	03.03.2010**
Untreated Control	182	195	162	178
Standard (Ref. prd.)	181	214	154	176
Glutex	175	207	183	195
Biplantol + copper	200	211	198	202
Armicarb	186	200	174	187

*sampling depth
**sampling date

Table 6. Persistence of distributed copper in leaves (I[st]and II[nd] year of activity); residue expressed in mg/kg (dry weight)

THESES	I[ST]YEAR OF ACTIVITY (2008) 06.06.2008 (fruit set)	11.08.2008 (veraison)	18.09.2008 (HARVEST)	THESES	II[ND]YEAR OF ACTIVITY (2009) 03.06.2009 (fruit set)	29.07.2009 (berry touch)	24.09.2009 (AFTER HARVEST)
Untreated Control*	25 a	37 a	31 a	Untreated Control*	52 a	72 a	19 a
Standard (Ref.prd.)	272 c	1237 c	869 c	Standard (Ref.prd.)	495 b	968 c	381 c
Glutex	143 b	532 b	437 b	Glutex	122 a	246 b	196 b
Biplantol + copper	50 a	76 a	61 a	Biplantol*	33 a	60 a	46 a
Armicarb*	32 a	58 a	49 a	Armicarb*	46 a	42 a	27 a

*copper presence due to not avoidable moderate distribution spread in field trials
Different letters indicate significant different valuesby Duncan test for P≤0.05

Table 7. Parameters relative copper persistence on leaves

Theses	Year of Activity	R_m (mg/kg Cu)	D_t (g/ha Cu)	R_m/D_t (10 m^2/kg)
Standard (Ref.prd.)	2008	876	5880	0.145
	2009	702	4532	0.155
Glutex	2008	395	2610	0.151
	2009	203	1691	0.120

Table 8. Determinations of copper on bunches (I[st]and II[nd] year of activity); residue expressed in mg/kg (dry weight)

Theses	18.09.2008 (harvest)	24.09.2009 (harvest)
Untreated Control *	6 a	3 a
Standard (Ref.prd.)	14 b	9 ab
Glutex	9 ab	6 ab
Biplantol + copper	9 ab	4 a
Armicarb*	5 a	3 a

*copper presence due to not avoidable moderate distribution spread in field trials
Different letters indicate significant different values by Duncan test for P≤0.05

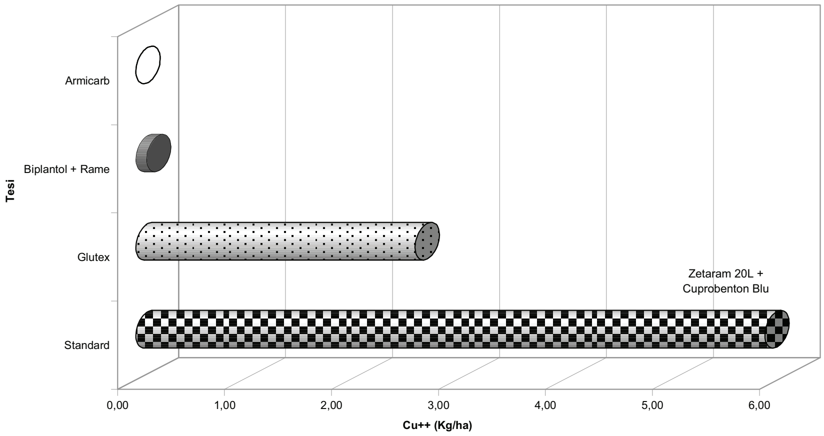

Figure 1. Quantity of metal copper (kg/ha) distributed by the treatments (I[st] year of activity)

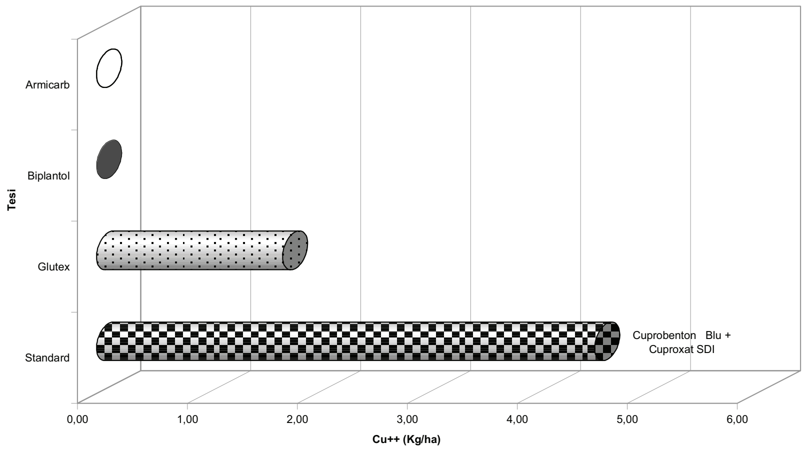

Figure 2. Quantity of metal copper (kg/ha) distributed by the treatments (II[nd] year of activity)

DISCUSSION

In two-years field trials we tested the effectiveness of different formulations in condition of high and medium pressure of *P. viticola*.
Both the copper compounds and the natural products were able to guarantee a satisfactory protectionin condition of medium pressure of downy mildew (2009). In 2008, due to the high pressure of *P. viticola*, only the copper reference product

performed an adequate protection while the alternative products were not effective.
The economic evaluations showed that the lowest overall cost was obtained with Glutex formulation in 2008 and with copper compounds used on Standard thesis in 2009.
The best cost/effectiveness ratio was obtained with copper compounds (Standard) both in 2008 and in 2009 (Table 4).
Glutex guaranteed good effectiveness in the control of grape downy mildew with a low contribution of metal copper.
During the trial, the phitotoxicity phenomenon was not highlighted on the plants of the considered thesis.
With regard to the quantitative presence of copper in soil (Table 5), we observed an apparent annual increase in copper contamination for all the theses, included those not directly treated with cupric products: possible existence of phenomena of random drift of metal during distributions must be considered. The values are averaged over sets of 9 determinations presenting a remarkable relative standard deviation. As a consequence, all the observed increases were not statistically significant at Duncan's test for $P \leq 0.05$. This can be also valid for Standard and Glutex theses,although the 18% increase over experimental annuity has been calculated (sampling depth: 0 to 20 cm). Then, it would be needed to extend this accumulation analysis over for more years with a greater number of representative samples for thesis in order to have a better knowledge about the behaviour of copper in the vineyard.
For the persistence of the metal on leaves (Table 6),it can be observed that a maximum accumulation of Cu has been reached in the medium term: this could be caused by different frequencies of treatments and by the effect of rainfall during the culture cycle, as well by chance in leaf area. When comparing the values of the ratio R_m/D_t (Table 7), it can be highlighted a different indication of percentage of deposition and persistence of applied foliar copper.
For the year 2008,the calculated R_m/D_t value, both for Standard and Glutex, was comparable; for the following year,this value has resulted to be quite constant only forthe first thesis, while the ratio was significantly lower for the second one.This indicates aninferior deposition and/or persistence of copper on the leaves of Glutex during year 2009. At harvest in all theses (Table8), the copper residual values on grapes were within the limits allowed by law (50 mg/kg).

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