GRAPEVINE DOWNY MILDEW CONTROL USING REDUCED COPPER AMOUNTS IN ORGANIC VITICULTURE

A. LA TORRE, V. POMPI, C. MANDALÀ and C. CIOFFI
Agricultural Research Council (CRA), Plant Pathology Research Center
Via C.G. Bertero 22, IT-00156 Rome, Italy
Correspondingauthor E-mail: anna.latorre@entecra.it

SUMMARY

Copper is an essential natural micronutrient. However, copper used as a plant protection product may have long-term consequences due to its accumulation in the soil. Limitations on copper use have therefore been defined in organic farming (Regulation EC 889/2008). In the light of new developments and evidence, the European Commission has planned to assess whether further restrictions are needed in the quantities of copper permitted.
A two-year field trial was therefore set up with new copper formulations to evaluate the possibility of reducing the copper quantities applied with treatments and consequently to reduce copper soil residues. Plots were prepared, each containing 12 plants and repeated four times in randomized blocks. The test organism was *Plasmopara viticola* (Berk. and M.A. Curtis) Berl. and De Toni. Cupric formulations characterised by a low metallic content (Glutex CU 90 and Labicuper) were tested in comparison with a reference product (standard) andan untreated control. Evaluations of treatments were carried out periodically on 100 leaves and 100 bunches for each replicate. Data obtained were subjected to statistical analysis. Chemical analyses were performed to determine copper residues on leaves, grapes and soil. Samplings of leaves and grapes were carried out for eachreplicate. Soilsamples were taken from 0-20 cm and 20-40 cm depth. Total copper was determined using spectrophotometry in atomic absorption by acetylene-air flame (FAAS at λ = 324.8 µm).
The results showed that the tested productswere effective in controlling downy mildew with a lower copper dosage than with the cupric formulations used as astandard. Glutex CU 90 formulation led to an annual input of copper that was a little more than a third comparedto the standard and Labicuper about a fifth or a sixth.
At harvest, copper levels in grapes were much lower than RML (fixed at 50 mg/kg). With regard to the impact of cupric treatments on organic vineyard soil, no statistically significant differential increase in Cu residue was observed in soil between tested products versus untreated control.
In conclusion, the environmental impact of copper in organic viticulture could be minimized through the new cupric formulations developed by agrochemical companies.

Key words:copper formulations, grapevine downy mildew, organic farming, copper residues

INTRODUCTION

Copper is an essential natural micronutrient (Van Assche and Clijsters, 1990; Harrison *et al.,* 1999). However, when copper is used as a plant protection product this may have long-term consequences due to its accumulation in the soil(Geoffrion, 1975; Woolhouse and Walker, 1981; Balsberg-Pahlson, 1989; Rhoads *et al*., 1989; Deluisa *et al*., 1996; Giller *et al*., 1998; Moolenaar and Beltrami, 1998; Brunt *et al*., 2003; Scaglione *et al*., 2008). The EU Commission Directive 2009/37/EC included copper compounds in Annex 1 of the Directive 91/414/EEC (concerning the placing of plant protection products on the market) but established that the status of copper compounds should be reviewed in the light of any new data becoming available. Directive 2009/37/EC stipulated that “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". In organic farming the conditions for the use of copper are restricted by fixing a ceiling of permissible copper amounts to a maximum of 6 kg per hectare per year (Regulation EC 889/2008). This ceiling has been reduced even further in Germany, Austria and the Czech Republic (Hofmann, 2002; Hofmann *et al*., 2006; Keil *et al*., 2008) and the European Commission plans to implement further restrictions should there be any new evidence.

MATERIALS AND METHODS

The trials were carried out over a two-years period (2009–2010) in accordance with the EPPO/OEPP PP1/31 (3) guidelines in an organic vineyard located near Rome, Italy. Plotscontained 12 plants and repeated four times in randomized blocks. In order to avoid products drifting from one plot to another, each plot was separated from its neighbour by a row of untreated plants. Data related to the farm are reported in Table 1.The test organism was *Plasmopara viticola* (Berk. and M.A. Curtis) Berl. and De Toni. A weather station was placed at the trial site to record weather data.

We compared low metalliccupric formulations (Glutex CU 90 and Labicuper) with a reference product (standard) and an untreated control.Table 2 reports the characteristics of products investigated. In order to evaluate how well the products performed, the responses of the plants to downy mildew disease were analysed regularly at each growth stage (BBCH-identification keys of grapevine), following the developmental scale described by Lorenz *et al*. (1994). Assessments were carried out on 100 leaves and 100 bunches for each replicate, identified at random in the central row of each plot. The percentage of diseased leaves and bunches(disease incidence), the percentage of leaves and bunch areas showing symptoms of disease (disease severity) were all computed using a scale of 9 classes (0-8) according to the Townsend-Heuberger formula (Townsend and Heuberger, 1943) and the index effectiveness according to Abbott (Abbott, 1925).

In addition, analytical determinations were conducted to evaluate the residual levels of copper on the leaves, bunches and soil (Pietrzak and McPhail, 2004; Gessa and Ciavatta, 2005). Leaf sampling was carried out (10 leaves per replicate) on the followings dates: 17 June (BBCH 75 – Berries pea-sized, bunches hang), 23 July (BBCH 77 – Berries beginning to touch) and 9 September (BBCH 89 – Berries ripe for harvest). The leafsamples were taken to the laboratory, and submitted to desiccation in a thermo-ventilated heater to 38°C, for at least 48 hours.Dried leaves were then mineralized (micro-wave apparatus) with a chloronitric mixture. Total copper determination was carried out using Flame Atomic Absorption Spectrometry (FAAS) at awavelength of 324,4 nm.

At harvest a sampling of bunches (3 kg of bunches per replicate) was also taken. From every sample of bunches, 100 grapes were removed.This reduced sample was homogenized, mineralized, and submitted to analogous determination.

The soil samplings were carried out in two different periods: before the execution of the first year of treatment with cupric agrochemical products (March 2009) and around four months after the harvest of the second year (January 2011).Soil samples were submitted to desiccation in air for at least 3-4 days; reduced and then ground and sieved to a diameter of 2 mm.Mineralization was finally performed according to opportune parameters (thermal and timing cycle process) for soil samples. On the samples processed, copper determination was carried out by atomic absorption.

Statistics were performed using GraphPad InStat version 3.00 for Windows. Data recorded as percentages were arcsine transformed. Data were analysed using the parametric statistical method ANOVA and Tukey's test (P ≤ 0.05) for quantitative variables (disease incidence). For ordinal variables (disease severity) individual antifungal activity differences in products were compared by a Kruskal-Wallis test (a nonparametric test) with Dunn's post hoc test (P ≤ 0.05). For analytical determinations Duncan's multiple range test (P ≤ 0.05) was used.

RESULTS

Figures 1 and 2 show the climatic conditions registered in 2009 and 2010 respectively. Figure 3 reports the results of the trial at the 2009 harvest. Figure 4 reports the results of the trial at the 2010 harvest. The results from the two field trials showed that the products were effective in controlling downy mildew with fewer amounts of copper than in the cupric formulations used as astandard. Glutex CU 90 guaranteed almost the same efficacy level as the standard witharound a third of the quantity of copper compared to the standard. Labicuper wasslightly less effective than thestandard,with around a fifth or a sixth of the copper quantity compared to the standard (Figures 5 and 6). Although Glutex CU 90 is authorized for use in Italy, Labicuperis marketed as a foliar fertilizer and has not been authorized as plant protection product. No symptoms related to the presence of phytotoxic effects were noted on the leaves or bunches during the field trials.

The results relating to the analytical determinations of copper on leaves and bunches are reported in Table 3. Each value is the average of four replicates. The different treatments carried out with different cupric formulations showed quite a good degree of correlation (although not strictly linear) among the levels of copper residues on leaves and the quantities of metal contained in all the cupric formulations. The standard showed a higher peakthan 400 mg/kg on leaves, and Labicuperpeakedat about 200 mg/kg. Note that for Glutex Cu 90 there wasan anomalous datum in the second sampling (23 July) which gave a residue value about 300 mg/kg. It is not surprising to find a minimum value of cupric deposit (20-30 mg/kg) also on the leaves of the untreated control. These residue values are attributed to the inevitable drift during the application of products. Table 3 reports the residual trendsat three sample periods, the maximum value for all treatments is around the period when theberries are ripe for harvest (BBCH 89). Copper residues persisted until harvest time. This is the result of the balance over time of the total quantities of copper applied to the vineyard by treatments versus the treatments washed away by rains.The extent of this removal depends both on the intrinsic chemical and physical characteristics of the products and obviously on the frequency and intensity of the seasonal precipitations (during July-September 2010 there were very few precipitations).

The residual values determined on bunches at harvest were clearly tightly correlated to those found on leaf samples collected on the same day. However, the average residue on the grapes for the standard (the highest one being approximately 18 mg/kg) is well below the Maximum Residue Level (MRL= 50 mg/kg) admitted on grapes by law (Commission Regulation EC No 149/2008).

The results of the copper residues determined in the vineyard soil related to the 0-20 cm horizon are reported in Table 4. In order to verify possibleaccumulation during the two productive seasons, comparing different treatments (horizontal test), Table 4 highlights that only standard residues increased significantly in January 2011 compared to the March 2009 sampling.The vertical test (Table 4) showed that non-homogeneous cupric contaminations in the upper 20 cm soil horizon were found before the field trials started (March 2009). Contamination of the lower soil horizon(20-40 cm depth) was generally higher than in the upper level, however the residue values showed a minor variability. This paper does not report copper residual data determined in20-40 cm soil horizon because residues did not increase significantly after two years of trials even when standard was distributed.

Table 1. Layout of the trial

Years	2009-2010
Locality	Pavona (Rome, Italy)
Farm	Due Antichi Casali
Cultivar	Malvasia di Candia
Year of planting	1966
Training system	Tendone
Rootstock	*Vitis berlandieri* x *vitisriparia* Kober 5BB
Plant distance (m)	2,5 x 2,5
Experimental scheme	Randomized blocks
N° of replicates	4
N° plants per plots	12 on single rows
Mode of application	Electrostatic Sprayer Martignani KWH
Year of conversion to organic farming	1989

Table 2. Characteristics of tested products

Treatment	Copper compounds	Composition	Cu concentration (g/L or %)	Year of activity	Rate of application (mL/hL or g-L/ha)
Standard	Cuproxat SDI	Tribasic copper sulphate	195	2009	2,5
	Cuprobenton blu	Copper oxychloride	14		4000
	Cuproxat SDI	Tribasic copper sulphate	195	2010	3
	Bentoram	Copper hydroxide	115		2-3-4
Glutex Cu 90	Glutex CU 90	Copper hydroxide	90	2009	425-450
				2010	400-425
Labicuper	Labicuper	Copper gluconate	80	2009	250-300
				2010	200-250

Table 3. Copper determinations on leaves and bunches

Treatment	Sampling 17/06/2010 (BBCH[#] 75)		Sampling 23/07/2010 (BBCH 77)		Sampling 9/09/2010 (BBCH 89)		Sampling 9/09/2010 (BBCH 89)	
	Leaves		Leaves		Leaves		Bunches	
	mg/kg (dry weight)	*	mg/kg (dry weight)	*	mg/kg (dry weight)	*	mg/kg (homogenized)	*
Untreated Control	18	a	20	a	28	a	5	a
Standard	157	c	328	c	428	d	18	c
Glutex Cu 90	167	c	306	c	295	c	13	b
Labicuper	101	b	84	b	203	b	11	b

* Vertical test - Means with different letters are statistically different (Duncan P ≤0,05)
[#] Phenological growth stages and BBCH-identifications keys of grapevine: 75–berries pea-sized, bunches hang; 77-berries beginning to touch; 89-berries ripe for harvest

Table 4. Copper determinations (average of 12 replicates) in vineyard soil

(**)

Treatment	Sampling 4/03/2009		Sampling 19/01/2011	
	Soil (0-20 cm)		Soil (0-20 cm)	
	mg/kg (dry weight)	*	mg/kg (dry weight)	*
Untreated Control	230	b(a)	222	ab(a)
Standard	213	a(a)	255	c(b)
Glutex Cu 90	237	b(a)	247	bc(a)
Labicuper	204	a(a)	217	a(a)

* Vertical test - Means with different letters are statistically different (Duncan P≤ 0,05)
(**) Horizontal test - Means with different letter are statistically different (Duncan P ≤0,05)

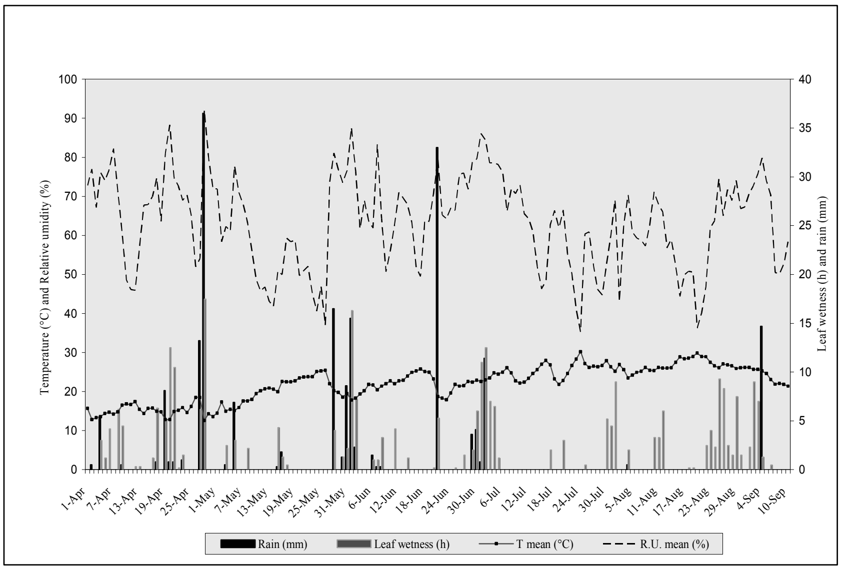

Figure 1. Climatic conditions registered during the trial (2009)

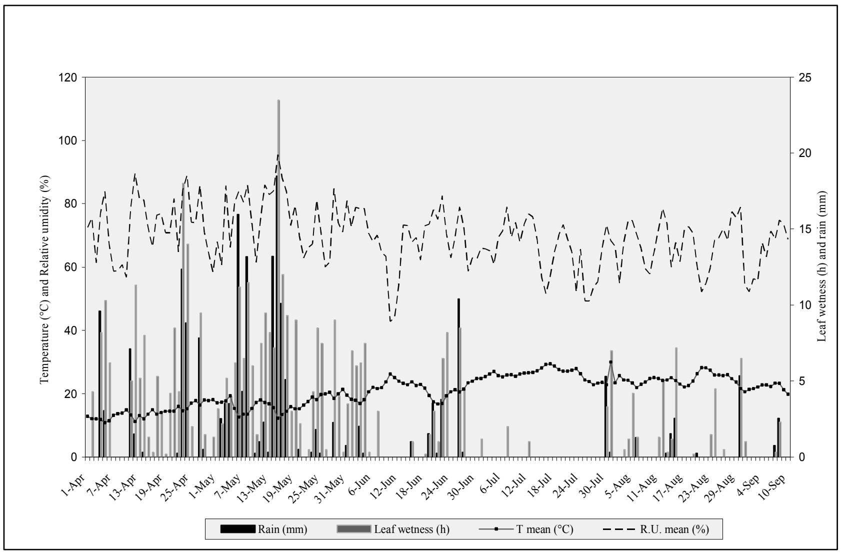

Figure 2. Climatic conditions registered during the trial (2010)

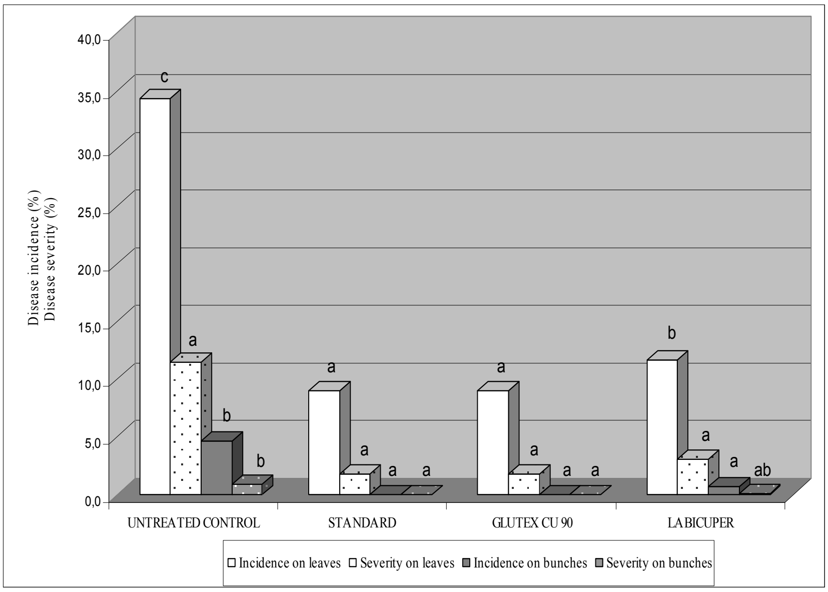

Figure 3. Disease incidence and disease severity on leaves and bunches at harvest (2009)

Means with different letters on the top of each bar type indicate significant differences between treatments according to the Tukey test at $P \leq 0.05$ (disease incidence).

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

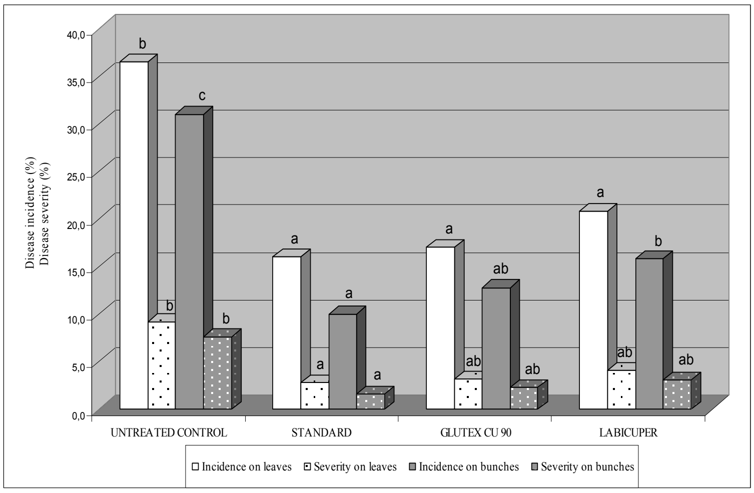

Figure 4. Disease incidence and disease severity on leaves and bunches at harvest (2010)
Means with different letters on the top of each bar type indicate significant differences between treatments according to the Tukey test at P ≤ 0.05 (disease incidence).
Means with different letters on the top of each bar type indicate significant differences between treatments according to the Kruskal–Wallis test followed by Dunn's multiple comparison test at P ≤ 0.05 (disease severity).

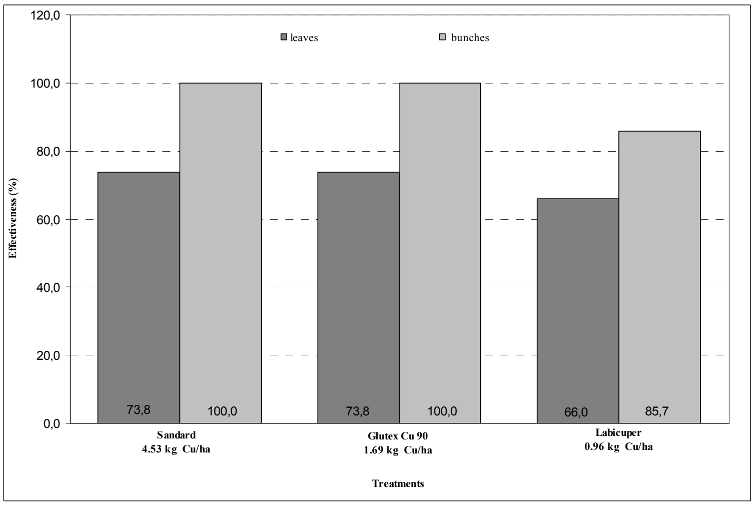

Figure 5. Effectiveness at the harvest of different products and total amount of copper applied with the treatments (2009)

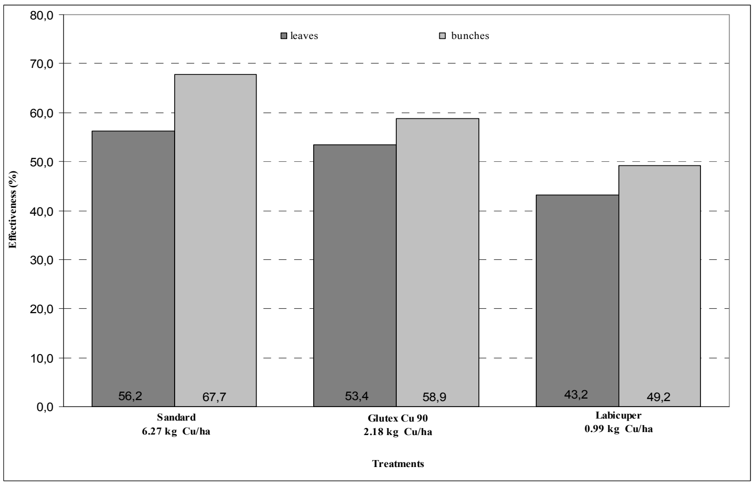

Figure 6. Effectiveness at the harvest of different products and total amount of copper applied with the treatments (2010)

DISCUSSION

Since natural alternative solutions to copper are not currently available, the use of copper is still indispensable for crop protection, especially in organic farming. Several copper alternatives, in fact, have not been shown to be effective against phytopathogenic fungi, especially in years with high disease pressure. Although copper cannot be fully replaced, it is actually possible to reduce the amount of copper using the new copper formulations developed by agrochemical companies.These new products have more copper ions available to provide disease control at a lower rate (Morando *et al*., 1997) and thus minimise the environmental impact. Results of analysis,in fact, indicated that cupric formulations with a lower metallic content (Glutex CU 90 and Labicuper) did not increase levels of copper in the upper 0-20 cm soil horizon after two annual field trials on already contaminated soil (upper 0-20 cm soil-horizon, previous contamination: 200-250 mg/kg). On the other hand in lower soil horizon(20-40 cm) even standard treatments not increased copper levels (previous contamination was 270-290 mg/kg in lower soil horizon).

Since these trials were carried out in a vineyard with a high copper soil contamination (this situation is very common in organic farming) further trials on soils with limited copper contamination should be carried out in order to demonstrate that a negligible Cu accumulation can generally be achieved by using formulations with a lower metallic content.

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REFERENCES

ABBOTT W.S.(1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*. **18:** 265-267.

BALSBERG PAHLSON A.M.(1989). Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Water, Air, and Soil Pollution*. **47:** 287-319.

BRUN L.A., LE CORFF J., MAILLET J. (2003). Effects of elevated soil copper on phenology, growth and reproduction of five ruderal plant species. *Environ. Pollut*. **122:** 361-368.

COMMISSION OF THE EUROPEAN UNION (2008). Commission regulation (EC) No 149/2008. *Official Journal of the European Union*, **L 58:** 1-2.

COMMISSION OF THE EUROPEAN UNION (2008). Commission regulation (EC) No 889/2008. *Official Journal of the European Union*, **L 250:** 1-84.

COMMISSION OF THE EUROPEAN UNION (2009). Commission directive 2009/37/EC. *Official Journal of the European Union*, **L 104:** 23-27.

DELUISA A., GIANDON P., AICHNER M., BORTOLAMI P., BRUNA L., LUPETTI A., NARDELLI F., STRINGARI G.(1996).Copper pollution in Italian vineyard soils. *Commun. Soil Sci. Plant Anal*. **27:** 1537-1548.

GEOFFRION R. (1975). L'altération des terres à vigne par une longue répétition des traitements à base de cuivre et de soufre. *Phytoma-Défense des végétaux,***267:** 14-16.

GESSA C.E. AND CIAVATTA C. (2005). Gli elementi nutritivi – Rame. In: "Fondamenti di chimica del suolo", Sequi P. (ed). Pàtron Editore, Bologna, pp:399-400.

GILLER K.E., WITTER E., MCGRATH S.P. (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil Biol. Biochem*. **30:** 1389-1414.

HARRISON M.D., JONES C.E., DAMERON C.T. (1999). Copper chaperones: function structure and copper-binding properties. *J. Biol. Inorg. Chem.***4:** 145-153.

HOFMANN U.(2002). Copper reduction and copper replacement-results and experiences of 12 years of on farm research. Proceedings of the 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing and Viticulture, 4-7 Febraury 2002, Weisberg, Germany, pp. 181-184.

HOFMANN U., JONIS M., LÉVITE D., TAMM L., HÄSELI A. (2006). Situazione in Germania, Francia e Svizzera. *Informatore agrario*. **6:** 62-65.

KLEIN, O. (2008). A Field Study to Evaluate the Effects of Copper on the Earthworm Fauna in Central Europe. Draft Interim Report 2007 – Year 4 Version 1, Study code: 20031343/G1-NFEw.

LORENZ D. H., EICHHORN K.W., BLEI-HOLDER H., KLOSE R., MEIER U., WEBER E.(1994). Phänologische Entwicklungsstadien der Weinrebe (*Vitis vinifera* L. ssp. *vinifera*). *Vitic. Enol. Sci*. **49:** 66-70.

MOOLENAAR S.W. AND BELTRAMI P.(1998). Heavy metal balances of an Italian soil as affected by sewage sludge and bordeaux mixture applications. *J. Environ. Qual*. **27:** 828-835.

MORANDO A., MORANDO P., BEVIONE D., LEMBO S. (1997). Vite e rame: l'impiego del rame fra pregi e difetti; il controllo della peronospora in vigneto con rameici a dosaggi ridotti. *Vignevini*, **24/7-8**: 53-57.

PIETRZAK U. AND MCPHAIL D.C. (2004). Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. *Geoderma*, **122:** 151-166.

RHOADS F.M., OLSON S.M., MANNING A. (1989). Copper toxicity in tomato plants. *J. Environ. Qual*. **18:** 195-197.

SCAGLIONE M., LA TORRE A., CORAMUSI A. (2008). I composti rameici in agricoltura biologica: stato attuale e prospettive*. Petria Giornale di Patologia delle Piante*. **18** (1): 89-135.

TOWNSEND G.R., HEUBERGER J.W. (1943). Methods for estimating losses caused by diseases in fungicide experiments. *Plant Dis. Rep*. **27:** 340-343.

VAN ASSCHE F., CLIJSTERS H. (1990). Effects of metals on enzyme activity in plant. *Plant Cell Environ.***13:** 195-206.

WOOLHOUSE H.W., WALKER S.(1981). The physiological basis of copper toxicity and tolerance in higher plants. In: Loneragan J.F., Robson A.D., Graham R.D. (eds.): *Copper in Soils and Plants*, pp 265–285. Academic Press, Sydney.