Response of potted grapevines to increasing soil copper concentration

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Abstract

Background and Aims: Copper accumulation in soil may promote phytotoxicity in grapevines. Nutritional implications of potted vines to increasing concentrations of copper (Cu) in either clay loam soil or clay loam soil mixed with 85% sand were tested on *Vitis vinifera* (L.) cv Sangiovese and crop toxicity threshold and symptoms determined.
Methods and Results: Soils were mixed at planting with Cu at the rates (mg Cu/kg) of 0 (control, native soil Cu only), 50, 100, 200, 400, 600, 800 and 1000, and non-bearing vines were grown in these for two seasons. Reduction of root growth was observed after addition of ≥400 mg Cu/kg to both soils; reduction of shoot growth, leaf number and chlorosis of leaf edges were detected only in sand-enriched soil. Root Cu concentration increased in response to soil Cu addition. Unlike that of leaf Cu and N, the amount of P and Fe (in both soils) and Mg and Ca (in sand-enriched soil only) were reduced by soil Cu.
Conclusion: Vines grown in sand-enriched soil tolerated lower concentrations of Cu than in clay loam soil, probably because of the lower nutritional status and the higher root Cu concentration.
Significance of the Study: Results provide information on the concentration of soil Cu that grapevine can tolerate and on the nutrients involved in the response to toxic levels of soil Cu in clay loam and sandy clay loam soils.

Keywords: *$CaCl_2$-extractable Cu, DTPA-extractable Cu, toxicity threshold,* Vitis vinifera *L.*

Introduction

Inorganic copper (Cu), a fundamental microelement for grape nutrition, is widely used in vineyards, either alone or mixed with agrochemicals, as a fungicide against such diseases as downy mildew (*Plasmopara viticola*) (Pearson and Goheen 1988), oidium (*Uncinula necator*), botrytis (*Botrytis cinerea*) and black-rot (*Guignardia bidwellii*). Intensive and long-term use of Cu salts to prevent diseases (Garcia-Esperanza et al. 2006), along with the frequent supply of Cu-enriched amendments (McBride 1995, Mantovi et al. 2003), have promoted an accumulation of Cu in soil (Morgan and Johnston 1991, Moolenaar and Beltrami 1999). Copper is a heavy metal that is toxic to aquatic and soil organisms (Capri et al. 1999), bacteria, fungi (Fleming and Trevors 1989, Giller et al. 1998, Merrington et al. 2002) and plants (Krupa and Bazynski 1995) and also has a negative effect on human health (Turnlund et al. 2004). Copper in soil is restricted mainly to the top layer because of its ability to bind tightly with carbonates, clay minerals, hydrous oxides of Al, Fe and Mn and organic matter (Mengel and Kirkby 2001). However, soil cultivation and tillage may promote a deeper distribution of Cu. For instance, in a survey carried out in the vineyards of Italy's Emila-Romagna Region, the total Cu concentration was found to be 86.7 (±37) mg/kg dwt, with no difference found between the upper (0–20 cm) and the lower (21–50 cm) soil layers, while diethylentriaminepentaacetic acid (DTPA)-extractable Cu was found to be (25.2 mg/kg dwt) higher in the upper than in the deeper (14.5) layer (M. Toselli et al., pers. comm., 2008). Copper mobility, its bioavailability for root uptake, and consequent phytotoxicity threshold for crops depend on soil pH (Chaignon et al. 2002, 2003), cation exchange capacity (CEC), texture and quality of organic matter (Brun et al. 2001, Parat et al. 2002). Excess of soil Cu is responsible for leaf chlorosis in annuals (Marschner 1995) and woody plants (Heale and Ormrod 1982, Kuhns and Sydnor 1976) and reduction of root and shoot growth (Lexmond and van der Vorm 1981) of plants, including in grapevine (Woolhouse and Walker 1981).

The aims of this study were to test the response of potted grapevines to increasing soil Cu concentrations in sand-enriched and clay loam soils, to describe nutritional implications and to determine both toxicity thresholds by soil type and crop toxicity symptoms.

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Materials and methods

Plant material and experimental conditions

The trial was conducted outdoors in 2004 and 2005 at the Cadriano experiment station of the University of Bologna (44°, 35 North) on 1-year-old, non-bearing rooted grapevine (*Vitis vinifera* L.) cuttings of cv. Sangiovese grafted to S.O.4 (*Vitis berlandieri* × *Vitis riparia*). Sixty-four cuttings were potted in May 2004 in 7-L pots filled with two soils differing in sand content (Table 1). Thirty-two vines were planted into clay loam, Calcaric Cambisol (Food and Agriculture Organization 1990) soil, collected near Bologna; the other 32 vines were potted in a sand-enriched soil obtained by adding to the same riverbed sand at a final ratio of 15:85 soil : sand. At planting, the soils of four pots were mixed thoroughly with Cu (as $CuSO_45H_2O$, Sigma Aldrich, Milan, Italy) at 0 (control, only native Cu without additions), 50, 100, 200, 400, 600, 800 and 1000 mg/kg. The vines were pruned in winter to leave the same number of buds. Shoot length and weight were recorded. After budburst in 2005, vines were trained to two to three shoots and watered daily throughout the experiment with 130–260 mL of tap water per pot to return the evapo-transpirated, calculated by successive weight over 24-h intervals. All pots received the same amount of water; soil moisture (measured three times over the experiment) was between 20 and 25% in clay loam soil and between 9 and 12% in sand-enriched soil, with no difference induced by soil Cu-enrichment rates. Each vine was fertilised in June 2004 with 1.5 g of fertiliser N (12%), P (5%) and K (35%) and in April 2005 with 0.75 g of N (30%), P (12%), K (10%) and 90 mg of Fe as iron chelate to prevent Fe-chlorosis. The pots were covered with radiant-barrier insulating film to prevent excessive increases in pot temperature, which ranged between 22 and 26°C. Air temperature, light intensity (10% reduced by plastic netting to protect plants against hail) and relative humidity were in the normal range for the area.

Table 1. Selected physical and chemical characteristics of sand-enriched and clay loam soils measured at the beginning of the experiment.

Soil characteristic	Soil type	
	Sand enriched	Clay loam
Sand (%)	87	43
Silt (%)	8	27
Clay (%)	5	30
pH	8.12	7.78
Calcium carbonate (%)	2	9
Active calcium carbonate (%)	1	7
Organic matter (%)	0.3	2.5
CEC (meq/100 g dwt)	6.9	31.4

CEC, cation exchange capacity.

Soil chemical determinations

In August 2004, 3 months after planting, $CaCl_2$-extractable Cu, DTPA-extractable Cu and total Cu were determined in both soils; in addition, DTPA-extractable Cu was also evaluated at the end of the experiment, in June 2005. The fraction of $CaCl_2$-extractable Cu was measured with 0.05 M $CaCl_2$ (Canet et al. 1997, Chaignon et al. 2003) as follows: 5 g dry soil were shaken for 2 h at 60 cycles per min with 25 mL of 0.05 M $CaCl_2$ and the suspension was then centrifuged at 3000 rpm for 5 min; after supernatant collection, 25 mL of water were added to the soil before centrifuging and the solution was collected. The fraction of DTPA-extractable Cu was extracted after Lindsay and Norvell (1978) but modified as follows: 5 g dry soil were shaken for 2 h at 60 cycles per min with 25 mL of a solution made with DTPA 1.97 g/L, triethanolamine 14.9 g/L and $CaCl_2$ 1.46 g/L buffered to pH 7.3 with HCl (Leita and Petruzzelli 2000). The suspension was centrifuged as described for $CaCl_2$-extractable Cu to obtain 50 mL of final solution after soil rinsing. Total soil Cu was extracted by wet mineralisation after US EPA Method 3052 (Kingston 1988) by treating 0.5 g of dry soil with 8 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%) at 180°C in an Ethos TC microwave labstation (Milestone, Bergamo, Italy) and determined by atomic absorption spectrophotometry (AAS) (Varian AA200, Mulgrave, Victoria, Australia).

At the end of the trial, soil pH was measured by pH-meter electrode (Basic 20, Crison, Crison Instruments, Barcelona, Spain) after a 2-h shaking of 10 g dry soil in 25 mL of deionised water (Violante and Adamo 2000) and 30-min sedimentation. Soil texture, calcium carbonate, active calcium carbonate, organic matter and CEC were determined by an external laboratory (ARPA, Ravenna, Italy) as per the Italian Ministry of Agriculture, Food and Forestry and the International Union of Soil Sciences (Violante 2000). Soil texture was evaluated by pipette after treating the soil with sodium hexametaphosphate (40 g/L) and sodium carbonate (10 g/L); calcium carbonate by volumetric determination of carbon dioxide (CO_2) after HCl addition; active calcium carbonate by titration of the excess of ammonium oxalate; organic matter by C elemental analysis; and CEC by the barium chloride method.

Biomass determinations

At the end of June 2005, vines were harvested and divided into roots, stem, shoot axis and leaves and oven-dried, at 65°C for 96 h, and weighed. Samples of the finest (diameter lower than 1 mm) brown roots were carefully washed in deionised water, oven-dried, ground and analysed for Cu, Fe, Mn and Zn concentrations. White-root samples were also collected from vines treated with 0, 400 and 1000 mg Cu/kg, carefully washed in deionised water, freeze-dried, ground and analysed for total Cu. At harvest, 20 randomly selected leaves per vine were used to determine leaf area (Portable Area Meter, Li-Cor Inc., Lincoln, Nebraska, USA). Leaves were then rinsed three times, oven-dried, weighed, ground and analysed for N, P, K, Ca, Mg, Fe, Mn, Zn and Cu. Total N was measured by Kjeldahl (Schuman et al. 1973) by mineralising 0.5-g leaves with 18 mL of a 95:5 (v/v) H_2SO_4 : H_3PO_3 mixture at 420°C for 150 min and titration with 32% (v/v) NaOH and 0.1 M H_2SO_4. Phosphorous content was determined as follows (Saunders and Williams 1955): the extracts were mineralised with 96% (v/v) sulphuric acid and 35% (v/v) oxygen peroxide, neutralised with 0.1 M NaOH and enriched with 0.1 M ascorbic acid, 32 mM ammonium molybdate, 2.5 M sulphuric acid and 3 μM potassium antimonyl tartrate to develop a phospho-molybdic blue colour; P was spectrophotometrically quantified at 700 nm. All the metals in roots and leaves were extracted by wet mineralisation of 0.5 g dry matter and determined by AAS as noted above for soil.

Table 2. Effect of soil type and soil Cu addition on soil pH as measured at the end of the experiment in June 2005.

Soil Cu addition (mg/kg)	Soil pH	
	Sand enriched	Clay loam
0	8.12	7.78
50	8.14	7.78
100	8.10	7.89
200	7.97	7.74
400	7.92	7.72
600	7.81	7.81
800	7.69	7.75
1000	7.53	7.66
Interaction	*	
SEM	(± 0.07)	

*Interaction between soil type and soil Cu addition significant at $P \leq 0.05$. Values differing by ≥2 standard error of means (SEM) are statistically different.

Statistical analysis

The experiment was a complete randomised factorial experimental design with two factors: soil type (two levels) and soil Cu addition (eight levels) with four replications (single vine). Data were analysed using analysis of variance. Statistically significance ($P \leq 0.05$) differences between means were separated by Student Newman Keuls test; when interaction between soil type and soil Cu addition was significant, twice standard error of means was used as the minimum difference between two statistically different means (Saville and Rowarth 2008). Polynomial contrast analysis of the quantitative factors was performed to evaluate the function that best described the response to increasing soil Cu additions.

Results

Soil determinations

Soil type and soil Cu addition rates significantly interacted with soil pH (Table 2), in fact, the addition of sand increased the pH, but the contemporary addition of Cu (≥200 mg Cu/kg) decreased it to the level of the clay loam soil, where pH was unaffected by Cu treatments. All the fractions of Cu, determined in 2004 and 2005, increased with the rate of soil Cu addition (Table 3). For all determinations, polynomial contrast analysis showed a highly significant ($P \leq 0.001$) linear response to Cu addition rate (data not reported in table). Unlike $CaCl_2$-extractable fraction, DTPA-extractable Cu and total Cu were higher in clay loam than in sand-enriched soil (Table 3).

Plant growth

Dry weight of shoots removed under 2004 pruning was unaffected by soil Cu addition and ranged between

Table 3. Effect of soil type and soil Cu addition on $CaCl_2$-extractable Cu as measured in August 2004, on DTPA-extractable Cu as measured in August 2004 and June 2005 and on total Cu as measured in August 2004 (values are expressed in mg/kg dwt).

Soil Cu addition	$CaCl_2$-extract Cu		DTPA-extractable Cu				Total Cu	
	2004		2004		2005		2004	
(mg/kg)	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam
0	0.19	0.13	4.6	18.4	9.1	19.1	20.1	68.1
50	0.37	1.13	29.4	49.7	29.8	58.0	64.2	126.1
100	0.61	0.68	63.3	98.1	53.4	87.3	109.8	188.4
200	0.78	2.87	121.0	203.4	114.8	198.2	193.3	322.7
400	2.08	2.71	279.9	446.2	244.3	408.7	372.9	617.0
600	4.65	2.89	482.3	505.2	453.7	587.6	560.6	752.3
800	5.47	4.38	579.3	813.7	614.1	783.0	687.4	1159
1000	4.12	3.47	606.1	764.2	706.0	899.7	753.0	1084
Interaction	**		n.s.		n.s.		***	
SEM	(± 0.75)		(± 54)		(± 57)		(± 75)	

n.s., **, ***: effect not significant or significant at $P \leq 0.01$ or $P \leq 0.001$, respectively. Values differing by ≥2 standard error of means (SEM) are statistically different. DTPA, diethylentriaminepentaacetic acid.

Table 4. Effect of soil type and soil Cu addition on shoot (leaves + shoots) and root dry weight and leaf number per plant as measured at harvest, June 2005.

Soil Cu addition (mg/kg)	Shoot dwt (g)		Leaves per vine		Root dwt (g)	
	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam
0	16.2	27.5	28.2	30.0	14.7	27.5
50	14.1	24.6	27.7	25.7	13.9	24.8
100	17.1	23.9	26.7	26.2	15.6	21.0
200	16.8	30.0	22.5	27.0	12.8	25.8
400	6.61	29.4	15.2	27.7	7.58	22.4
600	4.89	22.7	13.7	28.2	5.36	17.8
800	4.64	23.1	16.2	22.2	6.12	18.6
1000	4.75	26.7	12.2	25.7	4.86	20.6
Interaction	**		*		n.s.	
SEM	(± 2.13)		(± 2.71)		(± 2.39)	

n.s., *, **: interaction between soil type and Cu addition not significant or significant at $P \le 0.05$ and $P \le 0.01$, respectively. Values differing by ≥2 standard error of means (SEM) are statistically different.

5.25 (in soil enriched with 1000 mg Cu/kg) and 6.37 g (untreated control), although shoot weight in clay loam soil (8.94 g) was statistically higher than in sand-enriched soil (3.06 g) (data not reported). In 2005, soil type and soil Cu addition significantly interacted with shoot growth and leaf number per vine, with both parameters being reduced by soil Cu addition (≥400 mg/kg) in sand-enriched soil as compared with untreated soil (Table 4). As the addition of soil Cu (indicated as x in the formulas) was well correlated to both $CaCl_2$-extractable Cu ($y = 0.0051x + 0.1427$, $R^2 = 0.83$) and DTPA-extractable Cu ($y = 0.6306x + 14.81$, $R^2 = 0.89$), it was possible to establish the maximum amount of Cu that grapevine can tolerate in sand-enriched soil with no reduction of shoot growth: 1.16 and 141 mg Cu/kg of $CaCl_2$- and a DTPA-extractable Cu, respectively.

Cu addition did not modify leaf area and specific leaf weight (data not reported) in either soil, although discoloration of leaf margins was observed in vines in sand-enriched soil treated with Cu ≥400 mg/kg soil. Root dry weight was depressed by soil addition ≥400 mg Cu/kg (Table 4). Note, however, that only in sand-enriched soil was the relation between $CaCl_2$-extractable Cu and plant growth described according to a logarithmic function that showed higher R^2 for root than shoot dry weight (Figure 1).

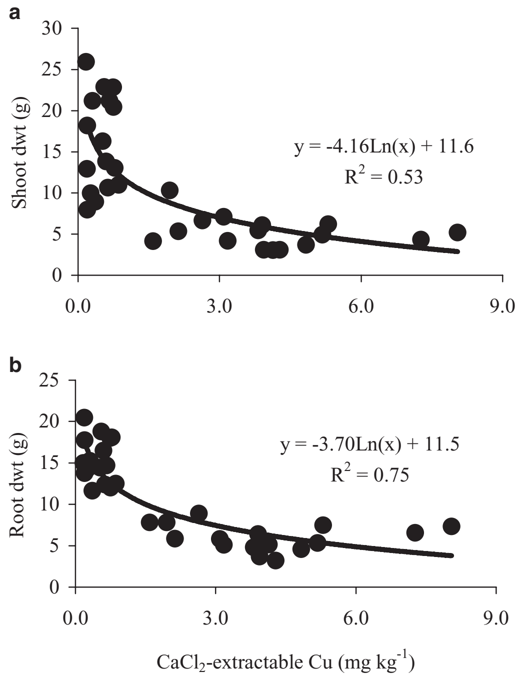

Figure 1. Relationship between $CaCl_2$-extractable Cu and shoot (a) or root (b) growth. Each point represents a single plant. DW, dry weight.

Mineral analysis

Soil type and Cu addition rates significantly interacted with leaf Ca and Mg content (Table 5). Leaf Ca and Mg content linearly decreased in sand-enriched soil as the rate of soil Cu addition increased but were not in clay loam soil; in few cases (400 and 800 mg Cu/kg of soil), leaf Ca content increased compared with untreated soil (Table 5). In both soil types, leaf P and Fe content linearly decreased under Cu-enriched soil (Table 5). Leaf N, K, Cu, Mn and Zn content was unaffected by soil Cu (Table 5). With few exceptions (i.e. Mn, Cu and Ca), all the other nutrients were higher in leaves from clay loam than sand-enriched soil (Table 5).

Soil type and Cu enrichment positively interacted with Cu concentration in both brown and white roots (Table 6). In both soils, with the exception of 800 mg Cu/kg, brown root Cu concentration increased with soil Cu concentration (Table 6) and, with the exception of 0 mg Cu/kg, was always higher in sand-enriched than in clay loam soil. In white roots, Cu concentration was much lower than in brown roots and increased in sand-enriched soil along with Cu metering rate. In clay

Table 5. Effect of soil type and soil Cu addition on content (mg leaf^{-1} dwt) of N, P, K, Ca, Mg, Cu, Fe, Mn and Zn in *Vitis vinifera* cv Sangiovese leaves.

Soil Cu addition (mg/kg)	N		P		K		Ca		Mg		Cu		Fe		Mn		Zn	
	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand-enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam
0	3.13	5.22	0.532	0.682	4.55	7.26	4.08	3.65	1.17	1.63	5.30	6.31	38.2	40.7	7.96	9.32	8.44	11.4
50	2.96	4.59	0.413	0.701	3.69	7.17	3.63	3.66	1.13	1.62	4.15	6.36	23.1	41.6	7.47	6.36	6.87	11.0
100	3.25	4.31	0.461	0.610	4.73	6.58	3.78	3.81	1.30	1.53	3.94	7.99	25.2	41.9	9.73	9.49	8.14	11.2
200	3.92	4.39	0.447	0.600	5.80	6.34	3.93	3.66	1.54	1.50	4.99	4.76	18.5	20.6	8.55	7.24	9.85	10.5
400	3.07	4.13	0.233	0.533	4.25	7.58	2.73	5.26	0.95	1.98	5.16	6.07	17.2	22.8	9.10	9.36	5.54	10.8
600	2.38	4.13	0.155	0.498	3.56	6.18	1.64	3.74	0.66	1.58	2.38	4.82	14.7	26.3	4.48	8.80	7.41	9.96
800	2.98	3.74	0.185	0.443	2.79	5.48	1.32	4.58	0.51	1.59	4.89	6.77	14.3	27.5	3.96	8.12	5.59	9.03
1000	2.97	4.37	0.161	0.431	3.82	5.54	2.01	3.17	0.78	1.40	3.99	4.20	15.9	18.1	4.39	7.70	5.92	9.33
Pol. contrast	–	–	linear***	linear***	–	–	linear***	n.s.	linear***	n.s.	–	–	linear**	linear**	–	–	–	–
Interaction	n.s.		n.s.		n.s		*		*		n.s.		n.s.		n.s.		n.s.	
SEM	(± 0.47)		(± 0.07)		(± 0.87)		(± 0.63)		(± 0.19)		(± 1.39)		(± 8.5)		(± 1.51)		(± 1.39)	

n.s., *, **, ***: effect not significant or significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. Values differing by ≥2 standard error of means (SEM) are statistically different.

Table 6. Effect of soil type and soil Cu addition on Fe, Mn, Zn and Cu concentration (mg/kg dwt) in brown roots and Cu concentration in white roots of *Vitis vinifera* cv Sangiovese, as measured at harvest, June 2005.

Soil Cu addition (mg/kg)	Brown roots								White roots	
	Fe		Mn		Zn		Cu		Cu	
	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam	Sand enriched	Clay loam
0	2655	2004	71.5	34.7	66.9	62.6	258	311	12.5	7.93
50	2711	3126	65.2	55.5	54.8	73.3	1862	1320	–	–
100	2782	2600	64.8	42.7	55.8	68.9	2672	1926	–	–
200	2361	2261	51.6	37.0	48.9	59.8	4684	2758	–	–
400	3331	2003	85.5	28.3	42.7	58.5	8469	3896	199.9	43.7
600	2743	1827	55.9	26.0	42.0	59.4	13 250	6021	–	–
800	1991	1765	38.3	22.7	46.3	44.8	12 246	5334	–	–
1000	1982	2559	37.9	36.5	42.6	47.2	15 713	7466	340.1	91.6
Pol. contrast	–	–	linear**	linear*	linear*	linear*	linear***	linear***	–	–
Interaction	n.s.		*		n.s.		*		***	
SEM	(± 448)		(± 8.35)		(± 8.10)		(± 664)		(± 22)	

n.s., *, **, ***: effect not significant or significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. Values differing by ≥2 standard error of means (SEM) are statistically different.

loam soil, it differed from control only at 1000 mg Cu/kg (Table 6). While root Fe concentration was unaffected (Table 6), Mn and Zn were linearly depressed by Cu dressing rates in both soils and, on average, Zn was higher in clay loam soil and Mn in sand-enriched soil.

Discussion

One initial response of vines to high soil Cu concentration was the reduction of root growth, which was observed in both soils. This has been observed in herbaceous species (McBride 2001, Sheldon and Menzies 2005) and woody plants (Påhlsson 1989), including grapevine (Woolhouse and Walker 1981), through inhibition of lateral root development (Patterson and Olson 1983). In our experiment, inhibition of root growth was followed by the reduced shoot growth, as already reported by Mozaffari et al. (1996) in citrus and Reichman et al. (2006) in ornamental trees, the year after planting, in sand-enriched soil treated only with ≥400 mg Cu/kg. The strong correlation (as supported by the coefficient of determination R^2 of 0.53) between soil-extractable Cu and shoot growth made it possible to establish a shoot inhibiting threshold of 1.16 mg of $CaCl_2$-extractable Cu and 141 mg of DTPA-extractable Cu/kg soil dry weight for potted grapevines in sand-enriched soil after 1 year of exposure. A similar logarithmic response of shoot and root dry weight to increasing Cu concentration in solution was observed in four Australian tree species by Reichman et al. (2006). DTPA-extractable and total Cu were always higher in clay loam compared with sand-enriched soil. We believe this is the result of a rapid leaching of supplied Cu as a consequence of constant watering to promote vine establishment immediately after planting, i.e. when Cu input was not stabilised in the soil.

Leaf Cu concentration did not increase in response to soil Cu supply, ranging between 10 and 20 mg/kg, which is considered sufficient for grape (Jones et al. 1991). Leaf P, Mg, Ca and Fe were among the nutrients affected by Cu soil application. The latter is closely related to chlorophyll synthesis and its lack is responsible for leaf chlorosis; Fe was reduced in both soils but particularly in soil enriched with sand where symptoms of chlorosis were observed. Magnesium leaf content decreased linearly under Cu dressing of sand-enriched but not clay loam soil, a finding that suggests that leaf chlorosis was related to this response. The low Ca level was probably the result of leaf chlorosis rather than its cause. In effect, Faust (1980) suggested a dependence of Ca uptake on the growth of healthy young root tips, which in turn depend on an appropriate photosynthesis rate. In addition, only a small amount of Ca is required for normal CO_2 assimilation, as reported by Terry and Huston (1975) in sugar beet. In rice, Lidon and Henriques (1993) showed no effect of Cu in the nutrient solution on shoot N and P concentration; however; when the data were expressed as amount of nutrient per plant, a reduction of both N and P plant content was found, as a consequence of the reduction of plant growth under high Cu concentration. Our responses were different as only P leaf content linearly decreased in response to the addition of Cu to the soil, while N, leaf size and weight were not affected by soil Cu. The different growing rate of the annual crop (rice) compared with the woody species (grapevine) may be the reason for the different results obtained here compared with those from rice. Leaf symptoms in response to Cu toxicity have been reported as interveinal chlorosis in *Lonicera tartarica* (Heale and Ormrod 1982) or basal chlorosis in *Cotoneaster divaricata* (Kuhns and Sydnor 1976) but not as a discolouration of leaf margins as found in our experiment.

Although the reduction of Zn in roots may be explained by the inhibitory effect of Cu found at very high concentration in roots, leaf Zn content was unaffected by treatments, confirming reports indicating the unclear responses of Zn to high soil Cu. Lidon and Henriques (1993) stressed a negative effect of Cu on both root and shoot Zn concentration in rice grown in nutrient solution, although the negative effect on shoots disappeared at the highest Cu addition rate, and Turner (1970) found that Cu and Zn did not interfere each other at the subcellular level of *Agrostis tenuis*. In our study, Fe leaf content was decreased as Cu addition rate increased although, at the same time, root Fe concentration remained unaffected. This response may indicate that high Cu soil concentrations were altering the translocation of nutrients. However, among the trace elements investigated, Cu and Fe showed root concentrations 1 or 2 orders of magnitude higher than Mn and Zn. This response may be partially explained by soil chemical composition. In fact, Fe concentration in our clay loam soil was found to be 33 mg/kg, Mn 10 and Zn 0.8 (data not reported), or it might be related to the higher affinity of Cu and Fe to cell-wall carboxylic groups.

Both $CaCl_2$ and DTPA-extractable Cu were found to be good indicators of the amount of soil Cu, the two fractions increasing linearly with Cu input rate. However, only in sand-enriched soil were extractable Cu fractions related to shoot growth. This response indicates that Cu bioavailability is hard to detect by chemical extraction and easier to evaluate by root Cu concentration (McBride 2001, Chaignon et al. 2003). Although lower Cu fractions were always detected in sand-enriched soil, root Cu concentration was higher there than in clay loam soil. In sand-enriched soil, we found a reduction of shoot growth at a root Cu concentration over 8400 mg/kg in brown roots and 200 mg/kg in white roots; in clay loam soil, these values were not reached and, consequently, no symptoms were observed. Our results support the hypothesis that root Cu is a more sensitive indicator of soil Cu bioavailability than shoot Cu concentrations, and leaf Cu is not a reliable tool to predict the potential of Cu toxicity (Alva et al. 1995, Chaignon et al. 2003, Reichman et al. 2006). With a few exceptions (i.e. Ca, Cu and Mn), the addition of sand decreased the leaf content of all macro- and micronutrients; this affected vine growth and probably increased the susceptibility of vines to excess of soil Cu. In fact, with a low nutrient concentration, the antagonistic effect of Cu was probably observed earlier than in vine with a sufficient nutritional status. In all the treatments, leaf concentrations of N (0.9–1.2%) and P (0.09–0.15%) were well below those recommended by the literature (Jones et al. 1991).

That root but not shoot growth was decreased in clay loam soil after Cu supply may indicate that in the longer term, symptoms of Cu toxicity in shoots should also be observed.

As stressed by Turner (1970), we believe that most of the root Cu was associated with cell walls (Iwasaki et al. 1990) and, more specifically, with the suberised esoderm as the difference between Cu concentration in brown (suberised) and white (not completely suberised) roots of the same diameter indicates. The high Cu concentrations found in brown roots were expected as the samples analysed were made of the thinnest roots (less than 1 mm in diameter) with a large surface-to-volume ratio and, consequently, a high amount of cell wall-bound Cu per root volume. Similar results are reported in rice (root Cu concentration of 3380 mg/kg) after 30 days of exposure to 6.25 mg Cu/L (Lidon and Henriques 1993), as well as in 2-year-old *Pinus resinosa* grown in culture solution enriched with 20 mg Cu/L, which showed a Cu concentration of 16 and 4000 mg/kg dry weight in needles and roots, respectively (Heale and Ormrod 1982).

Conclusions

Vines grown in clay loam soil can tolerate a high (more than 10-fold higher than natural concentration) amount of new added Cu with no reduction in shoot growth. On the other hand, in sand-enriched soil, which induced a low nutritional status, Cu toxicity threshold can be established at 200 mg/kg above which, a reduction of shoot growth and leaf chlorosis appeared after 2 years of Cu exposure. Root Cu concentration seemed to be the most appropriate indicator of Cu bioavailability in both soils. Considering the difficulties of Cu soil decontamination, attention must be paid to Cu management in soils of a very light texture. Moolenaar and Beltrami (1999) calculated that, with the common agricultural practices of Northern Italy, a soil content of 100 mg Cu/kg and over is reached in about 100 years. Along with a reduction of Cu inputs, the introduction of varieties tolerant to important diseases and proper cultivation rotation that includes vegetable crops are recommended.

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