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Research Paper

Soil-applied phosphorous is an effective tool to mitigate the toxicity of copper excess on grapevine grown in rhizobox

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ABSTRACT

The aim of the present study was to evaluate the effectiveness of P soil addition to mitigate the toxic effect of excess of Cu on grapevine on sandy non-calcareous soil.

Micro-propagated plants of 1103 Paulsen rootstock were grown according to a factorial experimental design with two factors: Cu addition (0, 100, 200 and 300 mg kg^{-1} dw) and P addition (0, 50 and 100 mg kg^{-1} soil dw). The experiment was conducted in the greenhouse and plants were grown on a sandy soil in rhizoboxes. At the end of the trial, plants were destructively harvested, the biomass and the nutrient concentration of organs were determined. Root growth was evaluated during the trial and the concentration of organic acid in root tissue and exudates was determined.

Shoot length and plant biomass declined as Cu concentration in soil increased; the application of P enhanced plant growth and nutritional status. Root biomass decreased at Cu $>$ 100 mg kg^{-1} and increased in P at 100 mg kg^{-1}. Phosphorous stimulated root length, diameter and the number of apexes. Root citric acid increased as a response of Cu at 300 mg kg^{-1}, root ascorbic acid decreased with the increase of Cu (200 and 300 mg kg^{-1}) and P at 100 mg kg^{-1}. The concentration of citric acid from root exudations was higher in Cu at 300 mg kg^{-1} and Cu at 200 mg kg^{-1} (compared to control) and P at 100 mg kg^{-1} soils.

The application of P fertilization seems to be a valuable strategy to overcome the toxicity of high concentration of Cu in soil.

1. Introduction

The reiterated use of copper (Cu)-based fungicides in viticulture to control diseases has resulted in an accumulation of Cu in the topsoil, reaching sometimes toxic levels that can cause plant stress and reduce soil fertility (Komárek et al., 2010). Copper is a fundamental microelement for grapevine nutrition since it is a constituent of several enzymes (Pilon et al., 2006; Kabata-Pendias, 2011) and it is involved in a number of metabolic processes (Kabata-Pendias, 2011). Nevertheless, Cu can be toxic to plants when highly concentrated in soils (Marschner, 1995). Excess of Cu reduces plant growth and nutrient uptake, it may alter membrane permeability, protein synthesis, photosynthetic and respiratory processes, enzyme activities and chromatin structure (Sandmann and Böger, 1980; Fernandes and Henriques 1991; Madejón et al., 2009; Toselli et al., 2009). Unlike N (Llorens et al., 2000), Ca, K, Mg, Fe and Mn uptake is negatively affected by Cu excess in soil (Wallace and Cha, 1989; Toselli et al., 2008).

Moreover, the increase of soil Cu concentration negatively affects, in terms of numbers and variability, the microbial communities present in vineyard soils (Dell'Amico et al., 2008; Lejon et al., 2008). This effect, not only impact adversely mineralization and nutrient availability, but it can also lead to the reduction of microorganism activity and consequently to lower mineralization rates of organic xenobiotics, such as pesticides.

Copper solubility greatly depends on soil pH; Cu becomes readily available at pH below 6 (Boudesocque et al., 2007). In acidic vineyard soils, Cu can migrate throughout soil profiles easily and thus cause groundwater pollution (Nóvoa-Muñoz et al., 2007). On the other hand, the mobility of Cu in soils can increase at pH higher than 7.5 through its solubilization and complexation by soluble organic matter (Arias et al., 2006).

Despite difficulties in calculating specific soil toxicity thresholds, as total soil concentrations rarely correlate to plant availability, critical total Cu levels depend on soil properties and in some areas have been

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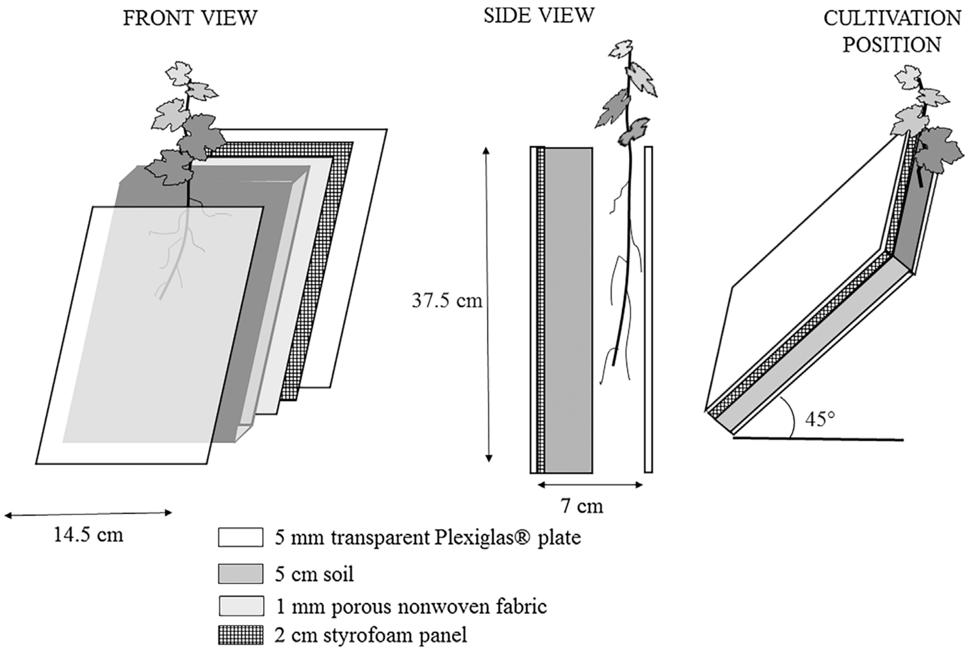

Fig. 1. Scheme of the rhizobox used in the experiment.

defined. In Australian and New Zealander sandy soils, for example, the toxic level of Cu is considered > 60 mg Cu kg^{-1}, in the Netherlands it is > 36 mg Cu kg^{-1}whereas in the European Community the range of toxicity is between 50 and 140 mg Cu kg^{-1}, depending on type of soil (Mackie et al., 2012). According to the Italian legislation (D.L. 152/2006, 2006) the threshold for Cu in agricultural soil is 100 mg Cu kg^{-1} and the yearly amount of elemental Cu permitted in viticulture is below 6 kg ha^{-1}.

The portion of Cu that falls onto the soil may accumulate in the topsoil leading to possible contamination of agricultural soils and induce plant toxicity. In addition, once Cu enters the food chain, it may affect human health (Basta et al., 2001; Ismail et al., 2005; Sipter et al., 2009). There are several options to achieve soil remediation from heavy metals including immobilization through pH alterations, organic matter addition, removal, sequestration, active mixing and phytoextraction techniques (Maier et al. 2009; Pietrzak and Uren, 2011; Mackie et al., 2012). A possible strategy to reduce the detrimental impact of Cu excess in soils is the application of phosphorous (P). Phosphate has been proven to effectively immobilize heavy metals in contaminated soils, mainly due to the ability of P in creating stable metal forms(Cao et al., 2002; Miretzky and Fernandez-Cirelli, 2008). The possible mechanisms of phosphate action in soil include ion exchange, surface complexation/sorption and precipitation (Theodoratos et al., 2002; Cao et al., 2004; Liu and Zhao, 2007). A significant reduction of lead mobility in solid was observed using phosphoric acid (Yang and Mosby, 2006), natural phosphate rocks (Ma et al., 1995) and synthesized apatites (Ryan et al., 2001). Those phosphate-containing compounds were also successfully applied to stabilize cadmium (Raicevic et al., 2005), zinc (Zn) (Wang et al., 2001; Chen et al., 2006) and Cu (Wang et al., 2001; Cao et al., 2004) in contaminated soils.

Phosphorous plays a fundamental role in energy generation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling and carbohydrate metabolism. The results of a study on three strains of *Scenedesmus acutus* (Twiss and Nalewajko, 1992) demonstrated the importance of cellular polyphosphate content in reducing the toxic effect of Cu on photosynthesis. According to the authors (Twiss and Nalewajko, 1992), the greater the cellular P content, the less inhibition of photosynthesis occurred during Cu exposure.

The biochemical pathways involved in cell organic acid metabolism are of fundamental importance in plant responses to stresses; in particular, roots increase the exudation of organic compounds (Parker and Pedler, 1998; Herrera-Estrella et al., 1999; Murphy et al., 1999; Pal and Rai, 2010). Plants are characterized by a higher organic acid concentration in tissues if compared with other organisms (Lopez-Bucio et al., 2000), that depends on species, age of the plant, tissue, type of soil and nutritional status of plants (Marschner, 1995). Root exudates include components (e.g. organic acids, phytosiderophores and phenolics) that play important roles in nutrient solubilization, restricting the passage of toxic metals across the root (e.g. citrate, malate, small peptides) and attracting beneficial microorganisms (e.g. phenolics, organic acids and sugars). Organic acids have a key role in the mechanisms that plants use to handle nutrient deficiencies, metal tolerance and plant-microbe interactions operating at the root-soil interface (López-Bucio et al., 2000).

The aim of the present study was to evaluate the effectiveness of P soil addition to mitigate the toxic effect of excess of Cu on grapevine on sandy non-calcareous soil.

2. Material and method

2.1. Plant material and experimental conditions

The study was carried out in 2012, in the greenhouse of the Department of Agricultural Sciences of the University of Bologna (44°30′54″N; 11°24′21″E) on micro-propagated Paulsen 1103 (*Vitis berlandier*i X *V. rupestris* L.) vines grown in rhizoboxes for 100 days. Five-months-old vines coming from a local nursery were acclimatized for 6 months in the greenhouse before transplant. One-year-old shoots were cut at 3 buds while roots were shortened at 5 cm of length and inserted at a depth of 3 cm. To support plant growth, a wood training stake was inserted next to each plant. The experiment was performed from March to June under natural light intensity and with temperature maintained between 20 ± 2 °C and 26 ± 2 °C (night:day). Pots were daily irrigated to restore the water lost by transpiration (determined by weighting each pot). The rhizoboxes (37.5 cm high, 14.5 cm wide and 5 cm thick) were made with wood and Plexiglas® and kept at a 45° angle from vertical to avoid preferential root growth along the box wall. To facilitate irrigation and gaseous exchange, styrofoam panel with small tunnels excavated with a herringbone pattern were put inside each rhizobox (on the opposite part of the transparent window) and covered with a nonwoven fabric (Fig. 1). Each rhizobox was covered with a