Photosynthesis and growth of young grapevines intercropped with native grasses in soils contaminated with copper

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Abstract

High copper (Cu) contents in vineyard soils due to long-term foliage-defense program based on Cu-containing fungicides may cause physiological and nutritional disorders in young grapevines, limiting plant growth and in some cases also compromising plant survival. This problem is particularly evident in viticultural areas of southern Brazil. Therefore, the study aimed to assess if the intercropping of grapevines with native grasses of southern Brazil can contribute to limit the soil Cu availability and thus the onset of toxicity symptoms (i.e., impairment of photosynthetic and growth parameters). In order to do this, we collected soil samples in the natural grassland of the Pampa Biome (southern Brazil). The samples were air-dried, the acidity, phosphorus and potassium levels were corrected and the samples were consequently incubated. We used three Cu levels – control (i.e., no Cu addition), 40 and 80 mg Cu kg^{-1}. Such Cu levels are normally found in vineyard soils of the region. The experimental design was completely randomized with three replications. At each Cu level, we set up three combinations of monocropping (Grapevine), and intercropping with *Paspalum plicatulum* Michx. (Grapevine + *Paspalum plicatulum*) and *Axonopus affinis* Chase (Grapevine + *Axonopus affinis*). In the intercropped treatments, two grass seedlings were transplanted into each experimental unit, 35 days before transplanting the grapevines. The experiment was conducted for 70 days. At 41 days after transplanting the grapevines, gas exchange (IRGA) was measured. At 70 days, plant height and total dry matter yield were determined and the relative growth rate was quantified. The addition of Cu caused phytotoxicity in the single crop (Grapevine), reducing photosynthetic carbon assimilation and plant growth. The cultivation of young grapevines intercropped with native grasses, especially *Paspalum plicatulum* Michx., promoted the growth of the grapevines in the control soil as well as in the moderately Cu-contaminated soils. This indicates that maintaining native vegetation in young vineyards can reduce Cu toxicity to transplanted grapevines.

Keywords: cover crops, phytotoxicity, heavy metals, biome pampa, *Vitis vinifera*

INTRODUCTION

The application of cupric fungicides for the control of foliar diseases in grapevines may lead to an increase in copper (Cu) contents in vineyard soils (Miotto et al., 2014; Cambrollé et al., 2015; Brunetto et al., 2016). High levels of Cu can cause toxicity to young grapevines planted in soils of eradicated old grapevines and to the cover crops that co-inhabit these vineyards (Miotto et al., 2014).

Copper is a micronutrient to plants, but its excessive acquisition by roots severely

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compromises plant growth by impairing the functioning of the photosynthetic apparatus, reducing the carbon fixation (Kabata-Pendias, 2011; Cambrollé et al., 2015; Tiecher et al., 2016). The symptoms of phytotoxicity caused by excess Cu depend on the available fraction in the soil solution and on the plant species; in fact, excess Cu might trigger different tolerance mechanisms depending on the plant species (Oyuela Leguizamo et al., 2017). Copper-tolerant plants either reduce the Cu availability in the rhizosphere by complexation reactions due to the release of ions and soluble organic compounds or by preventing Cu translocation to the shoots accumulating the metal in the root system (mainly in the apoplast) (De Conti et al., 2016; Brunetto et al., 2016). These tolerance mechanisms, can also favor the development of other plants grown in intercropping, due to the reduced bioavailability of the contaminant (Brunetto et al., 2016). Native species, such as the South American native grasslands, have mostly evolved in acidic and nutrient poor soils thanks to the ability of these plants to adapt to the conditions of low nutrient levels and the presence of toxic elements (Pallarés et al., 2005). The characteristics of these species, together with their natural occurrence in vineyards of southern Brazil, suggest great potential for the phytoremediation of these soils. Therefore, the study aimed at assessing if the intercropping of grapevines with native grasses of southern Brazil can contribute to limit the soil Cu availability and thus the onset of toxicity symptoms (i.e., impairment of photosynthetic and growth parameters).

MATERIAL AND METHODS

The soil used was a Typic Hapludalf (Soil Survey Staff, 2006) collected at 0-20 cm in an area of uncultivated grassland located in the Pampa Biome, southern region of Brazil (30°47'23.7"S and 55°22'7.3"W). The physical-chemical characterization of the soil is presented in Tiecher et al. (2016). After the collection, the soil was air dried, homogenized and passed through a 2 mm mesh sieve. Subsequently, soil acidity has been buffered adding a mixture of calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) with a 2:1 ratio at a concentration of 0.57 g kg^{-1} soil and applied 40 mg P kg^{-1} and 100 mg K kg^{-1} of soil, in the form of triple superphosphate and potassium chloride, respectively. The treatments consisted of three Cu levels: control (i.e., no Cu addition), and the addition of 40 and 80 mg Cu kg^{-1}, which are levels usually found in vineyards where grapevines have been grown for approximately 15 and 30 years, respectively (Miotto et al., 2014). The addition of Cu occurred 50 days after the application of the corrective, by applying a solution of $CuSO_4$. $5H_2O$. Subsequently, the soil was incubated again for 115 days, with soil moisture kept at 80% of the maximum water holding capacity (MWHC).

The experimental design was completely randomized with three replications. The experimental units were pots of 8 L containing 7 kg of dry soil. At each Cu level, we set up three combinations of monocropping (Grapevine), as well as intercropping with *Paspalum plicatulum* Michx. (Grapevine + *Paspalum plicatulum*) and *Axonopus affinis* Chase (Grapevine + *Axonopus affinis*). In November 2015, we transplanted one grapevine (*Vitis vinifera* L. 'Paulsen 1103') plant per pot, where it was grown for 70 days. In the intercropped treatments (Grapevine + *Paspalum plicatulum* and Grapevine + *Axonopus affinis*), two seedlings to native grasses were transplanted into each pot, 35 days before the transplanting of the grapevines. Soon after the transplanting, we determined the stem diameter at the ground level and the height of the grapevines. At 16 and 48 days after transplanting, we applied 20 and 10 mg N kg^{-1} of soil in the form of urea, respectively. In the intercropped treatments, the shoots of the cover crops were cut at 10 cm (height) and placed on the soil surface every 21 days, totaling three cuts along the cultivation of the grapevines. This management of the native grasses simulates the mowing typically used in managing cover crops in commercial vineyards. Soil moisture was maintained at 70% of the MWHC during cultivation, with daily weightings and the addition of distilled water when necessary.

At 41 days after transplanting the grapevines, gas exchange was measured in two fully expanded leaves (of the middle region), using an open system infrared gas analyzer (IRGA) (LI-6400XT LI-COR, Inc., Lincoln, NE, USA). Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, water use efficiency (WUE) and

instantaneous carboxylation efficiency (CE) were determined at an ambient CO_2 concentration of 400 µmol mol^{-1} at 20-25°C, 50±5% relative humidity and a photon flux density of 1000 µmol m^{-2} s^{-1}. After 70 days of the transplanting, we determined the stem diameter at the ground level and the height of the grapevines in order to estimate the relative growth rate (RGR/day) using the following equation (Sieverding, 1991):

$$(\pi r^2 \times h)/\text{plant age}$$

where r = 1/2 of the difference between the initial and final stem diameter, and h = the difference between the initial and final height of the grapevines. Afterwards, the grapevines were cut close to the soil surface and the roots were separated from the soil by hand, washed in running tap water to remove soil and then stored for the determination of dry matter (DM) in a forced air oven at ±65°C, until reaching constant dry matter.

The results obtained were submitted to analysis of variance (ANOVA) using Sisvar software, version 5.6 (Ferreira, 2011), with the following completely randomized bifactorial statistical model:

$$Y_{ijk} = \mu + C_i + Z_j + CZ_{ij} + \text{error } (i, j)$$

where µ = overall mean of the experiment, C = Cu levels (i = 1, 2, 3), Z = crops systems (j = 1, 2, 3) and error = experimental error. When the effects were significant, the means were grouped by the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

The net photosynthetic rate decreased significantly with increasing Cu concentration added to the soil in grapevines (monocropping) and in grapevines intercropped with *Axonopus affinis* Chase (Figure 1). In grapevine intercropped with *Paspalum plicatulum* Michx., the reduction in the net photosynthetic rate occurred only at the highest Cu concentration used (80 mg kg^{-1}, Figure 1). This behavior was also observed in stomatal conductance and instantaneous carboxylation efficiency (Figure 1). Furthermore, the intercropped systems had a higher net photosynthetic rate compared to the monocropping system (grapevine): in control and 40 mg kg^{-1} Cu-treated plants we observed an increase of 38.9 and 97.8% in grapevine + *Paspalum plicatulum* Michx., and 38.0 and 46.9% in grapevine + *Axonopus affinis* Chase, respectively (Figure 1). At these Cu levels, stomatal conductance, transpiration rate and instantaneous carboxylation efficiency were also higher in the intercropped systems (Figure 1). Intercellular CO_2 concentration and water use efficiency were slightly affected by Cu and did not differ between the cropping systems (Figure 1). These results show that the excess Cu compromises the functioning of the photosynthetic carbon assimilation pathway, reducing photosynthetic carbon assimilation. The excess Cu can further induce the reduction of the concentration of photosynthetic pigments and cause the degradation of the structure and the internal content of the chloroplast through the degradation of the membrane polar lipids (Maksymiec et al., 1995; Cambrollé et al., 2015).

The increase in the efficiency of the photosynthetic apparatus in the intercropped systems of the control and 40 mg kg^{-1} Cu treated plants is most likely due to the reduced Cu bioavailability and/or the increased bioavailability of other essential nutrients (Brunetto et al., 2016). Plant roots release in fact a myriad of compouds (i.e., root exudates) modifying the chemical characteristics of the rhizosphere. For instance, pH changes and the presence of organic ligands could increase the stable complexation of Cu^{2+} in the soil solution, thus reducing its bioavailability; such processes would favor plant growth, since the uptake of Cu by the roots occurs preferentially in the free form (McBride, 1994; De Conti et al., 2016).

Copper affected also plant growth both in terms of height and total dry matter, yet only in the monocropping systems and in the 80 mg kg^{-1} Cu treated intercropping systems (Figure 2). The intermediate Cu concentration (40 mg kg^{-1}) increased the plant height and total dry matter in the intercropping systems by 221 and 213% in grapevine + *Paspalum plicatulum* Michx., and 180 and 143% in grapevine + *Axonopus affinis* Chase, respectively. The relative

growth rate was higher in the intercropped systems compared to the monocropping/grapevine in the control and at the intermediate Cu concentration (Figure 2). The reduction in grapevine growth at high Cu levels is probably related to the lower photosynthetic carbon assimilation (Figure 1). Furthermore, the excess Cu can affect the function of membrane transporters and ion channels, reducing their selective capacity; the resulting nutritional imbalances might also contribute to the reduction of plant growth (Cambrollé et al., 2015).
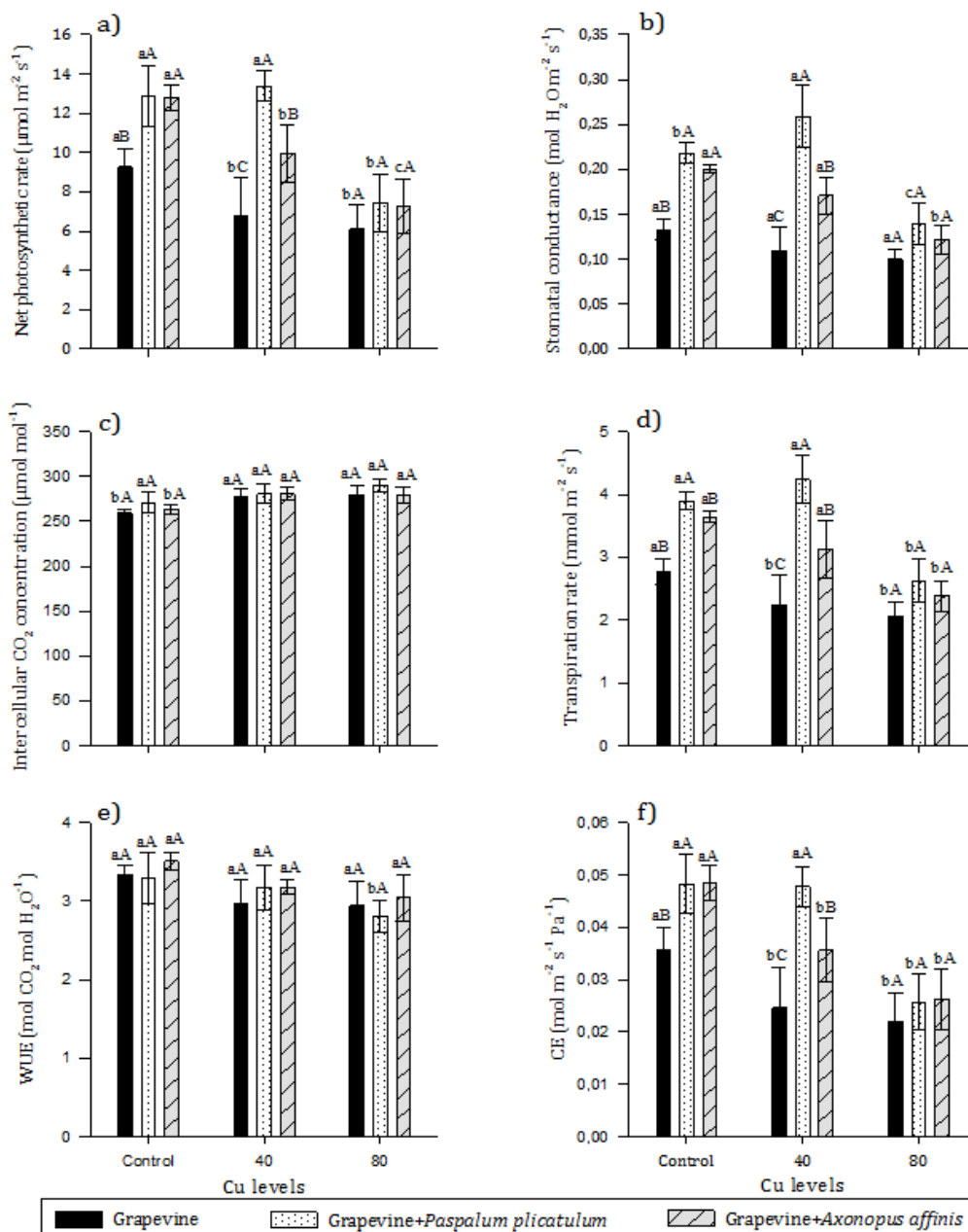

Figure 1. a) Net photosynthetic rate, b) stomatal conductance, c) intercellular CO_2 concentration, d) transpiration rate, e) water use efficiency and f) instantaneous carboxylation efficiency of young grapevines. Means followed by the same lowercase letter do not differ between Cu levels in the same cropping system. Means followed by the same uppercase letter do not differ between cropping systems in same Cu level (Scott-Knott test, $p<0.05$).
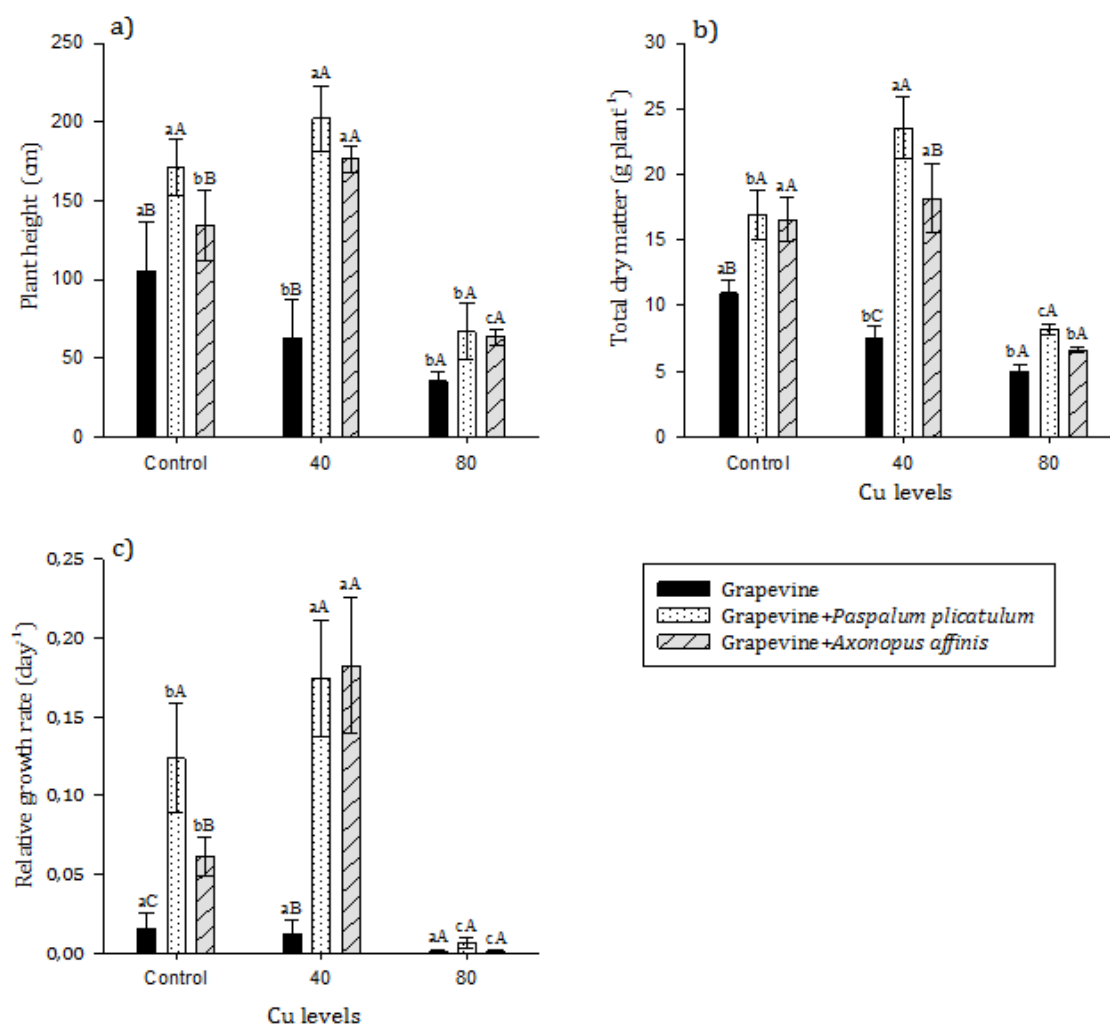
Figure 2. a) Plant height, b) dry matter yield, and c) relative growth rate of young grapevines. Means followed by the same lowercase letter do not differ between Cu levels in the same cropping system. Means followed by the same uppercase letter do not differ between cropping systems at the same Cu level (Scott-Knott test, $p < 0.05$).

CONCLUSION

The addition of Cu caused phytotoxicity in the single crop (Grapevine), reducing photosynthetic carbon assimilation and plant growth. The cultivation of young grapevines intercropped with native grasses, especially *Paspalum plicatulum* Michx., promoted the growth of the grapevines in the control soil as well as in the moderately Cu-contaminated soils. This indicates that maintaining native vegetation in young vineyards can reduce Cu toxicity to transplanted grapevines.

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