CIÊNCIA E TÉCNICA VITIVINÍCOLA

Ciência Téc. Vitiv. 38(1) 60-66. 2023

Article

ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION AS STRATEGY TO MITIGATE COPPER TOXICITY IN YOUNG FIELD-GROWN VINES

INOCULAÇÃO DE FUNGOS MICORRÍZICOS ARBUSCULARES COMO ESTRATÉGIA PARA ATENUAR A TOXICIDADE DO COBRE EM VIDEIRAS JOVENS CULTIVADAS NO CAMPO

Gustavo Brunetto[1], Anderson C. R. Marques[1,*], Edicarla Trentin[1], Paula B. Sete[2], Cláudio R. F. S. Soares[3], Paulo A. A. Ferreira[1], George W. B. de Melo[4], Jovani Zalamena[5], Lincon O. S. da Silva[1], Carina Marchezan[1], Isley C. B. da Silva[1], João P. J. dos Santos[1], Leticia Morsch[1]

[1]Federal University of Santa Maria (UFSM), Department of Soils, Santa Maria, RS, Brazil.

[2]Federal University of Santa Catarina (UFSC), Center for Agrarian Sciences, Florianópolis, SC, Brazil.

[3]Federal University of Santa Catarina (UFSC), Department of Microbiology, Immunology and Parasitology, Florianópolis, SC, Brazil.

[4]Embrapa Grape and Wine, Bento Gonçalves, RS, Brazil.

[5]Federal Institute of Education, Science and Technology of Rio Grande do Sul (IFRS), Restinga, RS, Brazil

***** Corresponding author: Tel.: (55) 9 99669293; e-mail: acrmarques@hotmail.com.br

(Received 10.2.2022. Accepted 27.04.2023)

SUMMARY

Frequent applications of foliar fungicides on grapevines increase copper (Cu) levels in soils, which may cause toxicity to young grapevines transplanted to eradicated old vineyards, especially because they are more sensitive. Therefore, it is necessary to adopt strategies to mitigate Cu toxic effects, such as grapevine seedlings inoculation with arbuscular mycorrhizal fungi (AMF), which establish symbiosis association with plants roots, preferably in field experiments. The present study we objective to evaluate AMF pre-inoculation effects on growth and mineral absorption of grapevine seedlings in a field experiment using sandy soil contaminated with Cu. The experiment was conducted in Santana do Livramento (RS), in a Typical Hapludalf soil. Initially, grapevine seedlings from 'Paulsen 1103' (*Vitis berlandieri* x *Vitis rupestris*) rootstock were AMP pre-inoculated and then transplanted into the field in area containing high Cu levels. Experimental design was a randomized complete block design, with a 4 x 2 factorial scheme: four seedlings pre-inoculation treatments (control = uninoculated seedlings; *Rhizophagus clarus* UFSC-14; *Rhizophagus intraradices* UFSC-32; *Dentisculata heterogama* UFSC-08); two soil Cu concentrations (62 mg/kg and 118 mg/kg (Mehlich-I), with five replications. Pre-inoculation did not favor growth of young vines in field after 316 and 500 days of transplantation (DAT). However, seedlings from pre-inoculated vines with *R. intraradices* UFSC-32 and *R. clarus* UFSC-14 presented lower Cu levels in leaves in the soils with high Cu levels. Thus, pre-inoculation of young vines seedling with selected AMF may represent a strategy to reduce Cu toxicity, contributing to plants establishment in contaminated soils.

RESUMO

Aplicações frequentes de fungicidas foliares em videiras aumentam os níveis de cobre (Cu) nos solos, o que pode causar toxicidade em videiras jovens transplantadas para vinhas velhas, especialmente porque são mais sensíveis. Por este motivo, é necessário adotar estratégias para mitigar os efeitos tóxicos do Cu, como a inoculação das novas plantas de videira com fungos micorrízicos arbusculares (FMA), que estabelecem associação de simbiose com as raízes das plantas, preferencialmente em ensaios de campo. Neste estudo nós objetivamos avaliar os efeitos da pré-inoculação de FMA no crescimento e absorção de minerais em plantas jovens de videira em ensaios de campo, em solo arenoso contaminado com Cu. O ensaio foi conduzido em Santana do Livramento (RS), em um solo Hapludalf Típico. Inicialmente, plantas jovens do porta-enxerto 'Paulsen 1103' (*Vitis berlandieri* x *Vitis rupestris*) foram pré-inoculadas com AMP e, em seguida, transplantadas para o campo, em área contendo altos teores de Cu. O delineamento experimental foi em blocos completos casualizados, com esquema fatorial 4 x 2: quatro tratamentos de pré-inoculação das plantas jovens (controle = plantas não inoculadas; *Rhizophagus clarus* UFSC-14; *Rhizophagus intraradices* UFSC-32 e *Dentisculata heterogama* UFSC-08); duas concentrações de Cu no solo (62 mg/kg e 118 mg Cu/kg (Mehlich-I), com cinco repetições. A pré-inoculação não favoreceu o crescimento de videiras jovens cultivadas em campo após 316 e 500 dias do transplante (DAT). No entanto, verificou-se que as plantas jovens de videiras pré-inoculadas com *R. intraradices* UFSC-32 e *R. clarus* UFSC-14 apresentaram menores teores de Cu nas folhas, quando instaladas em solos com altos teores de Cu. Assim, a pré-inoculação de plantas jovens de videira com FMA selecionados pode representar uma estratégia para reduzir a toxicidade do Cu, contribuindo para o estabelecimento de plantas em solos contaminados.

Keywords: Paulsen 1103, phytotoxicity, arbuscular mycorrhizae.
Palavras-chave: Paulsen 1103, fitotoxicidade, micorrizas arbusculares.

Article available at https://www.ctv-jve-journal.org or https://doi.org/10.1051/ctv/ctv20233801060

INTRODUCTION

Incidence of foliar fungal diseases, as mildew (*Plasmopara viticola*) is common in vines, and leads to reduced productivity and grape quality in traditional wine-growing regions, such as Rio Grande do Sul Gaucho Campaign (RS) in Brazil. Therefore, periodic foliar fungicides application, as bordeaux syrup ($Ca(OH)_2$+$CuSO_4$) or other fungicides, normally containing copper (Cu), is necessary (Miotto *et al*., 2014; Girotto *et al*., 2016; Brunetto *et al*., 2017). Over the years, successive applications of these compounds increased Cu concentration in vineyard soils, which causes toxicity to vines.

The Cu toxicity is commonly observed in young vines, especially when they are planted into old reformed vineyards of approximately 40 years-old because young grapes are sensitive to high levels of Cu. The soils of old vineyards before transplanting are usually mobilized for the incorporation of fertilizers. This practice accelerates the mineralization of organic matter as well as Cu availability. This occurs because a large part of Cu present in the soil is complexed in the organic matter (Komárek *et al*., 2010; Girotto *et al*., 2016).

Despite being a micronutrient, Cu presence in high levels in soil can cause anatomical and morphological changes in root system, as reduction in cellular mitotic division rate, impairing root growth, cellular plasmolysis and tissue darkening, shortening and increased root thickness, especially in apex region, impairing water and nutrients absorption, and reflecting in a lower growth of plants aerial part and altered nutrients concentration in tissues (Zambrosi *et al*., 2013; Ambrosini *et al*., 2015a). Besides, excess of Cu in the tissues can interfere with photosynthetic complex biosynthesis and enzyme activation, reducing photosynthetic activity, and may cause cell structure damage due to oxidative stress, as a result of increased reactive oxygen species concentration (ROS) (Zhang *et al*., 2014; Cambrollé *et al*., 2015).

Thus, it is necessary to adopt management strategies in order to reduce Cu's toxicity potential to growing young vines. One solution is the arbuscular mycorrhizal fungi (AMF) inoculation, which establishes a mutualistic association with plants root system (Smith and Read, 2008; Ambrosini *et al*., 2015b; Trouvelot *et al*., 2015; Brunetto *et al*., 2016). The AMF can develop strategies to tolerate soil high Cu levels, as organic substances production and release, as the protein, glomalin, which can complex with soil Cu, thus reducing its availability and, consequently its uptake (González-Chávez *et al*., 2004; Bedini *et al*., 2010; Ambrosini *et al*., 2015b). Also, AMF can store Cu in its structures, vesicles and spores, minimizing the toxicity to plant host (Cornejo *et al*., 2013). In addition, AMF can increase nutrients aerial absorption surface of roots, especially those nutrients with low soil mobility, such as P (Smith and Read, 2008; Ambrosini *et al*., 2015b; Santana *et al*., 2015). Studies with vines have already been performed using AMF to attenuate heavy metals, such as Cu. However, studies are usually performed under controlled conditions, such as in greenhouse (Karimi *et al*., 2011; Forgy *et al*., 2012; Ambrosini *et al*., 2015a). In this sense, studies carried out on vines grown in acid soils and with high Cu levels, and AMF pre-inoculated, are still scarce in international literature. In this study we objective to evaluate AMF pre-inoculation effects on grapevine seedlings growth and nutritional absorption in field experiment in a sandy soil contaminated with Cu.

MATERIALS AND METHODS

The experiment was performed in a vineyard located in an Sandy Dystrophic Red Argisoil soil (EMBRAPA, 2018), located in Santana do Livramento, in RS Gaucho Campaign region (Latitude 30º 48 '31' S; Longitude 55º 22 '33' ' W). The experimental area is derived from an eradicated vineyard with approximately 15 years of cultivation, followed by a two-year maintenance history with Pampa Biome native grasses. Before experiment installation, soil from 0-20 cm layer had the following physical-chemical characteristics: 140 g/kg clay; 8.0 g/kg organic matter; pH in H_2O (1:1) = 6.3; Ca, Mg and Al, 14.1, 4.5 and 0.0 cmolc/L, respectively (extracted by KCl 1 mol/L); 22.1 mg/L available P and 94.0 mg/L available K (both extracted by Mehlich-1); 62.11 mg/L Cu available (extracted by Mehlich-1). In August 2014, the experimental area was divided into two parts; in the first part, no Cu was added and, in the other half 125 kg of Cu sulfate ($CuSO_4.5H_2O$) was applied in order to increase soil Cu concentration to values close to those observed in vineyards with 30 years of cultivation and foliar Cu applications. Thus, in August 2015, soil from 0-20 cm layer artificially contaminated with Cu (+Cu) presented 118 mg Cu/kg, a toxic level for vines, according with Girotto *et al*. (2016), and Cu concentration in native soil (-Cu) of the order of 62 mg Cu/kg (both extracted by Mehlich-1).

'Paulsen 1103' grapevine rootstocks (*V. berlandieri* x *V. rupestris*) were harvested from cuttings in 200 mL tubes containing particulate substrate, sterilized at 121 °C for 40 minutes, based on thermophilic organic compound. The seedlings remained in a greenhouse with natural light and irrigation for 10 months, where they reached about 30 cm and 7 to 8 leaves.

For fungi were multiply in greenhouse on soil cultivated with *Brachiaria decumbens*. The soil-inoculum employed consisted of a mixture of colonized roots and AMF propagules, which contained 109, 350 and 17915 spores per 50 mL of soil-inoculum of *Dentisculata heterogama*, *Rhizophagus intraradices* and *Rhizophagus clarus*, respectively. For AMF spores counting in inoculum soil, the wet sieving method described by Gerdemann and Nicolson (1963) was used, using a stereoscopic microscope (40x).

In April 2015, rootstocks were pre-inoculated. For this purpose, a small part of soil was removed from tubes surface and 10 mL of soil-inoculum was added, mixing it with existing soil. Subsequently, another 10 mL of original vines substrate were added so as to cover applied soil-inoculum. At the end of the process, AMF not inoculated seedlings (control) and AMF inoculated seedlings with *R. clarus* - UFSC-14, *R. intraradices* - UFSC-32 and *D. heterogama* - UFSC-08 isolates were obtained. Isolates were provided by the Soil Microbiology Laboratory from Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina state, Brazil. Used AMF isolates were selected for benefiting vine growth under controlled conditions (Ambrosini *et al*., 2015b; Rosa *et al*., 2016).

Four months after inoculation, vines were transplanted to field using a 3.0 x 1.20 m spacing (2.778 plants/ha), in areas with different levels of Cu in the soil. The experimental design was a randomized block design, in a 4 x 2 factorial scheme (four treatments of seedlings pre-inoculation and two soil Cu concentrations), with five replications, and the vineyard was installed in a backrest system. Each replicate was composed of five plants, and evaluations were performed in the three central plants of each treatment repetition. At the time of experiment implementation, vineyard predominant vegetation was forkgrass (*Paspalum notatum*), while ryegrass (*Lolium multiflorum*) and white clover (*Trifolium repens*) predominated in the winter season. Vegetation on planting line was mowed to 10 cm height, with the residues deposited on soil. Applications of 29 g KCl (60% K_2O) and 48 g N (urea 44% N) per plant, equivalent to 48 kg K/ha and 1333 kg N/ha were carried out at 103 and 292 days after transplantation (DAT). Fertilizers were applied on soil surface in the canopy projection region, without incorporation.

At 316 DAT (1st evaluation) and 500 DAT (2nd evaluation) plant height was measured using a tape measure. In addition, leaves were collected from the middle third of the plants, dried in a greenhouse with forced air circulation at 65 °C until reaching a constant mass, ground and submitted to nitroperchloric digestion (EMBRAPA, 2009). In the resulting extract, Cu concentration was determined by atomic absorption spectrometer (EAA, Varian SpectrAA-600, Australia) and P in a spectrometer (Pro Analysis UV 51-00, Brazil) by the colorimetry method, with an absorbance of 882 nm, according to Murphy and Riley (1962) and Tedesco *et al*. (1995). Obtained results were submitted to analysis of variance by SISVAR v. 4.0 software (Ferreira, 2019), and, when significant, the Tukey averages comparison test was applied at 5% of error.

RESULTS AND DISCUSSION

Grapevine seedlings height was significantly influenced by pre-inoculation factors ($p<0.05$) and soil Cu contamination ($p<0.05$), while no significant effect of the interaction between these factors was verified (Table I). Regardless of pre-inoculation treatments, it was observed that plants height was 12.5% higher in -Cu soil compared to Cu supplemented soil (+ Cu). Increase Cu availability can cause plants toxicity, since it is known that Cu excess in soil can cause anatomical and morphological changes in root system, compromising water and nutrients absorption from soil, which can, in turn, hinder vines growth and development (Ambrosini *et al*., 2015a, b). Regardless of the addition of Cu to the soil, it was found that the seedlings of non-inoculated vines (control) presented heights on the order of 70 and 20% greater than the average of the pre-inoculated treatments at 316 and 500 DAT, respectively (Table I). Negative effects of AMF inoculation on plant growth are quite unusual (Moreira and Siqueira, 2006), and it is known that plant height is strongly influenced by environmental conditions, such as temperature, luminosity and soil moisture, during the plant cycle. Several works conducted under controlled conditions, including those employing Cu contaminated soils, demonstrated AMF inoculation benefits to plant growth when evaluating shoot and root biomass production (Ambrosini *et al*., 2015b; Rosa *et al*., 2016). Considering that the present study was conducted in the field and was intended to be used as a model for monitoring grapevine development in a long-term trial, it was not possible to collect plants in order to determine biomass production. In this trial, AMF isolates that benefited grapevine growth under field conditions were tested (Ambrosini *et al*., 2015b; Rosa *et al*., 2016), and it is expected that grapevine pre-inoculation leads to a greater root colonization, facilitating plants establishment after long exposure to Cu excess in the field.

Table I

Pre-inoculated vine seedlings height with arbuscular mycorrhizal fungi (AMF) at 316- and 500-days post-transplant (DAT) in the field in soils with (+ Cu) or without (-Cu) Cu supplementation

Treatments (AMF)	Height (cm)	
	1st evaluation (316 DAT)	2nd evaluation (500 DAT)
Control (not inoculated)	27.25 a[(1)]	52.15 a
Dentisculata heterogama X UFSC 08	16.95 b	41.15 c
Rhizophagus clarus XUFSC 14	16.24 b	44.13 bc
Rhizophagus intraradices X UFSC 32	14.90 b	45.57 b
+ Cu (118 mg Cu/kg)	-	43.06 b[(2)]
- Cu (62 mg Cu/kg)	-	48.44 a

[(1)] Means within the same column followed by different lowercase letters are significantly different ($p<0.05$) by Tukey averages comparison test. [(2)] No interaction between factors (treatments x with and without Cu addition).

However, it should be considered that, over time, there might be competition between introduced AMF and native AMF communities of low symbiotic efficiency, reducing the possible contribution of selected fungal isolates. All these aspects highlight the complexity involved in plant-climate-soil-microorganism interactions, justifying long-term studies as the present one.

Considering that AMF are known are known to assist P uptake by plants, Table II shows P and Cu concentrations in leaves from AMF pre-inoculated seedlings. The results reveal that, at 316 DAT, P concentrations in leaves were, on average, 37% higher in -Cu soil than in +Cu soil. In the second evaluation period (500 DAT), plants inoculated with *D. heterogama* and *R. clarus* present P concentrations similar to the control, but increasing the concentration of P in relation to the first period. Therefore, soil Cu contamination can reduce P absorption capacity by plants in the establishment of seedlings in the field in the initial stage, but over time the AMF may play an important role in supplying this nutrient to plants (Ferreira *et al.*, 2015). These outcomes may be related to the effect of mycorrhization on plant growth, which is predominantly nutritional. Such an effect is promoted by the branching of the hyphae and by the external mycelium of AMF, which increase the volume of soil explored in regions that the roots do not reach, thus increasing the absorption of nutrients, especially P, acting as a mechanism to attenuate Cu toxicity (Ferreira *et al.*, 2015). This behavior was observed for *R. clarus* - UFSC 14 and *R. intraradices* - UFSC 32 isolates in a study developed by Ambrosini *et al.* (2015a), on young vines in a greenhouse. Micorrhized vines seedlings grown in soils with excess Cu may have a massive root system, which may stimulate insoluble metal-phosphate complex formation, reducing P transport to shoot organs, including leaves (Soares and Siqueira, 2008; Zambrosi *et al*., 2013; Guimarães *et al*., 2016).

In general, Cu leaf concentrations at 500 DAT were higher than those observed at 316 DAT (Table II), evidencing the effects of soil contamination exposure on Cu absorption by grapevine seedlings. According to Kabata-Pendias (2011), Cu leaf concentrations between 5 and 20 mg/kg are considered normal, while levels between 20 and 100 mg kg/Cu are considered toxic (Silva *et al*., 2022; Kirkby, 2012). Thus, vine shoots prolonged exposure to Cu contaminated soils may lead to plant toxicity, and strategies to reduce tissue concentrations may favor seedlings survival in the field. In the present study, pre-inoculated vines seedlings were found to have lower leaf concentrations than those that were not inoculated, especially in *R. clarus* X UFSC 14 and *R. intraradices* X UFSC 32 treatments (Table II), corroborating results obtained by Ambrosini *et al*. (2015b), using the same fungal isolates on vines under controlled conditions.

The AMF associated benefits may be related to these fungi ability to tolerate high Cu levels in soils, especially mechanisms involved in the release of glycoproteins called glomalines, that are able to form complexes with Cu in soil.

Table II

Cu and P concentrations in leaves of pre-inoculated vine with arbuscular mycorrhizal fungal isolates (AMF) 316- and 500-days post-transplant (DAT) in soil with (+ Cu) or without (-Cu) supplementation.

Treatments (AMF)	Cu concentration in leaves (mg/kg)		P concentration in leaves (g/kg)	
	- Cu	+ Cu	- Cu	+ Cu
	1st evaluation (316 DAT)			
Control (not inoculated)	5,76 a(1)A(2)	5.52 aA	4.31 aA	3.78 aB
Dentisculata heterogama – UFSC 08	2.17 cA	1.86 cA	3.40 bA	2.31 bB
Rhizophagus clarus – UFSC 14	3.18 bB	4.44 bA	3.24 bA	1.82 cB
Rhizophagus intraradices – UFSC 32	3.28 bA	2.06 cB	2.30 cA	1.76 cB
	2nd evaluation (500 DAT)			
Control (not inoculated)	5.17 aB	7,60 aA	1.68 a(3)	
Dentisculata heterogama – UFSC 08	5.73 aA	5.57 bA	1.76 a	
Rhizophagus clarus – UFSC 14	4.09 bB	4.80 bcA	1.73 a	
Rhizophagus intraradices – UFSC 32	3.90 bA	4.55 cA	1.54 b	

(1) Means within the same column followed by different lowercase letters are significantly different ($p<0.05$) by Tukey averages comparison test. (2) Means within the same column followed by different uppercase letters are significantly different ($p<0.05$). (3) No interaction between factors (treatments x with and without Cu addition) and, therefore, only the difference between treatments were shown.

Additionally, AMF typical structures, as extraradicular hyphae, spores and vesicles, can act in retaining excess Cu (Cornejo *et al*., 2013), reducing its transfer to plants with consequent toxicity reduction.

CONCLUSIONS

Grapevine seedlings pre-inoculation with arbuscular mycorrhizal fungi (AMF) did not influence plant height at 500 days after transplanting to in Cu contaminated vineyards. On the other hand, pre-inoculation with selected AMF *Rhizophagus clarus* X UFSC 14 and *Rhizophagus intraradices* X UFSC 32 reduced significantly the Cu concentration after a long period of exposure. Thus, AMF pre-inoculation technique represents an apparently valid strategy for vine shoots establishment in Cu contaminated vineyards.

ACKNOWLEDGEMENTS

The authors thank Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (Fapergs, 17/2551-0000925-8), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Project 408318/2018-0) and Financiadora de Estudos e Projetos (Finep) for financial resources made available. The authors are also grateful to CNPq for first author’s Research Productivity Grant, to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and to CNPq for postgraduate grants, and to Fapergs for scientific initiation grants.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES

Ambrosini V.G., Rosa D.J., Corredor Prado J.P, Borghezan M, Bastos de Melo G.W., Soares C.R.F.S., Comin J.J., Simão D.G., Brunetto G., 2015a. Reduction of copper phytotoxicity by liming: A study of the root anatomy of young vines (*Vitis labrusca* L.). *Plant Physiol. Biochem.*, **96**, 270–280.

Ambrosini V.G., Voges J.G., Canton L., Couto R., Ferreira P.A., Comin J.J., Bastos de Melo G.W., Brunetto G., Roberto C., Sousa F., 2015b. Effect of arbuscular mycorrhizal fungi on young vines in copper-contaminated soil. *Environ. Microbiol.,* **1052**, 1045–1052.

Bedini S., Turrini A., Rigo C., Argese E., Giovannetti M., 2010. Soil Biology & Biochemistry Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice. *Soil Biol Biochem*., **42**, 758–765.

Brunetto G., Bastos de Melo G.W., Terzano R., Del Buono D., Astolfi S., Tomasi N., Pii Y., Mimmo T., Cesco S., 2016. Copper accumulation in vineyard soils: Rhizosphere processes and agronomic practices to limit its toxicity. *Chemosphere.,* **162**, 293–307.

Brunetto G., Ferreira P.A.A., Melo G.W., Ceretta C.A., Toselli M., 2017. Heavy metals in vineyards and orchard soils. *Rev Bras Frutic.*, **39**, 1–12.

Cambrollé J., García J.L., Figueroa M.E., Cantos M., 2015. Evaluating wild grapevine tolerance to copper toxicity. *Chemosphere.*, **120**, 171–178.

Cornejo P., Pérez-tienda J., Meier S., Valderas A., Borie F., Azcón-aguilar C., Ferrol N., 2013. Soil Biology & Biochemistry copper compartmentalization in spores as a survival strategy of arbuscular mycorrhizal fungi in Cu-polluted environments. *Soil Biol Biochem*., **57**, 925–928.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária., 2009. Manual de análises químicas de solos, plantas e fertilizantes. 627 p. EMBRAPA Informação Tecnológica, Brasília.

EMBRAPA., 2018. Sistema brasileiro de classificação de solos. 355 p. Embrapa Solos, Rio de Janeiro.

Ferreira D.F., 2019. SISVAR: A computer analysis system to fixed effects split plot type designs. *Brazilian J Biometrics*., **37**, 529–535.

Ferreira P.A.A., Ceretta C.A., Soriani H.H., Tiecher T.L., Soares C.R.F.S., Rossato L.V., Nicoloso F.T., Brunetto G., Paranhos J.T., Cornej P., 2015. Rhizophagus clarus and phosphate alter the physiological responses of Crotalaria juncea cultivated in soil with a high Cu level. *Appl. Soil Ecol.*, 91, 37-47.

Ferreira P.A.A., Tiecher T., Tiecher T.L., Rangel W.M., Soares C.R.F.S., Deuner S., Tarouco C.P., Giachini A.J., Nicoloso F.T., Brunetto G., Coronas M.V., Ceretta C.A., 2018. Effects of Rhizophagus clarus and P availability in the tolerance and physiological response of *Mucuna cinereum* to copper. *Plant Physiol. Biochem.*, 122, 46-56.

Forgy D., 2012. Arbuscular mycorrhizal fungi can benefit heavy metal tolerance and phytoremediation. *J Nat Resour Life Sci Educ*., **41**, 2008–11.

Gerdemann J.W., Nicolson T.H., 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Trans Br Mycol Soc*., **46**, 235–244.

Girotto E., Ceretta C.A., Rossato L.V., Farias J.G., Brunetto G., Miotto A., Tiecher T.L., de Conti L., Lourenzi C.R., Schmatz R., Giachini A., Nicoloso F.T., 2016. Biochemical changes in black oat (*Avena strigosa* schreb) cultivated in vineyard soils contaminated with copper. *Plant Physiol Biochem*., **103**, 199–207.

Guimarães P.R., Ambrosini V.G., Miotto A., Ceretta C.A., Simão D.G., Brunetto G., 2016. Black Oat (*Avena strigosa Schreb*.) growth and root anatomical changes in sandy soil with different copper and phosphorus concentrations. *Water, Air, Soil Pollut*., **227**, 192–202.

Kabata-Pendias A., 2011. Trace Elements in Soils and Plants. 520 p. CRC Press, Boca Ratón, Florida.

Karimi A., Khodaverdiloo H., Sepehri M., Rasouli-sadaghiani M., 2011. Arbuscular mycorrhizal fungi and heavy metal contaminated soils. *Afr. J. Microbiol. Res.*, **5**, 1571–1576.

Kirkby, E. (2012). Introduction, definition and classification of nutrients. *In Marschner's Mineral Nutrition of Higher Plants*. 3–5. Elsevier, EUA.

Komárek M., Eva Č., Chrastný V., Bordas F., Bollinger J., 2010. Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. *Environ Int*., **36**, 138–151.

Miotto A., Ceretta C.A., Brunetto G., Nicoloso F.T., Girotto E., Farias J.G., Tiecher T.L., De Conti L., Trentin G., 2014. Copper uptake, accumulation and physiological changes in adult grapevines in response to excess copper in soil. *Plant Soil*., **374**, 593–610.

Moreira F.M.S., Siqueira J.O., 2006. Microbiologia e bioquímica do solo. 744 p. Universidade Federal de Lavras, Minas Gerais.

Murphy J., Riley J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta*., **27**, 31-36.

Rosa D.J., Ambrosini V.G., Brunetto G., Roberto C., Sousa F., Borghezan M., Pescador R., 2016. Parâmetros fisiológicos em videiras 'Paulsen 1103' (*Vitis berlandieri* x *Vitis rupestris*) inoculadas com fungos micorrízicos arbusculares em solo contaminado com cobre. *Ciência Téc. Vitiv*., **31**, 14–23.

Santana N.A., Ferreira P.A.A., Soriani H.H., Brunetto G., Nicoloso F.T., Antoniolli Z.I., Jacques R.J.S., 2015. Interaction between arbuscular mycorrhizal fungi and vermicompost on copper phytoremediation in a sandy soil. *Appl Soil Ecol*., **96**, 172–182.

Silva, I.C.B., Somavilla, A., Soares, V.M. Tarouco, C.P., Schwalbert, R., Trentin, E., Quadros, F.L.F., Nicoloso. F.T., Ferreira, P.A.A., Brunetto, G. 2022. Potential phytoremediation of Pampa biome native and invasive grass species cohabiting vineyards contaminated with Cu in Southern Brazil. *Environ Sci Pollut Res*., **29**, 85376–85388.

Smith S.E., Read D.J., 2008. Mycorrhizal Symbiosis. 787 p. Academic Press, New York.

Soares C.R.F.S., Siqueira J.O., 2008. Mycorrhiza and phosphate protection of tropical grass species against heavy metal toxicity in multi-contaminated soil. *Biol. Fertil. Soils*., **44**, 833–841.

Tedesco M.J., Gianello C., Bissani C.A., Bohnen H., Volkweiss S.J., 1995. Análise de solo, plantas e outros materiais. 170 p. Departamento de Solos da Universidade Federal do Rio Grande do Sul, Porto Alegre.

Trouvelot S., Bonneau L., Redecker D., Tuinen D. Van., Adrian M., Wipf D., 2015. Arbuscular mycorrhiza symbiosis in viticulture: a review. *Agron Sustain Dev*., **40**, 1–11.

Zambrosi F.C.B., Mesquita G.L., Tanaka F.A.O., Quaggio J.A., Jr D.M., 2013. Phosphorus availability and rootstock affect copper-induced damage to the root ultra-structure of Citrus. *Environ. exp. bot.*, **20**, 1–27.

Zhang L., Pan Y., Lv W., Xiong Z., 2014. Physiological responses of biomass allocation, root architecture, and invertase activity to copper stress in young seedlings from two populations of *Kummerowia stipulacea* (maxim.) Makino. *Ecotoxicol Environ Saf*., **104**, 278–284.