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Technical Note

# Interactions of *Trametes versicolor*, *Coriolopsis rigida* and the arbuscular mycorrhizal fungus *Glomus deserticola* on the copper tolerance of *Eucalyptus globulus*

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ABSTRACT

The presence of high levels of Cu in soil decreases the shoot and root dry weights of *Eucalyptus globulus*. However, higher plant tolerance of Cu has been observed in the presence of the arbuscular mycorrhizal (AM) fungus *Glomus deserticola*. The hyphal length of *G. deserticola* was sensitive to low Cu concentrations, and the percentage of AM root colonisation and the metabolic activity of the AM fungus were also decreased by Cu. Therefore, a direct effect of Cu on the development of the AM fungus inside and outside the root cannot be ruled out. *E. globulus* colonised by *G. deserticola* had higher metal concentrations in the roots and shoots than do non-mycorrhizal plants; however, the absence of a higher root to shoot metal ratio in the mycorrhizal plants (1.70 ± 0.11) indicated that *G. deserticola* did not play a filtering/sequestering role against Cu. The saprobe fungi *Coriolopsis rigida* and *Trametes versicolor* were able to remove Cu ions from the asparagine–glucose growth media. However, plants inoculated with *C. rigida* and *T. versicolor* did not accumulate more Cu than non-inoculated controls, and the growth of the plant was not increased in the presence of these fungi. However, *C. rigida* increased the shoot dry weight, AM root length colonisation, and metabolic mycelial activity of plants colonised with *G. deserticola* in the presence of Cu; only this saprobe-AM fungus combination increased the tolerance of *E. globulus* to Cu. Inoculation with *G. deserticola* and *C. rigida* increased the *E. globulus* Cu uptake to levels reached by hyperaccumulative plants.



## 1. Introduction

Cu is essential for plant development and growth. However, excessive Cu can lead to root elongation and cause damage to root membranes. Moreover, it may cause toxicity by interfering with photosynthesis, respiratory processes, and protein synthesis (Marschner, 1995). White-rot fungi have to cope with toxic levels of metal ions such as Cu often during their growth in soil. Relatively few studies have been done using white-rot fungi in bioremediation of Cu-contaminated soils (Baldrian, 2003). The white-rot fungi belonging to the *Trametes* and *Coriolopsis* genera have been used to detoxify metal effluents from agroindustrial wastes (Barajas-Aceves et al., 2002). These saprobe fungi are able to remove heavy metals such as Cu by adsorbing them on their mycelia, and the degree of accumulation and tolerance of Cu from soil differs in different species of these fungi (Saglam et al., 1999). It is known that white-rot fungi increase the growth of plants, especially when plants are cultivated in soils contaminated with agroindustrial wastes (Aranda et al., 2006).

The arbuscular mycorrhizal (AM) fungi are a substantial component of the soil microbial biomass. This symbiosis benefits plant growth, particularly through enhanced phosphorus, water, and mineral nutrient uptake (Smith and Read, 1997). AM fungi improve plant resistance to the presence of high quantity of heavy metals such as Cu in the soil. However, the effect of AM fungi on the uptake of metals by plants is not yet totally clear. AM isolates can increase or decrease metal uptake and accumulation in shoots or in roots or can increase or reduce heavy metal translocation from roots to shoots (Joner and Leyval, 2001; Chen et al., 2003).

Phytoremediation, the use of plants to remove toxic metals from soils is emerging as a potential strategy for cost-effective and environmentally friendly remediation of contaminated soils (Glass, 2000). Some plants can accumulate high concentrations of heavy metals and have been used in experimental assays for the phytoremediation of contaminated soils (McGrath et al., 2002). Many of the accumulative plants used belong to the family *Brassicaceae*; this family does not form AM symbiosis. As many hyperaccumulating plant families are herbaceous and non-mycorrhizal, considerable scepticism exists about the functional importance of AM in highly tolerant hyperaccumulating plants, but nevertheless its functionality was recently confirmed (Regvar and Vogel-Mikus, 2008). However, these herbaceous plants produce little biomass, so

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they are of less interest than plants with higher productivity, such as trees (Greger and Landberg, 1999). *Eucalyptus* is a tree species with a wide plasticity to grow in impoverished or marginal soils and is able to accumulate high quantities of heavy metals (Arriagada et al., 2004). Studies of AM fungal symbiosis in trees are scarce (Wilkinson and Dickinson, 1995), but the *Eucalyptus* species were able to develop AM symbiosis (Arriagada et al., 2004). To our knowledge, there are few reports on the effect of AM fungi on Cu phytoextraction by high-biomass crops such as maize and *Eucalyptus* from Cu-contaminated soils (Arriagada et al., 2004; Wang et al., 2007). Cultivation of non-hyperaccumulating, highly mycorrhizal plants that produce large amounts of biomass, such as *Eucalyptus* and *Populus* trees, on contaminated soil is recommended as a phytoremediation practice to prevent food chain contamination because of their capacity to accumulate heavy metals in the stemwood (Leep and Dickinson, 1998; Arriagada et al., 2004; Komarek et al., 2008; Lingua et al., 2008).

In addition, it is known that soil microorganisms such as saprobe fungi affect AM symbiosis. Some experimental results confirm the existence of synergistic, neutral, and antagonistic effects of saprobe fungi on plant root colonisation by AM fungi (Fracchia et al., 1998).

The aim of this work is to determine if the interaction between AM and saprobe fungi increases the tolerance of *Eucalyptus* to high concentrations of Cu in soil.

## 2. Materials and methods

### 2.1. Arbuscular mycorrhizal (AM) fungi

The AM *Glomus deserticola* (Trappe, Bloss and Menge) from the Instituto de Investigaciones Agrobiológicas de Galicia was used.

### 2.2. Saprobe fungi

The saprobe fungi *Coriolopsis rigida* and *Trametes versicolor* were isolated by the particle washing method using a multichamber washing apparatus (Widden and Bisset, 1972). These fungi were classified as described by McAllister (1992). Strains are kept at the fungal culture collection of the Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera in Temuco, Chile. Both saprobe fungi were transferred to tubes of 39 g $L^{-1}$ potato dextrose agar (PDA, DIFCO) and 2% malt extract at 4 °C as stock culture.

### 2.3. In vitro experiments

The effect of Cu on spore germination and hyphal length of *G. deserticola* was tested in 9-cm diameter plastic Petri dishes. The spores of *G. deserticola* were surface-sterilised as described by Mosse (1962). Ten surface-sterilised spores per plate were placed 1 cm from the edge of a Petri dish with 10 mL of 10 mM 2-(N-morpholin) ethane sulphonic acid buffer (pH 7) plus 0.04 g of Gel-Gro (ICN Biochemicals, Aurora, OH, USA). The quantity of 39.6, 79.2, 118.8, 237.6 and 396.5 mg of $CuSO_4{\cdot}5H_2O$ were added to Petri dishes before the solidification medium to reach a final concentrations of 0, 10, 20, 30, 60, and 100 mg Cu $L^{-1}$. The plates were incubated at 25 °C in the dark for 21 d and were sealed to reduce dehydratation and contamination. Hyphal length of the germinated *G. deserticola* spores was determined under a binocular stereo microscope (Olympus SZ-PT) at 40× magnification at the end of the experiment using the gridline intersect method (Marsh, 1971). Ten replicates petri dishes with 10 spores each were used and all the fungal mycelia were measured.

An aqueous suspension in sterile distilled water containing mycelium of the saprobe fungi was prepared from cultures grown in PDA for 1 week at 27 °C. Two ml of this suspension were added to 250-mL flasks containing 125 mL of sterile AG liquid medium (Galvagno, 1976) in a shaker at 28 °C. The AG medium consisted of 1 g glucose, 0.4 g asparagine, 0.05 g $MgSO_4$, 0.05 g $KH_2PO_4$ and 100 mL distilled water. The quantity of 0.039, 0.39, 1.98 and 3.96 g of $CuSO_4{\cdot}5H_2O$ were added to AG medium to reach a final concentration of 0, 10, 100, 500, and 1000 mg Cu $L^{-1}$. The concentration of Cu was analysed in the AG medium after 2 week culture of *C. rigida* and *T. versicolor* by atomic absorption spectroscopy (Perkin-Elmer 5380, Norwalk, CT, USA) after microwave digestion with a mixture of $H_2SO_4$ and $H_2O_2$ (Mingorance, 2002). Ten replicates were used in these experiments.

### 2.4. Greenhouse experiments

The experiments were carried out using Eucalyptus (*Eucalyptus globulus* Labill) as test plants. Seeds were surface-sterilised with $HgCl_2$ for 10 min and thoroughly rinsed with sterilised water and sown in moistened sand. After germination, uniform seedlings were planted in 0.3-L pots (One seedling per pot), filled with a mixture of sterilised sand:soil at a proportion of 1:1 (v:v). The soil, classified as an Andisol (Acrudoxic Hapludands), is moderately acidic (pH 5.5) with good drainage and water infiltration. Plants were grown in a greenhouse with supplementary light provided by Sylvania incandescent and cool-white lamps, 400 E $m^{-2}$ $s^{-1}$, 400–700 nm, with a 16/8 h day/night cycle at 25/19 °C and 50% relative humidity. Plants were watered from below and fertilised every week with 10 mL of a nutrient solution plus 50 mg $L^{-1}$ of P (Hewitt, 1966). The AM fungal inoculum was a root-and-soil inoculum consisting of rhizosphere soil containing spores (approximately 1000 spores per 100 $g^{-1}$) and colonised root fragments of *Medicago sativa*. The inoculation plants were in amounts of 8 g of soil inoculum per pot, an amount that was predetermined to enable high levels of root colonisation. In order to restore the microbial population present in the soil inoculum, uninoculated plants were given a filtrate (Whatman No. 1 paper) of the inoculum containing common soil microflora that was free of AM fungal propagules.

We inoculated *E. globulus* pots with: (1) *G. deserticola*, (2) *C. rigida* or *T. versicolor*, and (3) *C. rigida* or *T. versicolor* plus *G. deserticola,* as well as we kept some seedlings as controls. Plants were inoculated at the time of transplanting (after 3 week of growth). The saprobe fungi were inoculated at the same time as was *G. deserticola*. The quantity of 0.039, 0.39, 1.98, 3.96 and 7.93 g of $CuSO_4{\cdot}5H_2O$ were applied to *E. globulus* pots to reach the concentrations of 0, 10, 100, 1000, up to 2000 mg Cu $kg^{-1}$ of sand:soil. Ten replicate pots per treatment and Cu concentration were used.

Plants were harvested after 12 week and dry mass was determined. After the harvest, two samples of fresh weight were taken from the entire root system at random. One of the samples was cleared and stained (Phillips and Hayman, 1970), and the percentage of root length colonisation with AM fungus was measured by the gridline intersect method (Giovannetti and Mosse, 1980). In the other sample, succinate dehydrogenase (EC 1.3.99.1) (SDH) activity was measured in fungal mycelia by the reduction of tetrazolium salts (Natrium blue tetrazolium from Sigma Chemicals) at the expense of added succinate (Succinic acid disodium salt from Fluka Analytical), (MacDonald and Lewis, 1978); the percentage of AM fungal mycelia with SDH activity was determined under a compound microscope (Ocampo and Barea, 1985).

We measured the following response variables; total Cu content in the root and shoot of 10 *E. globulus* seedlings per treatment. Cu concentrations were measured by atomic absorption spectroscopy (Perkin-Elmer 5380, Norwalk, CT, USA) after microwave digestion with a mixture of $H_2SO_4$ and $H_2O_2$ according to the procedure of Mingorance (2002).

We studied the following three main factors and their respective levels as follows AM fungal (control and *G. deserticola*), Sap-