The effect of mycorrhization with *Pisolithus tinctorius* on field growth of micropropagated plants of *Arbutus unedo* L.

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Fig. 1 – *Arbutus unedo L.* (strawberry tree)

INTRODUCTION

•*Arbutus unedo* L. (strawberry tree) grows spontaneously in Mediterranean ecosystems. Fruit production represents the major income for farmers. Adult plants have been selected for fruit production (Fig. 1) and micropropagated (Gomes & Canhoto 2009). Mycorrhizae can improve plant adaptation and tolerance to several types of biotic and abiotic stresses.

•Species of *Arbutus* (Ericaceae) form arbutoid mycorrhizae with several fungi which are also able to form ectomycorrhizae in association with other plant hosts (Massicotte *et al.*, 1993). In this work the effect of mycorrhization with *Pisolithus tinctorius* on field growth of micropropagated plants was evaluated.

MATERIALS AND METHODS

•Fungal isolates of *Pisolithus tinctorius* were obtained from sporocarps tissues collected in forest stands of *Quercus suber* and *Eucalyptus globulus*.

•Isolations were carried out through the culture of sporocarps tissues on Modified Melin Norkrans (MMN) agar culture medium at 30ºC (Jacob *et al.* 2001). Pure cultures were obtained in MMN liquid medium (glucose 1% w/v) at 30ºC. The mycelium was diluted in water (1:20; v/v), and then applied to the substrate (Fig. 2 - d, e, g).

•*In vitro* cloned plants of *A. unedo* through shoot proliferation were inoculated in the nursery with two inoculation treatments and compared to control plants (Tab. 1).

•Micropropagated plants (clone C1) were watered with either *P. tinctorius* mycelium (pure cultures in MMN liquid medium) or dry sporocarps (Tab. 1).

• At the nursery, before plantation roots were checked for the presence of mycorrhizae development.

•A field trial was established to test all the nursery inoculation treatments and fertilized plants: seedlings and 3 micropropagated clones (Fig. 2 - h).

•To study the effect of the inoculation treatments on plant growth, height was evaluated 2 and 4 months in the nursery and 20 months after plantation (Fig. 2 - i).

Table 1 – Inoculation treatments with *Pisolithus tinctorius* and the control .

	Type of inoculum tested *in vitro* clonal plants (C1)
C1M	Vegetative inoculum (mycelium) from sporocarps from a *Quercus suber* stand
C1S	Dry sporocarps from a *Eucalyptus globulus* stand
C1C	Control micropropagated plants

RESULTS

•Two months after inoculation no significant differences were found between mycorrhizal treatments and control plants in the nursery (Tab. 2).

•After 4 months in the nursery best results were achieved with dry sporocarps treatment (C1S) ($P<5\%$; Tab. 2). The control plants showed the lowest height increment value (ΔH; Tab. 2).

•At the nursery, before plantation the plants inoculated (C1M and C1S) showed mycorrhized roots.

•The survival rate in the field trial was about 96.8%, without significant differences between treatments (12 months after plantation).

•Twenty months after field trial establishment both mycorrhizal treatments tested in nursery improved plant growth (Tab. 3), but results were not significantly different.

•Seedlings and clone AL2 showed the lowest height average, even though they have been fertilized.

•Control plants (C1C) showed the lowest average of height increment after 20 months (Table 3; $P>0.05$).

•Inoculated plants in nursery (C1M and C1S) showed averages of height (H) and height increment (ΔH) higher than the control plants ($P>5\%$; Tab. 3).

CONCLUSIONS

•Addition of fertilizers is a common agricultural practice in field plantations by using a slow release fertilizer. Mycorrhization showed to be a more interesting option since it is cheaper, cleaner and improves the physiological conditions of the plants.

• Further studies are needed for fungal strains selection for their aggressiveness under nursery and field conditions, which is a requirement for a successful implementation of these methods.

•Mycorrhizal synthesis with edible fungi can improve not only the success of plant growth, but can also account for another source of income, for farmers.

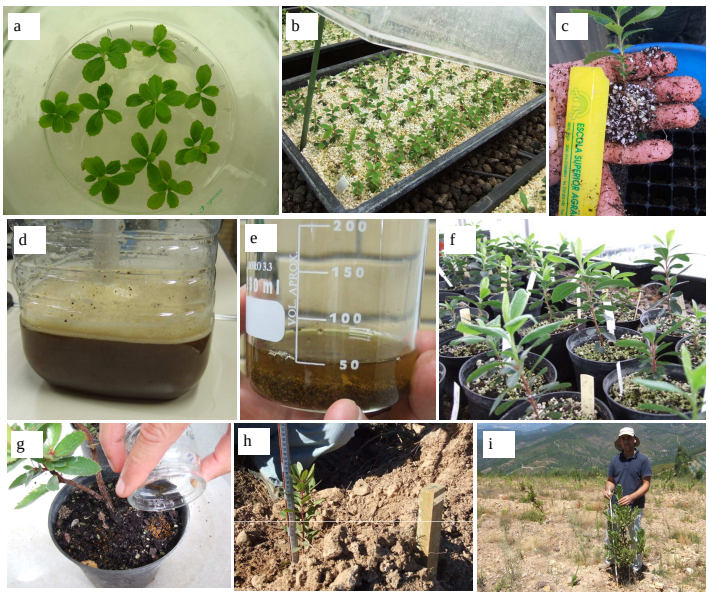

Fig. 2 - Inoculation of micropropagated plants with *P. tinctorius* in nursery. *A. unedo* cultures were *in vitro* multiplied (a). Plantlets were acclimatized (b) and then transferred to containers (c). Following growth of *P. tinctorius* vegetative inoculum, the mycelium and liquid medium were mixed (d) and diluted with water (e) for application to the acclimatized plants (f). After mycorrhization treatments (g) a field trial was established (h) and plant growth was evaluated (i).

Table 2 – Effect of different inoculation treatments with *P. tinctorius* on plants' height increment (ΔH) evaluated 2 and 4 months after inoculation under nursery conditions.

Treatments	Δ H 2 months (cm)	Δ H 4 months (cm)
C1 C - control plants	10.7±0.4[a]	14.4±0.7[b]
C1 M - with mycelium *in vitro* produced	11.0±0.4[a]	16.0±0.6[b]
C1 S - dry sporocarps water mixed	10.8±0.3[a]	19.1±0.7[a]

In each column values (mean ± SE) followed by different letters are significantly different ($P\leq0.01$).

Table 3 – Effect of the different treatments on plants height and height increment (ΔH) evaluated 20 months after field trial establishment

Mycorrhizal treatments	H (20 months) mean ± SE (cm)	Δ H ($H_{20} - H_0$) mean ± SE (cm)
AL1 - selected clone	76.6 ± 7.2 [a]	58.2 ± 7.7 [a]
AL2 - selected clone	68.6 ± 5.8 [a]	55.1 ± 5.8 [a]
AL3 - selected clone	75.3 ± 5.7 [a]	63.2 ± 6.0 [a]
SE - seedlings	68.6 ± 5.3 [a]	57.7 ± 4.8 [a]
C1 C - control plants	76.2 ± 6.7 [a]	54.8 ± 6.9 [a]
C1 M - mycelium *in vitro* produced	83.0 ± 4.7 [a]	62.0 ± 4.6 [a]
C1 S - dry sporocarps water mixed	84.7 ± 5.1 [a]	60.8 ± 5.0 [a]

In each column values (mean±SE) followed by same letters are not significantly different ($P\leq0.05$).

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