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Enhanced growth of wild cherry using micropropagated plants and mycorrhizal inoculation

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Abstract – Mycorrhizal inoculation is a promising, sustainable technique to enhance plant growth. We evaluated the effects of mycorrhizal inoculation and of the use of two substrates, soil and peat, on the growth of wild cherry, *Prunus avium* L., on the weaning and post-weaning. After weaning, plants were grown for 13 weeks in a greenhouse on either 40% soil or 40% peat at two levels of fertiliser: 2 or 4 g m⁻³ of a 16:9:12 slow-release fertiliser. They were subsequently kept for a further 120 days in a frost-free greenhouse before outplanting to the field. The results show that enhanced plant growth after seven months in the field was associated with increased peat and fertiliser levels in the substrates during the post-weaning growth phase, and with prior mycorrhizal inoculation by a *G. deserticola* isolate, which compensated for less favourable substrate conditions. Plants inoculated with *G. intraradices* had more branches positioned in the lower half of the stem, while plants inoculated with *G. deserticola* had more branches in the upper half of the stem.

arbuscular mycorrhiza / plant architecture / wild cherry / field outplanting

1. INTRODUCTION

Microbial inoculation is a promising, sustainable technique to enhance plant growth (e.g. Rodríguez-Romero et al., 2005; Barneix et al., 2005; Fauzia et al., 2006). Production of micropropagated plants is one of the most promising fields for application for arbuscular mycorrhizal fungus (AMF) inoculation (Lovato et al., 1996; Azcón-Aguilar et al., 1997). Micropropagated plants are produced in axenic conditions and generally weaned in artificial and/or disinfected substrates, therefore requiring reintroduction of the symbiotic fungi for enhanced plant growth, especially in artificial or disinfected soils/substrates (Pons et al., 1983; Saggin and Lovato, 1999), or for protection against root pathogens (Cordier et al., 1996, 1998). There can also be an effect of AMF inoculation on flowering (Scagel, 2004) and root architecture (Azcón-Aguilar et al., 1996; Locatelli et al., 2002), which can have a significant effect on plant yield and quality. Whilst several reports have shown enhanced growth and development of micropropagated plants due to AMF inoculation (Lovato et al., 1996), most of them are restricted to the weaning phase or to very early stages of growth. The few investigations that have been conducted under field conditions (Vestberg, 1992; Siqueira et al., 1997) showed greater growth and yield in mycorrhizal plants, which probably resulted from advantages obtained from AMF inoculation during early growth.

Substrate composition and AMF isolates can differentially determine subsequent development of micropropagated plants (Estaún et al., 1999; Zemke et al., 2003). Concentrations of nutrients, sugar and hormones used during weaning or in the post-weaning periods can also interact with the establishment of the mycorrhizal association (Dutra et al., 1996; Souza et al., 1996). In a previous work, we reported that beneficial effects of mycorrhizal inoculation on growth of micropropagated wild cherry and ash during the weaning phase disappeared when plants were transferred to substrates with fertiliser levels used in commercial nurseries (Lovato et al., 1994). This was probably due to the high levels of nutrients in the growth media. We evaluated interactions between substrate composition and arbuscular mycorrhizal inoculation in wild cherry plants grown under controlled conditions, and their effect on field growth of the same plants in a non-disinfected soil, containing indigenous AMF propagules.

2. MATERIALS AND METHODS

Wild cherry (*Prunus avium* L.) cv. F12/1 plants were micropropagated as previously described (Hammatt and Grant, 1993). Microplants with one or two root primordia were transferred to trays containing a 2:1:1 (v:v:v) mixture of clay loam soil, perlite and gravel (Lovato et al., 1994). The soil, from

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Domaine d'Époisses (Dijon, France) had the following properties: pH 6.8; 26 g kg⁻¹ organic matter; 203 mmol_c dm⁻³ CEC; 106 mg kg⁻¹ Olsen-P). In mycorrhizal treatments, soil was replaced by soil-based inocula of *Glomus intraradices* Schenck and Smith (BEG 141), or *Glomus deserticola* Trappe, Bloss and Menge (BEG 73, supplied by C. Azcón-Aguilar, Zaidin, Spain). Inoculum potential, evaluated by the Most Probable Number Method (MPN), was 6600 (1904–22880) propagules kg⁻¹. Plants were weaned for 21 days in a controlled environment room (12 h/day, 220 µmol m⁻² s⁻², 19–22 °C, 70% relative humidity), and then transferred to 2-L pots in a greenhouse (July–October, 19–24 °C, light supplemented to 16 h a day). Two steam-disinfected substrates and two levels of fertilisation, equivalent to those adopted by nurserymen, were used. One substrate consisted of 40% clay loam (Époisses) soil (v:v), 20% commercial-grade black sphagnum peat, 20% wood chips (Sylvagrest, France) and 20% gravel, and the other substrate of 20% clay loam soil (v:v), 40% peat, 20% wood chips and 20% gravel. Fertiliser levels corresponded to 2 and 4 kg m⁻³ of slow-release fertiliser (Osmocote®, Sierra Chemical, The Netherlands) with a final NPK composition of 16:9:12 (1:1 mixture of 3- to 4- and 8- to 9- month release time types). All substrates received 1.5 kg m⁻³ of a 3:2 mixture of CaCO₃ and MgCO₃ and 150 g m⁻³ NH₄NO₃.

After 13 weeks, the plants were transferred to a frost-free greenhouse (t > 4 °C). These conditions caused the plants to lose their leaves, and after 120 days they were outplanted, with bare roots, to the field into a non-disinfected clay loam soil (Domaine d'Époisses, INRA-Dijon, France) with 440 (133–1452) AMF propagules kg⁻¹, as calculated by the Most Probable Number (MPN) method. By that time the plants had an average height of 1.08 ± 0.09 m and an average number of 23 ± 3.5 buds. Plant spacing in the field was 1.0 m × 1.0 m. Treatments were arranged in the field in randomised blocks with five replicates. Plant growth was evaluated by stem length increase and lateral branch extension, in order to determine the effects of the treatments. Total length of branches was recorded separately for the upper half and the lower half of the stems. After 26 weeks, the plants were harvested and stem diameter, total stem and branch length and shoot dry weight were determined. Root samples were cleared and stained (Phillips and Hayman, 1970) and mycorrhizal colonisation was assessed according to Trouvelot and Kough (1986).

Due to the limited number of plants there was an unbalanced design among the factors substrate composition, fertiliser level and inoculation treatments. Therefore, in a first step, each combination of factors was considered as a different qualitative treatment. Data were subjected to ANOVA, and means were compared by the Newman-Keuls test at $P \leq 0.05$. In a second step, data were analysed following an over-parameterised model with type-IV decomposition (<http://www.statsoft.com/textbook/stathome.html>), and contrasts were calculated for each factor combination of interest.

3. RESULTS AND DISCUSSION

In the field phase, the highest values in shoot dry weight (Tab. I) were attained by wild cherry plants that had been post-weaned and overwintered in high-peat, high-fertiliser substrate.

Table I. Shoot dry weight (g plant⁻¹) of field-grown wild cherry plants weaned in substrates with 40% soil or 40% peat, with two fertiliser levels, uninoculated (NM) or inoculated with *Glomus intraradices* (Gi) or *G. deserticola* (Gd).

Substrate	Fertiliser level	Inoculation		
		NM	Gi	Gd
40% soil	low	578c	661b	600c
40% peat	low	668b	639b	727a
40% soil	high	657b	644b	–
40% peat	high	725a	725a	–

* Means followed by different letters are significantly different ($P \leq 0.05$) as indicated by the Newman-Keuls test; – : treatment non existent.

Plants showed no difference among treatments, in height or leaf numbers during the post-weaning phase, as previously reported (Lovato et al., 1994), and during that stage mycorrhizal colonisation in the inoculated treatments ranged between 70 and 80%. In this experiment, mycorrhizal root colonisation varied between 50 and 60%, with no difference among treatments. Shoot dry weight was increased by previous inoculation with *G. intraradices* in high-soil, low-fertiliser substrate and with *G. deserticola* in the high-peat substrate with low fertiliser level (there were no *G. deserticola*-inoculated plants in high-fertiliser level substrates). The wild cherry plants priorly inoculated with *G. deserticola* and grown in the high-peat substrate with the low fertiliser level also had the highest increase in main stem length, attaining a total length of 99 cm, doubling their height after seven months of growth in the field. Plants from all other treatments were 78–91 cm in height, an increase of between 70 and 90% by the end of the growing season, with no significant differences among them, but their growth pattern differed among treatments.

Differences in number, size and position of branches in field-grown plants were evident (Fig. 1), and were related to the substrate composition and to prior mycorrhizal inoculation in pots. The effects of prior inoculation were more evident in the first three months of growth in the field (i.e., until mid-June), and diminished towards the end of the growth season. In the early phases of field growth, branches appeared earlier in the plants inoculated with *G. deserticola* and *G. intraradices*. However, by the end of the experiment there was still a clear effect of the substrates used during greenhouse growth. Figure 2 shows branch length, as recorded separately for the lower and upper halves of the main stem, with no significant differences between treatments as analysed separately. However, there was a significant effect of substrate composition, as evaluated by contrast calculation. Plants previously grown in substrate with high peat and high fertiliser levels had a significantly larger proportion of total length of branches in the upper position as compared with all other treatments (Tab. II).

Shoot biomass accumulation of micropropagated wild cherry plants after seven months in the field was related to substrate and inoculation conditions during the greenhouse post-weaning phase. Higher peat and fertiliser levels in substrates used during the post-weaning phase, and inoculation with *G. deserticola* during the weaning phase enhanced plant growth

Figure 1. Wild cherry plants after 12 weeks of growth under field condition, uninoculated (NM) or inoculated at the weaning stage with *Glomus intraradices* (Gi) or *Glomus deserticola* (Gd). Plants shown are from 40% soil, low fertilizer substrates.

Figure 2. Total length (cm) of lateral branches located in the upper half and in the lower half of the main stem of wild cherry plants non inoculated (NM), or inoculated with *Glomus intraradices* (Gi) or *Glomus deserticola* (Gd) grown under field condition after hardening and growth in greenhouse conditions in substrates with 40% soil or 40% peat, with low or high level of fertiliser. Mean of five replicates. Bars represent standard errors of the mean.

and development after outplanting to the field. The results suggest an interaction between substrate composition, fertilisation and AMF inoculation, as previously reported for micropropagated woody plants (Estaún et al., 1999), since *G. deserticola* and *G. intraradices* only improved growth of plants previously cultivated with lower amounts of fertiliser in high- and low-peat substrates, respectively. Differences in growth promotion of micropropagated plants have been reported for different species of perennial plants (e.g., Monticelli et al., 2000; Taylor and Harrier, 2000; Yano-Melo et al., 1999). Main stem and lateral

branch growth was also differentially affected by substrates and AMF inoculation.

Wild cherry plants inoculated with *G. deserticola* in low-fertiliser substrates had longer length of lateral branches located in their upper parts, a characteristic which can confer higher commercial value upon the plants, whereas plants inoculated with *G. intraradices* had more and longer branches in the lower half of the stem. These differences were evident up to harvesting. The high-peat, high-fertiliser substrate caused non-inoculated and *G. intraradices*-inoculated plants to have more

Table II. Ratio of low/high position-lateral branch length of field-grown wild cherry plants weaned in substrates with 40% soil or 40% peat, with low- or high-fertiliser levels, and uninoculated (NM) or inoculated with *Glomus intraradices* (Gi) or *G. deserticola* (Gd).

Substrate	Fertiliser level	Inoculation		
		NM	Gi	Gd
40% soil	low	0.51a	0.61a	0.28b
40% peat	low	0.18c	0.39b	0.13c
40% soil	high	0.62a	0.30b	–
40% peat	high	0.03d	0.04d	–

* Means followed by different letters are significantly different ($P \leq 0.05$) as indicated by the Newman-Keuls test; – : treatment non existent.

branches towards the top of the plant, whereas *G. deserticola*-inoculated plants and non-inoculated plants in high-peat, low-fertiliser substrate were in an intermediary position. The variations in plant development could be due to a different hormonal balance caused by the mycorrhizal association, as previously observed (Souza et al., 1996; Shaul-Keinan et al., 2002). Effects of interactions between auxins and AM mycorrhizas on growth have been demonstrated in woody perennial plants (Dutra et al., 1996). Sugar allocation could also be a determinant factor, since the presence of mycorrhizas has been reported to change sugar distribution in micropropagated pear plants (Raparini et al., 1994), where mycorrhizal plants tended to concentrate more sugar in leaves, and later in roots, than non-mycorrhizal ones. Differential allocation and storage of sugars in roots and shoots may affect plant development of mycorrhizal plants (Estaún et al., 1999). The *G. deserticola* isolate used in this experiment previously showed greater effects on the growth of wild cherry and another woody plant (common ash) in the early stages of plant growth (Lovato et al., 1994), as compared with two other AMF isolates.

It should be emphasised that the effects reported here were evident several months after plants had been removed from the substrates and outplanted, with bare roots, to the same non-disinfected field soil, rich in mycorrhizal fungal propagules (Gianinazzi-Pearson et al., 1985). One possible reason for the differences between inoculated plants after outplanting to the field may be that plants were colonised differentially by the two fungi introduced in the weaning phase, and/or they differed in their ability to compete with the indigenous fungi present in the field soil.

These results indicate that combinations of substrates and AMF inoculation should be judiciously selected for improving micropropagated plant growth after outplanting, since they showed physiological effects on plants under field conditions.

4. CONCLUSIONS

Higher peat and fertiliser levels in substrates used during the post-weaning phase enhance plant growth and development after outplanting to the field. Main stem and lateral branch growth are differentially affected by substrate composition and by AMF inoculation.

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