Long-term effects of nursery starter substrate and AM inoculation of micropropagated peach x almond hybrid rootstock GF677

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Note technique

Long-term effects of nursery starter substrate and AM inoculation of micropropagated peach × almond hybrid rootstock GF677

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Abstract – The peach × almond hybrid GF677 was inoculated in three different substrates: sandy soil, peat and a commercial peat–bark compost mix, immediately after the weaning stage. After 3 months of growth under greenhouse conditions, plants were transplanted in a microplot set-up. At transplanting, differences in growth and in mycorrhizal colonisation were significantly related to the starter substrate used; plants grown in peat were taller than plants grown in soil or in the compost–peat mix. Although both soil-less growing media were less conducive than soil to AM colonisation, the compost–peat mix resulted in higher colonisation percentages. At the end of the first growing season, there were significant interactions between starter substrate and inoculation treatments affecting plant growth. After two growing seasons, the level of AM root colonisation was similar for all inoculated plants, and all plants presenting the symbiosis were bigger than those that had not been inoculated, irrespective of the substrate used in the nursery. (© Inra/Elsevier, Paris.)

arbuscular-mycorrhiza / early AM inoculation efficiency / field transplantation / fruit rootstocks / soil-less culture

Résumé – Effet du substrat initial et de l'inoculation avec des champignons mycorrhizogènes à arbuscules sur la croissance du porte-greffes hybride GF677 micropropagé. Le porte-greffes hybride d'amandier × pêcher GF677 a été inoculé avec le champignon mycorrhizogène *Glomus intraradices*, immédiatement après le stage d'acclimatation. Trois substrats ont été comparés comme support pour la mycorrhization sous conditions de pépinière en serre. Après trois mois de croissance, les plantes ont été transplantées dans des pots de 6 L contenant un sol sableux. Ces pots ont été enterrés formant un système de microparcelles avec les différents traitements distribués au hasard. Au moment du transplantation les différences en croissance et en colonisation des racines par *G. intraradices* étaient significativement

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dépendantes du substrat initial utilisé. À la fin de la première saison de croissance il y avait des interactions significatives entre le substrat utilisé et les traitements d'inoculation. Les différences de colonisation initiale des racines par *G. intraradices* n'étaient plus un facteur à considérer après deux saisons de croissance. Cependant, toutes les plantes inoculées étaient plus grandes que celles qui n'avaient pas été inoculées, indépendamment du substrat utilisé en pépinière. (© Inra/Elsevier, Paris.)

mycorrhize à arbuscules / culture hors-sol / micropropagation / porte-greffe / *Prunus* sp.

1. Introduction

In field conditions fruit trees from the *Rosaceae* family form the arbuscular mycorrhiza (AM) symbiosis [7]. The occurrence of this symbiosis improves the nutritional status of the plants by enhancing mineral absorption [15] by improving water relations [5] and by increasing tolerance to soil-borne pests and diseases [9, 22].

Prunus sp. rootstocks are produced in great quantities using micropropagation techniques. These procedures procure uniform and disease-free plantlets which are usually transferred from in vitro agar-based growing media to soil-less organic substrates, devoid of mycorrhizal propagules [19]. Although pre-transplant inoculation of container-grown plantlets with selected AM fungi has been considered as one of the most cost-effective ways of establishing the symbiosis [24], information on the cultural practices required for raising mycorrhizal plantlets in containers is limited [12, 14]. The effectiveness and efficiency of a particular AM fungus can vary with the substrate used as potting media [2, 4]. There is no long-term information on the post-transplant response due to both inoculation and substrate used, once the rootstocks have been transplanted into the field under harsher conditions. Pre-transplant AM colonisation of fruit trees is likely to shorten the production period necessary to obtain a sufficient stem diameter for grafting and increase survival after transplant [6]. However, the effects of the AM symbiosis on early plant growth can be partly substituted by the use of organic substrates amended with large quantities of nutrients (especially of N and P). The advantages of AM inoculation are, therefore, not always evident in the short term. The long-term performance of the rootstocks transplanted in the field might respond better to the existence of an effective AM symbiosis.

The purpose of this investigation was to evaluate the effects of early inoculation of the prunus rootstock GF677 with the AM fungus *Glomus intraradices* in three different substrates. The main effects and the interactions between inoculation and substrate used was assessed over two growing seasons after transplanting.

2. Materials and methods

The peach–almond (*Prunus persica* × *Prunus amygdalus*) hybrid GF677 was used as the host plant. This hybrid is the most popular rootstock for peaches in Spain and France because of its good adaptation to dry climatic conditions, resistance to clorosis and vigour [3]. The plantlets were micropropagated and obtained from a Spanish commercial nursery (Agromillora Catalana S.A., San Sadurní d'Anoia, Barcelona). Following the weaning stage, plantlets were transferred to 100-mL containers with three different potting media: autoclaved sandy loam soil (80 % sand, 18 % silt, 2 % clay), sphagnum peat (TKS-1 Floragard GMbH) and a commercial mixture of pine bark compost, peat and volcanic coarse sand (BVU Prodeasa Products Ltd.) (*table I*). During the transfer the plantlets were inoculated, or not, with 10 g of *Glomus intraradices* (BEG no. 72) soil inoculum placed below the roots during transplant. The inoculum consisted of rhizospheric soil from leek (*Allium porrum* L.) pot cultures containing heavily colonised root fragments with many internal spores. The plants not receiving mycorrhizal inoculum received a filtrate of soil inoculum free from AM propagules. After 3 months of growth under greenhouse conditions, plant roots were sampled to assess mycorrhizal colonisation

Table I. Physicochemical properties of the starter substrates used.

	Sandy loam soil	TKS peat	BVU compost mix
pH (H_2O)	7.5	6.87	6.40
EC 25°C ($dS \cdot m^{-1}$)	0.11	0.95	0.25
N (Kjedhal) (ppm)	3.92	140	65
P (Olsen) (ppm)	9.05	120	32
K (ppm)	46	220	16

and were then transplanted into 6-L pots containing pasteurised (80 °C) sandy loam soil.

The experimental design was a 3 × 3 factorial with 15 plants per experimental group. The experimental groups resulted from the combination of two factors. i) Inoculation with three treatments: a) inoculated with *G. intraradices*; b) non-inoculated non-amended control; and c) non-inoculated P-amended control. ii) Starter substrates with three treatments: a) sandy loam soil; b) sphagnum peat; and c) BVU compost mix.

The 6-L containers were buried in the soil spaced 80 cm apart in a completely randomised bucket microplot set-up [1] with 60 % shade in field conditions until the conclusion of the study. Plants were watered as needed and fertilised weekly with a modified Hoagland's [15] nutrient solution low in P (0.10 g KH_2PO_4 L^{-1}). In the non-inoculated P-amended treatment, plants received a double dose of P (0.20 g KH_2PO_4 L^{-1}). Stem height was measured at the beginning of the experiment, at the time of transplant into the 6-L pots, after 3 and 7 months (total growth of the first growing season) and at harvest, after 14 months of growth (two seasons) in the micro-plot set-up. Stem diameter, shoot and root dry weight were measured at harvest. Arbuscular mycorrhizal colonisation of a root subsample of all plants (inoculated and non-inoculated) was assessed after staining [16] using the grid line intersect method [8] at the time of transplant and at harvest.

3. Results

At the beginning of the experiment all plants were uniform with a shoot length of 8.4 ± 1.2 cm. At the time of transplanting, 3 months after the inoculation with *G. intraradices*, there were no significant interactions between the two factors studied: mycorrhizal treatment and starter substrate considering plant height (*table II*).

Table II. Plant height of the *Prunus* rootstock GF677 inoculated with *G. intraradices*, non-inoculated control and non-inoculated P-amended control. At transplant (time 0) and after 3 months (time 1), 7 months (time 2) and 14 months (time 3) growth under field conditions.

Treatments		Plant height (cm)			
Mycorrhiza	Starter substrate	Time 0	Time 1	Time 2	Time 3
NM		10.83	27.62	45.54	177.73
NM+P	soil	11.70	30.39	46.10	188.54
M		14.30	32.30	81.67	280.36
NM		11.56	31.30	47.30	182.45
NM+P	compost	11.56	31.15	47.18	204.36
M		13.10	31.70	72.11	237.27
NM		15.30	37.62	46.54	180.27
NM+P	peat	16.10	38.85	53.00	209.18
M		16.67	34.77	46.89	223.36
LSD (0.05)		**2.77**	**2.18**	**17.68**	**35.98**
Analysis of variance					
Starter substrate (S)		***	***	ns	ns
Mycorrhiza (M)		***	***	***	***
S × M		ns	***	***	***

NM = Non-mycorrhizal, NM+P = Non-mycorrhizal P-amended, M = inoculated with *Glomus intraradices*.
*** $P = 0.001$, ns = non-significant.

The starter substrate was a highly significant factor in this first sampling. The plants grown in peat were over 20 % larger than those grown in the peat–compost mix or in soil. The inoculation treatment was also significant: plants inoculated with *G. intraradices* were taller than the non-inoculated controls although there were no differences with the plants amended with P. The assessment of the root colonisation at this stage showed that the starter substrate influenced the degree to which the fungus colonised the root: in the soil plants achieved 60 % root colonisation, while in the BVU peat–compost mix the colonisation was 45 % and in peat the colonisation was only 17 % (*figure 1*).

Three months after transplanting (time 1) the survival was 100 % for all treatments and, when

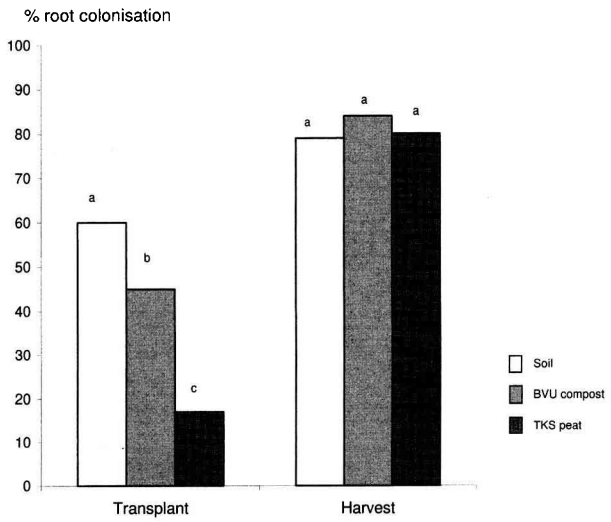

Figure 1. Root colonisation of the peach × almond hybrid GF677 inoculated with *Glomus intraradices* in three different starter substrates: sandy soil, peat (TKS) and compost–peat mix (BVU). At transplant (3 months after inoculation) and at harvest after two seasons growth.

considering plant height, both factors and their interactions showed significant differences (*table II*). Independently of the inoculation treatment, plants started in peat were larger than plants started in soil or in BVU peat–compost mix. Plants previously grown in soil showed a positive response to mycorrhization. Plants started in the BVU mix showed no differences attributable to inoculation, and control and fertilised plants started on peat were significantly taller than the inoculated ones on the same substrate.

After 7 months of growth (time 2), by the end of the first growing season, the interactions between the two factors considered were significant; however, there were no differences that could be solely adsorbed to the effects of the starter substrate (*table II*). The inoculation with *G. intraradices* was the most significant issue in this sampling time. *G. intraradices*-inoculated plants were over 40 % taller than both sets of control plants when plants had been started in soil or in BVU mix. Peat-started plants did not show a positive effect of AM inoculation and the initial advantage conferred by this substrate had disappeared.

After 14 months of growth (time 3: two growing seasons), at harvest (time 3), there were no differences in growth due to the starter substrate; however, the interactions persisted in some of the measured parameters (*tables II* and *III*). When considering the height (*table II*) of plants grown previously in soil, mycorrhiza-inoculated plants were significantly taller than non-inoculated control and P-amended plants. Plants started on peat showed no differences between mycorrhizal and P-fertilised treatments, both being significantly taller than control plants. In the compost, there was an intermediate situation. Inoculated plants were bigger than control and fertilised plants although the differences with the fertilised plants were not significant.

Stem diameter is a substantial characteristic of the rootstock because the thickness of the stem sets the time for grafting. In our experiment, stem diameter followed the same trend as plant height (*table III*), mycorrhizal plants had significantly thicker diameters than non-inoculated plants and there was a significant interaction between inoculation and the starter substrate used. When examining shoot dry weight (*table III*) no interactions between factors were found. Inoculated plants were significantly heavier than non-mycorrhizal, non-amended control plants, although no significant differences were detected between AM plants and non-mycorrhizal P-amended plants. Root dry weights were similar for all treatments considered, despite the differences found when measuring the other parameters. The comparison of root/shoot weights showed that this ratio was lower for mycorrhizal plants, irrespective of the starter substrates used, when compared to non-mycorrhizal, non-amended control plants; nevertheless, AM and non-mycorrhizal P-amended plants did not present significant differences.

Plants, inoculated in all three starter substrates studied, had similar levels of root colonisation by *G. intraradices*, after 14 months of growth, (*figure 1*).

4. Discussion

The AM colonisation levels at transplant varied with the substrate used as potting medium in the post-weaning phase. Organic substrates have been found less conducive to AM establishment and development than soil. Graham and Timmer [10] found that *G. intraradices* colonisation of container-grown *Citrus jambhiri* roots was reduced in peat soil-less mixes compared to soil and suggested an effect of organic matter on the AM development. Calvet et al. [2] found that certain types of peat and composted substrates had a negative effect on the establishment of the AM symbiosis, although the AMF germination and early mycelial growth were not affected suggesting a biological cause for the inhibition. Vidal et al. [25], noted that soil–peat mixes were more conducive for the establishment of the symbiosis in micropropagated avocado than sand–peat mixes, and also suggested a biological cause for the difference. Vestberg [23] found that sand fertilised with bone meal was superior to peat-based substrates in initiating rapid AM colonisation. Therefore, in our experiment, a reduced AM root colonisation was expected in both organic substrates. Although the use of peat as a growing medium decreased AM colonisation by 71 % compared to the levels achieved in the soil, the BVU peat–compost mix only reduced AM root colonisation by 25 %. Graham and Timmer [11] showed differences in AM root colonisation in soil-less peat-based media and related these differences to the amount and the source of phosphorus used as amendment. In our study, the peat used had high amounts of P that could explain the inhibitory effect of this substrate when compared to the BVU mix. Onguene and Habte [20], working with soil fertilised with different levels of phosphorus and nitrogen, found that high levels of both fertilisers, but especially of N, decreased AM root colonisation *of Leucaena leucocephala*. In our experiment, both organic substrates used had lower degrees of root colonisation when compared to soil, but peat, with higher levels of P and also of N and K, was less conducive to the establishment of the symbio-

Table III. Plant growth of the *Prunus* rootstock GF677 inoculated with *G. intraradices*, non-inoculated control and non-inoculated P-amended control. At harvest, after 14 months of growth under field conditions.

Mycorrhiza	Starter substrate	Shoot dry weight (g)	Root dry weight (g)	Stem diameter (mm)	Root/shoot (R/S) ratio
NM		17.28	11.60	6.77	0.68
NM+P	soil	18.17	11.33	6.43	0.63
M		23.25	11.77	8.20	0.55
NM		18.61	11.54	6.66	0.63
NM+P	compost	19.80	11.53	7.00	0.59
M		20.95	11.78	7.47	0.59
NM		15.96	11.26	6.47	0.71
NM+P	peat	20.75	12.73	7.23	0.62
M		19.70	12.71	7.09	0.65
LSD (0.05)		**2.28**	**1.75**	**0.64**	**0.065**
		Analysis of variance			
Starter substrate (S)		ns	ns	ns	ns
Mycorrhiza (M)		***	ns	***	***
S × M ns		ns	***	ns	

NM = Non-mycorrhizal, NM+P = Non-mycorrhizal P-amended, M = inoculated with *Glomus intraradices*.
*** $P = 0.001$, ns = non-significant.

sis than the compost mix. However, the existence of a concomitant biological effect cannot be discarded.

After two growing seasons, the effects of the substrate on the AM root colonisation disappeared and all inoculated plants had similar levels of AM root colonisation. McGonigle and Fitter [18] when studying post-transplant *Trifolium repens* performance in the field, also found that the differences in percentage AM root colonisation disappeared with time. However, *T. repens* showed no differences in growth or P-inflow attributable to the AM colonisation, while in our study, the effects on plant growth of early colonisation were persistent and noticeable after 7 months of growth, when the percentage of AM roots at transplant was a more important factor in plant growth than the starter substrate used. Onguene and Habte [20] working with *L. leucocephala* found that at transplant inoculated plants grown in fumigated soil with low fertilisation were comparable in their height to non-inoculated plants grown in heavily fertilised soil. In our study plants inoculated in soil were smaller than non-inoculated plants grown in peat. Pre-transplant treatments were affecting post-transplant growth up to 47 days after transplant, when the initial effects of the substrates and their level of nutrients disappeared. After 14 months of growth the differences in early colonisation were no longer a relevant factor; however, all inoculated plants were taller than those that had not been inoculated, irrespective of the substrate used in the nursery.

These results underscore the importance of an early establishment of the symbiosis for plant growth in the field. This effect would undoubtedly be enhanced if the plants were transplanted to harsher environments with biotic or abiotic constraints, especially in soils subjected to replant situations, so common in Mediterranean environments. Pinochet et al. [22] reported how the early inoculation with AM fungi can increase the tolerance of cherry rootstocks when established in soils infested with the lesion nematode *Pratylenchus vulnus*. Estaún et al. [5] found an increased transplant efficiency and growth of inoculated plants under drought conditions. Graham and Timmer [11] showed how *Citrus* trees subjected to high P amendments developed a P-induced Cu deficiency that was counterbalanced by AM colonisation, similarly Lopez et al. [17] found a better nutrition in microelements, which tend to be immobilised in calcareous soils in AM pear rootstocks established in nematode-infested soil.

The fact that even in very rich substrates the symbiosis can be established, although without appraisable results in the first stages of growth, implies the feasibility of the inoculation in technologically advanced nursery operations where peat-based potting mixes are used. AM inoculation is shown to be more efficient than high fertilisation for plant growth after transplanting and under unfavourable conditions. AM inoculation might reduce transplant stress and enable the plants to adapt more successfully to field situations in perennial high cash crops.

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