Tree Physiology 16, 757–763
© *1996 Heron Publishing—Victoria, Canada*

Influence of arbuscular mycorrhizae and phosphate fertilization on shoot apical growth of micropropagated apple and plum rootstocks

P. FORTUNA,[1] A. S. CITERNESI, [2] S. MORINI, [1] C. VITAGLIANO [2] and M. GIOVANNETTI[3]

[1] *Università di Pisa, Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sezione di Coltivazioni Arboree, via del Borghetto 80, Pisa, Italy*

[2] *Scuola Superiore di Studi Universitari e Perfezionamento S. Anna, via Carducci 40, Pisa, Italy*

[3] *Università di Pisa, Istituto di Microbiologia Agraria, via del Borghetto 80, Pisa, Italy*

Received September 26, 1995

Summary We studied the effects of phosphate fertilization and inoculation with the arbuscular mycorrhizal fungi *Glomus mosseae* (Nicol. and Gerd.) Gerdmann and Trappe, *Glomus intraradices* Schenck and Smith or *Glomus viscosum* Nicolson on shoot apical growth of plantlets that had been micropropagated from MM 106 apple (*Malus pumila* L.) and Mr.S. 2/5 plum (*Prunus cerasifera* Ehrh.) rootstocks. Unfertilized and non-mycorrhizal plantlets showed no apical growth during the post *in vitro* acclimation phase, whereas P fertilization induced early resumption of shoot apical growth. Growth enhancement and percentage of actively growing apices of mycorrhizal-inoculated plantlets were comparable to those obtained in plantlets fertilized with P. Furthermore, tissue P concentrations of mycorrhizal plantlets were similar to those of plantlets fertilized with P. We conclude that mycorrhizal inoculation can be used as a biotechnological tool to overcome blocked apical growth and to reduce chemical inputs, especially P inputs, to micropropagated fruit trees.

Keywords: micropropagation, mycorrhizal fungi, phosphorus.

Introduction

Many fruit tree species are dependent on arbuscular mycorrhizal (AM) infection for survival and growth (Timmer and Leyden 1978, Covey et al. 1981, Powell and Santhanakrishnan 1986, Schubert and Cammarata 1986). Moreover, mycorrhizal fruit trees have enhanced tolerance to biotic and abiotic stresses (Menge et al. 1978, Davis and Menge 1980, Guillemin et al. 1994*a*, 1994*b*). Improved growth of mycorrhizal plants is often related to more efficient uptake of nutrients, especially phosphorus (P), from soil (Gianinazzi-Pearson and Gianinazzi 1986, Bolan 1991). Higher rates of P uptake and higher tissue P content have been reported in mycorrhizal plants compared to controls (Sanders and Tinker 1973, Smith 1982, Giovannetti et al. 1988, Son and Smith 1988, Morin et al. 1994). Depending on host plant–AM fungus combinations and pedoclimatic conditions, different amounts of P are necessary to obtain growth increments comparable to those observed in mycorrhizal plants (Hoepfner et al. 1983, Geddeda et al. 1984, Johnson 1984).

Micropropagation is an excellent tool for rapid production of homogeneous, genetically improved fruit trees (George and Sherrington 1984). However, this technique reduces or even eliminates the population of beneficial microorganisms in the soil, such as mycorrhizal fungi and plant growth-promoting rhizobacteria, at least during the early stages of post *in vitro* acclimation. This phase is a critical step in the micropropagation cycle (Morini and Concetti 1984, Debergh and Zimmerman 1992), and the lack of beneficial microorganisms can adversely affect survival and growth of *in vitro*-produced plantlets (Pons et al. 1983). Previous studies have shown that inoculation with AM fungi at the time that micropropagated plantlets are transplanted from axenic to *in vivo* conditions significantly improves survival and growth (Ravolanirina et al. 1989*a*, 1989*b*, Guillemin et al. 1991, Branzanti et al. 1992, Fortuna et al. 1992, Hooker et al. 1994, Sbrana et al. 1994). However, the mechanism by which mycorrhizae enhance survival and growth of plantlets is not known. We have tested the hypothesis that mycorrhizae promote renewed shoot apical growth of micropropagated plants by improving P nutrition.

Materials and methods

Plant material

In vitro-derived shoots of Mr.S. 2/5 plum rootstock, a selection of *Prunus cerasifera* Ehrh., were propagated on an MS proliferation medium (Murashige and Skoog 1962), modified as previously described (Morini et al. 1990). Rooting was obtained by reducing macronutrient concentration by half and replacing growth regulators in the medium with 0.4 mg l^{-1} IBA. *In vitro*-derived shoots of MM 106 apple rootstock, a selection of *Malus pumila* L., were propagated on DKW proliferation medium (Driver and Kuniyuki 1984). Rooting was obtained by reducing macronutrient concentration by half and adding 162 mg l^{-1} phloroglucinol to the substrate (James and Thurbon 1981); growth regulators were replaced as described for Mr.S. 2/5. After 3 weeks in rooting medium, Mr.S. 2/5 microplants with 3–5 roots each 1–2 cm in length and MM 106 microplants with 5–7 roots each 2–3 cm in length were transplanted to 0.1-liter pots containing autoclaved sandy soil. My-

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024

corrhizal inoculation was performed by replacing a third of the soil with crude inoculum of an AM fungal species. During this initial post *in vitro* acclimation phase, all plantlets were maintained in glass boxes under controlled conditions (19 ± 1 °C, 16-h photoperiod, PPFD of 60 μmol m^{-2} s^{-1} supplied by cool white fluorescent tubes); relative humidity was 90–100% during the first week after transplanting and was gradually reduced to 60–70% during the following weeks. Plantlets were watered daily with tap water that did not contain P.

Soil

All trials were carried out in a sandy soil containing 16.4 ppm available P_2O_5 (Olsen), 19.1 ppm available K_2O (ammonium acetate), 0.2% total N (Kjeldahl) , 0.56% organic matter; pH (H_2O) was 7.3. The soil was sterilized for 40 min at 121 °C to kill native mycorrhizal endophytes.

Mycorrhizal inoculum

Mycorrhizal fungi used were *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe (Kent isolate), *Glomus intraradices* Schenck and Smith (isolated from a local serpentine soil) and *Glomus viscosum* Nicolson (isolated from a nursery substrate). Voucher specimens of the three isolates were deposited in the herbarium of the Department of Botanical Science, University of Pisa, *Herbarium Horti Botanici Pisani* (PI) as PI-HMZ 4, PI-HMZ 9 and PI-HMZ 10, respectively. Inoculum was produced in pot cultures of *Helianthus annuus* L. in the greenhouse collection of the Institute of Agricultural Microbiology, University of Pisa, Italy. Growth substrate was the same sandy soil as used in the experiments and pots were maintained in individual sunbags (Sigma, USA) to avoid external contaminations. After four months, pot cultures were harvested and the infested soil, containing infected root fragments, extramatrical mycelium and fungal spores as infective propagules, was checked for purity. The inoculum potentials of the different fungal species were balanced by using a large quantity of inoculum, corresponding to a third of the pot substrate (Lioi and Giovannetti 1987).

Mr.S. 2/5 micropropagated plantlets

We studied the effects of three P concentrations and mycorrhizal inoculation with *G. mosseae* or *G. intraradices* on renewed shoot growth of Mr.S. 2/5 micropropagated plantlets. During the initial post *in vitro* acclimation phase in the glass boxes, control and inoculated plants received 5 ml per pot of Hoagland solution without phosphate. Three groups of uninoculated plants were fertilized with Hoagland solution containing 31, 62 or 93 ppm of P. Each treatment consisted of 30 replications and each replication consisted of one plant per pot. The pots were arranged randomly in the glass boxes. After 2 months, eight plants from each treatment were harvested and the remaining plants were transplanted to 1-liter pots containing autoclaved sandy soil. The pots were randomly arranged in a naturally illuminated greenhouse. Each pot was fertilized once a week with 50 ml Hoagland solution containing the designated concentration of P. Thus, during the 4-month acclimation period in the greenhouse, each plant received 1.5 (P1), 3.1 (P2) or 4.6 (P3) mg of P per week. Four months after the plants were transferred to the greenhouse, 20 plants per treatment were harvested. The experiment was performed during the spring–summer season.

In another study, the effect of P fertilization on shoot apical growth was examined by increasing the frequency of fertilization from once to twice a week. In addition, the plantlets were transferred from the glass boxes to the greenhouse after 1 month. Therefore, during the acclimation phase in the greenhouse, each plant received 3.1 (P4), 6.2 (P5), or 9.3 (P6) mg of P per week. Each treatment consisted of 20 replications and each replication consisted of one plant per pot. The pots were arranged randomly in the greenhouse. Three months after the plants were transferred to the greenhouse, the final harvest was carried out on 10 plants per treatment. The experiment was performed during the summer season.

MM 106 micropropagated apple plantlets

In an experiment similar to those described for Mr.S. 2/5 micropropagated plantlets, we studied the effects of three P concentrations and mycorrhizal inoculation with *G. mosseae* or *G. viscosum* on renewed shoot growth of MM 106 micropropagated apple plantlets. Phosphorus fertilization was applied twice per week and the plantlets were transplanted to the greenhouse after a 1-month post *in vitro* acclimation period in glass boxes. Thus, during the 5-month acclimation period in the greenhouse, each plant received 3.1 (P4), 6.2 (P5) and 9.3 (P6) mg of P per week. Twenty replicate plants (one plant per pot) were used for each treatment and the pots were arranged randomly on the greenhouse bench. Six months after the beginning of the trial, the final harvest was carried out on 10 plants per treatment. The experiment was performed during the summer season.

Measurements

The plantlets consisted of a stem with leaves and no laterals. After transplanting to the greenhouse, the percentage of plants with actively growing apices (forming new leaflets) in each treatment was recorded every month. At each harvest, shoot and root fresh weights (g), shoot height (cm), leaf area (cm^2) and number of leaves were recorded, and root and shoot dry weight determined after drying to constant weight at 70 °C. Percentage of mycorrhizal root length was assessed using the grid-line intersect method (Giovannetti and Mosse 1980) after staining the roots with 0.05% trypan blue in lactic acid (modified from Phillips and Hayman 1970). Samples of dry material (1 g) were wet-digested with H_2SO_4 and $HClO_4$ and phosphorus content was determined spectrophotometrically by the phosphomolybdic method (Watanabe and Olsen 1965). Three replicate determinations were conducted. Experimental data were analyzed statistically by a one-way analysis of variance and means were separated by Duncan's test. Survival percentage and percentage of actively growing apices were analyzed by the χ^2 test.

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024

Results

Effects of P and mycorrhizal inoculation on renewed shoot apical growth of Mr.S. 2/5 plum rootstock

Survival of plantlets of Mr.S. 2/5 plum rootstock was not affected by mycorrhizal inoculation or P fertilization. After one month in the greenhouse, survival rate of plantlets was 100% in all treatments, except in plantlets inoculated with *G. intraradices*, where it was 93.3%.

After two months in glass boxes, all growth parameters of mycorrhizal and fertilized Mr.S. 2/5 plum rootstock plants, except shoot height, were statistically higher than those of control plants. Shoot fresh and dry weights and leaf area were higher in plants receiving 4.6 mg of P per week (P3 treatment) than in mycorrhizal plants (Table 1).

During the early stage of acclimation, many Mr.S. 2/5 plants showed no shoot apical growth, and even after 2 months, the percentage of plants with actively growing apices was low in all treatments (Figure 1). Subsequently, percentages of actively growing apices increased both in mycorrhizal plants and in P2- and P3-treated plants, whereas values in control and P1-treated plants remained low throughout the 6-month experimental period. After one month in the greenhouse, the percentages of actively growing apices in *G. mosseae*- and *G. intraradices*-colonized plants were 90 and 100%, respectively, whereas the percentages of actively growing apices in P2- and P3-treated plants were 35 and 57%, respectively. In subsequent months, only the mycorrhizal plants showed apical growth renewal and the percentage of actively growing apices decreased markedly in all P-treated plants.

After 4 months in the greenhouse, fresh and dry weights of shoots, shoot height and leaf number were significantly greater in mycorrhizal plants than in plants of other treatments (Table 2). Although the growth parameters of P-treated plants were greater than those of control plants, they were significantly lower than those of mycorrhizal plants. In contrast, root fresh and dry weights of P2- and P3-treated plants were comparable to those of *G. intraradices*- and *G. mosseae*-colonized plants, respectively. Growth responses of plants colonized with *G. mosseae* were greater than those of plants colonized with

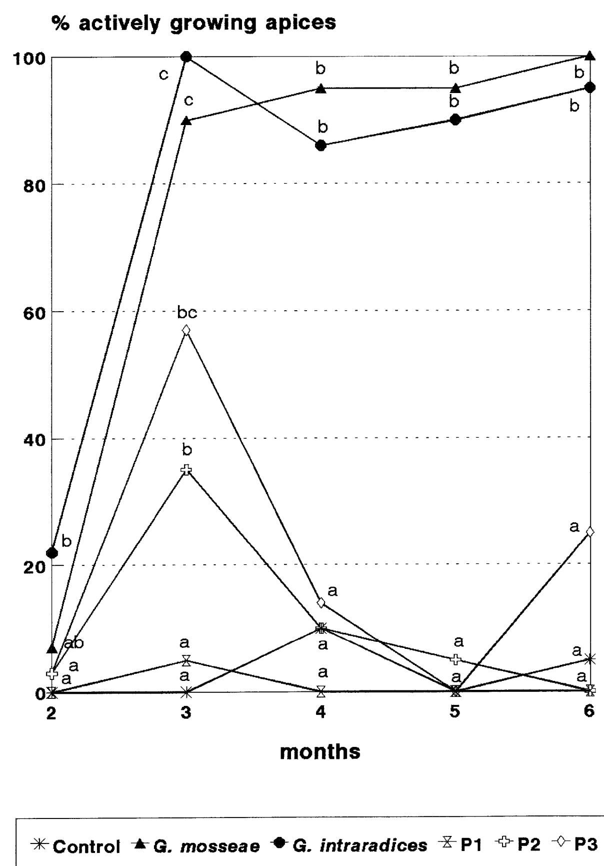

Figure 1. Percentage of shoot apices actively growing during greenhouse acclimation of Mr.S. 2/5 micropropagated rootstocks inoculated with AM fungi or fertilized with 0, 1.5, 3.1 or 4.6 mg of P per plant per week (Control, P1, P2 and P3 treatments, respectively). For each harvest, values associated with different letters are significantly different at $P = 0.05$ (χ^2 test).

G. intraradices, although percentage mycorrhizal infection did not differ between the treatments.

Among the treatments, mycorrhizal plants had the highest shoot P concentration and *G. mosseae*-colonized plants had

Table 1. Growth parameters and percentage AM colonization 2 months after *in vivo* transplanting of Mr.S. 2/5 micropropagated plants inoculated with *Glomus mosseae* or *Glomus intraradices* or fertilized weekly with 0 (Control), 1.5 (P1), 3.1 (P2) or 4.6 (P3) mg of P per plant per week. Values in columns followed by the same letter do not differ significantly ($P = 0.05$).

Treatment	Fresh weight (g)		Dry weight (g)		Shoot height (cm)	Leaf number	Leaf area (cm^2)	% AM colonization
	Shoot	Root	Shoot	Root				
Control	0.308 d	0.344 b	0.112 d	0.082 c	3.6 a	5 b	3.45 c	0
G. mosseae	0.786 c	0.747 a	0.222 c	0.130 b	4.3 a	8 a	5.23 b	51.8 a
G. intraradices	1.077 bc	0.928 a	0.311bc	0.214 a	4.6 a	9.7 a	5.87 b	66.2 a
P1	0.791 c	0.826 a	0.245bc	0.155 ab	3.5 a	8 a	5.23 b	0
P2	1.295 ab	0.897 a	0.340 b	0.158 ab	4.0 a	9.2 a	8.02 a	0
P3	1.590 a	0.970 a	0.448 a	0.189 ab	4.7 a	10 a	9.09 a	0

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024

Table 2. Growth parameters, phosphorus concentration and percentage AM colonization 6 months after *in vivo* transplanting of Mr.S. 2/5 micropropagated plants inoculated with *Glomus mosseae* or *Glomus intraradices* or fertilized weekly with 0 (Control), 1.5 (P1), 3.1 (P2) or 4.6 (P3) mg of P per plant per week. Values in columns followed by the same letter do not differ significantly ($P = 0.05$).

Treatment	Fresh weight (g)		Dry weight (g)		Shoot height (cm)	Leaf number	Shoot P conc. (%DW)	%AM colonization
	Shoot	Root	Shoot	Root				
Control	0.30 e	0.45 c	0.09 f	0.09 d	3.7 e	5.2 e	0.060 d	0
G. mosseae	15.92 a	8.55 a	4.89 a	1.50 a	61.8 a	35.8 a	0.201 a	74.5 a
G. intraradices	13.52 b	5.50 b	3.74 b	1.20 b	47.0 b	32.7 b	0.193 b	83.2 a
P1	1.25 e	2.65 c	0.51 e	0.55 c	3.5 e	4.0 e	0.073 cd	0
P2	2.94 d	5.07 b	1.13 d	1.10 b	7.9 d	8.3 d	0.077 cd	0
P3	5.47 c	7.75 a	2.00 c	1.59 a	16.0 c	14.6 c	0.100 c	0

higher tissue P concentrations than *G. intraradices*-colonized plants. There were no statistically significant differences in tissue P concentrations between control and P1- and P2-treated plants (Table 2).

Doubling the availability of P had no significant effect on survival of Mr.S. 2/5 micropropagated plants one month after transplanting. Only about 20% of the plants had actively growing apices (Figure 2). In the following months, renewed apical growth was observed in mycorrhizal plants and in P5- and P6-treated plants. Shoot apices of control and P4-treated plants did not resume growth during the 3-month period in the greenhouse. By September, the control plants appeared dead and were not harvested. The final harvest was performed at the end of September, when the plants of all treatments were fully dormant. The P5 and P6 treatments enhanced growth to about the same extent as observed in plants inoculated with mycorrhiza (Table 3).

The increase in tissue P concentration paralleled the amount of P supplied and was similar in P5- and P6-treated plants and in mycorrhizal plants (Table 4). Shoot and root P concentrations were significantly higher in *G. mosseae*-colonized plants than in *G. intraradices*-colonized plants, whereas the growth responses were significantly greater in *G. intraradices*-colonized plants than in *G. mosseae*-colonized plants.

Effect of P and mycorrhizal inoculation on renewed shoot apical growth of MM 106 apple rootstock

Use of phloroglucinol in the rooting medium stimulated rooting. The percentage of rooted microcuttings was 97.4%, but there was abundant production of callus (James and Thurbon 1981), which caused high mortality (about 40%). However, there were no significant differences in survival between treatments. After one month in the glass boxes, only about 20% of the plants were actively growing (Figure 3). Shoot apices of control plants failed to resume growth and only about 15–25% of P4-treated plants showed actively growing apices during the period in the greenhouse. In contrast, 2–3 months after the beginning of the trial, a high percentage of plants inoculated with *G. mosseae* or *G. viscosum* had actively growing apices. Near the end of the growth period in the greenhouse, the P5-

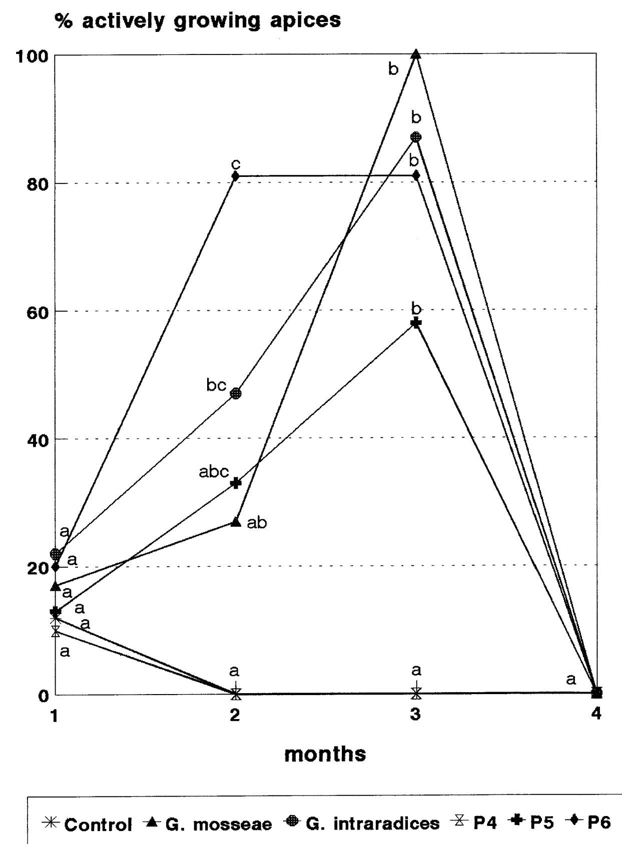

Figure 2. Percentage of shoot apices actively growing during greenhouse acclimation of Mr.S. 2/5 micropropagated rootstocks inoculated with AM fungi or fertilized with 0, 3.1, 6.2 or 9.3 mg of P per plant per week (Control, P4, P5 and P6 treatments, respectively). For each harvest, values associated with different letters are significantly different at $P = 0.05$ (χ^2 test).

and P6-treated plants had the highest number of actively growing shoot apices.

At the final harvest, shoot fresh and dry weights of P5-and P6-treated plants were similar to those of *G. mosseae*- and *G. viscosum*-colonized plants (Table 5), whereas root fresh and

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024

Table 3. Growth parameters and percentage AM colonization 4 months after *in vivo* transplanting of Mr.S. 2/5 micropropagated plants inoculated with *Glomus mosseae* or *Glomus intraradices* or fertilized weekly with 0 (Control), 3.1 (P4), 6.2 (P5), 9.3 (P6) mg of P per plant per week. Values in columns followed by the same letter do not differ significantly ($P = 0.05$).

Treatment	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Shoot height (cm)	Leaf number	Leaf area (cm^2)	% AM colonization
G. mosseae	4.77 bc	2.75 b	1.32 bc	18.7 b	22.6 a	10.9 b	62.8 a
G. intraradices	6.33 ab	4.35 a	1.87 a	26.1 a	17.3 bc	17.4 a	71.1 a
P4	3.42 c	3.21 b	0.91 c	13.4 c	14.6 c	10.7 b	0
P5	5.79 ab	3.13 b	1.65 ab	20.6 b	17.6 bc	15.2 a	0
P6	6.98 a	3.51 ab	1.76 ab	26.9 a	20.0 ab	15.9 a	0

Table 4. Shoot and root phosphorus concentrations (% dry weight) of Mr. S. 2/5 and MM 106 micropropagated plants 4 and 6 months after *in vivo* transplanting, respectively. Plants were either inoculated with a fungus or fertilized weekly with 0 (Control), 3.1 (P4), 6.2 (P5), or 9.3 (P6) mg of P per plant per week. Values in columns followed by the same letter do not differ significantly ($P = 0.05$).

Treatment	Mr.S. 2/5		MM 106	
	Shoot	Root	Shoot	Root
Control	–	–	0.085 b	0.076 c
G. mosseae	0.225 a	0.255 a	0.172 a	0.148 ab
G. intraradices	0.191 bc	0.171 b	–	–
G. viscosum	–	–	0.181 a	0.160 a
P4	0.117 d	0.109 c	0.153 a	0.131 b
P5	0.171 c	0.210 b	0.148 a	0.146 ab
P6	0.201 ab	0.204 b	0.150 a	0.163 a

dry weights of *G. mosseae*-colonized plants were significantly larger than those of plants in all other treatments. Growth parameters of control and P4-treated plants were not statistically different. Percentage of mycorrhizal infection by the two endophytes was similar.

Root P concentration of P-treated plants increased with increasing supply of P, whereas shoot P concentration did not differ among the P treatments (Table 4). Concentrations of P in mycorrhizal and fertilized plants were significantly higher than in control plants.

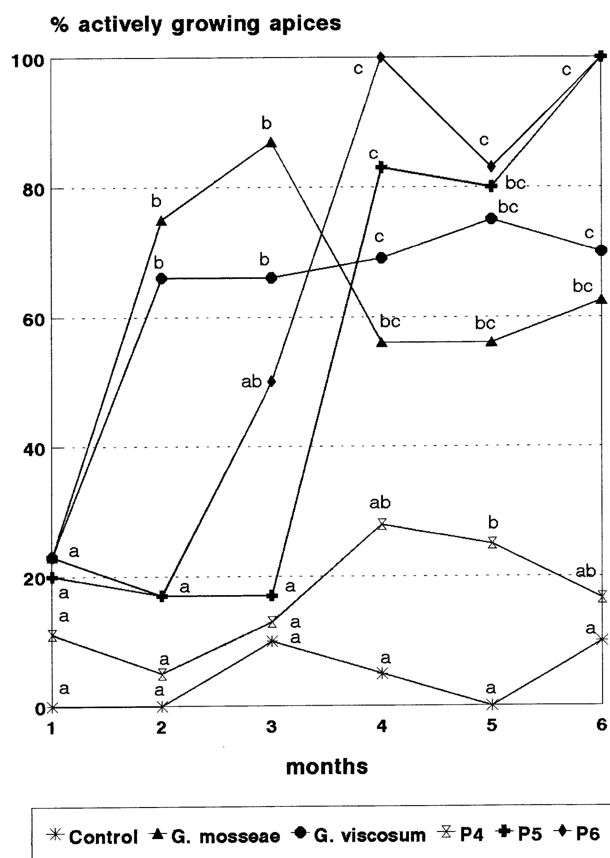

Figure 3. Percentage of actively growing apices during greenhouse acclimation of MM 106 micropropagated rootstocks inoculated with AM fungi or fertilized with 0, 3.1, 6.2 or 9.3 mg of P per plant per week (Control, P4, P5 and P6 treatments, respectively). For each harvest, values associated with different letters are significantly different at $P = 0.05$ (χ^2 test).

Discussion

Mycorrhizal symbiosis induced early resumption of shoot apical growth during the post *in vitro* acclimation phase of micropropagated Mr.S. 2/5 plum and MM 106 apple plants. This effect was associated with improved P nutrition. Previous studies have shown that arbuscular mycorrhizal fungi can induce morphological (Berta et al. 1993, Berta et al. 1995), physiological (Johnson 1984) and biochemical (Allen et al. 1980, 1982) modifications in host plants. Recently, the influence of AM symbiosis on shoot apical growth of micropropagated fruit trees was reported (Fortuna et al. 1992, Sbrana et al. 1994), although the mechanisms involved were not investigated.

Only plants fertilized with high amounts of phosphate (6.2 and 9.3 mg of P per plant each week, P5 and P6 treatments, respectively) showed renewal of shoot apical growth comparable to that observed in mycorrhizal plants (cf. Hoepfner et al. 1983, Geddeda et al. 1984). Because mycorrhizal plants had

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024

Table 5. Growth parameters and percentage AM colonization 6 months after *in vivo* transplanting of MM 106 micropropagated plants inoculated with *Glomus mosseae* or *Glomus viscosum* or fertilized weekly with 0 (Control), 3.1 (P4), 6.2 (P5), 9.3 (P6) mg of P per plant per week. Values in columns followed by the same letter do not differ significantly ($P = 0.05$).

Treatment	Fresh weight (g)		Dry weight (g)		Shoot height (cm)	Leaf number	% AM colonization
	Shoot	Root	Shoot	Root			
Control	0.748 c	1.012 b	0.318 b	0.146 b	4.24 c	2.6 c	0
G. mosseae	2.172 ab	2.338 a	0.994 a	0.455 a	7.10 b	8.3 b	51.8 a
G. viscosum	1.666 b	1.338 b	0.782 a	0.270 b	6.70 b	8.0 b	40.1 a
P4	0.796 c	0.868 b	0.310 b	0.118 b	4.10 c	3.6 c	0
P5	2.002 ab	1.282 b	0.870 a	0.212 b	8.80 ab	9.0 b	0
P6	2.578 a	1.286 b	1.038 a	0.212 b	9.80 a	14.6 a	0

the same tissue P concentrations as P5- and P6-treated plants, we conclude that renewal of shoot apical growth was related to tissue P concentration. In Mr.S. 2/5 plum plants, growth increments, percentage of actively growing apices and tissue P concentration increased with increasing P availability, and renewed shoot apical growth was observed when shoot P concentration ranged between 0.117 and 0.171% of dry weight, suggesting that the tissue P concentration must attain a threshold value for resumption of apical growth. In MM 106 apple plants, tissue P concentration of P-treated plants was similar regardless of the amount of P supplied, although tissue P concentration was greater in all P-treated plants than in control plants. It is possible that the difference between plantlets of Mr.S. 2/5 and MM 106 rootstocks in response to P fertilization is associated with different rates of uptake of phosphate. Uptake of phosphate differs among plant species and may also differ among cultivars of the same species (Barber and Thomas 1972, Brown et al. 1977). However, the involvement of other physiological processes in shoot apical growth renewal of mycorrhizal and P-treated plants, such as hormonal relationships, cannot be excluded (Allen et al. 1980, 1982, Edriss et al. 1984, Radin 1984).

Different species of AM fungi differ in infectivity and effectiveness (Lioi and Giovannetti 1987, Ravnskov and Jakobsen 1995). The AM fungus *G. intraradices* induced larger growth responses than *G. mosseae* in Mr.S. 2/5 plants two and four months after colonization. Six months after inoculation, however, *G. mosseae*-colonized plants had the greatest tissue phosphorus concentrations and growth responses. *Glomus mosseae* and *G. viscosum* differed markedly in their ability to improve root growth of MM 106 plants, but no differences were found in other growth parameters or in tissue P concentration.

We conclude that mycorrhizal inoculation in nursery production may represent a useful tool to overcome both inhibition of apical activity and stunted growth of plantlets and to reduce chemical inputs during the acclimation phase of micropropagated fruit trees.

References

Allen, M.F., T.S. Moore Jr. and M. Christensen. 1980. Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae: cytokinin increases in the host plant. Can. J. Bot. 58:371–374

Allen, M.F., T.S. Moore Jr. and M. Christensen. 1982. Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae: II. Altered levels of gibberellin-like substances and abscisic acid in the host plant. Can. J. Bot. 60:468–471.

Barber, W.D. and W.I. Thomas. 1972. Evaluation of the genetics of relative phosphorus accumulation by corn (*Zea mays* L.) using chromosomal translocation. Crop Sci. 12:755–758.

Berta, G., A. Fusconi and A. Trotta. 1993. VA Mycorrhizal infection and the morphology and function of root systems. Environ. Exp. Bot. 33:159–173.

Berta, G., A. Trotta, A. Fusconi, J. Hooker, M. Munro, D. Atkinson, M. Giovannetti, S. Morini, P. Fortuna, B. Tisserant, V. Gianinazzi-Pearson and S. Gianinazzi. 1995. Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera* L. Tree Physiol. 15:281–293.

Bolan, N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134:189–207.

Branzanti, B., V. Gianinazzi-Pearson and S. Gianinazzi. 1992. Influence of phosphate fertilization on the growth and nutrient status of micropropagated apple infected with endomycorrhizal fungi during the weaning stage. Agronomie 12:841–845.

Brown, J.C., R.J. Clark and W.E. James. 1977. Efficient and inefficient use of phosphorus by sorghum. Soil Sci. Soc. Am. J. 41:747–750.

Covey, R.P., B.L. Koch and H.J. Larsen. 1981. Influence of vesicular-arbuscular mycorrhizae on the growth of apple and corn in low-phosphorous soil. Phytopathology 71:712–715.

Davis, R.M. and J.A. Menge. 1980. Influence of *Glomus fasciculatus* and soil phosphorus on *Phytophthora* root rot of Citrus. Phytopathology 70:447–452.

Debergh, P.C. and R.H. Zimmerman. 1992. Micropropagation. Technology and application. Kluwer Acad. Publ., London, pp.71–93.

Driver, A. and A.H. Kuniyuki. 1984. *In vitro* propagation of Paradox walnut rootstock. Hortscience 19:507–509.

Edriss M.H., R.M. Davis and D.W. Burger. 1984. Influence of mycorrhizal fungi on cytokinin production in sour orange. J. Am. Soc. Hortic. Sci. 109:587–590.

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024

Fortuna P., S. Citernesi, S. Morini, M. Giovannetti and F. Loreti. 1992. Infectivity and effectiveness of different species of arbuscular mycorrhizal fungi in micropropagated plants of Mr.S. 2/5 plum rootstock. Agronomie 12:825–829.

Geddeda, Y.I., J.M. Trappe and R.L. Stebbins. 1984. Effects of vesicular-arbuscular mycorrhizae and phosphorus on apple seedlings. J. Am. Soc. Hortic. Sci. 109:24–27.

George E.F. and P.D. Sherrington. 1984. Plant propagation by tissue cultures. Exegetic Ltd., Eversley, U.K, pp. 39–72.

Gianinazzi-Pearson V. and S. Gianinazzi. 1986. The physiology of improved phosphate nutrition in mycorrhizal plants. *In* Mycorrhizae: Physiology and Genetics. 1st ESM, Dijon, INRA, Paris, pp. 101–109.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489–500.

Giovannetti, M., A. Schubert, M.C. Cravero and L. Salutini. 1988. Spore production by the vesicular-arbuscular mycorrhizal fungus *Glomus monosporum* as related to host species, root colonization and plant growth enhancement. Biol. Fertil. Soils 6:120–124.

Guillemin, J.P., S. Gianinazzi and V. Gianinazzi-Pearson. 1991. L'endomycorhization de vitroplants d'*Ananas comosus*: mis en évidence d'un effet mycorhizien. Fruits 46:355–358.

Guillemin, J.P., S. Gianinazzi, V. Gianinazzi-Pearson and J. Marchal. 1994*a*. Contribution of arbuscular mycorrhizas to biological protection of micropropagated pineapple (*Ananas comosum* (L.) Merr.) against *Phytophthora cinnamomi* Rands. Agric. Sci. Finland 3:241–251.

Guillemin, J.P., S Gianinazzi., V. Gianinazzi-Pearson and J. Marchal. 1994*b*. Control by arbuscular endomycorhizae of *Pratylenchus brachyurus* in pineapple microplants. Agric. Sci. Finland 3:253–262.

Hoepfner, E.F., B.L. Koch and R.P. Covey. 1983. Enhancement of growth and phosphorous concentration in apple seedlings by vesicular-arbuscular mycorrhizae. J. Am. Soc. Hortic. Sci. 108:207–209.

Hooker, J.E., S. Gianinazzi, M. Vestberg, J.M. Barea and D. Atkinson. 1994. The application of arbuscular mycorrhizal fungi to micropropagation systems: an opportunity to reduce chemical inputs. Agric. Sci. Finland 3:227–231.

James, D. and I.J. Thurbon. 1981. Shoot and root initiation *in vitro* in the apple rootstock M 9 and the promotive effects of phloroglucinol. J. Hortic. Sci. 56:15–20.

Johnson, C.R. 1984. Phosphorus nutrition in mycorrhizal colonization, photosynthesis, growth and nutrient composition of *Citrus aurantium*. Plant Soil 80:35–42.

Lioi, L. and M. Giovannetti. 1987. Variable effectivity of three vesicular-arbuscular mycorrhizal endophytes in *Hedisarum coronarium* and *Medicago sativa*. Biol. Fertil. Soils. 4:193–197.

Menge, J.A., R.M. Davis, E.L.V. Johnson and G.A. Zentmyer. 1978. Mycorrhizal fungi increase growth and reduce transplant injury in avocado. Calif. Agric. 4:6–7.

Morin, F., J.A. Fortin, C. Hamel, R. L. Granger and D. L. Smith. 1994. Apple rootstock response to vesicular-arbuscular mycorrhizal fungi in a high phosphorus soil. J. Am. Soc. Hortic. Sci. 119:578–583.

Morini, S. and S. Concetti. 1984. *In vitro* propagation of P.S.B2 peach rootstock. Acta Hortic. 173:205–210.

Morini, S., P. Fortuna, R. Sciutti and R. Muleo. 1990. Effect of different light-dark cycles on growth of fruit tree shoots cultured *in vitro*. Adv. Hortic. Sci. 4:163–166.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissues cultures. Physiol. Plant 15: 473–497.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158–161.

Pons, F., V. Gianinazzi-Pearson, S. Gianinazzi and J.C. Navatel. 1983. Studies of VA mycorrhizae *in vitro*: mycorrhizal synthesis of axenically propagated wild cherry (*Prunus avium* L.) plants. Plant Soil 71:217–221.

Powell, C.L. and P. Santhanakrishnan. 1986. Effect of mycorrhizal inoculation and phosphorus fertilizer on the growth of hardwood cuttings of kiwifruit (*Actinidia deliciosa* cv. Hayward) in containers. N.Z. J. Agric. Res. 29:263–268.

Radin, J.W. 1984. Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants. Plant Physiol. 76:392–394.

Ravnskov, S. and I. Jakobsen. 1995. Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. New Phytol. 129:611–618.

Ravolanirina, F., B. Blal, S. Gianinazzi and V. Gianinazzi-Pearson. 1989*a*. Mise au point d'une méthod rapid d'endomycorhization de vitroplants. Fruits 44:165–170.

Ravolanirina, F., S. Gianinazzi, A. Trouvelot and M. Carre. 1989*b*. Production of endomycorrhizal explants of micropropagated grapevine rootstocks. Agric. Ecosyst. and Environ. 29:323–327.

Sanders, F.E. and P.D. Tinker. 1973. Phosphate flow into mycorrhizal roots. Pestic. Sci. 4:385–391.

Sbrana, C., M. Giovannetti and C. Vitagliano. 1994. The effect of mycorrhizal infection on survival and growth renewal of micropropagated fruit rootstocks. Mycorrhiza 5:153–156.

Schubert, A. and S. Cammarata. 1986. Effect of inoculation of different endophytes on growth and P nutrition of grapevine plants growth in pots. *In* Mycorrhizae: Physiology and Genetics. Eds V. Gianinazzi-Pearson and S. Gianinazzi. INRA, Paris, pp 327–331.

Smith, S.E. 1982. Inflow of phosphate into mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different levels of soil phosphate. New Phytol. 90:293–303.

Son, C.L. and S.E. Smith. 1988. Mycorrhizal growth responses: interaction between photon irradiance and phosphorus nutrition. New Phytol. 108:305–314.

Timmer, L.W. and R.F. Leyden. 1978. Stunting of Citrus seedlings in fumigated soils in Texas and its correction by phosphorus fertilization and inoculation with mycorrhizal fungi. J. Am. Soc. Hortic. Sci. 103:533–537.

Watanabe, F.S. and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Proc. 677–678.

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024

Downloaded from https://academic.oup.com/treephys/article/16/9/757/1697009 by guest on 18 April 2024