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Apple Rootstock Response to Vesicular-arbuscular Mycorrhizal Fungi in a High Phosphorus Soil

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Abstract. **A 12-week greenhouse experiment was undertaken to test the efficiency of inoculation of vesicular-arbuscular mycorrhizal fungi on four apple (*Malus domestica* Borkh) rootstock cultivars: M.26, Ottawa 3 (Ott.3), P.16, and P.22. The plants were grown in soil from an apple rootstock nursery, containing high levels of extractable P (644 kg Bray/1 ha^{-1}). Inoculation treatments were *Glomus aggregatum* Shenck and Smith emend. Koske, *G. intraradix* Shenck and Smith, and two isolates of *G. versiforme* (Karsten) Berch, one originally from California (CAL) and the other one from Oregon (OR). Mycorrhizal plants were taller, produced more biomass, and had a higher leaf P concentration than the uninoculated control plants. Mycorrhizal inoculation also significantly increased the leaf surface area of 'M.26' and 'Ott.3' compared to the control. *Glomus versiforme*(CAL)-inoculated plants generally had the best nutrient balance, the greatest final height and shoot biomass, and produced an extensive hyphal network. All the mycorrhizal plants had similar percentages of root colonization, but the size of the external hyphal network varied with fungal species. *Glomus versiforme*(OR) had a larger extramatrical phase than *G. aggregatum* and *G. intraradix*. Mycorrhizal efficiency was associated with a larger external hyphal network, but showed no relation with internal colonization. Despite the high P fertility of the soil used, growth enhancement due to mycorrhizal inoculation was attributed to improved P nutrition.**

Apple trees show a strong dependency on mycorrhizae (Covey et al., 1981; Koch et al., 1982), and in orchards they form symbioses with the naturally occurring vesicular-arbuscular mycorrhizal (VAM) flora (Dalpé et al., 1986; Miller et al., 1985a). Mycorrhizae benefit apple plants by improving growth and nutrition (Geddeda et al., 1984; Hoepfner et al., 1983; Miller et al., 1985b; Plenchette et al., 1983ab) involving mainly P and, in some cases, other immobile nutrients such as Zn and Cu (Gnekow and Marschner, 1989). Other beneficial effects include improved resistance to drought (Runjin, 1989) and diseases caused by soilborne pathogens (Dehne, 1982).

Under unsterilized low P field conditions, Plenchette et al. (1981) showed that inoculation of greenhouse-produced apple seedlings, before field planting, can significantly increase growth, at least the first season after planting, as compared to both phosphorus fertilized and unfertilized naturally mycorrhizal controls. These results indicate the benefit of planting apple plants pre-colonized with a highly compatible VAM-fungus, even when plants become colonized by the indigenous VAM-flora once they are in the field.

Host plants show inter- and intraspecific variations in their ability to form and benefit from association with a VAM fungus. Experiments on wheat (Azcon and Ocampo, 1981; Manske, 1990; Young et al., 1985), soybean (Heckman and Angle, 1987), maize (Toth et al., 1984), millet (Krishna et al., 1985), and groundnut (Kesava et al., 1990) showed percentage differences in VAM colonization and growth response between parents and progenies tested, suggesting the possibility of breeding plants for improved mycorrhizal associations (Gianinazzi and Gianinazzi-Pearson, 1990).

The fungal species or isolate also influences the efficiency of the symbiosis in apple (Geddeda et al., 1984; Miller et al., 1985b). For instance, Benson and Covey (1976) reported that inoculation of apple seedlings with *G. fasciculatum* (Thaxter) Gerd. αvδ Trappe emend. Walker αvδ Koske, in a fumigated soil, caused a greater growth stimulation than that of *G. mosseae* (Nicholson αvδ Gerdemann) Gerdemann αvδ Trappe. Similarly, Miller et al. (1985b) showed that in a low P soil, seven isolates had different effects on apple growth.

Mycorrhizal efficiency depends not only on the host plant and the VAM-fungus, but also on edaphic conditions, particularly on soil P fertility. For instance, Hoepfner et al. (1983) found that infection levels of *G. mosseae* (Nicholson αvδ Gerdemann) Gerdemann αvδ Trappe for apple seedlings ranged from 11% to 41 %, depending on the soil tested. Hence, any experiment testing the practical use of inoculation must take the soil properties into consideration as well as the mycorrhizal partners. As apple orchard soils often are fertile or fertilized, evaluation of mycorrhizal effects on apple would be better conducted under conditions of higher fertility.

Specific combinations of fungal isolates and apple cultivars could influence growth more than other combinations. As the

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benefit of the symbiosis to the plant comes from the more efficient exploration of the soil volume by mycorrhizal hyphae as compared to roots (Gnekow and Marschner, 1989), the best isolate-cultivar combination could be characterized by the production of abundant extramatrical mycelium and a high shoot : root ratio. This experiment was undertaken to test the efficiency of four isolates of three VAM-fungi on four apple rootstock cultivars grown in a P rich soil, and to determine the relationship between efficiency and extent of mycorrhizal colonization with particular reference to external hyphal network.

Materials and Methods

A factorial experiment (five inoculation treatments × four rootstock cultivars) was conducted under greenhouse conditions for 12 weeks. Four apple (*Malus domestica* Borkh) rootstock cultivars [Malling 26 (M.260), Ottawa 3 (Ott.3), P.16, and P.22] were either not inoculated or were inoculated with each of four VAM-isolates. The experiment was a split-plot in which the experimental units, consisting of pots containing one plant, were randomized in five complete blocks. The rootstock cultivars were randomized to the main plots and the inoculation treatments were randomized to the subplots.

A total of 100 micropropagated, nonmycorrhizal apple rootstocks were planted individually in 15-cm plastic pots, in a Champlain sandy clay soil taken from the top 20 cm of the soil profile in an apple rootstock nursery, at St-Jean-Baptiste-de Rouville, Quebec. The soil contained 2.2% organic matter and the fertility status at the beginning of the experiment was pH=7.3, 644 kg of P-Bray/1 ha^{-1} (Bray and Kurtz, 1945), 2380 kg of K/ha^{-1}, 3090 kg of Ca/ha^{-1}, 697 kg of Mg/ha^{-1}, 1.6 ppm B, 2.2 ppm Cu, 360.1 ppm Fe, 34.8 ppm Mn, and 12.8 ppm Zn extractable with the Mehlich-3 solution (Mehlich, 1984). The soil was passed through a 5-mm sieve and steam pasteurized at 72C for 5 h. To alleviate the loss of texture due to manipulation, the soil was amended with grade 16 washed silica sand (1 sand : 3 soil).

Inoculation. The fungi tested included the following: two isolates of *G. versiforme* (Karsten) Berch, one from California (CAL), the other from Oregon (OR), both isolated from unknown host plants; *G. aggregatum* Shenck αvδ Smith emende. Koske isolated from an alfalfa field in Lapocatière, Quebec, Canada; and *G. intraradix* Shenck αvδ Smith, isolated from an ornamental plant production field in Pont-Rouge, Quebec, Canada. The inocula consisted of leek (*Allium porrum* L.) roots colonized with one of each of the above fungi. One gram (fresh mass) of inoculum was placed at the bottom of the transplanting hole, immediately before planting. Control plants received 1 g of an autoclaved mixture of all of the above inocula. To account for effects of nonmycorrhizal organisms potentially found in the inocula, all plants, including the uninoculated treatment, also received 25 ml of filtered (Whatman GF/D, 2.7 μm) washings of a mixture of the fungal inocula.

Throughout the experiment, plants were given small amounts of water to avoid leaching. No additional fertilizers were applied.

Parameters studied. Every 2 weeks, plant height was measured to the closest 0.5 cm. As plants branched very little, height was a good nondestructive estimate of treatment effects on plant growth. At the end of the experiment the plants were harvested, and the leaf area of each plant was measured with an area meter system (Delta-T Devices Ltd., Cambridge, England). Roots, leaves, and stems were dried at 70C for 48 h and weighed.

The dried leaves were ground and subsamples were ashed for mineral analysis. To determine N concentrations, tissue samples were digested and analyzed colorimetrically according to the method of Isaac and Johnson (1976). The concentrations of P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn were determined using an inductively coupled argon plasma spectrometer (Donohue and Aho, 1992). Leaf mineral contents were analyzed by DRIS (Diagnosis and Recommendation Integrated System) which, based on nutrient balance ratios, is a useful tool in diagnosing nutrients limiting to yield (Parent and Granger, 1989).

A fresh root sample of each plant was cleared with 2.5% KOH and stained with acid fuschin (Brundrett et al., 1984) before estimating their mycorrhizal colonization status. The percentage of root length containing vesicles was assessed by the grid-intersect method (Giovanetti and Mosse, 1980).

After harvesting the roots, the soil from each pot was collected separately and mixed thoroughly to homogenize the extramatrical hyphae. A 5-g soil sample containing hyphae was suspended in 200 ml water and a 15-ml subsample of the soil suspension was collected on a filter (model no. 1; Whatman). The external hyphae were stained with an acid fuschin solution (2% in 85% lactic acid) and rinsed with acidified water. The length of non-septate hyphae was measured by the modified line-intersect method of Tennant (1975), based on three subsamples per pot. As it is not yet possible to distinguish mycorrhizal hyphae from other non-septate contaminants, the measurements obtained overestimate the length of external mycorrhizal hyphae. Hyphal length was also measured in uninoculated soil to estimate the amount of nonseptate contaminants.

Statistical analysis. An analysis of variance (ANOVA) was run on the data, and the differences between the treatment means were further determined with the Bonferroni multiple range test (SAS Institute, 1987). Data for the leaf mineral concentrations were analyzed after arcsin transformation, except for B, Zn, and Mn, which did not require such transformation according to Bartlett's test for homogeneity of variance (Steel and Torrie, 1980).

Results

Plant height. After 6 weeks, plants inoculated with *G. aggregatum*, *G. versiforme*(OR), and *G. intraradix* were taller than the controls (Fig. 1). Plants inoculated with *G. versiforme*(CAL) were significantly taller than the controls at the 8th and the 10th week. At the 8th week, the mycorrhizal plants were twice as tall as

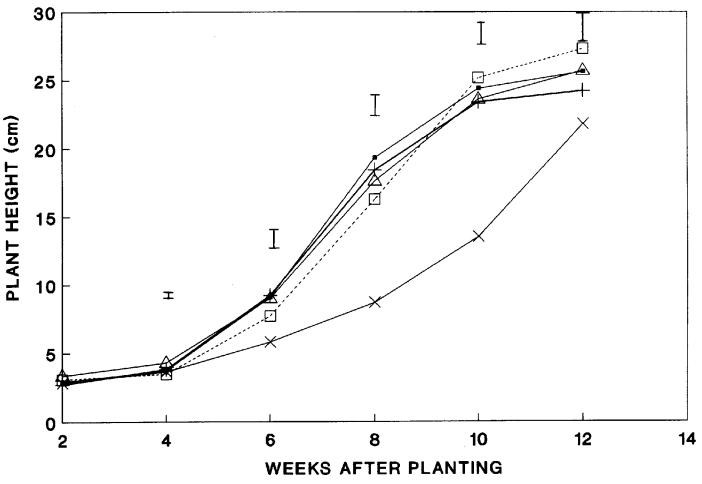

Fig. 1. Height of four apple rootstock cultivars, grown in a nursery soil under greenhouse conditions over 12 weeks. Plants were inoculated with the following: Δ, *Glomus versiforme*(OR); + , *G. aggregatum;* ■, *G. intraradix;* ❑, *G. versiforme* (CAL); ×, non mycorrhizal. Values are means of 20 replicates. The bar represents the range at $P < 0.05$ (Bonferroni test).

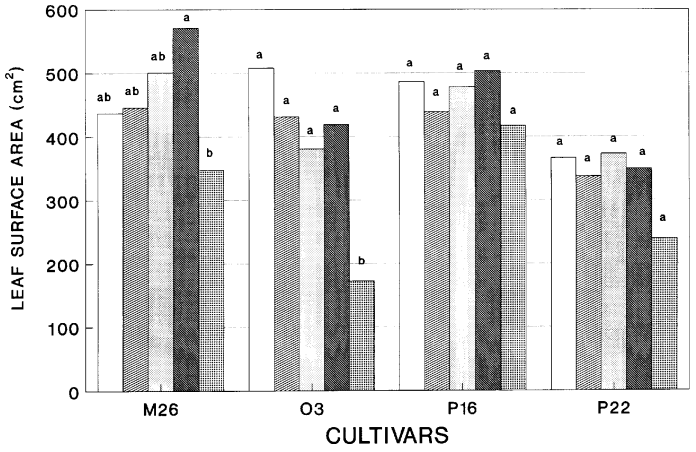

Fig. 2. Leaf surface area of four apple rootstock cultivars, grown in a nursery soil under greenhouse conditions for 12 weeks. Plants were inoculated with: white bars, *Glomus versiforme*(OR); lighter stripe shade, *G. aggregatum*; darker dot shade, *G. intraradix*; darker stripe shade, *G. versiforme*(CAL), lighter dot shade, non mycorrhizal. Values are means of five replicates. Within rootstocks, bars with the same letter are not significantly different ($P < 0.05$, Bonferroni test).

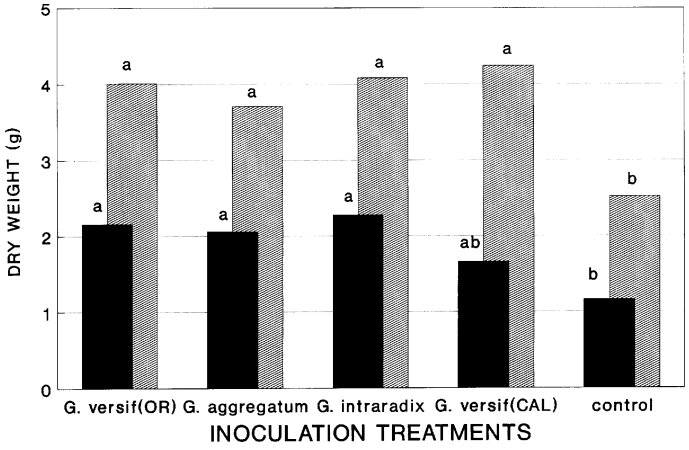

Fig. 3. Shoot (striped shade) and root (black shade) dry weights of apple rootstock cultivars, inoculated with different VAM isolates and grown in a nursery soil, under greenhouse conditions for 12 weeks. Values are means of 20 replicates. Within a series, bars with the same letter are not significantly different ($P < 0.05$, Bonferroni test).

the control plants. For example, at the 8th week the average height was 17.9 cm compared to 8.7 cm, respectively, for inoculated plants and controls. By the end of the experiment, the growth of the mycorrhizal plants had reached a plateau, with the result that plants inoculated with *G. intraradix* and *G. aggregatum* were no longer significantly taller ($P > 0.05$) than the control plants. *G. versiforme*(CAL)-inoculated plants overall had the greatest final height.

At the 4th and 12th week, a significant interaction ($P < 0.05$) between cultivar and inoculation treatment indicated a better response of 'M.26' rootstock to inoculation, as compared to the other cultivars, as the difference in height between mycorrhizal and non-mycorrhizal plants was greatest ($P < 0.05$, Bonferroni multiple range test) for 'M.26' rootstock than for 'P.16' (data not shown).

Leaf surface area. A significant ($P < 0.01$) rootstock cultivar × inoculation interaction indicated that the leaf area of 'Ott.3' and 'M.26' rootstocks could be increased by inoculation, while that of 'P.16' and 'P.22' was not affected (Fig. 2). 'M.26' produced significantly greater leaf surface area only when inoculated with *G. versiforme*(CAL).

Dry mass. The fungal isolates had similar effects on plant root and shoot dry mass (Fig. 3), and generally, mycorrhizal plants produced significantly greater dry weights than the control plants. Independent of the cultivar, plants inoculated with *G. versiforme*(CAL) had a greater ($P < 0.05$) shoot : root dry mass ratio than that of *G. intraradix* and *G. aggregatum* (data not shown). There was no difference between the shoot : root ratios of plants inoculated with the two *G. versiforme* isolates.

Mineral analysis. No rootstock cultivar × inoculation interaction effect was found in plant leaf concentration data. The N, Mg, S, and Fe leaf concentrations of mycorrhizal plants were similar among the mycorrhizal treatments, but significantly lower than that of the control plants (Table 1). In contrast, the P concentrations of control plants were significantly lower than that of the mycorrhizal plants and were below the critical value of 1.3 $mg \cdot g^{-1}$ determined by Shear and Faust (1980). Nitrogen was deficient in plants of all mycorrhizal treatments as the concentrations were below the critical value of 15 $mg \cdot g^{-1}$ (Shear and Faust, 1980). The negative DRIS indices (Table 1) emphasized that N was limiting (Kelling and Schulte, 1986). Though plant K and Ca concentrations were above their respective critical level, the DRIS indices showed that they tended to be limiting in the control treatment. Magnesium was in the sufficiency range for optimum

Table 1. Leaf mineral concentrations and DRIS[z] indices of apple rootstock cultivars inoculated with different VAM isolates.

Inoculation treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
				$mg \cdot g^{-1}$					$\mu g \cdot g^{-1}$		
G. versiforme(OR)	13.0[y]a	1.90 a	23.1 a	10.6 a	3.37 a	1.67 a	55.4 a	16.0 a	142.9 a	48.6 a	41.4 a
G. aggregatum	12.7 a	2.30 a	21.6 a	10.8 a	3.18 a	1.54 a	57.1 a	18.0 a	142.4 a	48.0 a	43.6 a
G. intraradix	12.4 a	2.01 a	21.8 a	10.6 a	3.23 a	1.65 a	56.6 a	15.7 a	137.8 a	50.2 a	46.2 a
G. versiforme(CAL)	14.3 a	1.96 a	22.7 a	10.2 a	3.37 a	1.72 a	55.8 a	17.2 a	138.8 a	47.2 a	40.6 a
Control	18.9 b	1.24 b	23.1 a	10.8 a	3.84 b	2.04 b	60.5 a	15.2 a	180.4 b	50.3 a	43.7 a
			DRIS index[x]				Total DRIS index				
G. versiforme(OR)	–36	9	0	–14	41		100				
G. aggregatum	–36	9	5	–12	35		97				
G. intraradix	–36	9	3	–14	38		100				
G. versiforme(CAL)	–33	7	0	–13	38		91				
Control	–15	–5	–18	–18	56		112				

[z]DRIS = Diagnosis and Recommendation Integrated System.
[y]Values are means of 20 replicates. Within a column, means followed by the same letter are not significantly different ($P < 0.05$, Bonferroni test).
[x]The most negative index is for the element most required and the most positive index gives the element least required (Kelling & Schulte, 1986).

yield by either approach, for all the treatments. Overall, the mycorrhizal plants had a better nutrient balance as shown by the lower sum of DRIS-index values (Jones et al., 1986) as compared to the control plants. The plants colonized by *G. versiforme*(CAL) showed the best nutrient balance of all inoculation treatments.

Mycorrhizal colonization. At the end of the experiment, 'P.16', 'M.26', and 'Ott.3' were highly mycorrhizal, with over 90% of their root length colonized, while 'P.22' was significantly ($P <$ 0.05) less colonized than 'M.26', 'P.16', and 'Ott.3', with 81% root colonization (Fig. 4).

Plants of mycorrhizal treatments had similar percentages of root colonization, which varied from 88% to 96% (Fig. 5). No mycorrhizal colonization was observed in the non-inoculated control plants.

Extramatrical hyphal length. Cultivars had similar ($P > 0.05$) lengths of external hyphae (Fig. 4), but *G. versiforme* produced more external mycelium than *G. intraradix* and *G. aggregatum* and there was no significant difference between the two isolates (Fig. 5). Although it is not possible to discriminate between VAM fungi and other fungal non-septate contaminants, the amount of hyphae present in the soil of the controls should represent the probable amount of mycelium other than mycorrhizal, as no intraradical colonization was found in the uninoculated control plants. The amount of hyphae in the soil of the control pots was small compared to that of the inoculated plants (<10%).

Discussion

Despite the high P availability of our soil, all mycorrhizal isolates improved leaf P concentration and apple tree growth, compared to the non-inoculated controls. This positive effect on plant growth is in agreement with the studies of Gnekow and Marschner (1989) and Plenchette et al. (1983a, 1983b), in which mycorrhizal growth-enhancement of apple remained significant in substrates containing high levels of extractable P. In contrast with the latter studies, our mycorrhizal plants contained a higher leaf P concentration than the non-mycorrhizal plants, while Zn and Cu concentrations remained statistically similar to that of the control plants. Such an increase in leaf P concentration is commonly observed in mycorrhizal plants growing in P-deficient soils (Koch et al., 1982; Plenchette et al., 1981), but is not expected under

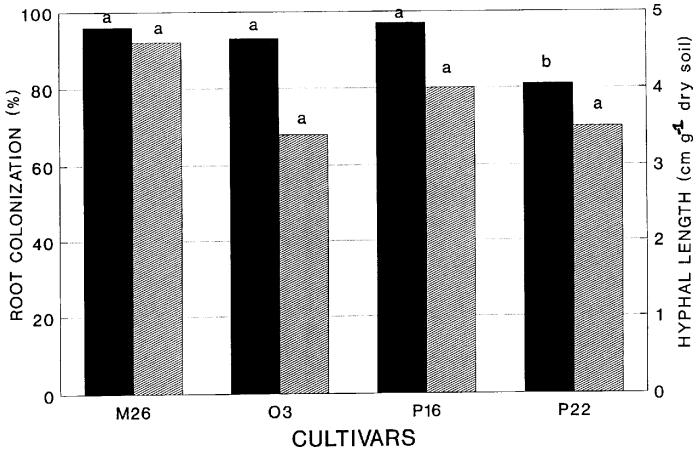

Fig. 4. Percentage root colonization (black shade) and extramatrical hyphal length (stripe shade) of four apple rootstock cultivars, grown in a nursery soil, under greenhouse conditions. Statistical analysis for root colonization does not consider non-mycorrhizal controls, since all values were zeros. Bars are means of 25 replicates. Within a series, bars with the same letter are not significantly different ($P < 0.05$, Bonferroni test).

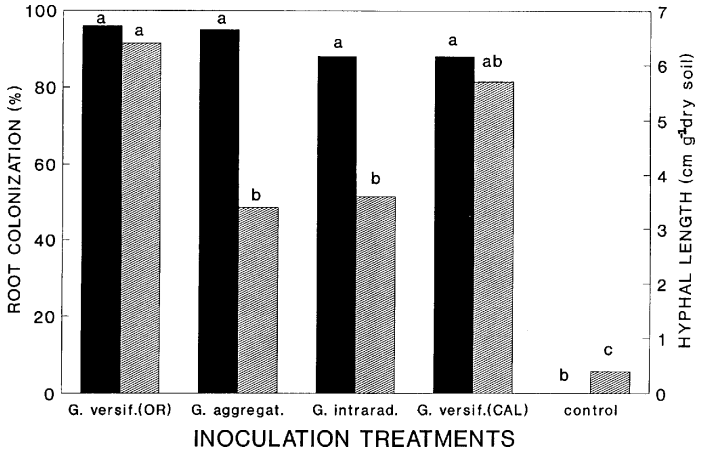

Fig. 5. Percentage root colonization (black shade) and extramatrical hyphal length (stripe shade) of four apple rootstocks inoculated with different VAM isolates. Values are means of 20 replicates. Within a series, bars with the same letter are not significantly different ($P < 0.05$, Bonferroni test).

conditions of high P fertility. Nevertheless, in spite of the high P availability of our soil (644 kg·ha^{-1}), the improved growth of the mycorrhizal plants was probably due to improved P nutrition, as indicated by the leaf mineral analysis. Atkinson (1986) and Neilsen et al. (1990) reported that P can limit fruit tree growth. As the plant needs a high P inflow rate and as P has a low mobility in soil, P depletion zones are likely to develop in the soil around apple roots because P is absorbed faster than it can diffuse toward the root surface. Our results emphasize that the P nutrition of apple trees is very dependent on the mycorrhizal fungi that extend the absorbing surface of plant root systems (Bolan et al., 1987). Therefore, responsiveness to mycorrhizal colonization is a characteristic that could be considered in the evaluation of apple rootstocks.

Glomus versiforme(CAL) was the best fungal isolate. It produced an extensive extramatrical phase and the plants inoculated with this isolate had the best nutrient balance (lowest total DRIS index), and the greatest final height, shoot dry mass, and shoot : root ratio. The large shoot : root ratio of *G. versiforme*(CAL)-inoculated plants can be related to their extensive extramatrical hyphal network, which lowered the need for root production. Sanders et al. (1977) also reported a decrease in the onion shoot : root ratio with effective endophytes, which they related to improved P nutrition.

It is unlikely that the initial delay of *G. versiforme*(CAL) in promoting plant growth, compared to the other mycorrhizal fungi, was a result of a delayed onset of colonization, as studies on the colonization process have shown that the most efficient VAM-species produced the highest and most rapid rate of colonization (Abbott and Robson, 1981; Miller et al., 1989; Reich, 1988). The initial delay in plant response may have been due to a greater drain on photosynthates by *G. versiforme*(CAL) during the development of a particularly extensive external mycelium, which finally was found to be very efficient. This hypothesis, however, needs to be verified.

As far as we know, mycorrhizae-induced growth depression of apple tree has never been reported. Sewell et al. (1988) conducted bioassays in which apple seedlings were grown in pots to predict the need of fumigation against apple replant disease. The results indicated that seedling height was generally greater when soil P availability was high in fumigated soils, but that in untreated soils it was mostly independent of P. This observation seems to suggest that, as mycorrhizal colonization is affected by soil P availability, mycorrhizal colonization with its potentially associated C drain is

not involved in replant disease. The authors concluded, rather, that mycorrhizae were very efficient in retrieving P when there was little available.

The depressed growth rate of mycorrhizal plants observed near the end of the experiment was probably due to a N deficiency, as a result of a dilution effect, i.e., plant growth surpassed N uptake. Because the initial growth of mycorrhizal plants was more vigorous, the plants could have depleted soil N, which became limiting. In contrast, it is possible that the P-deficient control plants did not grow enough to reach this level of soil N depletion, at least during the course of the experiment. The nutrient analysis data clearly show that, in spite of the high P availability of the soil, the P levels of non-mycorrhizal plants were lower than that of mycorrhizal plants.

At the end of the experiment, the fungal isolates differed in the length of their extramatrical hyphae, while they did not show any significant difference in their percentage of internal root colonization. Abbott and Robson (1985), working with clover, also found that VAM-species differed in the length of extramatrical hyphae produced per cm of colonized root.

Though 'P.22' had a significantly lower colonization percentage than 'M.26', 'P.16', and 'Ott.3', all the rootstocks showed similar growth responses to mycorrhizal inoculation. These results indicate that growth enhancement is related more to the extent of the extramatrical phase than to internal root colonization. Mycorrhizal efficiency seems characterized by an extensive external hyphal network and it appears that fungal isolates should be selected on the basis of this character, probably along with other factors, including their ability to colonize plants rapidly (Abbott and Robson, 1981).

In conclusion, in our P-rich soil (644 $kg \cdot ha^{-1}$), the benefit to mycorrhizal inoculation was attributed to improved P nutrition. Although 'M.26' rootstock tended to respond better to mycorrhizal inoculation, the interaction was rather weak. *G. versiforme*, especially the California isolate, was best for growth promotion and nutrition of all apple rootstocks. Large extramatrical mycelium development was associated with mycorrhizal efficiency.

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