Use of Vesicular-Arbuscular Mycorrhizae in Boston Fern Production: II. Evaluation of Four Inocula

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Abstract. Rooted plantlets of in vitro micropropagated Boston fern [*Nephrolepis exaltata* (L.) Schott var. *Whitmanii*] were transferred to pots containing a brown peat-based mix and simultaneously inoculated with one of four species of *Glomus*. *Glomus intraradices* and *G. clarum* formed rapid and extensive infection in *Nephrolepis exaltata* roots, while *Glomus vesiculiferum* and *G. versiforme* showed a significantly slower rate of infection. The high P fertilized control performed better than the other treatments, except in the number of fronds, which was similar. From the four mycorrhizal treatments, plants inoculated with *Glomus vesiculiferum* showed the most significant increase in growth when compared with the low P fertilization control. These results led us to re-examine vesicular-arbuscular mycorrhizal inoculation as an alternative to higher P fertilization in horticultural Boston fern production.

It would be questionable to talk about specificity with vesicular-arbuscular mycorrhizal (VAM) fungi because of their wide range of host plants, but the concept of specificity in this symbiotic association is also related to the degree to which the host plants derive benefit from the association. Isolates of VAM fungi differ in their ability to improve plant growth under greenhouse conditions. This has been demonstrated for several host plants (Carling and Brown, 1980; Mosse, 1973; Plenchette et al., 1982).

Since a VAM fungus exists as a continuous entity extending from the roots into the soil, it performs its function by virtue of its spread in the soil (Hayman and Mosse, 1972; Sanders and Tinker, 1971). Based on this assumption, Mosse (1972) stated that because of the wide host range of VA mycorrhiza, its specificity maybe determined more by the interactions between a fungal strain and the soil than between the fungus and its host. Therefore, in a previous experiment we selected a peat-based mix in which the mycosymbionts seemed to be more effective in their ability to improve growth of Boston fern (Ponton et al., 1990). In this work, four *Glomus* spp. were compared for their ability to colonize roots of Boston fern and to stimulate fern growth.

Micropropagation and substrate. Boston fern was micropropagated (at the "Serres Dion", Boisbriand, Québec, Canada) using in vitro techniques described by Henny et al. (1981). Before introduction to greenhouse conditions, plantlets were transferred to 60-cm³ pots and underwent 2 weeks of acclimatization under intermittent mist at 100% to 70% RH. Plantlets were maintained in these

Table 1. Percentage of *Nephrolepis exaltata* root segments containing arbuscules after 12 and 20 weeks.²

Fungus	Time of examination	
	12 weeks	20 weeks
<i>Glomus intraradices</i>	31 a	70 a
<i>Glomus vesiculiferum</i>	6 b	39 b
<i>Glomus clarum</i>	32 a	65 a
<i>Glomus versiforme</i>	2 c	11 c

²For each harvest, values with the same letters are not significantly different at $P < 0.05$, using Duncan's multiple range test.

Table 2. Analysis of variance of the percent of root segments containing arbuscules.

Source of variation ²	df	Sum of squares	Pr > F
Blocks	9	327	<0.0452
Harvests (H)	1	16,817	<0.0001
Fungi (F)	3	26,981	<0.0001
Fungi 3 vs. 5	1	45	<0.1148
Fungi 4 vs. 3 and 5	1	9,751	<0.0001
Fungi 6 vs. 3, 4, and 5	1	17,186	<0.0001
H × F	3	2,684	<0.0001
Error	63	1,102	
Total	79	47,911	

²Fungi 3: *Glomus intraradices*; fungi 4: *Glomus vesiculiferum*; fungi 5: *Glomus clarum*; fungi 6: *Glomus versiforme*.

Table 3. Number of fronds, frond height, and number of stolons of *Nephrolepis exaltata* var. *Whitmanii* harvested after 20 weeks².

Treatment	Number of fronds	Frond length (cm)	Number of stolons
Control (L)	46 a	19 c	19 bc
Control (H)	46 a	28 a	26 a
<i>G. intraradices</i>	41 a	22 b	18 bc
<i>G. vesiculiferum</i>	46 a	21 b	21 b
<i>G. clarum</i>	43 a	23 b	18 bc
<i>G. versiforme</i>	37 a	19 c	14 c

²For each criterion, values with the same letters are not significantly different at $P < 0.05$, using Duncan's multiple range test.

pots for 5 weeks before their transfer to 600-cm³ pots. Whatever the pot size during the trial, each fern plantlet was cultivated in the same mix of Canadian sphagnum peat (Premier Brands, Rivière-du-Loup, Québec). The mix used in this study was 75% brown peatmoss (Super SL) and 25% vermiculite.

Growth environment and fertilization. Fern plantlets were grown in the greenhouse with

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Table 4. Analysis of variance of number of fronds, number of stolons, frond height, frond dry weight, and root dry weight.

Source of variation ^a	df	Sum of squares	Pr > F
Number of fronds			
Blocks	9	2810	<0.0389
Harvests (H)	1	2159	<0.0003
Fungi (F)	5	984	<0.2668
Fungi 1 vs. 6	1	250	<0.2005
Fungi 3 vs. 4	1	148	<0.3234
Fungi 5 vs. 3 and 4	1	103	<0.4108
Fungi 2 vs. 3, 4, and 5	1	479	<0.0776
Fungi 1 and 6 vs. 2, 3, 4, and 5	1	5	<0.8625
H × F	5	1399	<0.1085
Error	99	14901	
Total	119	22253	
Number of stolons			
Blocks	9	279	<0.1912
Harvests (H)	1	3445	<0.0001
Fungi (F)	5	432	<0.0027
Fungi 1 vs. 6	1	53	<0.1233
Fungi 3 vs. 4	1	8	<0.5445
Fungi 5 vs. 3 and 4	1	1	<0.8154
Fungi 2 vs. 3, 4, and 5	1	158	<0.0084
Fungi 1 and 6 vs. 2, 3, 4, and 5	1	211	<0.0025
H × F	5	536	<0.0005
Error	99	2168	
Total	119	6860	
Frond length			
Blocks	9	34.32	<0.2308
Harvests (H)	1	941.92	<0.0001
Fungi (F)	5	501.14	<0.0001
Fungi 1 vs. 6	1	0.36	<0.7234
Fungi 3 vs. 4	1	0.04	<0.9036
Fungi 5 vs. 3 and 4	1	6.96	<0.1223
Fungi 2 vs. 3, 4, and 5	1	312.82	<0.0001
Fungi 1 and 6 vs. 2, 3, 4, and 5	1	180.96	<0.0001
H × F	5	159.86	<0.0001
Error	99	283.72	
Total	119	1920.96	
Frond dry weight			
Blocks	9	0.46	<0.1233
Harvests (H)	1	71.61	<0.0001
Fungi (F)	5	14.22	<0.0001
Fungi 1 vs. 6	1	0.04	<0.2610
Fungi 3 vs. 4	1	0.12	<0.0506
Fungi 5 vs. 3 and 4	1	0.00	<0.9463
Fungi 2 vs. 3, 4, and 5	1	10.13	<0.0001
Fungi 1 and 6 vs. 2, 3, 4, and 5	1	3.92	<0.0001
H × F	5	10.77	<0.0001
Error	99	3.15	
Total	119	100.22	
Root dry weight			
Blocks	9	0.41	<0.3725
Harvests (H)	1	64.21	<0.0001
Fungi (F)	5	10.84	<0.0001
Fungi 1 vs. 6	1	0.01	<0.5738
Fungi 3 vs. 4	1	0.14	<0.0720
Fungi 5 vs. 3 and 4	1	0.00	<0.8796
Fungi 2 vs. 3, 4, and 5	1	6.78	<0.0001
Fungi 1 and 6 vs. 2, 3, 4, and 5	1	3.90	<0.0001
H × F	5	8.36	<0.0001
Error	99	4.16	
Total	119	87.98	

^aFungi 1: control type L; fungi 2: control type H; fungi 3: *Glomus intraradices*; fungi 4: *Glomus vesiculiferum*; fungi 5: *Glomus clarum*; fungi 6: *Glomus versiforme*.

day/night averages of 26/21C (±3C). High-pressure sodium vapor lamps were used to provide 15 klx light to extend the daylength to 16 hr. Plants were watered as needed and fertilized weekly with a commercial solution that contained in each liter: 480 mg of MgSO₄·7H₂O, 750 mg of Ca(NO₃)₂·4H₂O,

31.4 mg of 10N-52P-10K, and 550 mg of 12N-0P-44K (Plant Products Co., Bramalea, Ontario). This nutrient solution is based upon the nutrient solution of Long Ashton (Hewitt, 1966) and is particularly weak in P. Pots of 60 cm³ and 600 cm³ received 6 ml·week⁻¹ and 60 ml·week⁻¹ of nutrient so-

lution, respectively. The mix was limed in order to obtain a pH between 5.8 and 6.2.

VAM inoculation. *Glomus intraradices* Schenck & Smith, *G. vesiculiferum* (Thaxter) Gerdemann & Trappe, *G. clarum* Nicolson & Schenck, and *G. versiforme* (Karsten) Berch were taken from VAM inocula produced by the Centre de Recherche Premier, Ltée. (Rivière-du-Loup) using the pot culture method of Plenchette et al. (1982). Samples of these four *Glomus* spp. have been deposited at the National Mycology Herbarium, Ottawa, Ont., and are respectively identified with the numbers DAOM 197918, DAOM 198531, DAOM 193873, and DAOM 196672. Experimental pots were inoculated with 1 g fresh weight of leek roots colonized by the appropriate fungus. Inoculations were performed at the onset of the acclimatization period when plantlets were provided with about 10 roots of 0.5 to 1.0 cm length. Each of the two types of uninoculated controls received 1 g of nonmycorrhizal leek roots. Type L control received low P fertilization consisting of the basal nutrient solution that also was given to inoculated plants. Type H control received high P fertilization consisting of the basal nutrient solution supplemented with 114 mg·liter⁻¹ of ON-44P-0K (Sudbury Laboratory, Sudbury, Mass.).

Experimental design. A randomized complete-block (10 blocks) design was used to study the effect of VAM on Boston ferns in the selected peat-based substrate. Each plant was an experimental unit. There were six mycorrhizal treatments: inoculated with mycorrhizal leek roots containing chlamydospores of *Glomus intraradices* (Gi); inoculated with *G. vesiculiferum* (Gvm); inoculated with *G. clarum* (Gc); inoculated with *G. versiforme* (Gve); type L uninoculated control; and type H uninoculated control. There were two replicates of each treatment in each block.

Harvesting and measurements. One random replicate of each treatment in each block was harvested 12 and 20 weeks after inoculation. The percentage of root segments containing arbuscules was determined using the gridline intersect method described by Giovannetti and Mosse (1980) after clearing and staining the roots according to Brundrett et al. (1984). The fronds and roots were oven-dried for 3 days at 65C before the dry weight was recorded. The total number of fronds and stolons was evaluated at each harvest. For the estimation of total height we took the average of the five longest fronds for each plant.

Statistical analysis. The homogeneity of variances for all variables was tested using the Burr-Foster procedure (Anderson and McLean, 1974). The variances for the percentage of root segments containing arbuscules were analyzed using analysis of variance (ANOVA) (5% level of significance) procedures for a 2 × 4 factorial model, whereas those for the other characteristics were analyzed for a 2 × 6 factorial model (Steel and Torrie, 1980). Duncan's multiple range test ($P < 0.05$) was used in order to illustrate differences between means.

Fig. 1. Frond dry weight of *Nephrolepis exaltata* inoculated with *Glomus intraradices* (Gi), *G. vesiculiferum* (Gvm), *G. clarum* (Gc), or *G. versiforme* (Gve) in the selected peat-based mix. Measurements were made 12 and 20 weeks after inoculation. Noninoculated controls received low (L) and high (H) P fertilization. For each harvest, values with the same letters are not significantly different (Duncan's multiple range test, $P < 0.05$).

Fig. 2. Root dry weight of *Nephrolepis exaltata* inoculated with *Glomus intraradices* (Gi), *G. vesiculiferum* (Gvm), *G. clarum* (Gc) or *G. versiforme* (Gve) in the selected peat-based mix. Measurements were made 12 and 20 weeks after inoculation. Noninoculated controls received low (L) and high (H) P fertilization. For each harvest, values with the same letters are not significantly different (Duncan's multiple range test, $P < 0.05$).

Vesicle development remained rather low, with $<18\% \pm 5\%$ of root segments containing vesicles after 20 weeks (data not shown). After 12 and 20 weeks, Gi and Gc colonized fern roots better than Gvm, which in turn showed a better colonization than Gve (Table 1). The percentage of arbuscules increased in the second harvest in all the *Glomus* spp. These significant effects of fungi (F) and harvest (H) were also shown by the ANOVA of the percent root segments containing arbuscules (Table 2).

Growth characteristics of Boston fern were evaluated after 20 weeks (Table 3). Control H performed better than the other treatments except for the number of fronds, for which differences were nonsignificant. The ANOVA of the number of fronds indicated that it was influenced by the time of harvest (Table 4). The mean frond length was similar for all treatments at the first harvest (data not shown). Significant differences appeared after 20 weeks (Table 3). On a frond-length basis, all of the mycorrhizal treatments, except Gve, performed better than control L. These significant effects of fungi (F) and harvest (H), as well as the variation in the

effect of fungi on the frond length with the time of harvest ($H \times F$), were also shown by the ANOVA of the frond length (Table 4).

The ANOVA revealed that the frond dry weight was more affected by the time of harvest than by the fungi (Table 4). At the first harvest (12 weeks), the treatments showed little significant effect on frond dry weight (Fig. 1). After 20 weeks, control H performed better than any other treatment. Of the four mycorrhizal treatments, only plants inoculated with Gvm showed a significant increase in frond dry weight when compared with control L. Similar results were obtained for the root dry weight (Table 4). Except for plants inoculated with Gve, all the other treatments showed a significant, but small, increase in root dry weight when compared with control L after 12 weeks (Fig. 2). At the second harvest (20 weeks), plants inoculated with Gvm and Gc showed a significant increase from control L. Control H performed better than the other treatments.

Results of the present study and those of the previous one (Ponton et al., 1990) show that, after 20 weeks, vesicle development re-

mained low in Boston fern roots. The inoculum used in this experiment consisted of leek roots colonized by the appropriate fungus. In leek root inoculum of identical age, the same fungal strains produced almost exclusively vesicles. Since the morphology and the colonization pattern of endomycorrhizas is influenced by host plant (Daniels-Hetrick et al., 1985; Jaquelinet-Jeanmougin and Gianizazzi-Pearson, 1983; Lackie et al., 1987), root physiological status could explain such a low vesicle development in Boston fern roots.

Glomus spp. in the present study are divided into two groups according to the percentage of fern root segments in which they formed arbuscules. *Glomus vesiculiferum* and *G. versiforme* had a significantly slower rate of infection than Gi and Gc. It is well-recognized that difference of root colonization by various VAM fungi is attributable to both edaphic conditions and host-fungus interactions (Harley and Smith, 1983). However, Gvm and Gi were found to colonize fern roots with the same intensity in previous work (Ponton et al., 1990). The only major difference between these two experiments was that one was conducted in spring-summer seasons and the other in fall-winter (present study). Thus, the timing of VAM fungus inoculation on host plants appears to be very important and could affect the ability of propagules to germinate and initiate primary infections on fern roots and consequently could have an effect on their rate of infection. This effect would be more or less important depending upon the endophyte life cycle. Field and greenhouse studies by Boullard (1957) and Iqbal et al. (1981) also reported that VAM fungal colonization of other fern plants was affected by seasonal variations.

These differences in the ability of the fungi to colonize fern roots had little effect on the number of fronds or stolons and a limited effect on frond length (Table 3). Stamps and Johnson (1984) reported that frond number of leatherleaf fern was reduced when inoculated with Gi. According to our results, VAM inoculation had no significant effect on frond number of *Nephrolepis exaltata* after 20 weeks. They also noted that inoculated plants produced heavier fronds. Among the fungal isolates tested under our experimental conditions, only plants inoculated with Gvm tended to have a significantly higher frond dry weight.

Inoculation with Gvm was also the best mycorrhizal treatment in terms of root dry weight, in spite of its slow rate of colonization after 20 weeks (Figs. 1 and 2). Benefit from a particular strain was not always correlated with the extent of root infection (Mosse, 1972). Abbott and Robson (1985) found that VA mycorrhizal fungi differed in the length of external hyphae produced per centimeter of infected root. They also demonstrated that a VAM fungus can produce larger amounts of external mycelium than another one, even with a lower intensity of infection spread. Graham et al. (1982) found that the difference between the capacity of different isolates of VAM fungi to enhance

growth of *Citrus* was more a function of the amount of external hyphae that had developed than the degree to which they colonized the roots. According to our present results and those previously obtained (Ponton et al., 1990), Gvm performed better than other *Glomus* spp. with Boston fern. This interesting potential could be attributable to *G. vesiculiferum*'s capacity to spread easily in our peat-based mixture.

In the present study and the previous one (Ponton et al., 1990), mycorrhizal treatments are compared with two types of uninoculated controls in order to evaluate the growth effects of VAM on Boston fern plantlets. Type L controls received low P fertilization that was also given to inoculated ferns, whereas type H controls received a rich supplement of P. Both studies convincingly showed that growth of inoculated plants was higher than control L but lower than that of control H. It appears that a substantially richer P fertilization improves the production of Boston fern more than endomycorrhizal inoculation. However, Ramade (1982) reported that with the present rate of consumption in P, this resource would be exhausted at the end of the next century. VAM inoculation in horticultural production of Boston ferns could be the first step toward a fern more efficient in absorbing P, which would be a compromise between the overconsumption of this mineral and a faster-growing plant.

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