VA Mycorrhizal Inoculation of Landscape Trees and Shrubs Growing under High Fertility Conditions[1,2]

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Abstract

This study investigated the effects of vesicular-arbuscular mycorrhiza (VAM) inoculation on the growth of landscape trees and shrubs under high-fertility nursery growing conditions. Four species of 1 year old trees, and rooted cuttings of nine species of shrubs, were inoculated with *Glomus intraradices*, or *Glomus fasiculatum*, or served as non-inoculated controls. The trees were transplanted to two high fertility, non-sterile field locations. Inoculation significantly increased the level of colonization in *Acer platanoides, Sorbus aucuparia, Malus*, and *Fraxinus pennsylvanica*, but did not enhance growth. The shrubs were containerized in a peat and bark medium with two levels of controlled release fertilizer. VAM inoculation significantly increased the level of colonization in *Spiraea* × *bumalda, Syringa* × *chinensis, Prunus* × *cistena, and Cornus alba*, while *Weigela, Cotoneaster dammeri*, and *Potentilla parvifolia* became well colonized without inoculation. *Forsythia ovata* and *Viburnum opulus* did not become significantly colonized. The growth of *Syringa* was stimulated by VAM inoculation during consecutive seasons, irrespective of fertilizer level. The growth of *Prunus* at the lower fertilizer level was significantly stimulated by inoculation even though control plants became highly colonized without VAM inoculation. Two years after inoculation, five species were transplanted to a second, non-sterile, field site to monitor the effect of inoculation on post transplant growth. *G. intraradices* significantly enhanced *S. aucuparia* caliper growth in the second year post-transplant.

Index words: *Glomus fasiculatum, Glomus intraradices*, phosphorus tolerance

Species used in this study: Norway Maple (*Acer platanoides* L.); Green Ash (*Fraxinus pennsylvanica* Marsh.); Mountain Ash (*Sorbus aucuparia* L.); Crabapple (*Malus* Mill. var. Rudolph); Cotoneaster (*Cotoneaster dammeri* C.K. Schneid. 'Coral Beauty'); Silveredged dogwood (*Cornus alba* L. 'Elegantissima'); Forsythia (*Forsythia ovata* Nakai. 'Northern Gold'); Cinquefoil (*Potentilla parvifolia* Lehm. 'Goldfinger'); Spiraea (*Spiraea* × *bumalda* Burv. 'Anthony Waterer'); Viburnum (*Viburnum opulus* L.); Weigela (*Weigela* Thunb. 'Bristol Ruby'); Lilac (*Syringa* × *chinensis* Willd. 'Sanguinea'); Purple leafed sandcherry (*Prunus* × *cistena* N.E. Hansen).

Significance to the Nursery Industry

Vesicular-arbuscular mycorrhizas (VAM) are naturally occurring soil fungi shown to enhance the growth of several woody species grown under low to moderate fertility. This study addresses the response of 4 field-grown landscape trees and 9 container-grown shrubs to inoculation with VAM under high fertility conditions. None of the tree species showed growth enhancement due to VAM-inoculation following 2 years of growth; however, after transplant to a secondary field site, inoculated *S. aucuparia* did exhibit enhanced growth. The response of the containerized shrubs ranged from a strong growth enhancement due to inoculation in *Syringa* to growth suppression in *P. parvifolia* and *C. alba*.

The results in this study demonstrate that growth responses to VAM inoculation can be observed in woody ornamental stock at nursery fertility levels and with competition from indigenous mycorrhizae; furthermore, inoculated stock which does not show a growth response in the nursery may exhibit a growth response upon transplant to a second field site. Despite these observations, most of the species tested exhibited no growth response or a negative growth response to the inocula used in this study. In order for VAM inoculation to be of significant benefit to landscape plant production inoculum performance must be improved substantially.

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Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi are obligate biotrophs which occur naturally in soil and enter into symbiotic relationships with an estimated 90% of higher plants (15). The symbiosis imparts a wide range of benefits to the host plant including increased nutrient uptake (2, 3, 18), increased resistance to soil pathogens (4, 5, 24), increased transplant survival (13), increased drought tolerance (11, 21), and decreased toxicity of soil pollutants (8). Increased uptake of phosphorus and other immobile nutrients

is considered the principal benefit of the symbiosis, and other benefits are indicated to be an indirect result of improved phosphorus nutrition (10).

Nursery field soils may have low levels of indigenous VA mycorrhizas because of the low density of host plants and high fertility. Many of the tree species cultivated in nursery fields, including birches, beeches, and pines, are not hosts for VAM fungi (17). Thus, the infectivity of nursery soils would be expected to be inconsistent, preventing transplants from being colonized quickly and uniformly. In many nurseries, landscape plants are often produced in containers using VAM-free artificial media. The prospect of limited VAM populations in nurseries suggests inoculation as a strategy for improving plant growth.

In general, the effects of VAM inoculation on plant growth have been studied in media of low fertility, whereas production fields and containers are often highly fertile. Nursery stock may become colonized with VAM even without inoculation, but neither the natural levels in a wide species range, or the effects of additional colonization, by inoculation, have been thoroughly studied. This study was designed to determine the effect of VAM inoculation on a range of landscape shrub and tree species growing under high fertility, nursery production conditions.

Materials and Methods

Experiment 1. Field grown landscape trees. Sixty, one year old *Acer platanoides L., Fraxinus pennsylvanica* March., *Sorbus aucuparia* L. and fifty *Malus* Mill. var. Rudolph (of similar age) were obtained from a source nursery. The *A. platanoides, S. aucuparia,* and *Malus* had mean stem diameters (± standard deviation) of 11.6 ± 1.6 mm (0.464 ± 0.064 in), 11.6 ± 1.4 mm (0.464 ± 0.056 in), and 9.0 ± 1.0 mm (0.36 ± 0.04 in) respectively. Measurements were made at 30 cm (12 in) above the graft union or root crown. *F. pennsylvanica* had a mean stem diameter of 3.2 ± 0.5 mm (0.13 ± 0.02 in) at 20 cm (8 in) above the root crown.

Root samples were collected randomly from twenty trees in each species to assay for mycorrhizal colonization. The root samples were cleared, stained (16), and assayed by the plate estimation method (9). The percentage of the root length which contained VAM was estimated according to an index describing degrees of infection from 0 to 10 where 0: No VAM, 1: >10% of the root length colonized, 2: 10–20%, 3: 20–30%, 4: 30–40%, 5: 40–50%, 6: 50–60%, 7: 60–70%, 8: 70–80%, 9: 80–90%, 10: 90–100%.

A sandy loam, and a clay loam field (each 450 m^2, or 4800 ft^2) were prepared for transplanting. The sandy loam field had been fallowed the previous year, while the clay loam field had been fallowed for two years. Both fields had been plowed and harrowed the summer prior to transplanting. Both plots were fertilized with 39 kg N/ha (35 lb N/acre) as ammonium nitrate each spring. Average available phosphorus levels, one year after transplanting, were found to be 267 ± 27 kg P/ha (238 ± 24 lb P/acre) in the sandy loam and 247 ± 53 kg P/ha (221 ± 47 lb P/acre) in the clay loam. The Nova Scotia Department of Agriculture and Marketing Soil Testing Laboratory rated both fields as high in phosphorus.

Before transplanting, three to five soil samples were collected from the top 15 cm (6 in) of soil within each plot. Dilution assays (19, 22) established that the sandy loam and clay loam soils had average inoculum potentials of 12 propagules/g (340 prop/oz) (st. dev. = 7 prop/g) and 0.8 prop/g (20 prop/oz) (st. dev. = 0.9 prop/g) respectively.

The trees were transplanted in late April. At each field site three treatments were applied to each tree species: inoculated with *Glomus intraradices* Schenk and Smith, inoculated with *Glomus fasiculatum* Thaxter Gerd. and Trappe, and non-inoculated (both isolates were kindly provided by Dr. Valentin Furlan, Agriculture Canada). The trees were transplanted in rows, with only one tree species per row. The roots of each tree were dipped in a 0.3% slurry of a hydrophilic polymer (potassium propenoate copolymer; Agrigel, Nepera Inc., Harriman, NY) and inoculum was sprinkled onto tree roots as the trees were lowered into the furrow. Each inoculated tree received 5g (0.18 oz) of *G. fasiculatum* or 4g (0.14 oz) of *G. intraradices* inoculum. The inoculum was prepared by non-flowing hydroponic culture of VAM infected *Latuca sativa* var buttercrunch and *Phaseolus vulgaris* var Pinto. The roots and attendant extramatrical mycelium in the hydroponic cultures were chopped up and mixed 1:2 (by weight) with neutralized, dried peat. Inoculum infectivity was determined by the most probable number method to be 9200 prop/g for *G. fasiculatum* and 5400 prop/g for *G. intraradices*.

For each tree species, the experimental design was a completely randomized arrangement of 3 inoculum treatments at 2 field sites. The experimental unit was a pair of trees, planted 4 feet (1.2 m) apart, receiving the same inoculum. Each tree species included 30 experimental units, 24 for *Malus*. Experimental units were separated by 6 feet (1.8 m) within rows, and 4 or 8 feet (2.4 m) between rows.

The trees in both plots were observed for 17 months (through August of the second growing season). The plots were manually weeded several times during each spring and summer. In late August of both years, soil cores were collected from around the base of each tree in each treatment. Roots were separated from the soil samples, stained, and assayed for mycorrhizal colonization using the plate estimation method. Stem diameter measurements were taken at the same point on the stem as had been used initially.

Experiment 2. Containerized landscape shrubs. Rooted cuttings of *Cotoneaster dammeri* C.K. Schneid. 'Coral Beauty', *Cornus alba* L. 'Elegantissima', *Forsythia ovata* Nakai. 'Northern Gold', *Potentilla parvifolia* lehm. 'Goldfinger', *Spiraea* × *bumalda* Burv. 'Anthony Waterer', *Viburnum opulus* L., *Weigela* Thunb. 'Bristol Ruby', *Syringa* × *chinensis* Willd. 'Sanguinea', and *Prunus* × *cistena* N.E. Hansen were obtained from a commercial propagator. Root samples from ten cuttings of each species were stained and assayed for mycorrhizal infection by the plate estimation method.

Cuttings were inoculated and transplanted into 15 cm (6 in) pots containing moist sand. The plants were inoculated by coating all roots with either *G. intraradices* (5400 prop/g = 154,000 prop/oz), *G. fasiculatum* (1100 prop/g) or a non-mycorrhizal control inoculum. The non-mycorrhizal control inoculum was prepared by making a 1:1 slurry of a mixture of the *G. intraradices* and *G. fasiculatum* inoculum, and sterile distilled water. The slurry was allowed to stand for a few hours and then filtered through #1 Whatman filter paper (pores size of 11 μm). A dilution assay of the control inoculum confirmed it contained no mycorrhizal propagules.

The plants remained in the greenhouse for six weeks prior to being transplanted into the permanent growing medium. The cuttings were selected for uniformity based on fresh weight and thirty plants of each species in each inoculated treatment were transplanted into peat based potting media. Root samples were collected from the discarded plants and assayed to determine the degree of mycorrhizal colonization. Two potting media of different fertility were used for the transplants such that there were fifteen plants of each species per fertilizer level/inoculum treatment combination. Both media consisted of a 1:1:1 mixture of peat:bark:sand, augmented with slow release fertilizers (Nutricote and Nutritrace; Chisso-Asahi Fertiler Co. Ltd., Tokyo, Japan). The high fertility medium contained 5kg/m^3 (8.3lb/yd^3) of Nutricote 16N:4.4P:8.1K (16-14-10), 0.5 kg/m^3 (0.83 lb/yd^3) of Nutritrace (a slow release trace elements fertilizer) and 4.6 kg/m^3 (7.6 lb/yd^3) of dolomite. The lower fertility medium contained the same amount of dolomite, but only 50% of the Nutricote and Nitritrace. Each plant was transplanted into a separate 3 l (#1) container and allowed to grow outside from May to November. The plants were segregated by species and fertilizer level on a uniform standing ground to eliminate shading effects. For each species and fertilizer level, the three inoculum treatments were randomized within each block. The pots were manually weeded and watered as necessary.

In early October, five plants were selected at random from each of the shrub species/fertilizer level/inoculum treatment combinations and harvested. The potting medium was washed from the roots, and a representative root sample of each plant was collected. The roots were stained and assayed according to the plate estimation method. Roots and shoots were dried at 60°C, and masses were determined. The remaining ten plants in each treatment were stored in a plastic house over the winter.

The following spring, five of the shrub species, *C. alba, P. parvifolia, Weigela, Syringa*, and *Prunus* were chosen for continued study. These shrubs were transplanted into 12 l (#5) containers in late May and organized in a complete randomized block design. The entire contents of the original 3 l (0.8 gal) containers were placed into the larger containers, which were then filled with fresh medium. The fresh potting medium was as that prepared the previous spring except that it contained 5.8 kg/m^3 (9.6 lb/yd^3) Nutricote, 0.58 kg/m^3 (0.96 lb/yd^3) Nutritrace, and 4.6 kg/m^3 (7.6 lb/yd^3) dolomite at the 100% fertilizer level. The 50% fertilizer level contained the same amount of dolomite, but half the Nutricote and Nutritrace. Only dead branch tips were cut off during pruning.

In late September of the second growing season, five of the remaining plants in each treatment combination were randomly selected and harvested. The plants were processed as in the previous year, with dry masses and degrees of mycorrhizal infection being determined.

Experiment 3. Post-transplant effects of inoculation. Eight *Malus, S. aucuparia*, and *F. pennsylvanica* from each inoculation treatment were selected from the clay loam field for transplant to a second field site. The trees were selected to minimize the variance of stem diameters around the treatment mean. In late April the trees were transferred, bare-root, to the transplant site. During transport, the trees were shaded, and roots were coated in Agrigel, to minimize dehydration. At the transplant site, the trees were transferred to holes dug on 1.2 m (4 ft) centers. Each species was planted in a different row, with treatments randomized within rows.

Four *P. parvifolia* and *Spiraea* shrubs from each treatment in the first phase of the study (a factorial combination of 2 fertilizer levels by 3 inoculum levels) were also transplanted to the second site. The entire volume of each #5 container was placed into holes dug on 1.2 m (4 ft) centers. Each shrub species also received its own row, with the order of treatments randomized within the rows.

Stem diameters of the trees were measured at transplant, and the measuring points remarked on all trees. Root samples taken from the trees immediately prior to transplanting (while bare-root) were stained and assayed for VAM infection. Stem diameters of the trees were remeasured in September at the end of the first growing season after transplant, and in October at the end of the second growing season. Dry masses of the shrub shoots were determined at the end of the second growing season after transplant.

For all experiments, results from each plant species and each year were analyzed separately. Within individual species and years, growth and colonization data were subjected to analyses of variance. Where indicated, Tukey's multiple comparison test was used to separate means.

Results and Discussion

Experiment 1. Field grown landscape trees. Ninety percent of the trees were colonized by VAM fungi prior to inoculation. Of the infected trees, 55% had less than 2% infection, 32% had 2 to 10% infection, and the remaining trees (all *A. platanoides* and *S. aucuparia*) had infection levels between 10 and 20% infection. The level of infection was similar in all species.

In year 1, inoculated *A. platanoides* trees showed less caliper growth in both mycorrhizal treatments than in the control treatment (Table 1).

In year 2, there was no significant difference in caliper growth between inoculum treatments. Inoculated trees had significantly higher levels of VAM infection than the controls in year 1, but there were no differences in year 2. Between years 1 and 2, VAM infection remained about the same in the controls, but decreased in the inoculated trees.

Growth suppression in the inoculated *A. platanoides* trees may reflect a mildly parasitic response. Indeed, the magnitude of the growth depression was greater in the sandy loam field where phosphorus fertility was higher, and control growth was greater than at the clay loam field. By the second year, levels of VAM infection decreased in inoculated trees and the growth suppression was no longer evident. The reduced intramatrical infection between years 1 and 2 probably reduced the mycorrhiza's carbohydrate utilization to a level compensated by the benefits of the symbiosis. This negative relationship between intramatrical VAM infection and plant growth at elevated levels of phosphorus fertility has been observed previously (1, 20). Both studies concluded that growth suppression resulted when phosphate fertility was high enough that enhanced phosphorus nutrition due to VAM did not offset endophyte photosynthate utilization.

There was no significant effect of inoculation on *Malus* caliper growth in the first year in either field (Table 2); however, in the second year, caliper growth of inoculated trees was less than that of the controls in sandy loam, but

Table 1. ***Acer platanoides*** **stem diameter (caliper) growth and VAM infection for two years at two field locations.**

Year	Field	Control		G. fasiculatum		G. intraradices	
		Caliper growth[w]	VAM infection[z]	Caliper growth	VAM infection	Caliper growth	VAM infection
1	Clay loam[x]	4.7 a[y]	0.5 A	4.2b	2.0 B	4.2 b	2.3 B
2		14.9 a	0.9 a	13.6 a	1.4 a	11.5 a	1.2 a
1	Sandy loam	5.8 a	0.9 A	4.2 b	1.3 B	3.7 b	1.8 B
2		10.6 a	0.7 a	10.4 a	0.9 a	10.7 a	1.1 a

[z]Level of VAM infection is classified on a scale from 0 to 10.

[y]Mean separation within rows for caliper growth or VAM infection by Tukey's multiple comparison test. P=0.05 (lowercase letters) or 0.01 (uppercase letters).

[x]No interactions between field and treatment were observed.

[w]Caliper growth in mm. 1 mm = 0.04 in.

Table 2. Malus stem diameter (caliper) growth and VAM infection for two years at two field locations

Year	Field	Control		G. fasiculatum		G. intraradices	
		Caliper growth[w]	VAM Infection[z]	Caliper growth	VAM infection	Caliper growth	VAM infection
1	Clay loam	6.2	1.2	5.9	2.3	5.0	3.5
2		13.8	1.1	17.9	2.1	12.9	2.0
1	Sandy loam	4.7	2.6	4.0	1.6	4.3	2.6
2		6.8	2.2	4.9	3.2	5.4	4.1

Significance	Year 1	Year 2
Caliper growth	Inoculum: NS[y]	Inoculum: NS
	Field: **	Field: ***
	Fieldxlnoc.: NS	Fieldxlnoc.:**
	SE (8,42[x]): 0.59	SE (8,42): 0.99
VAM infection	Inoculum: NS	Inoculum: NS
	Field: NS	Field: *
	Fieldxlnoc.: NS	Fieldxlnoc.: NS
	SE (8,42): 0.69	SE (8,42): 0.67

[z]Level of VAM infection is classified on a scale from 0 to 10.

[y]NS, *, ** Nonsignificant or significant at P=0.05, or 0.01, respectively.

[x]SE: Standard error of the mean (number of replicates, degrees of freedom).

[w]Caliper growth in mm. 1 mm=0.04 in.

either greater than (*G. fasiculatum*) or equal to (*G. intraradices*) that of the controls in clay loam. When fields were considered separately, these effects were statistically significant. In sandy loam, VAM infection of all inoculated plants increased between the first and second years, perhaps causing a shift towards mycorrhizal parasitism and reduced growth rates as discussed in the case of *A. platanoides*. In clay loam, on the other hand, VAM infection of inoculated plants declined (*G. intraradices*) or remained the same (*G. fasiculatum*), suggesting a more effective host regulation of the symbioses. Reasons for the influence of the two soil types on the colonizing ability of the mycorrhizae and regulatory capacity of the host are unclear.

The inoculant mycorrhizas appeared to be commensal with *F. pennsylvanica* and *S. aucuparia* at both field sites as no treatment effects were detected in either year (data not shown; however as with *Malus*, VAM infection levels in the second year generally increased at the sandy loam field and decreased at the clay loam field relative to their levels in the first year.

Taken together, these data suggest that VAM inoculation of trees growing in non-sterile, high fertility soils establish parasitic, or more frequently, commensal symbioses. Our studies revealed a high incidence of VAM infection without inoculation, and although inoculation usually increased the level of infection (at least transiently), there was little evidence that VAM inoculation would enhance tree growth under these production conditions. In fact, the ability of the inoculant mycorrhizas to become established under high fertility conditions may negatively affect the growth of some tree species as indicated for both *A. platanoides* and *Malus*.

Experiment 2. Containerized landscape shrubs. There was no mycorrhizal infection detected in the rooted cuttings upon receipt from the source nursery, with the exception of *C. dammeri, P. parvifolia and Spiraea*. All sampled *C. dammeri* and *Spiraea* were colonized, with average infection levels of 3.1 and 1.4 respectively. Six of ten *P. parvifolia* had traces of infection.

After six weeks, the incidence of infection was increased by inoculation in all species which were previously observed to be uninfected. In most species, infection levels in in-

oculated plants remained around 1 on the scale; however, infection levels in *P. parvifolia*, *Spiraea*, and *C. dammeri* rose to means of 5.8, 5.6, and 2.4. Control plants of species which were previously uncolonized remained uncolonized except for some *F. ovata* and *Prunus* control plants in which very low levels of infection (<2% of the root length) were detected. These mycorrhizae were probably present but undetected in the initial screening.

Inoculation was required for substantial colonization of *Prunus* in year 1 (Table 3); however, infection levels in inoculated treatments generally dropped in year 2, while infection levels of the controls increased. There were no significant treatment or fertilizer level effects on total masses in year 1. In the second year, if the 50% fertilizer level is considered separately, the *G. intraradices* inoculated plants were significantly larger than the uninoculated plants. Indeed, inoculated plants at the 50% fertilizer level were as large as plants grown at the 100% fertilizer level. This indicates that inoculation can substitute for 50% of the fertilizer present in typical nursery container medium, even when the control plants become colonized without inoculation. Thus the mycorrhizas in the inoculants were capable of stimulating *Prunus* growth significantly beyond the level achieved bv the mycorrhizas present in the control plants.

VAM infectio levels were significantly lower at the higher fertilizer level in both years; furthermore, VAM infection levels in the non-inoculated plants dropped to a greater degree than levels in inoculated plants with the increase in fertilizer level. This suggests the inoculant mycorrhizas were more fertilizer tolerant than the indigenous mycorrhizas.

In *Syringa*, total dry masses were significantly larger in the *G. intraradices* inoculated treatments than in the control treatment in the first year (Table 4). In the second year, both inoculated treatments had significantly larger total masses than the controls. Although all VAM infection levels were low, inoculated plants had significantly higher infection levels than the controls in the first year. In the second year, the differences were not significant.

Syringa plants showed a statistically significant growth response to VAM inoculation in each of two years despite low levels of VAM infection. Growth responses to VAM infection are often associated with higher levels of colonization, but, even a small volume of colonized root might support enough extramatrical hyphae to effectively explore the entire container volume (23).

Plant sizes were significantly larger at the higher fertility level in the first year, but the fertilizer effect disappeared in the second year. Coupled with the consistent growth response to inoculation, this indicates that after two years, VAM had a larger effect on *Syringa* growth than did doubling the fertilizer regime, over the range studied. It seems that *Syringa* remains highly mycorrhizal dependent even at high medium fertility and low levels of root colonization.

In the first year, masses of *G. intraradices* inoculated *C. alba* plants were less than those of the controls when grown at the 100% fertilizer level, but exceeded the controls at the 50% level (Table 5). *P. parvifolia* masses showed a similar tendency (Table 6). In both cases the interaction between fertilizer level and inoculum treatment was statistically significant. *C. alba* plants inoculated with *G. intraradices* and grown at the 100% fertilizer level showed 3 times as much VAM infection as controls, or plants inoculated with *G. fasiculatum* in the first year. However, there were no significant differences in degree of infection at the 50% fertilizer level. Again, *P. parvifolia* showed very similar trends.

Taken together these results suggest that, in *P. parvifolia* and in *C. alba*, *G. intraradices* was initially beneficial at the lower fertilizer level, but parasitic at the higher level. These trends are similar to those observed by Yeager et al (28) with *Podocarpus*. At the manufacturer's recommended fertilizer level, *G. intraradices* inhibited *Podocarpus* growth,

Table 3. Prunus dry masses and VAM infection data at two fertilizer levels for two years.

Year	Inoculum	100% fertilizer medium		50% fertilizer medium	
		Total dry mass (g)[w]	VAM infection[z]	Total dry mass (g)	VAM infection
1	Control	37.5	0.2	35.1	1.0
	Glomus fasiculatum	40.0	2.4	34.7	3.2
	Glomus intraradices	32.7	2.4	30.8	4.6
2	Control	82.7	0.6	43.3	3.8
	Glomus fasiculatum	80.6	2.4	89.7	2.4
	Glomus intraradices	94.6	1.2	108.2	2.2

Significance	Year 1	Year 2
Total dry mass	Inoculum: NS[y]	Inoculum: NS
	Fertilizer: NS	Fertilizer: NS
	Fert.xInoc.: NS	Fert.xInoc.: NS
	SE[x] (5, 24): 3.84	SE (5, 24): 18.5
VAM infection	Inoculum: ***	Inoculum: NS
	Fertilizer: *	Fertilizer: *
	Fert.xInoc.: NS	Fert.xInoc.: NS
	SE (5, 24): 0.57	SE (5, 24): 0.85
	SE (5, 24): 0.57	SE (5, 24): 0.85

[z]Level of VAM infection is classified on a scale from 0 to 10.

[y]NS, *, **, *** Nonsignificant or significant at P=0.05, 0.01, 0.001.

[x]SE: Standard error of the mean (number of replicates, degrees of freedom).

[w]1 g = 0.035 oz.

Table 4. *Syringa* **dry masses and VAM infection data at two fertilizer levels for two years.**

Year	Inoculum	100% fertilizer medium		50% fertilizer medium	
		Total dry mass (g)[w]	VAM infection[z]	Total dry mass (g)	VAM infection
1	Control	8.4	0	5.6	0.2
	Glomus fasiculatum	10.4	1.0	7.8	1.0
	Glomus intraradices	14.8	1.0	6.5	1.0
2	Control	66.7	0.4	62.1	0.4
	Glomus fasiculatum	142.7	0.6	114.7	0.8
	Glomus intraradices	116.1	0.8	110.1	0.2

Significance	Year 1	Year 2
Total dry mass	Inoculum: *	Inoculum: *
	Fertilizer: ***	Fertilizer: NS
	Fert.xInoc.: NS	Fert.xInoc.: NS
	SE[x] (5, 24): 1.4	SE (5, 24): 25.8
VAM infection	Inoculum: ***	Inoculum: NS
	Fertilizer: NS	Fertilizer: NS
	Fert.xInoc.: NS	Fert.xInoc.: NS
	SE (5, 24): 0.08	SE (5, 24): 0.22

[z]Level of VAM infection is classified on a scale from 0 to 10.
[y]NS, *, **, *** Nonsignificant, P = 0.05, 0.01, 0.001, respectively.
[x]SE: Standard error of the mean (number of replicates, degrees of freedom).
[w]1 g = 0.035 oz.

Table 5. *Cornus alba* **dry masses and VAM infection data at two fertilizer levels for two years.**

Year	Inoculum	100% fertilizer medium		50% fertilizer medium	
		Total dry mass (g)[w]	VAM infection[z]	Total dry mass (g)	VAM infection
1	Control	23.8	1.0	18.7	2.6
	Glomus fasiculatum	22.7	1.0	17.1	1.2
	Glomus intraradices	11.1	3.2	35.0	3.0
2	Control	404.5	1.2	577.8	3.8
	Glomus fasiculatum	375.7	1.8	455.3	4.4
	Glomus intraradices	493.4	2.6	574.1	4.8

Significance	Year 1	Year 2
Total dry mass	Inoculum: NS[y]	Inoculum: NS
	Fertilizer: NS	Fertilizer: NS
	Fert.xInoc.: *	Fert.xInoc.: NS
	SE[x] (5, 24): 6.2	SE (5, 24): 83.6
VAM infection	Inoculum: **	Inoculum: NS
	Fertilizer: NS	Fertilizer: ***
	Fert.xInoc.: NS	Fert.xInoc.: NS
	SE (5, 24): 0.49	SE (5, 24): 0.63

[z]Level of VAM infection is classified according to a scale from 0 to 10.
[y]NS, *, **, *** Nonsignificant, P = 0.05, 0.01, 0.001, respectively.
[x]SE: Standard error of the mean (number of replicates, degrees of freedom).
[w]1 g = 0.035 oz.

while at lower fertilizer regimes inoculation was stimulatory. The ability of *G. intraradices* to colonize these plants under high fertility conditions suggests that the mycorrhiza is phosphorus tolerant, an attribute considered in the selection of mycorrhizal strains for agricultural use (7, 25, 26); however, the host growth depression observed upon VAM colonization at higher fertilizer regimes suggests that fertilizer tolerance can predispose the mycorrhiza to parasitism.

C. alba masses in the second year were unaffected by inoculation or fertilizer level despite the fact that plants generally became well colonized by VAM. In both *C. alba* and *P. parvifolia, G. intraradices* infection decreased at the 100% fertilizer level in the second year and the growth depression disappeared. *G. intraradices* infection at the 50% fertilizer level increased in both species in the second year and the growth enhancement effect disappeared. This suggests a similar dynamic in carbon partitioning between host and mycorrhizae as discussed with reference to *A. platanoides*.

VAM inoculated and control *Weigela* became well co1-

Table 6. ***Potentilla parvifolia*** **dry masses and VAM infection data at two fertilizer levels for two years.**

Year	Inoculum	100% fertilizer medium		50% fertilizer medium	
		Total dry mass (g)[w]	VAM infection[z]	Total dry mass (g)	VAM infection
1	Control	23.8	0.6	9.2	2.6
	Glomus fasiculatum	19.7	1.4	18.1	2.6
	Glomus intraradices	16.0	1.8	17.2	2.2
2	Control	196.7	0.8	156.9	1.8
	Glomus fasiculatum	119.1	1.0	100.5	1.8
	Glomus intraradices	147.0	0.8	144.1	4.6
	Significance	Year 1		Year 2	
	Total dry mass	Inoculum: NS[y]		Inoculum: NS	
		Fertilizer: *		Fertilizer: NS	
		Fert.xlnoc.: *		Fert.xlnoc.: NS	
		SE[x] (5,24): 2.62		SE (5,24): 27.1	
	VAM infection	Inoculum: NS		Inoculum: NS	
		Fertilizer: *		Fertilizer: **	
		Fert.xlnoc.: NS		Fert.xlnoc.: NS	
		SE (5,24): 0.62		SE (5.24): 0.70	

[z]Level of VAM infection is classified according to a scale from 0 to 10.
[y]NS, *, **, *** Nonsignificant, P = 0.05, 0.01, 0.001, respectively.
[x]SE: Standard error of the mean (number of replicates, degrees of freedom).
[w]1 g = 0.035 oz.

onized at both fertilizer levels, but there was no evidence of any growth effects due to inoculation during either year of study (data not shown), suggesting a commensal relationship between host and inoculant mycorrhizas. The other species, *C. dammeri, Spiraea, F. ovata*, and *V. opulus* were studied for only the first year (data not shown). Control and inoculated *C. dammeri* and *Spiraea* became well colonized whereas no *F. ovata* or *V. opulus* became colonized to a considerable degree irrespective of inoculation. None of these species showed a growth response to inoculation. This is in contrast to other studies which have shown positive growth responses of *Viburnum* to inoculation, but at lower, and probably growth limiting, fertilizer levels (6, 14, 27).

Experiment 3. Post-transplant growth response to inoculation. The post transplant growth of *Syringa* and *P. parvifolia* was unaffected by inoculation (data not shown).

In the first year after transplant, there was no significant effect of nursery inoculation on caliper growth in any tree species (Table 7). In the second year there was again no effect on *F. pennsylvanica* and *Malus* caliper growth, but *G. intraradices* inoculated *S. aucuparia* grew significantly ($P<0.001$) more than *G. fasiculatum* inoculated or uninoculated trees; *S. aucuparia* exhibited no growth effect of inoculation during nursery production (data not shown). Inoculated *S. aucuparia* had significantly higher levels of mycorrhizal infection that uninoculated *S. aucuparia* upon bare-root transplant, but the fungus may have needed a year to establish extramatrical hyphae in the soil. Improved post production landscape performance would be a significant benefit of VAM inoculation in the nursery, even without early growth enhancement.

The 100% fertilizer regime did not yield significantly larger plants than the 50% fertilizer regime in *C. alba, V. opulus, F. ovata,* or *Spiraea*. Although researchers emphasize the potential to decrease fertilizer application using mycorrhizae, it is clear that even without VAM, fertilizer rates can often be reduced substantially without affecting plant growth.

Where plants are colonized by indigenous VAM, inoculation can increase the level and uniformity of mycorrhizal colonization, and can introduce superior VAM strains to maximize the potential benefit from mycorrhizal associa-

Table 7. VAM infection levels at transplant, and post-transplant growth (mm) in tree diameter (n = 8).

		Control		G. fasiculatum		G. intraradices	
		Caliper growth[w]	VAM infection[z]	Caliper growth	VAM infection	Caliper growth	VAM infection
Fraxinus	Year 1	5.7 a[y]	2.1 a	5.7 a	2.7 a	4.8 a	2.4 a
	Year 2	4.6 a	N.D.	4.6 a	N.D.	4.7 a	N.D.
Malus	Year 1	2.7 a	1.1 a	3.6 a	1.9 a	3.8 a	1.2 a
	Year 2	4.9 a	N.D.	4.7 a	N.D.	4.9 a	N.D.
Sorbus	Year 1	7.0 a	0.4 a	6.8 a	1.4 b	6.7 a	3.0 b
	Year 2	5.2 A	N.D.	5.0 A	N.D.	5.9 B	N.D.

[z]VAM infection is classified on a scale from 0 to 10 in which 0 corresponds to no infection and 10 corresponds to >90% of the root length colonized.
[y]Mean separation for caliper growth or VAM infection data by Tukey's multiple comparison test. P = 0.05 (lowercase letters) or 0.01 (uppercase letters).
[w]Caliper growth in mm. 1 mm = 0.04 in.

tion. The use of non-sterile fields, and pre-colonized shrubs, provided an opportunity to consider plant responses to inoculation with controls being exposed to indigenous VAM populations. Most of the trees and shrubs which were not inoculated with VAM did become colonized, often to a similar degree as the inoculated stock; however, inoculation of *F. pennsylvanica, S. aucuparia, Weigela*, and *P. parvifolia* increased the level of VAM infection relative to non-inoculated plants after two years. More importantly, the growth responses of *Prunus* and *Syringa* demonstrate that inoculation can stimulate plant growth despite high fertility and competition with indigenous mycorrhizas.

VAM tolerance of phosphorus (or fertilizer) may result from different traits which may or may not be desirable in inoculants. For instance, fertilizer tolerance arising by a fungal ability to evade the direct inhibition of fungal growth by ambient phosphorus (12) may be a positive trait because it increases the potential for symbiosis. Conversely, a fungal ability to evade plant regulation which limits mycorrhizal development at high fertilizer regimes would be a negative attribute as it might give rise to a parasitic symbiosis. Thus the basis for VAM fertilizer tolerance may be crucial in determining a mycorrhiza's potential for parasitism. The parasitism observed at the higher fertilizer regimes used in this study suggests that fertilizer tolerance can be a negative attribute.

While many studies have focused on plant responses to mycorrhizal colonization under low fertility conditions, it is clear from the present work that there is still much to understand about plant/mycorrhizal relationships in highly fertile soils. Mycorrhiza researchers must de-emphasize demonstrations of positive plant growth responses to inoculation, and study all levels of fertilization if we are to develop a full and realistic picture of the potential horticultural benefits of VAM inoculation.

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