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Growth Response of *Podocarpus* and *Ligustrum* to VA Mycorrhizae and Fertilizer Rate[1]

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Abstract

Container-grown *Podocarpus macrophyllus* (Thunb.) D. Don and *Ligustrum japonicum* Thunb. inoculated or noninoculated with the mycorrhizal fungus *Glomus intraradices* Schenck & Smith were fertilized with Lesco 20N-2.6P-10K (20-6-12) at either 0, 4.3, 8.6, 17.3 or 34.6 g (0, 0.15, 0.30, 0.60 or 1.20 oz) per 6-*l* (#2) container. Plants were fertilized at potting and again 3.5 months later for each of 2 consecutive applications. Seven months after potting, *P. macrophyllus* shoot dry weights were greater for noninoculated than inoculated plants when fertilized with the manufacturer's recommended fertilizer rate of 17.3 g per container. *L. japonicum* shoot dry weights were not different due to inoculation at the 17.3 g fertilizer rate. Mean heights for *P. macrophyllus* were greater for the inoculated than noninoculated plants and plants fertilized with one-half the recommended fertlizer rate (8.6 g) were ranked superior to noninoculated *P. macrophyllus* receiving the manufacturer's recommended rate (17.3 g). One year after plants were transplanted to a simulated landscape, *P. macrophyllus* and *L. japonicum* shoot dry weights were not different due to inoculation.

Index words: Container production, dry weight, landscape plant establishment, *Glomus* inoculum

Significance to the Nursery Industry

The ability to produce mycorrhizal plants with less fertilizer than nonmycorrhizal plants can vary with genus of plant. Container-grown *P. macrophyllus* that were inoculated with the mycorrhizal fungus *G. intraradices* and received one-half the manufacturer's fertilizer rate that was applied to noninoculated plants, were taller and ranked superior after one growing season. However, the response for *L. japonicum* was different in that inoculated plants that received one-half the quantity of fertilizer as noninoculated plants, were not ranked comparable to noninoculated plants. Subsequent evaluation revealed that inoculated and noninoculated plants of both genera were of a similar size one year after transplanted to a simulated landscape.

Introduction

Agronomic and horticultural crops colonized by vesicular-arbuscular (VA) mycorrhizae generally exhibit greater growth or yield responses than nonmycorrhizal plants when grown in substrates with low available P (2, 5, 16). VA mycorrhizae facilitate P uptake from substrates with low P availability (5, 15, 19) and also enhance growth responses to N and K fertilizer applications. Johnson et al. (12) determined that 750 kg/ha/yr (668 lb/ac/yr) of N resulted in greater fresh weights of mycorrhizal *Podocarpus macrophyllus* than 250 or 1250 kg/ha/yr (223 or 1113 lb/ac/yr), while the highest rate of N resulted in the greatest fresh weight of nonmycorrhizal plants. Coxwell and Johnson (3) observed that fresh weight of *Pittosporum tobira* was greater for mycorrhizal than nonmycorrhizal plants regardless of NH_4:NO_3 ratio applied. A 25:75 ratio (NH_4:NO_3) resulted in higher leaf tissue N and P for mycorrhizal *P. tobira*, while leaf tissue K was not different. Davies (4) determined that K uptake and shoot dry weights of *Rosa multiflora* were greater for mycorrhizal than nonmycorrhizal roses. Johnson et al. (12) found no difference in mycorrhizal *P. macrophyllus* fresh weights with K rates of 250, 750 or 1250 kg/ha/yr (223, 668, 1113 lb/ac/yr). Fresh weight of *Juniperus chinensis* 'Sargent' was greater for mycorrhizal than nonmycorrhizal plants grown in a clay loam soil : washed river sand : milled pine bark (4:1:1 by vol) medium amended with a 10N-4.4P-8.3K (10-10-10) fertilizer at 220 or 110 μg/g of medium (25).

Previous research indicated that mycorrhizal plants were more tolerant of water stress than nonmycorrhizal plants and thus are more desirable for post-production handling and transplanting (10, 11, 21). Johnson and Hummel (11) determined that 6 months after transplanting, mycorrhizal *Poncirus* × *Citrus* had greater stem and leaf dry weights than nonmycorrhizal plants regardless if the plants were moisture stressed or nonstressed. However, stem and leaf dry weights were greatest for nonstressed mycorrhizal plants. Sweatt and Davies (28) found that mycorrhizal geranium grown with a low moisture regime had greater dry weights and N uptake than nonmycorrhizal plants with a high moisture regime. Phosphorus uptake was not different. Mycorrhizal geranium plants also exhibited greater recovery to water stress than nonmycorrhizal plants.

The aforementioned research indicates that mycorrhizal plants have increased nutrient uptake, require lower fertility levels and are less likely to suffer moisture stress associated with transplanting. The purpose of this research was to determine if less fertilizer applied to container-grown *P. macrophyllus* and *Ligustrum japonicum* inoculated with the mycorrhizal fungus *Glomus intraradices* resulted in comparable growth to noninoculated plants at high fertility rates and to determine the growth response of these plants in a simulated landscape.

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Materials and Methods

Two-hundred single stem rooted cuttings of *P. macrophyllus* and *L. japonicum* were potted January 30, 1985 in a fired montmorillonite clay growth medium in 12 cm (5 in) diameter (700 cm^3 or 23.7 oz) plastic containers. One-half of the plants for each genera were inoculated during potting with the mycorrhizal fungus *Glomus intraradices*, Schenck and Smith, a common mycorrhizal species in Florida (27), using a 10 g mixture of media, chlamydospores (100 spores/g), hyphae and infected roots. An inoculum filtrate was applied to roots of noninoculated plants. The plants were grouped according to mycorrhizal inoculation and maintained in a glass greenhouse [21°C (70°F) minimum] with 50% light exclusion and the dark period interrupted by incandescent lighting (14 $\mu mole/m^2/s$) from 2300 to 0200 HR. On April 9, *P. macrophyllus* and *L. japonicum* root colonization by *G. intraradices* was 32 and 38%, respectively, as determined by the clearing and staining procedures of Phillips and Hayman (22) and a modified gridline intersect method (6). The plants were transported to Gramling Nursery Co., Plant City, Fla. (latitude 28° 00′N, longitude 82° 07′W) and potted in 6-*l* (#2) containers with a sedge peat : pine bark : cypress sawdust : builders' sand (10:5:3:2 by vol) growth medium. The medium contained 2 *G. intraradices* spores per 200 cm^3 (6.6 oz) and was amended with dolomitic limestone to adjust pH to 6.0 to 6.2 and 1.8 kg/m^3 (3.0 lb/yd^3) of Step (trademarked micronutrient fertilizer of O. M. Scott & Sons Co., Marysville, Ohio). Lesco 20N-2.6P-10K (20-6-12, a trademarked fertilizer of Lakeshore Equipment Co., Elyria, Ohio) was surface applied at potting to mycorrhizal and nonmycorrhizal plants at either 0, 4.3, 8.6, 17.3, or 34.6 g (0, 0.15, 0.30, 0.60 or 1.20 oz) per container. The plants were placed on black plastic in a split plot arrangement with randomized main plots of inoculation and fertilizer rate as subplots. Each fertilizer rate was replicated 4 times (1 plant per experimental unit) in each of 5 blocks per genera. The plants received 823 ml of water as needed [1 inch (28 oz) per application] by overhead irrigation. Three and one-half months after potting growth medium samples were taken. A composite sample was obtained by removing a 2.5 × 15 cm (1 × 6 in) core of medium from the outer edge of 5 to 10 containers for each of the 8.6 and 17.3 g fertilizer rates for inoculated and noninoculated *P. macrophyllus* and *L. japonicum*. Two growth medium samples were obtained for each fertilizer rate and inoculation combination for each genera. Nitrate-N, P, K and pH of the growth medium were determined according to the procedures of the University of Florida Soil Testing Laboratory (9). Fertilizer was reapplied to the growth medium surface following sample collection.

Seven months after potting, growth medium samples were taken again as previously described and *P. macrophyllus* and *L. japonicum* heights were determined. The 2 greatest widths perpendicular to each other were determined for *L. japonicum*. The plants of each treatment were rated by 33 nursery operators based on size, color and form. A 1.4 × 15 cm (0.5 × 6.0 in) core of medium was randomly removed from 10 containers of inoculated *P. macrophyllus* and *L. japonicum* for each fertilizer rate and 3 containers per fertilizer rate were sampled for noninoculated plants of each genus. Root segments were separated from the medium and colonization by *G. intraradices* determined as previously described. Stems were severed above the uppermost roots for 3 of the replicate plants of each fertilizer rate within a block and root and shoot dry weights determined after drying tissue for 48 hr at 70°C (158°F) in a forced air oven. Shoot tissue N, P and K for 1 replicate of each block for the 8.6 and 17.3 g fertilizer rates of inoculated and noninoculated *P. macrophyllus* were determined by standard analyses (13). Daily minimum and maximum air temperatures for the 7-month experimental period averaged 20°C (68.6°F) and 32°C (90.0°F), respectively (1).

The single replicate for each fertilizer rate per block for each genus was planted April 29, 1986 in an Arrendondo fine sand (loamy, siliceous, hyperthermic, grossarenic paleudulp) in Gainesville, Fla. (82° 21′W longitude, 29° 31′N latitude). Mehlich 1 extractable P, K, Ca and Mg were 32, 22, 36 and 394 ppm, respectively. The pH was 6.4. A completely randomized factorial design was used with 5 blocks for each fertilizer rate. The plants were fertilized immediately after planting with the same fertilizer and respective rates as applied to the container plants. Fertilizer was distributed evenly over the root ball area. Each plant was mulched with cypress shavings [5 cm (2 in) deep × 60 cm (24 in) diameter] and received 8 *l* (2 gal) of water immediately after planting, 4 *l* every other day for 7 days and weekly thereafter for 1 month when rainfall did not exceed 1.3 cm (0.5 in). Plants were subsequently watered (1 gal per application) a total of 4 times during periods of extreme drought. Four months after planting, fertilizer was reapplied as previously described but placed on the mulch surface.

P. macrophyllus and *L. japonicum* heights and the 2 greatest widths of *L. japonicum* perpendicular to each other, were determined 1 year after planting. A 20 to 30 ml (1 oz) composite sample of *P. macrophyllus* roots was taken at random near the base of 2 replicate plants for each of the 8.6 and 17.3 g rates and 1 replicate for the 0 g rate for inoculated and noninoculated plants. Fifty ml composite soil samples were taken similarly, then centrifuged in deionized water and 40% sucrose and sieved (43 μm) to isolate spores. Root colonization was determined as previously described (6, 22) and spore identity determined according to the keys of Schenck and Perez (26). Stems of all plants were severed above uppermost roots and shoot dry weights determined. Daily minimum and maximum ambient temperatures during the experimental period averaged 15°C (59.5°F) and 28°C (81.9°F), respectively (1, 20).

Data were subjected to an analysis of variance with *t* tests performed according to Little and Hills (17). Regression analyses were utilized for the data of *P. macrophyllus* and *L. japonicum* grown in the Arrendondo fine sand.

Results and Discussion

There was a significant inoculation and fertilizer interaction for *P. macrophyllus* shoot and root dry weights; therefore, means for inoculated and noninoculated plants were compared for each fertilizer rate. Shoot dry weights for the noninoculated *P. macrophyllus* (Table 1) were larger than inoculated plants for the 4.3 and 17.3 g fertilizer rates while shoot and root dry weights were larger for the inoculated plants at the 34.6 g fertilizer rate. Mycorrhizal colonization of *P. macrophyllus* roots averaged 44% with a maximum of 61% of roots colonized for the 8.6 g fertilizer rate and a minimum of 31% colonized at the 34.6 g rate.

Mean heights of *P. macrophyllus* were greater for the

Table 1. Shoot and root dry weights, heights and rankings of *Podocarpus macrophyllus* inoculated or noninoculated with *Glomus intraradices* and grown 7 months in a soilless container medium.

Fertilizer (g per container)	Dry weights (g)				Heights (cm)	
	Shoots		Roots		Shoots	
	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated
0.0[z]	4.2	2.9	2.6	2.1	18.9 (8)	15.4 (10)
4.3	10.3	16.2*[y]	4.4	5.1	37.7 (6)	29.7 (8)
8.6	18.9	20.2	7.2	6.3	47.1 (3)	36.3 (7)
17.3	15.7	22.4*	6.2	5.8	54.1 (1)	43.9 (4)
34.6	17.5	12.9*	6.7	4.1*	55.8 (2)	47.9 (4)
Mean[x]					42.7	34.6*

[z]Lesco 20N-2.6P-10K surface applied to each 6-liter container immediately after potting and 3.5 months later.

[y]Means for inoculated and noninoculated followed by an * are significantly different at the 5% level by *t* test for either shoots or roots for each fertilizer rate.

[x]Mean height for inoculated and noninoculated, averaged over fertilizer rates, is significantly different at the 5% level by *t* test. Inoculation × fertilizer interaction was nonsignificant for height.

Numbers in parentheses are rankings based on 33 individual ratings (1 = best).

inoculated plants (nonsignificant inoculation × fertilizer interaction) and the response was similar for each fertilizer rate (Table 1). Heights increased substantially with increasing increments of fertilizer to the 17.3 g rate, which is the manufacturer's recommended rate. The benefit of more than 17.3 g of fertilizer per container is questionable due to the diminished height response above the 17.3 g rate.

Inoculated *P. macrophyllus* fertilized with the recommended rate (17.3 g) were ranked superior to the other treatments (Table 1). However, inoculated *P. macrophyllus* receiving one-half the recommended fertilizer rate (8.6 g) were ranked superior to noninoculated plants that received the recommended rate (17.3 g). *P. macrophyllus* ratings were based primarily on heights because of minimal branching, although heights were not directly related to the shoot dry weights for the 4.3 and 17.3 g treatments.

Growth medium extract NO_3-N, P, K and pH levels for *P. macrophyllus* and *L. japonicum* were similar for each sampling date and inoculation. However, *P. macrophyllus* heights for inoculated plants exceeded noninoculated plants, concurring with previous research that indicated a beneficial effect of mycorrhizal plants grown in low fertility substrates (19). *P. macrophyllus* growth medium extract levels for the 8.3 and 17.3 g rates for both sampling times averaged 3, 1 and 5 ppm for NO_3-N, P and K, respectively, and pH averaged 6. Nitrate-N, P and K levels were below optimum (29) as would be expected since the manufacturer recommends fertilizer reapplication every 3–4 months. *P. macrophyllus* shoot tissue N, P and K levels for the 8.6 and 17.3 g fertilizer rates were similar regardless of inoculation and averaged 1.1, 0.3 and 0.7%, respectively.

There was a significant inoculation and fertilizer interaction for *L. japonicum* shoot and root dry weights; therefore, means for inoculated and noninoculated plants were compared for each fertilizer rate. Shoot dry weights for the inoculated plants (Table 2) were larger than noninoculated plants for the 4.3 and 8.6 g fertilizer rates. Root dry weights (Table 2) were larger for the noninoculated plants at the 17.3 g rate, and for the inoculated plants at the 34.6 g rate. Root colonization percentages were not obtained for *L. japonicum* because an unidentified root constituent limited stain penetration of the roots and thus accurate colonization

Table 2. Shoot and root dry weights, growth index and rankings of *Ligustrum japonicum* inoculated or noninoculated with *Glomus intraradices* and grown 7 months in a soilless container medium.

Fertilizer (g per container)	Dry weights (g)				Growth index[z]	
	Shoots		Roots		Shoots	
	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated
0.0[y]	12.7	8.0	12.1	10.0	0.72 (9)	0.61 (10)
4.3	68.0	39.1*[x]	67.7	33.0	0.79 (7)	0.75 (8)
8.6	114.2	83.6*	100.1	75.9	0.94 (5)	0.74 (6)
17.3	102.3	107.7	107.1	192.2*	0.87 (3)	0.87 (4)
34.6	135.3	134.4	189.0	129.6*	1.00 (1)	0.94 (2)
Mean[w]					0.86	0.78*

[z]Average growth index = [(width 1 + width 2)/2]/height.

[y]Lesco 20N-2.6P-10K surface applied to each 6-liter container immediately after potting and 3.5 months later.

[x]Means for inoculated and noninoculated followed by an * are significantly different at 5% level by *t* test, for either shoots or roots for each fertilizer rate.

[w]Mean growth index for inoculated and noninoculated, averaged over all fertilizer rates, is significantly different at 5% level by *t* test. Inoculation × fertilizer interaction was nonsignificant.

Numbers in parentheses are rankings based on 33 individual ratings (1 = best).

Table 3. Growth parameters for *Ligustrum japonicum* and *Podocarpus macrophyllus* after 1 year in a simulated landscape.

Fertilizer (g per plant)	*Ligustrum* Dry weight[z] (g)	*Ligustrum* Growth[zy] index	*Podocarpus* Dry weight[z] (g)	*Podocarpus* Height[z] (cm)
0.0[x]	56	0.92	21.7	47.0
4.3	112	1.00	42.9	56.4
8.6	141	0.94	56.3	64.1
17.3	199	0.96	81.6	75.8
34.6	262	0.89	87.4	83.7
Significant effects	L*[w] Q*	L[ns] Q[ns]	L* Q[ns]	L* Q*

[z]Data averaged over inoculation treatments. Inoculation × fertilizer interaction was nonsignificant.

[y]Average growth index = [(width 1 + width 2)/2]/height.

[x]Lesco 20N-2.6P-10K surface applied to each plant immediately after planting and 4 months later.

[w]Linear (L), quadratic (Q) or nonsignificant (ns) at 5% level.

percentages were not determined for all treatments, but colonization averaged 12% for the 0 and 4.3 g fertilizer rates.

Growth indices (Table 2) exhibited a similar relationship to the shoot dry weights with the mean growth index greater for inoculated than noninoculated plants. The inoculation × fertilizer interaction was nonsignificant. Growth index for inoculated *L. japonicum* at the 8.6 g fertilizer rate was numerically higher than noninoculated plants receiving twice the fertilizer; however, the latter treatment received a superior ranking. Regardless of inoculation, *L. japonicum* fertilized with 34.6 g was ranked superior to the other treatments (Table 2) indicating that 17.3 g of fertilizer was not adequate for maximum *L. japonicum* shoot growth. *L. japonicum* shoot dry weight was not enhanced by inoculation at the 17.3 and 34.6 g fertilizer rates (Table 2).

L. japonicum shoot dry weights and growth indices were not different due to inoculation and there was no interaction with fertilizer rate when plants were grown for 1 year in the Arrendondo fine sand of the simulated landscape (Table 3). Plants that received the highest fertilizer rate had the greatest shoot dry weights, but growth indices were not different due to fertilizer rates.

One year after planting *P. macrophyllus* in the Arrendondo fine sand, shoot dry weights and heights were not different due to inoculation and there was no interaction with fertilizer rate. Shoot dry weights and heights exhibited a linear relationship with fertilizer rate (Table 3). Spores of 10 species of VA mycorrhizal fungi, including *G. intraradices*, were observed in soil samples taken in April 1987 at the base of *P. macrophyllus* plants, but presence of spores does not imply a particular species colonized a root. Irrespective of species of mycorrhizal fungi and fertilizer rates, colonization of *P. macrophyllus* roots averaged 24% for noninoculated plants and 19% for inoculated plants. The colonization of noninoculated plants and low colonization for inoculated plants may be due to indigenous mycorrhizae; however, the relationship between spore numbers and colonization (14) and colonization and growth (7, 14, 23) is not clearly established. Ponder (24) noted that mycorrhizal walnut seedlings grown under moist conditions and transplanted to drier sites, may become quickly colonized by indigenous mycorrhizae. Graham and Syvertsen (8) indicated that a nonmycorrhizal citrus tree receiving adequate P prior to transplanting, performs as well as a mycorrhizal plant for about 9 months after transplanting, at which time the citrus roots become colonized by indigenous mycorrhizae. Indigenous mycorrhizae species were not documented in the transplanting study of Johnson and Crews (10), but they determined that mycorrhizal azalea plants were larger than nonmycorrhizal plants after 4 months in the landscape.

Data from this research indicated that *P. macrophyllus* inoculated with *G. intraradices* and fertilized with 8.6 g of Lesco 20N-2.6P-10K (20-6-12) per 6-*l* (#2) container were ranked superior 7 months later to noninoculated plants fertilized with the manufacturer's recommended rate of 17.3 g. Inoculated container-grown *L. japonicum* plants were ranked superior to noninoculated plants for each fertilizer rate. *P. macrophyllus* and *L. japonicum* shoot dry weights were not different regardless of inoculation, 1 year after transplanted to a simulated landscape. Future evaluations of mycorrhizal plants transplanted to the landscape should consider the influence that indigenous mycorrhizae have on plant growth. Combinations of plant species and VA mycorrhizal fungi that provide a beneficial plant response need to be established (15, 18) and utilized (15) when selecting plants for minimal maintenance landscapes.

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