HRI's Mission:

To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

The use of any trade name in this article does not imply an endorsement of the equipment, product or process named, nor any criticism of any similar products that are not mentioned.

Colonization and Growth Effects of the Mycorrhizal Fungus *Glomus intraradicies* in a Commercial Nursery Container Production System[1]

F.T. Davies, Jr.[2], J.A. Saraiva Grossi, L. Carpio, and A.A. Estrada-Luna
Department of Horticultural Sciences
Texas A&M University, College Station, TX 77843-2133

Abstract

The objectives of this research were to demonstrate that mycorrhiza can survive in a commercial nursery container production system, and enhance plant productivity. Four species were used as host plants [*Nandina domestica* 'Moon Bay', *Loropetalum chinense* variety Rubrum 'Hinepurpleleaf' Plumb delight®, *Salvia gregii*, and *Photinia fraseri*]. Plants were inoculated with arbuscular mycorrhizal fungi, *Glomus intraradices*, and grown in a commercial nursery in Texas. For the first 5.5 months, plants were grown in #1 cans containing either 3 kg cu m (5 lbs cu yd) or 4.2 kg cu m (7 lbs cu yd) 24N–4$P_2$0$_5$–8$K_2$0. For the final 6.5 months of the study, plants were in larger containers, all of which contained 4.2 kg cu m (7 lbs cu yd) 24N–4P_2O_5–8K_2O. The commercial inoculum of *Glomus intraradices* only enhanced growth of *N. domestica*. The shoot dry mass of mycorrhizal *N. domestica* plants at 3 kg cu m was the same as non-colonized plants at the higher fertility level of 4.2 cu m. Intraradical hyphae development and colonization (total arbuscules, vesicles/endospores, hyphae) of *L. chinense*, *N. domestica*, and *S. gregii* increased at the higher fertility levels. *S. gregii* had the greatest mycorrhizal development and a 216% increase in hyphae development and colonization at the higher fertility level.

Index words: colonization, arbuscular mycorrhiza, endomycorrhiza, plant growth, fertility, best management practices.

Species used in this study: *Nandina domestica* 'Moon Bay', *Loropetalum chinense* variety Rubrum 'Hinepurpleleaf' Plumb delight®, *Salvia gregii*, and *Photinia fraseri*.

Significance to the Nursery Industry

New production systems are being developed that emphasize the use of slow-release fertilizers, minimize the use of pesticides and soluble herbicides, and more efficiently utilize water. There are excellent opportunities to incorporate arbuscular mycorrhizal fungi (AMF) in nursery production systems that help reduce fertility and pesticide usage, and enhance crop vigor, productivity, and plant survival rates during transplanting to field conditions. This study demonstrates that AMF can survive in a commercial nursery production system. Moderately higher fertility levels stimulated AMF by increasing intraradical hyphae development and total colonization (total arbuscules, vesicles/endospores, hyphae) in three of the four plant species utilized: *Loropetalum chinense*, *Nandina domestica*, and *Salvia gregii*. AMF enhancement of plant growth was greatest with the shrub, *N. domestica*, which had one of the higher arbuscule levels and was intermediate in overall colonization. The nursery industry wants to reduce chemical costs and usage, improve plant quality, and command a higher price for their products. The value-added benefits of AMF in producing superior plants needs to be demonstrated so that the nursery industry can market and command a higher market price for more stress-resistant mycorrhizal nursery crops.

Introduction

Important production factors impacting the U.S. nursery industry include water quality and availability, the need to reduce pesticide and fertilizer usage for recycling water, and minimizing nonpoint source pollution (8, 11). Because of environmental legislation and economic incentives, nurseries in Texas, California, Florida, Oregon and throughout the southern United States are incorporating best management practices (BMP) to recapture and recycle irrigation runoff water (22). New production systems are being developed that emphasize the use of slow-release fertilizers, minimize the use of pesticides and soluble herbicides, and more efficiently utilize water. As a BMP practice, utilizing arbuscular mycorrhizal fungi (AMF) technology can potentially impact nursery production systems in many ways.

In general, AMF enhance plant water and nutrient relations and help minimize environmental stress. Mycorrhizal associations can also increase drought resistance, nutrient relations and temperature stress tolerance of herbaceous and woody plant species (2, 5, 6, 7, 10, 12, 13, 14, 20, 21). AMF can also increase plant resistance to pathogens (1, 16), and improve transplant survivability under severe outplanting conditions such as strip mine and highway revegetation sites (3).

The objectives of this research were to demonstrate that AMF can survive in a commercial nursery container production system, and that the growth of AMF plants is better than non-colonized (Non-AMF) plants under a moderate and moderately-high level of slow-release fertilizer. Four plant species were used as host plants: *Nandina domestica* 'Moon Bay', *Loropetalum chinense* variety Rubrum 'Hinepurpleleaf' Plumb delight®, *Salvia gregii*, and *Photinia fraseri*. The plants were propagated and grown in a commercial nursery in Texas and later evaluated for AMF colonization and plant growth.

Materials and Methods

Plant culture. Four plant species were used as host plants: *Nandina domestica* Thunb. 'Moon Bay' (shrub), *Loropetalum chinense* (R. Br.) Oliv. variety Rubrum 'Hinepurpleleaf'

[1]Received for publication June 21, 2000; in revised form September 25, 2000.

[2]This study was supported in part by **The Horticulture Research Institute, 1250 I Street, NW, Suite 500, Washington, DC 20005**, and Premier Tech, Rivière-du-Loup, Québec, Canada. We thank Tom Foley, Jr. for supplying the rooted liners and use of the production facilities at Hines Nurseries, Fulshear, Texas. We thank J.N. Egilla for critical review of the manuscript.

Plumb delight® (shrub), *Salvia gregii* A. Gray. (herbaceous perennial), and *Photinia fraseri* Dress (small tree). All plants were propagated from shoot cuttings (9) and rooted under an intermittent mist system in liner pots containing a peat: perlite (1:1 by vol) medium at Hines Nurseries, Fulshear, TX. Clearys® (Hummert, Earth City, MO) was used to clean the beds that the propagation flats were placed on to minimize any contamination. Rooted liners were transplanted into #1 containers containing a band of commercial inoculum of the AMF *Glomus intraradicies* Schenck & Smith at 750 propagules per container mixed with a perlite:peat carrier (MYCORISE®, Premier-Tech, Rivière-du-Loup, Québec, Canada). The AMF band was placed in the bottom one-third of each container medium, i.e. 400 ml of inoculum was banded in #1 containers. The container medium was a 20% sand: 80% milled, composted pine bark (by vol), which was not pasteurized. Two fertility levels, 3 cu m (5 lbs cu yd) and 4.2 kg cu m (7 lbs cu yd) of Osmocote $24N–4P_20_5–8K_20$ formulation were preincorporated with a trace element formulation, Micromax at 0.9 kg cu m (1.5 lbs cu yd), and 2.7 kg cu m (4.5 lbs cu yd) of dolomitic limestone. The experiment was established on October 13, 1997, at Hines Nursery, Fulshear, TX. Plants were grown outdoors under full sun in #1 containers on gravel beds. On May 29, 1998, *L. chinense* variety Rubrum 'Hinepurpleleaf' Plumb delight®, *S. gregii*, and *P. fraseri* were shifted (transplanted) from #1 containers into #5 containers, while *N. domestica* 'Moon Bay' was transplanted into #3 containers. For all treatments, the final container transplanting medium was 20% sand: 80% milled, composted pine bark (by vol), amended with a trace element formulation, Micromax at 0.9 kg cu m (1.5 lbs cu yd) , and 2.7 kg cu m (4.5 lbs cu yd) of dolomitic limestone, and one fertilizer level: Osmocote $24N–4P_20_5–8K_20$ at 4.2 kg cu m (7 lbs cu yd). Roughly after one year of production, plants were evaluated and destructively harvested on October 9, 1998.

Mycorrhizal measurements and plant biomass. Measurements of mycorrhizal (AMF) colonization included arbuscule formation, vesicle, intraradical hyphae, and total colonization. For AMF analysis of roots, 1-cm root segments from 10 plants per treatment were sampled at harvest and pooled to assess colonization percentage through clearing and staining of the root samples (18). Ten 1-cm (0.39 in) stained root pieces were placed on each slide and three microscopic observations per 1-cm root piece at 40× were made at the top, the middle and the bottom of each root piece; there were 10 slides per treatment (n = 300 per treatment). The presence of arbuscules, vesicles, and hyphae were recorded and the data were statistically analyzed. Plants were evaluated for root and shoot dry mass (DM) and root/shoot ratios ($g\ g^{-1}$). Each containerized plant was an individual unit (n = 25).

Statistical design. For mycorrhizal and plant growth data, the experiment was a 2 (± AMF) × 2 fertility (3 and 4.2 kg cu m of Osmocote $24N–4P_2O_5–8K_2O$) factorial in a completely randomized design. All data were analyzed using Analysis of Variance (ANOVA) [19].

Results and Discussion

This study demonstrates that AMF can survive in a commercial container nursery. Among the four plant species, AMF colonization either increased or was not depressed at the higher fertility rates (Table 1). With *L. chinense, N. domestica* and *S. gregii*, intraradical hyphae development and colonization (total arbuscules, vesicles/endospores, hyphae) increased at the higher fertility levels. The greatest increase occurred with *S. gregii*, which had a 216% increase in hyphae development and colonization at the higher fertility level. Arbuscules were only detected in root samples of *N. domestica* and *S. gregii*.

In an unrelated study using the same AMF isolate in commercial greenhouse production of prenuclear minitubers of potato, both growth and disease resistance benefits were reported with only 0.4% colonization (15). The potato plants were subjected to normal pesticide and fungicide applications, which depressed colonization levels, but did not eliminate the symbiosis. Our colonization levels were considerably higher, ranging from 7.7 to 42.7%.

There were species differences in AMF stimulation of plant growth. AMF enhancement of plant growth occurred only with the shrub, *N. domestica*, which was intermediate in colonization levels (10 to 16%). However, *N. domestica* had one of the highest arbuscule levels, which is indicative of active AMF development (20). There were no AMF growth enhancement of *S. gregii*, which had the highest colonization levels. A limited number of ornamental species are non-mycorrhizal. In an unrelated study, *Cuphea hyssopifolia* 'Allison', which is a short-lived perennial subshrub in the Lythraceae family, would not colonize with *Glomus intraradices.*

The commercial inoculum of *Glomus intraradices* (AMF) had no significant effect on the growth of *P. fraseri, L. chinense* or *S. gregii*. Only AMF colonized *Nandina domestica* 'Moon Bay' had growth enhancement (Table 2). AMF increased the shoot DM and root/shoot ratio at both fertilizer levels: 3 kg cu m and 4.2 kg cu m of Osmocote $24N–4P_2O_5–8K_2O$. AMF also enhanced the root DM at 3 kg cu m. The shoot DM of AMF plants at 3 kg cu m was the same as non-colonized plants (Non-AMF) at 4.2 kg cu m.

Among the four species, plant biomass and the root/shoot ratio were generally greatest at the higher fertilizer level. While the shoot mass of *S. gregii* was greater at the higher fertility regime, the root mass and root/shoot ratio were unaffected. The containerized plants of *L. chinense* were inadvertently pruned at the nursery prior to our final harvest, hence we were unable to take to shoot data and determine subsequent root to shoot ratios.

For the first 5.5 months of our nursery study, plants were placed on fertility regimes of either: 3 kg cu m or 4.2 kg cu m Osmocote $24N–4P_20_5–8K_20$ formulation, both of which were preincorporated with a trace element formulation, Micromax at 0.9 kg cu m, and 2.7 kg cu m of dolomitic limestone. For the remaining 6.5 months, plants from all treatments were transplanted into larger containers that had the higher fertility level: Osmocote $24N–4P_2O_5–8K_2O$ at 4.2 kg cu m (7 lbs cu yd), amended with a trace element formulation, Micromax at 1.5 lbs cu yd (0.9 kg cu m), and 2.7 kg (4.5 lbs cu yd) of dolomitic limestone. Since all treatments were exposed to the same fertility levels during the final 6.5 months of the experiment, this most likely minimized any fertility differences than if the same moderate to moderately-high fertility regimes had been maintained throughout the duration of the experiment. However, it was our intent to maintain commercially acceptable plant growth rates. Nonetheless there was still significant fertility growth effects with

N. domestica and the shoot growth of *S. gregii*. At the commercial nursery where this study was conducted, 4.2 kg cu m Osmocote 24N–4$P_2 0_5$–8$K_2 0$ is commonly used, although fertility regimes will vary with plant materials. It is important to document which plants will colonize at moderate to moderately-high fertility levels. While AMF enhancement of growth may be more dramatic at low fertility, it is important that a commercial crop be produced as quickly as possible to obtain a marketable size.

It has also been our observation that AMF are sometimes found with nursery liner plants. In part, this may be due to commercial nurseries not sterilizing their propagation and production media. However, though colonization can naturally occur, it is not necessarily with mycorrhiza that enhance plant growth or increase resistance to abiotic and biotic stresses. In our study, no colonization was detected with Non-AMF plants.

It is important to rethink that the main benefits of AMF are solely producing larger plants or increasing phosphorus uptake (17). In a commercial nursery where fertility is generally not a limiting factor, AMF growth differences are frequently more minimal as our study shows. However, during commercial production, if there is less plant stress [i.e., greater resistance to high temperature (12, 13, 14)] that occurs because of AMF colonization, which subsequently helps reduce the usage of pesticides and fungicides—then producers can gain in environmental and economic savings.

Though not part of this study, the long-term goal of this research is to evaluate other woody and herbaceous perennial nursery crop species from propagation to production to transplanting and establishment in the landscape. The nursery industry wants to reduce chemical costs and usage, improve plant quality, and command a higher price for their product. There are excellent opportunities to incorporate AMF in nursery production systems that help reduce levels of fertility and pesticide usage, and enhance crop vigor and productivity with recycled irrigation water. The value-added benefits of AMF in producing superior plants needs to be

Table 1. Effect of mycorrhiza (AMF) and fertility on colonization of four ornamental species grown in a commercial nursery.

Plant Species	AMF	Fertility (kg cu m)	Arbuscles (%)	Vesicles and endospores (%)	Hyphae (%)	Colonization (%)
Nandina Domestica 'Moon Bay'						
	Yes	3	5.7 ± 2.2[z]	3.3 ± 0.7	7.0 ± 1.4	10.0 ± 1.5
	No		0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Yes	4.2	5.3 ± 1.6	0 ± 0	15.7 ± 1.7	16.0 ± 1.6
	No		0 ± 0	0 ± 0	0 ± 0	0 ± 0
Significance[y]						
AMF			**	**	**	**
Fertility			NS	**	**	**
AMF × Fertility			NS	**	**	**
Photinia fraseri						
	Yes	3	0	1.3 ± 0.7	9.3 ± 1.1	9.3 ± 1.1
	No		0	0 ± 0	0 ± 0	0 ± 0
	Yes	4.2	0	1.0 ± 0.7	10.0 ± 2.6	10.0 ± 2.6
	No		0	0 ± 0	0 ± 0	0 ± 0
Significance						
AMF			NS	**	**	**
Fertility			NS	NS	NS	NS
AMF × Fertility			NS	NS	NS	NS
Salvia gregii						
	Yes	3	4.7 ± 1.8	0.3	19.7 ± 3.5	19.7 ± 3.5
	No		0 ± 0	0	0 ± 0	0 ± 0
	Yes	4.2	2.7 ± 0.8	0	42.7 ± 4.3	42.7 ± 4.3
	No		0 ± 0	0	0 ± 0	0 ± 0
Significance						
AMF			**	NS	**	**
Fertility			NS	NS	**	**
AMF × Fertility			NS	NS	**	**
Loropetalum chinense variety Rubrum 'Hinepurpleleaf' Plumb delight®						
	Yes	3	0	0	2.7 ± 0.8	2.7 ± 0.8
	No		0	0	0 ± 0	0 ± 0
	Yes	4.2	0	0	7.7 ± 1.5	7.7 ± 1.5
	No		0	0	0 ± 0	0 ± 0
Significance[y]						
Mycorrhiza (AMF)			NS	NS	**	**
Fertility			NS	NS	**	**
AMF × Fert			NS	NS	**	**

[z]Means with standard error and ANOVA; n = 300.

[y]NS = nonsignificant, * = significant at 5% level, ** = significant at 1% level.

Table 2. Effect of mycorrhizal fungi (AMF) and fertility on growth and development of four ornamental species grown in a commercial nursery.

AMF	Fertility (kg cu m)	Shoot DM (g)	Root DM (g)	Root/Shoot Ratio (g g^{-1})
Nandina domestica 'Moon Bay'				
Yes	3	48.6 ± 4.5[z]	18.4 ± 2.9	0.38 ± 0.01
No		38.5 ± 3.5	13.2 ± 1.9	0.34 ± 0.01
Yes	4.2	55.9 ± 2.6	23.4 ± 5.1	0.50 ± 0.02
No		46.5 ± 4.6	22.1 ± 1.9	0.40 ± 0.01
Significance[y]				
AMF		NS	NS	**
Fertility		*	*	**
AMF × Fertility		*	*	**
Photinia fraseri				
Yes	3	68.9	11.4	0.17
No		74.3	12.8	0.16
Yes	4.2	90.3	11.4	0.13
No		77.4	13.8	0.18
Significance				
AMF		NS	NS	NS
Fertility		NS	NS	NS
AMF × Fertility		NS	NS	NS
Salvia gregii				
Yes	3	38.6 ± 3.8	25.8	0.73
No		38.8 ± 3.7	21.3	0.54
Yes	4.2	46.1 ± 3.4	24.9	0.54
No		48.2 ± 5.0	23.1	0.49
Significance				
AMF		NS	NS	NS
Fertility		*	NS	NS
AMF × Fertility		NS	NS	NS
Loropetalum chinense variety Rubrum 'Hinepurpleleaf' Plumb delight®				
Yes	3	—	26.9	—
No		—	22.9	—
Yes	4.2	—	30.6	—
No		—	27.5	—
Significance				
AMF			NS	
Fertility			NS	
AMF × Fertility			NS	

[z]Means with standard error and ANOVA; n = 25.

[y]NS = nonsignificant , * = significant at 5% level, ** = significant at 1% level.

demonstrated so that the nursery industry can market and command a higher market price for mycorrhizal nursery corps.

Literature Cited

1. Bethlenfalvay, G.J. and R.G. Linderman. 1992. Mycorrhizae in sustainable agriculture. American Society of Agronomy, Madison, Wisconsin. ASA Special Publication No. 54.

2. Davies, F.T., Jr. 1987. Mycorrhizal fungi, fertility and media effects on growth and nutrition of *Rosa multiflora*. Plant and Soil 104:31–35.

3. Davies, F.T., Jr. and C.A. Call. 1990. Mycorrhizae, survival and growth of selected woody plant species in lignite overburden in Texas. Agriculture, Ecosystems and Environment 31:243–252.

4. Davies, F.T. Jr., Y. Castro-Jimenez, and S.A. Duray. 1987. Mycorrhizae, soil amendments, water relations and growth of *Rosa multiflora* under reduced irrigation regimes. Scientia Horticulturae 23:261–267.

5. Davies, F.T., Jr., J.R. Potter, and R.G. Linderman. 1992. Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. J. Plant Physiol. 139:289–294.

6. Davies, F.T., Jr., J.R. Potter, and R.G. Linderman. 1993. Drought resistance of mycorrhizal pepper plants independent of leaf P concentration—time course response of gas exchange and water relations. Physiol. Plantarum 87:45-53.

7. Davies, F.T., Jr., S.E. Svenson, J.C. Cole, L. Phavaphutanon, S.A. Duray, V. Olalde-Portugal, C.E. Meier, and S.H. Bo. 1996. Non-nutritional stress acclimation of mycorrhizal woody plants exposed to drought. Tree Physiol. 16: 985–993.

8. Goode, M. 1998. Irrigation is essential to the survival of horticulture. Texas Nurseryman 28(2):10–14.

9. Hartmann, H.T., D.E. Kester, F.T. Davies, Jr., and R.L. Geneve. Plant Propagation—Principles and Practices. 1997. 6th edition. (Prentice Hall). Englewood Cliffs, New Jersey. 778 pp.

10. Henderson, J.C. and F.T. Davies, Jr. 1990. Drought acclimation and the morphology of mycorrhizal *Rosa hybrida* L. cv. 'Ferdy' is independent of leaf elemental content. New Phytologist 115:503–510.

11. Hottovy, S.A. 1994. Monrovia's response to new environmental restrictions. Proc. Intern. Plant Propagators' Society 44:161–164.

12. Newman, S.E. and F.T. Davies, Jr. 1987. High soil temperature and water relations of endomycorrhizal nursery crops. J. Environ. Hort. 5:93-96.

13. Newman, S.E. and F.T. Davies, Jr. 1988a. High root-zone temperatures, mycorrhizal fungi, and water relations and root hydraulic conductivity of selected container grown woody plants. J. Amer. Soc. Hort. Sci. 113:138–145.

14. Newman, S.E. and F.T. Davies, Jr. 1988b. Influence of field bed position, ground surface color, mycorrhizal fungi and high root-zone temperature in woody plant container production. Plant and Soil 112:29–35.

15. Niemira, B.A., G.R. Safir, R. Hammerschmidt, and G.W. Bird. 1995. Production of prenuclear minitubers of potato with peat-based arbuscular mycorrhizal fungal inoculum. Agronomy J. 87: 942–946.

16. Pfleger, F.L. and R.G. Linderman (eds). 1994. Mycorrhizae and Plant Health. American Phytophathological Society Press. St. Paul, MN.

17. Phavaphutanon, L., F.T. Davies, Jr., S. Hla Bo, and S.A. Duray. 1996. Growth, root alteration, and nutrient uptake of Neem tree (*Azadirachta indica* A. Juss) seedlings in response to vesicular- arbuscular mycorrhizal fungi and phosphorus nutrition**.** International Tree Crops J. 9:59–67.

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

19. SAS Institute, Inc. 1988 SAS/STAT User's Guide, Version 6.03. SAS Institute, Cary, NC.

20. Smith, S.E. and D.M. Read. 1997. Mycorrhizal symbiosis. 2nd Edition. Academic Press Inc., London.

21. Svenson, S.E., F.T. Davies, Jr., and C.E. Meier. 1991. Ectomycorrhizae and drought acclimation influences water relations and growth of loblolly pine seedlings. HortScience 26:1406–1409.

22. Yeager, T., C.D. Fare, C. Gilliam, A. Niemiera, T. Bilderback and K. Tilt. 1997. Best Management Practices Guide for Producing Container-Grown Plants. Southern Nursery Association, Marietta, GA.