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Effectiveness of Commercial Mycorrhizal Inoculants on the Growth of *Liquidambar styraciflua* in Plant Nursery Conditions[1]

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Abstract

The effectiveness of several commercial mycorrhizal inoculants on the growth and development of *Liquidambar styraciflua* (sweetgum) was evaluated. Plants were grown in a nursery potting mix and were inoculated with the mycorrhizal products at the manufacturer's recommended rate. The growth response of mycorrhizal and nonmycorrhizal plants was analyzed at two harvests (8 and 14 weeks after transplanting). Significant differences were found in the growth of *L. styraciflua* to mycorrhizal colonization with the different commercial products. Fourteen weeks after transplanting, inoculation with products 1 (Earth Roots), 2 (MycoApply endo), and 3 (VAM 80) enhanced the growth of sweetgum relative to the nonmycorrhizal plants. However, plants inoculated with products 2 and 3 had greater leaf area, dry mass and relative growth rates than those inoculated with product 1. Plants of *L. styraciflua* inoculated with product 4 were less responsive to mycorrhizal colonization and only increased their leaf area relative to the non-inoculated controls. Testing both the infectivity and effectiveness of mycorrhizal fungi is recommended for the successful application of mycorrhizal technology in horticultural practices.

Index words: commercial mycorrhizal inoculum, mycorrhizal colonization, leaf area, relative growth rate.

Species used in this study: *Liquidambar styraciflua* L. [sweetgum].

Significance to the Nursery Industry

The suppliers of commercial mycorrhizal inoculants advertise that the incorporation of arbuscular mycorrhizal (AM) fungi in horticultural practices will enhance plant growth and performance (29, http://mycorrhiza.ag.utk.edu/). However, it is well known that different species and geographic isolates of AM fungi elicit different plant growth responses (7, 8, 23). We tested the effects of several commercial mycorrhizal inoculants on the growth and development of *Liquidambar styraciflua*. Significant differences were found in the mycorrhizal responsiveness of sweetgum to the different products. Approximately three-fold growth increases were obtained in plants inoculated with product 1, while five-fold growth increases were obtained for plants inoculated with products 2 and 3. Plants of *L. styraciflua* inoculated with product 4 were less responsive to mycorrhizal colonization and only increased their leaf area relative to the non-inoculated controls. We recommend that plant nurseries test both, the infectivity and effectiveness of mycorrhizal inoculants for the successful application of mycorrhizal technology in horticultural practices.

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Introduction

The number of plant nurseries interested in the implementation of mycorrhizal technology is increasing (3, 8, 14, 22). The suppliers of commercial mycorrhizal inoculum advertise that the incorporation of arbuscular mycorrhizal (AM) fungi in their management practices will enhance plant quality and performance while reducing fertilizer and pesticide requirements (29, http://mycorrhiza.ag.utk.edu/). The advantages of mycorrhizal colonization on plant propagation and growth, drought tolerance, and resistance to pathogens have been demonstrated (4, 21). However, it is also known that not all the combinations of plant hosts and AM fungi species are functionally compatible (20, 28). Plant responses to mycorrhizal colonization are mediated by plant species, AM fungi species and growing medium interactions (5, 16, 17).

Most of the commercial mycorrhizal inoculants available in the U.S. market contain highly infective AM fungi species (e.g., *Glomus intraradices*) (10). However, infectivity (rate of mycorrhizal colonization) does not always control effectiveness (positive growth responses to mycorrhizal colonization) (15); different species and ecotypes of AM fungi elicit different effects on plant growth (2, 6, 13, 20, 23).

In a previous investigation, we tested the infectivity of commercial mycorrhizal inoculants in nursery conditions (10). In this study, several products were selected to evaluate their effect on the growth and development of *Liquidambar styraciflua*, one of the most important commercial hardwoods in the southeastern United States, which is highly dependent on mycorrhizal fungi (12, 18, 30).

Materials and Methods

Effects of several commercial mycorrhizal inoculants on the growth and development of *Liquidambar styraciflua* were

tested under nursery conditions. The experiment was conducted in the greenhouse of the Tree of Life Nursery in San Juan Capistrano, CA, from March to May 2003. Average high/low temperatures during this time were 29/7C (79/46F), respectively.

Growing medium. The growing medium was a standard nursery potting mix composed of redwood bark, pine sawdust, calcined clay and sand (1:2:1:1 by vol). This medium has previously been shown to be suitable for mycorrhizal colonization (10). After steam pasteurization at 70C (158F) for three hours on two consecutive days, it was amended with 1.17 kg/m^3 (2 lb/yd^3) of dolomite, and 0.28 kg/m^3 (0.5 lb/yd^3) of Sierra Micromax® trace element mix. Before the incorporation of 0.6 kg/m^3 (1 lb/yd^3) of 18N–6P_2O_5–12K Osmocote® slow release fertilizer, its content of NO_3, NH_4, PO_4 and K was 66, 91, 10 and 640 ppm, respectively, according to the growth medium analysis determined at the Soil and Plant Laboratory, Inc. in Orange County, CA (major elements by sodium chloride extraction; phosphorus by sodium bicarbonate extraction).

Growth experiment. L. styraciflua seeds were obtained from Ojai Valley seeds, Ojai, CA. They were surface sterilized with 5% bleach for ten minutes prior to planting in a mixture of perlite and vermiculite. Eight days after seedling emergence, uniform seedlings were transplanted to 160 ml Super Cells (21 cm (8.2 in) deep, 3.8 cm (1.5 in) diameter, Steuwe and Sons, Corvallis, OR) ¾ filled with sterile potting mix. At the time of transplanting, plants were inoculated with seven different commercial mycorrhizal inoculants at the manufacturer's recommended rate. In most cases, the roots of the seedlings were placed directly on the layer of inoculant and covered with sterile potting mix. Some products came in a liquid carrier and they were applied directly onto the root system of each seedling. There were 20 replicates per mycorrhizal inoculum treatment and 20 non-inoculated (nonmycorrhizal) controls. To avoid product cross contamination, the Super Cells of each treatment were placed in separate racks that were rotated weekly in the greenhouse bench.

Ten randomly selected replicates were harvested 8 and 14 weeks after transplanting. Stems, leaves and roots were separated and the stem height was recorded. Leaf area was measured with a Li-Cor LI 30100 leaf area meter. Stems and leaves were oven-dried at 70C (158F) and their dry mass was recorded. The root system was divided in two parts and the fresh mass was recorded on both. One part of the root system was oven dried and used to calculate root dry mass based on fresh to dry mass relations. Total dry mass (shoot and root dry mass) was used to determine the relative growth rate [(RGR), increase in total dry mass as g/g/day (9)], and the mycorrhizal responsiveness [(total dry mass of mycorrhizal plants minus total dry mass of nonmycorrhizal plants) / dry mass of mycorrhizal plants (26, 27)].

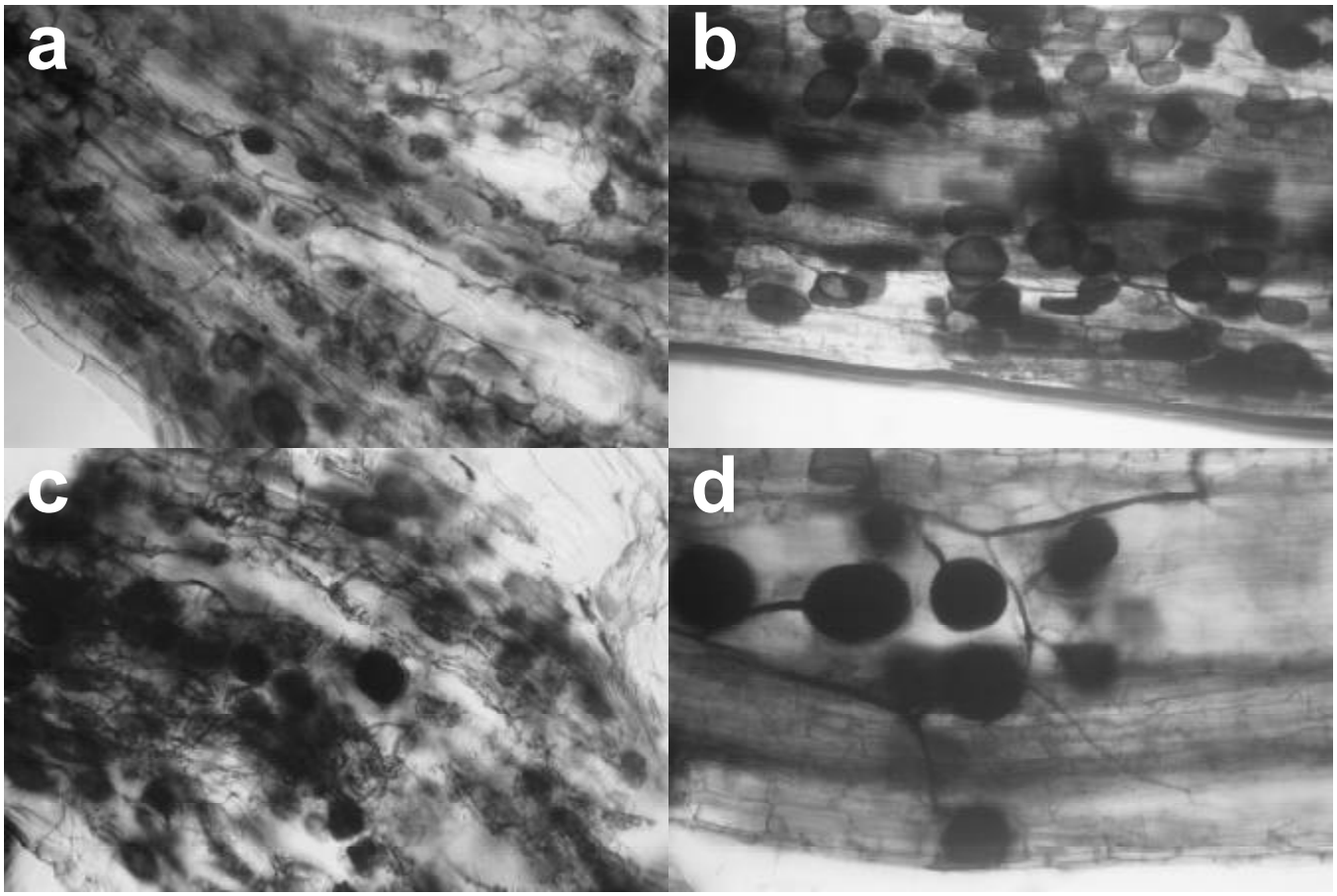

Fig. 1. Mycorrhizal colonization in *Liquidambar styraciflua.* Arbuscules and/or vesicles in plants inoculated with Earth Roots, MycoApply endo and VAM 80 (a, b, c, respectively). Spores in plants inoculated with product 4 (d). Pictures taken with a Nikon microphot light microscope with Nomarski interferential contrast.

Table 1. Mycorrhizal colonization of *Liquidambar styraciflua* inoculated with four commercial mycorrhizal inoculants eight and fourteen weeks after transplanting (first and second harvest, respectively).

	Mycorrhizal inoculum	Total percentage of mycorrhizal colonization	Percentage of arbuscules	Percentage of vesicles
First harvest	1[z]	19.57[y] ± 2.6a[x]	19.2 ± 2.3a	4.3 ± 1.6a
	2	41.02 ±12.2a	12.2 ± 1.6a	33.7 ±13.4b
	3	43.99 ± 3.6a	25.3 ± 5.1a	34.0 ± 5.2b
	4	0.40 ± 0.4b	0.4 ± 0.4b	0.0c
Second harvest	1	67.18 ± 9.0a	51.6 ±14.4a	30.1 ± 2.5a
	2	79.45 ± 3.1a	9.7 ± 2.2b	67.5 ± 5.0b
	3	89.97 ± 2.6a	22.1 ± 8.7a	79.9 ± 4.3b
	4	13.33 ± 6.4b	4.9 ± 2.2b	5.4 ± 2.6c

[z]Products 1, 2 and 3 are Earth Roots, MycoApply endo and VAM 80, respectively (disclosed with permission of the manufacturer).

[y]Data represent the Mean ± the Standard error of 10 replicates.

[x]Different lower case letters (within columns) indicate significant differences among commercial mycorrhizal inoculants at $P \leq 0.05$.

The fresh root pieces were cleared and stained using the technique of Koske and Gemma (19), and fifty 1 cm segments were mounted on microscope slides to determine the percentage of mycorrhizal colonization by the magnified intersection method of McGonigle et al. (24).

One way ANOVA was performed on shoot height, leaf area, total dry mass, RGR, mycorrhizal responsiveness and AM colonization (percentage of root length occupied by arbuscules, vesicles, coils and hyphae). Prior to statistical analysis, data were tested for normality with the Kolmogorov-Smirnov test and AM colonization percentages were arcsine-square root transformed. Mean contrasts were performed using Fisher's protected least significant difference (PLSD) with $P < 0.05$ as the level of significance (31).

Results and Discu\ssion

Mycorrhizal colonization was found in plants of *Liquidambar styraciflua* inoculated with products 1 (Earth Roots), 2 (MycoApply endo), 3 (VAM 80) and 4 (Fig. 1). Plants inoculated with product 4 had considerably lower percentages of mycorrhizal colonization than plants inoculated with products 1, 2 and 3, at both, first and second harvests (Table 1). No mycorrhizal colonization was evident in plants inoculated with three products possibly due to either low density of viable AM fungal propagules or incompatibility with testing conditions.

Plant growth response of *L. styraciflua* to mycorrhizal colonization was influenced by the source of inoculum (Fig. 2; Table 2). Plants inoculated with product 1 and 3 were taller, had double the leaf area, and considerably greater dry mass and RGR than the nonmycorrhizal controls, eight weeks after transplanting. Inoculation with product 2 increased the shoot height and the leaf area, and with product 4 the shoot height, but there were no significant differences between the dry mass of *L. styraciflua* plants inoculated with these products (2 and 4) and the nonmycorrhizal controls at the first harvest (Table 2).

At the second harvest (14 weeks after transplanting), inoculation with products 1, 2 and 3 notably enhanced the dry mass of *L. styraciflua* (Table 2); however, there were significant differences in sweetgum mycorrhizal responsiveness to the different commercial inoculants (Fig. 3). Plants inoculated with product 2 and 3 were more responsive (Fig. 3), had greater leaf area, leaf dry mass, total dry mass and RGR than plants inoculated with product 1 and 4 (Table 2). Plants

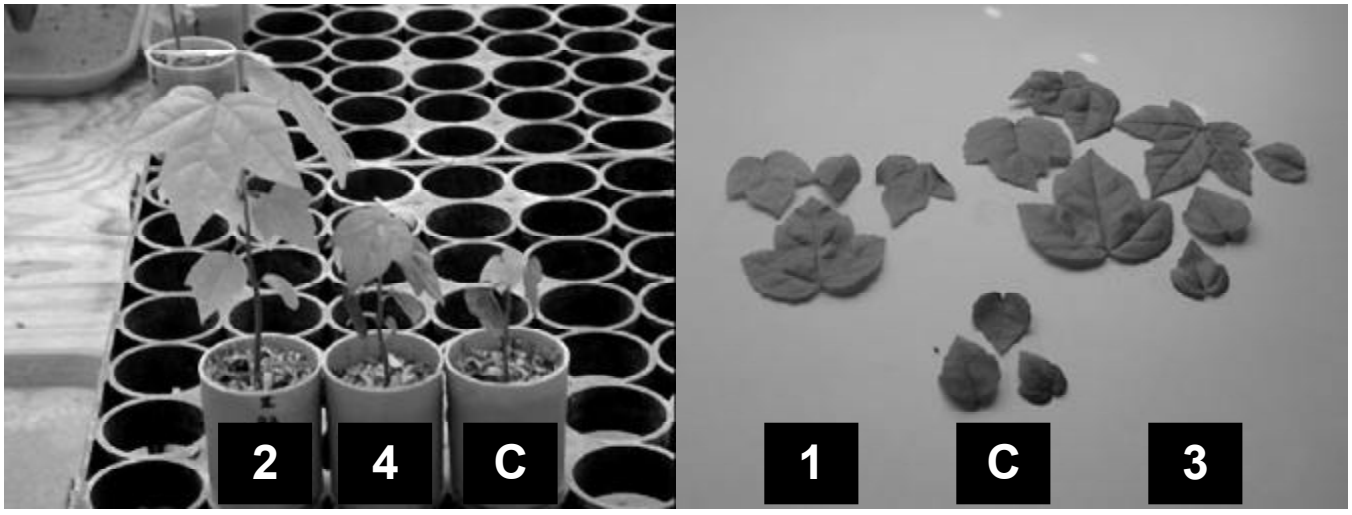

Fig. 2. Plants of *Liquidambar styraciflua* inoculated with different commercial mycorrhizal inoculants and non-inoculated control. Numbers denote inoculation with product 1 (Earth Roots), 2 (MycoApply endo), 3 (VAM 80), 4 and nonmycorrhizal control (C).

Table 2. Effects of different commercial mycorrhizal inoculants (products 1, 2, 3, 4) and nonmycorrhizal control on the growth response of *Liquidambar styraciflua*.

		Control	1[z]	2	3	4
First harvest	Shoot height (cm)	6.58[y] ± 0.310a[x]	10.02 ± 0.360b	7.93 ± 0.300c	8.49 ± 0.430c	7.55 ± 0.430c
	Leaf area (cm^2)	12.02 ± 1.260a	24.28 ± 2.160b	18.21 ± 1.280c	22.16 ± 2.970bc	16.14 ± 1.120a
	Leaf dry mass (g)	0.04 ± 0.005a	0.09 ± 0.010b	0.06 ± 0.006a	0.07 ± 0.009b	0.06 ± 0.005a
	Total dry mass (g)	0.11 ± 0.014a	0.21 ± 0.017b	0.15 ± 0.025a	0.18 ± 0.024b	0.14 ± 0.014a
	RGR	0.04 ± 0.001a	0.05 ± 0.001b	0.04 ± 0.002c	0.05 ± 0.001c	0.04 ± 0.001ab
	Root:Shoot	0.64 ± 0.053a	0.49 ± 0.044a	0.50 ± 0.178a	0.55 ± 0.049a	0.49 ± 0.042a
Second harvest	Shoot height	6.58 ± 0.328a	11.31 ± 0.473b	10.97 ± 0.644b	12.11 ± 0.617b	6.94 ± 0.348a
	Leaf area	10.38 ± 0.902a	44.04 ± 4.556b	65.10 ± 8.467c	74.87 ± 6.949c	15.61 ± 1.225b
	Leaf dry mass	0.05 ± 0.006a	0.19 ± 0.022b	0.27 ± 0.037c	0.29 ± 0.035c	0.06 ± 0.008a
	Total dry mass	0.16 ± 0.002a	0.45 ± 0.045b	0.70 ± 0.107c	0.71 ± 0.099c	0.20 ± 0.020a
	RGR	0.02 ± 0.0004a	0.03 ± 0.0004b	0.04 ± 0.001c	0.04 ± 0.00 d	0.03 ± 0.002a
	Root:Shoot	1.30 ± 0.33a	0.49 ± 0.037b	1.26 ± 0.273a	1.17 ± 0.320a	1.05 ± 0.120a

[z]Products 1, 2 and 3 are Earth Roots, MycoApply endo and VAM 80, respectively (disclosed with permission of the manufacturer).

[y]Data represent the Mean ± Standard error of ten replicates.

[x]Different lower case letters (across rows) indicate significant differences among commercial mycorrhizal inoculants at $P \leq 0.05$.

inoculated with product 4 were the least responsive (Fig. 3) and only increased the leaf area of sweetgum slightly, compared to the nonmycorrhizal controls (Table 2).

Although the advantages of mycorrhizal colonization are not restricted to plant growth, it is possible that the growing conditions were unsuitable for product 4 optimum performance. It has been demonstrated that the infectivity of commercial mycorrhizal inoculants can be affected by the growing medium (10). No colonization was detected in most of the plants inoculated with product 4 at the first harvest, and the percentages of mycorrhizal colonization were lower than those obtained in plants inoculated with products 1, 2 and 3, at the second harvest (Table 2). While the benefits of mycorrhizal colonization have been related to early colonization (1), a higher infectivity level does not always guarantee plant growth improvement, beneficial responses have been reported with only 0.4 percent of mycorrhizal colonization (25). Furthermore, it is well known that different species and ecotypes of AM fungi promote different plant growth responses (7, 8, 23). In fact, previous studies have already shown that some AM fungi species are more beneficial than others for the growth of *L. styraciflua.* Plants of sweetgum inoculated with *Glomus fasciculatum* were larger than those inoculated with another species of *Glomus* and/or a mixture of AM fungi although they showed lower or similar percentages of mycorrhizal colonization (17, 18).

Testing the effectiveness of commercial mycorrhizal inoculants is as important as testing their infectivity for the successful application of mycorrhizal technology in horticultural practices.

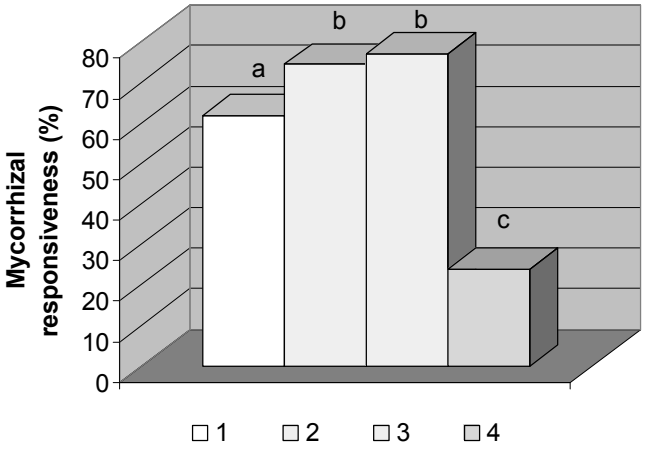

Fig. 3. Mycorrhizal responsiveness of plants of *Liquidambar styraciflua* inoculated with four commercial mycorrhizal inoculants (product 1 (Earth Roots), 2 (MycoApply endo), 3 (VAM 80) and 4). Different letters above bars indicate significant differences among products at $P \leq 0.05$.

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