

# Performance of Mycorrhizal Products Marketed for Woody Landscape Plants<sup>1</sup>

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## Abstract

Commercial products containing propagules of arbuscular mycorrhizal fungi (AMF) are widely marketed to improve woody plant performance in the landscape. However, the infectivity of these products has rarely been subjected to independent testing. We evaluated commercial AMF inoculants in a series of greenhouse experiments using corn (*Zea mays*), sorghum (*Sorghum bicolor*), trident maple (*Acer buergerianum*), and sweetbay magnolia (*Magnolia virginiana*) as host plants. In corn and sorghum, colonization rarely exceeded 5% when plants were treated with commercial inoculants. In contrast, viable lab-cultured inoculant of similar species composition yielded mean colonization percentages of 38 to 61%. Despite the near absence of colonization, commercial inoculants generally improved shoot growth and increased soil nutrient concentrations in a dose-dependent manner. Commercial inoculants had no effect on mycorrhizal colonization or shoot growth of trident maple or sweetbay magnolia liners. Product-treated magnolias grown from seed also developed little or no mycorrhizal colonization, whereas plants treated with a lab-cultured inoculant were 74% colonized. If commercial AMF inoculants are to receive broad acceptance as landscape soil amendments, manufacturers must demonstrate that their products can promote mycorrhizal colonization under the conditions of their intended distribution and use.

**Index words:** corn (*Zea mays*), sorghum (*Sorghum bicolor*), trident maple (*Acer buergerianum*), sweetbay magnolia (*Magnolia virginiana*), mycorrhizal colonization, arbuscular mycorrhizal fungi.

## Significance to the Nursery Industry

Commercial arbuscular mycorrhizal fungal (AMF) products are heavily marketed to the nursery, landscape, and arboricultural industries. Product manufacturers make a variety of performance claims, including greater transplant success, enhanced plant growth, and improved appearance. However, previous research has shown little benefit of AMF product application under typical nursery and landscape conditions. Unlike most soil amendments, AMF inoculants contain living propagules that are sensitive to storage duration and conditions; poor product performance may therefore reflect loss of viability during storage and shipping. In this series of experiments, commercial mycorrhizal products

consistently failed to promote mycorrhizal colonization in corn, sorghum, trident maple, and sweetbay magnolia. In contrast, viable lab-cultured inoculant of similar species composition promoted high levels of AMF colonization. Commercial products did enhance plant growth and soil fertility, suggesting that non-mycorrhizal ingredients were acting as fertilizers and/or plant growth regulators. While plant professionals may value this practical outcome, it should be noted that high soil fertility commonly inhibits AMF colonization. Nutritional ingredients are therefore counterproductive to the intended function of AMF inoculants. Before AMF inoculants can be broadly recommended, manufacturers must better demonstrate that their products are compatible with current retail distribution methods and can promote mycorrhizal colonization under the conditions of their intended use.

## Introduction

Commercial products containing propagules of arbuscular mycorrhizal fungi (AMF) are widely marketed to improve woody plant performance in the landscape. Product manufacturers offer a three-fold rationale for using mycorrhizal inoculants. First, the abundance and infectivity of native AMF are thought to be reduced when soils are disturbed by land development and construction. Evidence suggests that

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soil disturbance associated with cultivation, strip mining, and urbanization can negatively impact AMF populations (5, 17, 22, 23, 36; but see 43). Second, AMF colonization of transplanted trees and shrubs may be low or absent due to fertilization and fungicide application in the nursery (11, 24, 26, 29, 38). Finally, AMF exhibit relatively low host specificity: a product containing one or more AMF species should successfully inoculate a wide range of woody plant hosts (7, 8).

In highly controlled experiments with lab-cultured inoculum, AMF inoculation has generally improved the mycorrhizal colonization, survival, and growth of woody plants (4, 9, 16, 18, 21, 30, 35, 37, 44). However, AMF inoculation of woody plants under typical nursery and landscape conditions has yielded inconsistent results (1, 2, 3, 13, 14, 26, 29). Failure of AMF inoculation to improve woody plant performance in the field has been attributed to numerous factors: low plant carbohydrate status at transplant, AMF-inhibiting soil conditions, fungus-host incompatibility, pre-existing AMF colonization, and absence of significant plant stress (1, 2, 15).

In the case of commercial AMF inoculants, the viability of the products themselves must also be considered (1). Unlike most soil amendments, inoculants contain living propagules that are sensitive to storage duration and conditions (6, 32, 34, 40, 42). Manufacturers of commercial inoculants seem to be aware of these limitations, and product labels frequently contain explicit storage instructions and expiration dates. However, many commercial inoculants are available only through mail-order retailers, raising the possibility that they could lose infectivity during storage and shipping.

The infectivity of commercial AMF inoculants has rarely been subjected to independent testing. Appleton (1) conducted an infectivity trial of ten commercial inoculants but did not elaborate on their performance beyond stating that some products provided successful colonization while others did not. Manufacturers' voluntary product samples evaluated by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi possessed infectivity comparable to lab-cultured inoculum; however, results from products acquired through anonymous consumer channels were less favorable (20).

Corkidi *et al.* (11) tested the infectivity of ten commercial inoculants provided by their manufacturers and found that four out of ten products did not promote mycorrhizal colonization of corn roots (*Zea mays*) in a standardized greenhouse bioassay. Furthermore, only two products achieved colonization levels greater than 10% in a soil-based medium. Similarly, Tarbell and Koske (39) found that five of eight commercial inoculants failed to colonize corn roots when applied to a sand/peat putting green medium at the manufacturers' recommended rate.

The objective of the current study was to independently evaluate commercial AMF inoculants purchased through typical consumer channels. A series of greenhouse experiments was conducted using both standard bioassay host plants and woody landscape species. The effects of the inoculants on mycorrhizal colonization, plant growth, plant nutrition, and soil fertility were evaluated.

## Materials and Methods

**General methods.** All experiments were performed in a controlled-environment greenhouse at Clemson University in

Clemson, SC. Commercial AMF inoculants were purchased anonymously through typical mail-order transactions and stored at 5°C (41°F) for less than 4 weeks prior to use (Table 1). Unless otherwise indicated, successive experiments were conducted with newly-purchased products.

The soil medium used in all experiments was prepared by mixing multi-purpose silica sand with sandy loam field soil at a 2:1 volume ratio and steam-pasteurizing at 82°C (180°F) for seven hours to eliminate native AMF propagules. The field soil (Cecil series: fine, kaolinitic, thermic Typic Kanhapludult) was collected from the Ap horizon of a fallow pasture near Clemson, SC. Unless indicated otherwise, a new batch of soil medium was prepared for each experiment. Following pasteurization, a sample of each soil batch was submitted to the Clemson University Agriculture Service Laboratory for standard nutrient analysis via Mehlich-1 extraction and inductively-coupled plasma spectroscopy (Table 2).

During the experiments, plants were manually watered as needed. Unless otherwise indicated, supplemental light at 490  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}$  (2,459 ft-c) intensity was provided when ambient light fell below 685  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}$  (3,439 ft-c) during a controlled 16-hour photoperiod. Greenhouse temperature and relative humidity were maintained at 25°C (77°F) and 50% throughout all experiments.

Initial infectivity tests were performed with standard mycorrhizal bioassay host plants: corn (*Zea mays*) and sorghum (*Sorghum bicolor*). These species are readily colonized in the presence of viable AMF inoculant and are commonly used in mycorrhizal research (19, 28). Further experiments used woody landscape species that form AMF associations: trident maple (*Acer buergerianum*) and sweetbay magnolia (*Magnolia virginiana*).

For each experiment, the treatment main effect was assessed using analysis of variance. When necessary, values of dependent variables were transformed prior to analysis to satisfy normality and homogeneity of variance assumptions. When the treatment main effect was significant, dependent multiple comparisons were made between treatment groups using either Dunnett's procedure or Tukey's HSD procedure ( $\alpha = 0.05$ ).

**Experiment 1: Corn host.** Eight commercial AMF inoculants were purchased anonymously through consumer channels and shipped to the home address of a laboratory member. Six granular products and two root-dip products were evaluated (Table 1). Each granular product was thoroughly mixed with pasteurized soil medium at the manufacturer's recommended rate as given in Table 1. The prepared soil mixtures were poured into 164 ml (5.5 oz) plastic containers (Ray Leach Cone-tainer™, Stuewe and Sons, Inc., Corvallis, OR), and three fungicide-free corn seeds (*Zea mays* 'Viking', Albert Lea Seedhouse, Albert Lea, MN) were sown in each container. Containers for control and root-dip treatments were filled with pasteurized soil medium only. Root-dip inoculants were applied to the radicals of three-day-old corn seedlings according to the manufacturers' directions; one seedling was then transplanted into each container as described above.

At planting, each container received 150 ppm of 13-2-13 (N-P-K) fertilizer, 227 ppm of sulfate-of-potash magnesium fertilizer, and 650 ppm of calcitic lime. Amendments were supplied to correct pH and macronutrient deficiencies (except phosphorus) identified in the soil analysis (Table 2).

Table 1. Characteristics of commercial AMF products evaluated in a series of greenhouse experiments.

Inoculant ID	Product	Manufacturer	Ingredients <sup>z</sup>	Mycorrhizal content <sup>z</sup>	Manufacturer's application rate <sup>z</sup>	1:10 v/v application rate	Experiments in which evaluated
Granular 1	AgBio-Endos	AgBio, Inc., Westminster, CO	Organic potting mix containing six <i>Glomus</i> and one <i>Gigaspora</i> arbuscular mycorrhizal fungi (AMF) species	131 propagules/g	3.0 g/L	57.4 g/L	1, 2, 3
Granular 2	AM 120	Reforestation Technologies International, Salinas, CA	Solid carrier containing three <i>Glomus</i> AMF species	152 propagules/g	3.0 g/L	79.2 g/L	1, 2, 3, 4, 5
Granular 3	BEI	BioOrganics, Santa Maria, CA	Solid carrier containing six <i>Glomus</i> , one <i>Gigaspora</i> , and one <i>Paraglomus</i> AMF species	76 spores/g	1.8 g/L	65.9 g/L	1, 2, 3, 5
Granular 4	BioGrow Endo	Mycorrhizal Applications, Inc., Grants Pass, OR	Organic compost containing three <i>Glomus</i> AMF species, <i>Trichoderma</i> fungus, and vitamins	44 propagules/g	3.0 g/L	81.0 g/L	1, 2, 3,
Granular 5	DieHard Endo Starter	Horticultural Alliance, Inc., Sarasota, FL	Six <i>Glomus</i> and one <i>Gigaspora</i> AMF species; nine ectomycorrhizal fungi species; <i>Trichoderma</i> fungus; biostimulants; plant growth enhancers; beneficial bacteria; micronutrients; hydrogel	Not provided	1.2 g/L	70.7 g/L	1, 2, 3, 5
Granular 6	MycorMax	JH Biotech, Inc., Ventura, CA	Solid carrier containing two <i>Glomus</i> AMF species and five ectomycorrhizal fungi species	22 (endo) & 52 (ecto) propagules/g	1.2 g/L	88.4 g/L	1, 2, 3, 4, 5
Granular 7	Mycor Nursery/Media Mix	Plant Health Care, Inc., Pittsburgh, PA	Organic biostimulants and soil amendments mixed with beneficial rhizosphere bacteria and live spores of several AMF species	88 spores/g	3.0 g/L	76.0 g/L	2, 3, 5
Root-dip 1	MycorTree Root Dip	Plant Health Care, Inc., Pittsburgh, PA	Five species of ecto-/endomycorrhizal fungi; organic biocatalysts; water management gel	Not provided	Mix with water	—	1, 4
Root-dip 2	Root Dip Universal	Tree Pro, West Lafayette, IN	AMF spores combined with humic acids, biostimulants, beneficial bacteria, soluble sea kelp, and yucca plant extracts; water management gel	Not provided	Mix with water	—	1, 4

<sup>z</sup>As described in product literature.

**Table 2.** Chemical properties of soil media used in a series of greenhouse experiments evaluating commercial AMF products. Soil media were prepared by mixing washed sand and field-collected topsoil in a 2:1 volumetric ratio followed by steam pasteurization.

	pH*	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Mn (ppm)
Experiment 1	5.3	3	10	60	12	6	10
Experiment 2	6.8	4	10	157	14	4	13
Experiment 3	5.5	9	10	223	18	6	26
Experiment 4	6.3	6	13	153	11	4	18

\*Soil pH was determined from dried, screened soil samples using a 1:1 ratio of soil weight to water volume.

Soil phosphorus concentrations were kept low (3 to 9 ppm) to encourage AMF colonization. Each container was thinned to one plant at three days post-germination, and 300 ppm of 13–2–13 fertilizer was applied at two and four weeks after sowing.

Nine inoculant treatments (control and eight AMF products) and three plant harvest intervals (two, four, and eight weeks) were replicated eight times in a completely randomized design, totaling 216 experimental units. Multiple harvest intervals were chosen to ensure adequate time for AMF colonization to develop while minimizing the possibility of secondary colonization (11, 20, 28). Whole plants were harvested at the given intervals and separated into shoots and roots. Shoots were oven-dried at 75C (167F) and weighed. Roots were preserved in 50% ethanol and stored at 5C (41F) for AMF colonization assessment.

Approximately 2 g (0.07 oz) fresh weight of fine roots was collected from each preserved root system. Roots were cleared and stained with trypan blue (25) and mounted on microscope slides. AMF colonization was assessed using the magnified intersections method with a compound microscope (110×) and cross-hair reticle (27). For each sample, 50 root intersections were assessed for the presence of fungal hyphae. AMF colonization was calculated as the percentage of 50 root intersections at which fungal hyphae were also present. It was assumed that all hyphae observed in harvested roots were AMF. The experimental soil media were steam-pasteurized, and colonization levels of control plants were extremely low throughout all experiments, suggesting that this assumption was justified. Nonetheless, it is possible that this assumption was violated, particularly in the case of Product 4, which contained propagules of a non-mycorrhizal fungal species.

*Experiment 2: Corn host + multiple application rates.* Seven granular AMF inoculants were purchased anonymously through consumer channels as described above. New batches of six of the seven granular products from Experiment 1 and one new granular product were evaluated (Table 1). Fungicide-free corn seeds were used as the experimental host. Each AMF product was tested at five application rates: 1×, 5×, 10×, 20×, and 40× the manufacturers' recommended rate. Three replicate samples of soil medium were prepared for each product × application rate combination and analyzed for mineral nutrient content.

All cultural practices of Experiment 1 were duplicated in Experiment 2, and each treatment combination was

replicated seven times for a total of 252 experimental units. To correct nutrient deficiencies (except phosphorus), each container received 150 ppm of 13–2–13 (N–P–K) fertilizer and 227 ppm of sulfate-of-potash magnesium fertilizer at planting. An additional application of 150 ppm of 13–2–13 fertilizer was made three weeks after sowing. All plants were harvested and evaluated four weeks after treatment as in Experiment 1. In addition, shoots from three replicate plants of each treatment combination were randomly sampled and analyzed for foliar nutrient content.

*Experiment 3: Positive control + additional host species.* New batches of seven granular inoculants were purchased anonymously (Table 1). At the same time, a lab-cultured inoculant containing mycorrhizal propagules of *Glomus intraradices*, *G. etunicatum*, and *G. clarum* was obtained from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM; Morgantown, WV) for use as a positive control. Fungicide-free corn and sorghum seeds (Outsidepride.com, Inc., Salem, OR) were used as the experimental hosts.

A completely randomized experiment was performed with two application rates of the commercial inoculants (1× and 10× manufacturer's rate) and nine treatments (seven AMF inoculants and positive and negative controls). Each treatment combination was replicated eight times with both plant species, totaling 256 experimental units. The granular AMF treatments and negative control were prepared as in Experiment 1. The positive control was prepared by thoroughly mixing the cultured inoculant with the steam-pasteurized soil medium at a 1:10 volume ratio, the standard protocol used by the culturing lab for evaluating inoculant infectivity.

To obtain sufficient shoot and root biomass, each sorghum experimental unit was established by sowing five seeds into a single container. All cultural practices of Experiment 1 were duplicated in Experiment 3. To correct pH and nutrient deficiencies, soil amendments were applied to each container as follows: 600 ppm of calcitic lime, 227 ppm of sulfate-of-potash magnesium fertilizer, and 150 ppm of 13–2–13 (N–P–K) fertilizer five days after sowing, and 150 ppm of 13–2–13 fertilizer at 3 weeks after sowing. All plants were harvested and evaluated as in Experiment 1 at four weeks after treatment.

*Experiment 4: Woody plant hosts.* Bare-root sweetbay magnolia seedlings and peat-plugged trident maple seedlings were acquired from a nursery liner producer (Heritage Seedlings, Inc., Salem, OR). Neither species had been deliberately inoculated with AMF during propagation. Whole-plant fresh weight was recorded, and a fine root sub-sample was collected and preserved from each plant prior to treatment. Two granular inoculants and two root-dip inoculants were evaluated (Table 1). The granular inoculants had been stored in the dark at 5C (41F) for 3 months whereas the root-dip inoculants were newly purchased.

The granular inoculants and soil medium were mixed as in Experiment 1 and poured into 500 ml (17 oz) plastic containers (Rootrainer™, Hummert International, Earth City, MO); a single seedling was then transplanted into each container. Root-dip inoculants were applied to seedlings before transplant according to the manufacturers' instructions. Root-dip treated and control plants were transplanted to containers filled with steam-pasteurized soil medium only.



Five inoculant treatments (control and four AMF products) and two plant species were replicated six times in a completely randomized design, totaling 60 experimental units. The plants were watered as needed and received no supplemental light or fertilization. The experiment was conducted in the summer when the maximum light intensity observed in the greenhouse at solar noon was approximately 1480  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}$  (7,430 ft-c). A fine root sample was collected from each plant at four and eight weeks after treatment, and all plants were harvested and evaluated as in Experiment 1 at 16 weeks after treatment.

**Experiment 5: Woody plant host + positive control.** Eight inoculant treatments (six AMF products at manufacturers' recommended rates and positive and negative controls) were prepared as in Experiment 3 and evaluated using sweetbay magnolia seedlings as the plant host (Table 1). Surplus inoculants from Experiment 3, having been dry-stored in the dark at 5C (41F) for 2 months since purchase, were used. Prior to treatment application, cold-stratified, open-pollinated sweetbay magnolia seeds were germinated in steam-pasteurized soil medium for four weeks. At the cotyledon stage, the seedlings were lifted, weighed, and randomly transplanted to individual 164 ml (5.5 oz) plastic containers. Each inoculant treatment was replicated eight times, totaling 64 experimental units.

Plants were illuminated and irrigated as in Experiment 1. They received no supplemental fertilization and were harvested six weeks after treatment. Measurements at harvest included leaf area (LI-3100 Area Meter, LI-COR Environmental, Lincoln, NE), leaf + stem dry weight, and percent hyphal colonization.

## Results and Discussion

**Experiment 1: Corn host.** Commercial AMF inoculants had little effect on corn root mycorrhizal colonization when obtained anonymously through typical consumer channels and applied at the manufacturers' recommended rates. AMF colonization of corn was extremely low for all treatments and harvest intervals (Table 3). At two weeks, only plants

treated with granular product 2 showed higher colonization than controls; at four weeks, only plants treated with granular product 1 showed higher colonization than controls. In both cases, the absolute magnitude of colonization was low (3.0 and 5.5% for granular products 2 and 1, respectively). A previous evaluation of commercial AMF inoculants used 20% colonization as the threshold criterion for product performance (20). Based on this criterion, the products evaluated in Experiment 1 failed to provide acceptable colonization. In a similar experiment with ten commercial inoculants provided by their manufacturers, Corkidi *et al.* (11) found that four products failed to promote colonization at all and only one product resulted in colonization over 20% in a soil-based medium.

The majority of granular commercial inoculants significantly increased shoot dry weight despite the near-absence of AMF colonization ( $p < 0.05$  treatment main effect on all dates; Table 3). At two weeks, plants treated with granular products 3 and 6 were larger than controls. At four weeks, plants treated with granular products 3, 4, and 6 were larger than controls, and at eight weeks, plants treated with granular products 1, 3, 4, 5 and 6 were larger than controls. Plants treated with these products were approximately twice as large as controls at eight weeks. The root-dip products had no effect on plant size.

Growth enhancement occurred independently of mycorrhizal colonization, perhaps in response to non-mycorrhizal auxiliary ingredients, which may have improved the fertility or physical properties of the soil media. A similar result was obtained by Corkidi *et al.* (11), who found that plant growth was promoted only by those commercial products that *did not* enhance mycorrhizal colonization. Root-dip products did not promote corn growth at any harvest interval. Due to their application method, root-dip products introduce a relatively small amount of material to the soil, which may explain the absence of a plant growth response.

**Experiment 2: Corn host + multiple application rates.** Despite application rates up to 40 times higher than manufacturers' recommendations, AMF colonization was negligible

**Table 3. Mycorrhizal colonization and shoot dry weight of corn plants treated with commercial AMF products and grown in steam-pasteurized soil medium. Plants were treated at the manufacturers' labeled rate and harvested at three intervals (n = 8 for each product  $\times$  harvest combination). Control was steam-pasteurized soil medium only. For mycorrhizal colonization, both the treatment mean and the maximum value from the sample are listed.**

	Control		Granular 1		Granular 2		Granular 3		Granular 4		Granular 5		Granular 6		Root-dip 1		Root-dip 2	
	Mycorrhizal colonization (%)																	
Harvest date	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
Two weeks	0.2	1.3	0.0	0.0	3.0*	6.7	0.5	2.7	0.7	1.3	0.0	0.0	1.2	4.0	1.2	4.0	0.2	1.3
Four weeks	0.0	0.0	5.5*	22.7	1.8	8.0	0.7	2.7	1.3	6.7	0.2	1.3	0.5	2.7	0.0	0.0	0.8	6.7
Eight weeks	0.0	0.0	1.5	8.0	2.0	16.0	3.2	13.3	0.2	1.3	0.0	0.0	1.8	4.0	0.3	2.7	0.0	0.0
	Shoot dry weight (g)																	
Harvest date	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Two weeks	0.18	0.02	0.21*	0.01	0.16	0.02	0.31*	0.01	0.22*	0.01	0.20*	0.01	0.26*	0.01	0.18	0.02	0.20	0.02
Four weeks	0.49	0.03	0.61*	0.05	0.38	0.05	0.86*	0.04	0.76*	0.04	0.55*	0.02	0.84*	0.04	0.43	0.03	0.43	0.03
Eight weeks	1.15	0.09	2.25*	0.24	1.16	0.07	2.83*	0.08	2.61*	0.24	1.97*	0.12	2.72*	0.19	1.37	0.11	1.43	0.10

\*Within a row, indicates that the treatment mean is significantly different from the control mean using Dunnett's multiple comparisons procedure ( $\alpha = 0.05$ ).

**Table 4.** Mycorrhizal colonization, shoot dry weight, extractable soil phosphorus, and foliar phosphorus content of corn plants treated with commercial AMF products applied at five levels of the manufacturers' labeled rate (for colonization and shoot weight,  $n = 7$  for each product  $\times$  rate combination; for soil and foliar phosphorus contents,  $n = 3$ ). Control was steam-pasteurized soil medium only. For mycorrhizal colonization, both the treatment mean and the maximum value from the sample are listed.

	Control		Granular 1		Granular 2		Granular 3		Granular 4		Granular 5		Granular 6		Granular 7	
	Mycorrhizal colonization (%)															
Application rate	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
1× application	1.1	8.0	1.3	4.0	1.0	4.0	0.9	4.0	0.3	2.0	0.6	2.0	0	0	1.4	6.0
5× application	— <sup>z</sup>	—	1.7	4.0	0.3	2.0	1.7	4.0	† <sup>y</sup>	†	0.3	2.0	1.1	4.0	0.3	2.0
10× application	—	—	2.6	4.0	0.6	2.0	0.9	2.0	†	†	1.1	6.0	0.3	2.0	0.7	4.0
20× application	—	—	0.6	4.0	2.9	10.0	0.7	2.0	†	†	0.3	2.0	1.1	4.0	1.2	4.0
40× application	—	—	0.9	4.0	8.3	24.0	0	0	†	†	0.9	4.0	0.9	4.0	†	†
	Shoot dry weight (g)															
Application rate	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1× application	0.53	0.04	0.41	0.05	0.49	0.05	0.51	0.04	0.82**	0.06	0.58	0.04	0.48	0.03	0.37*	0.04
5× application	—	—	0.50	0.05	0.92*	0.07	0.58	0.04	†	†	0.58	0.07	0.56	0.06	0.58	0.09
10× application	—	—	0.71	0.06	0.93*	0.09	0.69*	0.04	†	†	0.75	0.10	0.63	0.06	0.61	0.07
20× application	—	—	0.95*	0.08	1.06*	0.15	0.79*	0.06	†	†	0.87*	0.07	0.88*	0.09	0.66	0.05
40× application	—	—	1.14*	0.07	1.25*	0.08	1.03*	0.05	†	†	1.00*	0.11	1.07*	0.08	†	†
	Extractable soil phosphorus (ppm)															
Application rate	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1× application	3.67	0.17	4.50	0.29	5.67	0.60	4.00	0.29	12.67*	3.06	4.67	0.17	4.50	0.29	4.83*	0.17
5× application	—	—	6.00*	0.29	15.83*	2.73	5.83*	0.60	†	†	6.50*	0.76	4.67	0.17	10.33*	1.01
10× application	—	—	7.83*	0.73	21.33*	2.33	9.83*	0.33	†	†	8.67*	0.60	6.17*	0.33	17.67*	0.73
20× application	—	—	12.67*	1.59	48.50*	15.89	15.50*	1.26	†	†	15.67*	2.20	10.33*	0.88	31.50*	1.80
40× application	—	—	27.17*	2.40	84.00*	15.89	31.00*	1.61	†	†	33.00*	1.53	20.00*	4.25	†	†
	Foliar phosphorus content (%)															
Application rate	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1× application	0.11	0.00	0.11	0.02	0.13	0.00	0.12	0.01	0.12	0.00	0.11	0.01	0.13	0.01	0.15	0.01
5× application	—	—	0.11	0.01	0.14	0.00	0.11	0.00	†	†	0.11	0.01	0.13	0.02	0.14	0.00
10× application	—	—	0.11	0.01	0.12	0.01	0.12	0.01	†	†	0.11	0.01	0.11	0.01	§ <sup>w</sup>	§ <sup>w</sup>
20× application	—	—	0.11	0.01	0.15	0.01	0.12	0.00	†	†	0.13	0.01	0.12	0.01	0.24*	0.05
40× application	—	—	0.12	0.01	0.18*	0.02	0.12	0.00	†	†	0.17*	0.02	0.13	0.01	†	†

<sup>‡</sup>Not applicable.

<sup>†</sup>† no plants lived.

\*\*Within a row, indicates that the treatment mean is significantly different from the control mean using Dunnett's multiple comparisons procedure ( $\alpha = 0.05$ ).

<sup>w</sup>§ inadequate leaf tissue was available for P content analysis.

for all commercial products in Experiment 2 (Table 4). In this experiment, the treatment main effect on root colonization was not significant at any application rate.

As in Experiment 1, the treatment main effect on shoot dry weight was significant for all AMF products ( $p < 0.05$ ; Table 4). Shoot dry weight increased with product application rate in a dose-dependent manner, although in several cases the highest application rates appeared to be toxic (granular products 4 and 7).

Interestingly, the extractable phosphorus content of the soil media was significantly higher when AMF products had been added ( $p < 0.05$ ; Table 4). Mineral nutrient analysis of product-amended soil revealed that phosphorus content, like shoot dry weight, increased in a dose-dependent manner with the product application rate. While only two products (4 and 7) imparted significantly greater phosphorus content at the standard (1 $\times$ ) application rate, all products significantly differed

from the control at or above the 10 $\times$  rate. Similar results were obtained for soil nitrate, potassium, calcium, and magnesium (data not shown). In three cases, foliar P concentration was significantly higher in product-treated plants than controls: granular product 7 at the 20 $\times$  rate and granular products 2 and 5 at the 40 $\times$  rate (Table 4). Although nutrient content of the raw products was not analyzed (and product labels had no fertilizer analysis), it appears that the products improved soil fertility. These results are surprising given that high soil fertility levels commonly inhibit AMF colonization (33), and incorporation of nutritional ingredients seems counterproductive to the intended function of AMF inoculants.

*Experiment 3: Positive control + additional host species.* The roots of corn and sorghum plants treated with lab-cultured AMF inoculant (positive control) had much higher levels of mycorrhizal colonization than those of nega-

**Table 5.** Mycorrhizal colonization and shoot dry weight of corn and sorghum plants treated with commercial AMF products applied at two levels of the manufacturers' labeled rate ( $n = 8$  for each product  $\times$  rate combination). Negative control was steam-pasteurized soil medium only. Positive control was a lab-cultured inoculant composed of three *Glomus* species. Standard error of the mean is shown in parentheses.

Application rate	Neg. control	Pos. control	Granular 1	Granular 2	Granular 3	Granular 4	Granular 5	Granular 6	Granular 7
<b>Corn mycorrhizal colonization (%)</b>									
1 $\times$ application	1.3 (0.5)* <sup>z</sup>	38.0 (3.8)# <sup>y</sup>	2.8 (1.3)*	1.1 (0.6)*	2.6 (0.8)*	3.3 (1.4)*	1.3 (0.5)*	1.5 (1.0)*	2.0 (1.4)*
10 $\times$ application	— <sup>x</sup>	—	2.8 (0.9)*	2.5 (0.5)*	5.7 (2.8)*	3.5 (2.4)*	1.8 (0.7)*	3.3 (1.3)*	6.7 (5.8)*
<b>Sorghum mycorrhizal colonization (%)</b>									
1 $\times$ application	2.0 (0.9)*	61.7 (6.3)#	2.8 (0.9)*	2.8 (0.9)*	4.0 (2.0)*	3.8 (1.2)*	2.0 (1.1)*	4.3 (1.8)*	4.3 (1.3)*
10 $\times$ application	—	—	7.3 (2.7)*	6.0 (1.7)*	0.9 (0.6)*	† <sup>w</sup>	9.4 (2.7)*#	7.7 (2.4)*	6.6 (2.3)*
<b>Corn shoot dry weight (g)</b>									
1 $\times$ application	0.33 (0.03)	0.40 (0.01)	0.36 (0.02)	0.36 (0.03)	0.31 (0.02)	0.48 (0.02)#	0.35 (0.02)	0.30 (0.02)	0.37 (0.02)
10 $\times$ application	—	—	0.43 (0.04)	0.36 (0.03)	0.43 (0.03)	0.40 (0.07)	0.40 (0.02)	0.37 (0.02)	0.22 (0.04)*
<b>Sorghum shoot dry weight (g)</b>									
1 $\times$ application	0.04 (0.00)	0.06 (0.01)	0.04 (0.00)	0.04 (0.01)	0.05 (0.01)	0.07 (0.00)#	0.05 (0.01)	0.05 (0.00)	0.05 (0.01)
10 $\times$ application	—	—	0.08 (0.01)#	0.07 (0.01)#	0.06 (0.00)	†	0.07 (0.01)#	0.08 (0.01)#	0.05 (0.01)

<sup>z</sup>\*Within a row, denotes significant difference from the positive control using Dunnett's multiple comparisons procedure ( $\alpha = 0.05$ ).

<sup>y</sup>#Within a row, denotes significant difference from the negative control using Dunnett's multiple comparisons procedure ( $\alpha = 0.05$ ).

<sup>x</sup>Not applicable.

<sup>w</sup>†No plants lived

tive controls and product-treated plants ( $p < 0.05$ ; Table 5). Positive control corn and sorghum roots averaged 38.0 and 61.7% AMF colonization, respectively, compared to 1.3 and 2.0% for negative control roots. Despite marked differences in AMF colonization, negative and positive controls did not differ in shoot dry weight (Table 5).

In only one case did a commercial inoculant produce greater AMF colonization than the negative control (granular product 5 on sorghum at the 10 $\times$  rate). Shoot growth response to product treatment was not as pronounced as in previous experiments. In corn, inoculation increased shoot dry weight ( $p < 0.05$ ) relative to the negative control for one out of seven products (Table 5). In sorghum, inoculation increased shoot weight for one product at the 1 $\times$  rate and four products at the 10 $\times$  rate (Table 5). Variability in shoot growth response across experiments may have been due to the practice of acquiring new product batches for each experiment.

Incorporation of multiple host species and viable lab-cultured inoculant provided evidence that poor performance of commercial inoculants was not the result of plant host-AMF incompatibility or inappropriate experimental techniques. AMF species present in the positive control inoculant were of the same genus (*Glomus*), and in many cases the same species, as those found in the commercial inoculants. It is therefore unlikely that the positive control inoculant possessed greater host compatibility than commercial inoculants. Tarbell and Koske (39) also observed marked differences in colonization between commercial AMF products and a lab-cultured inoculant. In their experiment, five of eight products produced no colonization at the manufacturer's application rate whereas the cultured inoculant produced 60% colonization.

In previous experiments, the manufacturer of our positive control inoculant found that AMF colonization of corn

treated with lab-cultured inoculant at a 1:10 v/v dilution was similar to that of corn treated with commercial products (20). However, favorable results with commercial inoculants only occurred with voluntary product samples from the manufacturers. Samples obtained through typical consumer channels produced less favorable results (Dr. Joseph Morton, personal communication).

Despite far higher levels of AMF colonization, shoots of positive control plants were not significantly larger than those of negative controls. This result contradicts the assumption that greater mycorrhizal colonization necessarily enhances plant growth. In fact, a number of previous studies refute this assumption. In particular, the work of Peng *et al.* (31) showed conclusively that mycorrhizae exact a carbon cost and can reduce plant growth under high fertility conditions.

**Experiment 4: Woody plant hosts.** Although not intentionally inoculated at the nursery, peat-plugged trident maple seedlings were already colonized by AMF upon arrival, and pre-treatment colonization averaged about 20% (Table 6). In general, treatment with granular or root dip AMF products had no effect on subsequent AMF colonization. An exception occurred at four weeks when plants treated with granular product 2 had significantly lower colonization levels than controls ( $p = 0.0234$ ; treatment main effect at four weeks).

When the maples were destructively harvested at 16 weeks, there were significant treatment effects on whole-plant fresh weight and root fresh weight ( $p = 0.0129$  and  $p = 0.0051$ , respectively). In general, treated plants were smaller than control plants; however, only maples treated with root-dip product 1 were significantly smaller than controls (data not shown).

The bare-root sweetbay magnolia seedlings had limited fine root development when they arrived from the nursery. To

**Table 6.** Mycorrhizal colonization of trident maple (*Acer buergerianum*) and sweetbay magnolia (*Magnolia virginiana*) treated with commercial AMF products and grown in steam-pasteurized soil medium. Plants were treated at the manufacturers' labeled rate and harvested at three intervals ( $n = 6$  for each product  $\times$  harvest combination). Control was steam-pasteurized soil medium only.

Harvest date	Control		Granular 2		Granular 6		Root-dip 1		Root-dip 2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>Trident maple mycorrhizal colonization (%)</b>										
Pre-treatment	22.9	7.5	15.1	6.8	18.0	4.0	21.8	9.2	20.7	6.6
Four weeks	42.7	4.9	16.9 <sup>z</sup>	4.0	31.6	3.6	28.9	5.2	24.4	7.2
Eight weeks	25.6	4.8	15.6	2.7	15.3	4.2	17.6	2.2	23.1	3.8
Sixteen weeks	20.7	4.4	15.8	2.2	22.7	2.8	15.1	3.9	16.2	3.3
<b>Sweetbay magnolia mycorrhizal colonization (%)</b>										
Pre-treatment <sup>y</sup>	—	—	—	—	—	—	—	—	—	—
Four weeks	33.6	7.1	38.1	6.7	26.7	8.3	32.2	11.3	33.9	8.3
Eight weeks	23.6	6.1	46.7	1.7	39.3	12.2	30.2	13.3	38.7	9.4
Sixteen weeks	50.4	5.9	40.5	5.6	42.7	8.2	49.8	4.0	46.4	6.4

<sup>z</sup>\*Within a row, indicates that the treatment mean is significantly different from the control mean using Dunnett's multiple comparisons procedure ( $\alpha = 0.05$ ).

<sup>y</sup>There was insufficient root length on the plants to permit sampling.

avoid further root loss, AMF colonization was not evaluated prior to treatment. All magnolia seedlings were colonized by AMF fungi when evaluated at four, eight and 16 weeks, but there were no differences between treated and control plants (Table 6).

At 16 weeks, treatment had no effect on the fresh weight or dry weight of any plant parts (data not shown).

Similar results were obtained by Appleton (1), whom, despite multiple trials and varying substrates, was unable to induce AMF colonization in seedlings of several woody plant species using commercial inoculants. However, Davies (13) successfully enhanced AMF colonization of three woody plant species using a commercial inoculant in a commercial nursery production system.

It has been suggested that pre-existing mycorrhizal colonization may reduce the benefits of commercial inoculant application (2, 15). Recent experiments have shown that an established AMF mycorrhizal symbiosis can suppress further colonization in herbaceous plants (10, 41). In the current experiment, pre-existing AMF colonization of the seedlings may have limited colonization enhancement. Increased soil fertility following product application may also have suppressed AMF colonization in treated plants (see Experiment 2).

**Experiment 5: Woody plant host + positive control.** Six weeks after treatment, sweetbay magnolias that received positive control inoculant had a mean AMF colonization of 74% (Table 7). Plants in other treatment groups developed

little or no mycorrhizal colonization, with the exception of a single plant treated with granular product 8 that possessed 94% colonization. Whether this colonization resulted from viable product or from positive-control contamination could not be assessed. There were no other significant differences among treatments.

Six weeks after treatment, AMF product affected shoot dry weight ( $p = 0.0087$ ; Table 7). However, none of the product-treated plants differed in size from negative or positive controls. The treatment effect arose from a significant difference in size between plants treated with granular products 3 and 8. Results for leaf area were similar (data not shown).

In this series of experiments, commercial AMF inoculants consistently failed to promote mycorrhizal colonization of mycotrophic host plants. It seems unlikely that individual non-infective product batches were consistently responsible for the poor results because numerous batches were purchased over the course of three years. Almost all of the commercial inoculants were purchased from retailers and had undergone shipping, handling, and storage following manufacture. It is possible that the products lost viability during this time due to inappropriate storage conditions or prolonged shelf time.

It is possible that the manufacturers' recommended application rates for the products tested were simply too low. The recommended application rate was 3.0 g-liter<sup>-1</sup> or less for the majority of granular products. Based on visual inspection, this propagule density appeared inadequate to ensure rapid root contact. When mixed with the soil medium, propagules

**Table 7.** Mycorrhizal colonization and shoot dry weight of sweetbay magnolia (*Magnolia virginiana*) treated with commercial AMF products applied at the manufacturers' labeled rate ( $n = 8$ ). Negative control was steam-pasteurized soil medium only. Positive control was a lab-cultured inoculant composed of three *Glomus* species. Standard error of the mean is shown in parentheses.

	Neg. control	Pos. control	Granular 2	Granular 3	Granular 4	Granular 5	Granular 6	Granular 7
Mycorrhizal colonization (%)	0.3 (0.3)b <sup>a</sup>	74.3 (11.1)a	0.0 (0.0)b	0.5 (0.3)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	12.0 (11.7)b
Stem + leaf dry weight (g)	0.20 (0.01)ab	0.19 (0.00)ab	0.18 (0.01)ab	0.21 (0.01)a	0.18 (0.02)ab	0.17 (0.01)ab	0.18 (0.01)ab	0.15 (0.01)a

<sup>a</sup>Within a row, values followed by different letters are significantly different (Tukey's multiple comparisons procedure;  $\alpha = 0.05$ ).



were highly dispersed. However, increasing the application rate to as much as forty times the recommended rate failed to promote colonization in these experiments.

Commercial AMF inoculants promoted growth of test plants independent of mycorrhizal colonization, suggesting that non-mycorrhizal ingredients were acting as fertilizers and/or plant growth regulators. The variety of non-mycorrhizal ingredients in commercial inoculants is quite broad, ranging from potting soil and clay granules to organic bio-stimulants and rhizosphere bacteria. In the present study, product selection was purposefully limited to those which contained a minimum of auxiliary ingredients. Nonetheless, given the wide variety of product formulations, it is not possible to determine which product component(s) were responsible for the observed growth effects.

This research also called into question one of the common rationales for AMF product application: the lack of colonization in nursery-grown plants. Woody liners purchased in this experiment were colonized when they arrived from the producer, despite not having been intentionally inoculated. However, other studies have shown that nursery-grown woody seedlings can exhibit low, erratic levels of mycorrhizal colonization (12, 29). The extent to which AMF colonization typically develops in nursery-grown woody plants is an important question for future research.

Clearly, if manufacturers of commercial AMF inoculants desire large-scale acceptance of mycorrhizal technology, they must better demonstrate that their products are compatible with current retail distribution methods and can promote mycorrhizal colonization under the conditions of their intended use. The application of viable mycorrhizal inoculant may have a place in the nursery and landscape industry. However, improved methods of production, distribution, and application are necessary before commercial AMF inoculants can be broadly recommended.

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