Arbuscular Mycorrhizal Inoculation Affects Root Development of *Acer* and *Magnolia* Species[1]

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Abstract

The effects of arbuscular mycorrhizal fungi (AMF) inoculation on fine root development of four woody landscape plants were studied during the first year after transplant. Test species included two members of obligately mycorrhizal Magnoliaceae (*Magnolia virginiana* and *Magnolia stellata*) and two members of facultatively mycorrhizal Aceraceae (*Acer* × *freemanii* and *Acer buergerianum*). Field-grown, balled and burlapped plants were treated with a commercial inoculant containing *Glomus* spp. and *Gigaspora* spp. mycorrhizal fungi and transplanted to a piedmont field site. Root architecture and demographics were evaluated *in situ* using minirhizotrons. One year after transplant, AMF colonization levels had increased in three of the four species regardless of whether they had been intentionally inoculated. AMF-treated *M. virginiana* and *A. buergerianum* had significantly lower standing root crops (total root length visible on minirhizotrons) than control plants, and a similar trend was observed in *Acer* × *freemanii*. Inoculated *M. virginiana* roots exhibited reduced branching and shorter life spans, but were less likely to develop brown pigmentation. Species-specific effects of inoculation on root longevity and browning were also observed in the maples. AMF inoculation had no effect on above ground growth or foliar nutrient concentrations. Investment of photosynthate in the growth and maintenance of AMF may represent a more efficient nutrient acquisition strategy than root proliferation, leading to lower fine root production in heavily mycorrhizal plants.

Index words: arbuscular mycorrhizal fungi, fine root demography, minirhizotron, mycorrhizal colonization, mycorrhizal inoculation.

Species used in this study: *Acer buergerianum* Miq. [trident maple], *Acer* × *freemanii* E. Murray [Freeman maple], *Magnolia stellata* (Siebold & Zucc.) Maxim. [star magnolia], *Magnolia virginiana* L. [sweetbay magnolia].

Significance to the Nursery Industry

Horticulturists constantly seek methods to improve growth and development of woody landscape plants. Arbuscular mycorrhizal fungi (AMF) are key symbionts of most of these plants. As such, considerable effort has been focused on studying and cultivating this important symbiosis. AMF effects on root development of woody landscape plants have not been studied extensively under field conditions. In this study, we used minirhizotrons to explore responses of four field-grown species to AMF inoculation. In general, inoculation depressed fine root branching, life span, production, standing crop (total root length visible on minirhizotrons), and mortality. Despite dynamic below ground responses, inoculation had no effect on above ground growth or foliar nutrition. Commercial AMF inoculants are generally marketed on the claim that they will increase root and shoot growth after transplant. Our results add to the growing body of evidence on woody landscape plants that does not support this claim. Horticulturists are urged to independently evaluate commercial products before widely adopting the practice of AMF inoculation.

Introduction

Commercial products containing propagules of arbuscular mycorrhizal fungi (AMF) are widely marketed to improve woody plant performance in landscapes. Marketing literature for such products often suggests that AMF inoculation will improve plant performance and enhance fine root growth following transplant. Nonetheless, these effects have rarely been tested experimentally (25), and AMF inoculation of woody plants under typical nursery and landscape conditions has yielded inconsistent results (2, 3, 6, 18, 19, 37, 40).

AMF form symbiotic associations with the majority of known terrestrial plant species, enhancing mineral nutrition of their hosts under conditions of reduced soil fertility (47). Evidence suggests that AMF colonization can also alter fine root production, architecture, and life span in a number of plant species (24, 32, 50). However, the direction of these effects is by no means consistent: both increased and decreased root branching has been reported in response to AMF colonization (9, 11, 29, 30, 33, 41, 42, 45, 50, 51). AMF colonization increased fine root life span in drought-stressed, field-grown *Citrus* (20), but had the opposite effect on *Populus* cuttings in a growth chamber (34).

Variability in fine root responses to AMF may reflect differences in species' dependence on the AMF symbiosis. Plants can be categorized as obligately mycorrhizal, facultatively mycorrhizal, or non-mycorrhizal (13). Obligately mycorrhizal plants represent the ancestral state and consistently possess high levels of AMF colonization, few root branching orders, minimal root hair development, and long root life spans (12). The Magnoliaceae represent one such plant family (7). Facultatively mycorrhizal plants, such as the Aceraceae, possess intermediate levels of AMF colonization that depend on soil fertility levels. Their finer and more highly branched roots can acquire adequate soil P without significant mycorrhization under high fertility conditions, yet they retain the capacity for mycorrhization in infertile soils.

In this investigation, we treated young *Magnolia* and *Acer* with AMF inoculant and assessed differences in fine root development and longevity during the first year after

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transplant. Our objectives were threefold: (1) to determine whether initial levels of AMF colonization could be increased by inoculation under field conditions, (2) to assess the effect of increased AMF colonization on root architecture and demographics, and (3) to determine whether belowground changes associated with AMF inoculation were accompanied by changes in aboveground growth. Commercial AMF inoculants are marketed to horticulturists and arborists based on the largely untested assumption that inoculants will increase mycorrhizal colonization and root growth following transplant. We hoped to provide insight into the validity of this assumption.

Materials and Methods

Research site. The investigation was conducted at the Musser Fruit Research Center located approximately 10 km (6 mi) southwest of Clemson University in the piedmont region of South Carolina (USDA Hardiness Zone 7). Average annual rainfall is approximately 1362 mm (54 in), and the typical frost-free period lasts from the first week of March through the first week of November. The experimental planting was established in a former agricultural field that had lain fallow for several years. Soil on this site was Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludult). During the experiment, soil pH averaged 5.2 ± 0.1 and Mehlich-1 extractable phosphorus concentration was low (10.0 ± 1.7 ppm).

Experiment installation. In April 2002, thirty-two plants each of Freeman maple (*Acer* × *freemanii* E. Murray), trident maple (*Acer buergerianum* Miq.), sweetbay magnolia (*Magnolia virginiana* L.), and star magnolia (*Magnolia stellata* [Siebold & Zucc.] Maxim.) were purchased from a local wholesale nursery. All plants were field-grown and procured in 51 cm (20 in) diameter burlapped root balls two weeks prior to transplanting. Maples had an average trunk caliper of 3.7 cm (1.5 in). Magnolias were multi-stemmed with an average height of 1.2 m (3.9 ft).

Plants were randomized within a complete block experimental design with four species, two mycorrhizal treatments (inoculated and non-inoculated), and eight blocks for a total of 64 experimental units. Blocks consisted of eight rows spaced 5 m (16.4 ft) apart and oriented perpendicular to the slope of the field. Within a block, plants were spaced 3.1 m (10.2 ft) apart and each experimental plant was paired with a buffer plant of like treatment, resulting in 16 plants per row.

Planting holes were dug with a 61 cm (24 in) auger to a depth of 46 cm (18 in) and manually widened to 102 cm (40 in) diameter using a shovel. A root observation tube (minirhizotron) was placed against the planting hole sidewall of each experimental plant (64 total). Minirhizotrons were oriented 30° from vertical and situated between each experimental plant and its adjacent buffer plant to prevent exposure to roots of adjacent experimental plants. Minirhizotrons were constructed of clear cellulose-acetate butyrate tubing [5 cm (2 in) inside diameter] cut to a length of 60 cm (24 in) and permanently sealed on one end with a plastic plug. Three vertical transects were etched on the exterior of each minirhizotron at 120° angles from one another. Each transect was divided into thirty-one 1.8 × 1.4 cm (0.7 × 0.5 in) windows. After removing the burlap and wire basket, the root ball was placed in the planting hole approximately 25 cm (10 in) from the minirhizotron, and both root ball and minirhizotron were covered with sieved backfill soil. The exposed portion of the minirhizotron was wrapped in black electrical tape to minimize light infiltration and capped with a white aluminum can to minimize radiant heating.

AMF-treated plants received 250 ml (8.5 fl oz) of inoculant (Bio-Organics Endomycorrhizal Inoculant, Bio-Organics, Santa Maria, CA) sprinkled over the entire surface of moistened root balls prior to placing them into their planting holes. The inoculant consisted of 50 spores/ml (1478 spores/fl oz) of seven AMF species (*Gigaspora margarita*, *Glomus brasilianum*, *G. clarum*, *G. deserticola*, *G. intraradices*, *G. monosporus*, and *G. mosseae*) suspended in an inert clay-based carrier. No additional fertilizers or biostimulant ingredients were present in the inoculant. To ensure inoculation success, AMF-treated plants were reinoculated in June 2002 by removing mulch from the root zone, sprinkling 58 ml (2 fl oz) of inoculant onto the root ball surface, irrigating, and reapplying mulch. A third AMF inoculation was performed in March 2003 by mixing the inoculant with water and making twenty 1 liter soil injections, each containing approximately 60 AMF propagules, within a 60 cm (24 in) radius around each plant. Control plants received the same manipulations as treated plants, but without inoculant application.

After transplant, a continuous 1.5 m (5 ft) wide strip of shredded hardwood mulch was applied along each row to suppress weeds, and an irrigation system consisting of drip tubing and spray emitters was installed to provide water during establishment. Two directional micro-emitters were situated on opposite sides of each plant to provide even irrigation coverage over the root zone [about 61 cm (24 in) radius]. During the first growing season, each plant received approximately 38 liter (10 gal) of water per week in absence of typical rainfall [about 2.5 cm (1 in) per week]. Total annual precipitation during the experiment was normal, measuring 1423 mm (56.03 in) in 2002 and 1415 mm (55.69 in) in 2003. April 2002 was abnormally dry (75% below 10-year average) while September 2002, December 2002, and May 2003 were abnormally wet (all 80% above 10-year average).

Data collection and analysis. Prior to planting and treatment, a fine root sample was collected from each plant for assessment of pre-transplant AMF colonization. Approximately 20 g (0.7 oz) of live roots were randomly sampled from the interior of each root ball after gently scraping away the surface soil. Fine root samples were collected again in June 2003 to assess effects of inoculant treatment on AMF colonization. Three 5 cm (2 in) diameter soil cores were collected equidistant around the perimeter of the original root ball to a depth of 15 cm (6 in). Cores were mixed together for each plant, and approximately 20 g (0.7 oz) of live roots were randomly sampled from the mixture.

All root samples were washed with distilled water, soaked in 10% KOH at 75C (167F) for six hours to clear pigments, and stained with Trypan blue at 75C (167F) for 30 minutes to reveal AMF structures (14). Mycorrhizal colonization was assessed using the magnified intersections technique (38); approximately 2 g (0.07 oz) of stained roots were randomly selected from each stained sample, mounted on slides, and assessed with a compound microscope (110 ×) equipped with a cross-hair reticle. Colonization was calculated as percentage of assessed root intersections possessing internal and/or external AMF structures. Seventy-five root intersections were randomly selected and assessed for each sample.

Pre-dawn leaf water potential was measured on all experimental plants at two-week intervals during June, July, and August 2002. An undamaged mature leaf was randomly sampled at a uniform height from the canopy of each plant and measured using a portable plant water status console (Soilmoisture Equipment Corp., Santa Barbara, CA). Maple trunk caliper and magnolia crown volume were measured immediately after transplant and again at the end of the 2002 and 2003 growing seasons. Crown volume of the multi-stemmed magnolias was estimated from measurements of crown height and width (8). Shoot elongation data were obtained for all four species at the end of the 2003 growing season by averaging terminal internode length of four randomly selected branches.

Foliar nutrient content of Freeman maples, trident maples, and sweetbay magnolias was assessed in August 2003. Approximately 20 g (0.7 oz) of young, fully expanded leaves were collected from the crown of each plant, oven-dried at 70C (158F) for 2 days, and submitted to the Clemson University Agricultural Services Laboratory for standard mineral nutrient analysis. Star magnolias possessed sparse foliage due to disease and were not sampled for nutrient analysis.

Minirhizotron observations of fine roots were made from May 2002 through June 2003 as trees established after transplant. Videotape footage was collected approximately every two weeks during the growing season and approximately every four weeks during the dormant season. A BTC-100X camera (Bartz Technology, Santa Barbara, CA) and 8 mm digital video recorder (DCR-TRV17; Sony Corporation, Tokyo, Japan) were used to record images of roots growing against the outer surface of the minirhizotrons. Numbered windows etched onto minirhizotron surfaces provided a visual reference for repeated imaging of the same roots through time. Digital still images were created from video footage using Cleaner 5.0 software (Terran Interactive, Montreal, Canada). For each videotaping session, root data were acquired from digital still images using RooTracker software (Duke University Phytotron, Durham, NC).

Individual root variables measured included root length, diameter, branching order, life span, and time to browning. Root length was measured for each root on each date that it was present. Root diameter was defined as maximum diameter exhibited by each root during its lifetime. In general, fine roots exhibited maximum diameter on the date when they first appeared and declined in diameter by as much as 50% with age. Root branching order was classified on the basis of branching patterns discernable within each minirhizotron frame. A root was classified as first order if it did not visibly originate from another root within the frame. A root originating from a first order root was classified as second order, and a root visibly originating from a second order root was classified as third order. No roots with an apparent order greater than three were observed in this experiment. Note that this classification scheme differs from that employed in Wells *et al.* (52, 53).

Root life span was defined as the number of days between the date when the root first appeared and the date when it disappeared. Roots were classified as dead when they disappeared from the minirhizotron or when they exhibited a blackened, shriveled appearance. Time to browning was defined as the number of days between the date on which the root appeared and the date on which brown pigmentation became apparent. Root pigmentation has been attributed to accumulation of condensed tannins (39) that precedes root cortical cell death (44, 48). While pigmentation is not indicative of root death *per se*, cortical cell death eliminates the site of mycorrhizal development within the root (1, 26) and is associated with reduced rates of metabolic activity (15).

Other belowground variables measured in this experiment included standing root crop, root production, and root mortality. Standing root crop was defined as the total root length observed on a minirhizotron tube on a given observation date. Root production was defined as the new root length observed on a minirhizotron on a given date that was not present on the previous date. Root mortality was defined as the root length that died and/or disappeared from a minirhizotron between two sampling dates.

All statistical analyses were performed using SAS 8.2 (SAS Institute, Cary, NC, USA). When necessary, values of dependent variables were transformed prior to analysis to satisfy normality and homogeneity of variance assumptions. The effect of AMF inoculation on aboveground growth, mycorrhizal colonization, and root architecture was assessed for each plant species using one-way analysis of variance and PROC GLM. Multiple comparisons of treatment means among plant species were performed using Tukey's HSD procedure. The effect of AMF inoculation on root standing crop, production, and mortality was assessed using repeated measures analysis of variance performed with PROC MIXED.

Cox proportional hazards regression (PROC PHREG) was used to examine relationships between fine root life span and a number of covariates including AMF treatment, root depth in the soil profile, season of root appearance, root color, root diameter, and root order. Fine root survivorship curves were also constructed using PROC PHREG. Multiple comparisons of root lifespan among plant species were performed using the TEST statement of PROC PHREG. Tests for all dependent variables were conducted at the $\alpha = 0.05$ significance level, with the exception of tree-level belowground variables, which were evaluated at the $\alpha = 0.1$ level because of the marked spatial variability of such data.

Results and Discussion

Fine root characteristics by genus. Within the non-inoculated control group, fine roots of obligately mycorrhizal magnolias tended to be thicker, less branched, and more highly AMF-colonized than those of facultatively mycorrhizal maples (Table 1). In contrast to common claims that nursery stock lack mycorrhizae, we found that AMF colonization at transplant ranged from 8.4% in Freeman maple to 48.4% in sweetbay magnolia, with sweetbay magnolia having significantly higher colonization than all other species ($P < 0.0001$). Fine root diameter ranged from 0.48 mm (0.019 in) in trident maple to 0.90 mm (0.035 in) in star magnolia, with magnolia roots having approximately twice the diameter of maple roots. More than 16% of maple fine roots belonged to higher branch orders (see Methods), whereas only 1.4% (star) and 6.0% (sweetbay) of magnolia roots were of higher order. These results are consistent with root traits of obligately (*Magnolia*) and facultatively (*Acer*) mycorrhizal genera.

Differences in root standing crop and longevity between non-inoculated magnolias and maples were less pronounced. In general, magnolias had lower maximum standing root crops than maples, but only trident maple and star magnolia

Table 1. Fine root attributes of non-AMF inoculated plants from four species. Initial AMF colonization was assessed at time of transplant. Other attributes were assessed during the first year after transplant using minirhizotrons. With exception of median life span, each value is the mean of eight replicates ± standard error.

	Initial AMF colonization %	Root diameter mm	High order roots[z] % total	Maximum standing crop[y] mm·cm^{-2}	Median life span[x] days
Maple					
Freeman maple	8.4 ± 2.2b[w]	0.51 ± 0.02b	16.2 ± 1.9a	2.7 ± 0.7ab	266b
Trident maple	11.9 ± 2.3b	0.48 ± 0.03b	17.0 ± 3.3a	3.6 ± 0.6a	315a
Magnolia					
Sweetbay magnolia	48.4 ± 5.2a	0.81 ± 0.04a	6.0 ± 1.5b	2.3 ± 0.9ab	301a
Star magnolia	19.7 ± 4.0b	0.90 ± 0.19a	1.4 ± 0.7b	1.0 ± 0.4b	230b

[z]Percent of roots classified as second or third order (see Methods).

[y]Maximum root length observed per cm^2 of minirhizotron viewing area.

[x]Median life span derived from survival probabilities calculated using Cox proportional hazards regression; means separations performed using TEST statement of PROC PHREG ($\alpha = 0.1$).

[w]Values followed by different letters within a column are significantly different using Tukey's HSD procedure ($\alpha = 0.1$).

differed significantly ($P = 0.012$) from one another (Table 1). Median root life spans ranged from 230 days (star magnolia) to 315 days (trident maple), but there was no clear relationship between life span and genus. Data from the star magnolias must be interpreted cautiously since these plants suffered extensive, prolonged defoliation because of transplant stress and powdery mildew infection. Because they were acutely stressed throughout much of the experiment, only limited data on the star magnolias are presented here.

Objective 1 — increasing AMF colonization of field-grown plants. All plants were colonized by AMF upon arrival from the nursery; plants assigned to the control and inoculated groups did not differ in pre-transplant colonization levels (data not shown). One year after transplant, colonization levels had increased in all species (except star magnolia) regardless of whether they had been intentionally inoculated (Fig. 1). Despite inoculating the AMF-treated plants on three separate occasions, inoculation had only a modest effect on AMF colonization. Only in sweetbay magnolia was the difference between control and AMF-treated plants significant ($P = 0.038$). Post-treatment colonization was measured only once (June 2003), and it is possible that significant differences in AMF colonization between treated and control maples existed at earlier time points.

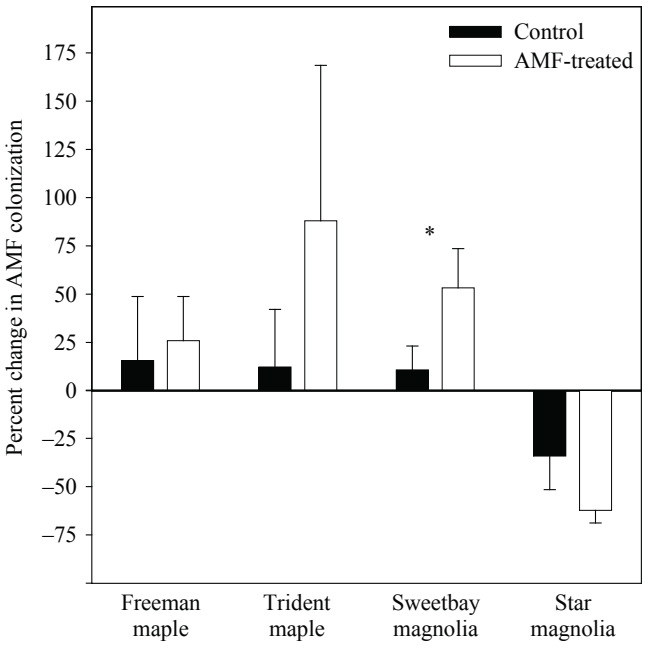

Fig. 1. Percent change in AMF colonization of four woody plant species one year after transplant. Each value is the mean of eight replicates. Error bars depict standard error of mean. Within a species, asterisk (*) denotes significant difference in treatment means ($\alpha = 0.1$).

Objective 2 — root responses to AMF inoculation. Balled-and-burlapped plants lose a large fraction of their fine root length at transplant; we were therefore interested in quantifying the extent of fine root re-growth in control and AMF-treated plants. We also measured how long new roots lived, extent to which they branched, and how much time elapsed before they underwent cortical browning and senescence.

During the first year after transplant, AMF-treated sweetbay magnolias had significantly lower standing root crop than controls on 12 out of 17 sampling dates (Fig. 2). Standing crop of inoculated sweetbays averaged 0.24 mm·cm^{-2} (0.06 in·in^{-2}), whereas that of control sweetbays averaged 1.49 mm·cm^{-2} (0.38 in·in^{-2}) — approximately a six-fold difference. AMF-treated trident maples had significantly lower standing root crop than control trident maples on 7 out of 17 sampling dates (Fig. 2), with inoculated plants averaging 1.87 mm·cm^{-2} (0.47 in·in^{-2}) and controls averaging 2.71 mm·cm^{-2} (0.69 in·in^{-2}). There were no significant treatment-related differences in standing root crop in Freeman maple or star magnolia.

AMF inoculation delayed post-transplant production of fine roots in sweetbay magnolia (Fig. 3). Control sweetbays began producing large amounts of fine roots immediately after transplant, but AMF-treated sweetbays produced no measurable fine roots until late summer. Control sweetbays also produced more fine roots on several dates in the fall, winter, and subsequent spring. Root mortality was significantly greater in control sweetbays on four sampling dates (Fig. 3) — a result that likely reflected larger amounts of root length present in controls. Similar trends in fine root production and mortality were observed in trident maple (Fig. 3), although differences in root production and mortality were significant on only one sampling date. There were no significant

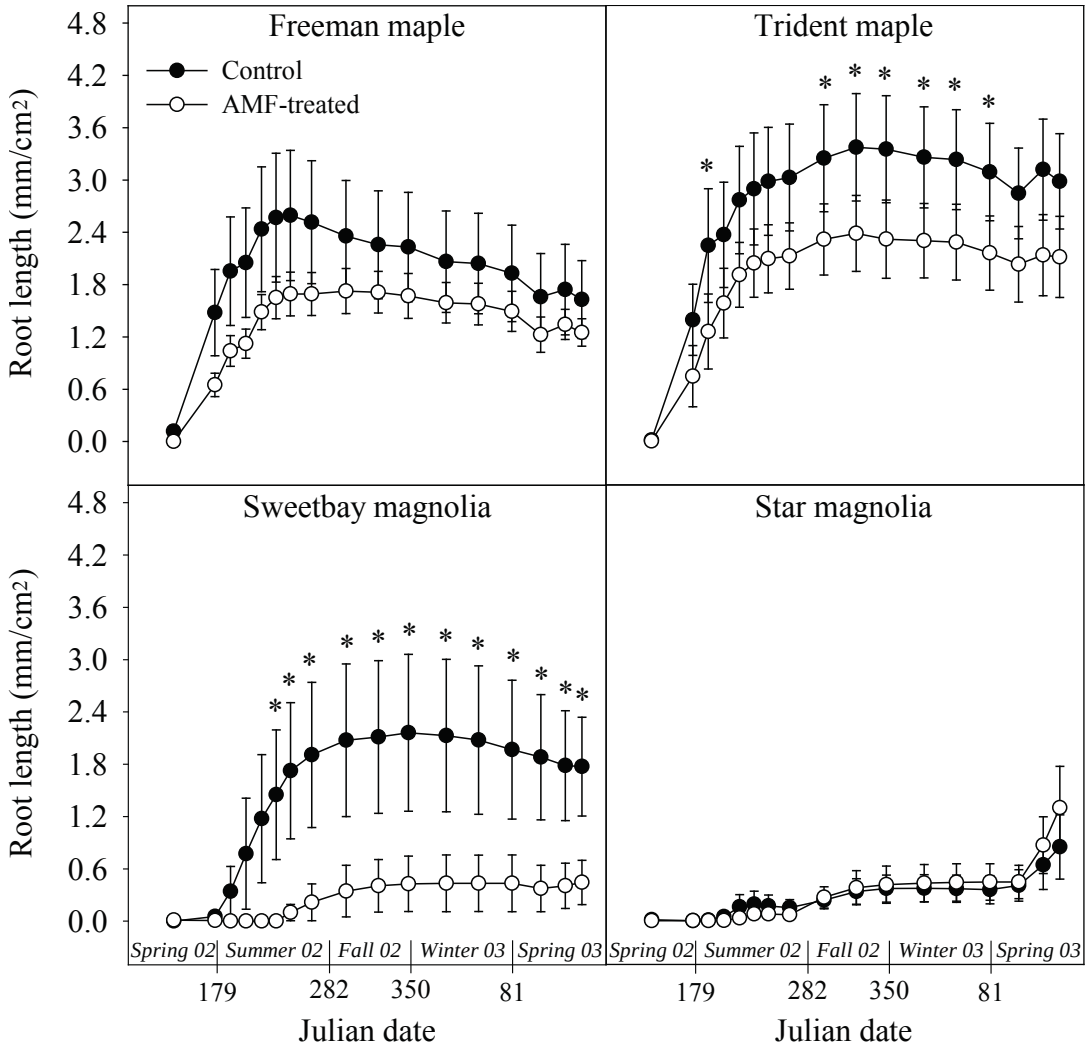

Fig. 2. Standing root crop (total root length visible on minirhizotrons) of four woody plant species during the first year after transplant. AMF inoculant was applied to half of the replicates on three dates (dashed vertical lines in fourth panel). Each value is the mean of eight replicates. Error bars depict standard error of mean. Within a sampling date, asterisk (*) denotes significant difference in treatment means (α = 0.1).

treatment-related differences in root production and mortality in Freeman maple or star magnolia (data not shown).

Our results contrast with previous work in which AMF-inoculated tree seedlings in sterilized soil produced greater root biomass than non-mycorrhizal controls (17, 35, 36, 46). However, these experiments compared mycorrhizal and non-mycorrhizal seedlings. Greater root growth simply reflected improved phosphorus nutrition and greater overall growth in mycorrhizal plants. Conditions in the present study were qualitatively different. Trees of identical size and phosphorus nutrition were compared at two levels of mycorrhization under field conditions. These conditions accurately simulate a typical landscape scenario and remove potentially confounding effects of plant size and nutrition.

Reduced root growth in response to AMF inoculation has been observed in experiments where nutritional differences between mycorrhizal and non-mycorrhizal plants were minimized. For example, Gavito *et al.* (24) reported that mycorrhizal *Pisum sativum* plants exhibited lower standing root crop and production than non-mycorrhizal plants. Reduced root:shoot ratios in mycorrhizal plants have been reported in a number of other species (9, 11, 16). It has been suggested that an energetic investment in AMF hyphae is a more efficient nutrient acquisition strategy than root proliferation (7, 22), which may explain lower root production in heavily colonized plants.

AMF inoculation significantly affected fine root life span of sweetbay magnolia (P = 0.003) and Freeman maple (P = 0.016), but not trident maple (Table 2). In sweetbay, median root life span of AMF-treated plants was 243 days, compared to 301 days in control plants (Fig. 4). In Freeman maple, AMF treatment had a non-proportional effect on root life span (time × treatment interaction). Although median life spans were quite similar (261 days for AMF-treated vs. 266 days for control), roots of AMF-treated plants were at significantly greater risk of mortality beyond age 250 days (Fig. 4). Previous reports cite increased (4, 20), decreased (34), or unchanged (4, 31) root life span in response to AMF inoculation. Effects of mycorrhizal colonization on root longevity clearly vary with plant species and experimental design, and it is likely that the degree of mycorrhization and species of fungal symbiont are also relevant. Bearing in mind that the number of studies is small, shorter root life spans have been observed in three out of five AMF-inoculated woody

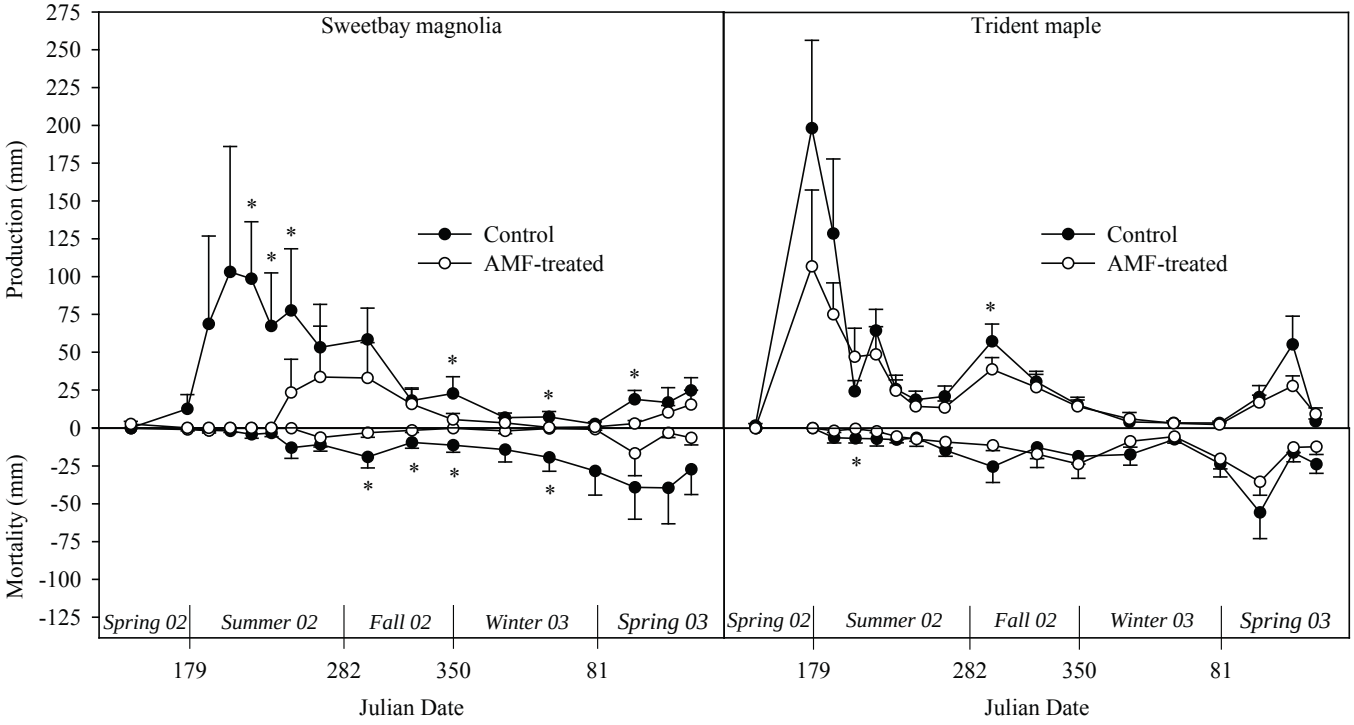

Fig. 3. Fine root production and mortality of two woody plant species during the first year after transplant. AMF inoculant was applied to half of the replicates on three dates (dashed vertical lines). Each value is the mean of eight replicates. Error bars depict standard error of mean. Within a sampling date, asterisk (*) denotes significant difference in treatment means (α = 0.1)

species studied (present work, 34) while increased root life span has been observed in one, i.e. mycorrhizal citrus roots under drought conditions (20).

Mycorrhizal inoculation delayed development of fine root pigmentation in sweetbay magnolia and trident maple, but had the opposite effect on Freeman maple (Table 2). Root pigmentation has been attributed to accumulation of condensed tannins (39) that precedes root cortical cell death (44, 48). The cortex is the site of mycorrhizal development within roots and senescence of this tissue likely precludes continued colonization of the root segment (1, 26). It may be advantageous for AMF to delay root browning in order

Table 2. Results of Cox proportional hazards regression analysis of effects of AMF treatment, root diameter, and depth in soil on fine root life span and time to browning in three woody plant species during the first year after transplant.

	Variable	DF	Parameter estimate	SE	Chi-square	*P*-value	Hazard ratio
Sweetbay magnolia							
Lifespan	AMF treatment	1	0.651	0.221	8.70	0.0032	1.918
	Root diameter	1	–1.503	0.293	26.40	0.0001	0.222
	Depth in soil	1	–0.078	0.154	0.25	0.6141	0.925
Time to browning	AMF treatment	1	–0.941	0.187	25.39	0.0001	0.390
	Root diameter	1	–1.614	0.225	51.64	0.0001	0.199
	Depth in soil	1	–0.069	0.122	0.32	0.5703	0.933
Trident maple	AMF treatment	1	–0.042	0.091	0.21	0.6460	0.959
Lifespan	Root diameter	1	–1.408	0.218	41.88	0.0001	0.245
	Depth in soil	1	–0.163	0.093	3.09	0.0783	0.849
Time to browning	AMF treatment	1	–0.160	0.092	3.01	0.0826	0.852
	Root diameter	1	–1.792	0.229	61.38	0.0001	0.167
	Depth in soil	1	–0.075	0.090	0.69	0.4052	0.928
Freeman maple							
Lifespan	AMF treatment	1	–0.217	0.090	5.79	0.0161	0.805
	Root diameter	1	–1.481	0.186	63.50	0.0001	0.227
	Depth in soil	1	–0.078	0.085	0.84	0.3591	0.925
Time to browning	AMF treatment	1	0.289	0.076	14.33	0.0002	1.335
	Root diameter	1	–0.378	0.136	7.74	0.0054	0.685
	Depth in soil	1	–0.287	0.073	15.31	0.0001	0.751

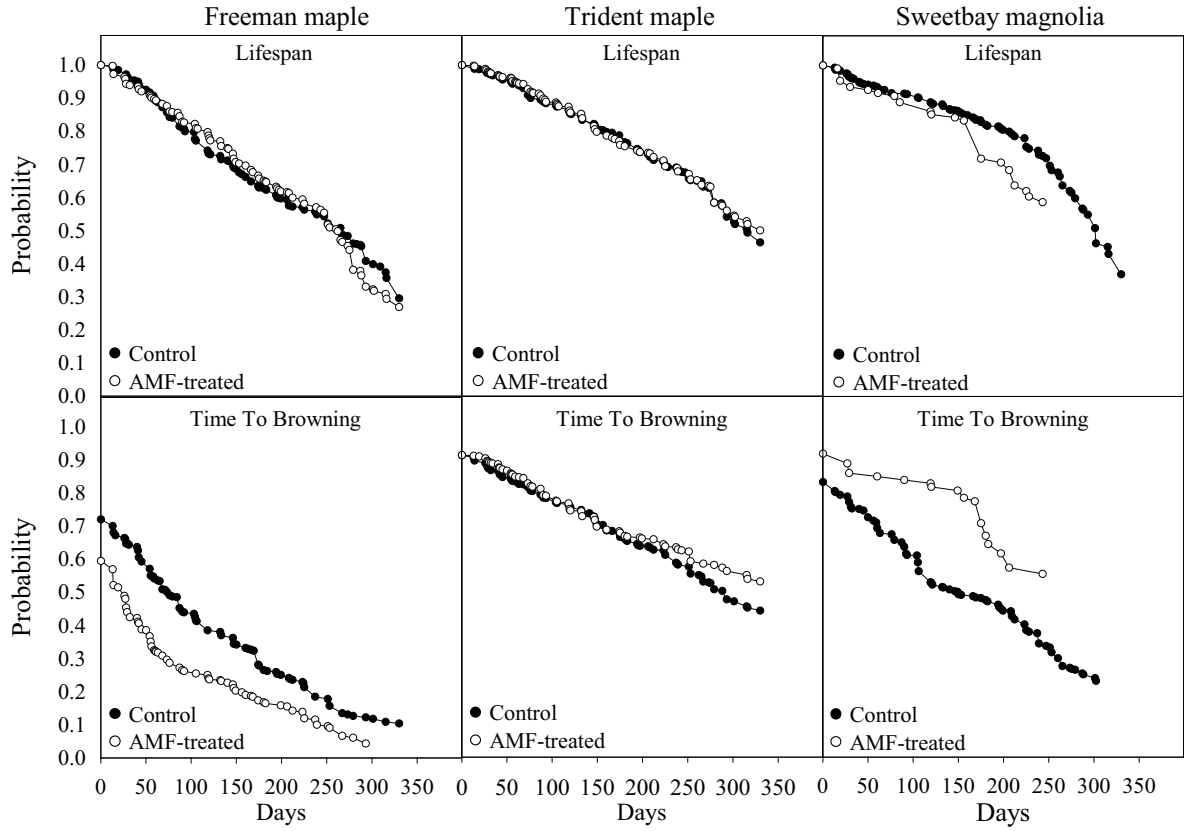

Fig. 4. Fine root survivorship and time to browning in three woody plant species during the first year after transplant. Survivorship curves were derived using Cox proportional hazards regression.

to extend the life span of the symbiosis, if not the life span of the root itself.

In all species, larger diameter roots lived longer and remained white longer than smaller diameter roots ($P < 0.01$; Table 2). Depth in the soil did not affect root life span or browning in sweetbay magnolia. However, roots deeper than 23 cm (9 in) lived longer in trident maple ($P = 0.078$) and remained white longer in Freeman maple ($P = 0.0001$).

AMF inoculation had no effect on root branching in Freeman maple and trident maple, but significantly reduced branching in sweetbay magnolia ($P = 0.047$; Fig. 5). This result was unexpected because most past research has shown increased root branching as a result of AMF inoculation (11, 33, 50). However, reduced branching in response to mycorrhizal inoculation has been noted in several species (29, 30, 42). AMF inoculation was shown to inhibite root branching in mycorrhizal-dependent warm season grasses, but had no effect on less mycorrhizal-dependent cool-season grasses — a result similar to that presented here. In the present study, root production and standing crop were greatly reduced in inoculated sweetbay magnolia. Because fine root systems increase in size through branching, reduced branching in inoculated sweetbay may have simply reflected a much smaller root system size. Alternately, changes in root morphology may arise from changes in plant growth regulator synthesis in colonized roots and/or synthesis of plant growth regulators by fungal symbionts themselves (5, 10).

Objective 3 — above ground responses to AMF inoculation. Three months after transplant, AMF treatment had no significant effect on foliar concentrations of phosphorus

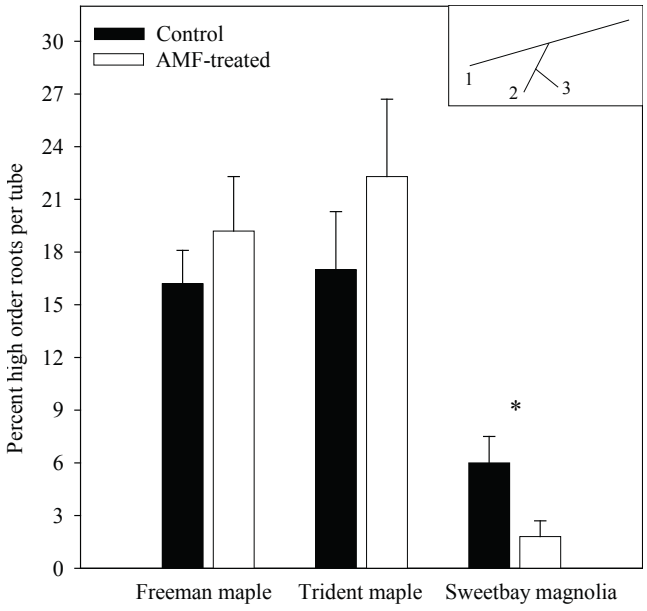

Fig. 5. Percent high order roots produced by three woody plant species during the first year after transplant. Fine root populations were assessed using minirhizotrons from May 2002 to June 2003. Each value was calculated by summing the number of roots classified as orders two and three (see inset), dividing by total number of roots, and multiplying by 100. Each value is the mean of eight replicates. Error bars depict standard error of mean. Within a species, asterisk (*) denotes significant difference in treatment means ($\alpha = 0.1$).

Table 3. Foliar nutrient concentrations of three AMF-treated woody plant species three months after transplant. Each value is the mean of eight replicates ± standard error.

	Freeman maple			Trident maple			Sweetbay magnolia		
	Control	AMF-treated[z]	*P*-value[y]	Control	AMF-treated	*P*-value	Control	AMF-treated	*P*-value
N (%)	2.31 ± 0.06	2.39 ± 0.04	0.3181	2.43 ± 0.06	2.48 ± 0.07	0.6235	2.12 ± 0.12	2.15 ± 0.12	0.8421
P (%)	0.22 ± 0.03	0.18 ± 0.01	0.2480	0.15 ± 0.01	0.14 ± 0.01	0.4044	0.14 ± 0.01	0.14 ± 0.02	0.7522
K (%)	0.98 ± 0.04	0.95 ± 0.08	0.7516	0.98 ± 0.08	1.11 ± 0.10	0.3413	0.62 ± 0.09	0.68 ± 0.16	0.7186
S (%)	0.18 ± 0.01	0.18 ± 0.01	0.9154	0.19 ± 0.01	0.19 ± 0.01	0.6927	0.17 ± 0.01	0.18 ± 0.01	0.5219
Ca (%)	0.80 ± 0.04	0.72 ± 0.05	0.2481	0.88 ± 0.03	0.85 ± 0.06	0.6968	0.82 ± 0.04	0.76 ± 0.07	0.4446
Mg (%)	0.29 ± 0.02	0.29 ± 0.03	0.8467	0.14 ± 0.01	0.15 ± 0.01	0.6680	0.26 ± 0.01	0.27 ± 0.02	0.5110

[z]Plants were inoculated with arbuscular mycorrhizal fungi at transplant, one month after transplant, and one year after transplant.

[y]P-values calculated with two-sample t-tests comparing control and AMF treatment groups within each species.

Table 4. Size and growth of three AMF-treated woody plant species at transplant and following two growing seasons. Each value is the mean of eight replicates ± standard error.

	Pre-treatment			2002 Growing season			2003 Growing season		
	Control	AMF-treated[z]	*P*-value[y]	Control	AMF-treated	*P*-value	Control	AMF-treated	*P*-value
Freeman maple									
Stem diam. (mm)	33.1 ± 1.0	33.5 ± 0.7	0.7979	40.0 ± 1.4	40.0 ± 0.9	0.9793	68.8 ± 3.8	74.5 ± 3.0	0.3354
Internode[x] (cm)	—	—	—	—	—	—	60.3 ± 7.7	90.3 ± 9.5	0.0671
Trident maple									
Stem diam. (mm)	39.6 ± 1.3	41.3 ± 1.9	0.4722	47.7 ± 1.7	48.3 ± 2.3	0.8338	79.7 ± 6.9	87.2 ± 4.9	0.4600
Internode (cm)	—	—	—	—	—	—	92.8 ± 7.9	113.7 ± 14.4	0.2695
Sweetbay magnolia									
Crown vol. (m^3)	4.7 ± 0.5	3.8 ± 0.5	0.2255	6.2 ± 0.8	4.5 ± 0.8	0.1690	10.3 ± 1.9	8.0 ± 2.1	0.4307
Internode (cm)	—	—	—	—	—	—	32.4 ± 10.6	23.6 ± 4.8	0.4759

[z]Plants were inoculated with arbuscular mycorrhizal fungi at transplant, one month after transplant, and one year after transplant.

[y]P-values calculated with two-sample t-test comparing control and AMF treatment groups within each species.

[x]Internode measurements were not taken prior to treatment or after 2002 growing season.

or any other nutrient in Freeman maple and trident maple (Table 3). This was expected since AMF inoculation failed to increase mycorrhizal colonization in these species. However, no differences were observed in sweetbay magnolia foliar nutrients either, despite a significant increase in colonization of AMF-treated plants. Other researchers have noted a lack of improvement in woody plant foliar nutrition despite successful AMF inoculation (27, 49). These results may suggest that a minimum colonization threshold exists, below which nutritional benefits are negligible.

There were no significant differences in initial plant size between treatment groups, and AMF inoculation had no significant effect on subsequent growth in any species (Table 4). AMF-treated Freeman maple did show a trend towards greater shoot elongation in the 2003 growing season, although the difference was not significant ($P = 0.067$; Table 4). Bi-weekly pre-dawn water potential measurements showed no differences in water status between treated and control trees during the summer after transplant (data not shown). In similar studies, mycorrhizal inoculation has also failed to provide growth benefits to landscape trees (3, 21, 23, 25, 37), despite being used successfully in reforestation applications (28, 43, 46).

Commercial AMF inoculants are generally marketed based on assumptions that they will increase root and shoot growth after transplant. In the present experiment, inoculation tended to decrease the former and had no effect on the latter. Inoculation effects were most pronounced in obligately mycorrhizal sweetbay magnolia and much more subtle in the two facultatively mycorrhizal maple species. Our results differ qualitatively from those obtained with non-mycorrhizal seedlings evaluated in sterilized soils, underscoring the importance of field trials with typical nursery stock. Clearly, additional research is needed to determine exactly how site conditions, inoculant content, and host species interact to influence root growth and plant performance.

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