STIMULATION OF GREEN ASH GROWTH BY GLOMUS ETUNICATUM AND PHOSPHORUS FERTILIZATION[1]

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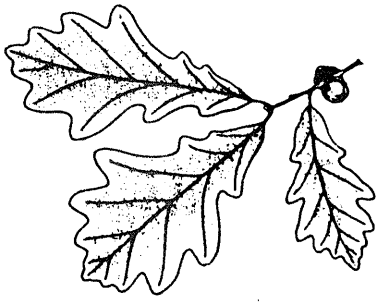

ABSTRACT.--This study examined the growth and internal phosphorus (P) concentration of mycorrhizal and nonmycorrhizal green ash (Fraxinus pennyslvanica) supplied with four levels of P. Green ash were grown for 10 weeks with and without Glomus etunicatum in washed sand. Seedlings grew significantly more in response to both P fertilization and mycorrhizal colonization. At all levels of P fertilization, inoculated seedlings were larger than noninoculated seedlings. Most of the increased growth in mycorrhizal plants could be explained by improved P nutrition. The data suggests the potential for using mycorrhizal inoculation with G. etunicatum in place of, or in conjunction with, P fertilization as an effective means of improving nursery production of green ash.

INTRODUCTION

When commercially valuable hardwood tree species form vesicular-abruscular (V-A) endomycorrhizal associations with members of the genus Glomus, improved growth results (Pope, et al., 1983; Schultz, et al. 1981; Kormanik, et al., 1982; and Ponder, 1984). The importance of vesicular-arbuscular mycorrhizae in promoting seedling establishment and growth potential has been recognized in recent years. Relatively little is known, however, about the physiology of hardwood mycorrhizae, despite the abundance of literature characterizing the improved growth, nutrient uptake and water uptake of VA mycorrhizal grasses and agronomic species (Hayman, 1983). The potential for replacing phosphorus (P) fertilization with mycorrhizal fungi has been investigated in Citrus by Menge, et al. (1978). They found that inoculation with Glomus fasciculatum effectively substituted for 56 and 278 ppm P fertilization of Troyer citrange and Brazilian sour orange, respectively. Phorphorous fertilization of mycorrhizal green ash (Fraxinus pennsylvanica Marsh.) has produced variable results, depending on the fungal species and experimental conditions employed (Schultz et al., 1981; Egel and Pope, 1982). Vesicular-arbuscular mycorrhizal plant associations frequently result in improved plant nutrition regardless of the amount of fertilizer applied, (Stribley, et al., 1980), making physiological evaluation of the benefits of mycorrhizal associations difficult to analyze.

The objectives of this study were two fold: 1) examine the specific growth responses of green ash seedlings to P fertilization following inoculation with G. etunicatum; 2) and determine whether addition of moderate levels of P would be sufficient to eliminate differences in growth and tissue P concentrations between V-A mycorrhizal and nonmycorrhizal plants.

MATERIALS AND METHODS

Green ash seeds (Fraxinus pennsylvanica lanceolota, F.W. Schumacher Co., Sandwich, Mass.) were surface sterilized for 2 min in 0.5% NaOCl, thoroughly rinsed with deionized water, and germinated in moist vermiculite. Washed sand (pH 7.1, 150 ppm 2N HCl extractable phosphorus, 2.2 ppm available P) was sterilized with methyl bromide and ventilated for 3 days prior to use. Approximately 2.8 kg of sterilized sand was added

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to 15 x 15 cm sterilized plastic pots. One-half of the pots received 300 spores per pot of G. etunicatum (Native Plants Inc., 417 Wakara Way, Salt Lake City, Utah 84108), added at 5 and 10 cm below the surface of the sand. Spores were collected from axenic cultures of endomycorrhizal sudan grass (Sorghum spp.). Inoculum filtrate ($\leq$45 uM) was added to nonmycorrhizal pots to standardize soil microflora. Following radical emergence, three seeds were carefully transplanted to each pot.

Following a two week establishment period, seedlings were thinned to 1 per pot and fertilization treatments were implemented. Four levels of (P) fertilization were used: level 0: no phosphorous was added during the experiment; level 1: 1.55 mg P as KH_2PO_4 added to each pot weekly from week 2 through 4 and 7 through 9 (9.3 mg total); level 2: 4.65 mg P added to each pot weekly from week 2-4 and 7-9 (27.9 mg total); and level 3: 9.29 mg P added to each pot weekly from week 2 through week 9 (74.3 mg total). Phosphorous was not added in levels 1 and 2 during weeks 5 and 6 in order to prevent excess P from accumulating in these soils. The experiment was conducted as a 2x4 factorial (4 levels of applied P and 2 levels of mycorrhizal inoculation) with 7 replications per treatment. Phosphorus was added in combination with 200 ml 1/2X modified Hoaglands solution (Hoagland and Arnon, 1950, minus P) at concentrations of 0.25, 0.75, and 1.5 mM KH_2PO_4 for levels 1, 2, and 3, respectively. Hoaglands solution was added to all pots weekly from week 2 to week 9 regardless of the level of P. Seedlings received 200 ml deionized water once each week, midway between fertilizer applications.

Pots were randomly arranged in a growth chamber with a 16 hr photoperiod and 25/20 ± 0.2 C day/night temperatures. Photon flux density approximated 400 uEinsteins of photosynthetically active radiation.

Height growth was measured weekly (Table 1). Four seedlings were harvested from each treatment after 10 weeks and plant growth variables were analyzed. A subsample of each root system was weighed and stored in FAA (formalin, alcohol and acetic acid). The remainder of the root system was weighed, dried for 24 hr at 70 C and reweighed. Total root dry weight was calculated, based on the percent water content found in the dried portion. Leaf area and number, stem and leaf dry weights, and diameter at the root collar were also recorded.

Lateral root subsamples were removed from FAA, stained according to the methods of Phillips and Hayman (1970), and examined under the dissecting microscope to confirm the presence of VA mycorrhizal colonization. Colonization was quantified according to the methods of Bildusas (1984). Root subsamples were divided along each segment and scored 0 or 1 based on the presence of fungal structures. Each seedling was then assigned a score: 0=no infection on any of the root segment subsamples; 1=less than 25% of root segment subsamples infected; 2=between 25-50% of root segment subsamples infected; 3=between 50-75% of root segment subsamples infected; and 4-greater than 75% of the segment subsamples infected.

Leaf and root phosphorus concentrations were analyzed colorimetrically using the methods of John (1970).

Analysis of variance and Duncan's multiple-range test were used to test statistical significance between individual treatment means (p=0.05).

RESULTS

Phosphorus fertilization resulted in increased seedling height after 5 weeks, and by week 6 both P fertilization and mycorrhizal colonization by G. etunicatum stimulated a significant shoot growth response (Table 1). Differences in height between mycorrhizal and nonmycorrhizal treatments were most pronounced at week 10. From week 8 until harvest, P fertilization at all levels was insufficient to eliminate height differences between mycorrhizal treatments (Table 1).

Leaf and root dry weights, leaf areas and stem diameters were increased by fungal inoculation at all levels of P, but the differences between mycorrhizal treatments within a P level were not always significant (Table 2). Phosphorus fertilization also stimulated all measures of plant growth, especially leaf area and root dry weight. Growth responses to inoculation were most pronounced in levels 1 and 2 P fertilization. After 10 weeks, there were no significant differences between mycorrhizal and noninoculated plants at the highest level of P fertilization. Levels of P fertilization used in this experiment had no apparent inhibitory effect on VA mycorrhizal colonization (Table 2); average ratings ranged from 1.5 to 2.0.

Root-shoot ratios in noninoculated plants were approximately two times those of mycorrhizal plants except at the highest level of P fertilization where root-shoot ratios did not differ significantly between mycorrhizal treatments (Figure 1). Phosphorous fertilization did not influence root-shoot ratios of inoculated plants. Root-shoot ratios were closely related to root P concentrations in both mycorrhizal and noninoculated plants (Figure 2).

Phosphorous concentrations in leaves and particularly in roots were increased by mycorrhizal colonization (Table 3). In root tissue, P concentrations in mycorrhizal seedlings were significantly greater than in noninoculated seedlings at fertilization levels 2 and 3. Foliar concentrations of P were similar between inoculation treatments at high levels of P fertilization.

Root dry weight increased as phosphorus concentrations in roots increased (Figure 3). The response curve was similar for both mycorrhizal and noninoculated seedlings, suggesting that root dry weight is primarily a function of internal

TABLE 1.--Mean weekly height (cm) from week 4 to week 10 of mycorrhizal (M) (G. etunicatum) and nonmycorrhizal (NM) green ash seedlings grown at 4 levels of P. fertilization. Each value is the mean of 7 seedlings.

Level of P Fert.	WEEK 4		5		6		7		8		9		10	
	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM
0	8.2	7.8	9.0	8.2	10.0 abc	8.5 a	11.9 bc	8.6 a	12.7 b	8.6 a	14.1 c	8.7 a	14.7 c	8.8 a
1	7.8	7.8	9.0	8.3	11.0 bc	8.7 a	13.6 cd	8.9 a	16.0 c	9.0 a	18.7 d	9.1 ab	20.4 d	9.3 ab
2	8.8	7.7	10.6	8.9	13.0 d	9.6 ab	16.0 e	10.6 ab	18.8 d	11.0 ab	21.8 de	12.0 bc	23.5 d	12.6 bc
3	8.1	8.6	9.2	9.9	11.6 cd	10.9 bc	15.3 de	13.2 cd	18.9 d	16.2 c	23.6 e	19.9 d	27.2 e	22.1 d

* Values followed by the same letter within a week are not significantly different (p=0.05) using Duncan's multiple-range test.

TABLE 2.--Mean root and leaf dry weights, leaf area, stem diameter and infection rating for mycorrhizal (M) (G. etunicatum) and nonmycorrhizal (NM) seedlings grown for 10 weeks at 4 levels of P fertilization. Each value is the mean of 4 seedlings.

Total P Added mg/pot	Root Dry Weight (g)		Leaf Dry Weight (g)		Leaf Area (cm^2)		Stem Diameter (mm)		Infection Rating	
	M	NM	M	NM	M	NM	M	NM	M	NM
0.0	0.141 ab	0.062 a	0.183 ab	0.025 a	50.68 ab	8.00 a	1.87 bc	1.29 a	1.5	0.0
9.3	0.240 bc	0.059 a	0.353 bc	0.032 a	100.40 bc	12.10 a	2.42 d	1.44 ab	2.0	0.0
27.9	0.254 bc	0.115 a	0.402 c	0.078 a	119.35 c	24.28 a	2.33 cd	1.60 ab	1.5	0.0
74.3	0.325 c	0.255 bc	0.575 c	0.417 c	150.13 c	108.90 c	2.28 cd	2.26 cd	1.8	0.0

* Values followed by the same letter within a plant variable are not significantly different (p=0.05) using Duncan's multiple-range test.

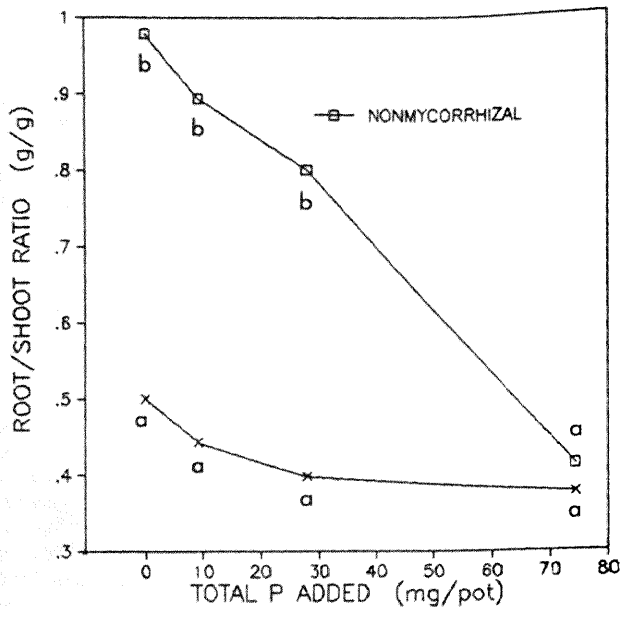

FIGURE 1.--Root/shoot ratios of mycorrhizal and nonmycorrhizal green ash seedlings grown for 10 weeks at 4 levels of P fertilization. Each point represents the mean of 4 seedlings.

* Points followed by the same letter are not significantly different (p=0.05) using Duncan's multiple range test.

TABLE 3.--Mean leaf and root P concentrations (% oven dry weight) of mycorrhizal (M) and nonmycorrhizal (NM) seedlings grown at 4 levels of P fertilization for 10 weeks. Each value represents the mean of 4 seedlings.

Total P Added (mg/pot)	Leaf P (%ODW)		Root P (%ODW)	
	M	NM	M	NM
0	0.061 ab	0.048 a	0.091 ab	0.056 a
9.3	0.083 c	0.050 a	0.117 abc	0.052 a
27.9	0.087 cd	0.074 bc	0.146 bc	0.070 a
74.3	0.112 e	0.102 de	0.267 d	0.167 c

* Values followed by the same letter within each variable are not significantly different (p=0.05) using Duncan's multiple-range test.

root P concentration. Concentrations of P in the foliage also increased as total leaf weight increased; nonmycorrhizal seedlings, however, had higher P concentrations than inoculated seedlings per unit dry weight (Figure 4).

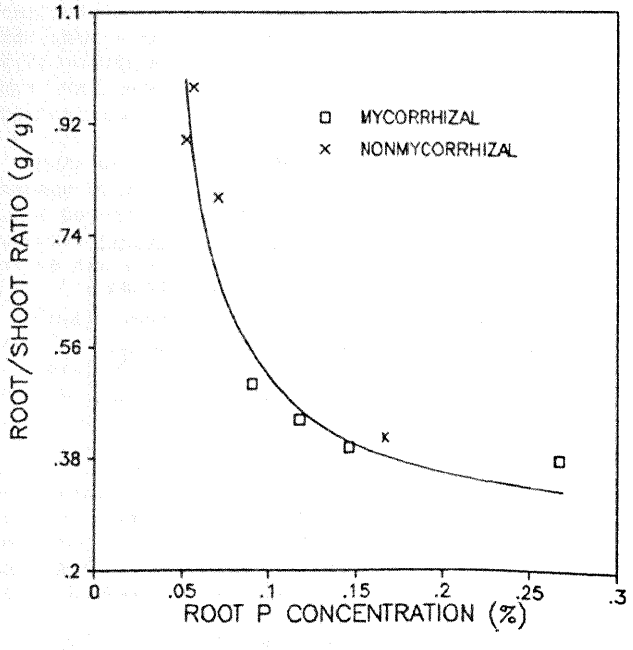

FIGURE 2.--Relationship between root P concentration (% oven dry weight) and root/shoot ratio in 10 week old seedlings. Each point represents the mean of 4 seedlings. The curve is hand fitted.

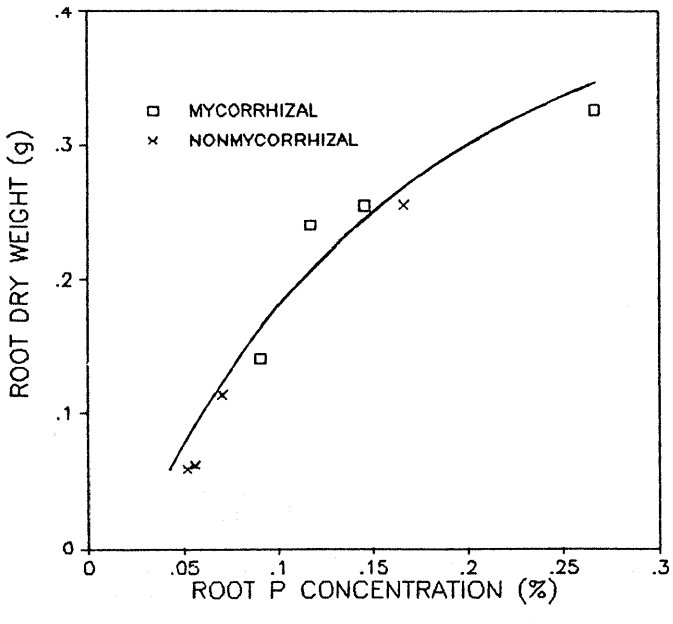

FIGURE 3.--Root phosphorus concentration (% oven dry weight) versus total root dry weight. Each point represents the mean of four seedlings. The curve is hand fitted.

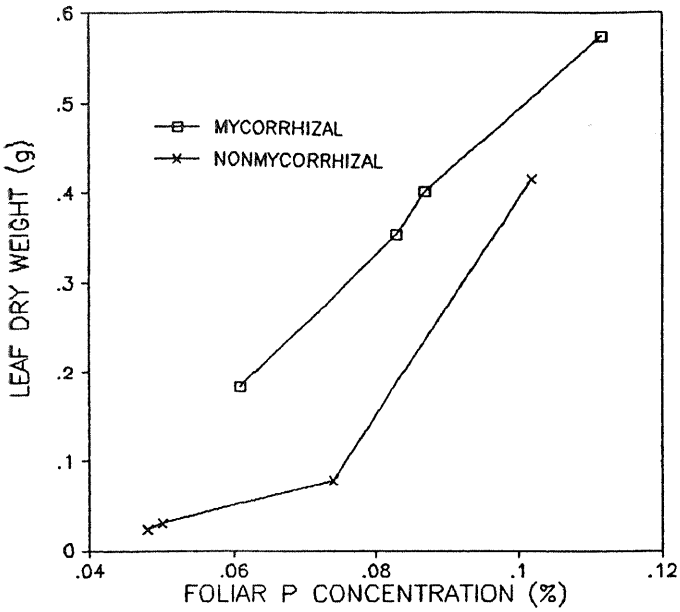

TABLE 4.--Leaf phosphorus (% oven dry weight) versus total leaf dry weight. Each point is the mean of four seedlings.

DISCUSSION

The seedlings colonized by G. etunicatum were larger by every measure (Table 2). Such promotion of hardwood seedling growth following endomycorrhizal colonization is well documented for green ash and other hardwood species (Ponder, 1984; Pope, 1980; Pope et al., 1983; and Kormanik, et al., 1982). The response of green ash seedlings to V-A mycorrhizal colonization has varied with the fungal species. Pope et al. (1983) found that G. macrocarpum stimulated green ash growth more than G. mosseae, G. fasciculatum, G. etunicatum, or G. epigaeum. Kormanik et al. (1982) found only slight differences in green ash growth between seedlings inoculated with G. fasciculatum and those inoculated with a mixture of G. mosseae and G. etunicatum.

The difference in height between mycorrhizal and noninoculated seedlings was first significant 6 weeks after planting (Table 1). Douds (1984) found a significant increase in green ash shoot weights after 5 weeks in soil inoculated with G. macrocarpum. In other plant species, temperature, moisture and nutrient availability influence timing (Hayman, 1983). Onions (Allium sp.) can show growth responses as early as 2 weeks after inoculation (Hayman, 1983). In the present experiment, fertilization with P stimulated growth of both mycorrhizal and nonmycorrhizal plants. These results differ considerably from those of Schultz, et al. (1981), who found decreased root, stem and leaf dry weights in mycorrhizal green ash (mixture of G. mosseae and G. etunicatum) when 10-10-10 fertilizer was applied at the high rate of 1120 kg/ha. Egel and Pope (1982) found a significant green ash response to phosphorus fertilization but no additional mycorrhizal (G. macrocarpum) response above 10 ppm available soil P.

Decreased mycorrhizal colonization resulting from increased soil fertility levels has been observed in sweetgum (Liquidambar styraciflua) (Kierman, et al., 1981), green ash (Egel and Pope, 1982), and in grasses (Rabatin, 1979). There was no apparent reduction in colonization by increasing P fertilization in this experiment, however, differences in height growth at the highest level of P did not become significant until week 8, which may represent a slight delay in root colonization resulting from fertilization (Table 1). In contrast, Douds and Chaney (1982) found increased colonization of green ash by G. macrocarpum when Hoaglands nutrient solution was increased from one-half to double strength.

Root-shoot ratios in this experiment decreased with P fertilization and P concentration in roots (Figure 1 and 2). Root-shoot ratios in mycorrhizal plants were less affected by P fertilization than those of nonmycorrhizal plants, but were similarly related to root P. Douds and Chaney (1982) similarly found larger root-shoot ratios in nonmycorrhizal green ash seedlings but could not attribute the respones solely to a physiological change resulting from mycorrhizal colonization. Hall, et al. (1984) found reduced root-shoot ratios in mycorrhizal ryegrass (Lolium perenne).

Root dry weight in mycorrhizal and nonmycorrhizal seedlings was positively related to root phosphorus concentration, and followed the same response curve (Figure 3). Douds (1984) found higher root P concentrations in mycorrhizal than nonmycorrhizal green ash but he did not indicate if there was any relation between root P and total root weight. To the best of our knowledge, this is the first report linking root P concentrations and increased root biomass in V-A mycorrhizal hardwood seedlings.

Leaf dry weight was related to foliar P concentrations in green ash seedlings (Figure 4). Mycorrhizal plants had lower concentrations of foliar P per unit dry weight of leaf than noninoculated seedlings (Figure 4). These results differ from those generally reported (Menge, et al., 1978; Stribley et al., 1980). Mycorrhizal green ash seedlings had more leaf growth than nonmycorrhizal seedlings for a given level of internal leaf P, which suggests a physiological response to the mycorrhizae independent of leaf P. Increased mycorrhizal efficiency of P utilization in leaves is one possible explanation.

In this experiment inoculation with G. etunicatum stimulated plant growth and improved P uptake in green ash. Most of the growth differences could be attributed to improved P nutrition resulting from mycorrhizal colonization. Further experiments utilizing different nutrient combinations will help determine whether, as with Citrus (Menge, et al., 1978), G. etunicatum can be used in place of or in combination with fertilizers to improve nursery production.

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