GROWTH OF CONTAINERIZED YELLOW-POPLAR AS AFFECTED BY DIFFERENT VESICULAR-ARBUSCULAR MYCORRHIZAL MYCOBIONTS[1/]

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Abstract.--Super 45 rootrainers containing media amended with pot culture inocula of Glomus mosseae, G. fasciculatus, G. mosseae plus G. fasciculatus, soil from a natural yellow-poplar stand, or uninoculated were planted with yellow-poplar seedlings and grown in greenhouse culture. Seedlings were harvested at 3, 5, 7, 9, 12, and 16 weeks after planting. Uninoculated seedlings were the smallest and were not mycorrhizal. Seedlings infected by inoculum from the natural stand were larger than the control seedlings but smaller than those infected by Glomus spp. The seedlings exposed to a single Glomus inoculum were larger than those seedlings infected by the Glomus mixture. Growth acceleration was preceded by an increase of vesicular-arbuscular mycorrhizae in the root cortex.

Keywords: Liriodendron tulipifera, Glomus, Glomus mosseae, Glomus fasciculatus, growth rates, colonization rates, infection rates

INTRODUCTION

Yellow-poplar (Liriodendron tulipifera L.), a valuable hardwood of the Appalachian region, commonly occupies moist, well-drained, loose-textured soils (Olson 1969). Wood qualities are favorable for construction timber, plywood, pulp, and composite board. Due to its fast growth, yellow-poplar plantation culture is possible. However, soil factors which affect the quality and quantity of rooting volume, especially soil moisture supplying capacity during the growing season, limit growth rates of yellow-poplar. Soil factors reported to be important include depth to tight subsoil (Auten 1945), fragipan (Gilmore et al. 1968), and mottling (Tyron et al. 1960).

Hardwood plantings frequently have problems not common in pine plantations. Survival of hardwoods, for example, is usually low after planting on old fields (Boyette 1970). Seedlings that do survive often do not grow well for the first several years, perhaps due to a poor quality root system at lifting (Kormanik 1986) or poor soil structure and weak mycorrhizal development (Clark 1963). Russell (1977) stated that the majority of yellow-poplar outplantings failed because of inferior planting stock and poor site preparation.

Containerized seedlings have the potential to overcome some problems associated with hardwood plantings. According to Graber (1978), containerized seedlings survive better and grow faster than bare-rooted seedlings, especially if the site is droughty. The planting season for containerized seedlings can be extended beyond that for bare-rooted seedlings. Inoculation with mycobionts is easily accomplished in containerized seedling culture. Vesicular-arbuscular mycorrhizae (VAM) on yellow-poplar increase the effective surface area for nutrient and water absorption after transplanting. These fungi also increase seedling growth in containers creating a larger, more desirable seedling (Ford 1978).

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The use of containerized yellow-poplar seedlings in reforestation is new. Appropriate growing medium, container size, and culture techniques are not yet fully determined. Conditions that promote VAM development in containerized yellow-poplar are not known at this time. The effects that various species of VAM fungi may have on yellow-poplar growth have not been tested. The objectives of this study were to determine: (1) when VAM fungi infect roots of yellow-poplar in containers, (2) if successful infection causes increases in seedling growth, and (3) the effect of mycobiont species on infection and seedling growth.

MATERIALS AND METHODS

Yellow-poplar seeds were collected from McMinn County, Tennessee, by Tennessee Division of Forestry personnel and stratified 90 days at 5 C. The seeds were surface sterilized for three minutes in a 0.5% (w/v) sodium hypochlorite solution and thoroughly rinsed in tap water. After rinsing, the seeds were then planted into flats containing perlite and masonry sand (50:50). The seeded flats were illuminated and heated with 150 watt lamps to maintain temperatures between 20 C to 30 C.

Mycorrhizal treatments consisted of: (1) no inoculum added, (2) natural inoculum, (3) Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe, (4) Glomus fasciculatus (Thaxter sensu Gerd.) Gerd. & Trappe, and (5) a mixture of the two Glomus spp. Natural inoculum was unsterilized soil and humus from a yellow-poplar stand at the University of Tennessee, Oak Ridge Forestry Field Station. Glomus inocula were originally obtained from The Institute for Mycorrhizal Research and Development, U.S.D.A. Forest Service, Athens, Georgia, and they were maintained on grain sorghum (Sorghum bicolor (L.) Moench) in pot cultures.

Germinants were transplanted into Spencer-Lemaire "Super-45" rootrainers, a book type, polystyrene container consisting of three cavities of 45 cubic inches (290 cm^3) each. Ten rootrainers were packaged into a compact bundle, comprising one replication which contained six seedlings (two filled rootrainers from each treatment). There were 16 replicates. Harvests were planned at 3, 5, 7, 9, 12, and 16 weeks after transplanting.

The growth medium was an equal mixture of milled pine bark, masonry sand, and silt loam soil. The pH of the medium was 6.9, phosphorus concentration was 5.44 ppm, and the potassium concentration was 326.4 ppm. The medium was sterilized by methyl bromide fumigation. VAM inoculum was mixed with the sterile medium (200 ml:9500 ml) and was placed in the rootrainers on top of a glass wool plug, inserted to hold the medium in place. Treatments were assigned randomly to rootrainers, and healthy seedlings of uniform size were transplanted. Leachate, which had been filtered through Whatman No. 4 Filter Paper, of all fungal treatments combined was added to the control group to standardize the nonmycorrhizal microflora of the rhizosphere.

Gro-lux™ fluorescent lights were used to extend the daily photoperiod to 16 hours. The seedlings were watered as needed, and an application of liquid fertilizer (12-24-10) was made every three weeks. Benomyl was applied at recommended rates to the foliage at eight weeks to control powdery mildew.

At harvest, stem length and root collar diameter were measured. Stems were dried at 60 C at least 48 hours and weighed. Roots were stored in formalin-ethanol-acetic acid and stained as described by Phillips and Hayman (1970) using trypan blue (C.I. No. 477, Lot 11003). After staining, roots were stored in lactophenol until assayed for VAM infection.

VAM colonization was assessed using a method developed by Barnhill (1977). One centimeter segments were cut from each root and mounted in lactophenol. Fifty microscopic fields (about 3 mm^2 at magnification 100x) were sampled at random from each root segment using from 8 to 10 sections per root system. Presence of free hyphae, intracellular hyphal coils, arbuscules, and vesicles was recorded. A percentage of microscopic fields containing each structure was calculated as an index of VAM colonization.

Analysis of variance was conducted using single-degree of freedom, orthogonal contrasts to determine treatment differences. The design was treated as a randomized complete block. Orthogonal comparisons consisted of: (1) uninoculated (Treatment 1) vs. inoculated (Treatments 2-5), (2) natural inoculated (Treatment 2) vs. Glomus inoculated (Treatments 3-5), (3) mixed Glomus inoculated (Treatment 5) vs. pure Glomus inoculated (Treatments 3-4), and (4) G. fasciculatus inoculated vs. G. fasciculatus inoculated (Treatment 4) vs. G. mosseae inoculated (Treatment 3).

RESULTS

Table 1 summarizes the results of the single-degree of freedom, orthogonal comparisons for seedling growth variables for weeks 12 and 16. There were no significant differences prior to week 12. Uninoculated seedlings were significantly smaller than inoculated seedlings for all measurements (alpha <0.01). Seedlings inoculated with soil from a natural stand were significantly shorter and had less biomass at 12 and 16 weeks than seedlings inoculated with Glomus spp. (alpha <0.01). The seedlings grown in natural inoculum also were significantly smaller in diameter at the root collar than the Glomus-inoculated seedlings at 16 weeks (alpha <0.01). The mixed Glomus-inoculated seedlings

were shorter at 12 and 16 weeks, and they had a smaller root collar diameter at 16 weeks than those inoculated with either G. mosseae or G. fasciculatus. There were no significant differences between seedlings inoculated with either Glomus spp.

Table 1.--Orthogonal comparisons of yellow-poplar stem measurements made at 12 and 16 weeks of age.

Harvest	Stem/Leaf Biomass	Total Height	Root Collar Diameter
	- - - - - -Probability > F- - - - -		
Week 12			
C1[1/]	0.0001	0.05	0.05
C2	0.01	0.001	NS
C3	NS[2/]	0.05	NS
C4	NS	NS	NS
Week 16			
C1	0.01	0.001	0.01
C2	0.05	0.01	0.01
C3	NS	0.01	0.05
C4	NS	NS	NS

1/Comparisons are C1 = uninoculated vs. inoculated, C2 = Glomus inoculated vs. inoculum from natural stand, C3 = Pure Glomus inoculated vs. mixed Glomus inoculated, C4 = G. fasciculatus vs. G. mosseae.

2/Nonsignificant

Seedling response to VAM colonization was not manifested until after the nine weeks measurement (fig. 1-3). By the 12th week, there was a separation of seedling height, diameter, and biomass growth. Those seedlings infected by either G. mosseae or G. fasciculatus were consistently and significantly larger.

Results of the orthogonal comparisons of VAM structures for root cortex are presented in Table 2. Figures 4-7 show root colonization with hyphae, arbuscules, and vesicles through 16 weeks of seedling development. Control seedlings were not infected by VAM. Due to fungal activity at five weeks, mycorrhizal structures in inoculated seedlings were significantly greater than in the noncolonized controls. Seedlings inoculated with VAM fungi from the natural stand had the highest percentage of infection at week 7, but the Glomus treatments had more infection at weeks 12 and 16. Vesicles were uniformly present in all VAM-infected seedlings and absent from the controls. Intracellular hyphal coiling and arbuscules in Glomus-infected seedlings occurred significantly more than in seedlings colonized by fungi from the natural stand at 7, 12, and 16 weeks and 7 and 12 weeks, respectively.

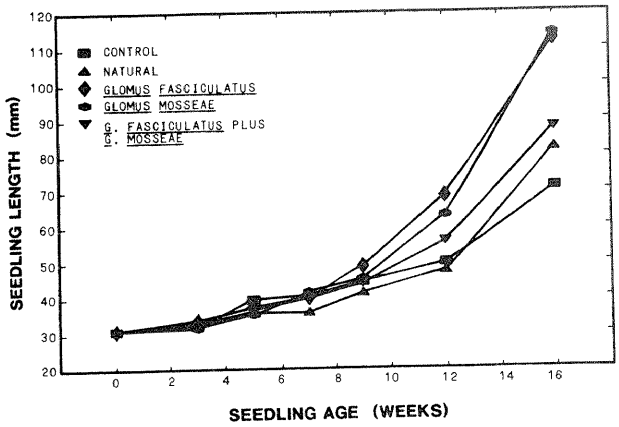

Figure 1.--Stem length of yellow-poplar seedlings inoculated with different sources of vesicular-arbuscular mycorrhizal inocula.

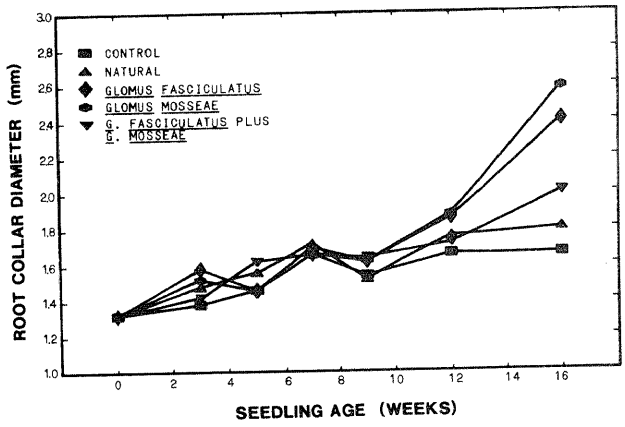

Figure 2.--Diameter at root collar of yellow-poplar seedlings inoculated with different sources of vesicular-arbuscular mycorrhizal inocula.

DISCUSSION

VAM fungi stimulated growth of inoculated seedlings compared to growth of uninoculated seedlings. Clark (1963), Smith (1974), and Gerdemann (1965) reported similar results. The differential effects on growth among different inoculum treatments indicated that inoculum source may be important in producing larger seedlings in a short time. Growth rates of seedlings inoculated with a single endophyte were quite smaller. However, seedlings inoculated with a combination of the two endophytes had a slightly slower growth rate.

Barnhill (1977) found similar results in a another greenhouse study with yellow-poplar. Competition between the two endophytes and the effect on seedling growth are not understood. Based on this evidence, a single species of VAM fungus should be used to inoculate containerized seedlings for increased growth at least through the 16th week. The duration of the competition effect, however, is not known.

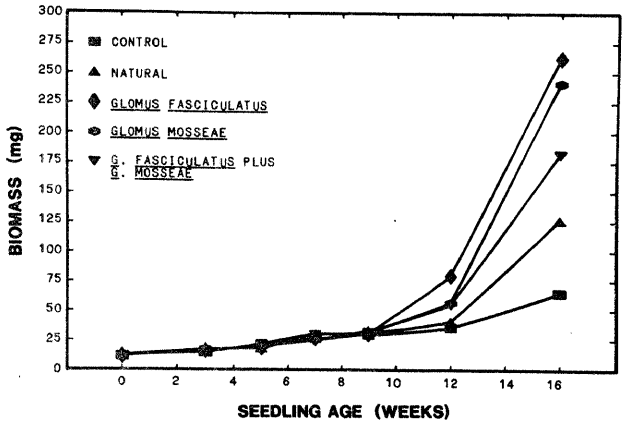

Figure 3.--Above-ground biomass of yellow-poplar seedlings inoculated with different sources of vesicular-arbuscular mycorrhizal inocula.

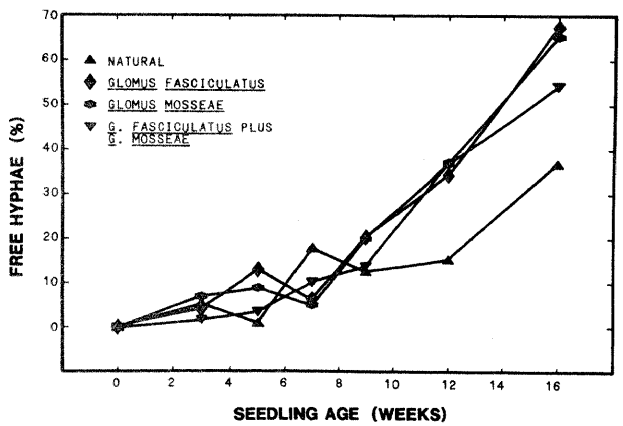

Figure 4.--Percentage of yellow-poplar seedling root tissue containing free hyphae for different sources of inocula.

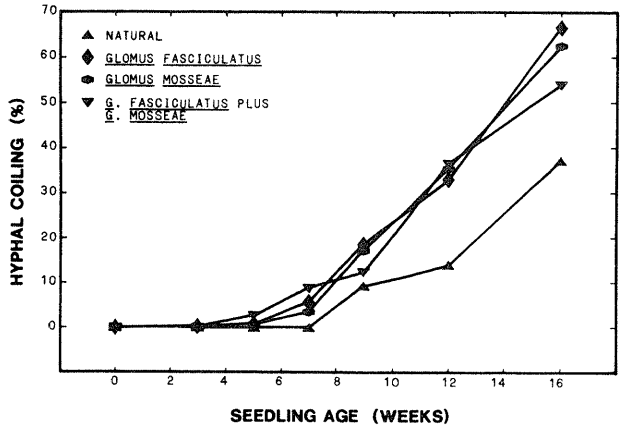

Figure 5.--Percentage of yellow-poplar seedling root tissue containing intracellular hyphal coiling for different sources of vesicular-arbuscular mycorrhizal inocula.

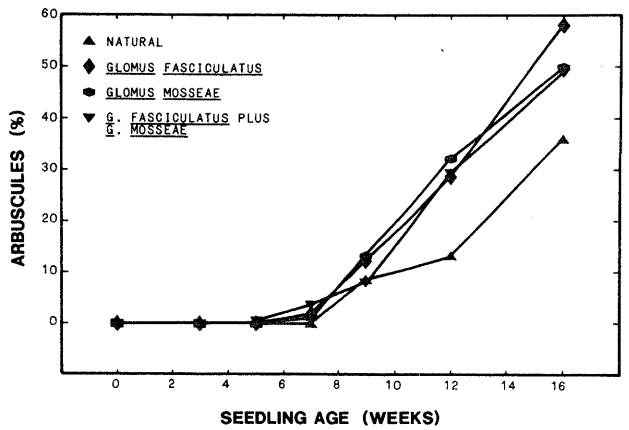

Figure 6.--Percentage of yellow-poplar seedling root tissue containing arbuscules for different sources of vesicular-arbuscular mycorrhizal inocula.

Table 2.--Results of orthogonal comparisons of amount of yellow-poplar root tissue colonized by vesicular-arbuscular mycorrhizae.

Harvest	Free Hyphae	Vesicles	Hyphal Coiling	Arbuscules
	- - - - - - - - - - -Probability > F- - - - - - - - - - -			
Week 3				
C1[1/]	0.05	NS	NS	NS
C2	NS[2/]	NS	NS	NS
C3	NS	NS	NS	NS
C4	NS	NS	NS	NS
Week 5				
C1	NS	NS	NS	NS
C2	0.05	NS	NS	NS
C3	NS	NS	NS	NS
C4	NS	NS	NS	NS
Week 7				
C1	0.05	0.0001	0.05	NS
C2	0.01	NS	0.05	0.001
C3	NS	NS	NS	NS
C4	NS	NS	NS	NS
Week 9				
C1	0.001	0.05	0.05	0.01
C2	NS	NS	NS	NS
C3	NS	NS	NS	NS
C4	NS	NS	NS	NS
Week 12				
C1	0.0001	0.0001	0.0001	0.0001
C2	0.01	NS	0.001	0.01
C3	NS	NS	NS	NS
C4	NS	NS	NS	NS
Week 16				
C1	0.0001	0.05	0.0001	0.0001
C2	0.001	NS	0.001	NS
C3	NS	NS	NS	NS
C4	NS	NS	NS	NS

[1/] Comparisons are C1 = uninoculated vs. inoculated, C2 = *Glomus* inoculated vs. inoculum from natural stand, C3 = Pure *Glomus* inoculated vs. mixed *Glomus* inoculated, C4 = *G. fasciculatus* vs. *G. mosseae*.

[2/] Nonsignificant

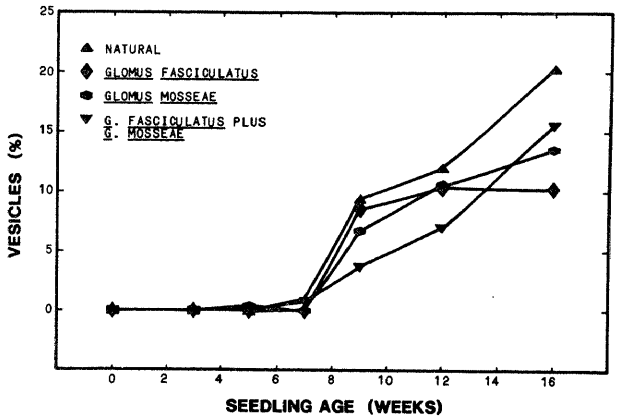

Figure 7.--Percentage of yellow-poplar seedling root tissue containing vesicles for different sources of vesicular-arbuscular mycorrhizal inocula.

Seedlings inoculated with soil from a natural stand did not show increased growth comparable to those inoculated with a single Glomus spp. Endophyte competition may have also played a role in these seedling growth differences, but other factors such as plant parasites and hyperparasites of the VAM fungi may have been more important. No attempt was made to identify the mycobionts in the natural inoculum. Nematodes and various septate and aseptate fungi were also observed on the roots of seedlings inoculated with soil from the natural stand. Ross and Daniels (1982) discussed the potential negative impact of hyperparasites of VAM fungi. Although hyperparasites were not detected in this study, their presence in the natural inoculum could account for lower growth rates of the fungi as well as lower infection rates. Conn (1980) found similar growth differences between natural inoculum and Glomus inoculum on yellow-poplar seedlings grown in a nursery.

There appeared to be an increase in VAM infection before seedling growth increased. Infection began to increase at an increasing rate between seven and nine weeks for most treatments. Seedling growth did not show a dramatic increase until after 10 weeks. Yellow-poplar seedlings need to be infected by VAM fungi to obtain adequate growth in containers. Since VAM infection always preceded significant growth increases and uninoculated seedlings grew significantly less than inoculated seedlings, yellow-poplar seedlings need VAM fungi.

Comparisons of figure 3 with figures 4, 5, 6, and 7 demonstrate that, in this study, mycorrhizal colonization increased exponentially four weeks prior to a corresponding seedling growth. This is particularly evident in comparing seedling biomass growth (fig. 3) with the amount of hyphal coiling (fig. 5) and the amount of arbuscular development (fig. 6). This result agrees with the assumption that the arbuscule is the site of mineral and nutrient transfer and that hyphal coiling accompanies arbuscular development in yellow-poplar (Gerdemann 1965). According to figures 3 and 6, the roots must be about 30 percent colonized by arbuscules, employing the method used in this study, in order for yellow-poplar to begin to grow at an exponential rate. More data are needed to verify if this amount of colonization is critical before rapid seedling growth can begin. Many workers have shown the benefits that colonized plants have over noncolonized ones. No study found by these authors has shown the relationship of time of colonization and critical colonization levels to corresponding growth increases.

The infection sequence of the VAM fungi in yellow-poplar in this study followed the pattern reported by Gerdemann (1965) and later described by Brown and King (1982) and Kormanik, et al. (1977). Hyphae occurred as early as three weeks after exposure to the inoculum. Intracellular hyphal coiling was observed at five weeks and an increase in arbuscules was noted at seven weeks. The dynamics of arbuscule formation, though interesting and complex, will not be elaborated here. Results of this study supported other workers who found that hyphal coiling occurs before arbuscule formation. Arbuscule formation preceded formation of vesicles. Arbuscules have been considered as the nutrient exchange site and vesicles as storage organs. To be able to store carbohydrates, infection must occur to obtain the raw materials; vesicle formation must follow arbuscule formation.

CONCLUSION

Seedlings with VAM fungi were larger than noncolonized seedlings. The use of a natural inoculum did increase seedling growth over controls, but other unexplained factors prevented the seedlings growing in the natural inoculum to reach the size of seedlings inoculated with Glomus cultures. Neither G. mosseae or G. fasciculatus proved superior in stimulating growth of yellow-poplar seedlings, but seedlings inoculated with a single VAM fungus grew faster than those inoculated with a mixture of the two endobionts. An increase of colonization was noted to occur four weeks prior to an increase in growth, and a rating of 30 percent of the root colonized appeared to be the critical level before an exponential growth rate can occur. More data are needed to verify this critical colonization level.

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