GROWTH OF ENDOMYCORRHIZAL YELLOW-POPLAR SEEDLINGS IN FUMIGATED NURSERY SOIL

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Abstract.--Main treatments included various combinations of base fertilizer plus urea top-dressings upon which four endomycorrhizal treatments were superimposed, e.g. *Glomus mosseae*, *G. fasciculatus*, natural inoculum from a yellow-poplar stand, and no inoculum (control). Seedlings were grown in the Tennessee Division of Forestry at Pinson. Watering, weeding, and other operations followed normal nursery schedules.

Height, diameter at the root collar, and taproot diameter were greatest when at least two top-dressings of urea were applied during the growing season, regardless of the endomycorrhizal inoculum source. Soil phosphorus content exceeded 100 ppm thereby masking many initial benefits of mycorrhizae.

Top dry weight and taproot fresh weight were greatest for *Glomus mosseae*-infected seedlings. *Glomus mosseae*-infected roots had the greatest arbuscule frequency. The number of hyphal coils in both species of *Glomus*-infected roots was nearly equal, but these were significantly greater than for other inocula sources. Natural inoculum-infected seedlings had more vesicles than other seedlings.

Index words: Yellow-poplar, endomycorrhizae, nursery-culture, seedlings, *Glomus*.

INTRODUCTION

In the southeastern United States, pines have reforested much abandoned pasture and farm land. Originally, these lands supported angiosperm forests, but poor land management practices have caused site deterioration to such an extent that pines have been the only practical species to assure outplanting success. On those sites that have angiosperm potential, however, one silvicultural possibility for diversifying forest management is to plant acceptable hardwood species.

The technology of producing seedlings that will survive and grow well when outplanted has advanced significantly in the last few years. Recent forestry research on mutually symbiotic relationships between certain species of fungi(mycobionts) and tree roots promises to increase outplanting success. These fungus-root associations, known as mycorrhizae, have been found to occur naturally on almost all plants. In the absence of mycorrhizal fungi, especially

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in low-fertility situations, most plants do not grow well (Kormanik, et al., 1977a). Marx (1977) obtained significant growth increases when mycobionts were chosen for specific pines on specific sites. Perhaps similar benefits could be obtained through hardwood seedling culture.

Mycorrhizae and yellow-poplar (Liriodendron tulipifera L.) have not been thoroughly studied. However, mycorrhizal inoculation increased yellow-poplar growth in several studies where seedlings were grown in containers (Clark, 1963; 1964; 1969). Ford (1978) found marked growth increases when yellow-poplar seedlings were first infected by Glomus endomycorrhizae in Spencer-Lemaire rootrainers. Mosse, et al. (1969) demonstrated that if sweetgum (Liquidambar styraciflua L.) seedlings were mycorrhizal when planted in unsterilized soil, they maintained a significant growth advantage over similar non-mycorrhizal seedlings for a period of at least nine weeks. It has not yet been demonstrated that yellow-poplar will perform similarly.

Unfortunately, yellow-poplar seedlings growing in a nursery may have few naturally occurring endomycorrhizae due to the common practice of yearly fumigation of nursery beds. Endomycorrhizal fungal spores are not airborne as are the spores of the ectomycorrhizal fungi associated with pines, thus endomycorrhizal fungi are slow to recolonize nursery beds after soil fumigation.

The major objective of our study was to produce healthy seedlings, suitable for outplanting, using standard nursery techniques, while specifically testing:

1. The effect of varying fertilizer regimes on yellow-poplar seedling growth,

2. The effect of mycorrhizal infection on seedling growth, and

3. The interaction effects between fertilizer and mycorrhizae treatments.

PROCEDURES

Experimental Design

A split plot design was used; fertilizers were the main treatments and mycobionts were imposed upon them.

The fertilizer treatments were (1) no fertilizer, (2) base fertilizer applied as 500 kg/ha of 15-15-15 before seeding, (3) base fertilizer plus two urea topdressings of 100 kg/ha of 48-0-0 each, applied during the growing season and (4) base fertilizer plus four urea topdressings similarly applied.

The mycorrhizae treatments were (1) no inoculum, (2) natural inoculum, (3) Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe, and (4) Glomus fasciculatus (Thaxter) Gerd. and Trappe. Natural inoculum was unsterilized

forest soil and plant roots obtained from a nearby, pure, yellow-poplar stand. Glomus inoculum was obtained from greenhouse pot cultures of sorghum (Sorghum vulgare var roxburghi) as a mixture of hyphae, roots, spores, and soil. These components were thoroughly chopped, mixed and applied to the nursery beds at a rate of 2 liters per square meter of bed surface.

Study Site

The study was done at the Tennessee Division of Forestry Nursery at Pinson, Tennessee. Nursery soils were generally of the Lexington-Memphis-Smithdale associations. The soil type in the experimental beds was Lexington silt loam, which is deep, well-drained, and has high available water-holding capacity. These soils are easily tilled, have good permeability, and are moderately to strongly acidic. Lexington soils are low in phosphorus and potassium unless these nutrients have been added, which had occurred here.

Bed Preparation, Seeding, and Inoculation

An area approximately 8 meters by 135 meters was allocated for the study. After the area was thoroughly disked, 5 seed beds were fumigated with methyl bromide at standard rates. The second and fourth beds were seeded to yellow-poplar in the spring, and the buffer-strips were seeded to a sorghum-sudan grass hybrid.

Each of the yellow-poplar beds contained two replications of the study. In each replication, four fertilizer treatments were established at random and base fertilizer was applied on the appropriate plots. Within each of these treatment areas four mycorrhizae treatments were established. Various bed lengths served as buffers between mycorrhizae and fertilizer plots.

Beds were then seeded with stratified yellow-poplar seed sufficiently dense to cover about 90 percent of the surface. Seeds were mulched with a thin layer of clean sawdust and watered from an elevated irrigation system. Urea topdressings were applied throughout the growing season to complete the prescribed treatments.

Data Collection

In June, July, August, and October, heights and root collar diameters were measured on every third seedling in a 30 cm wide strip across the center of each mycorrhizal plot. Final growth measurements and seedling harvest took place in December, after seedling growth had ceased for the season. At that time random samples of harvested seedlings were selected from the sampled strip in each plot for additional measurements. Root and top (exclusive of leaves) dry weights of four seedlings were measured. Root/shoot ratios were calculated on the basis of those weights. Taproot diameter, fresh weight of the taproot, and fresh weight of the root system exclusive of the taproot, i.e. the secondary, tertiary, and smaller roots, were determined from four other seedlings. A ratio of taproot fresh weight to root weight exclusive of the taproot was calculated for each seedling in this group.

Root samples of known fresh weight were taken from one seedling in the bed interior and one seedling from the bed edge in each plot. These root system samples were cut into 4 mm segments and fixed in a formalin-ethyl alcohol-acetic acid solution (FAA). After clearing the host cytoplasm, the root tissue was stained with trypan blue in lactophenol using a modified version of the Phillips and Hayman (1970) method. After staining, roots were stored in glycerin.

Estimates of total root length in the stored samples as well as the length of roots colonized by endomycorrhizal hyphae, vesicles, arbuscules, and hyphal coils were obtained by using the Ambler and Young (1977) method, modified in that the total area viewed on each slide was slightly reduced. Knowing the percentage of a sample evaluated on a slide and the percentage of roots exclusive of the taproot taken in the original sample, the root length estimates for each slide were expanded to give estimates for the recovered root system of each seedling.

Analysis

Covariate analyses were used to test the significance of fertilizer, mycorrhizae, bed position, and replication effects, plus various interaction effects. Seedling density in the beds was the cofactor since it appeared to be related to several of the growth variables measured. The analysis was assisted by use of the SAS 79, General Linear Models procedure.

RESULTS

Soil Analysis

Prior to experimental establishment, the soil in the nursery beds was quite uniform in nitrate-nitrogen, phosphorus, potassium, magnesium, calcium, and pH. Soil nitrates were found to be low to moderate, averaging 9 ppm. Phosphorus levels in the beds were extremely high, averaging 98.3 ppm by the weak Bray method and 106 ppm by the strong Bray method (Ankerman and Large, 1978). Potassium was also abundant, with a mean value of 116 ppm. Calcium and magnesium levels in the beds were low, with mean values of 575 ppm and 54 ppm respectively. The soil in the beds was moderately acidic with pH ranging from 4.9 to 5.2.

Main Treatments--Fertilizer

Seedlings receiving the base plus two topdressing had the largest plot height sums, i.e., the combined height of the total number of seedlings on the sampled strip (Figure 1). Plot height sums provided an estimate of growth that accounted for differences in bed density and treatment. Summed heights of seedlings receiving four topdressings were significantly less than those for the two topdressing treatment ($p = .10$), with values of 300 and 320 cm respectively. Seedlings receiving base fertilizer and no fertilizer ranked third and fourth. The summed heights of seedlings in the no fertilizer treatment were less than those of the base only treatment ($p = .01$), just as the summed heights for the base only treatment were less than those of the base plus urea treatments ($p = .05$).

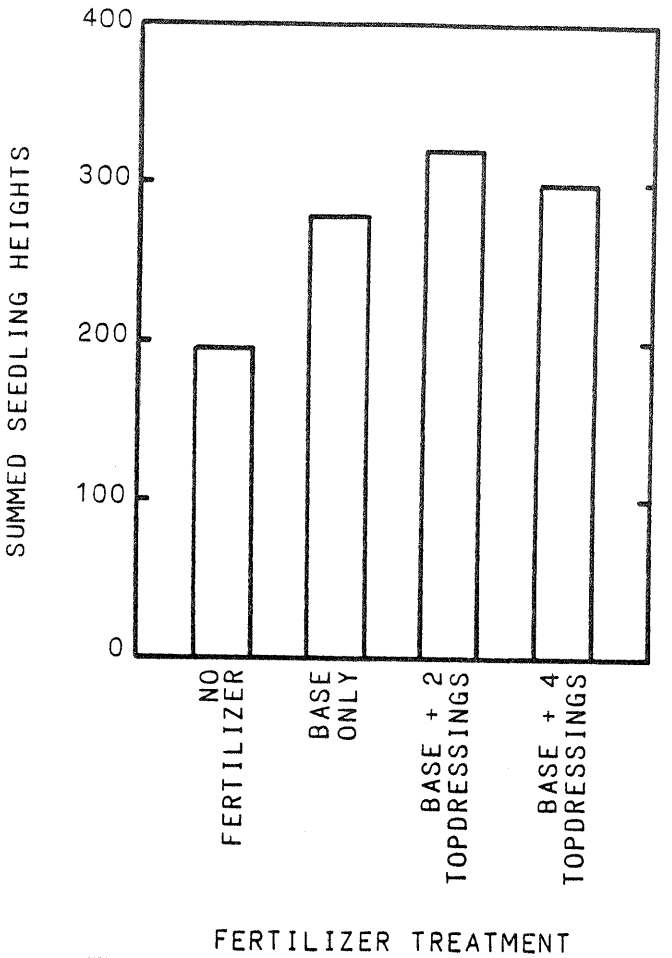

Figure 1. Heights of yellow-poplar seedlings as influenced by fertilizer treatments after one growing season in the Pinson Nursery, Tennessee.

Summed root collar diameters for seedlings in the base plus two topdressing treatment were the largest, averaging 52 mm (Figure 2). Diameter growth differences were not as pronounced among the different fertilizer treatments as were the height differences. Seedlings receiving base fertilizer, with a mean of 47 mm, had smaller root collar diameters than seedlings that received two urea topdressings ($p = .05$). Root collar diameter was the same for seedlings receiving no fertilizer and base only, and the two and four topdressing treatments produced seedlings with the same diameters.

Seedlings in the base plus two urea topdressing treatment had the largest taproot biomass. Four urea topdressings produced slightly smaller taproot biomass with means of 9.5 and 9.0 g, respectively, ($p = .10$). Seedlings that received just the base fertilizer ranked third in taproot biomass; these roots were significantly smaller than those produced with two topdressings ($p = .05$). Without fertilizer, taproot biomass was 7.6 g and the same as that produced through base fertilizer treatment.

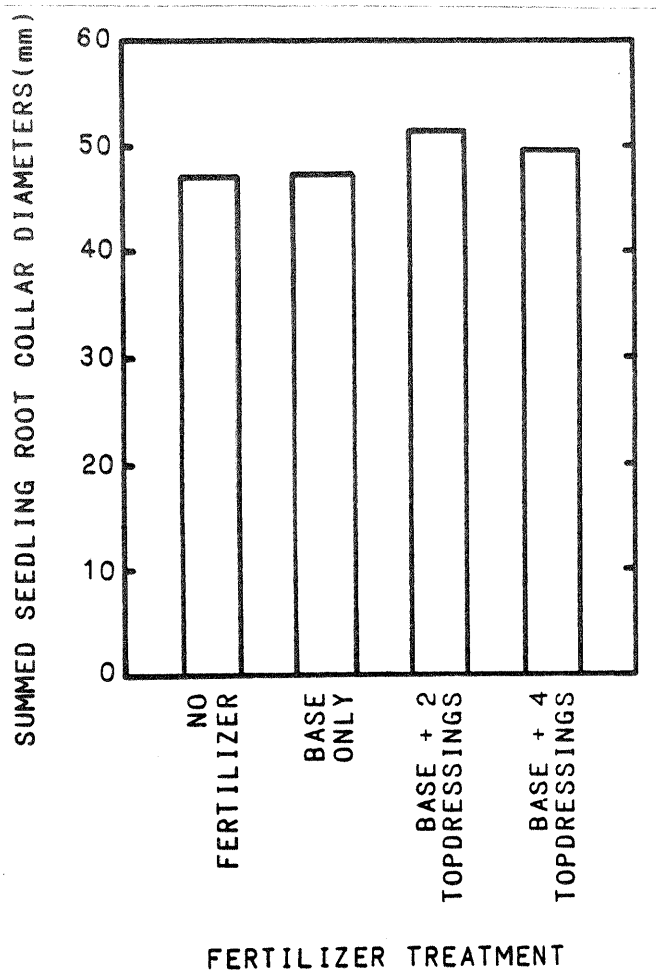

Figure 2. Root collar diameters for yellow-poplar seedlings after one growing season in the Pinson Nursery, Tennessee. Values are expressed as the sum of root collar diameters for all stems on each treatment plot.

Split Treatments--Mycorrhizae

Dry weight of tops was greatest for seedlings inoculated with *Glomus mosseae*, (Figure 3). *Glomus fasciculatus*-inoculated seedlings produced the second greatest top dry weight, with a mean of 2.9 g. Mean top dry weights for the two *Glomus* treatments were not significantly different ($p = .10$). Seedlings receiving natural inoculum had lower top weights (2.2 g) than seedlings with no inoculum source, but neither of these were significantly different from the *Glomus* infected seedlings.

Seedlings inoculated with *G. mosseae* had the greatest taproot fresh weights, followed by those with natural inoculum, no inoculum, and *G. fasciculatus*. Taproot fresh weights of the *G. mosseae* seedlings were greater than those from *G. fasciculatus* seedlings ($p = .05$), but weights of seedling tap-

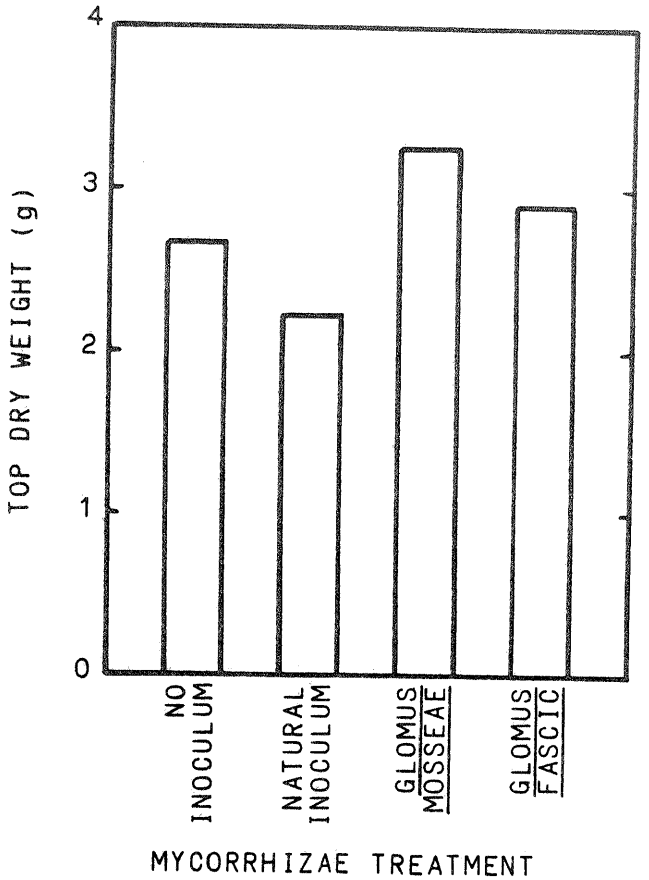

Figure 3. Top dry weight of yellow-poplar seedlings according to mycorrhizal treatments after one growing season in the Pinson Nursery, Tennessee.

roots treated with natural inoculum and control seedling taproots did not differ from those of G. fasciculatus. Meaningful inferences from lateral roots were not possible due to the harvesting technique.

Presence and abundance of endomycorrhizal structures were analyzed in the cleared and stained root tissue. Seedlings treated with the natural inoculum had the greatest occurrence of vesicles, but G. mosseae was a close second. G. fasciculatua had the lowest incidence of vesicle-infected root tissue; nearly seven times fewer than the natural inoculum seedlings. Arbuscules were most common in G. mosseae, with G. fasciculatua next. The Glomus treatments were about equally effective in producing hyphal coils and much more effective than other treatments. In each case, the no-inoculum control roots had some endomycorrhizal structures, either from contamination or from natural introduction in the beds.

Bed Position

Seedlings from the interior portion of the beds were significantly taller

(p = .01) than seedlings from the edge of the beds. The fertilizer and mycorrhizal treatments influenced how tall the seedlings were but the pattern across the beds remained similar. Although no measurements of stem branching were attempted, seedlings on the edge appeared to have more lateral branches than seedlings from the center of the beds.

Density Interactions

For seedling height and root/shoot ratio, there were significant fertilizer and seedbed density interactions (p = .01). In all fertilizer treatments, seedling height increased as bed density increased. Unfertilized seedlings also increased in height with increasing bed density, but considerably less so than fertilized seedlings. Unfertilized seedlings had the highest root/shoot ratios at high bed densities, but they had the lowest root/shoot ratios at low bed densities. Fertilization caused root/shoot ratios to decrease slightly with increasing bed density.

Seedbed density significantly interacted with the amount of lateral roots produced in some mycorrhizal treatments. *G. mosseae* infected seedlings produced more lateral root mass than any inoculum source with less than 7 seedlings per square foot of bed, but at bed densities greater than 10 seedlings per square foot *G. mosseae* seedlings had the least lateral root mass. *G. fasciculatus* did not show a correlation of lateral root weight to bed density; the weights remained constant at bed densities from 1 to 15 seedlings per square foot.

DISCUSSION

Several traits in our opinion, should characterize a quality yellow-poplar seedling ready for outplanting. It should have a well-developed, wide-spreading lateral root system to absorb nutrients and water in sufficient quantities to supply the above-ground portions of the developing seedling. Moreover, the root system should have sufficient mycorrhizae to make full use of the limited soil resources that might be encountered after removal from the nursery. A quality seedling needs to be tall enough to be competetive, but not so tall that the roots cannot supply enough water and nutrients to the top. A quality seedling should have a large root collar diameter to insure enough vascular tissue for translocation. Furthermore, a quality seedling should have enough leaf surface area to produce sufficient photosynthate for growth.

Desirable hardwood seedlings are not generally produced using operational techniques standard in most pine nurseries. It may be possible to produce large hardwood seedlings by using large amounts of fertilizers and watering frequently, but seedlings produced by such practices might perform poorly when outplanted on sites of less than highest quality. Large tops do not always mean large, well-developed root systems with adequate lateral roots to generate new roots after outplanting. Frequently the roots of forced seedlings are smaller than desired and if such seedlings survive outplanting, they usually grow slowly until a balanced root/shoot ratio develops.

Using hardwood seedlings with all the necessary characteristics elabora-

ted through research, silviculturists and land managers should experience good outplanting success on a range of site qualities. Those sites that had been delegated as "pine only" might now be planted to appropriate angiosperms. Silvicultural diversity is an important characteristic and need for much of the land in the Central Hardwood Region.

We think that the best way to produce angiosperm seedlings in nurseries is to insure thorough endomycorrhizal infection of their roots, reduce the amount of inorganic fertilizers applied annually, and only fumigate the soil to control a specific pest problem. Although our study almost raised more questions than it answered, it did illuminate some truths and pointed toward some very important trends. The main problem we experienced was the high levels of phosphorus; the positive growth effects of endomycorrhizal infection are muffled when soil phosphorus is high. To be sure, our seedlings were endomycorrhizal, but the urea topdressings had more to do with size differential than mycorrhizae. In a low phosphorus soil seedling growth likely would have shown a greater influence from the Glomus.

Endomycorrhizal Inoculum

Most nursery soils are not heavily inoculated with endomycobionts if they are regularly and frequently fumigated with methyl bromide. Endomycobionts must be increased and maintained through initial inoculation and cover crops. Fumigation should be used only to combat weed or disease problems.

Several endomycorrhizal inoculants are available commercially and they need to be tested for the specific species desired and specific nursery situation at hand. The inoculants can be increased through greenhouse culture by growing the fungi on sorghum or grass to minimize the initial investment. By using a sorghum or sudex cover crop during the first year in the nursery after inoculation, the level of endomycobionts should be increased to insure thorough infection of tree seedling root systems. Thereafter, a periodic cover crop can be seeded to increase the endomycobiont level after serious depletion due to harvest of infected root systems. Perhaps a mixture of several endomycobionts within the nursery soil would be best; there may be host specificity between tree and fungal species.

Fertilizers

Once the level of endomycobionts is sufficient to thoroughly infect tree seedling root systems, the amounts of N, P, and K necessary to produce a good seedling crop decrease substantially. Phosphorus levels should be medium to low; in the Pinson Nursery soils, 10 to 20 ppm is adequate to insure good seedling growth. In our experiment, we were dealing with 100 ppm, thereby making it impossible to see much effect of mycobiont infection. The differences in needed phosphorus and available phosphorus were created by annual applications of triple-15 fertilizers. Such practices should cease; fertilizers are approximately $250 per ton. Careful soil testing combined with endomycorrhizal inoculation must undergird modern hardwood nursery management practices.

Nitrogen fertilization at low levels will still be necessary in angiosperm seedling nursery culture. Our seedlings responded postively to two topdressings

of urea (100 kg/ha) during the growing season. Nitrogen fertilization rates and schedules required in a low-phosphorus soil that is well inoculated with endomycobionts was not developed. We're sure, however, that N requirements in the presence of endomycobionts will be lower than previously used in nursery management.

Some Trends

Recently, attention has been focused on the type and shape of seedling root systems that grow best after outplanting (Kormanik et al., 1977b). Even with endomycorrhizal infection there can be considerable variation in the size and shape of the root system. Elevation of seedling root system quality is necessary; it's not enough to just measure height or root collar diameter and plant a carrot-shaped taproot with few laterals.

Previous mention has been made of the importance of a well-developed lateral root system for increased water and nutrient absorption and new root generation immediately after outplanting. It was quite apparent in our study that many *Glomus*-infected seedlings had prolific lateral root systems. This trend was not apparent in our controls or in the yellow-poplar the nursery had grown commercially; however, it was impossible to statistically verify this obvious trend. Too many lateral roots were broken and left in the beds during seedling lifting.

Frequently, control seedlings had root systems that resembled a carrot, i.e., a large, well-developed taproot with few lateral roots. These seedlings would be easy to outplant, even with a planting bar. *Glomus*-infected seedlings most often had a large mass of lateral roots, in spite of damage during lifting. To outplant these would require a shovel or an auger (we used the latter) in order to adequately place the roots in the soil.

The use of endomycobionts in yellow-poplar seedling production seems desirable, both from an economic and biological standpoint in the nursery. The fact that positive mycorrhizal effects were evident in this study, in spite of the high soil phosphorus levels, was testimony to the potential benefits that might be realized under more favorable circumstances. *G. mosseae* was the best symbiont tested. More work needs to be done, however, with other endomycobionts to see if there might be species possessing better growth benefits.

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