THIRD-YEAR SEED PRODUCTION IN OUTPLANTED SWEETGUM RELATED TO NURSERY ROOT COLONIZATION BY ENDOMYCORRHIZAL FUNGI

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Abstract.--A sweetgum plantation was established from nursery seedlings grown in soil infested with vesicular-arbuscular mycorrhizal (VAM) fungi and amended with phosphorus at 25 ppm (Bray II); control seedlings were grown without VAM fungi and with soil P adjusted to 800 ppm. At the end of the third year, seed production was observed on 78 young trees, 75 from the VAM treatment and 3 from the control. In another plantation established from seedlings grown in VAM fungus-infested nursery soil and outplanted on a site that received different fertilization regimens with sewage sludge, heavy seed production was observed in both the fourth and fifth years. Observations in both plantations indicate that early seed production can be attributed to both VAM fungal root colonization and high levels of available soil P on the planting site.

Additional keywords: Vesicular-arbuscular mycorrhizae, early seed production, soil phosphorus, Liquidambar styraciflua L.

For practical purposes one can generalize that almost all plants require mycorrhizae to survive and grow in a natural environment. Most agricultural plants can be, and frequently are, fertilized with phosphorus and nitrogen compounds so that growth and yield are not noticeably improved by endomycorrhizae. In forestry, we cannot afford to apply high rates of fertilizer to maintain phosphorus above the soil phosphorus threshold level[2] for forest trees on routine forest sites; trees must rely on mycorrhizae to obtain needed nutrients. Trees with mycorrhizae will grow well in soils with phosphorus below the threshold level. In many forest soils, available soil phosphorus is so low that important tree species symbiotic with endomycorrhizal fungi would probably never become established in the absence of mycorrhizae (Marks and Kozlowski 1973, Kormanik and others 1977).

Fortunately, endomycorrhizal fungi are ubiquitous in nature and are well represented in natural forest soils. Because they are widespread, their importance has been underestimated or overlooked by many forest scientists. In the Southeastern United States forest soils seldom contain more than 10 ppm of available phosphorus (Bray II); most contain less than half this amount. In such soils endomycorrhizal fungi are indispensable to their tree hosts.

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[2] Soil phosphorus threshold level is that concentration of available soil phosphorus at which a nonmycorrhizal plant will grow as large as a mycorrhizal plant at the same phosphorus concentration.

Since 1975, scientists at the Institute for Mycorrhizal Research and Development (IMRD) in Athens, Georgia, have been investigating the importance of vesicular-arbuscular endomycorrhizal (VAM) fungi to growth and development of some of the more important commercial hardwoods. Initially, we were interested in determining whether VAM fungi benefit seedling production in hardwood nurseries by improving seedling quality similar to that found with ectomycorrhizal manipulation in pine nurseries (see Marx and others 1982). Early in the program it became apparent that the VAM technology developed in our experimental nursery had great potential for improving seedling quality of sweetgum (Liquidambar styraciflua L.) (Kormanik and others 1977).

In our early nursery experiments, we found significant and consistent improvement in growth of half-sib sweetgum seedlings from selected families if we maintained moderate. levels of available soil phosphorus (25 ppm, Bray II) and had adequate VAM fungal inoculum in the nursery soil (Bryan and Kormanik 1977, Kormanik and others 1977). We were unable to test the performance of these seedlings on outplanting sites because of the great differences between the size of the VAM and the nonmycorrhizal seedlings.

After 5 years of testing we determined the phosphorus threshold level for sweetgum that enabled us to produce nursery-grown nonmycorrhizal sweetgum seedlings equal to or larger than VAM seedlings grown at low to moderate soil phosphorus levels. This threshold level is approximately 40 to 50 ppm of available P (Bray II) for sweetgum as well as for other selected hardwoods examined in our research. Sweetgum seedlings from most half-sib seedlots grown in soils below the threshold level needed VAM to develop beyond the primary leaf stage (Bryan and Kormanik 1977, Kormanik and others 1977). As available P exceeds this threshold level (up to about 250 ppm P) nonmycorrhizal seedlings grow well, but family growth response and root colonization by VAM fungi are often erratic.

Nursery experiments and outplanting trials are underway to determine family response when seedlings with and without VAM are grown at given increments above the phosphorus threshold level. Following leads developed in citrus research (Menge and others 1978), early in our program we increased P to very high levels and found we could grow large nonmycorrhizal seedlings when available soil P was approximately 800 ppm. These nonmycorrhizal seedlings were compared with VAM seedlings (i.e., those inoculated with selected VAM symbionts and grown in soil with 25 ppm P) in an outplanting experiment. Although this study will not be completed until after the fifth growing season (1984), observations of differences between treatments in early seed production are reported.

STUDY INSTALLATION

Seed were collected from four mother trees on the Scull Shoals Experimental Forest in northeast Georgia during the last week of September 1977. Two of the selections (77-14U and 77-12U) were growing on typical, infertile upland piedmont sites, and the other two (76-2B and 77-5B) were selected from fertile river bottom sites.

In May 1978, seed were sown in three nursery beds at the IMRD experimental nursery maintained at the University of Georgia's Whitehall Forest. Each bed (1.22 m x 18.3 m) contained 22.3 m^2 of forest soil which had been fumigated

with methyl bromide (Dowfume MC-2). Two beds contained soil with available soil phosphorus standardized at 50 ppm; one was infested with the VAM fungus Glomus etunicatus and one with G. fasciculatus. The third bed was not inoculated and soil phosphorus was adjusted to 800 ppm. Each bed was divided into four equal compartments; each seed lot was randomly planted in a compartment. Seed were planted in 30 evenly spaced rows within each compartment for a final seedling density of approximately 60/m^2 (6/ft2).

Beginning in May 1978, all seedlings were top dressed with a total of 560 kg/ha of nitrogen applied as NH_4NO_3 in 10 equal amounts of 56 kg/ha of N every 2 weeks. The beds were watered as needed throughout the growing season. Seedlings were lifted in late January 1979, measured, placed in cold storage, and outplanted by hand during the first week of February on a site at the Savannah River Plant near Aiken, South Carolina.

The study was installed on an upland piedmont site with a site index between 80 and 85 for loblolly pine (age 50). In 1974, a natural loblolly pine stand had been harvested from this site and natural vegetation consisting of grasses, hardwood sprouts, and dense thickets of blackberry (Rubus spp.), all of which are good host plants for VAM fungi, became established. In the fall of 1978, the site was prepared with a KG blade and root rake; debris was windrowed and the site double disked prior to planting. An assay of 20 soil samples, randomly collected from the site, revealed VAM fungal spore counts in excess of 3500/100 cc of soil.

The study had five blocks, each containing 12 plots (3 mycorrhizal conditions x 4 mother trees). Each plot was hand-planted to 25 permanent test trees spaced at approximately 3 m x 3 m (10 ft x 10 ft). An additional 15 seedlings per plot were planted approximately 0.3 m from preselected permanent test seedlings. Five of these extra seedlings per plot were excavated each year for 3 years to assess VAM development.

In April 1979, the study area was fertilized with 280 kg/ha of diammonium phosphate and was fertilized annually for three additional years with 280 kg/ha of ammonium nitrate. The plantation was disked three times during the first growing season, twice during the second and third growing seasons, and once, after fertilization, early in the fourth growing season.

RESULTS AND DISCUSSION

Initiation of flower buds was first observed at the end of the second growing season (January 1981). The swelling and characteristic shapes of the flower buds were noted, but no buds were removed and dissected. Staminate flowers were first observed in April 1981. We decided not to tag these flowering trees because we felt they would abort early in the growing season. In mid-July, we observed that some developing seedballs on these trees had aborted but others appeared to be developing as well as those from mature trees in the adjacent natural stand.

In late July 1981, 78 trees that had seedballs were tagged. Only three of these trees were from the nonmycorrhizal, high P nursery treatment. In early October, all tagged trees produced mature seed. We then collected seed from the original mother trees at the Scull Shoals Experimental Forest to

compare germination percentages of these seed with those from the young, half-sib test trees. Only 30 of the 3-year-old seedlings produced 10 or more seedballs. The other 48 tagged seedlings produced less than five seedballs each. Since so few sound seed were produced on these trees, testing was restricted to those seed collected from the 30 trees bearing 10 or more seedballs (Table 1). In 1981, seed from the young 77-14U and 12U half-sib progeny germinated significantly better than the seed from their original mother trees. In 1978, seed from these same mother trees had germination percentages of 86 and 72, respectively. There were no significant differences in seed germination between the 76-2B and 77-5B half-sib progeny and their original mother trees. In 1978, seed germination percentages for these mother trees were 72 and 78, respectively.

Table 1.--Germination of seed collected from 3-year-old sweetgum half-sib progeny and original mother trees, 1981.

Family	Percent germination	
	Progeny	Mother tree
77-14U	96	72
77-12U	90	75
76-2B	79	75
77-5B	80	89

In January 1982, buds from more than 100 young trees in this plantation were sampled. Immature flowers were found in more than 30 percent of them, however, no seed production was recorded because a killing frost in late April destroyed all the flowers, as well as the 1982 sweetgum seed crop throughout the piedmont of Georgia and North Carolina. We did employ the same fertilization schedule (280 kg/ha NH_4NO_3 (250 lg/A) applied in April) for an additional year to improve chances for a good seed crop in 1983.

At the end of the first growing season, approximately 85 percent of all feeder root samples from the 180 seedlings excavated were heavily colonized by VAM fungi. Sampling of roots showed that it was not until late August that the initially nonmycorrhizal/high phosphorus seedlings had significant VAM fungal colonization. Therefore, these seedlings received no benefit from VAM until late in the first growing season. The fact that 75 of the 78 trees producing seed at the end of the third growing season had VAM at planting suggests that VAN affects host metabolic functions in sweetgum that can have lasting effects not currently recognized or, at least, poorly understood.

The presence of VAM only partly accounts for this stimulation of early seed production. In other plantations that we observed, seedlings with VAM at planting failed to produce seed even after 5 years in the field; however, additional P was not applied after planting. In another field study with sweetgum, sewage sludge was used as an amendment. After 5 years, evidence of heavy seed production during the fourth and fifth growing seasons was noted on trees growing in soil with 47 to 75 ppm available P. Apparently, levels of available P in the soil 4 to 8 times that normally found in nonamended soil (10 ppm) can have a direct effect on early seed production of sweetgum.

In this present study, 30 to 35 ppm available P was applied in 0.75 to 1 m (2 to 3 ft) width bands (ca 60 lb/A of available P) when the trees were initially fertilized. This means of application provided considerably more than 30 to 35 ppm available P in the immediate root zone of seedlings. After the third growing season, assay of soil samples showed that 30 ppm P (Bray II) was available in a 1-m circle around the trees. We have not observed early seed production in any of our young sweetgum plantations where soil phosphorus is below 10 ppm.

In April 1983, just 2 weeks before the two killing frosts of April 18-19, we examined all trees in this plantation for flower development. All trees were equally well colonized by VAM fungi during the entire growing season in which flowers were initiated. As yet there is no assessment of the possible frost damage, but before the frost there were no differences in flower production based upon the initial nursery treatments.

It is commonly recognized that sweetgum seed orchards are not nearly as productive as they need to be. Observations over the past 3 years suggest that, perhaps, seed orchard fertility trials should be established to clarify the role of soil phosphorus availability and seed production for this species. The soil phosphorus levels should not be so high that they interfere with or reduce the mycorrhizal dependency of the selected trees. This type of research is well within the capabilities of our present VAM technology and should be encouraged.

Seedlings characteristically grow slowly during the first 3 or 4 years in sweetgum plantations on upland sites. Increment growth in this study, however, has been excellent. At the end of the fourth growing season the average height was more than 3 m (9 ft) and the average DBH was ca 3.5 cm (1.4 inch); lateral branches of adjacent rows were frequently touching along the lower 1.5 m (4.9 ft). Crown closure should occur sometime during the fifth growing season. This is remarkable growth when one considers that during the first 2 years of plantation establishment this area experienced the two driest years on record. In the second year, less than 7 inches of rain fell on the site from mid-May until the end of August. We feel strongly that the plantation performance is correlated with the exceptionally high levels of mycorrhizal spores in the soil that were available for rapid and effective root colonization. Spore levels were 10 to 20 times greater than what we normally observe in cutover forest stands. Undoubtedly, the high spore level can be attributed to the clearcut and revegetation by VAM plants during the 4 years that the site lay fallow. During this fallow period the plant succession was primarily plant hosts that stimulated the VAM fungal populations.

Also, it should be noted that this plantation was cultivated more than is normal in an operational outplanting. Extra cultivation was needed to allow seasonal excavation of seedlings and assay of roots for infection by VAM fungi. During excavation we noted that cultivation enhanced lateral root development. Many roots damaged by cultivation tended to develop massive, fan-shaped clusters of feeder roots. Closer examination of these new roots showed that they were well colonized with VAM fungi. This additional root development certainly improved nutrient water uptake.

Another interesting observation was that newly planted sweetgum seedlings do not initiate significant new root growth until the leaves are fully expanded in late June or early July. The same pattern of root growth has been

observed in three other plantations recenly established. It appears, therefore, that intact and functional roots on planting stock must sustain the newly planted seedling until at least midsummer.

We have concluded that VAM development on seedlings at planting, high levels of available P in soil, and cultivation during the early years of plantation establishment may affect precocious seed production in sweetgum.

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