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Influence of Endomycorrhizae on Growth of Sweetgum Seedlings From Eight Mother Trees

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ABSTRACT. Sweetgum seedlings from eight mother trees were grown in fumigated soil with or without the endomycorrhizal fungus *Glomus mosseae* at four levels of soil fertility for one growing season in nursery microplots. Nonmycorrhizal seedlings of all families died or failed to exceed 5 cm in height regardless of soil fertility. Endomycorrhizal seedlings suffered little mortality, averaged about 36 cm in height, and fertility level did not significantly influence their biomass. These results demonstrate that to increase the percentage of plantable seedlings in nurseries sweetgum seedlings must be endomycorrhizal. The data further suggests that adequate endomycorrhizal inoculum in nursery beds can allow the use of less fertilizer than has been customary for production of sweetgum seedlings. FOREST SCI. 23:500–505.

ADDITIONAL KEY WORDS. *Liquidambar styraciflua, Glomus mosseae,* fertilizer.

SWEETGUM (*Liquidambar styraciflua* L.) is a commercially important hardwood species in the southern United States where it competes successfully in natural stands with many other hardwoods and with southern pines. However, attempts to artificially regenerate sweetgum have often failed because of slow height growth for several years after planting. This slow erratic growth begins in the nursery where in spite of high fertility levels, it is difficult to regulate seedling quality (Webb 1969).

Growth of sweetgum seedlings can be enhanced with endomycorrhizae (Bryan and Ruehle 1976, Bryan and Kormanik 1977). However, endomycorrhizal inoculum may be limiting in forest nursery soils during a considerable portion of the growing season because of fumigation and other cultural practices considered essential to control weeds and destructive root pathogens. Poor early season development of sweetgum seedlings may be related to a lack of endomycorrhizal inoculum in the zone of effective fumigation.

Many plants whose growth is enhanced by endomycorrhizae make acceptable growth if higher rates of soil fertility are maintained when endomycorrhizae are absent (Gerdemann 1968, Smith 1974). This interaction of soil fertility and endomycorrhizae has not been clarified for sweetgum seedling development. The purpose of this study was to determine whether different soil fertility rates could enhance growth of mycorrhizal sweetgum seedlings and to determine if artificial inoculation with the endomycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) (Gerd. and Trappe) of soils at various fertility levels would increase the size of seedlings produced by eight sweetgum families.

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Methods

Seeds were collected from eight trees growing on the Scull Shoals Experimental Forest in Northeast Georgia. Five of the trees were from fertile bottomland sites and three from dry upland sites. The seeds were stratified in water at 2°–4°C for 24 days prior to sowing.

The study was carried out in 32 microplots in an experimental nursery at Athens, Georgia. The microplots were about 1 meter square × 0.3 meter deep and were constructed of redwood. The empty microplots were positioned on recently tilled soil and fumigated under plastic with methyl bromide (Dowfume MC-2, Dow Chemical Co.). The plastic was removed after 48 hours. The microplots were then filled with a similarly fumigated soil mixture consisting of equal volumes of sandy loam forest soil, sand, and finely ground pine bark. Analysis of this mixture revealed the following amounts of extractable ions in kg/ha: NO_3-N, 39.2; P, 25.8; K, 77.3 and Ca, 366.2. Hydrated lime (CaO) was added to the mixture in all microplots to bring elemental calcium up to 1120 kg/ha.

All microplots were inoculated with 270 grams of coarsely chopped sorghum roots from either pure pot cultures of sorghum containing *Glomus mosseae* or noninfected sorghum control pots. The top 3–10 cm of soil were removed and the inoculum was spread uniformly over the surface and the soil replaced. Root-washings from the pot cultures were passed through a 45 mesh sieve (openings smaller than the spore diameter of *G. mosseae*) and filtered through Whatman No. 1 paper to standardize the rhizosphere microflora at the time of sowing. The *G. mosseae* microplots received washings from noninfected sorghum pot cultures and the control microplots received washings from the sorghum pot cultures of *G. mosseae.*

Microplots were randomly assigned to four fertilizer treatments—140, 280, 560, and 1120 kg/ha of 10-10-10 fertilizer. These were incorporated into the top 7 to 10 cm of soil when the soil was removed for placement of inoculum. In addition, all microplots received 560 kg/ha of NH_4NO_3 three times during the growing season.

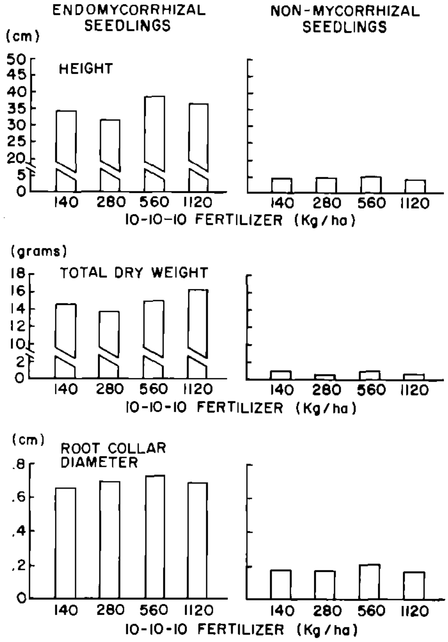
Seeds from eight half-sib sweetgum families were sown in the microplots during the third week of April. The experimental design was a factorial with 8 families, 4 fertilizer treatments, and 2 endomycorrhizal conditions. There were 40 planting locations in each microplot and each of the eight families were randomly assigned 5 of those locations. Four to six seeds were planted at each spot and the microplots were lightly covered with fumigated pine needle mulch. After germination, seedlings were thinned to one per planting spot.

The seedlings were harvested in mid-October. The presence or absence of infection by *G. mosseae* was determined from root samples from each microplot (2 seedlings/family) using the chloral hydrate acid-fuchsin clearing and staining procedure of Phillips and Hayman (1970). Heights and root rollar diameters were measured, and root and top weights were obtained after drying to constant weight at 70°C.

Analysis of variance and Tukey's W-procedure (Steel and Torrie 1960) were used to identify significant responses. A mean separation test was also done on the pooled upland (families 2, 8, 9) and bottomland seed sources (families 4, 5, 6, 7, and 51).

Results

Endomycorrhizal Effects.—Examination of the cleared and stained root samples from seedlings growing in plots containing *G. mosseae* showed that all were endomycorrhizal after 6 months and that control seedlings were nonmycorrhizal. The seedlings inoculated with *G. mosseae* outgrew the nonmycorrhizal ones (Fig. 1).



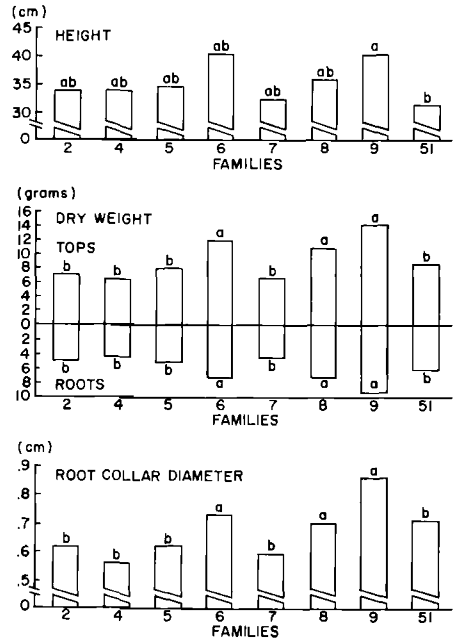


FIGURE 1. Pooled growth responses of endomycorrhizal and nonmycorrhizal sweetgum seedlings of eight families grown for 6 months in a soil-sand-bark mixture containing 140, 280, 560, and 1120 kg/ha of commercial 10-10-10 fertilizer. Bars for endomycorrhizal and nonmycorrhizal seedlings represent the means of approximately 120 and 50 seedlings, respectively.

FIGURE 2. Heights, dry weights, and root collar diameters of eight families of 6-month-old mycorrhizal sweetgum seedlings. Bars labeled with the same letter are not different at the 95 percent confidence level.

The values in Figure 1 are the pooled means of all families within fertilizer treatments. About 60 percent of all the nonmycorrhizal seedlings died during the growing season. This mortality and minimal growth of the surviving nonmycorrhizal seedlings precluded family comparisons between nonmycorrhizal and endomycorrhizal seedlings.

Fertilizer Effects.—Height growth was the only one of the six parameters tested that was significantly affected by fertilizer level (Table 1). Fertility level did not significantly influence growth of progeny from any of the eight mother trees.

Family and Ecotype Effects.—There were highly significant differences among families in growth of endomycorrhizal seedlings (Table 1, Fig. 2). Means of all growth parameters other than seedling height were significantly greater for the pooled upland families than for the bottomland families (Table 2).

DISCUSSION

The beneficial effects of the endomycorrhizal symbiont, *G. mosseae*, to sweetgum seedlings were not altered by high soil fertility. The endomycorrhizal seedlings grew equally well at the lowest and highest levels tested (Fig. 3). The nonmycorrhizal seedlings from all eight half-sib families failed to develop normally even with

TABLE 1. Summary of F*-tests from analyses of variance performed on sweetgum seedlings of eight families inoculated with* Glomus mosseae.

Source of variation	Total weight	Root weight	Stem weight	Leaf weight	Root collar diameter	Height
Replication	**[1]	*[2]	*	**	NS[3]	**
Family	**	**	**	**	**	**
Fertilizer	NS	NS	NS	NS	NS	*
Family-fertilizer interaction	NS	NS	NS	NS	NS	NS

[1] ** *F* significant at 1 percent level.
[2] * *F* significant at 5 percent level.
[3] NS *F* not significant.

the application of 1120 kg/ha of 10-10-10 plus side dressings of NH_4NO_3 totaling 1680 kg/ha.

Most reports of plant responses to endomycorrhizal symbionts have been concerned with agronomic field or truck crops (Gerdemann 1968). Inoculation of these plants with endomycorrhizal fungi can increase growth and yield significantly. This increased productivity is greatest at low levels of soil fertility. In soils with optimum or higher fertility levels, nonmycorrhizal controls often develop as well as the endomycorrhizal plants (Gerdemann 1968). However, the greatest increase in growth by nonmycorrhizal plants occurs with the addition of high rates of phosphorus to the growing media. At the highest fertility level in this study, the nonmycorrhizal sweetgum seedlings had approximately 55–60 ppm of available P as well as adequate levels of the other elements. The concentration of P, although fairly high, was apparently not sufficient to alter the endomycorrhizal requirement of sweetgum. This lack of response by sweetgum to high levels of fertilization differs from that of most agronomic plants.

The uniform response of both the endomycorrhizal and nonmycorrhizal sweetgum seedlings to different fertility levels has practical applications in nursery management. The fertility levels tested approximate the normal range in forest tree nurseries growing hardwoods. The poor development of the nonmycorrhizal seedlings suggests that high fertility levels alone are not sufficient for good seedling production. However, even the lower and moderate rates applied to a naturally fertile soil may be adequate to grow sweetgum seedlings if endomycorrhizal symbionts are plentiful in the nursery soils. Unfortunately there is no information on the distribution and abundance of these essential root symbionts in nursery soils.

TABLE 2. Growth parameters for pooled upland and bottomland families of mycorrhizal sweetgum seedlings.[1]

Families	Total weight (gm)	Root weight (gm)	Stem weight (gm)	Leaf weight (gm)	Root collar diameter (cm)	Height (cm)
Upland	18.5	7.2	5.4	5.9	0.74	37.3
Bottomland	14.0	5.4	4.3	4.5	0.64	34.8

[1] Means from upland and bottomland families are significantly different for all growth parameters, except height, at the 5 percent level.

FIGURE 3. Left picture shows the responses of the best family (74-9) and the poorest family (74-51) to 140 kg/ha (125 lbs/a) of 10-10-10 fertilizer. Right picture shows the responses of the same two families to 1120 kg/ha (1000 lbs/a) of 10-10-10 fertilizer. For each family, a typical nonmycorrhizal seedling is at left, the smallest endomycorrhizal seedling is in the middle, and the largest endomycorrhizal seedling is at right.

This and earlier work (Bryan and Kormanik 1977, Bryan and Ruehle 1976) indicate that endomycorrhizae can have a significant effect on the size of sweetgum seedlings as well as reducing the number of substandard individuals. The erratic growth and consistent high percentage of substandard sweetgum seedlings produced in forest nurseries is apparently not from improper nutritional levels but from the lack of sufficient endomycorrhizal inoculum as a result of soil fumigation. Periodic fumigation is used in hardwood nurseries to control destructive pathogens and to control weeds. This practice can also destroy the endomycorrhizal inoculum in the zone of effective fumigation. Until the fumigated zone becomes naturally re-infested with viable inoculum or the roots grow below it and into the proximity of viable inoculum, sweetgum seedlings will not develop endomycorrhizae and may not develop properly. Since our control seedlings were not exposed to natural mid-season infection, because of thorough fumigation of the whole soil medium, they remained stunted throughout the season and did not exhibit late summer growth so commonly found in production nurseries. This absence of midseason infection may account for the high mortality observed here and earlier (Bryan and Kormanik 1977).

In addition to contributing to the enhancement of nutrient uptake, endomycorrhizae have also been shown to be beneficial in water uptake and transport by plants (Safir and others 1971, 1972). We observed that cessation of diurnal leaf wilting is an indicator of mycorrhizal formation. For 2 or 3 weeks after germination, the leaves of seedlings wilt during the day. Soon after the seedlings cease wilting, height growth normally commences and leaves expand rapidly. In the nonmycorrhizal plots, seedlings continue to exhibit diurnal wilting and leaves eventually turn red or purple.

Additional observations are required before much significance can be placed upon ecotypic adaptations to endomycorrhizal infection. The half-sib progeny from mother tree upland selections did grow significantly better than progeny from bottomland parents. However, except for the progeny from mother trees 4 and 7, both bottomland selections, the average root collar diameters of all families exceeded that deemed necessary (0.763 cm) by Belanger and McAlpine (1975) for acceptable outplanting performance. These results and other work in progress indicate that it may be practical to evaluate selected mother trees as to their potential for producing a high percentage of plantable sized seedlings when nursery soils are infested with specific endomycorrhizal symbionts. It is not unreasonable to assume from these data that the significant ecotype response was nothing more than a mother tree response unrelated to planting site.

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These are the proceedings of a symposium consisting of 24 papers, an introduction, and a review of resolutions and recommendations. The symposium was arranged by the Commonwealth Forestry Institute, Oxford University, on behalf of the Linnean Society of London, and the International Union of Forestry Research Organizations for the following purposes according to the "Report on resolutions and recommendations":

- (*i*) to examine existing knowledge and current activities in variation, breeding and conservation of tropical forest trees,
- (*ii*) to evaluate their application to practical tropical forestry programmes, in particular the development of plantations of fast growing species for industrial purposes, and
- (*iii*) to consider methods of genetic conservation, both *in situ* and *ex situ* in their widest context.

Strongest emphasis fell on topics *i* and *iii*, with very little on plantation culture, beyond the point of producing genetically improved seed. Plantation culture will undoubtedly