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ENDOMYCORRHIZAE INFLUENCE GROWTH OF BLACKGUM SEEDLINGS IN FLOODED SOILS¹

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ABSTRACT

Nyssa sylvatica seedlings grown for a year under flooded conditions established endomycorrhizal associations with *Glomus mosseae*. Lowland ecotypes with endomycorrhizae showed a significant increase in biomass over non-mycorrhizal controls; the greatest difference was in aboveground biomass. Upland ecotypes survived flooding poorly and established fewer endomycorrhizae than lowland ecotypes and did not show any enhanced growth over controls. In general, most endomycorrhizae were formed near main roots and significantly decreased in abundance with distance from the main axes of the root system. It is suggested that under flooded conditions internal oxygen transport may be limiting to mycorrhizae in the more distal roots.

THE IMPORTANCE of mycorrhizal associations between roots and fungal symbionts is well known. The vesicular-arbuscular (VA) endomycorrhizal types occur on more plant species and are more widely distributed geographically than any other type. They are considered to be ubiquitous in soils, with one exception, viz., continuously flooded sites. Recent studies indicate that some aquatic plants may form mycorrhizal associations (Sondergaard and Laegaard, 1977; Bagyaraj, Manjunath, and Patil, 1979); more often though, aquatics and emergent plants growing in wet places are non-mycorrhizal. In these latter plants, it seems that wet growing conditions are responsible for the non-mycorrhizal habit since such plants often become mycorrhizal if the water table drops or if they are transplanted to well-drained soil (Gerdemann, 1968; Filer, 1975).

As part of a larger study of flood tolerance in ecotypes of the deciduous hardwood *Nyssa sylvatica* (Blackgum), this study was initiated to answer the following questions: 1) Do blackgum seedlings establish mycorrhizal associations under continuously flooded conditions? 2) Do mycorrhizae influence the growth of blackgum seedlings? and 3) Are there differences in these responses between upland and lowland ecotypes?

MATERIALS AND METHODS—*Plant and fungal species*—*Nyssa sylvatica* Marshall seeds were collected from trees growing on well-drained, upland sites and from trees growing on waterlogged lowland sites in the piedmont of Georgia. Previous studies have shown that

these upland and lowland plants are ecotypically differentiated with respect to numerous physiological and morphological traits (Keeley, 1979). Rhizosphere inoculum of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, a VA endomycorrhizal fungus, was obtained from Dr. D. H. Marx (USDA Forest Service, Institute for Mycorrhizal Research and Development, Forest Science Lab., Athens, Georgia). (Since this work was completed, the culture of *G. mosseae* has been found to also contain *Glomus etunicatus* Becker and Gerdemann.) *Glomus mosseae* was considered appropriate since, under field conditions, upland ecotypes of *Nyssa sylvatica* will form VA mycorrhizae (Driver, 1950) and under drained conditions in a greenhouse both upland and lowland ecotypes will form mycorrhizal associations with *G. mosseae* (Keeley, unpubl.). Also, VA endophytes are not considered to be highly host specific (Mosse, 1973).

Experimental techniques—Prior to inoculation of the blackgum seedlings, a mycorrhizal and a non-mycorrhizal inoculum were prepared for cross-inoculation (to account for the introduction of other microorganisms) as follows. Sorghum seeds were germinated in 15-cm pots filled with a sandy loam soil of relatively low fertility which was steamed three times on alternate days, each time for 8–10 hr at ca. 85 C. Half of the sorghum seedlings were inoculated with *G. mosseae*; after 3 months, 100-ml samples of soil plus rhizosphere from these pots constituted the mycorrhizal inoculum. Similar samples from the non-inoculated seedlings were the non-mycorrhizal inoculum. After inoculation, the other associated rhizosphere microorganisms were standardized in all pots as follows. Leachates of mycorrhizal and non-mycorrhizal rhizosphere soil from sor-

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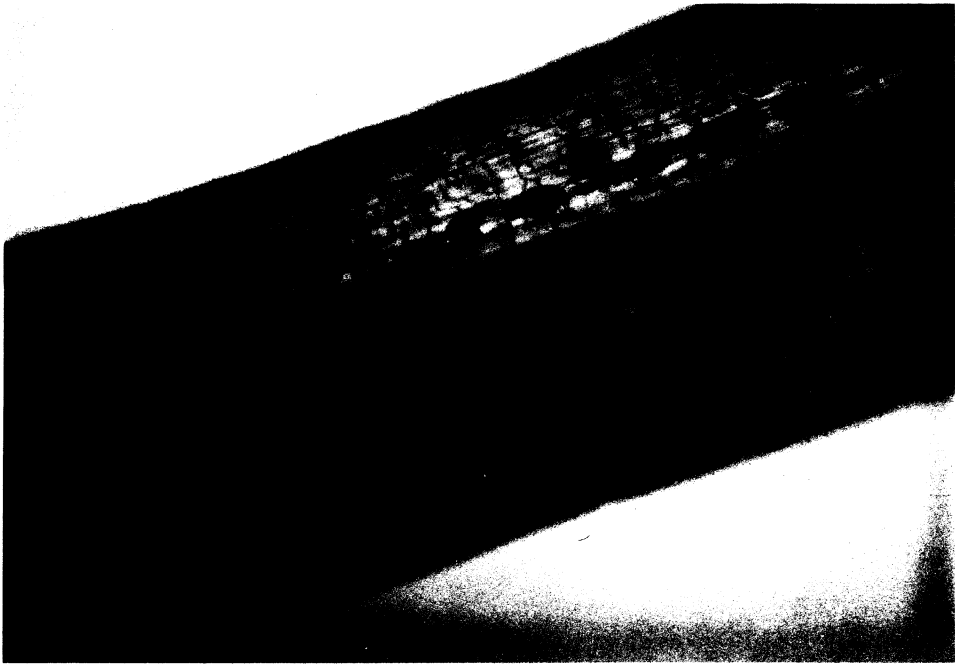


Fig. 1. Medium infection of *Endogone mosseae* in lowland *Nyssa sylvatica* root after 1 year flooded conditions.

ghum were prepared by incubating soil (1%, v/v) with distilled water for 18 hr and filtering them twice through filter paper and a 44- μ m-pore screen. Filtering removed the *Glomus* spores from the mycorrhizal leachate but did not remove other microorganisms (Marx, Bryan, and Campbell, 1971). Test pots with non-mycorrhizal sorghum roots received 50 ml of mycorrhizal leachate, and test pots with mycorrhizal sorghum roots received 50 ml of non-mycorrhizal leachate.

Blackgum seeds from upland and lowland ecotypes were each sown in 24 pots (same soil and steam treatment as above). Twelve pots of each ecotype were inoculated with *Glomus* and placed in a 30-cm high plastic-lined flood tank; twelve pots without *Glomus* were placed in a separate but identical tank to minimize possible contamination. At the time of sowing, the water level in the tanks was at the soil surface. After one month seedlings were thinned to two per pot and the water level was raised to 3–5 cm above the soil surface.

After one year in the greenhouse, without supplemental lighting or fertilizer treatments, all plants were harvested; root samples were fixed, cleared and stained with a modified version of Phillips and Hayman's (1970) procedure, and the remaining roots, stems and foliage were oven-dried (100 C) to constant weight and weighed.

RESULTS—All blackgum seedlings from soil inoculated with *G. mosseae* were endomycorrhizal (Fig. 1). All control seedlings were non-mycorrhizal. Several forms of infection were observed, e.g., vesicles, hyphal coils, and arbuscules; however, vesicles were the most prevalent form of infection (Fig. 1). To quantify the extent of infection for each ecotype, 250 1-cm sections were randomly selected from six seedlings and tallied as high, medium or low infection (where Fig. 1 represents a medium infection). In lowland plants 32% of the roots were highly infected and 45% with low infection. The upland plants had only 5% highly infected and 90% with low infection.

One observation which was unexpected was that in the lowland roots, mycorrhizal fungi tended to be most abundant near the main axes of roots and less abundant in the more distal ones. To quantify this, long secondary roots (<1 mm diam.) were tallied for frequency of highly infected 1-cm sections vs. distance from the point of origin with the next higher order root (>1 mm diam.). Spearman's rank correlation coefficient showed a highly significant ($r_s = 0.78$, $P < 0.01$) positive correlation between frequency of highly infected cells and proximity to the main axes of the root system.

A comparison of growth for mycorrhizal and non-mycorrhizal seedlings (Table 1) shows that: 1) lowland ecotypes have significantly

TABLE 1. Biomass (g oven-dry weight) of 1-year-old *Nyssa sylvatica* ecotypes continuously flooded with and without mycorrhizae (each treatment was begun with 24 seedlings)

		Control			Mycorrhizal		
		$\bar{x} \pm \text{S.E.}$	N	t^a	$\bar{x} \pm \text{S.E.}$	N	
Lowland	Aboveground	0.86 \pm 0.11	23	**	1.93 \pm 0.38	24	
	Belowground	1.05 \pm 0.16	23	*	2.07 \pm 0.48	24	
	Total	1.91 ^b \pm 0.25	23	*	4.00 ^c \pm 0.86	24	
Upland	Aboveground	0.39 \pm 0.05	24	NS	0.56 \pm 0.11	20	
	Belowground	0.52 \pm 0.006	24	NS	0.66 \pm 0.13	20	
	Total	0.95 ^b \pm 0.11	24	NS	1.25 ^c \pm 0.24	20	

^a Variances were significantly heterogeneous (F_{\max} test) so treatments were compared with the approximate t -test (Sokal and Rohlf, 1969, p. 374–375).

* $P < 0.05$.

** $P < 0.01$.

^{b,c} $P < 0.01$ that the lowland ecotype is greater than the upland ecotype.

NS $P > 0.05$.

greater biomass under flooding when mycorrhizae are established, 2) this difference in ecotypes is most pronounced in aboveground parts (stems and leaves), and 3) upland ecotypes do poorly under flooded conditions and mycorrhizae do not significantly influence their growth.

DISCUSSION—It is apparent from these results that *Glomus mosseae* will establish mycorrhizal associations with *Nyssa sylvatica* roots under continuously flooded conditions and that for lowland ecotypes this can have a significant effect on growth. This enhanced growth is most obvious in the aboveground parts as the result of a lower root/shoot ratio in mycorrhizal plants (1.07 vs. 1.22). Upland plants survive flooded conditions poorly, mycorrhizae are only sparsely established and have no discernible effect on growth.

In lowland roots the diminishing extent of infection with distance from the main axes is very likely related to the availability of oxygen. Mycorrhizal fungi are aerobic and thus under flooded conditions would be dependent upon their host for oxygen (Read and Armstrong, 1972). Under flooded conditions, *Nyssa sylvatica* transports oxygen from the stems to the roots (Keeley, 1979). The process is undoubtedly one of gaseous diffusion through intercellular spaces (Coutts and Armstrong, 1976), so the extent to which a root will be oxygenated will depend upon the oxygen concentration gradient and the distance between the root and stem. Consequently, more distal roots should be less oxygenated than ones closer to the main axes.

The report here of mycorrhizal establishment in flooded blackgum roots is somewhat at variance with field studies. For example,

Filer (1975) found that sweetgum (*Liquidambar styraciflua*) trees growing in periodically flooded reservoirs did not have mycorrhizae in roots initiated under short periods of flooding. Also, preliminary findings by Dr. R. Best (Institute of Ecology, Univ. of Georgia, Athens, pers. commun.) indicate that in the Okefenokee Swamp of south Georgia, *Nyssa sylvatica* roots are non-mycorrhizal. There are two likely explanations for these differences observed between field and greenhouse studies. One hypothesis is that they reflect the difference between seedlings (used in this study) and mature trees (used in field studies). Mechanistically, this difference could arise because the pathway of oxygen diffusion, from stem to roots, increases as the tree enlarges; as a consequence, the oxygen concentration in the distal roots of large trees may be too low to support mycorrhizal fungi. Another hypothesis is suggested by the observation that increased soil nutrients (particularly phosphate) will tend to reduce mycorrhizal formation since the plant is less dependent on the fungus for obtaining adequate nutrient supplies (Mosse, 1973). It may be that the soil nutrient pools in flooded lowland sites are high enough to preclude mycorrhizal formation. There are several reasons why this might be true. Whereas the soil used in this study was of quite low fertility, and nutrients were not added, many lowland swamps act as nutrient sinks (Kitchens et al., 1975). Since plants in flooded soils have the added cost of supplying oxygen to the fungus, it may be that just slightly enhanced nutrient conditions are sufficient to shift the cost:benefit relationship against mycorrhizal maintenance. This seems particularly likely in light of the demonstration that reducing conditions, brought on by flooding, may greatly increase

phosphate concentrations in the soil solution (Patrick and Mahapatra, 1968); this would minimize the benefit of maintaining mycorrhizal fungi under flooded conditions.

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