FOOD RESERVES IN MOUNTAIN LONGLEAF PINE ROOTS DURING SHOOT ELONGATION

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Abstract—Survival and growth of longleaf pine seedlings depends upon a well-developed root system. Soil moisture is also critical for the seedling to emerge from the grass-stage. When longleaf pine seedlings emerge from the grass stage, they grow rapidly in height and diameter. Branches are often few in number and, if present, may have low photosynthesis rates. This growth pattern is seen on all longleaf pine sites, including low fertility mountain soils. Root growth patterns on poor soils suggest that biochemical adaptations are occurring when compared to those of Coastal Plain soils. Our results show that roots of mountain longleaf pine have a normal anatomy but also have unusual amounts of starch when compared to loblolly pine roots growing during phenologically equivalent time periods. Longleaf pine roots from mountain soils appeared large in diameter and appeared to grow much nearer the soil surface than roots we observed from Coastal Plain longleaf pine. Among the variables examined to determine root food reserves, numbers of starch grains were found to be easiest to quantify. Starch grains were large in size and uniformly filled root cells. Nuclear staining served to verify the observed root cells were healthy. These results yield methodology potentially useful in assessment of health and productivity of longleaf pine.

INTRODUCTION

Longleaf pine (*Pinus palustris* Mill.) is considered as resistant or highly tolerant to many diseases and insects that adversely affect other southern pine species (Derr 1966, Mann 1969). Under some conditions, prescribed burning has been associated with increased mortality of mature longleaf pine (Otrosina and others 2000). The increased mortality is also associated with presence of certain root infecting fungi (Ophiostomatoid fungi such as *Leptographium* species and the root rot pathogen *Heterobasidion annosum* (Fr.)Bref.) not previously thought to be pathologically important in this tree species but may be indicators of ecosystem stress. This study is part of an ongoing project designed to measure and explain root mortality in longleaf pines that receive prescribed fire infrequently or have prescribed fire reintroduced after a long interval. To accomplish this goal, we are employing histological and statistical methods to identify variables in roots that are associated with this mortality. We attempt to quantitatively and qualitatively characterize fine root tissues we define as healthy; those roots having high food reserves, low mortality, and normal cortical cell nuclei. Means for histological variables we investigated can become standards to evaluate efficacy, effect, and consequences of fire and other silvicultural practices as well as to interpret other root pathological activity reported previously (Otrosina and others 2000).

MATERIALS AND METHODS

Longleaf pine saplings used in this study were approximately 10 years old and located in the Talledega National Forest (figure 1). Roots from 12 saplings undergoing rapid height growth were chosen because they must develop rapidly and extensively to support height growth rates of 1.0 to 2.0 meters per year. The stand of several thousand sapling longleaf pine was approximately 3.5 m in height. The randomly sampled trees ranged from 6.0 to 8.0 cm d.b.h. (diameter at breast height). Buds had not expanded at the time of sampling.

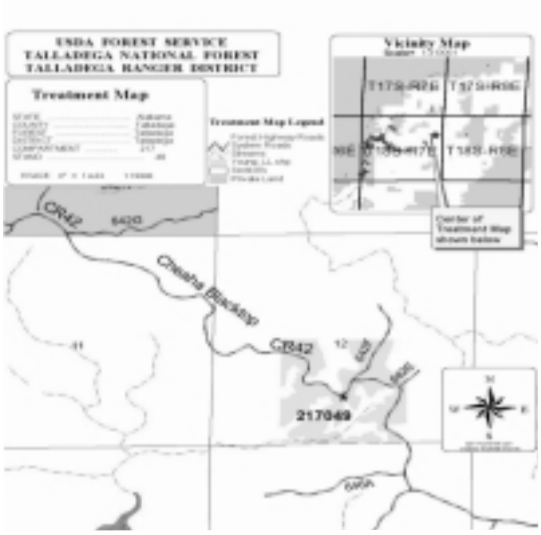

Figure 1—Location of longleaf pine saplings used in this study.

Roots were also sampled from randomly selected adult longleaf pine in northwestern South Carolina (Savannah River Site, New Ellenton SC). Trees sampled at this

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Table 1—Description of variables used in longleaf pine root histological studies

VARIABLE	P-VALUE [a]	DEFINITION
Abnormal Cambium	0.500	Cambial initials reduced in number or out of alignment. Necrotic derivations present.
Bark Formed	0.0001	Intact layer of bark cells similar to those found in the stem encompasses the root. Protects the root from injuries and microbial invasion.
Dead Roots	0.231	Cells with ruptured membranes. Tannin adhere to cell walls. Chromatin abnormal. Starch grains may or may not be present.
Large Root	0.0112	Roots more than 3 mm in diameter.
Nuclear Stain (abnormal)	0.231	Indicates degeneration (pyknosis) of chromatin.
Number Of Starch Grains	0.0189	Number of starch-containing plastids per cell viewed at a single focus per cell length at 100 to 500 diameters.
Small Roots	0.0326	Actual measurement of roots less than 3 mm.
Size of Starch Grains	0.0008	Size of starch grains scored as 1, 2, or 3 for each cell. Range in actual size was 0.5 to 4.0 microns.
Starch Use	0.0001	Starch grains 50 percent or more hydrolyzed.
Tannin	0.5393	Accumulation of excess tannin-containing cells in the cortex, rays and inner xylem.

[a] From ANOVA that compared values for variables in roots of 12 saplings.

Table 2–Variation in starch grain number, size, and use in fine roots of sapling longleaf pines

Sapling	Number of roots	Number of grains per cell	Relative size of grains [a]	Starch use index [a]
1	21	20.2	2.67	0.048
2	14	21.2	2.78	0.071
3	23	17.3	2.30	0.087
4	15	16.3	2.40	0.067
5	16	15.1	2.25	0.125
6	14	17.1	2.50	0.214
7	13	17.5	2.08	0.231
8	19	21.3	2.68	0.579
9	14	18.1	2.43	0.0714
10	18	18.5	2.89	0.444
11	24	17.0	2.71	0.083
12	19	18.5	2.74	0.211

[a] See table 1 for definitions.

location were from unburned plots that are part of a prescribed burning study previously reported (Otrosina and others 2000). The Alabama and South Carolina locations are at equivalent latitudes and under management by the USDA Forest Service. The adult trees ranged from 25 to 35 cm d.b.h. Buds had not expanded at the time of sampling.

From both locations, roots within 8.0 cm of the soil surface were collected from the main laterals and immediately placed in formalin:acetic acid:alcohol (FAA) fixative solution (Sass 1951). After several weeks in FAA, root pieces 3-4 mm long were rinsed thoroughly with 70 percent ethanol, dehydrated, in 100 percent ethanol, paraffin embedded, and sectioned and 8-15 microns. Staining involved a number of acidic and basic dyes (Hass 1980, Horobin and Bancroft 1998, Preece 1972). See references for pertinent literature regarding detailed histological procedures.

Root tissue mortality was scored when nuclear stains were abnormal and expressed as percentage of fine roots examined. Abnormally staining nuclei appeared as grey to dull brown rather than red or blue-grey in a microscopic field of about 100 microns in radius. Other variables measured are in table 1.

RESULTS

Roots from the 12 mature longleaf pines had less starch, more tannin, and higher mortality than that of saplings:

VARIABLE	SAPLING [a]	MATURE [a]
No. starch grains	15.1 - 21.3	1.60 - 6.40
Tannin [b]	.048 - .261	0.54 - 0.90
Mortality [b]	.000 - .071	0.10 - 0.67

[a] Values indicate range.

[b] Proportion of roots with this variable.

Nuclei stained normally in roots from 11 of the 12 saplings and in 50 percent of the roots from adult trees. A similar result was obtained in evaluating cambial condition: 96.2 percent were normal in sapling roots and only 76.2 percent

were normal for the mature trees. Means for the roots from the 12 saplings were not significantly different for the following variables: abnormal cambium, root mortality, nuclear stain, small roots, and tannin accumulation. Also, for the saplings, variables with the largest and statistically significant differences among means occurred in size of starch grains and starch grain use (tables 1 and 2). Roots from sapling number 8 had significantly (α =0.05) higher numbers of starch grains (21.3) and greater use of starch (0.579) than the other root specimens. Moreover, the proportion of tannin-containing cells in roots from this tree was only 0.0526 compared to an over all mean of 0.124.

DISCUSSION

Roots of saplings appear to be models for healthy tissues in longleaf pines. They contain high numbers of large starch grains and have active nuclei. Only one root died and the proportion of roots with excess tannins was much lower compared to mature trees. Roots from the adult trees reflect a number of processes that appear to be minimal in the saplings. For example, the high numbers of dead roots in adult trees might imply a much greater turnover rate relative to the young saplings.

Thus, studies involving root metabolism interactions with silvicultural treatments should take advantage of sapling root vigor as a standard reference point for comparison. The variable, starch use, was particularly sensitive for comparing roots on different saplings. Although we did not measure the sink for the glucose that results from starch hydrolysis, large quantities of simple carbohydrates would be needed to sustain growth of the root system and for rapid top growth (1.0 to 2.0 m per year) (Allen and Scarbrough 1969). This sensitivity can also confound data interpretation as they related to site productivity and silvicultural regime, and underscores the necessity of further comparative studies.

On the other hand, the dramatically lower starch concentration in mature tree roots may imply a degree of stress and unthriftiness. This is supported by our data on the high amount of infected roots present on this site (Otrosina and others 2000). Assessment of root vigor and health by application of these standard histological procedures will contribute to evaluation of various silvicultural treatments and their effects on forest health and productivity such as being conducted by Haywood (2000).

CONCLUSION

This study begins to address a void in our knowledge of the histological parameters that can be useful in evaluating effects of silvicultural treatments in longleaf pine and other pine species. Further comparative studies are needed over a wide range of sites, silvicultural treatments, age classes, and pathological conditions. Once baselines and key variables are established, these techniques will permit forest health assessment over wide geographic areas.

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