ASSESSMENT OF LOBLOLLY PINE DECLINE IN CENTRAL ALABAMA

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Abstract—Loblolly pine (*Pinus taeda* L.) decline has been prevalent on upland sites of central Alabama since the 1960's. The purpose of this study was to compare Forest Health Monitoring (FHM) standards and protocols with root health evaluations relative to crown, stem, and site measurements. Thirty-nine 1/6 acre plots were established on loblolly decline sites in nine central Alabama counties. Sites were selected on federal, state, and private industrial lands to measure variables of decline symptoms, age classes and management procedures. A two-root sampling procedure, selective media, and soil baiting assay methods were used to isolate pathogenic root infecting fungi. Pitfall traps collected root-feeding insects from which *Leptographium* species were recovered. FHM indicators of tree crown conditions were recorded on all pines in the plots. Preliminary results showed a significant correlation between live crown ratio and incidence of *Leptographium* spp. We recovered *Leptographium* from damaged roots in eighty-four percent of plots. The pine basal area was significantly reduced with increased incidence of *Phytophthora cinnamomi* Rands, with *P. cinnamomi* being recovered from the soil root zone in 50 percent of the plots. Histological examination of root damage indicated a significant correlation between reduced growth and root wounding.

INTRODUCTION

A decline of loblolly pine, *Pinus taeda* L., has been observed on the Talladega National Forest in central Alabama since 1959 (Brown and McDowell 1968). The decline condition was initially referred to as "loblolly pine die-off," and was most frequent in sawtimber-size trees over age 50. During the 1960's and 1970's, studies were initiated to determine the cause of the die-off and the rate of spread. Twenty-four plots were established in loblolly pine stands on the Oakmulgee District to assess decline and mortality. Soil and root samples were analyzed for the presence of root pathogens including *Phytophthora cinnamomi* Rands, a primary factor in the development of littleleaf disease. There was some recovery of *P. cinnamomi* from the decline plots, but it was reported that root system deterioration of the die-off trees was more extensive than that found in littleleaf diseased trees (Brown and McDowell 1968). Although a specific cause was not determined, several observations and conclusions came out of this study. Lateral root deterioration preceded the presence of observable foliage symptoms. Symptoms included sparse crowns, chlorotic needles, reduced radial growth at age 40-50, and heavy cone crops occurring prior to mortality. Mortality occurred 2 to 6 years after onset of symptoms. Also, a large percentage of the fine roots died before tree mortality occurred (Brown and McDowell 1968).

The decline symptoms were most severe on the Oakmulgee Ranger District near Centreville, AL, which falls within the Upper Gulf Coastal Plain Province. During the 1940's and 1950's, other surveys in the Upper Coastal Plain reported damage to shortleaf pine (*Pinus echinata* Mill.) stands caused by littleleaf disease. This disease was strongly associated with *P. cinnamomi* on sites with low fertility and poor internal drainage (Campbell and Copeland 1954, Roth 1954). Loblolly pine was also affected by littleleaf disease when associated with diseased shortleaf pine sites (Campbell and Copeland 1954).

The forests of the Oakmulgee were predominately longleaf pine (*Pinus palustris* Mill.) during the pioneer settlement era of the early 1800's. From 1908 until 1929, most of these trees were harvested for lumber, and the land was converted to agricultural use. During the 1930's and 1940's, federal acquisition programs relocated farm families and established National Forests (Johnson 1947). Abandoned farmland then regenerated to loblolly pine and shortleaf pine.

Management recommendations from the 1960's and 1970's for the Oakmulgee Ranger District were to prevent decline situations by reducing the rotation age of loblolly pine stands from 70 to 60 years and by maintaining basal area at 60 to 70 ft² per acre. Recommendations also

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Table 1—Number of plots by location, physiographic region, and land management ownership

Number of Plots	Soil Physiographic Regions Of Alabama	County	Land Management Ownership
9	Piedmont	Clay, Cleburne, Talladega	Talladega National Forest (Federal)
5	Ridge & Valley	Calhoun	Choccolocco State Park (State)
16	Coastal Plain	Bibb, Hale, Chilton, Perry	Talladega National Forest (Federal) (Oakmulgee R.D.)
9	Cumberland Plateau	Tuscaloosa	Gulf State Paper Corporation (Private-Industrial)

included the conversion of declining loblolly pine stands to longleaf pine. To date, approximately 20,000 acres have been converted to this species. However, there are still about 40,000 acres of loblolly stands on the Oakmulgee District in various stages of decline. A recent biological evaluation of decline sites on this District found that *P. cinnamomi* and *Pythium* sp. are predominant pathogens associated with loblolly pine fine root deterioration (Hess and others 1999).

Other areas of central Alabama have reported loblolly decline, including National Forest lands in the Anniston/ Heflin area of Alabama (Hess 1997). Forest industry land managers reported declining loblolly stands in Tuscaloosa and Bibb Counties (Allen 1994). Loblolly pine decline on littleleaf diseased sites on two National Forests within the Piedmont Physiographic Region of South Carolina has been found (Oak and Tainter 1988). In addition, loblolly pine decline associated with *P. cinnamomi* and *Pythium* species was also reported in Louisiana (Lorio 1966).

Since its inception in 1990, Alabama has participated in the USDA Forest Service's Forest Health Monitoring (FHM) Program. A main objective of this program is to develop and implement standards and protocols for assessing conditions of forest resources. Preliminary analyses of FHM (P3) plots and other survey data collected in Alabama and other southern states from 1995 through 1997 identified 30 percent of loblolly pine stands as having decline symptoms (Steinman and others 2000). This FHM Evaluation Monitoring Project represents further investigation of causal agents associated with the decline of loblolly pine within central Alabama. The purpose of this study was to compare the presence of pathogens in roots and soil with tree growth and FHM indicators on loblolly pine decline sites.

METHODS

Plot Description

Sites for plot establishment were selected on federal, state, and private industrial lands. Thirty-nine 1/6 acre sample locations in nine central Alabama counties were selected in the spring of 2000. Plot establishment followed the FHM guidelines (Dunn 1999), using a cluster of four 1/24 subplots. The plot locations fell within four Physiographic Regions of Alabama: the Piedmont, Ridge and Valley, Upper Coastal Plain, and Cumberland Plateau (table 1). At each location, root health assessment was accomplished by selecting three dominant, or co-dominant, symptomatic pines nearest the plot center of the center subplot. Root sampling was done with the modified two-root excavation method (Otrosina and others 1997).

Radial growth was measured by obtaining an increment borer core at breast height (BH) of each of the sample trees. With the aid of hand lenses, increment cores were measured for five and ten year radial growth increments and age.

FHM Indicators of Tree Crown Conditions

Tree crown conditions were measured on all pine trees (DBH ^{3} 5.0) within all 39 plots. The crown measurements included live crown ratio, crown light exposure, crown density, crown dieback, and foliage transparency. Live crown ratio is a measure of crown length and its relationship to total tree height. Crown light exposure and crown position are combined in analysis to determine stand and canopy structure. Once the live crown ratio, crown light exposure, and crown position are determined, the next step is to measure how much of a crown exists. Crown density, which includes foliage, branches and reproductive structures, measures the crown biomass. Crown dieback defines how much of the crown does not have foliage but has fine twigs, indicating a loss of vigor or growth potential. Foliage transparency estimates how dense the foliage is on branches, indicating a loss of vigor or stress due to foliage damage or defoliation (USDA Forest Service 2000).

Insect Interactions

Pitfall traps were installed on 15 of the 39 plots, and insects collected weekly from April 17th to June 5th, 2000. Traps were constructed of 20 cm lengths of 10 cm

Table 2—Sample plot means of tree measurements by types of forest ownership

Measurements	Public (n = 30 plots) Mean	S.E.	Industry (n = 9 plots) Mean	S.E.	Probability
All overstory pine trees					
Stand age (years)	46	2	36	2	0.0265
Total pine basal area (ft²/acre)	69.3	3.4	63.3	7.1	0.41
DBH (inches)	10.0	0.3	10.2	0.5	0.76
Live crown ratio (pct)	38	1	41	1	0.17
Foliage transparency (pct)	32	0	29	1	0.00
Crown dieback (pct)	1	0	0	0	0.67
Crown density (pct)	39	1	40	2	0.70
Plot sample trees					
DBH (inches)	11.4	0.5	11.0	0.7	0.67
Last 5-yr basal area increment (ft²)	0.09	0.01	0.11	0.02	0.18
Last 10-yr basal area increment	0.18	0.02	0.20	0.02	0.42
Live crown ratio (pct)	37	1	41	2	0.10
Foliage transparency (pct)	31	1	29	1	0.05
Crown dieback (pct)	1	0	0	0	0.71
Crown density (pct)	39	1	39	1	0.85

diameter drainpipe with eight entrance holes spaced around the pipe. The interior of each trap was coated with liquid Teflonä to prevent insect escape. Ends were capped with plastic lids and two holes were drilled in the bottom end for drainage. Traps were placed so that the entrance holes were slightly above ground level. Each trap was baited with two 8 ml glass vials, one containing 95 percent alcohol and one containing turpentine. Two freshly cut pine stems were also placed inside the traps (Klepzig and others 1991). Trapped insects were placed in sterile polyethylene specimen cups and maintained for two to three days at 4°C until isolations could be made. Insects were inventoried and rolled across cycloheximide-streptomycin amended malt extract agar (CSMA—2 percent MEA containing 800 ug/ml of cycloheximide and 200 ug/ml of streptomycin sulfate) and unamended malt extract agar (MEA) (Hicks and others 1980). Agar plates were incubated at 25°C and colonies resembling *Leptographium* were transferred to sterile plates or slants of MEA.

Processing Roots

Two primary roots from each sample tree were excavated using hand tools, beginning at the root collar and extending out to the tree drip line. The primary roots were then cut from the tree and removed. All soil samples were collected adjacent to the roots.

Root samples were collected during April, May, and June of 2000. The fine roots from each primary root were excised, bagged, labeled and maintained in the field on ice. The

Table 3—Correlations between crown vigor associated with plot sample trees

	Crown dieback (pct)	Crown Density (pct)	Foilage Transparency (pct)	Live Crown Ratio (pct)	BAI[1] Last 5 years (ft²/tree)	BAI Last 10 years (ft²/tree)
	- - - - - - - - Pearson correlation coefficient - - - - - - - -					
	- - - - - - - Probability of significance - - - - - - -					
BAI last 5 years	-0.15	0.38	-0.20	0.54		
(ft²/tree)	0.36	0.02	0.21	0.00		
BAI last 10 years	-0.23	0.43	-0.10	0.49		
(ft²/tree)	0.15	0.01	0.52	0.00		
Pine basal area	-0.10	-0.15	-0.19	-0.04	-0.27	-0.24
(ft²/acre)	0.55	0.35	0.25	0.79	0.09	0.13
Stand basal area	-0.06	-0.14	-0.02	-0.11	-0.25	-0.19
(ft²/acre)	0.71	0.40	0.88	0.50	0.13	0.26

[1] BAI = basal area increment

Table 4—Sample plot means of tree measures of vigor by incidence of *Leptographium* spp

Measurements	Incidence of *Leptographium* in roots or soil: 0 pct (n = 4 plots) Mean	S.E.	33 pct (n = 8 plots) Mean	S.E.	67 pct (n = 16 plots) Mean	S.E.	100 pct (n = 11 plots) Mean	S.E.	F Probability
Total pine basal area (ft²/acre)	73	16	70	7	63	3	73	6	0.52
Last 5-yr basal area increment (ft²)	0.11	0.04	0.10	0.01	0.09	0.01	0.07	0.01	0.32
Last 10-yr basal area increment (ft²)	0.20	0.05	0.20	0.02	0.20	0.02	0.15	0.02	0.34
DBH (inches)	12.4	1.9	11.1	0.7	11.2	0.6	11.4	1.0	0.86
Crown dieback (pct)	1	1	0	0	0	0	1	1	0.14
Crown density (pct)	38	2	41	2	41	1	37	2	0.24
Foliage transparency (pct)	29	1	29	1	32	1	30	1	0.14
Live crown ratio (pct)	42	3	42	2	37	2	35	1	0.06

primary roots were also randomly chipped or cut into pieces, bagged, labeled, and iced for transportation to the laboratory. Roots were excavated from 117 trees, with 234 primary roots sampled, along with collections of fine roots and soil samples from the root zones. Root samples for isolation of fungi from primary and fine roots were transported to Louisiana State University, Plant Pathology Laboratory, Baton Rouge, LA.

Isolation of Microorganisms

Phytophthora spp. and *Pythium* spp. were isolated from fine roots and soils using three methods. The first method used the selective medium PARP(H) (Ferguson and Jeffers 1999). Eight to ten pieces of fine roots (< 2mm diameter) were washed, dried, cut into 2 cm lengths, and plated on PARP(H). The specimens were incubated in the dark at 20°-25°C for 3 days. Subcultures were established from *Phytophthora*-like fungi growing from the roots. The second method of isolating was soil assay. Soil samples were assayed from a soil suspension on PARP(H) (Jeffers 2000). The plates were examined for *P. cinnamomi* after incubation for 48 to 72 hours in the dark. A third isolation method employed for *Phytophthora* and *Pythium* species was baiting. Soil samples collected during root excavation were incubated in Petri plates with fresh camellia, juniper, or pine stems (Jeffers 2000). Plates were incubated at 24 and 72 hours, after which we checked for characteristic *Pythium* or *Phytophthora* sporangia.

Isolation of Ophiostomatoid fungi from primary roots utilized selective media. Roots were cut into small pieces, rinsed in tap water, decontaminated in 10 percent commercial bleach, treated with 10 percent ethanol solution for one minute, rinsed again in tap water for three minutes, and blotted dry. Four pieces were placed in a Petri plate containing selective medium (CSMA) to isolate *Leptographium* species. Plates were incubated at 25°C and *Leptographium* isolates were subcultured from hyphal tips and conidial heads onto MEA. Subcultures were maintained in MEA slants and stored at 8°C until identified.

Table 5—Sample plot means of tree measures of vigor by incidence of *P. cinnamomi*

Measurements	-----Incidence of *P. cinnamomi* in roots or soil---------- 0 pct (n = 19 plots) Mean	S.E.	33 pct (n = 12 plots) Mean	S.E.	67 pct (n = 5 plots) Mean	S.E.	100 pct (n = 3 plots) Mean	S.E.	F Probability
Total pine basal area (ft²/acre)	80	4	55	4	56	5	63	9	0.00
Last 5-yr basal area increment (ft²)	0.09	0.01	0.10	0.02	0.08	0.01	0.11	0.04	0.70
Last 10-yr basal area increment (ft²)	0.18	0.02	0.19	0.03	0.18	0.01	0.20	0.07	0.96
DBH (inches)	11.8	0.6	11.1	0.8	10.5	0.7	11.3	2.1	0.8
Crown dieback (pct)	0	0	1	0	0	0	1	1	0.77
Crown density (pct)	39	1	39	2	39	4	41	2	0.95
Foliage transparency (pct)	30	1	31	1	33	2	30	3	0.55
Live crown ratio (pct)	38	1	38	2	39	2	34	6	0.72

Soil samples were analyzed for *Leptographium* sp. by removing a 10 g aliquot from thoroughly mixed soil previously collected near lateral roots. Root fragments were removed from the aliquot by sieving and suspending them in 40 mls of sterile, 0.5 percent water agar. One milliliter of this suspension was pipetted into ten petri dishes containing 10 mls of CSMA. Dishes were incubated at 25°C and examined daily for the presence of fungus. Isolates were transferred to MEA slants and stored at 8°C until they could be identified.

Root Damage Assessment

During the root excavation and sampling procedures, a sub-sample of 17 plots was chosen to evaluate fine root damage through histological examination. Random samples of unwashed fine roots were taken from the primary roots and placed in formalin/acetic acid/alcohol fixative (FAA) for 14 days (Sass 1951). Fixed root specimens were cut to 1 to 3 mm, dehydrated in an alcohol series, embedded in paraffin, and sliced into 7 to 10 µm transverse sections. Slides were stained with a variety of schedules, including Papanicolaou's hemotoxylin-eosin or an acid-Schiff procedure (Hass 1980, Horobin and Bancroft 1998). Stained sections where observed under a light microscope and then catalogued into damage categories (Walkinshaw and Tiarks 1997, Walkinshaw and others, 2001).

Data Analysis

Measures of stand structure, tree growth, and tree crown and damage conditions were used to summarize decline symptoms of loblolly pine for each plot. Values were compared between the 30 plots located on public land and the 9 plots on industrial ownership to determine if the presence of decline symptoms was related to different types of forest management these ownerships represent. Data were analyzed using T-tests.

Correlations between continuously scaled measurements of stand structure, crown conditions, radial growth, and root conditions were also calculated. These summaries were used to interpret physiological and pathological relationships among different indicators that express decline symptoms. We used the percentage of the three sample trees on each plot with pathogens present to define four categorical levels of pathogen incidence (0, 33, 67, or 100 percent). We then compared plot values for stand structure, tree growth, and tree crown conditions among the categories of pathogen incidence by analyses of variance.

Proportions of damaged roots, root mortality, and number of starch grains in cortical cells were recorded from histological examinations and paired with tree growth and crown variables. Correlations and regression analysis were conducted on these data (Walkinshaw and Otrosina 2001).

RESULTS

Analysis of tree crown condition indicators of the 39 sample plots, compared to the other loblolly pine Forest Health Monitoring plots, shows a difference in transparency of the tree crowns. Transparency (the amount of light filtering through the foliated portion of the tree crown) was considerably greater for our sample plots, as would be expected, as crowns decline and become sparse. Foliage transparency was greater on public land (table 2). Stand age on public land was ten years older than that of industrial land. Tree crown condition indicators showed a significant correlation between DBH growth, crown density, and live crown ratio (table 3). Live crown ratio was less on plots having a greater incidence of *Leptographium* sp. (table 4). On a plot basis, the incidence of *Leptographium* sp. from roots of the 39 sample plots was 84 percent from roots, and 33 percent from soils. Overall *Leptographium* isolation percentage was greater on public lands (93 percent) when compared to industrial lands (55 percent).

Using baiting procedures, *Phytophthora cinnamomi* was isolated from soils in 50 percent of the plots on public land and slightly higher on industrial land (55 percent). *P. cinnamomi* was not recovered from root isolations. Plots with *P. cinnamomi* had less pine basal area than those those plots where *P. cinnamomi* was not found by soil baiting (table 5).

Microscopic examination of 700 fine root pieces from the 17 selected plots showed high incidence of root injury and mortality. The number of starch grains in the cortical cells was reduced and the disposition of tannin was excessive (Walkinshaw and others 2001). The inverse relationship between proportion of roots with injuries and radial growth of the cambium in the last 5 years was significant ($r^2 > 0.50$). Damage to resin canals was also a useful variable in the interpretation of microscopic data (Walkinshaw and others 2001) and was consistent with the observed root pathological status.

DISCUSSION AND CONCLUSIONS

The majority of the plots in this study had trees with decline symptoms, and assessment of woody roots, fine roots, and soil demonstrated the presence of root pathogenic fungi. The results of this assessment are consistent with the observations of Brown and McDowell (1968) who characterized fine root deterioration in 40 to 50 year-old trees prior to the onset of severe decline symptoms. A notable difference between their study and ours lies in the recovery of pathogenic root infecting fungi. Isolation and detection procedures for Pythiaceous and Ophiostomatoid fungi from roots and soil have become more efficient since the Oakmulgee studies of the 60's and 70's (Tainter and Baker 1996). Even though the edaphic parameters of this assessment are not complete, the abundant recovery of *Leptographium* sp. and *P. cinnamomi* from primary woody and fine roots, respectively, and the associated soils, coupled with the ability to evaluate the crown symptoms with established FHM protocols, help to further define the components of loblolly pine decline. *Leptographium* species are associated with pine decline and mortality in connection with root-feeding beetles and weevils that attack living trees (Harrington and Wingfield 1977, Otrosina and others 1997). Hess and others (1999) concluded that *P. cinnamomi* and *Pythium* sp. appeared to be the primary pathogens associated with the deterioration of loblolly pine fine root systems on the Oakmulgee. Although *P. cinnamomi* is considered a primary pathogen causing littleleaf disease, other factors such as poor soil aeration,

low fertility, periodic moisture stress, and other soil-inhabiting microorganisms are also damaging to fine roots (Oak and Tainter 1988). Loblolly pine, although affected by littleleaf disease, is considered less susceptible and was planted to replace shortleaf pine on sites within the historic range of littleleaf disease (Oak and Tainter 1988, Campbell and Copeland 1954). Littleleaf disease of loblolly pine generally has been reported on eroded Piedmont soils. This is in contrast to the somewhat deeper soil profiles we encountered in our initial soil examinations prior to this study. However, our data suggest decisions to plant loblolly pine on sites with similar characteristics and in similar physiographic areas should be approached with caution, especially if planning rotation ages greater than 35-40 years.

This assessment of loblolly decline included plots in four Physiographic Regions, encompassing a zone in central Alabama from the east (Cleburne and Clay counties) to the west (Tuscaloosa and Hale counties). The evaluation of site variables, including soil classification, bulk density, soil porosity and moisture capacity, and soil nutrient analysis will be a key to assessing the influence of soil and root pathogens recovered from these sites and their relationship to crown characteristics of symptomatic loblolly pines. The soil and site measurements will not be completed until late 2001, at which time a complete evaluation and analysis of all data, including site, root, soil, tree growth, crown indicators, and crown damage will be accomplished. The results of this preliminary study indicate: (1) Management on public lands shows that damage and mortality increases with age of the stands, especially after age 40. (2) Loblolly pine decline symptoms are the same as littleleaf disease of shortleaf pine, and preliminary results of our evaluation show a correlation between reduced radial growth and BA, declining crowns, root damage, and recovery of *P. cinnamomi* and *Leptographium* sp. (3) Loblolly decline is prevalent on sites within the historic range of littleleaf disease and is associated with sites and soils other than the heavy clay soils of the Piedmont Province.

Evaluation of loblolly pine decline in central Alabama is ongoing. The goal is to define the parameters of decline sites, develop a predictive risk model, and estimate amount of land affected. The evaluation of edaphic factors is continuing with soil classification, bulk density analyses, and soil porosity analyses in progress. Soil sample collections for nutrient analysis are scheduled for the summer of 2001. These soil variables will then be incorporated into an overall analysis linking management regimes, root pathological assessments, and root feeding insects, which will further define biological foundations of FHM protocols.

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