

**BIOLOGY AND ECOLOGY OF *LEPTOGRAPHIUM* SPECIES AND THEIR
VECTORS AS COMPONENTS OF LOBLOLLY PINE DECLINE**

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ABSTRACT

Loblolly pine (*Pinus taeda* L.) decline (LPD) has been present in upland sites of central Alabama since the 1960s. Symptoms of LPD (fine root deterioration, short chlorotic needles, sparse crowns, reduced radial growth) begin in the 30-40 yr age class, resulting in premature death at ages 35-50. Previously, declining loblolly was diagnosed as littleleaf disease (LLD); however, site conditions associated with LPD are different from LLD sites. Littleleaf disease only occurs on eroded, heavy clay soils and is secondarily associated with the fungus, *Phytophthora cinnamomi*. In contrast, LPD occurs on sandy, well-drained soils and is associated with *Leptographium* spp., as well as with root-feeding bark beetles and weevils.

In the present study, 17 species (eleven newly reported) of subcortical root- and lower-stem feeding beetles were identified as vectors of *Leptographium* species, of which *Hylastes salebrosus*, *H. tenuis*, *Hylobius pales* and *Pachylobius piciporus* were statistically more abundant ($F_{3,14}=13.90$, $p=0.003$) in LPD sites. *Leptographium terebrantis*, *L. procerum*, *L. lundbergii*, and *L. serpens* were isolated from the roots and insects. Pathogenicity studies suggested that *L. lundbergii* and *L. serpens*, fungi not previously reported in the U.S., were more virulent on loblolly pine.

Spatial analysis correlated LPD to site and stand physical factors. Slope and aspect were the predominant predictive variables of LPD in central Alabama. Convexity and elevation were predictive only in combination with other topographical factors. These analyses have allowed the creation of LPD risk maps to accurately predict areas of loblolly decline, providing a vital new tool for managing southern forests for predetermined purposes.

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

“Forests are complex dynamic communities of living and dead trees interacting among themselves and with an array of microbes, pests, environmental, human and other factors to continuously shape and reshape the community over time” (Manion and Lachance, 1992).

1.1 Forest Decline

“Decline” is a general term applied by forest pathologists to a reduction in tree vigor usually expressed by a series of symptoms beginning with foliage chlorosis, followed by retarded leaf and twig growth, then by branch dieback and reduced annual increment. The concurrent lack in vigor kill the trees, or increase their susceptibility to invasion by organisms, variously described as secondary, weak, opportunistic, facultative, or saprogenic. Such organisms are relatively innocuous in healthy, unstressed trees.

Reports of forest decline and mortality have increased in recent years, although they may have actually been occurring for a very long time (Manion and Lachance, 1992), and are presently considered to be a major threat in temperate ecosystems. Decline diseases are caused by interactions of abiotic predisposing factors and biotic agents. The organisms involved are generally opportunistic, able to compete either saprophytically or parasitically, and function as part of a complex, causing the decline to occur (Manion, 1991). Decline and/or dieback diseases have commonly been reported in many hardwood species such as, birch, ash, maple, oak, and sweetgum (Hepting, 1971). Decline syndromes also occur in some conifer species, including pole blight of western white pine (*Pinus monticola* Dougl.), decline of red pine (*Pinus resinosa*

Aiton), decline of ponderosa pine (*Pinus ponderosa* Laws.), and littleleaf disease of shortleaf (*Pinus echinata* Mill.) and loblolly (*Pinus taeda* L.) pines.

It is possible now to develop a relatively thorough understanding of factors leading to general declines. Spatial analysis of information available from geographical information system (GIS) databases as it relates to new information generated through research mandates a more holistic approach to understanding forest declines. Such an approach requires detailed consideration of all the factors discussed above, at the least. This dissertation is presented as a model of such an approach, and deals with the intricate interactions occurring among several organisms and the stresses that might lead to loblolly decline. Loblolly pine decline (LPD) (Fig. 1.1) has an unknown etiology, but has become a serious problem in central Alabama, where the dominant forest type in the presettlement era was longleaf pine (*Pinus palustris* Mill.). This area was extensively clearcut, and the land cultivated prior to establishment of the Talladega National Forest (Johnson, 1948). Beginning in the 1930's forestry practice emphasized watershed protection, and much of this area was replanted with loblolly pine. Initially, only a qualitative description of LPD symptomology was known. It is characterized by deterioration of fine roots, short chlorotic needles, sparse crowns and reduced radial growth followed by death (Lorio, 1966, as littleleaf disease), initially occurring when the trees reach the 40-50 yr age class. Affected trees decline slowly and die prematurely.

1.2 *Phytophthora Cinnamomi* and Littleleaf Disease of Shortleaf Pine

It has been assumed that LPD and littleleaf disease (LLD) of shortleaf pine, described by Campbell and Copeland (1954), are the same. The cause of LLD is primarily attributed to a



Figure 1.1. Declining loblolly pine stand in the Talladega National Forest, Oakmulgee Ranger District, AL.

combination of soils with poor internal drainage and secondarily to the presence of *Phytophthora cinnamomi* Rands (Campbell and Copeland, 1954; Roth, 1954). While the symptoms of LPD are very similar to those described for LLD of shortleaf pine, the site conditions are not. Littleleaf disease primarily affects shortleaf pine, but symptoms have occurred on loblolly pine when found in an area where the disease is particularly severe (Campbell and Copeland, 1954). The symptoms of LLD are those commonly associated with some sort of malnutrition, and first appear when trees reach the 30-50 yr age class. Losses progressively increase as stands become older. Affected trees have an overall sickly appearance, generally decline slowly, and die prematurely. In the early stages of littleleaf disease symptoms are commonly indefinite,

consisting of a slightly yellowing foliage with reduced shoot growth and abnormally short needles. The early symptoms are difficult to distinguish from growth variations resulting from numerous other causes. The next stage of LLD is quite distinct and striking, featuring sparse and tufted foliage resulting from the progressive annual shortening of needle and twig growth. The needles turn yellow green in the fall and winter, with a tendency to become a more normal green in spring and early summer. As the disease progresses, twigs and branches die throughout the crown and epicormic branches may develop on the trunk and branches. In the final stages of the disease, the scanty and chlorotic foliage is confined to the ends of the branches, and the trees produce heavy cone crops. The development of symptoms is usually gradual, although trees may decline in a single year. These are essentially the same symptoms described for LPD.

Symptomatic trees also display fine root mortality, which led to the hypothesis that littleleaf disease was caused by pathogenic soil organisms. Campbell and Copeland (1954) made thousands of fungal isolations, but they only isolated one known plant pathogen, *P. cinnamomi*. It was isolated from 2% of the 11 trees sampled, but 91% of the time from soil in the root zone of diseased trees. Surveys for *P. cinnamomi* outside the range of LLD yielded a 48% soil isolation rate. This soil survey of *P. cinnamomi* showed, that while it was ubiquitous in soils under pine stands, it was more prevalent in areas where LLD symptoms occurred. This may be accounted for simply by soil/site conditions; LLD is prevalent in areas that have very heavy, poorly drained soils, which enhance the growth and reproduction of *P. cinnamomi*. Greenhouse studies demonstrated that *P. cinnamomi* does act as a pathogen to shortleaf pine under continuously saturated soil conditions. Campbell and Copeland (1954) speculated that *P. cinnamomi* functions pathogenically by destroying the fine root systems of shortleaf pine. While this may be

true, *P. cinnamomi* was isolated only rarely from root systems and would be somewhat atypical for *P. cinnamomi*. This organism typically occurs on perennial angiosperms and hardwood trees (Erwin, 1996), often causing obvious cankers. The presence of *P. cinnamomi* in soil at high levels is not easily accounted for on the basis of its very low levels in the pine roots. However, the known ability of *P. cinnamomi* to parasitize roots of hardwood trees and woody bushes may account for its presence. A preliminary investigation of this possibility has been conducted (Appendix).

1.3 *Leptographium* Species and Loblolly Pine Decline

Otrosina *et al.* (1995, 1997) reported a statistically significant relationship between the occurrence of southern pine beetle and the presence of *Leptographium* from the roots of southern pine. *Leptographium* species had previously been associated with bark beetles (Wagener and Mielke, 1961; Goheen and Cobb, 1980; Christiansen *et al.*, 1987; Harrington and Cobb, 1988), but very little was known about the details of this association. At about the same time Hess *et al.* (1999) reported declining loblolly pine in Alabama that had a symptom range typical for a decline syndrome. This stimulated my interest in an investigation of the role of *Leptographium* spp. and/or *Phytophthora cinnamomi* in that decline, which came to be known as LPD.

During the past few decades, the importance of several species of the genus *Leptographium* and closely related genera (i.e., *Graphium*) as pathogens of trees has emerged (Harrington and Cobb, 1988). Several fungi in the genus *Leptographium* are important internationally as pathogens of conifers (Wingfield *et al.*, 1988); attention in North America has been focused on *Leptographium wageneri* (Kendrick) Wingfield, *L. procerum* (Kendrick) Wingfield, and *L. terebrantis* Barras and Perry, the diseases they are associated with, and their

means of transmission (Table 1.1). *Leptographium wageneri* is a severe pathogen causing black stain root disease (Table 1.1), a wilt disease of conifers found in the western United States and Canada. *Leptographium wageneri* can be transmitted via contact between diseased and healthy roots (Wagener and Mielke, 1961; Smith, 1967; Landis and Helburg, 1976), through continuous xylem in root grafts (Landis and Helburg, 1976; Hunt and Morrison, 1986), by short distance growth through the soil (Goheen and Cobb, 1978), and by insect vectors such as *Hylastes nigrinus* (Table 1.1). Trees are killed by blockage of water transport in the outer annual rings (Hessburg, 1984).

Leptographium terebrantis causes lesions in the phloem and resin-soaking of the xylem of wound-inoculated seedlings and mature trees of several conifers (Harrington and Cobb, 1983; Wingfield, 1983; Rane and Tattar, 1987; Klepzig *et al.*, 1996; among others). The pathogen may be important when vectors (Table 1.1) attack the bases of still-living pines, but colonization of the host by the pathogen is apparently restricted to those trees attacked by beetles, and then only in the vicinity of the beetle galleries.

The disease associated with *L. procerum* is commonly known as procerum root disease (white pine root decline), which occurs on *Pinus strobus* and *P. resinosa* in Virginia, Wisconsin and Quebec, Canada (Table 1.1). Symptoms include decreased shoot growth, delayed bud break, needle wilt, exudation of resin from the root collar area, and resin soaking of affected wood (Dochinger, 1967; Houston, 1969; Sinclair and Hudler, 1980; Swai and Hindal, 1981). In addition to being associated with these disease symptoms on white pine, *L. procerum* has been isolated from dying roots of many other conifer species in the United States (Towers, 1977; Livingston and Wingfield, 1982; Wingfield, 1983), Canada, and Sweden (Kendrick, 1962). In

these latter cases, the fungus did not appear to be the main cause of tree mortality.

Leptographium procerum has also been associated with growth reduction of ozone-sensitive trees (Lackner and Alexander, 1983). Trees dying of procerum root disease are often found on moist, poorly drained sites (Halambek, 1976; Towers, 1977; Shaw and Dick, 1980; Weaver and Stipes, 1983), usually scattered throughout a plantation and not found in discrete infection centers. This suggests that the disease affects single trees, does not spread from infected to adjacent healthy trees through soil (Lewis *et al.*, 1987) and may involve insect vectors.

Leptographium procerum and *L. terebrantis* are been commonly found in the southeastern United States; *L. wagnerii* only occurs in the western United States. The distribution and occurrence of other *Leptographium* spp., (i.e., *L. serpens* and *L. lundbergii*), are less well described.

Leptographium serpens has not previously been reported from the United States, but it has been associated with root disease of *P. pinea* in Italy (Lorenzini and Gambogi, 1976) and *P. radiata* and *P. pinaster* in South Africa (Wingfield and Knox-Davies, 1980). *Leptographium serpens* colonizes both the ray parenchyma and the tracheids, resulting in a wedge-shaped discoloration of infected wood (Wingfield *et al.*, 1988). Symptoms of the disease include scant, yellowish foliage in the upper crown of trees, reduced needle length, sudden marked decrease in height growth, and dark stained areas on roots (Wingfield and Marasas, 1983; Wingfield *et al.*, 1988).

1.4 Insect Associations

Insects are able to disperse fungi externally on their bodies, internally in their guts and occasionally in specialized mycangia. The best known examples of externally transmitted fungal

spores are those of blue stain fungi, the Ophiostomataceae, which have been studied intensively along with their insect vectors. Whitney and Blauel (1972) suggested that these fungi were adapted to adhering to their insect vectors and would not be washed off by rain, but ultimately would become detached in the resin-filled galleries of a new tree.

Insects are commonly associated with *Leptographium* spp. (Table 1.1), and there are two hypotheses proposed to explain the relationship between *Leptographium* spp. and insects. One is that these fungi are mostly transported with some primary benefit to the insects (Jacobs and Wingfield, 2001), either serving as a source of food for the insects or playing some role in the development of the brood (Jacobs and Wingfield, 2001). The second hypothesis is that the association of the insects and the fungi might be coincidental. The fungi would then be considered as “weeds” in the habitat of the beetles (Jacobs and Wingfield, 2001).

Leptographium spp. are associated with insects, especially bark beetles (Coleoptera: Scolytidae), on conifers. These insects can be primary bark beetles that attack and kill healthy trees, or secondary bark beetles that rarely kill their hosts. Although one species of insect may carry more than one species of *Leptographium*, most are quite specific to the fungal species that they carry (Jacobs and Wingfield, 2001). The insect introduces a fungus that can run the spectrum from a pathogen virulent enough to kill the tree to a saprophytic organism which may function only in metabolizing necrotic tissue on the dying tree, possibly providing food and/or brood material for the insects (Jacobs and Wingfield, 2001). Several studies also indicate that root disease and blue-stain fungi predispose the trees to further attack by bark beetles by diminishing tree defenses (Raffa and Smalley, 1988; Orosina *et al.*, 1997).

Table 1.1. Characteristics of *Leptographium* species found in North America.

<i>Leptographium</i> species	Geographical Range	Hosts	Hosts Common Name	Associated Disease	Insect Vectors	Insect Common Name
<i>L. wagneri</i>	CA, WA, OR, BC (3, 4, 11, 19, 21)	<i>Pseudotsuga</i> spp. <i>Pinus ponderosa</i> <i>Pinus contorta</i> <i>Tsuga mertensiana</i> <i>Tsuga heterophylla</i> (3, 4, 11, 19, 21)	Douglas-fir Ponderosa pine Lodgepole pine Mountain hemlock Western hemlock	Black stain root disease	<i>Hylastes nigrinus</i> (4, 5, 24) <i>Pissodes faciatu</i> s (4, 5, 24) <i>Steremnius carinatus</i> (4, 5, 24)	Root feeding beetle Root collar weevil
<i>L. terebrantis</i>	CA, MS, ID, MN, WI, MA, LA, BC (1, 4, 6, 23)	<i>Pinus thunbergiana</i> <i>Pinus sylvestris</i> <i>Pinus resinosa</i> (6, 9, 17)	Japanese Black pine Scots pine Red pine	Red Pine Decline (9)	<i>Dendroctonus terebrans</i> (1, 17) <i>Dendroctonus valens</i> (4, 9, 15) <i>Hylastes porculus</i> (9) <i>Hylobius pales</i> (9) <i>Hylobius radialis</i> (9) <i>Hylurgops porsus</i> (4, 9, 13, 23) <i>Pachylobius picivorus</i> (9)	Black turpentine beetle Red turpentine beetle Root feeding beetle Pales weevil Pine root collar weevil Pitch-eating weevil
<i>L. procerum</i>	VA, WI, Quebec (2, 7, 8, 9, 10, 14, 18, 20) as well as other countries	<i>Pinus strobus</i> <i>Pinus resinosa</i>	White pine Red pine	Procerum root disease (2, 7, 18) White Pine Decline (2, 7, 10, 14, 22) Red Pine Decline (9)	<i>Dendroctonus valens</i> (9) <i>Hylastes porculus</i> (9) <i>Hylobius pales</i> (9, 14) <i>Hylobius radialis</i> (9) <i>Pachylobius picivorus</i> (9, 12, 16, 17, 23) <i>Pissodes approximatus</i> (12, 16, 17, 23) <i>Pissodes nemorensis</i> (14)	Red turpentine beetle Root feeding beetle Pales weevil Pine root collar weevil Pitch-eating weevil Northern pine weevil Deodar weevil

References:

- | | | |
|----------------------------------|--------------------------------|---------------------------------|
| 1. Barras and Perry 1971 | 9. Klepzig <i>et al.</i> 1991 | 17. Rane and Tattar 1987 |
| 2. Dochinger 1967 | 10. Lackner and Alexander 1982 | 18. Sinclair and Hudler 1980 |
| 3. Harrington 1983 | 11. Leaphart and Gill 1959 | 19. Smith 1967 |
| 4. Harrington and Cobb 1983 | 12. Lewis and Alexander 1986 | 20. Towers 1977 |
| 5. Harrington <i>et al.</i> 1985 | 13. Mielke 1981 | 21. Wagener and Mielke 1961 |
| 6. Highley and Tattar 1985 | 14. Nevill and Alexander 1992 | 22. Weaver and Stipes 1983 |
| 7. Houston 1969 | 15. Owen <i>et al.</i> 1987 | 23. Wingfield 1983 |
| 8. Kendrick 1962 | 16. Raffa and Smalley 1988 | 24. Witcosky <i>et al.</i> 1986 |

1.5 *Leptographium* Species

Species within the genus *Leptographium* are dematiaceous hyphomycetes characterized by dark, robust mononematous and macronematous conidiophores that terminate in a penicillately branched conidiogenous apparatus (Fig. 1.2). The conidia are hyaline, aseptate, and produced in a slimy matrix through holoblastic extension of conidiogenous cells (Fig. 1.2) (Hughes, 1953; Kendrick, 1961, 1962; Wingfield *et al.*, 1993). Conidiation results in the accumulation of slimy conidial masses at the apices of conidiophores which facilitate insect dispersal (Wingfield *et al.*, 1993). These fungi are anamorphs of *Ophiostoma*, tolerant of high

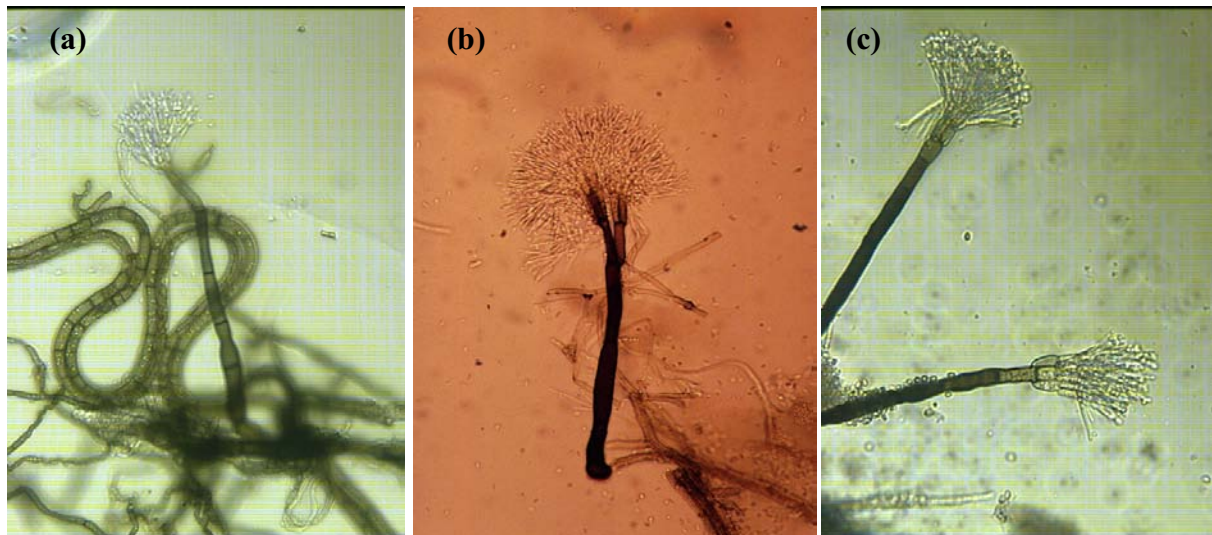


Figure 1.2. Three examples of *Leptographium* spp: (a) *L. serpens*, (b) *L. terebrantis*, (c) *L. procerum*.

concentrations of cycloheximide in culture media (Harrington, 1981), and have rhamnose in their cell walls (Jewell, 1974; Weijman and DeHoog, 1975; Horner *et al.*, 1986). These fungi are adapted to insect dispersal and are commonly associated with scolytid bark beetles (Coleoptera: Scolytidae), many of which infest conifers (Harrington and Cobb, 1983; DeHoog and Scheffer, 1984; Harrington, 1988, 1993a, 1993b). A number of *Leptographium* species have been

associated with root diseases of conifers (Hunt and Morrison, 1979; Mielke, 1981; Harrington and Cobb, 1987; Harrington, 1993b), and *L. wagneri* is the causal agent of black stain root disease, a serious problem in the western United States (Harrington, 1993b). *Leptographium* species have been associated with pine decline and mortality, primarily as associates of root feeding bark beetles and weevils that attack living trees, throughout the United States (Caird, 1935; Bramble and Holst, 1940; Reid *et al.*, 1967; Herbert *et al.*, 1975; Goheen and Cobb, 1980; Harrington, 1983; Livingston *et al.*, 1983; Ferrell and Parmeter, 1989; Klepzig *et al.*, 1991; Ferrell *et al.*, 1994; Nevill *et al.*, 1995; Orosina *et al.*, 1997).

1.6 Loblolly Pine (*Pinus Taeda* L.)

In the original forests of the southern United States, loblolly pine (Fig. 1.3) was a native, minor species both on the uplands, which were dominated by longleaf pine or mixed upland hardwoods, and in the wet river bottoms and swamps, which were dominated by mixed bottomland hardwoods. It was, however, an important species on moist sites that were not subject to regular burning. In the Coastal Plain, loblolly pine grew best mixed with hardwoods along stream margins and around swamps on sites that were not subject to long periods of flooding or to serious fire. Loblolly pine was also a minor component of the original Piedmont forests, which were largely composed of mixed hardwoods. Colonization, farming, and intensive logging in the 1800's, followed by fire control and extensive planting of loblolly pine in the 1900's, converted the southern pinery from predominantly longleaf pine to predominantly loblolly pine in less than 100 years (Schultz 1997).

Loblolly pine is an ideal tree for site restoration and forest management. It is the most hardy and versatile of all southern pines, in terms of its ability to reproduce and grow rapidly on

diverse sites. It seeds profusely, regenerates easily, provides large yields per hectare, provides many different marketable products at a relatively early age, and provides a good wildlife habitat when stands of many ages are growing in close proximity (Schultz 1997). It grows naturally in various combinations with longleaf pine, shortleaf pine and slash pine (*P. elliottii* Engelm.), with numerous annuals and perennials, and with most southern hardwoods. It reaches maturity by age 80 and rarely lives beyond age 300 even under the best conditions. In the absence of disturbance, succession results in nearly complete elimination of loblolly pine and the formation of mixed hardwood forests (Schultz 1997).



Figure 1.3. Healthy loblolly pine stand, Talladega National Forest, Oakmulgee Ranger District, AL.

The natural range of loblolly pine extends from Texas eastward to Florida and northward to Delaware, spanning 15 southern and mid-Atlantic states. This range is generally continuous, with the exception of the Mississippi River flood plain and a disjunct population in Texas called the “lost pines.” The extensive range of loblolly pine overlaps the natural ranges of longleaf pine, shortleaf pine, slash pine, pitch pine (*P. rigida* Mill.), pond pine (*P. serotina* Michx.), and Virginia pine (*P. virginiana* Mill.). Completely mixed stands occur in many areas, and natural hybridization is relatively common (Schultz 1997).

CHAPTER II

GENERAL METHODS

2.1 Plot Descriptions

Thirty-nine sites, in areas of central Alabama where loblolly decline had been observed, were identified in nine counties located in Choccolocco State Park, the Talladega National Forest (Shoal Creek and Oakmulgee Ranger Districts) and on Gulf State Paper Company lands (Table 2.1, Fig. 2.1). The sites were located within four physiographic regions of Alabama: the Piedmont, Ridge and Valley, Upper Coastal Plain and Cumberland Plateau (Table 2.1). Sites were chosen visually, using tree crown appearance, to select declining (trees with sparse, thinning crowns) and healthy (trees with thick, full crowns) plots. The loblolly pines on 32 of the sites showed obvious symptoms of decline (Fig. 1.1); seven sites had only mild or no symptoms of decline (Fig. 1.3). One central plot and three sub-plots identical to it were established at each selected site. The subplots were located 120 m away from the central plot at bearings of 120, 240, and 360 degrees (Fig. 2.2) (Dunn, 1999). Research plots were established in 1999 and monitored through September, 2002.

2.2 Root Sampling

Roots were collected from all 39 research plots in the spring and summer (April, May, June) of 2000, and provided data that enabled a more focused sampling scheme in 2001. Root samples were collected from a total of 16 plots in May of 2001 using a two-root excavation method (modified from Otrosina *et al.*, 1997) (Fig. 2.3), in which three dominant/co-dominant symptomatic trees nearest to the plot center were selected. Two lateral root segments > 3 cm dia

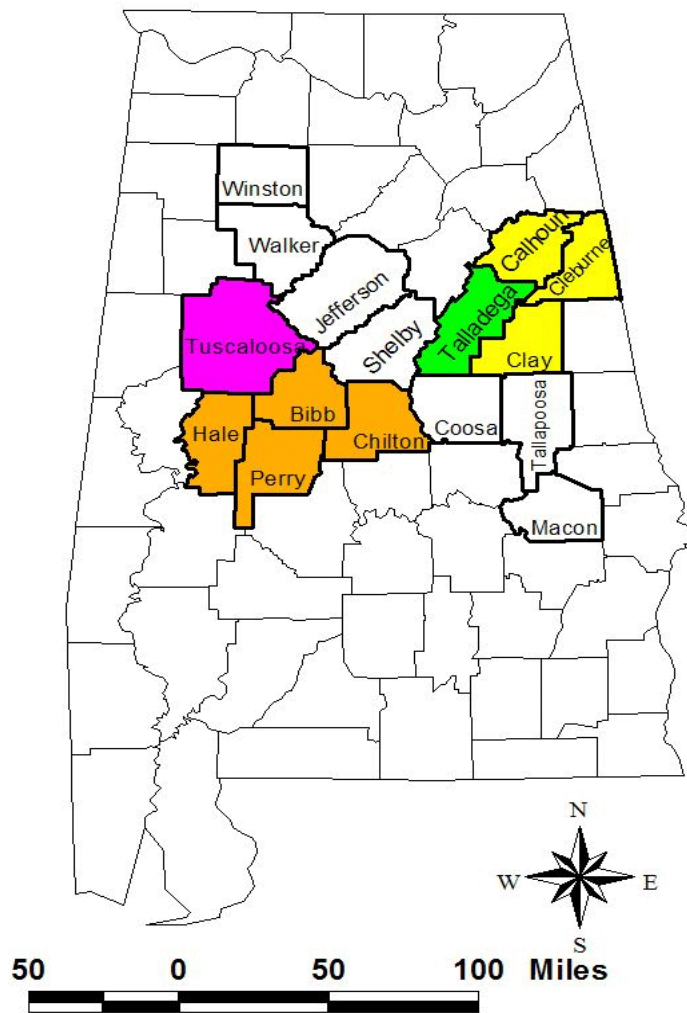


Figure 2.1. Loblolly pine decline study area in central Alabama. Yellow = Shoal Creek, Green = Talladega, Orange = Oakmulgee, and Pink = Gulf State Paper. Area outlined in bold black has decline symptomology.

Table 2.1. Locations and characteristics of sites used in the study of loblolly pine decline in Alabama

Site	County	Location	Physioregion	Soil Series ¹	Ownership
1	Clay	N 33 28.485 W 85 44.315	Piedmont	Madison sandy loam	Federal
2	Clay	N 33 28.194 W 85 44.276	Piedmont	Madison sandy clay loam, eroded	Federal
3	Clay	N 33 27.870 W 85 44.325	Piedmont	Madison sandy clay loam, eroded	Federal
4	Cleburne	N 33 30.444 W 85 42.114	Piedmont	Madison sandy loam	Federal
5	Cleburne	N 33 30.212 W 85 41.895	Piedmont	Madison sandy loam	Federal
6	Calhoun	N 33 42.826 W 85 40.920	Ridge & Valley	Decatur clay loam, eroded	State
7	Calhoun	N 33 42.797 W 85 40.100	Ridge & Valley	Fine-loamy, mixed, semi-active, thermic aquic hapludults	State
8	Clay	N 33 28.660 W 85 44.663	Piedmont	Louisa sandy loam	Federal
9	Clay	N 33 28.722 W 85 45.094	Piedmont	Louisa sandy loam	Federal
10	Talladega	N 33 23.384 W 85 56.898	Piedmont	Tallapoosa sandy loam	Federal
11	Talladega	N 33 23.316 W 85 57.303	Piedmont	Tallapoosa sandy loam	Federal
12	Bibb	N 32 58.802 W 87 27.123	Coastal Plain	Maubila flaggy sandy loam	Federal
13	Bibb	N 32 58.245 W 87 23.170	Coastal Plain	Maubila flaggy sandy loam	Federal
14	Bibb	N 32 57.928 W 87 24.715	Coastal Plain	Wadley loamy sand	Federal
15	Hale	N 32 59.835 W 87 29.780	Coastal Plain	Smithdale sandy loam	Federal
16	Hale	N 32 59.741 W 87 29.733	Coastal Plain	Riverview sandy loam	Federal
17	Bibb	N 32 57.850 W 87 22.933	Coastal Plain	Maubila flaggy sandy loam, eroded	Federal
18	Bibb	N 32 57.767 W 87 22.805	Coastal Plain	Maubila flaggy sandy loam	Federal
19	Bibb	N 32 55.911 W 87 23.147	Coastal Plain	Suffolk loamy sand	Federal
20	Bibb	N 32 54.484 W 87 22.836	Coastal Plain	Maubila flaggy sandy loam	Federal
21	Hale	N 32 55.818 W 87 25.595	Coastal Plain	Smithdale sandy loam	Federal
22	Hale	N 32 56.113 W 87 26.420	Coastal Plain	Maubila flaggy sandy loam	Federal
23	Tuscaloosa	N 33 24.806 W 87 26.657	Cumberland Plateau	Suffolk loamy sand	Industry
24	Tuscaloosa	N 33 24.697 W 87 26.900	Cumberland Plateau	Sweatman sandy loam	Industry
25	Tuscaloosa	N 33 23.436 W 87 26.202	Cumberland Plateau	Luverne sandy loam	Industry
26	Tuscaloosa	N 32 23.206 W 87 26.245	Cumberland Plateau	Sweatman sandy loam	Industry
27	Tuscaloosa	N 33 23.229 W 87 26.356	Cumberland Plateau	Suffolk sandy loam	Industry
28	Tuscaloosa	N 33 22.545 W 87 26.748	Cumberland Plateau	Smithdale sandy loam ²	Industry
29	Tuscaloosa	N 33 22.585 W 87 26.694	Cumberland Plateau	Smithdale sandy loam ²	Industry
30	Tuscaloosa	N 33 22.537 W 87 26.882	Cumberland Plateau	Smithdale sandy loam ²	Industry
31	Tuscaloosa	N 33 22.603 W 87 26.853	Cumberland Plateau	Smithdale sandy loam ²	Industry
32	Perry	N 32 47.302 W 87 01.456	Coastal Plain	Suffolk loamy sand	Federal
C1	Cleburne	N 33 28.696 W 85 45.673	Piedmont	Madison sandy loam	Federal
C2	Calhoun	N 33 42.889 W 85 34.725	Ridge & Valley	Fruithurst loam	State
C3	Cleburne	N 33 36.917 W 85 39.513	Piedmont	Fruithurst loam	Federal
C4	Bibb	N 32 58.511 W 87 20.751	Coastal Plain	Suffolk loamy sand	Federal
C5	Bibb	N 32 58.392 W 87 20.811	Coastal Plain	Wadley loamy sand	Federal
C6	Bibb	N 32 54.307 W 87 22.788	Coastal Plain	Wadley loamy sand	Federal
C7	Chilton	N 32 46.117 W 86 59.283	Coastal Plain	Smithdale sandy loam	Federal

¹ Data furnished by Dr. Art Goddard, National Forest of Alabama, Montgomery, AL.

² Data furnished by Bruce DeHaan, Gulf State Paper, Tuscaloosa, AL.

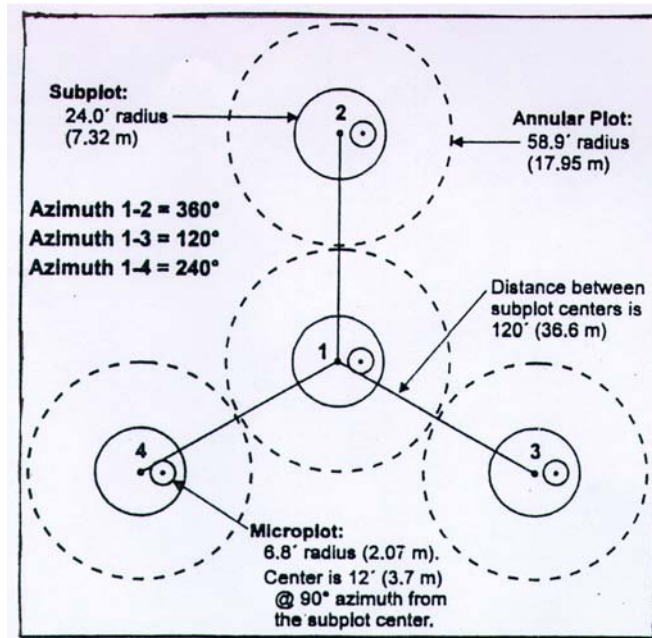


Figure 2.2. Loblolly study plot layout (Dunn, 1999).

from each of the three selected plot trees were excavated with hand tools from opposite sides of the root collar to the approximate crown drip line. Root depth was also recorded at this time. Roots were visually examined for primary root damage and fine root presence or absence, damage, and/or death before removal from soil. Primary roots were defined as the major lateral roots extending from the base of the tree to the drip line. All secondary and feeder roots were categorized as fine roots. Roots that were shriveled and dried were tallied as dead. Trees that had primary roots with no secondary root growth were tallied as having their fine roots absent.

Twenty cm-long sections of each root were cut beginning at 16 cm from the root collar of each tree, placed in plastic bags, and kept chilled in ice chests for transport to the laboratory. Roots were stored at 4°C until they could be processed (about 2-3 days). Roots were examined for insect damage and blue or black stain by removing bark with a flamed scalpel. The roots

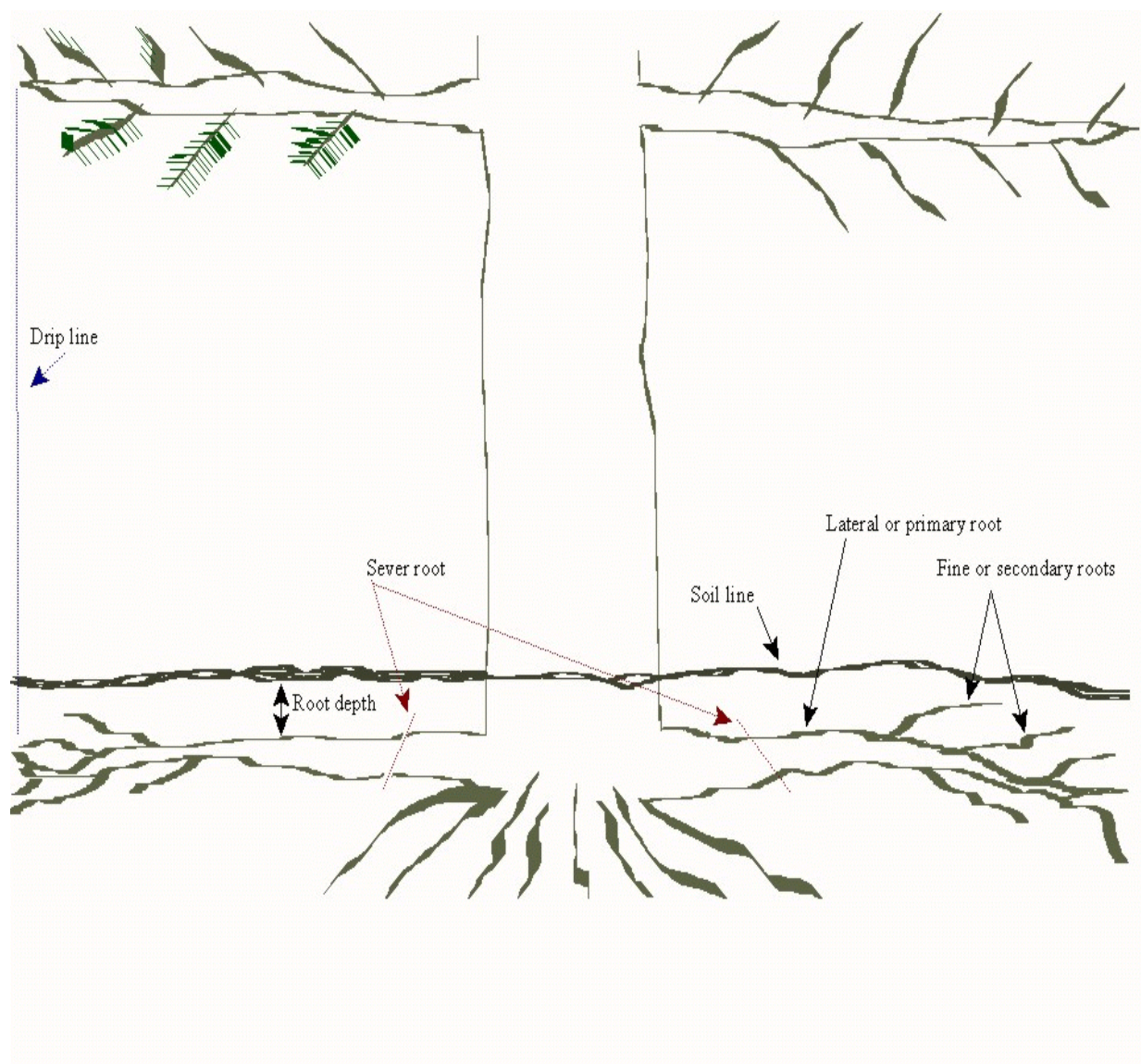


Figure 2.3. Lateral roots are excavated to drip line, depth recorded, severed and packaged.

were then cut into pieces, rinsed in tap water, surface sterilized in commercial bleach, EtOH and dH_2O (10:10:80 v/v) for one min, rinsed in tap water for 3 min, and blotted dry with sterile Kimwipes. The root pieces were plated (4 pieces per plate, 20 plates per sample) on MEA (2% malt extract agar) and CSMA (MEA, containing 800 mg/l of cycloheximide and 200 mg/l of streptomycin sulfate) (Hicks *et al.*, 1980) and incubated at 25°C under fluorescent lighting (460

$\mu\text{mol m}^{-2} \text{ s}^{-1}$) for 2 wks and then examined for fungal growth. *Leptographium*-like isolates were subcultured by transferring hyphal tips and/or spore heads of each isolate to sterile plates of MEA. Subcultured isolates were placed on cleared V-8 agar slants and were stored at 8°C for subsequent identification to species.

2.3 Soil Sampling

Soil samples were collected from all plots in 2000 and from 16 plots in 2001. Soil collections were made from around the selected lateral roots of the three dominant/co-dominant symptomatic trees nearest to center of the central plot in a specific collection pattern using a soil auger (Lewis *et al.*, 1987) (Fig. 2.4). The samples from each root were placed in separate plastic bags, kept on ice, transported to the laboratory and stored at 4°C for no more than 3 days. Each soil sample was thoroughly mixed, and a representative 10 g subsample was taken. The subsample was suspended in 40ml of sterile 0.5% water agar after sifting through a #12 sieve to remove root fragments. One ml aliquots were spotted onto each of five CSMA plates, and five more plates were swirled with 1 ml aliquots to distribute the sample evenly across the plates (adapted from Johnson and Curl, 1972). Plates were incubated at 25°C under fluorescent lighting ($460 \mu\text{mol m}^{-2} \text{ s}^{-1}$) for 2 weeks and then examined for fungal growth. *Leptographium*-like isolates were processed as described above.

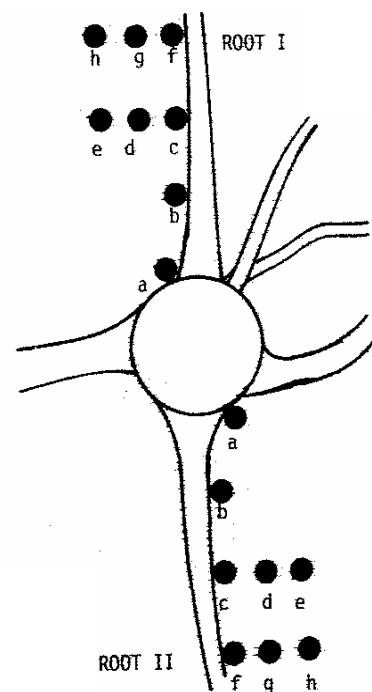


Figure 2.4. Soil Sampling scheme. Letters (a-h) indicate where 15.2 cm deep soil cores were taken. (Lewis *et al.*, 1987)

2.4 Insect Activity

2.4.1 Pitfall Trapping

Pitfall traps (adapted from Klepzig *et al.* 1991) for capturing crawling insects were employed continuously for an eight week period on 15 plots (10 asymptomatic and 5 symptomatic) during the spring of 2000, 2001 and 2002, to allow for best chance of capturing the emergence period of most bark beetles (Drooz, 1985). One trap was placed at the center of each sub-plot for each of the 15 plots (3 traps per plot). These traps consisted of 20 cm sections of 10 cm dia PVC plastic drain pipe with eight entrance holes equally spaced around the pipe circumference at one end (Fig. 2.5). The interior of each trap was coated with a thin layer of

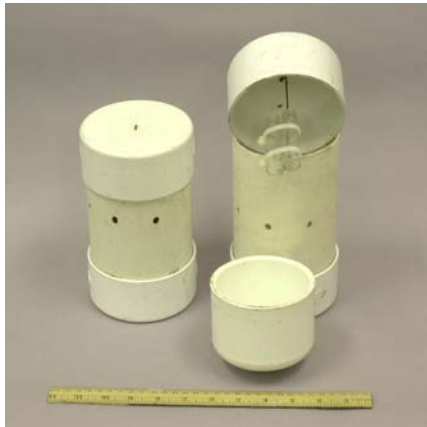


Figure 2.5. Pitfall trap used to capture crawling insects on plots. Photograph by G. Lenhard, Department of Entomology, Louisiana State University.

liquid TeflonTM (Northern Products, Woonsocket, RI) to prevent the escape of the captured insects. Both ends were capped with removable plastic lids, and two holes were drilled in the bottom lid for drainage. The traps were buried, leaving entrance holes slightly above ground level. Each trap was baited with two 8 ml glass vials, one containing 95% ethanol and one containing steam distilled southern pine turpentine (Hercules), and two cut pine stems approximately 5 cm long by 2 cm dia. Trapped insects were collected weekly and placed in sterile polyethylene specimen cups and refrigerated

at 4°C for no more than 3 days. These insects were identified and rolled non-destructively across MEA and CSMA. Plates were incubated at 25°C under fluorescent lighting (460 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 2wks and examined for fungal growth. *Leptographium*-like isolates were processed as above.

The stepwise regression procedure in SAS (2001) was used to determine if the number of individuals were significant in the model.

2.4.2 Duff Sampling

A 1 m² leaf litter/duff sample was collected from each subplot of 15 plots (3 subplots per plot, 45 total samples) over an eight week period in the spring of 2002. The leaf litter/duff was collected by placing a 1 m² wooden frame on the ground near plot center and raking up all material inside the frame down to the soil using a hand claw. These samples were placed in sealed plastic bags, weighed and kept cool for transport to the laboratory. Samples were then placed on a Berlese funnel for extraction of insects (Fig. 2.6). Each sample was left on the funnel until it was completely dry, about 7 to 14 days, and the collected insects identified to family. Carabid beetles were identified to species.



Figure 2.6. Berlese funnel with ethanol filled collection cup used to extract insect from duff sample.

2.4.3 Statistical Analysis

Insects known or suspected to have had an impact on the health of southern pines (i.e., Curculionidae and Scolytidae spp.) and found to carry *Leptographium* spp. were analyzed for correlations in abundance as related to decline and control areas. Precipitation and temperature were used in correlation analyses to determine any trends between insect presence and abundance and weather conditions (i.e, drought). Climatic data (annual precipitation and mean temperature) were obtained from the National Oceanic and Atmospheric Administration (NOAA). A weekly temperature average was determined by averaging all hourly readings for the month in °C.

Precipitation was calculated as totals for the month in millimeters. Insect tally data for years 2000, 2001, and 2002 showed no significant differences in abundance and were used for correlation analysis between weather trends and insect occurrences in decline and control areas. Significance in the model was determined using SAS proc GLM and proc REG STEPWISE option (SAS, 2001). SAS proc CORR Kendall was used to determine any correlations between temperature or rainfall and insect abundance.

2.5 *Leptographium* Identification

Cultures were single-spored, grown on MEA under a 12-hour photoperiod ($460 \mu\text{mol m}^{-2} \text{s}^{-1}$) and placed on silica gel (Dhingra and Sinclair, 1995) for long term storage at 4°C for later identification to species. Cultures were then plated on MEA and grown in the dark for comparison to species described in the literature. After identification, isolates were sent to M.J. Wingfield (FABI, Pretoria, South Africa) for confirmation.

2.6 Plot Characterization

2.6.1 *Leptographium* Incidence Rating (LIR).

Plots were rated for incidence of *Leptographium* as follows: 0 = target fungi were not recovered from the three trees sampled on the plot; 1 = target fungi were recovered from one of the three sampled trees on the plot; 2 = target fungi were recovered from two of the three trees sampled on the plot; and 3 = target fungi were recovered from all the trees sampled on the plot. Tree selection was as described above.

2.6.2 Vegetation Density Ratings

(VDR).

A vegetation density rating was devised to determine the thickness of the understory. Vegetation density was determined on all center and subplots, by counting the number of stems between 2.54 cm and 10.16 cm dia in each plot, and categorized as follows: Light (1) = 0 to 20 stems; Moderate (2) = 21 to 40 stems; and Heavy (3) 41 stems (Fig. 2.7). Plots with heavy vines and thicket-type vegetation were rated visually on a comparable scale.

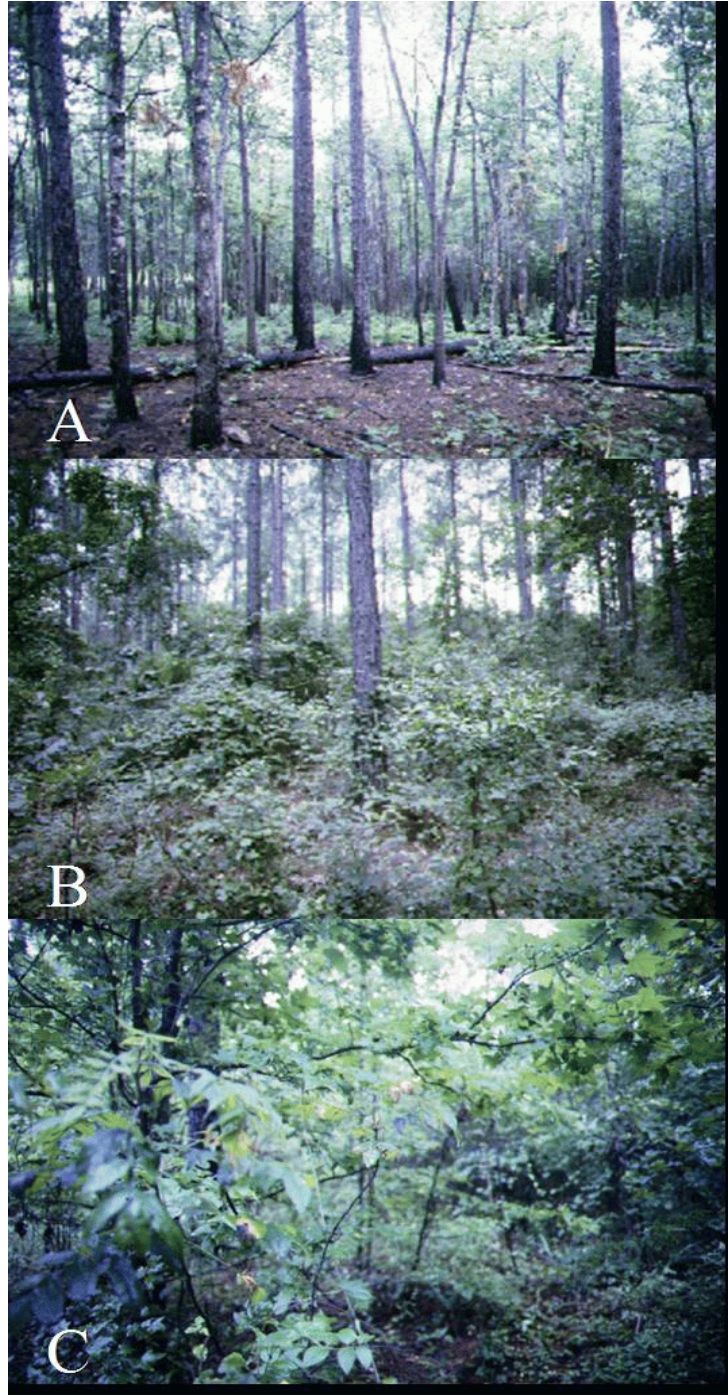


Figure 2.7. Vegetation Density Rating examples. A=Light, 0-20 stems/plot; B=Moderate, 21-40 stems/plot; C=Heavy, 41+ stems/plot.

CHAPTER III

ASSOCIATION OF AN INSECT-FUNGAL COMPLEX WITH LOBLOLLY PINE DECLINE IN CENTRAL ALABAMA

3.1 Introduction

Forest decline and mortality syndromes have been increasingly noted in the past twenty years, and pose a threat to some subtropical and temperate forest ecosystems. A number of biotic and abiotic stresses, both natural and anthropogenic, have been proposed as causal factors (Manion, 1991). Some of these syndromes involve complexes of closely associated, interdependent, pathogenic organisms. One of the best known of these declines in the southeast is littleleaf disease of shortleaf pine (*Pinus echinata* Mill), first reported in 1954 (Campbell and Copeland). This syndrome is characterized by a strong statistical relationship between a specific soil/site profile and symptom development. Campbell and Copeland (1954) also reported a correlation between littleleaf and the presence of *Phytophthora cinnamomi*, and were able to demonstrate the ability of this fungus to cause damage to shortleaf pine in a greenhouse study. Their work and others (Oak and Tainter, 1988), however, indicated that LLD could be explained on the basis of site conditions alone.

Loblolly pine decline is a syndrome of loblolly pine in the southeastern United States and is superficially similar to LLD of shortleaf pine. The initial hypothesis was that LPD and LLD of shortleaf pine were actually the same syndrome. Symptoms common to LPD and LLD of shortleaf pine include short chlorotic needles, sparse crowns, and reduced radial growth followed by mortality (Lorio, 1966; Hess *et al.*, 1999). The onset of symptoms in both syndromes is associated with older trees. Affected trees decline slowly

and die prematurely, typically accompanied by symptoms caused by several insect and fungal species, including various stem and root-feeding bark beetles, and blue-stain fungi (Table 1.1). Loblolly pine is the most extensively planted pine in the southeastern United States because of its ability to reproduce and grow rapidly on diverse sites (Schultz, 1997). Loblolly pine decline is, therefore, potentially very serious due to mortality among the older timber trees and because weakened trees lead to an increased incidence of southern pine beetle, which threatens the younger pulpwood plantations (Otrosina *et al.*, 1997).

Several *Leptographium* spp. are important internationally as pathogens of conifers (Wingfield *et al.*, 1988). In North America, attention has focused on *L. wageneri* (Kendrick) Wingfield, *L. procerum* (Kendrick) Wingfield, and *L. terebrantis* Barras and Perry, the diseases with which they are associated, and their means of transmission (Table 1.1). *Leptographium* spp. have been associated with pine decline and mortality, primarily as associates of root feeding bark beetles and weevils that attack living trees (Caird, 1935; Bramble and Holst, 1940; Reid *et al.*, 1967; Herbert *et al.*, 1975; Goheen and Cobb, 1980; Harrington, 1983; Livingston *et al.*, 1983; Ferrell and Parmeter, 1989; Klepzig *et al.*, 1991; Ferrell *et al.*, 1994; Nevill *et al.*, 1995; Otrosina *et al.*, 1997). The biological basis for these relationships is variable (Harrington, 1993a, 1993b), and the relationships among beetles, fungi, and trees are still unclear. In the southeastern United States, loblolly pine stands affected by root disease appear to be more vulnerable to attack by southern pine beetle and are more likely to contain *Leptographium* spp. within their root systems than apparently healthy stands (Otrosina *et al.*, 1997; Hess *et al.*, 1999, 2002).

Leptographium wageneri, in the western United States and Canada, causes black stain root disease (Table 1.1), which kills the trees by blocking water transport in the outer

annual rings (Hessburg, 1984); its teleomorph, *Ophiostoma wagneri* (Goheen and Cobb) Harrington, is associated with *Hylastes macer* LeConte (Coleoptera: Scolytidae) (Goheen and Cobb, 1980). *Leptographium wagneri* can be transmitted via contact between diseased and healthy roots (Wagner and Mielke, 1961; Smith, 1967; Landis and Helberg, 1976), through continuous xylem in root grafts (Landis and Helberg, 1976; Hunt and Morrison, 1986), by short distance growth through the soil (Goheen and Cobb, 1978), and by insect vectors (Table 1.1).

Leptographium terebrantis causes lesions in the phloem and induces resin-soaking of the xylem of wound-inoculated seedlings and mature trees (Harrington and Cobb, 1983; Wingfield, 1983; Rane and Tattar, 1987; Klepzig *et al.*, 1996). This may be important when insect vectors attack the base of a living pine, but colonization of the host by the pathogen apparently is restricted to those trees attacked by the bark beetles, and then only in the vicinity of the beetle galleries.

The disease associated with *L. procerum* is commonly known as procerum root disease (=white pine root decline). Symptoms include decreased shoot growth, delayed bud break, needle wilt, exudation of resin from the root collar area and resin soaking of affected wood (Dochinger, 1967; Houston, 1969; Sinclair and Hudler, 1980; Swai and Hindal, 1981). In addition to being associated with these disease symptoms on white pine, *L. procerum* has been isolated from dying roots of many other conifer species in the United States (Towers, 1977; Livingston and Wingfield, 1982; Wingfield, 1983; Alexander *et al.*, 1988), Canada and Sweden (Kendrick, 1962), although it has never been implicated as the main cause of tree mortality. *Leptographium procerum* has also been associated with growth reduction of ozone-sensitive trees (Lackner and Alexander, 1983). Trees that die of

procerum root disease are often found on moist, poorly drained sites (Halambek, 1976; Towers, 1977; Shaw and Dick, 1980; Weaver and Stipes, 1983), usually scattered throughout a plantation and not found in discrete infection centers. This suggests that the disease affects single trees, does not spread from infected to adjacent healthy trees through soil (Lewis *et al.*, 1987), and may involve insect vectors.

Leptographium procerum and *L. terebrantis* have been commonly found in the southeastern United States, but the distribution and occurrence of congenics, such as *L. lundbergii* Lagerb. and Melin, and *L. serpens* (Goid.) Wingfield, are not well known. *Leptographium serpens* has been associated with root disease of *Pinus pinea* in Italy and *P. radiata* and *P. pinaster* in South Africa. Wingfield *et al.* (1988) concluded that the combined effects of feeding by insects and subsequent colonization by the fungus may result in tree death, although Zhou *et al.* (2002) concluded in recent pathogenicity studies that *L. serpens* and *L. lundbergii* are not serious tree pathogens in South Africa.

The symptoms of LPD are very similar to those described for LLD of shortleaf pine (Campbell and Copeland, 1954; Lorio, 1966), but data obtained in the present study do not support the involvement of *P. cinnamomi* with LPD, and the site conditions in declining trees are very different from those associated with LLD. Therefore, this study was directed toward evaluating whether or not the incidence of *Leptographium* spp. and their insect vectors (along with other physical and chemical factors) were correlated with LPD.

3.2 Materials and Methods

Plots were installed and roots, soil and insects sampled as described in Chapter II, General Methods.

3.2.1 Plot Characterization

3.2.1.1 Plot Measurements

Measurements taken on all center and subplots included: tree species composition (pines and hardwoods), diameter at breast height (dbh, approx. 137 cm above ground), basal area (10 factor) for the loblolly pines, and total trees present (Dunn, 1999). Additional measurements of sampled trees included age and growth increment (5 and 10 yr) (Dunn, 1999). These measurements provided a measure of site conditions, stand density and influence of external stresses.

3.2.1.2 Crown Ratings

Live crown ratio, crown light exposure, crown position, crown density, crown dieback and foliage transparency are Forest Health Monitoring (FHM) crown/ damage indicators that were recorded for all loblolly pines with dbh 12.7 cm or greater to describe relative tree health (USDA, 2001). Trees with high scores for live crown ratio, density and diameter and low scores for dieback and foliage transparency have increased potential for carbon fixation, nutrient storage and increased potential for survival and reproduction (USDA, 2001). Crown evaluations quantitatively assess current tree conditions and provide an integrated measure of site conditions, stand density and influence of external stresses.

3.2.1.3 *Leptographium* Incidence Rating (LIR)

Completed as described in Chapter II, General Methods.

3.2.1.4 Resin Sampling (Tree Vigor)

One hundred twenty trees were sampled (30 per LIR) on the south side of each tree, by punching a hole approximately 137 cm above ground with a 1.9 cm diameter arch punch

(No. 149) (Osbourne). A bead of silicon caulk (ACE silicone sealant, Oak Brook, IL) was placed under each hole to direct resin into a pre-weighed plastic bag tacked onto the tree (Fig. 3.1). The bags were collected 24 hr later and stored on ice for transport to the laboratory. Resin weights were determined and samples were stored at -70°C until gas chromatography analysis



Figure 3.1. Resin sampling of loblolly pine.

(GC) of toxic terpenoid levels could be completed (performed by Jolie Mahfouz) at the USDA, Forest Service, Southern Research Station, Pineville, LA).

The resin analysis was done with a 6890 GC equipped with a 5973 MS (Hewlett Packard corp., Palo Alto, California) and an HP 5MS column, 30 m length x 250 µm ID x .25 µm thickness. The temperature program was 60°C for 1 min, then 6°C/min to 200°C, then 15°C/min to 250°C. Flow rate was 0.7 ml/min with an injector temperature of 200°C. Monoterpenes were analyzed by extracting 100mg of oleoresin in 5 ml pentane. The pentane was spiked with 0.1% diphenylmethane as an internal standard to quantify the compounds. Compounds were quantified by their mass spectra and retention time matches with known standards.

3.2.1.5 Vegetation Density Ratings (VDR)

Completed as described in Chapter II, General Methods (Fig. 2.7).

3.2.2 Insect Damage

Damage caused by insects was determined by direct observation at the time of root sampling on every pine on all center and sub-plots. The presence and number of *Dendroctonus terebrans* Olivier (Coleoptera: Scolytidae) were estimated by counting the



Figure 3.2. *Dendroctonus terebrans* pitch tubes on loblolly pine.

number of pitch tubes found around the circumference of each tree in the lower 1 m of the trunk and immediately below the soil line (Fig. 3.2). Infestation and damage caused by *Hylastes salebrosus* Eichoff (Coleoptera: Scolytidae), *Hylastes tenuis* Eichoff (Coleoptera: Scolytidae), *Hylobius pales* Herbst. (Coleoptera : Curculionidae), and *Pachylobius picivorus* (Germar) (Coleoptera : Curculionidae) were estimated by sweeping soil away from the root collar and lateral roots and looking for entrance/exit holes and pitching on bark. Damage was also assessed in the laboratory by

peeling the bark from the roots and observing the presence of galleries.

3.2.3 Statistical Analysis

Because sampling location criteria and data collection procedures were identical for all plot locations, the data were combined for analysis. Dummy coding was used for the categorical variables. Presence and absence of *Leptographium* spp. was entered as a binary variable (i.e., a value of 0 for absent, 1 for present). Understory vegetation density, crown density, foliar transparency, live crown ratio, crown light exposure, crown position, crown dieback, root condition (percent damage and staining), resin flow, average tree age and size, 5 yr and 10 yr radial growth, total and pine basal area, and insect numbers were entered as continuous variables (i.e., their actual measured values).

Percent of trees infected in a sample stand was the dependent variable, which was treated statistically as the number of successful events (infected trees) per number of trials (trees sampled) at each sample site. The stepwise regression procedure was performed with

the data set using PROC REG STEPWISE option in SAS (2001) to determine which variables were statistically significant and relevant in the model.

3.3 Results

3.3.1 Root and Soil Isolations

Leptographium terebrantis, *L. procerum*, and *L. serpens*, were frequently isolated from roots of symptomatic trees in all locations, but *L. lundbergii* was isolated only from Choccolocco State Park. Other fungi isolated from roots included *Graphium* spp. and *Ophiostoma* spp. as well as non-stain fungi such as *Aspergillus* spp., *Aureobasidium* spp., *Cladosporium* spp., *Curvularia* spp., *Gliocladium* spp., *Mortierella* spp., *Mucor* spp., *Penicillium* spp., and *Trichoderma* spp. The overall proportion of stain fungi isolated was significantly higher ($F_{5,38} = 24.82$, $p = 0.0003$) in symptomatic plots than in asymptomatic plots, and when all *Leptographium* spp. were pooled, they were found significantly more often ($F_{5,38} = 20.14$, $p = 0.001$) in symptomatic than in asymptomatic trees, as were *Graphium* isolates (Table 3.1). Of the four identified *Leptographium* species, *L. procerum*, *L. terebrantis* and *L. serpens* were present in significantly more symptomatic trees (Table 3.1). Cycloheximide-amended and unamended MEA were equally effective in isolating blue-stain fungi, although CSMA plates contained far fewer non-target organisms. Only *L. procerum* was isolated from soil samples, and was more common in soil from symptomatic (57%) than asymptomatic plots.

3.3.2 Root System Characteristics

Root system damage was statistically greater ($F_{5,38} = 14.47$, $p = 0.002$) in symptomatic than in asymptomatic plots (Table 3.2). Symptomatic trees had consistently fewer fine roots, more fire and/or insect damage, and staining to the primary root systems

than did asymptomatic trees (Table 3.2). Root system damage was positively correlated with the number of insects ($F_{3,14} = 13.90$, $p = 0.003$) and the incidence of *Leptographium* ($F_{3,14} = 12.37$, $p = 0.004$). All symptomatic plots had been prescription burned within the last 6 years.

Table 3.1. Isolation percentages of blue-stain fungi from asymptomatic vs. symptomatic trees in 2000 and 2001

Fungal Species	Asymptomatic	Symptomatic	P-value ¹
	- % fungal isolation -		
<i>Leptographium procerum</i>	43.0	87.7	0.003
<i>Leptographium lundbergii</i>	14.0	14.0	1.0
<i>Leptographium serpens</i>	14.0	42.8	0.001
<i>Leptographium terebrantis</i>	40.1	82.5	0.0001
Pooled <i>Leptographium</i> species	41.5	86.5	0.001
<i>Graphium</i> species	53.5	71.0	0.05
Pooled species	53.6	94.8	0.0003

¹P-values are for stepwise regression comparisons of asymptomatic (N=27) vs symptomatic (N=138) trees for each species category.

3.3.3 Insect Activity and Climate Variables

No significant differences were found for monthly rainfall totals during years 2000, 2001, and 2002 ($F_{2,14} = 8.47$; $p = 0.079$), nor for average temperatures over the three year study ($F_{2,14} = 6.47$; $p = 0.059$). Because insect trapping was only employed from February to May, there were no correlations found between insect abundance and climatic data. There was a considerable cold snap in March, 2001 when temperatures dropped below freezing for a period of one week, but trapping numbers for that year were not significantly affected.

Table 3.2. The comparative condition of primary and fine roots from asymptomatic vs. symptomatic loblolly pine in 2000 and 2001

	Asymptomatic	Symptomatic	P-value ¹
Fine Roots	- % of roots -		
Present (Alive)	85	35	0.02
Present (Dead)	10	32.5	0.04
bsent	5	32.5	0.04
Primary Roots			
Alive	100	100	1.0
Damaged ²	15/10.5	58.5/48.5	0.03/0.02
Dead	0	0	1.0
Stained	15	74.5	0.01

¹ P-values are for stepwise regression comparisons of asymptomatic (N=7) vs symptomatic (N=32) plots for each category.

²The first value indicates insect damage, the value following the slash indicates fire damage.

3.3.4 Insect Damage and Activity

Insects were much more abundant in symptomatic (N=10) ($F_{3,14} = 13.90$, $p = 0.003$) than in asymptomatic (N=5) plots. Symptomatic plots showed large spikes in insect populations during the trapping periods (2000 - 2002), while asymptomatic plot catches stayed at low steady levels (Fig. 3.3). Several insect species were positively correlated with the incidence of loblolly decline. Of the 17 species trapped, six had previously been associated with *Leptographium*, but the other 11 are new reports (Table 3.3, Fig. 3.4 and Fig. 3.5). Ninety-one percent of the seventeen insect species trapped carried *Leptographium* spp. at a rate greater than or equal to 50% (Table 3.3). All 17 insect species monitored by traps were more abundant in symptomatic stands, but only *H. salebrosus*, *H. tenuis*, *P. picivorus* and *H. pales* were statistically significantly

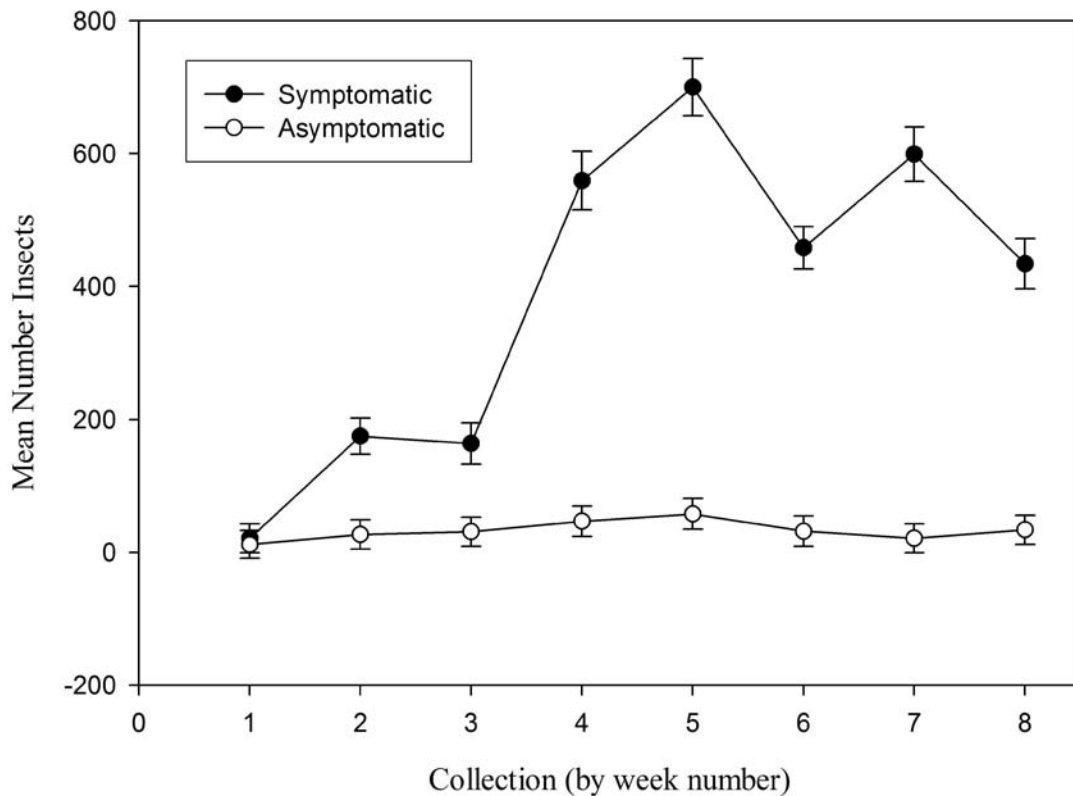


Figure 3.3. Mean insect catches observed from 2000-2001 from pitfall traps on symptomatic (N=10) and asymptomatic (N=5) plots in Alabama.

($F_{3,14} = 13.90$, $p = 0.003$). Insect numbers were inversely related to vegetation densities and significantly higher catches were made where there were light to moderate vegetation densities ($F_{3,14} = 7.74$, $p = 0.04$) (Fig. 3.6), but only *H. salebrosus*, *H. tenuis*, *P. picivorus* and *H. pales* were statistically significant (Table 3.4). *Hylastes* spp. damage was evident on the roots of living trees sampled on symptomatic plots (Table 3.2), as was *Dendroctonus terebrans* damage at the base of these trees. Trees on symptomatic plots (LIR=3) with severe decline symptoms and damage from both *Hylastes* and *Dendroctonus* spp. eventually became infested with *Ips* spp. and woodborer (Cerambycidae) and did not survive. This is similar to results found by Klepzig *et al.* (1991) for red pine decline in Wisconsin.

Leptographium terebrantis, *L. procerum*, and *L. serpens* were consistently isolated from *P. picivorus*, *H. pales*, *H. salebrosus* and *H. tenuis* (Table 3.3). *L. lundbergii* was isolated only from insects trapped on the Choccolocco State Park. An undescribed species morphologically similar to *L. peucophilum* Jacobs and Wingfield (DNA sequence analysis pending) was isolated from 78 % of the *Colopterus unicolor* (Say) (Coleoptera: Nitidulidae). *Leptographium peucophilum* has only been reported to be associated with a root feeding conifer swift moth (*Korscheltellus gracillus*) on red spruce (*Picea rubens*) in and near Vermont (Jacobs *et al.*, 2001). Its pathogenicity is unknown, although large areas of discolouration are usually associated with the feeding wounds caused by moth larvae (Jacobs *et al.*, 2001). Other fungi isolated from collected insects and root samples included *Graphium* spp. and non staining fungi such as *Aspergillus* spp. *Aureobasidium* spp., *Gliocladium* spp., *Penicillium* spp., and *Trichoderma* spp.

Interesting trends were also seen when insect trap catches were compared to timing of prescribed burns. Increased root-feeding bark beetle and weevil catches were seen during the same year of burning and the year following, but once the plots were three years past burn date, plot insect catches suggested a low steady population as seen in unburned plots (Fig. 3.7) as well as asymptomatic plots. Also, non-pest insect trap catches were inversely related to pest insect catches (see Chapter VI) (Fig. 3.8). Higher pest insect catches during this post burning period allows for a potentially greater number of point inoculations of vectored *Leptographium*, which can accumulate over time, resulting in stressed and declining loblolly pines.

Table 3.3. Isolation percentages of *Leptographium* spp. from insects trapped in 2000 - 2002

Insects Species	<i>Lt</i> ¹	<i>Lp</i>	<i>Ls</i>	<i>Ll</i>	<i>L</i> pool	<i>G</i>	<i>L + G</i> pool
<i>Corthylus punctatissimus</i> ^{2,5}	-	29	14	-	29	14	31
<i>Dendroctonus frontalis</i> ³	12	-	-	-	17	3	18
<i>Dendroctonus terebrans</i>	90	-	-	-	90	75	100
<i>Gnathotrichous materiarius</i> ²	-	-	67	-	67	-	-
<i>Hylobius pales</i>	78	66	-	-	91	8	92
<i>Hylastes salebrosus</i> ²	25	30	57	2	87	11	91
<i>Hylastes tenuis</i> ²	20	25	51	1	84	9	87
<i>Ipps pini</i> ³	95	-	-	-	95	-	-
<i>Monarthrum mali</i> ²	-	-	64	-	64	29	75
<i>Colopterus unicolor</i> ^{2,4}	17	15	33	4	78	6	82
<i>Orthotomicus caelatus</i> ²	-	-	50	-	50	-	-
<i>Pachylobius picivorus</i>	92	14	-	-	97	12	98
<i>Pissodes nemorensis</i>	75	100	-	-	98	-	-
<i>Rhizophagus</i> spp. ²	40	20	55	-	93	-	-
<i>Xyleborinus saxesini</i> ²	-	-	70	-	70	-	-
<i>Xylosandrus compactus</i> ^{2,5}	-	-	78	-	67	-	-
<i>Xylosandrus crassiusculus</i> ^{2,5}	-	-	68	-	67	-	-

¹*Lt* = *L. terebrantis*, *Lp* = *L. procerum*, *Ls* = *L. serpens*, *Ll* = *L. lundbergii*, *L* pool = all *Leptographium* spp pooled, *G* = *Graphium* spp, *L+G* = all *Leptographium* spp + *Graphium* spp pooled.

²Not previously associated with a *Leptographium* species.

³Only found on plot C2 which was adjacent to a southern pine beetle (SPB) spot that was active 2000 and 2001. Spot was cut and trees removed 3 weeks prior to trapping in 2002.

⁴Seventy-eight percent of these insects also carried an undescribed *Leptographium* species that was recovered from hardwood roots.

⁵Not known or reported to utilize conifers as a primary host.

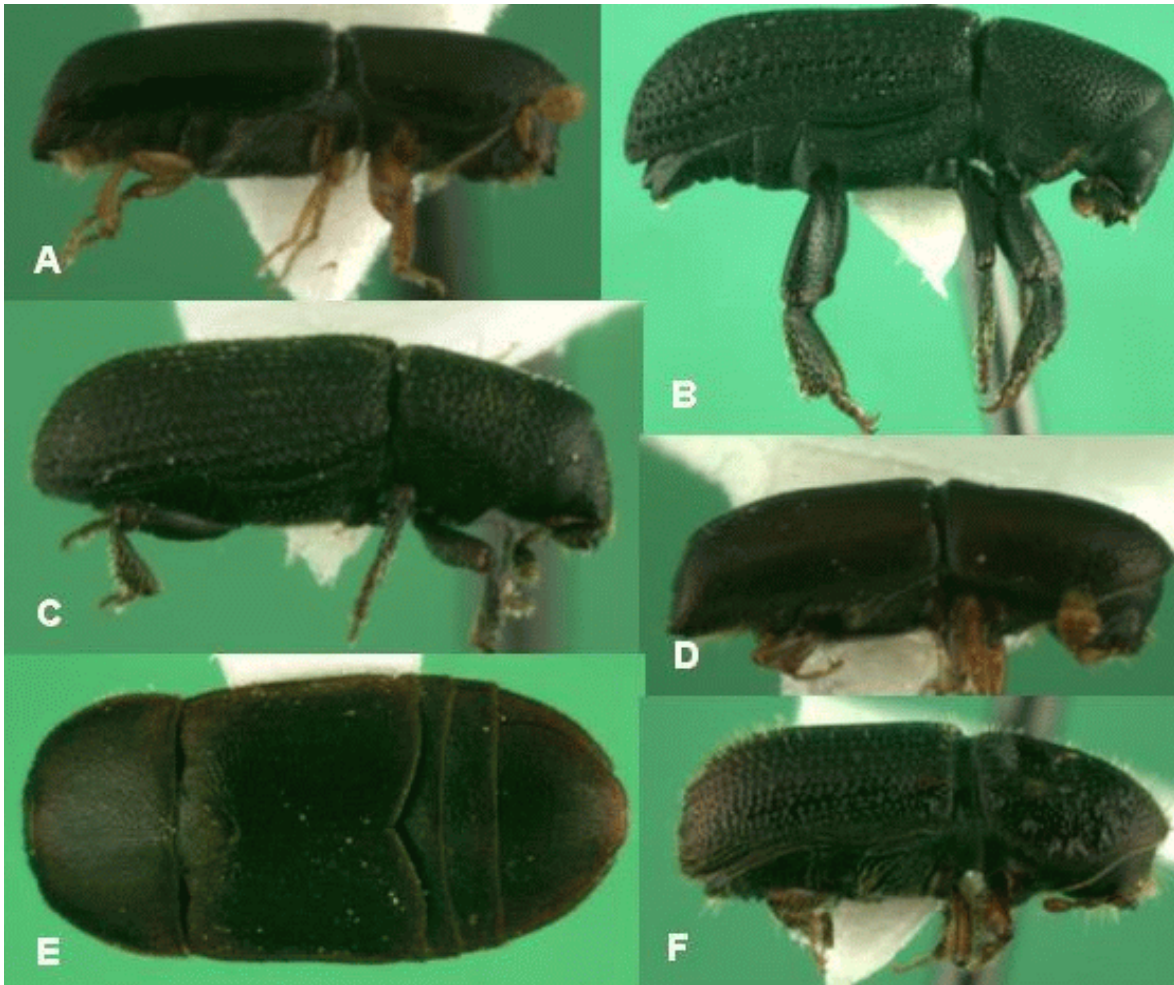


Figure 3.4. Insects not previously associated with *Leptographium* species. a. *Gnathotrichous materiarius*, b. *Hylastes salebrosus*, c. *Hylastes tenuis*, d. *Monarthrum mali*, e. *Colopterus unicolor*, f. *Orthotomicus caelatus*. Photos by G. Lenhard, Department of Entomology, Louisiana State University.



Figure 3.5. Insects not previously associated with *Leptographium* species. a. *Rhizophagous* species (undescribed), b. *Xyleborinus saxesini*, c. *Xylosandrus compactus*, d. *Xylosandrus crassiusculus*. Photos by G. Lenhard, Department of Entomology, Louisiana State University.

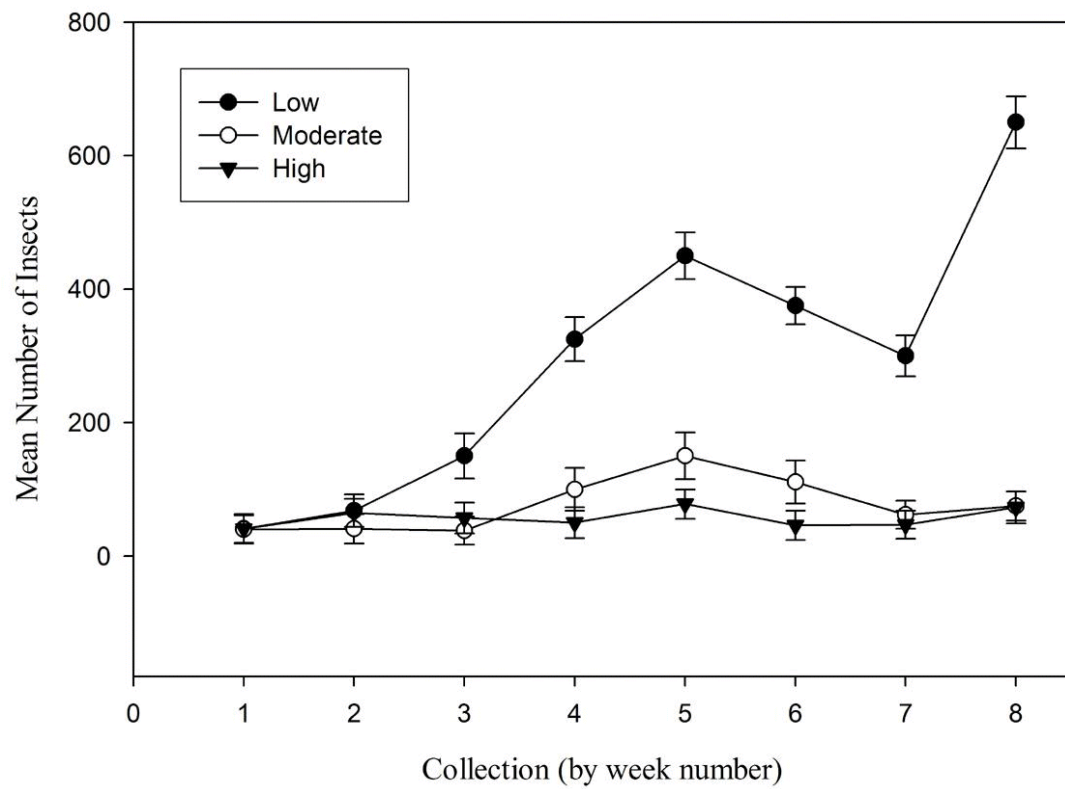


Fig 3.6. Mean insect catches from pitfall traps by vegetation density for 2000-20001. Low (N=5), Moderate (N=5), and High (N=5).

Table 3.4. Mean number (\pm S.E.) per plot of weevils, bark beetles and other beetles captured in pitfall traps operated from April 2000 to June 2000 and February 2001 to May 2001 and February 2002 to May 2002. The stands differed in the density of understory vegetation.

Insect Species	Vegetation Density ¹			F	P
	Low	Moderate	High		
Weevils					
H. pales	144 ± 21 a	12 ± 5 b	4 ± 2 b	4.54	0.041
H. picivorus	389 ± 47 a	75 ± 10 b	16 ± 3 b	11.3	0.003
P. nemorensis	10 ± 2	7 ± 1	8 ± 2	1.22	0.74
Phloem feeders					
C. punctatissimus ³	5 ± 2	0	0	2.32	0.25
D. frontalis ²	37 ± 4	0	0	3.04	0.29
D. terebrans	4 ± 2	1 ± 0	0	1.27	0.14
H. salebrosus	1827 ± 81 a	318 ± 49 b	122 ± 5 b	15.9	0.002
H. tenuis	998 ± 12 a	264 ± 23 b	97 ± 11 b	9.8	0.009
I. pini	20 ± 7	0	0	2.97	0.17
O. caelatus	2 ± 2	0	0	0.98	0.518
Xylem feeders					
G. materiarius	1 ± 2	0	0	1.21	0.365
M. mali	7 ± 3	3 ± 1	0	1.89	0.195
X. saxeseni	78 ± 9	21 ± 2	2 ± 2	3.56	0.074
X. compactus ³	7 ± 4	2 ± 1	0	2.17	0.117
X. crassiusculus ³	11 ± 4	9 ± 1	1 ± 2	3.47	0.077
Other beetles					
Rhizophagus spp.	7 ± 3	7 ± 1	0	2.84	0.364
C. unicolor	187 ± 23	98 ± 15	32 ± 6	1.82	0.153

¹Means within rows followed by the same letter are not significantly different according to the LSD procedure.

²Only found on plot C2 which was adjacent to a southern pine beetle (SPB) spot that was active 2000 and 2001. Spot was cut and trees removed 3 weeks prior to trapping in 2002.

³Not known or reported to utilize conifers as a primary host.

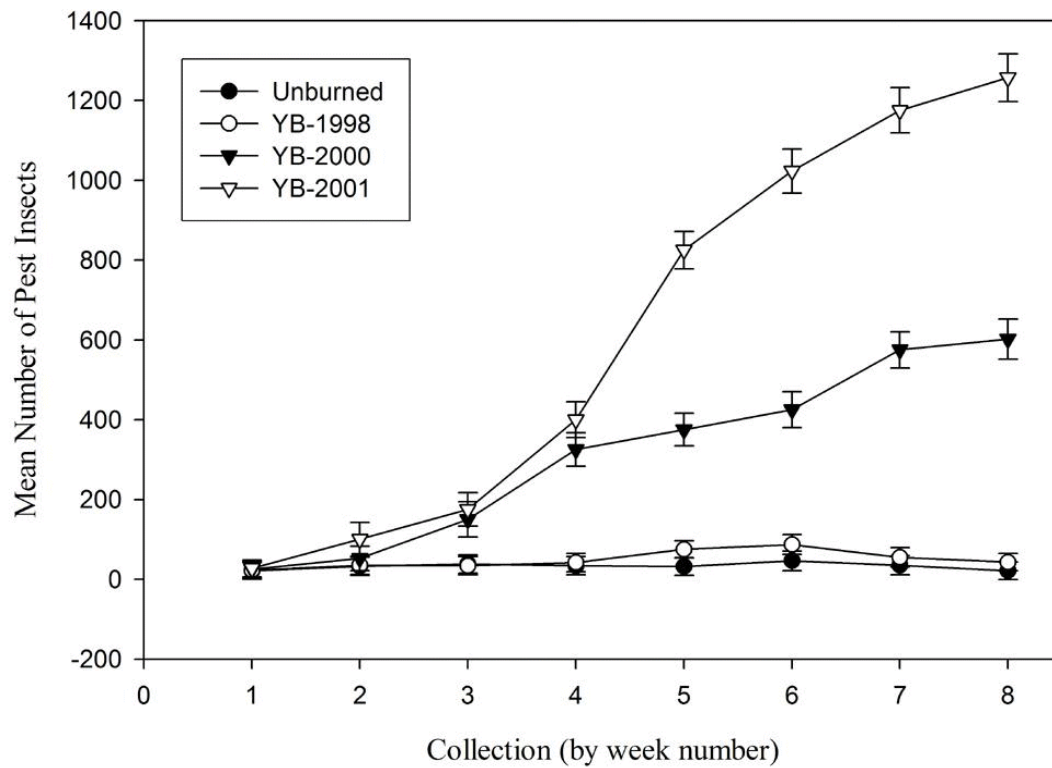


Figure 3.7. Mean pest insect catches from pitfall and duff collections from 2000-2002 by year of plot burning. YB= year burned; Unburned (N=3); YB-1998 (N=4); YB-2000 (N=4); and YB-2001 (N=4).

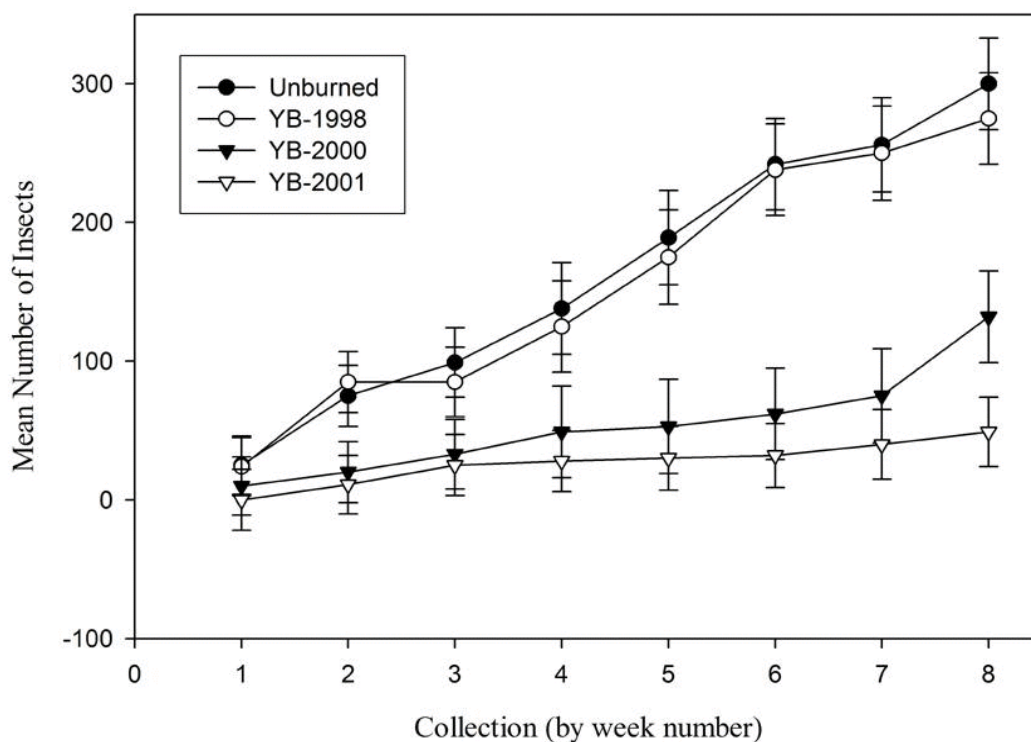


Figure 3.8. Mean number of insects collected (minus pest insects) from pitfall and duff collections from 2000-2002 by year of plot burning. YB=year burned; Unburned (N=3); YB-1998 (N=4); YB-2000 (N=4), and YB-2001 (N=4).

3.3.5 Plot Characterization

3.3.5.1 Plot Composition

Competing species of pine, including longleaf, shortleaf and Virginia pine were present as well as hardwood species such as dogwood (*Cornus florida* L.), southern red oak (*Quercus falcata* Michx.), white oak (*Quercus alba* L.), water oak (*Quercus nigra* L.), chestnut oak (*Quercus peinus* L.), blackjack oak (*Quercus marilandica* Muench. and *Quercus incana* Bartr.), post oak (*Quercus stellata* Wangenh.), cottonwood (*Populus* spp.), southern sugar maple (*Acer barbatum* Michx.), yellow poplar (*Liriodendron tulipifera* L.), American sweetgum (*Liquidambar styraciflua* L.), black gum (*Nyssa sylvatica* Marsh.), and hickory (*Carya* spp.). A heavy understory of woody plants including sassafras (*Sassafras albidum* [Nutt] Nees.), sourwood (*Oxydendrum arboreum* [L.] DC.), lowbush blueberry (*Vaccinium angustifolium* Ait.), mountain laurel (*Kalmia latifolia* L.), black huckleberry (*Gaylussacia baccata* [Wangenh.] K. Kock), and vines such as Alabama supplejack vine (*Berchemia scandens* [Hill] Koch), greenbrier (*Smilax* spp.), southern bush honeysuckle (*Diervilla sessilifolia* Buckl.), kudzu (*Pueraria* spp.) and poison ivy (*Toxicodendron radicans* [L.] Kunté) was also present in many plots. The parasitic plant, *Senna seymeria* (*Seymeria cassioides* Blake) was also present on some plots.

3.3.5.2 Plot Measurements

Increased age ($F_{5,38} = 4.86$, $p = 0.034$), reduced 10-yr radial growth ($F_{5,38} = 12.04$, $p = 0.002$), and lower vegetation density ($F_{5,38} = 7.59$, $p = 0.009$) relate significantly to LPD. Trees in symptomatic plots had a reduction in radial growth over the previous 10 years, which may be related to the presence of disease or competition, although, plots with a higher density understory had healthier trees and a lower LIR (Table 3.5 and Fig. 3.9). Total basal

area appears to be biologically meaningful in field observations, although there was not a statistical significance ($F_{5,38} = 3.72$, $p = 0.07$), between asymptomatic and symptomatic plots. Field observations suggest that pines appear to be healthier when stands have a hardwood component. The lower vegetation densities appear to be related to the occurrence and history of prescription burns.

3.3.6 Symptomology

Poor root systems and staining were positively correlated with low values for crown density ($r^2 = 0.89$, $p < 0.001$) and high scores for foliage transparency ($r^2 = 0.91$, $p < 0.0001$). These foliar symptoms were also positively correlated with increased insect capture, decreased vegetation density, and increased *Leptographium* incidence. Trees with declining symptoms had very poor root systems and obvious staining compared to apparently healthy stands, which had intact and healthy root systems essentially free of debilitating stain and damage (Table 3.2). There were significant differences in resin flow among *Leptographium* infected trees ($F_{3,116} = 246.50$, $p < 0.0001$) (Table 3.5) and an indirect and direct positive correlation between resin flow and foliar symptoms and the incidence of *Leptographium* (Table 3.5 and Fig. 3.9). Gas chromatography analysis of the resin indicated that *Leptographium* infected trees had increased production of monoterpenes (alpha-pinene and beta-pinene), which are toxic to fungi (Bridges, 1987) (Fig. 3.10).

3.4 Discussion

Leptographium spp. were consistently associated with declining trees in symptomatic, but not asymptomatic, stands, and the damage in these root systems was statistically higher in symptomatic trees. Symptomatic trees consistently had fewer fine

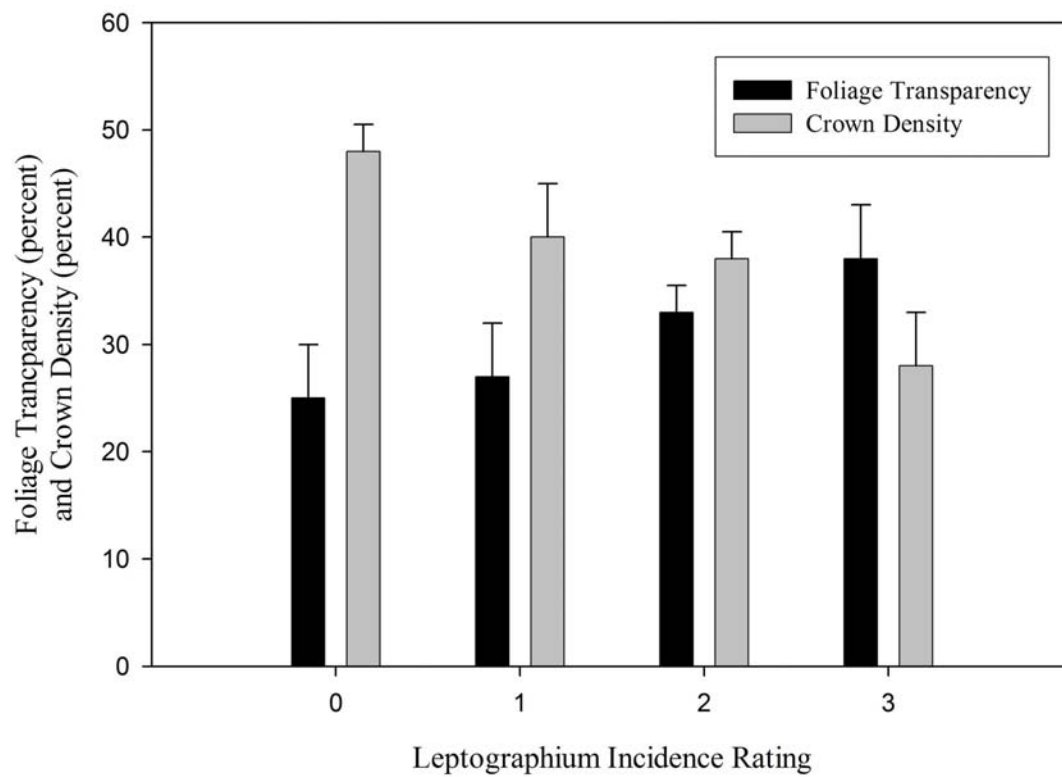


Figure 3.9. Incidence of *Leptographium* spp., foliage transparency, and crown density in loblolly pine decline and control sites.

Table 3.5. Comparison of mean resin flow for classes of LIR.

<i>Leptographium</i> Incidence Rating	Mean Resin, g ¹	S.E.
0	5.84 a	± 0.63
1	3.66 b	± 0.61
2	2.04 c	± 0.49
3	1.31 d	± 0.41

¹ Numbers followed by the same letter within a column are not significantly different at P=0.05, using Tukey's W.

roots and generally more insect and fire damage and staining to the primary root systems than asymptomatic trees. Root system damage was positively correlated with insect abundance, increased *Leptographium* incidence and increased root damage. Insect damage alone was not found to seriously affect the trees, but the resulting colonization by the vectored *Leptographium* spp. was extensive. In longleaf pine (*Pinus palustris* Mill.), prescribed burns have an effect on fine root growth by affecting mineral nutrition and root carbohydrate metabolism (Kuehler, 2003). Fire has a long-lasting effect as a result of the damage it does to shallow roots, making them more vulnerable to infection by a variety of imperfect fungi (Gara *et al.*, 1985). This also could be a factor in loblolly pine since loblolly is less tolerant to fire than longleaf pine (Boyer, 1990). Prescribed fire by a variety of imperfect fungi (Gara *et al.*, 1985). This also could be a factor in loblolly pine since loblolly is less tolerant to fire than longleaf pine (Boyer, 1990). Prescribed fire could, consequently, be one of the causes of fewer fine roots on the trees in these areas.

Symptomatic plots also had lower vegetation ratings, apparently due to prescribed burns, than asymptomatic plots. The increased insect populations may be due to low

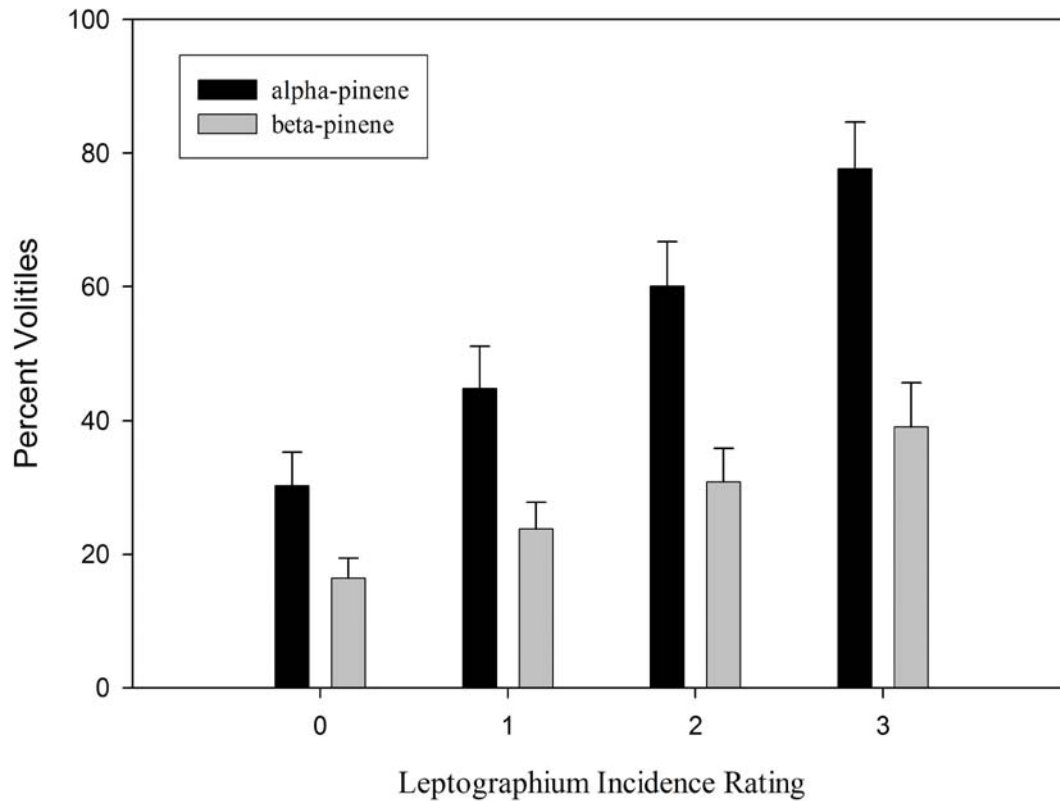


Figure 3.10. Percentage of volatiles detected in resin from loblolly pines on decline and control sites in Alabama.

vegetation density, which results in a less obstructed flight path and a lower overall allelochemical profile. Plots with low vegetation ratings and high insect numbers also had high LIR's that were correlated with poor crown ratings, decreased resin flow, increased root stain and poor root condition. The above-ground symptoms of radial growth reduction, increased foliar transparency, and decreased crown density within symptomatic plots, as compared to controls, corresponded to root debilitation and is consistent with work done in other pines associated with *Leptographium* spp. (Leaphart and Gill, 1959; Wagener and

Mielke, 1961). *Leptographium procerum* was the only species recovered from the soil and it may be less dependent upon vectors to initiate root infection. It is possible that wounding caused by root initiation, or presence of dead or decaying lateral roots could serve as openings for infection by this fungus (see Chapter IV). Damaged roots were frequently observed in the field during excavations, but *L. procerum* was also found on the insects in this study and presumably is also vectored by them. This study also provides the first report of *L. serpens* and *L. lundbergii* within the United States. In South Africa, *L. serpens* behaves as a typical root-infecting fungus, spreading to adjacent trees through root contacts, colonizing ray parenchyma and tracheids, and resulting in wedge-shaped patterns of discoloration (Wingfield and Knox-Davies, 1980; Wingfield and Marasas, 1980). In the present study, two root feeding insects, *H. salebrosus* and *H. tenuis*, were associated with this fungus and apparently acted as vectors (Table 3.3).

Four insect species (*H. picivorus*, *H. pales*, *H. salebrosus* and *H. tenuis*) occurred in higher numbers in symptomatic than in asymptomatic stands. This is similar to the increased levels of vector activity within stands associated with the incidence of *Leptographium* spp. in red pine decline in Wisconsin (Klepzig *et al.*, 1991) and black stain root disease caused by *L. wagneri* (Hansen, 1978; Harrington *et al.*, 1985). These insects were also consistently associated with *L. terebrantis*, *L. procerum*, and *L. serpens* and appear to serve as vectors of these fungi (Table 3.3), as well as similar fungi in other disease complexes (Table 1.1). While *L. lundbergii* was only isolated from insects in the Choccolocco State Park, these insects were consistently associated with this species. Other insects that occurred in lower numbers [*Ips pini* L. (Coleoptera: Scolytidae), *Dendroctonus frontalis* (Zimmermann) (Coleoptera: Scolytidae), *Pissodes nemorensis* Germar (Coleoptera: Scolytidae) *Xyleborinus*

saxeseini (Ratzeburg) (Coleoptera: Scolytidae), *Xyleborus affinis* (Eichhoff), *Xylosandrus compactus* (Eichhoff) (Coleoptera: Scolytidae), *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Scolytidae), an undescribed *Rhizophagus* spp., *Gnathotrichus materiarius* (Fitch) (Coleoptera: Scolytidae), *Orthotomicus caelatus* Eichhoff (Coleoptera: Scolytidae), *Corthylus punctatissimus* (Zimmermann) (Coleoptera: Scolytidae) and *Monarthrum mali* (Fitch) (Coleoptera: Scolytidae)], were detected only in symptomatic plots. This study provides the first report of an association between *H. salebrosus*, *X. saxeseini*, *X. compactus*, *X. crassiusculus*, the undescribed *Rhizophagus* spp., *G. materiarius*, *O. caelatus*, *C. punctatissimus*, *M. mali*, *C. unicolor* and *Leptographium* spp. *Colopterus unicolor* (sap beetle) is an opportunistic insect that is attracted to fungal mats and may spread *Leptographium* inadvertently. This was the only species to have an association with the undescribed *Leptographium* species, which was also isolated from hardwood roots (Appendix A). All the members of the genus *Colopterus* are known to be subcortical and generalized sap feeders and are found on both pines and hardwoods (Parsons, 1943). Nitidulid beetles also have been reported to be associated with the overland transmission of *Ceratocystis fagacearum* (Oak wilt) by the attraction to damaged areas where sap may be present and/or to sweet smelling fungal mats (Himelick and Curl, 1958; McMullen *et al.*, 1960; Appel *et al.*, 1986; Juzwik *et al.*, 1999; Cease and Juzwik, 2001; Juzwik, 2001). Both *Corthylus punctatissimus* and *M. mali* are considered hardwood pests that breed in maple and dogwood (USDA, 1985), which are abundant in the Alabama study plots, but *M. mali* has been observed previously on pines (USDA, 1985; Hanula *et al.*, 2002). *Xylosandrus compactus* and *X. crassiusculus* are not known or reported to utilize conifers as a primary host.

An important question to be considered in LPD, then, is what factors affect insect population and behavior? All of the insect species in this study were present in large numbers when understory species were controlled by prescription burns. It appears that fire could be a catalyst in LPD by stressing and damaging trees, which stimulates production of terpenoid compounds, resulting in increased attacks by pestiferous insects. Prescription burns over time leads to multiple attacks by root feeding insects that repeatedly vector *Leptographium* spp. into the host root system. This relationship needs further investigation, particularly because it may have significant implications for current forestry management practices. Hanula *et al.* (2002) reported that bark beetles, ambrosia beetles and weevils exhibited a significantly increased abundance in burn areas as compared to controls. They found also that trees in these burned areas were infected with one or more species of *Leptographium* and/or *Graphium* species, and no such fungi were recovered from unburned stands.

Littleleaf disease, as described in the literature (Campbell and Copeland, 1954) and LPD are not the same disease. Littleleaf disease typically occurs in poorly drained clay soils, but LPD occurs on relatively well drained sandy loam/loamy sand soils. In contrast, the healthiest loblolly stands occurred in low lying wet areas (Appendix A). Littleleaf disease is associated with the occurrence of *P. cinnamomi*, and no *P. cinnamomi* could be isolated from roots of LPD trees (Ann Weber, unpublished). Also, soil bulk density was generally higher in decline plots, but not significantly higher, especially in subsurface layers below 40 cm. Concomitant with increased bulk density, a reduction in total porosity was evident in decline sites. However, bulk density and total porosity values did not reach growth limiting levels (personal communication, Dr. Emily Carter, USFS, Southern Research Station,

Auburn, AL). Soil nutrient analysis showed the soil pH range to be between 3.73 and 5.59 with aluminum levels ranging from 7 - 234 ppm, which has been shown to reduce the virulence of *Phytophthora* spp. (Erwin, 1996).

The role of *P. cinnamomi* in LLD was attributed to its ability to kill fine roots (Campbell and Copeland, 1954) and thus inhibit root growth and nutrient absorption. Fine roots generally live from 1 to 4 yrs, and mortality is associated with cold weather, excessive soil moisture, poor soil drainage and aeration, drought, attacks by insects, fungi and other organisms, and foliage loss (Zimmerman and Brown, 1971; Torrey and Clarkson, 1975). It is also known that loblolly pine is not as susceptible to *P. cinnamomi* as is shortleaf pine (Fraedrich and Tainter, 1989; Fraedrich *et al.*, 1989). *Phytophthora cinnamomi* was found in the soils of only a few plots. While *P. cinnamomi* could be a factor of fine root death, it was not present in the fine or larger roots of the trees sampled, and presence of *P. cinnamomi* within soils was not correlated with any of the measures of LPD. However, occurrence of this fungus in soil was correlated with the presence of hardwood species known to be hosts in the plots (Appendix A). These data indicate that *P. cinnamomi* does not play a role in LPD (Ann Weber, personal communication). The data from this research strongly suggests that LPD as a disease syndrome, is distinct from LLD.

CHAPTER IV

PATHOGENICITY OF FOUR *LEPTOGRAPHIUM* SPECIES ASSOCIATED WITH LOBLOLLY PINE DECLINE IN CENTRAL ALABAMA

4.1 Introduction

The associations of *Leptographium* spp. and root feeding insects with root disease has been demonstrated in several systems (Table 1.1). In the southeastern United States, loblolly pine stands affected by root disease appear to be more vulnerable to attack by southern pine beetle and are more likely to contain *Leptographium* species within their root systems than are apparently healthy stands (Hess *et al.*, 1999; Otrrosina *et al.*, 1997).

Data on the pathogenicity of *Leptographium* spp. to pines (Harrington and Cobb, 1983; Lackner and Alexander, 1983; Wingfield, 1983; Raffa and Smalley, 1988; Nevill *et al.*, 1995) are minimal. Only *Leptographium procerum* (Kendrick) Wingfield and *L. terebrantis* Barras and Perry have been tested for pathogenicity on loblolly pine (Lackner and Alexander, 1983; Cook and Hain, 1985, 1988; Nevill *et al.*, 1995). *Leptographium terebrantis* killed seedlings, while *L. procerum* did not. *Leptographium terebrantis* is also pathogenic to several conifer species that occur in the northeastern and western U.S. (Harrington and Cobb, 1983; Highley and Tatter, 1985; Wingfield, 1986; Owen *et al.*, 1987; Rane and Tatter, 1987; Raffa and Smalley, 1988). In recent pathogenicity studies, Zhou *et al.* (2002) concluded that *L. serpens* and *L. lundbergii* are not serious tree pathogens in South Africa.

Leptographium serpens (Goid.) Wingfield has recently been reported in central Alabama in loblolly pine (see Chapter III) and Louisiana in longleaf pine (Bauman *et al.*, 2001).

Leptographium lundbergii Lagerb. and Melin. has recently been reported in central Alabama in

loblolly pine (see Chapter III). These are the first reports of these two species in the southeastern United States. *Leptographium procerum*, *L. terebrantis*, *L. serpens* and *L. lundbergii* (see Chapter III) were isolated from roots and soil of trees exhibiting loblolly pine decline symptoms in central Alabama. They were used to conduct a series of inoculations to determine their potential as pathogens on loblolly pine.

4.2 Materials and Methods

4.2.1 Inoculation Studies

Three inoculation studies (one field, two greenhouse) were conducted to determine the potential pathogenicity of selected *Leptographium* species to loblolly pine and provide preliminary information on modes of infection and root colonization by these fungi. All seedlings used in these experiments were supplied by the LDAF (Louisiana Department of Agriculture and Forestry), Office of Forestry. The *Leptographium* spp. used for inoculations were collected as described in Chapter II.

Roots were inoculated with one isolate of *L. procerum* (Table 4.1) from collected samples and one isolate of *L. procerum* (253,CMW) provided by M.J. Wingfield (FABI, Pretoria, South Africa). Both were grown in liquid ME (2% malt extract) for two weeks and then macerated in a blender for 15 seconds to form a slurry into which bare roots were dipped. *Leptographium terebrantis*, *L. serpens*, *L. procerum* and *L. lundbergii* were also grown on MEA plates, from which 3-mm plugs were cut for stem inoculations as described in Table 4.1.

Ninety freshly lifted bare-root seedlings were inoculated immediately after lifting, or were potted in the greenhouse and inoculated after two or four weeks by removing the seedlings, dipping the roots in the *Leptographium* slurry (adapted from Hessburg and Hansen, 2000) (Fig.

Table 4.1. Sources of *Leptographium* isolates used for inoculation studies.

Isolate	Isolate #	Collection Site	Host Source
<i>Leptographium serpens</i>	LOB-R-00-309	Talladega National Forest, Shoal Creek Ranger District	Loblolly pine root
<i>L. terebrantis</i>	LOB-R-00-805	Gulf State Paper Company Land	Loblolly pine root
<i>L. procerum</i>	LOB-I-00-456	Talladega National Forest, Oakmulgee Ranger District	<i>P. picivorus</i> ¹ from infected tree
<i>L. procerum</i>	LOB-S-00-525	Talladega National Forest, Shoal Creek Ranger District	Soil from loblolly pine root zone
<i>L. procerum</i> ²	253, CMW	Italy	<i>Pinus nigra</i>
<i>L. lundbergii</i>	LOB-R-00-57	Choccolocco State Park	Loblolly pine root

¹ *Pachylobius picivorus* (Coleoptera : Curculionidae), pitch-eating weevil.

² Isolate provided by Dr. M.J. Wingfield, FABI, Pretoria, South Africa.

4.1) and repotting in 2.5L containers containing Jiffy Mix Plus™. Pots were randomized in the greenhouse and watered from above to saturation three times each week for nine weeks (December - March, and repeated March - July). Seedlings were destructively sampled 34, 44, 54, 64, and 74 days after inoculation, with six pots sampled each time, including pots with seedlings showing even the slightest indication of wilting. At the time of destructive sampling, seedlings were removed from the pots, root systems gently washed, wrapped in a wet



Figure 4.1. Dip inoculation procedure employed for loblolly pine seedlings.

paper towel, and stored in plastic bags at 4°C for no longer than 24 h. Dissections were made under a binocular dissecting microscope by systematically removing the bark from the root collar to the root ends. Once the bark was removed, the remaining tissue was repeatedly misted with a spray bottle containing a sterile dilute solution of distilled water and lemon juice to keep the wood moist and discourage phenolic discoloration (Hessburg and Hansen, 2000). Root initials were counted, examined and dissected to determine infection. Infection was determined by staining of root tissue and the observation of mycelial growth using a dissecting scope. The

number of infections was tallied for each seedling. Number of root initials infected and present were analyzed using a SAS (2001) one-way ANOVA and Proc Mixed. Tukey's W was used for mean separation. Re-isolation of the fungi was attempted from all inoculated and uninoculated roots.

Fifty bare-root seedlings were potted in the greenhouse and stem inoculated after 12 weeks. Seedlings were prepared for inoculation by pruning needles from the lower portion of the stem and surface disinfesting the area with 70% ethanol. A sterile razor blade was used to cut a small slit 2-7 mm long in the bark 2 cm above the soil line. A 3 mm dia plug of MEA colonized by the fungus was inserted into the wound, wrapped with sterile cotton, moistened with sterile water, and secured with parafilm. Controls consisted of an identical inoculation with a sterile plug of MEA. The seedlings were held in a greenhouse for up to 3 months to allow symptom development. Seedlings were watered three times per week; surviving seedlings were examined and lesions were measured and recorded. Lesion length was analyzed using a SAS (2001) one-way ANOVA and Proc Mixed. Tukey's W was used as a Post-ANOVA technique. Re-isolation of the fungus was attempted from the inoculation area including 1 cm above and below the observed lesion.

4.2.2 Tree Inoculation

Twenty trees from a 21 year old stand in the Johnston Tract, Palustris Experimental Forest, Kisatchie National Forest, LA were selected for inoculation studies. Height and diameter at breast height (dbh) were recorded for each tree. Inoculations closely followed the procedure described by Klepzig *et al.*, (1995). Each tree was inoculated at four equidistant points with the respective fungus growing on MEA as described above with a fifth point receiving only

mechanical damage as a control. A preliminary study showed no difference between inoculation with sterile MEA and mechanical damage. Each tree was inoculated once with each of four fungi: *Leptographium procerum*, *L. terebrantis*, *L. serpens* and *L. lundbergii*, in random sequence. Inoculation points were prepared by smoothing the bark with a sterile machete around the stem at breast height. The inoculations were made by punching holes (using a 1.9 cm diameter leather punch) into the xylem, placing a 1 cm diameter plug of colonized MEA into each hole with a sterile spatula, and sealing the holes by placing the bark plugs back in and then covering with duct tape.

Inoculations were destructively sampled after three weeks. The trees were de-barked in the inoculation zone, sprayed with a dilute solution of lemon juice and the lesions traced onto overhead transparencies. The total area of resinosis was measured for each lesion using a digital planimeter. Lesion area was analyzed using a SAS (2001) one-way ANOVA and Proc Mixed. Tukey's W was used as a Post-ANOVA technique. Lesion area also was analyzed for correlations with tree height and dbh, employing simple linear regression.

4.3 Results

4.3.1 Seedling Root Inoculation

There were no significant differences between repetitions and data were combined for subsequent analysis. There were significant differences among treatments for total root mass, total root initials produced and number of root initials infected (Fig. 4.2). Only four seedlings died during the course of the experiment. Symptoms, yellowing and browning of foliage, occurred one to two weeks earlier in the time zero and two week inoculations than in the four week inoculations and was more prevalent in these seedlings.



Figure 4.2. Root mass and seedling growth comparison between (a) control, (b) Lp-W (isolate # 253, CMW, Table 3.1), (c) Lp (isolate # LOB-S-00-525).

Leptographium procerum inoculated seedlings produced significantly ($F_{2,267} = 746.10$, $p < 0.0001$) fewer root initials leading to smaller root mass with a higher rate of infection ($F_{2,267} = 799.06$, $p < 0.0001$) than controls (Table 4.2 and Fig. 4.4). There was no significant difference between either *L. procerum* isolate. *Leptographium procerum* had an adverse effect on loblolly seedling roots, causing smaller root mass, deteriorated and dead roots and root initials. These results are similar to the effect described of *Phytophthora cinnamomi* on shortleaf pine by Campbell and Copeland (1954).

Re-isolation from the roots revealed that all test fungi colonized the xylem adjacent to the infection site (root initial). Fungi were also re-isolated from the stem area at least 2 cm above the soil line. None of the inoculated fungi were recovered in sections beyond 2 cm above the soil line. There were no fungi recovered from control seedlings.

Table 4.2. Mean root initials, mean percent infected, mean percent fungal recovery, mean percent mortality for loblolly pine seedlings sampled 34, 44, 54, 64, and 74 days after inoculation with fungi obtained from roots soils adjacent to declining loblolly pine. n=60 for all treatments.

Inoculation Time	Isolate	Mean Root Initials	Mean Percent Infected	Mean % Fungal Recovery	Mean Percent Mortality
Initial	Control	423 a ¹	0 a ¹	0 a ¹	0 a ¹
	Lp-W	149 b	100 b	100 b	0 a
	Lp	161 b	100 b	100 b	3 a
2 week	Control	522 ac	0 a	0 a	0 a
	Lp-W	168 b	94 b	100 b	7 a
	Lp	163 b	93 b	100 b	0 a
4 week	Control	682 c	0 a	0 a	0 a
	Lp-W	216 b	76 b	100 b	0 a
	Lp	227 b	76 b	100 b	3 a

¹ Numbers followed by the same letter within a column are not significantly different at P=0.05, using Tukey's W.

4.3.2 Seedling and Tree Stem Inoculation

There were significant differences among all treatments for lesion length ($F_{4,45} = 278.97$, $p < 0.0001$) and vertical occlusion of xylem ($F_{4,45} = 532.25$, $p < 0.0001$) but not for mortality. Only eight seedlings, one control and seven inoculated, died during the study. Inoculations with all isolates produced expanding resin-soaked lesions. All *Leptographium* species tested produced significantly longer lesions than did controls, but *L. lundbergii* and *L. serpens* were the longest (Table 4.3 and Fig. 4.5). Lesions produced in mature trees by *L. serpens*, *L. lundbergii*, *L. terebrantis* and *L. procerum* were significantly larger than control inoculations ($F_{1,95} = 107.35$, $p < 0.0001$) (Table 4.4 and Fig. 4.6).

Re-isolation from the inoculation site of seedlings revealed that all test fungi colonized the xylem adjacent to the inoculation site. Fungi also were re-isolated beyond the 1 cm section adjacent to the inoculation point when the lesion or occluded tissue extended into the next 1 cm section. None of the inoculated fungi were recovered in the 1 cm sections beyond the last section with occluded tissue.

Table 4.3. Mean length (and standard error) of lesion and linear extent of sapwood occlusion of loblolly pine seedlings 4 months after inoculation with fungi obtained from loblolly pines with loblolly decline symptoms.

Inoculum	Mean Lesion Length (mm)¹	Mean Occluded Length (mm)¹
<i>Leptographium lundbergii</i>	34.09 (4.51) a ²	52.67 (4.75) a ²
<i>Leptographium serpens</i>	30.02 (1.46) b	45.47 (1.12) b
<i>Leptographium terebrantis</i>	19.95 (1.71) c	33.62 (1.53) c
<i>Leptographium procerum</i> ³	9.69 (0.46) d	18.96 (0.96) d
Control	7.00 (0.00) e	11.57 (0.96) e

¹ Mean lesion length and mean occluded sapwood include 7 mm inoculation wound.

² Numbers followed by the same letter within a column are not significantly different at P=0.05, using Tukey's W.

³ Isolate # LOB-I-00456.

Table 4.4. Mean area (and standard error) of lesion of sapwood occlusion of loblolly pine trees three months after inoculation with fungi obtained from loblolly pines with loblolly decline symptoms.

Inoculum	Mean Lesion Area (cm)¹
<i>Leptographium serpens</i>	44.15 (9.53) a ²
<i>Leptographium terebrantis</i>	34.77 (9.60) b
<i>Leptographium procerum</i>	22.64 (4.95) c
<i>Leptographium lundbergii</i>	55.36 (12.17) d
Control	4.78 (0.49) e

¹ Mean lesion area include 4.7 cm inoculation wound.

² Numbers followed by the same letter within a column are not significantly different at P=0.05, using Tukey's W.

4.4 Discussion

The pathogenicity of the *Leptographium* species examined in this study was consistent with pathogenicity studies previously conducted on conifers (Harrington and Cobb, 1983; Wingfield 1983; Paine 1984; Cook and Hain, 1985; Wingfield, 1986; Owen *et al.*, 1987; Cook and Hain, 1988; Parmeter *et al.*, 1989, Parmeter *et al.*, 1992; Smalley *et al.*, 1993; Klepzig *et al.*, 1995; Nevill *et al.*, 1995). Seedling mortality was not a significant factor in this study, although it has been in other pathogenicity studies (Harrington and Cobb, 1983; Lackner and Alexander, 1983; Wingfield 1983; Owen *et al.*, 1987). Differences in mortality between this and other studies may be due to differences in host species, inoculum dose, or inoculation technique. Mortality rates could have been higher if the experiment had run longer.

Of the fungi tested, *L. lundbergii* and *L. serpens* caused the longest lesions in both the seedling and tree inoculations. Lesion length has been used to compare pathogenicity of different blue-stain fungi (Molnar, 1965; Safranyik *et al.*, 1983; Paine, 1984), but Solheim (1988) found that the most virulent fungus, associated with the ambrosia beetle *Ips typographus*, *Ceratocystis polonica*, caused shorter lesions than the less pathogenic *Ophiostoma penicillatum*. Lesion lengths may not be the best all-round measure of pathogenicity of blue-stain fungi, and it should be used carefully. Lesion length may reflect host resistance, with small lesions indicating a rapid and effective defense response of resistant trees with less resistant trees producing longer lesions in response to inoculation (Horntvedt, 1988; Solheim, 1988, 1995). In this study, tree resistance was accounted for by inoculating all fungi into the same tree, therefore, all differences were due only to the virulence of the fungi. The fungi in this study appear to be weak pathogens that attack trees weakened by marginal site parameters, leading to predisposition to attacks by other pathogenic fungi and insects.

The causal relationship between *Leptographium* spp. and coniferous root diseases must be interpreted carefully. *Leptographium* spp. are often secondary pathogens introduced by bark beetles to trees that are already stressed by other pathogens or environmental factors (Harrington and Cobb, 1983; Wingfield, 1986). They are easily isolated and could overgrow the other pathogens when diseased tissue is cultured. The distinct lesions these fungi produce in inoculated roots are significant, however. The *Leptographium* spp. are introduced by root feeding bark beetles, such as *Hylastes* spp. (Wingfield, 1986), that feed on apparently healthy or slightly stressed roots (Goheen and Cobb, 1978; Harrington *et al.*, 1983; Harrington *et al.*, 1985; Witcosky and Hansen, 1985). This suggests a complex of interacting factors which may impact the tree synergistically. This report confirms the basic ability of *Leptographium* species to attack loblolly pine and supports their possible role as primary pathogens involved in LPD.

CHAPTER V

***HYLASTES SALEBROSUS* AND *HYLASTES TENUIS* (COLEOPTERA:SCOLYTIDAE): VECTORS OF PATHOGENIC FUNGI (OPHIOSTOMATALES) ASSOCIATED WITH LOBLOLLY PINE DECLINE**

5.1 Introduction

Loblolly pine decline symptoms have been observed with increasing frequency at various locations in Alabama, Louisiana and South Carolina within 40-50 year old loblolly pine (*Pinus taeda* L.) stands (Campbell and Copeland, 1954; Lorio, 1966; Brown and McDowell, 1968; Oak and Tainter, 1988; Hess *et al.*, 1999). The symptoms associated with loblolly pine decline are non-specific and common to decline diseases in general, including littleleaf disease (Lorio, 1966). They include short chlorotic needles, sparse crowns, and reduced radial growth followed by death (Hess *et al.*, 1999, 2002; Lorio, 1966). Root systems of declining trees exhibit high rates of mortality and infection with the vascular stain fungi *Leptographium procerum* (Kendrick) Wingfield, *L. terebrantis* Barras and Perry, *L. lundbergii* Lagerb. and Melin, and *L. serpens* (Goid.) Wingfield (see Chapter III).

Five species of root and lower stem infesting insects, the pales weevil, *Hylobius pales* (Herbst), the pitch eating weevil, *Pachylobius picivorus* (Germar), the black turpentine beetle, *Dendroctonus terebrans* Olivier, *Hylastes salebrosus* Eichoff and *H. tenuis* Eichoff have been associated with LPD (see Chapter III). Other insects associated with LPD include *Xyleborinus saxesini*, *Xylosandrus crassiusculus*, *Gnathotrichus materiarius*, and *Monarthrum mali*. These insects are also newly reported vectors of *Leptographium* species (see Chapter III), but little is known about their biology and host preference.

Ophiostomatoid fungi are consistently associated with bark beetle species (Paine *et al.*, 1997), yet their roles in the life cycles of these insects remain poorly understood. Initial research on stain fungi-bark beetle interactions focused on the role of the beetles as vectors of these fungi, but pathogenicity tests of bark beetle associated fungi have produced mixed results. Some stain fungi are capable of killing seedlings (Rane and Tattar, 1987) while others are associated with the death of mature trees when accompanied by mass wounding and inoculation (Mathre, 1964; Horntvedt *et al.*, 1983), but most inoculation experiments result in restricted host defensive reactions (Shrimpton, 1973; Raffa and Berryman, 1982; Raffa and Berryman, 1983a, b; Cook and Hain, 1986; Paine and Stephen, 1987; Cook and Hain, 1988; Raffa, 1991; Lieutier *et al.*, 1993; Raffa and Smalley, 1995; Paine *et al.*, 1997). Harrington (1993b) has gone so far as to conclude that most bark beetle-associated stain fungi apparently weaken trees by lowering host resistance.

The ecological relationships of stain fungi and bark beetles are unclear, but the most extensively studied systems indicate that these fungi reduce reproductive success of the beetles (Barras, 1970; Yearian *et al.*, 1972; Klepzig and Wilkens, 1997; Robins and Reid 1997; Klepzig *et al.*, 2001) by reducing brood production and/or causing larval avoidance of stained regions. There is also evidence that, rather than killing trees, the stain fungi may reduce the exposure of colonizing beetles to plant defensive chemicals to tolerable levels (Hemingway *et al.*, 1977; Christiansen and Horntverdt, 1983; Raffa and Berryman, 1983a). Based on this body of evidence, Raffa (1995) proposed that the net impact of ophiostomatoid fungi on their bark beetle vectors may vary with the conditions of the host, ranging from negative in dead logs to positive in healthy well-defended trees. A direct test of this model is difficult with aggressive beetles because mass attacks are required to colonize trees, and the beetles cannot develop without

killing their hosts. However, less aggressive beetles (e.g., *Hylastes*) associated with compromised hosts are more easily studied. *Hylastes* spp. are root feeding bark beetles that typically attack unhealthy, declining, wounded, or even dead pines (Wood, 1982; Klepzig *et al.*, 1991) and have been associated with decline diseases in pines (Klepzig *et al.*, 1991). Therefore, the purpose of this experiment was to determine the effectiveness of the vector and to determine whether or not *Leptographium* spp. have any role (mutualistic or antagonistic) in the development of the beetle.

5.2 Materials and Methods

5.2.1 Vectoring Capability and Insect Reproduction

Loblolly pine roots approximately 5-7 cm dia. were removed from healthy pines at the Palustris Experimental Forest (Rapides Parish, LA) and tested by plating (as described in Chapter II) and visual examination to ensure that they were free of *Leptographium* spp. Roots were then cut into 12 sections 30 cm in length (adapted from Six and Payne, 1998). Six root sections were drilled with entrance holes to facilitate and/or induce entry by *H. salebrosus* or *H. tenuis* and six additional roots were left undrilled. The severed ends of each root were dipped in paraffin wax to retard dessication and buried under moist, sterilized sand in plastic boxes (one root segment per box) (Fig. 5.1). One hundred and twenty *H. salebrosus* and 120 *H. tenuis* were collected as described in Chapter II, surface-sterilized with commercial bleach, EtOH, and distilled water solution (10:10:80 v/v) for 1 min. and gently rolled on CSMA (2% malt extract agar containing 800 mg/l of cycloheximide and 200 mg/l of streptomycin sulfate) to verify absence of viable *Leptographium* spp. propagules. Sixty *H. salebrosus* and 60 *H. tenuis* were then placed on growing cultures of *L. terebrantis* or *L. serpens* (isolated from trapped insects) for approximately

12 hours to allow inoculum acquisition. Sixty inoculated and 60 uninoculated adults, of each species were introduced into the plastic boxes containing the root sections (5 males and 5 females per box), and covered with cheese cloth to prevent escape. Boxes were kept at 25°C under an 8-10 hour photoperiod ($56 \mu\text{mol m}^{-2}\text{s}^{-1}$), and watered with distilled H₂O every other day to keep the sand moist. Root sections were visually examined every 2-3 days to detect adult entrance; after nine weeks roots were stripped to visually determine adult mating, larval survival, staining



Figure 5.1. Loblolly root sections partially buried under moist, sterilized sand in plastic boxes.

and presence of fungal fruiting structures. Parent adult insects were removed and not included in determinations of brood productions. Samples from galleries of inoculated and sterilized beetles were plated on CSMA and MEA to determine the presence or absence of *Leptographium* spp. and other fungi. Number of emerging brood, pupae, and larvae were compared among treatments using the SAS (2001) ANOVA procedure and Tukey's W as a Post-ANOVA technique.

5.3 Results

5.3.1 Insect Recovery

Because beetle reproductive success was significantly affected by species of *Hylastes* and *Leptographium* spp. ($F_{1,32}=86.56$, $p<0.0001$), the two insect species were considered separately in subsequent analyses. Neither the creation of wound courts ($F_{1,22}=0.16$, $p<0.07$) nor the species of *Leptographium* used ($F_{1,22}=0.03$, $p<0.87$) significantly affected the reproductive success of *H. salebrosus* (Fig. 5.2). However, the presence of *Leptographium* spp. on *H. salebrosus* adults did

significantly increase their reproductive success ($F_{1,22}=691.74$, $p<0.0001$) (Fig. 5.3); brood production was almost 2.5 times higher in *Leptographium* infected roots than in controls.

Drilling to provide entrance courts for *H. tenuis* ($F_{1,22}=0.17$, $p=0.69$), nor the species of *Leptographium* used significantly affected the reproductive success of *H. tenuis* (Fig. 5.4). However, the presence of *Leptographium* spp. on *H. tenuis* adults did significantly increase their reproductive success ($F_{1,22}=49.83$, $p<0.0001$) by 19% (Fig. 5.5). Out of a subsample of 174 emerging *H. salebrosus* and 152 *H. tenuis*, 162 and 152 were females, respectively. This is similar to results obtained by Klepzig (1994) with laboratory colonies of *Hylastes porculus*, and indicates the strong possibility of parthenogenic reproduction in this genus.

5.3.2 Fungal Recovery

H. salebrosus and *H. tenuis* both vectored *L. terebrantis* and *L. serpens* to 100% of the roots into which they were introduced and neither were found in control root sections. *Leptographium terebrantis* and *L. serpens* were also recovered from 100% of adults and larval *H. salebrosus* and *H. tenuis* emerging from *Leptographium* infested root sections. These root sections also exhibited the extensive staining (Fig. 5.6) typical of *Leptographium* infected roots. Fungi were recovered from entrance and exit holes, galleries, and pupal chambers (Fig. 5.7) of vector inoculated roots, but not from control roots.

5.4 Discussion

In this study, reproduction by two species of *Hylastes* was increased due to the influence of *Leptographium* fungi. This is the first report that I am aware of, in which stain fungi positively affected non-aggressive beetles. Raffa and Smalley (1995) reported that although phytopathogenic fungi can assist bark beetles in killing trees, trees respond to the presence of

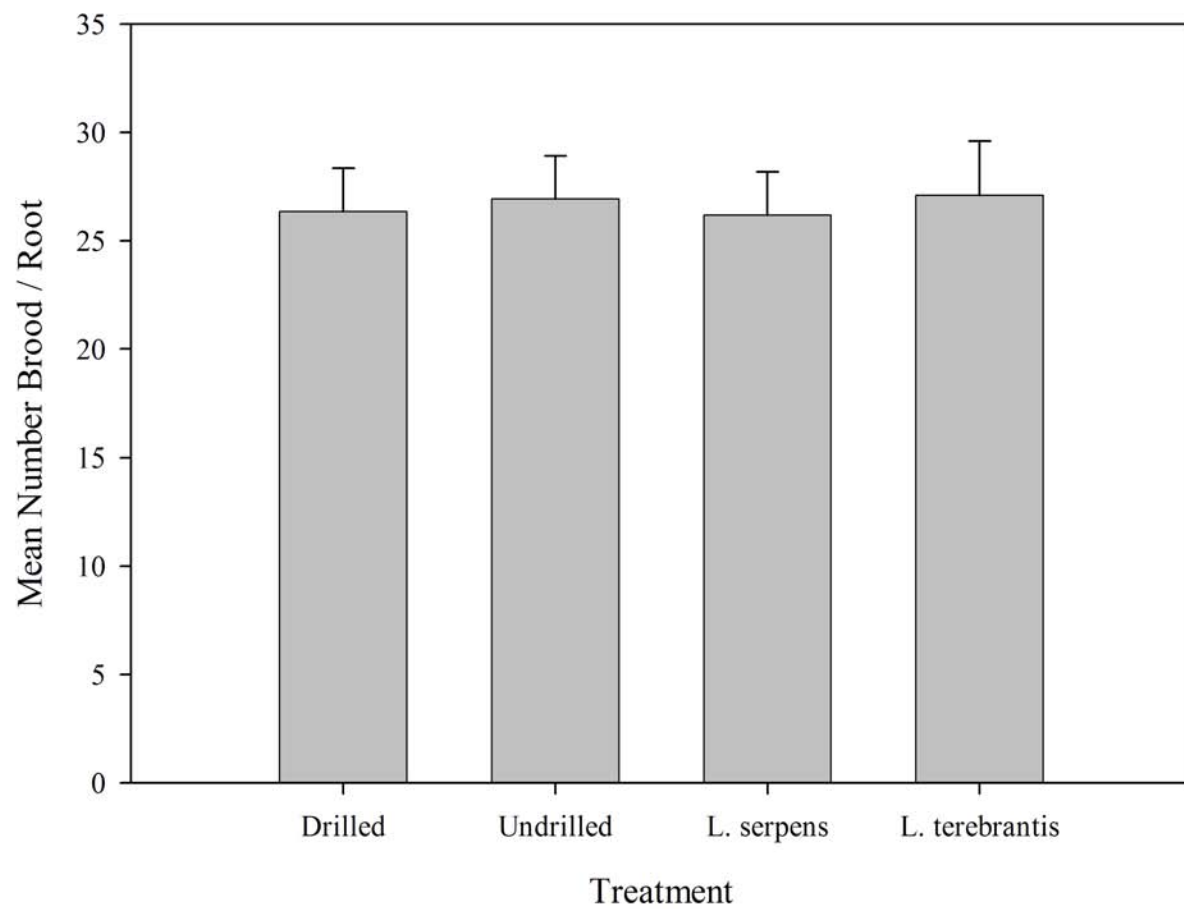


Figure 5.2. Effects of wound and fungal treatments on *H. salebrosus* brood production in *P. taeda* roots. Bars indicate standard error.

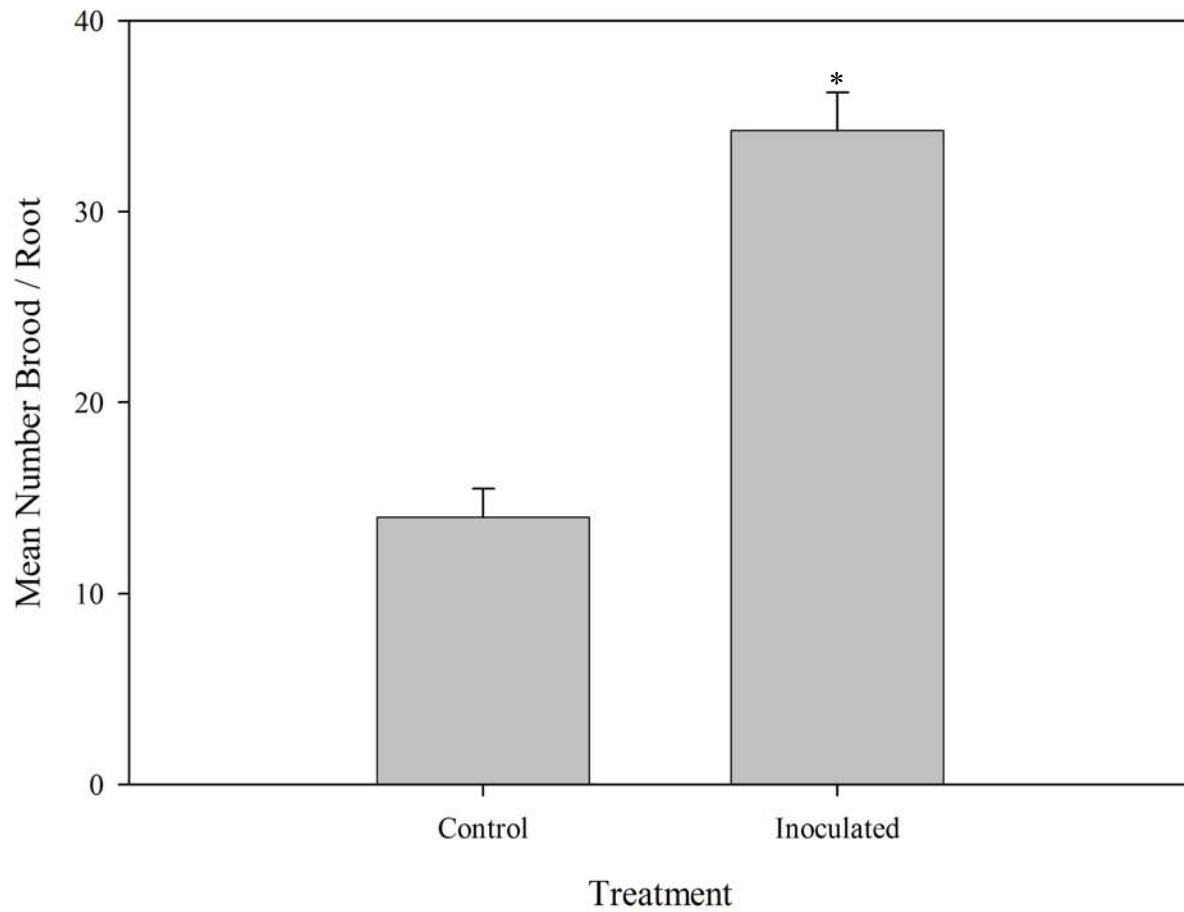


Figure 5.3. Effects of *Leptographium* spp. on *H. salebrosus* brood production in *P. taeda* roots. Bars indicate standard error. * = significant difference from control ($p < 0.0001$).

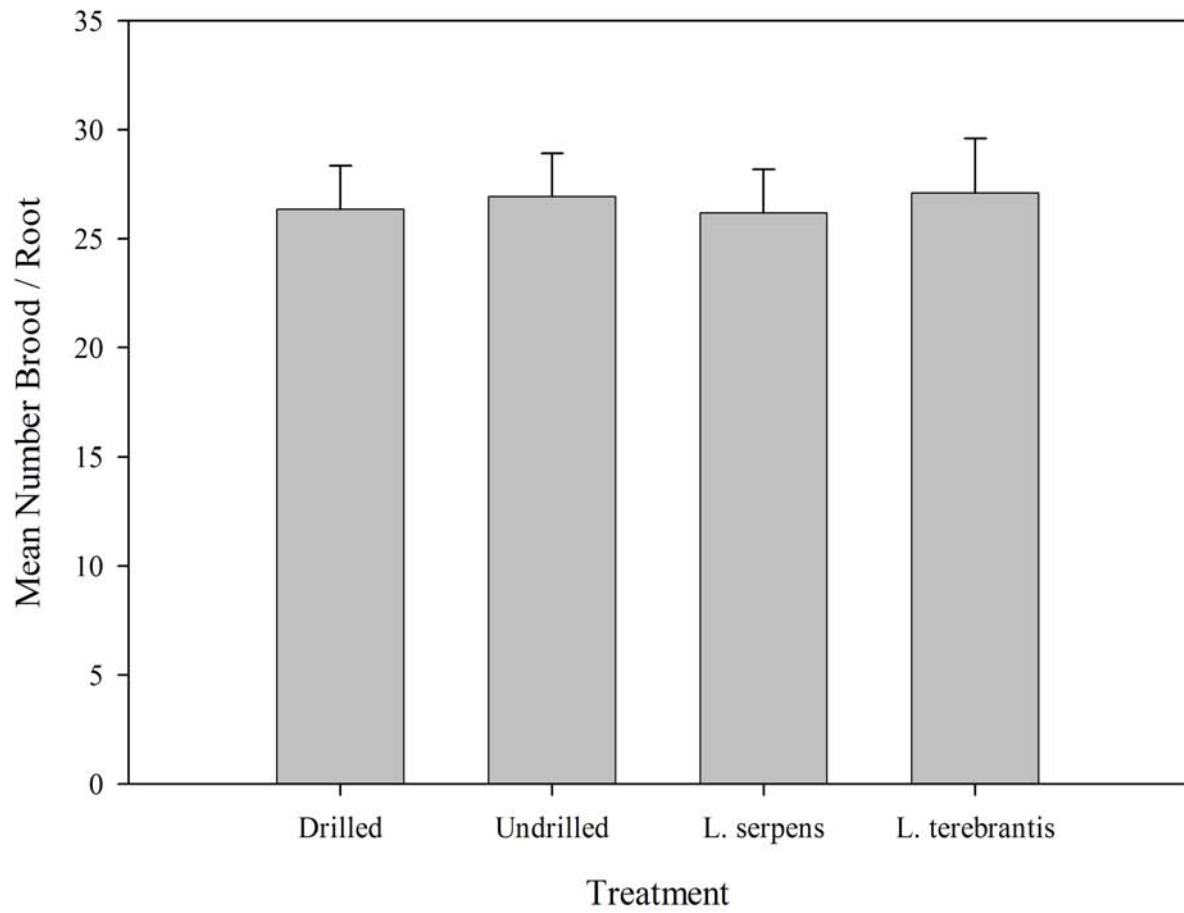


Figure 5.4. Effects of wound and fungal treatments on *H. tenuis* brood production in *P. taeda* roots. Bars indicate standard error.

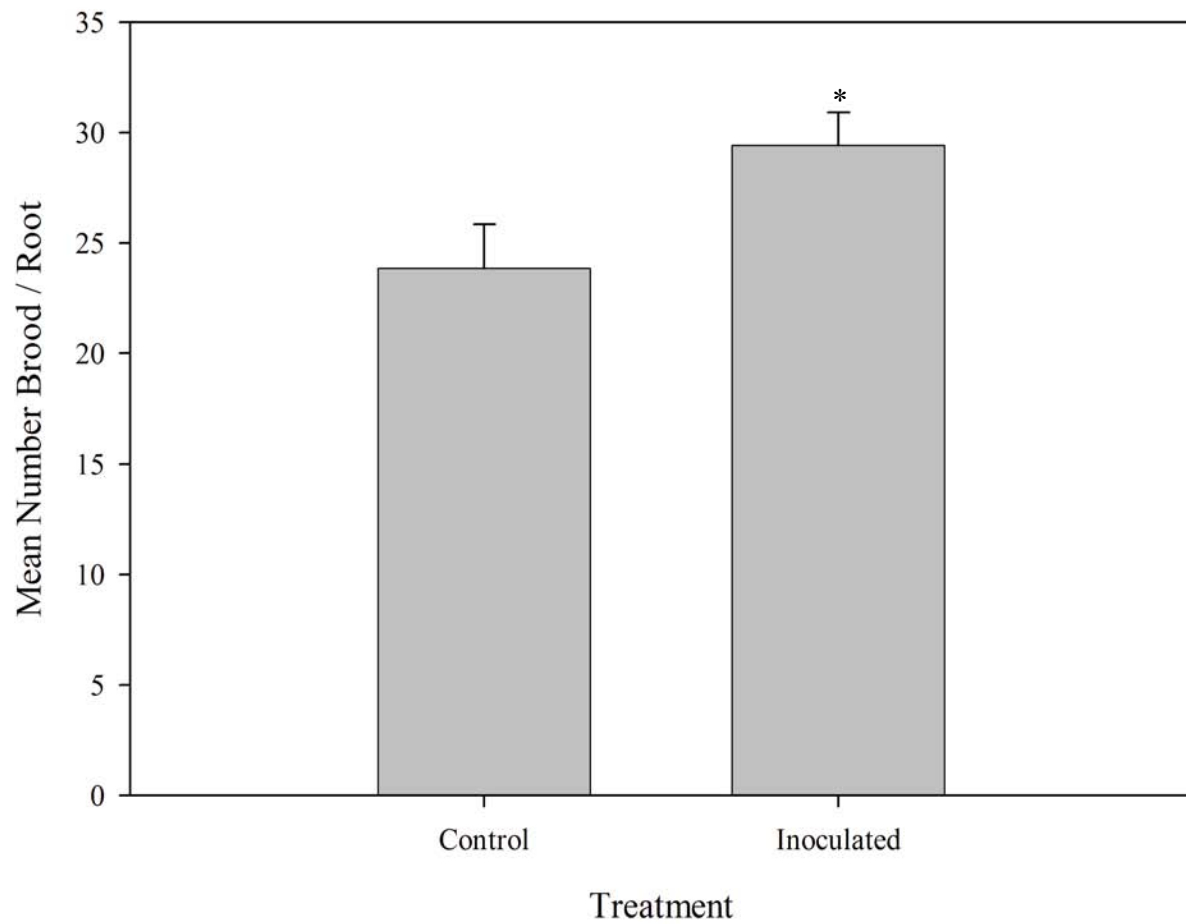


Figure 5.5. Effects of *Leptographium* spp. on *H. tenuis* brood production in *P. taeda* roots. Bars indicate standard error. * = significant difference from control ($p < 0.0001$).



Figure 5.6. *Pinus taeda* root sections infested with *Hylastes tenuis* root beetles. (a) surface-sterilized, uninoculated beetles. (b) beetles inoculated with *Leptographium* spp. Note extensive staining.

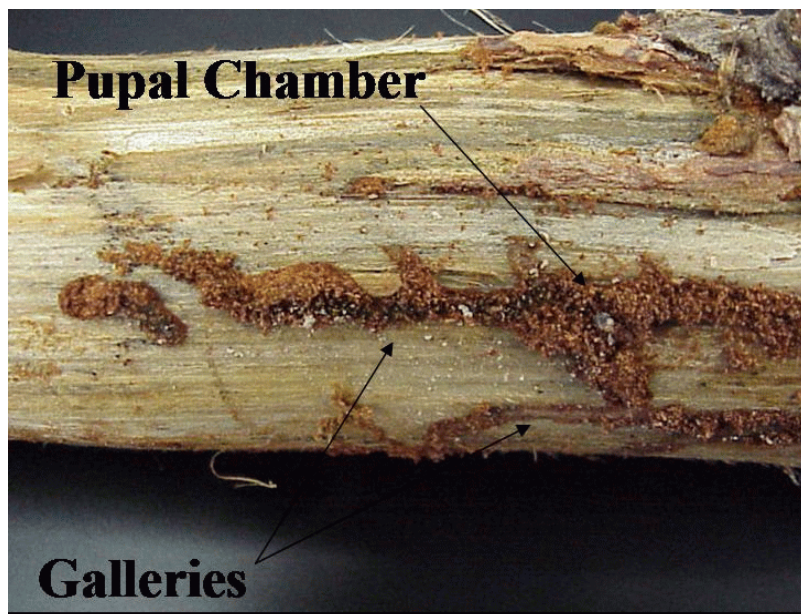


Figure 5.7. Root section showing *L. terebrantis* introduced by *H. salebrosus* growing in galleries and pupal chambers.

these fungi by accumulating allelochemicals to concentrations that adversely affect the beetle vector. Raffa suggested (personal communication) three possible, nonexclusive, mechanisms by which ophiostomatoid fungi could affect beetle populations: 1) Certain fungi may reduce host tree resistance against bark beetles. For example, *Ophiostoma piliferum* is used as a biopulping agent, primarily due to its ability to degrade diterpene acids (Blanchette *et al.*, 1992), which have allelochemical properties. 2) Some fungi may compete with developing larvae for host nutrients, or otherwise interfere with brood development (Barras 1970, 1973; Ayres *et al.*, 2000). The nitrogen content of phloem is about 0.38% in healthy loblolly; therefore, bark beetles must concentrate dietary nitrogen by 16-26 fold (Hodges and Lorio, 1969). Ayres *et al.* (2000) noted regions of high N concentration associated with colonies of mycangial fungi, perhaps because the hyphae of mycangial fungi extract N from phloem and concentrate it into the feeding chamber. In contrast, N concentrations were lower where blue stain fungi grew. These stain fungi are apparently out-competing the larvae for nitrogen. 3) Some fungi may compete with other fungi that either facilitate brood development (e.g. mutualistic mycangial fungi) or with those that compete with developing beetle larvae (antagonistic non-mycangial fungi) (Klepzig and Wilkens, 1997).

The first mechanism offers the best explanation for the results presented here. Certainly it did not appear that the *Leptographium* species competed with the insects within roots as in the second proposed mechanism. In this study, control roots did not contain contaminating fungi which could have affected results one way or the other, a requirement under the third scenario. Even though the root sections used were not the same as roots of living trees, there still could be

levels of allelochemicals present that otherwise might interfere with *Hylastes* feeding and development. I believe that the *Leptographium* species inoculated into the roots increased beetle success by making the host material more suitable for their insect vectors, either by detoxification of the host chemistry, or by the production of some metabolic by-products that the beetles found useful or nutritive. Further research is needed to determine if one or both of these mechanisms can account for these results.

The vectoring of the two root fungi most commonly associated with loblolly pine decline by the two most common root feeding bark beetles provides additional evidence for the involvement of these bark beetle/fungus complexes in this disease syndrome. Both beetles were able to transmit fungi to wounded and unwounded roots. In addition, beetles of closely related species may be attracted to wounds or host volatiles associated with wounds (Rudinsky and Zethner-Moller, 1967; Owen, 1985; Witcosky *et al.*, 1987; Phillips, 1990; Klepzig *et al.*, 1991; Hobson *et al.*, 1993). These data strengthen the putative role of *H. salebrosus* and *H. tenuis* as agents of LPD.

CHAPTER VI

THE RELATIONSHIP OF ARTHROPOD OCCURRENCE TO RELATIVE LEVELS OF ROOT FEEDING BARK BEETLES AND WEEVILS

6.1 Introduction

Terrestrial arthropods are an integral component of forest ecosystems. Most insects are beneficial to, or have little impact on, tree health, though few are pests capable of causing serious economic damage. Insects associated with loblolly pine decline have little impact on the trees they are attacking, but the fungi that they vector appears extensive in the root systems (see Chapter III). These beetles increase in population when trees are under stress and fungal inoculation is increased. Root feeding bark beetles and weevils, for example, were much more abundant in loblolly decline plots than asymptomatic plots (see Chapter III). The pestiferous populations in these sites showed large fluctuations during the year, while asymptomatic plot populations stayed at relatively low constant levels (Chapter III, Fig. 3.7 and Fig. 3.8). Symptomatic plots also exhibited lower vegetation densities due, in part, to prescribed fires (Chapter III, Table 3.5). It was hypothesized that the increased insect abundance observed was due to the lower vegetation density, enabling insects to more effectively find their tree hosts. Low predator populations (i.e., carabid beetles) and/or tree stress may also contribute to this pattern.

In this study, focus was specifically on the relative abundance of non-pest ground inhabiting beetles (Coleoptera) that were attracted to, or at least not deterred from the same cues that bark beetles and weevils are attracted to. This group was chosen for study because it

contained species that function as herbivores, predators, and fungivores, are easily sampled through a variety of passive-trapping methods, relatively easy to identify (Hutcheson and Jones, 1999) and are regarded as a good group with which to evaluate habitat change (Thiele, 1977; Gardner, 1991; Niemelä *et al.*, 1993). The majority of ground beetle species are predators of larvae and other small arthropods (Dillon and Dillon, 1961). Through patterns in their diversity and abundance, ground beetles can provide indirect information regarding the status of their prey and how alterations in habitat conditions affect them (Day and Carthy, 1988). Also, the polyphagous habits of carabids (Best and Beegle, 1977; Kirk, 1982), as predators of pest insects (Floate *et al.*, 1990; Winder, 1990) and consumers of plant material (Johnson and Cameron, 1969), have been well documented. The abundance of other predatory arthropods (centipedes, spiders, fire ants, scorpions) were also considered. My interest was focused on the interactions of ground beetles on root-feeding bark beetles and weevils although other arthropods were noted.

6.2 Materials and Methods

6.2.1 Insect Trapping

Insects were collected in two ways: pitfall trapping and duff sampling (as described in chapter II). Statistical analysis was performed as described below. Number of insects and number of families were analyzed for correlations with duff weight, vegetation density and LIR. Significance for the model was determined using SAS proc GLM and proc REG (SAS, 2001). SAS proc CORR Kendall was used to determine any correlations between vegetation density or LIR and insect abundance.

6.2.2 Vegetation Density Ratings (VDR)

Completed as described in Chapter II.

6.2.3 Feeding Experiments

Scarites subterraneus F. (Coleoptera: Carabidae) captured alive in pitfall traps and duff were placed in separate petri dishes: 78 were starved for 36 hours and 78 were not. Each insect was ultimately transferred to a petri dish with or without duff material: 1 predator/dish; 39 dishes/treatment. Different species of root feeding bark beetles (*Hylastes salebrosus*, *H. tenuis*, and *Dendroctonus terebrans*), weevils (*Hylobius pales* and *Pachylobius picivorus*), ambrosia beetles (*Xyleborinus saxesini*, *Xylosandrus compactus*, and *X. crassiusculus*) and other species trapped on the plots (*Corthylus punctatissimus*, *Gnathotrichous materiarius*, *Monarthrum mali*, *Nitidulid* spp. and *Orthotomicus caelatus*) were added to the dishes (3 dishes/insect/treatment). Dishes were checked every 2 hr to determine if the carabid had consumed the insect added to its dish. Sixty-six *Scarites substriatus* Say were also captured as described and treated similarly, except none were starved. Data were analyzed using SAS (2001) ANOVA procedure and Duncan's multiple range test ($P \leq 0.05$).

6.3 Results

6.3.1 Insect Trapping

Thirty-three arthropod families representing 12 orders were collected from the moderate to high vegetation rated plots (Table 6.1 and Table 6.2), while only 13 families representing 6 orders were collected from low vegetation density plots. There was a significant difference of duff weight/m² among different vegetation densities ($F_{2,42} = 151.91$, $p < 0.0001$) (Table 6.3) and a positive correlation between the number of families of arthropods collected and the vegetation density ($r^2 = 0.91$). There were 2.5 times as many families in the moderate to high vegetation rated plots for both pitfall traps and duff sampling. Eighteen species of carabids were collected from duff (Table 6.4) with a positive correlation between the number of carabid species collected

and the vegetation density ($r^2 = 0.91$), with five times as many species in the moderate to high vs. low vegetation rated plots for both pitfall traps and duff sampling. There were no beneficial insects and only six pestiferous insects caught on plot C2 in duff and pitfall traps in 2002, as compared to 27 and 147 in 2001, respectively, possibly because plot C2 was burned during January 2002, the stand on the eastern side burned February 2002 and stands to the north and south of the plot were clearcut in September 2000 and October 2001, respectively, due to southern pine beetle attacks. It is probable that habitat alteration adjacent to the trapping plot had an affect on insect populations within the plot.

A large number of spiders (Araneae) were also collected from the duff (Table 6.1 and Table 6.2), and more ground webs and spiders were observed on plots with higher density vegetation, although no counts were made. In the moderate to higher vegetation density, around pitfall traps, spiders commonly constructed webs that consistently trapped bark beetles. Many spiders are forest floor predators and feed on larvae and other small arthropods (Dillon and Dillon, 1961). Fire ant mounds (Hymenoptera: Formicidae: *Solenopsis invicta* Buren) also were observed to be more abundant on plots with higher density vegetation. Both spiders and fire ants may be considered predatory on ground insects such as root feeding bark beetles, as well as, beneficial ground insects (ie., carabid beetles). A large number of centipedes (Scolopendromorpha) were also collected from both pitfall traps and duff. Centipedes are venomous predators, generally feeding on earthworms, other insects and spiders.

Eighteen species of ground beetles were collected from the moderate to high vegetation plots, while only 3 (*Calosoma scrutator* and *Pasimachus punctulatus*, and *Harpalus pensylvanicus*) were collected from low vegetation density plots (Table 6.4). More species of carabids were collected from all plots in 2002 than in 2001.

Table 6.1. Arthropod families collected from pitfall traps during 2001 and 2002 and the numbers of individuals within each family from each vegetation density.

Order	Family	Vegetation Density			Total	% Total ¹
		Low	Moderate	High		
Araneae	Gnaphosidae	0	13	19	32	1.5
	Lycosidae	0	38	54	92	4.4
	Thomisidae	0	25	26	51	2.4
Blattaria	Blattidae	14	42	52	108	5.1
Coleoptera	Buprestidae	0	16	20	36	1.7
	Cerambycidae	0	25	17	42	2.0
	Curculionidae	382	123	115	620	-
	Elateridae	2	12	10	24	1.1
	Nitidulidae	0	100	179	279	13.3
	Platypodidae	174	0	0	174	8.3
	Rhizophagidae	0	10	5	15	*
	Scarabaeidae	0	28	50	78	3.7
	Scolytidae	2751	628	427	3806	-
	Staphylinidae	2	14	14	30	1.5
	Tenebrionidae	0	300	213	513	24.4
	Trogositidae	33	4	1	38	1.8
Dermaptera	Forficulidae	0	60	58	118	5.6
Hemiptera	Aradidae	0	8	9	17	*
	Largidae	3	14	10	27	1.3
	Pentatomidae	0	5	10	15	*
	Reduviidae	10	120	130	260	12.4
Hymenoptera	Formicidae	0	32	50	82	3.9
	Mutillidae	0	4	2	6	*
Opiliones	Sclerosomatidae	0	2	9	11	*
Orthoptera	Tettigoniidae	0	8	6	14	*
Scolopendromorpha	Scolopendridae	0	24	19	43	2.0
Scorpiones	Buthidae	1	4	7	12	*

¹ Percent total excluding Scolytidae and Curculionidae, because the trapping methods were biased toward these pest insects. * values < 1%

Table 6.2. Arthropod families collected from duff sampling (2002) showing numbers of individuals within each family from each vegetation density.

Order	Family	Vegetation Density			Total	% Total
		Low	Moderate	High		
Araneae	Agelenidae	13	23	31	67	2.6
	Gnaphosidae	0	23	29	52	2.1
	Lycosidae	0	28	34	62	2.4
	Thomisidae	2	38	29	69	2.7
Blattaria	Blattidae	4	22	35	61	2.4
Coleoptera	Buprestidae	0	10	20	30	1.2
	Carabidae	53	249	318	620	24.5
	Cerambycidae	0	21	10	31	1.2
	Curculionidae	18	10	5	33	1.3
	Elateridae	2	8	10	20	*
	Nitidulidae	0	10	19	29	1.1
	Scarabaeidae	0	38	60	98	3.9
	Scolytidae	21	8	0	29	1.1
	Silphidae	0	8	10	18	*
	Staphylinidae	100	300	307	707	27.9
	Tenebrionidae	0	20	33	53	2.1
Dermaptera	Forficulidae	0	40	58	98	3.9
Hemiptera	Aradidae	0	18	17	35	1.4
	Coriidae	0	3	4	7	*
	Largidae	3	12	10	25	1.0
	Pentatomidae	0	15	20	35	1.4
	Reduviidae	10	20	30	63	2.5
Hymenoptera	Formicidae	0	38	80	118	4.1
	Mutillidae	0	2	2	4	*
Isoptera	Rhinotermitidae	67	0	0	67	2.6
Opiliones	Sclerosomatidae	0	20	29	49	1.9
Orthoptera	Tettigoniidae	0	8	6	14	*
Scolopendromorpha	Scolopendridae	0	34	26	60	2.4
Scorpiones	Buthidae	1	6	8	15	*
Spirobolida	Spirobildae	0	2	4	6	*

* values < 1%

Table 6.3. Comparison of duff weights/m² among vegetation densities.

Vegetation Density Rating	Duff Weight (kg)
Low	1.09 (.32) a
Moderate	1.94 (.34) b
High	3.32 (.24) c

¹ Numbers followed by the same letter within a column are not significantly different at P=0.05, using Tukey's W; number in parenthesis is the S.E.

Table 6.4. Carabidae collected from duff sampling in 2002 and the numbers of individuals collected from each vegetation density grouping.

Species	Vegetation Density			Total	% Total
	Low	Moderate	High		
<i>Agonum decentis</i>	0	12	21	33	5.3
<i>Bothriopterus adstictus</i>	0	28	31	59	9.5
<i>Bothriopterus pensylvanicus</i>	0	31	48	79	12.7
<i>Calosoma calidum</i> F.	0	3	1	4	*
<i>Calosoma frigidum</i>	0	2	3	5	*
<i>Calosoma scrutator</i>	21	0	0	21	3.4
<i>Carabus sylvosus</i>	0	34	41	75	12.1
<i>Carabus serratus</i>	0	26	28	54	8.7
<i>Dicaelus sculptilis</i>	0	19	26	45	7.3
<i>Harpalus pennsylvanicus</i>	15	0	0	15	2.4
<i>Pasimachus punctulatus</i>	17	0	0	17	2.7
<i>Pterostichus femoralis</i> Kirby	0	2	3	5	*
<i>Pterostichus permundus</i> Say	0	8	9	17	2.7
<i>Scaphinotus elevatus</i>	0	2	2	4	*
<i>Scarites subterraneus</i> F.	0	22	28	50	8.1
<i>Scarites substriatus</i> Say	0	31	41	72	11.6
<i>Sphaeroderus lecontei</i>	0	23	31	54	8.7
<i>Synuchus impunctatus</i>	0	6	5	11	1.8

* values < 1%

6.3.2 Feeding Experiments

There was no significant effect of starvation on carabid predation of bark beetles ($F_{1,64} = 0.09$, $p < 0.77$), but consumption time was significantly affected by the presence of duff ($F_{10,65} = 27.17$, $p < 0.0001$ and $F_{11,65} = 25.08$, $p < 0.0001$) (Table 6.5 and Table 6.6, respectively). There was a positive correlation between insect size and carabid consumption in the presence of duff ($r^2 = 0.92$).

Table 6.5. Mean time (hours) until consumption of insect by *Scarites subterraneus* in the laboratory

Insect	Mean Time of Consumption	
	w/o duff	duff
<i>Xyleborinus saxesini</i>	2.0	11.3 a
<i>Xylosandrus crassiusculus</i>	2.3	11.3 a
<i>Xylosandrus compactus</i>	2.3	10.6 ab
<i>Hylastes tenuis</i>	2.6	10.3 abc
<i>Monarthrum mali</i>	2.0	10.0 abcd
<i>Orthotomicus caelatus</i>	2.3	9.3 bcd
<i>Corthylus punctatissimus</i>	2.0	8.6 cd
<i>Gnathotrichous materiorius</i>	2.3	8.3 d
<i>Colopterus unicolor</i>	2.0	6.6 e
<i>Hylastes salebrosus</i>	2.3	5.6 e
<i>Dendroctonus terebrans</i>	2.6	2.0 f
<i>Hylobius pales</i>	NC ²	NC
<i>Pachylobius picivorus</i>	NC	NC

¹ Numbers followed by the same letter within a column are not significantly different at $P=0.05$, using Duncan's multiple range test.

² NC = not consumed. Insects that were not consumed were not included in the SAS analysis.

Table 6.6. Insect choice and mean time until consumption of insect by *Scarites substriatus*

Insect Combination	Chosen Insect & Mean Time (hrs) of Consumption							
	w/o duff				duff			
<i>Xyleborinus saxesini</i> (Xs)+ <i>Dendroctonus terebrans</i> (Dt)	Dt	2.0 a ¹	Xs	2.0 a	Dt	2.0 a	Xs	12.0 a
	Xs		Dt		Dt		Xs	
	Dt		Xs		Dt		Xs	
<i>Xylosandrus crassiusulus</i> (Xc) + <i>Hylastes tenuis</i> (Ht)	Xc	2.5 a	Ht	2.0 b	Ht	10.0 a	Xc	11.0 a
	Ht		Xc		Ht		Xc	
	Ht		Xc		Ht		Xc	
<i>Xylosandrus compactus</i> (Xc) + <i>Xyleborinus saxesini</i> (Xs)	Xc	2.0 a	Xs	2.0 b	Xc	10.5 a	Xs	11.5 a
	Xc		Xs		Xs		Xc	
	Xs		Xc		Xc		Xs	
<i>Monarthrum mali</i> (Mm) + <i>Colopterus unicolor</i> (Cu)	Mm	2.5 a	Cu	2.5 c	Cu	6.0 a	Mm	11.0 a
	Mm		Cu		Cu		Mm	
	Cu		Mm		Cu		Mm	
<i>Hylastes tenuis</i> (Ht) + <i>Hylastes salebrosus</i> (Hs)	Ht	2.5 a	Hs	2.0 cd	Hs	5.0 a	Ht	12.0 a
	Hs		Ht		Hs		Ht	
	Ht		Hs		Hs		Ht	
<i>Orthotomicus caelatus</i> (Oc) + <i>Monarthrum mali</i> (Mm)	Oc	2.0 a	Mm	2.5e	Mm	8.5 a	Oc	6.5 b
	Mm		Oc		Oc		Mm	
	Oc		Mm		Mm		Oc	
<i>Gnathotrichous materiorius</i> (Gm) + <i>Orthotomicus caelatus</i> (Oc)	Gm	2.0 a	Oc	2.5 ce	Oc	6.0 a	Gm	8.0 bc
	Oc		Gm		Gm		Oc	
	Gm		Oc		Oc		Gm	
<i>Corthylus punctatissimus</i> (Cp) + <i>Monarthrum mali</i> (Mm)	Cp	2.5 a	Mm	2.0e	Mm	8.0 a	Cp	8.5 b
	Mm		Cp		Cp		Mm	
	Mm		Cp		Mm		Cp	
<i>Colopterus unicolor</i> (Cu) + <i>Xylosandrus crassiusulus</i> (Xc)	Cu	2.5 a	Xc	2.5 c	Cu	5.5 a	Xc	11.0 a
	Xc		Cu		Cu		Xc	
	Cu		Xc		Cu		Xc	
<i>Dendroctonus terebrans</i> (Dt) + <i>Hylastes salebrosus</i> (Hs)	Hs	2.0 a	Dt	2.5 ad	Dt	2.0 a	Hs	2.5 d
	Dt		Hs		Dt		Hs	
	Hs		Dt		Dt		Hs	
<i>Hylastes salebrosus</i> (Hs) + <i>Xylosandrus compactus</i> (Xc)	Hs	2.5 a	Xc	2.0 a	Hs	2.5 a	Xc	10.5 ac
	Xc		Hs		Hs		Xc	
	Xc		Hs		Hs		Xc	

¹ Numbers followed by the same letter within a column are not significantly different at P=0.05, using Tukey's W.

6.4 Discussion

Vegetation density had an affect on the number and species of ground beetles trapped or collected in this study. *Calosoma scrutator* is a species generally found in open forests, while *P. punctulatus* and *H. pennsylvanicus* are species typically found in open, grassy fields. Thompson and Allen (1993) suggest that finding ground beetle species, such as these, in forests is indicative of disturbances such as those caused by prescription burns in the study area. The presence of these species suggests that the prescription burns altered habitat conditions in these stands by changing the vegetation densities (see chapter III) and/or species composition (Connell, 1978). Fire appeared to affect habitat conditions so severely that species from unburned stands were restricted from burned stands, as seen on plot C2. Total arthropod and ground beetle abundance was significantly reduced in the low vegetation density plots ($F_{2,12} = 53.93$; $p < 0.0001$ and $F_{2,12} = 37.71$; $p < 0.0001$, respectively), which could be expected to have ramifications for other faunal groups. Other invertebrates that prefer open, disturbed conditions could be expected to increase in these low vegetation areas, along with species that use dead wood as a habitat or food source. In addition, those insect species (root feeding bark beetles and weevils) that take advantage of weakened or wounded trees clearly benefit if fire damage to the residual stand is great. Based on these data, it appears that the absence of herbaceous understory was directly or indirectly related to the populations of the ground beetle species examined and also probably lead to an increase in abundance of root feeding beetles and weevils. The increased abundance of carabids in the moderate to high vegetation densities suggested that management practices that favor higher vegetation density should be considered. However, these management techniques would cause more competition between pines and other vegetation, which is what prescription burning is used

to minimize. More research is necessary to develop management practices that balance the need to reduce competition to pine from high vegetation density with the need to maintain a vegetation density sufficient to stimulate high levels of beneficial insects.

CHAPTER VII

THE RELATIONSHIP BETWEEN TOPOGRAPHIC FACTORS AND LOBLOLLY PINE DECLINE IN CENTRAL ALABAMA

7.1 Introduction

Declining loblolly stands have been a management concern of forest managers at the Talladega National Forest in Alabama since the 1960's. The symptoms include sparse crowns, reduced radial growth, deterioration of fine roots, and decline and mortality by age 50 (Hess *et al.* 1999, 2001, 2002; Lorio 1966; Chapter II of this dissertation). These symptoms appeared to be more pronounced on areas that had slope and a southern aspect. This observed relationship in the field led to the hypothesis that topographic factors may influence the severity of loblolly pine decline.

Similar relationships have been reported in other tree species. Drohan *et al.* (2002) reported that in sugar maple decline in Pennsylvania declining plots were found more often at higher elevations, tended to be found on S, SW, W, and NW aspects, and dead sugar maple basal area increased in higher topographic positions. Horsley *et al.* (2000) also correlated sugar maple decline to topographic position on the Allegheny Plateau. Thomas *et al.* (2001) reported that in the Vosges mountains of France that altitude, slope and aspect were correlated with fir decline. In Argentina, Baccala *et al.* (1998) reported that the combination of altitude, annual precipitation and slope gradient appeared to be a fairly accurate indicator of decline incidence for *Austrocedrus chilensis*. They found that incidence of decline corresponded to high precipitation and moderate to low altitudes, while healthy trees were corresponded to sites with either low precipitation, or with the combination of high altitudes and/or steep slopes with moderate to high

precipitation. Terrain slope and precipitation were very important in determining the soil water characteristics of a site, which in turn seem to be associated with the decline incidence of *Austrocedrus chilensis*.

The purpose of this study was to analyze the geographic distribution of topographic features and the condition of loblolly pine trees. Available Geographic Information Systems (GIS) databases were combined with the biological data presented in previous chapters in order to identify and predict areas at high risk for loblolly pine decline (LPD).

7.2 Materials and Methods

7.2.1 Study Site

Thirty-nine plots (as described in Chapter II) were established from an area of 880 ha in central Alabama, which encompasses four physioregions (Fig. 7.1). The mean temperature is 16°C and mean yearly precipitation is 1346 mm. Elevation ranges from 83 to 397 m above sea level. The health of the pine trees at each plot was assessed during 1999 - 2001 as described in Chapter II. Additional data taken at each plot for this specific study included percent of slope (100% slope = 45°), aspect (direction which slope faces), convexity, and topographic position (i.e., toe-slope or ridge-top) of each plot.

The geology of each plot was determined by overlaying plot locations in a GIS database with a surface geology map of Alabama. Plot formation and dominant formation lithology were determined from map data tables. The substrate in the four physioregions is composed of marinesediments, clay and sand from the Upper Crustaceous (Upper Coastal Plain and Cumberland Plateau), mica schist and chloritic schist from the Precambrian/Paleozoic (Piedmont,

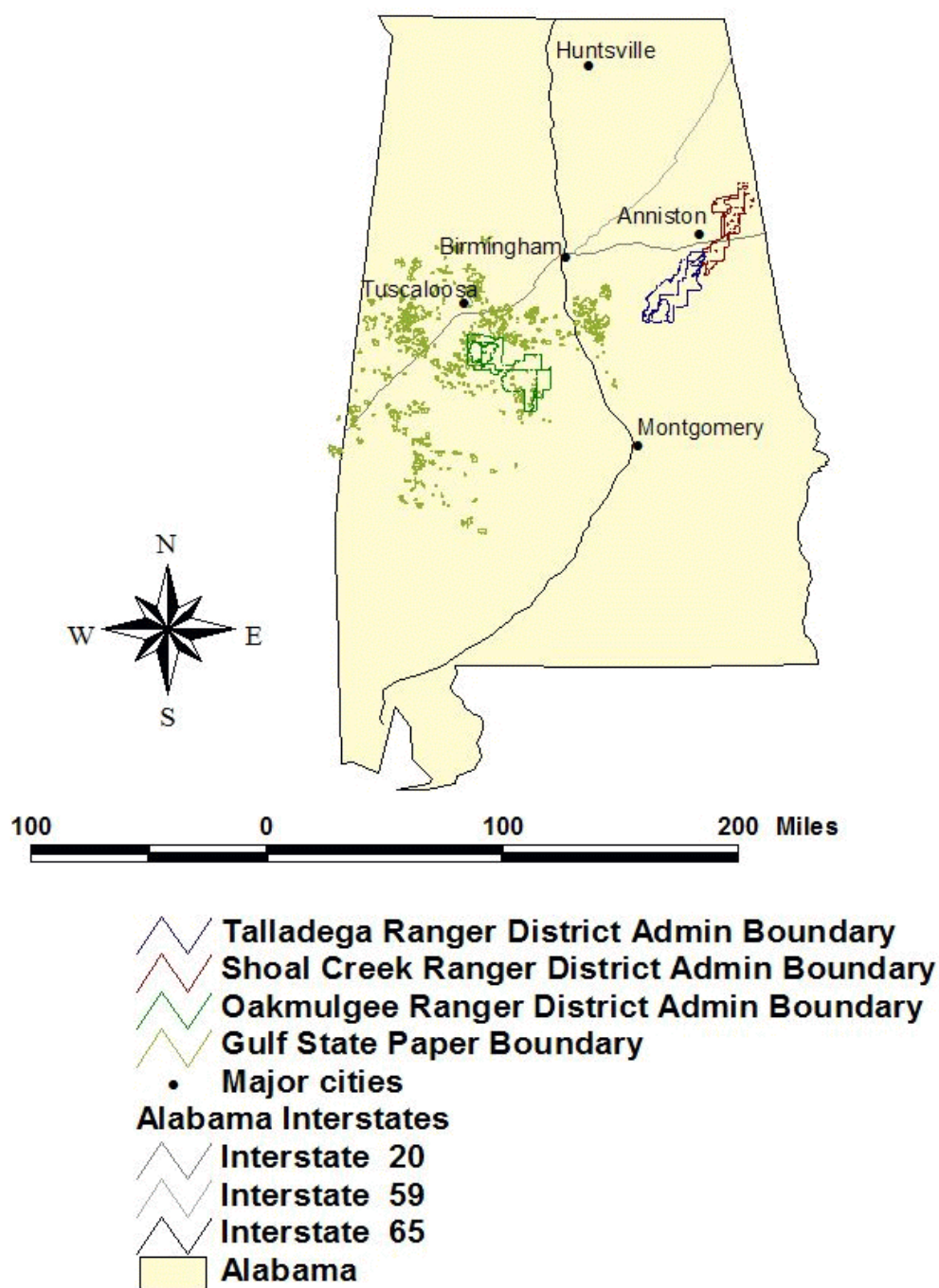


Figure 7.1. Study area in central Alabama. Green = Oakmulgee Ranger District, Red = Shoal Creek Ranger District, Blue = Talladega Ranger District, Gold = Gulf State Paper Company.

Ridge and Valley), limestone and shale from the Cambrian (Ridge and Valley), and slate and phyllite from the Silurian/Devonian (Piedmont).

7.2.2 Plot Topographic Feature and Attribute Characterization

Geographical data were obtained from the Geographical Survey of Alabama (GSA) website (<http://www.gsa.state.al.us/GSA/GIS/DATA>) and the United States Forest Service (USFS), Region 8, GIS web page (<http://www.r8web.com/GIS/alabama>). Topographic data were derived from 10 m and 30 m Digital Elevation Models (DEM) downloaded from the GeoCommunity GIS Data Depot database (<http://data.geocomm.com>). Several parameters were derived from the DEMs: (1) elevation, (2) slope, (3) aspect and (4) surface of aspect. Finally, climatic data (annual precipitation and mean temperature) were obtained from the National Oceanic and Atmospheric Administration (NOAA).

Talladega National Forest shape files delineating district boundaries, stands, compartments, roads, and streams for the Talladega National Forest were also obtained from the USFS, Region 8, GIS web page (<http://www.r8web.com/GIS/alabama>). Shape files for privately owned properties were obtained from Gulf State Paper Company (Bruce DeHaan, Research Forester). All data gathered were georeferenced and reprojected to UTM83 and thus constitute a geographic database of central Alabama.

7.2.3 Map Creation

Leptographium incidence ratings (LIR) developed from plot biological data (see Chapter III) were correlated with plot topographical features derived from DEMs. A range of classes for slope and aspect for each LIR was developed from the geographic table data produced from the merging of the biological data with the stand and CISC data provided by the districts. These

classes were used to create parameters to determine similar areas in the Oakmulgee, Shoal Creek, and Talladega Ranger Districts and Gulf State Paper Company (GSPC) lands. LIRs were converted to risk ratings (None, Low, Moderate, High) to be use for predictive application within the districts.

7.2.4 Statistical Analysis

Because sampling location criteria and data collection procedures were identical for all plot locations, the data were combined for analysis. Categorical independent variables, physioregion, convexity and topography were grouped to reduce the number of categories for model calculation (i.e., the topographic positions ridge-top and nose-slope positions were grouped into an up-slope class). Dummy coding was used for the categorical variables. Presence and absence of *Leptographium* spp. was entered as a binary variable (i.e., a value of 0 for absent, 1 for present). Elevation, slope, latitude (UTM northing), average age tree size, mean precipitation and temperature were entered as continuous variables (i.e., their actual measured value). The aspect measurement, which is azimuthal (circular data), was linearized by taking the cosine of the aspect in radians.

Percent of trees infected in a sample stand was the dependent variable, which was treated statistically as the number of successful events (infected trees) per number of trials (trees sampled) at each sample site. The stepwise regression procedure was performed with the data set using PROC REG STEPWISE option in SAS (2001) to determine which variables were statistically significant and relevant in the model.

7.2.5 Spatial Analysis Among Topographical Features and Between LIR and Topographical Features

To study the effect of topology on loblolly pine decline, all data were divided into classes, or information layers, which were geographically combined to form maps. Contingency tables were subsequently constructed using paired variables to determine the relative area occupied by the different *Leptographium* incident rated plots (see Chapter III) for each topographical feature.

The topographical features used include elevation, slope and aspect. Aspect was divided into eight directions: N, NE, E, SE, S, SW, W, NW; topography was divided into convexity or concavity; topographical position (ridge-top, nose-slope, side-slope, foot-slope, and toe-slope) (Boul *et al.*, 1989) was determined from USGS quadrangle maps and field observations. Because of insufficient sample size in some topographic position classes, the ridge-top and nose-slope positions were grouped into an up-slope class, and the foot-slope and toe-slope positions were grouped into a bottom-slope class.

ArcView 3.2 (ESRI, 1996) along with the Spatial Analysis extension (ESRI, 1996) was used to combine and analyze the different maps obtained by paired comparisons of each geographic, topological, and climatic feature with each LIR (0, 1, 2, 3). Each paired comparison was used to form a unique layer composed of basic polygons containing all the data from the combined layers. These layers were then used to construct contingency tables. The chi-square test (X^2) was applied to the contingency tables to assess the relationships between LIR, geographical, and topographical features, and the Contingency Coefficients (C) were used to measure the intensity of the relationships (SAS, 2001).

7.2.6 Ground-truthing

All data (slope, aspect, convexity, elevation) derived from DEMs for each study plots (39 center and 16 sub-plots) were visually verified in the field for accuracy, and compared to created GIS slope and aspect LIR maps. In addition, 62 sub-plots and 180 additional randomly selected points across all districts were visually checked to verify the condition of a stand as predicted by a combined map of slope and aspect. There was also a comparison of 29 loblolly points previously evaluated by trained forest health personnel with the created maps. All plots compared to combined maps were visually rated as follows: healthy, pre- to moderate decline, and severe decline. These ratings are more realistic for visual ground ratings of decline when it is impossible or impractical to collect root and other plot data.

7.3 Results

7.3.1 Description of Study Area

Among a total of 39 plots observed (see Chapter II), 18% were symptom free (LIR=0), 28% had a LIR rating of 1 (pre-decline, mostly root symptoms but crowns beginning to thin), 31% had a LIR rating of 2 (thinning crowns, short chlorotic needles), and 23% had a LIR rating of 3 (sparse crowns, short chlorotic needles, mortality). Plots were distributed across most slopes and aspects.

7.3.2 Relationships Between Topographic Features and LIR

Slope classes developed from the decline study data (see Chapter III) were found through ground-truthing to be predictive for visual observations in the field within and outside the study plots. Plots with a slope between 0 and 10 % were healthy (LIR=0 or no risk to low risk), between 10-15% had few decline symptoms (LIR=1 or low risk), between 15-20% exhibited

moderate decline symptoms (LIR=2 or moderate risk), and plot with a slope >20% were in severe decline (LIR=3 or high risk). Sixty-one percent of the 56 plots and sub-plots in this study were found most often on side-slope topographic positions. Anomalies were seen in trees located on bordering edges between healthy and decline areas. For example, trees located on the bottom of slopes near areas of severe decline were healthy even though the slope was still above 20%. Based upon field and statistical data, slope appears to have the most significant influence.

Aspect classes developed from the decline study data (see Chapter III) were also found through ground-truthing, predictive for health and decline in the field within and outside the study plots. Aspects of NW/N/NE were healthy. Plots with an east aspect exhibited few decline symptoms. Plots with a west aspect exhibited moderate decline symptoms, and aspects of SW/S/SE exhibited severe decline. Aspect also appeared to have a strong statistical influence. For example, on GSPC lands where there was minimum slope, aspect became the influential determinate indicating where decline was observed. Symptomology was more subtle, but was recognizable to the trained eye. On plots that had minimum slope and S/SW aspect, moderate decline symptoms were observed.

Minor factors, such as convexity and elevation, positively or negatively influenced the degree of symptoms in a particular area. For example, an area that had a concave landform exhibited fewer or more subtle decline symptoms than did an area having a convex landform. These factors play a more important role when paired with either slope or aspect. So, if an area had a convex landform and a S/SW aspect, it exhibited more distinct decline symptoms than a plot that had the same aspect, but was concave. Additionally, minor factors when in combination with both slope and aspect, have an additive affect. For example, plots located on the Shoal

Creek Ranger District which were the highest elevation in the study, exhibited severe decline symptomology when coupled with steep slope and S/SW aspect. These plots had a basal area of 20 with trees dead at age 35 yr. Alone, these factors have little to no effect on the severity of decline.

7.3.3 Statistical Analysis

The stepwise regression identified 7 variables that were potentially related to LIR. The most important variables in the model were slope ($r^2 = 0.92$, $p < 0.0001$) and aspect ($r^2 = 0.86$, $p < 0.0001$). Although other variables were also statistically significant, in combination, these variables accounted for a much smaller proportion of the variation in *Leptographium* incidence than the other variables.

7.3.4 Spatial Analysis

The analysis of contingency tables (Table 7.1) showed that slope was the most highly correlated variable with LIR ($C=0.87$). The proportion of declining loblolly increased as slope increased, and loblolly trees, in general, seemed to be more symptomatic on steep slopes ($> 20\%$ or 9°). Topographic position ($C=0.76$), which is directly related to slope, was also statistically linked to decline. Aspect ($C=0.78$) and convexity ($C=0.66$) were also statistically linked to decline symptoms, and LIR with convex areas with a S/SW aspect exhibiting the most severe decline symptoms. Increased elevation was also statistically linked to LIR ($C=0.67$) and symptom expression, but only in combination with another topographical factor when observed in the field. Older trees (40-62 yr) were found to show more pronounced symptoms than did younger trees (29-40 yr) ($C=0.82$). Physioregion also appeared to be significant ($C=0.62$), but only as a factor as related to elevation when observed in the field. The Ridge and Valley

physioregion located at the highest elevation exhibited the most severe symptoms. Geographical representation and analysis of slope (Fig. 7.2, Fig. 7.4, Fig. 7.6, Fig. 7.8) and aspect (Fig. 7.3, Fig. 7.5, Fig. 7.7, Fig. 7.9) represent what was found from the statistical analysis. The analysis of contingency tables (Table 7.1) also showed that slope and aspect have a strong additive effect ($C=0.92$) when both are present. Trees observed on steeper slopes with S/SW orientation had the most severe decline symptoms across all age classes studied. Elevation and convexity are statistically linked to slope ($C=0.73$ and $C=0.74$, respectively). Either one of these factors in combination with slope were associated with a higher LIR.

7.3.5 Ground-truthing

Ground-truthing verified data derived from DEMs. Data from 10m DEMs were more accurate than data derived from 30m DEMs. When derived data deviated more than 5 % from ground data, ground data were used in the analysis, but this only was a problem in the 30m DEM data. When study plots were ground-truthed against LIR slope and LIR aspect maps, 97% of the study plots agreed with the map data. Differences appear to be related to areas of intense prescribed burning. Randomly selected points and previously evaluated points checked against combined aspect-slope LIR maps (Fig. 7.10, Fig. 7.11, Fig. 7.12, Fig. 7.13) matched 99% of the trees.

7.4 Discussion

Slope, aspect and topographic position of trees on the landscape appear to be predominant “predictive” variables of loblolly decline in central Alabama. Convexity and elevation were predictive only when considered in combination with other topographical factors. Stand age statistically correlated with LIR, such that younger trees (29-40 yr) exhibited fewer symptoms.

Table 7.1. Contingency coefficients derived from contingency tables measuring the relationship between environmental variables and LIR in loblolly pines in Alabama. LIR = *Leptographium* incidence rating; Pr = physioregion; Ag = age of loblolly population; Elv = elevation; Sl = slope; As = aspect; Cv = convexity; Tp = topological position; P = precipitation; T = temperature.

	LIR	Pr	Ag	Elv	Sl	As	Cv	Tp	P	T
LIR	1.00									
Pr	0.62	1.00								
Ag	0.62	0.42	1.00							
Elv	0.67	0.85	0.20	1.00						
Sl	0.87	0.31	0.48	0.73	1.00					
As	0.78	0.41	0.51	0.65	0.92	1.00				
Cv	0.66	0.35	0.33	0.31	0.74	0.63	1.00			
Tp	0.76	0.61	0.39	0.71	0.90	0.64	0.75	1.00		
P	0.42	0.23	0.12	0.24	0.17	0.14	0.18	0.20	1.00	
T	0.12	0.18	0.16	0.31	0.20	0.19	0.17	0.17	0.14	1.00

Physioregion also appeared to be significant, but trees exhibiting all levels of decline were found in all areas.

High decline incidence corresponded to steep slopes (> 20%), while plots with low to no slope were healthy. Terrain slope (convexity and steepness) are very important in determining the soil water characteristics of a site and could increase effects during drought. While precipitation levels were not statistically significant, water moisture characteristics of the soil were not measured. Other authors have noted the importance of slope gradient and position in determining the distribution of water in soils, given certain characteristics of sites (soil type, amount of precipitation, etc.) (Gerardin and Ducruc, 1990; Lundin, 1995). Lower precipitation and steeper slopes would correspond to better drained soils, and higher precipitation and gentle slopes would probably indicate poor drainage conditions, at least during the period of more

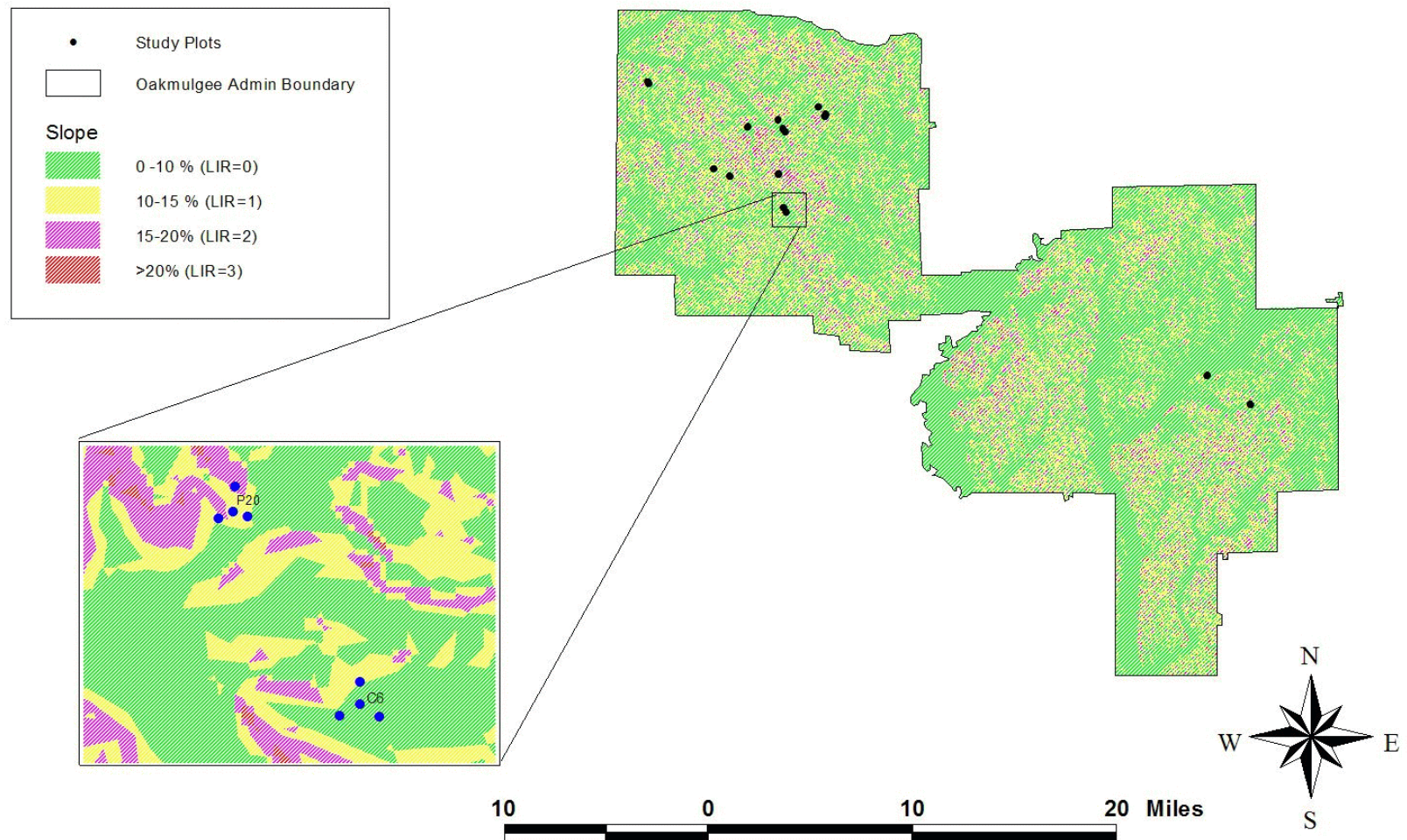


Figure 7.2. A spatial geographic layer representing LIR by slope of the Oakmulgee Ranger District, Talladega National Forest. P20 is a decline plot with a LIR of 3, but the map indicates a LIR of 1. This plot is on a small plateau on a steep slope and aspect plays a role in increasing the LIR to 2. C6, a control plot, has a LIR of 0; subplots of C6 that have slope are adjacent to a drainage and are healthy.

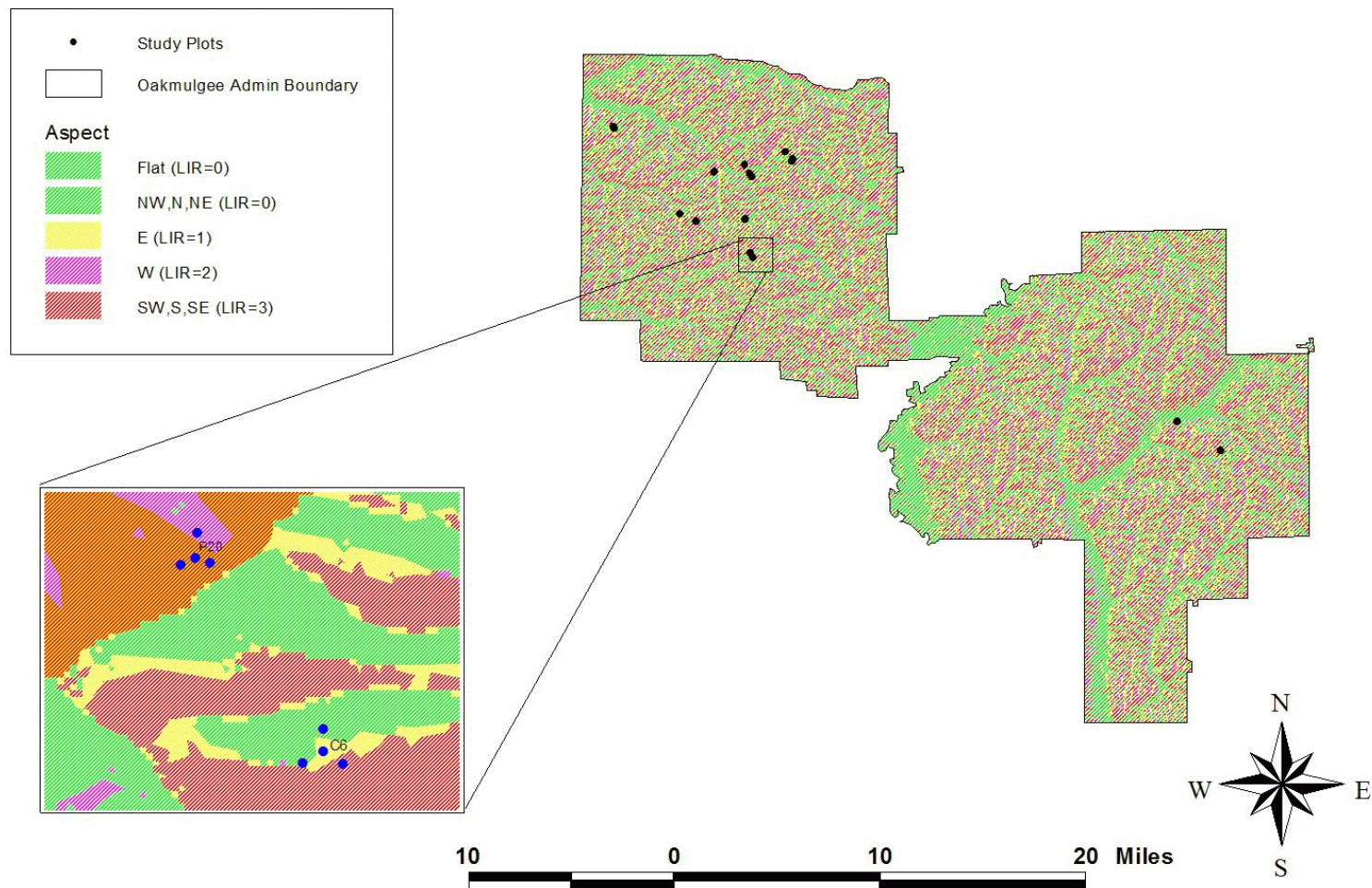


Figure 7.3. A spatial geographic layer representing LIR by aspect of the Oakmulgee Ranger District, Talladega National Forest. Collected data rated P20 as LIR = 3, which is the same as the rating by aspect. C6 rates as LIR = 0, the aspect for C6 is a LIR = 1. The center plot of C6 is in the drainage which lowers the risk of LPD. The C6 subplot in the red is again adjacent to a drainage and is healthy, as per visual ratings (no roots were dug on this subplot).

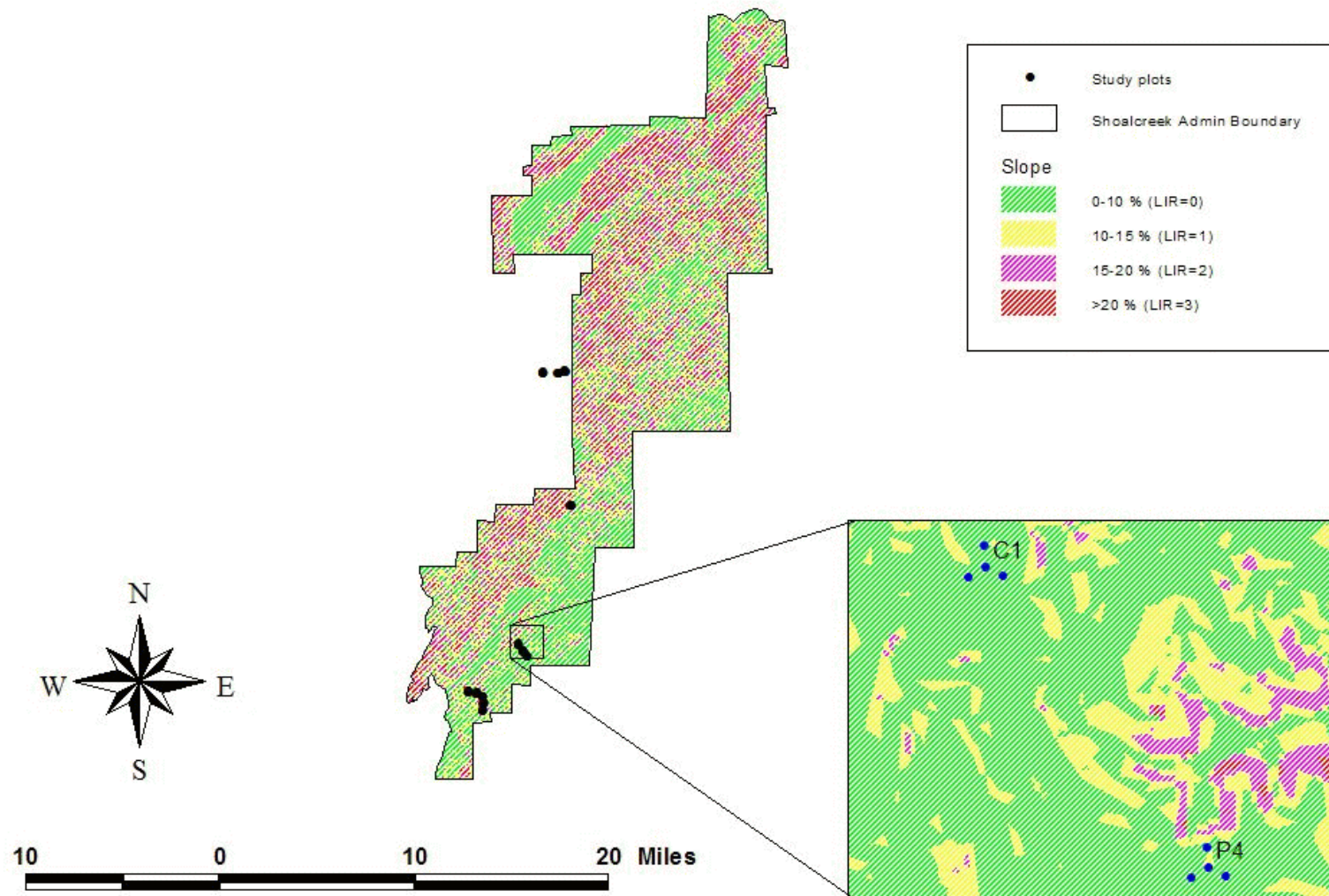


Figure 7.4. A spatial geographic layer representing LIR by slope of the Shoal Creek Ranger District, Talladega National Forest. P4 is a decline plot rated as LIR = 2 and C1 a control plot rated as LIR = 0.

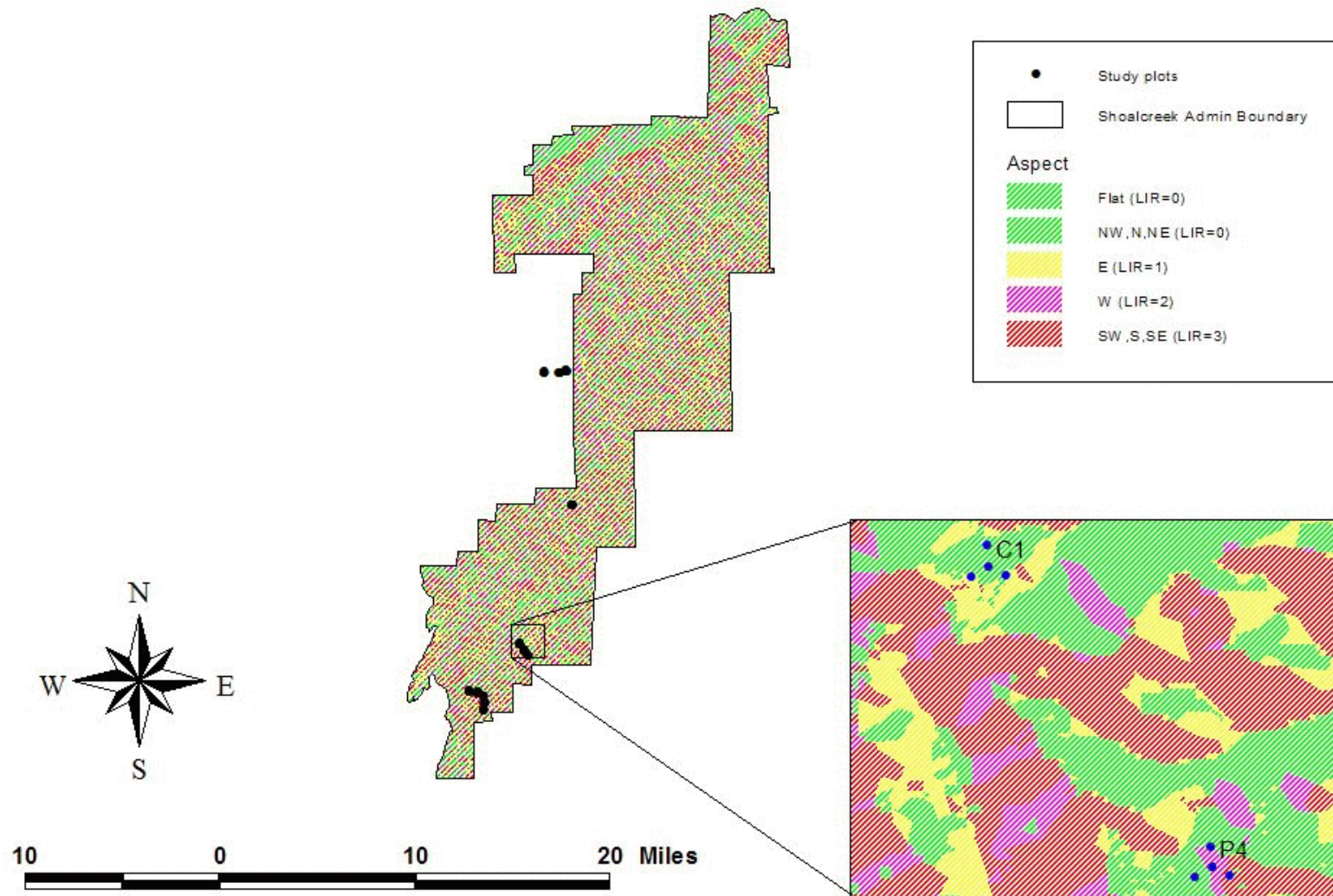


Figure 7.5. A spatial geographic representation of LIR by aspect of the Shoal Creek Ranger District, Talladega National Forest. P4 is a decline plot rated as LIR = 2 and C1 appears to have a severe aspect, but with slope of 2% and concave landform, the plot is healthy.

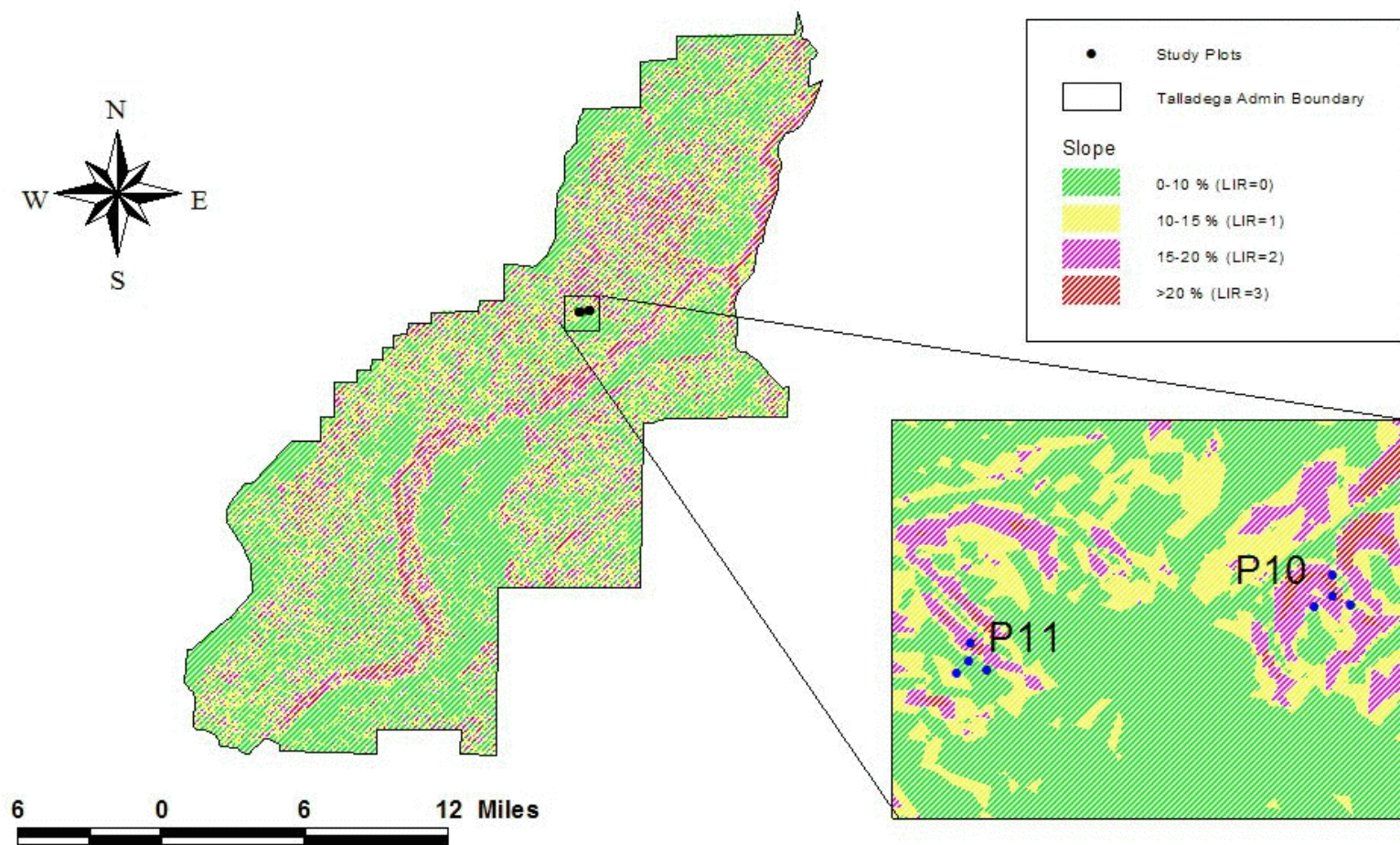


Figure 7.6. A spatial geographic layer representing LIR by slope of the Talladega Ranger District, Talladega National Forest. P10 and P11 are decline plots rated as LIR = 2.

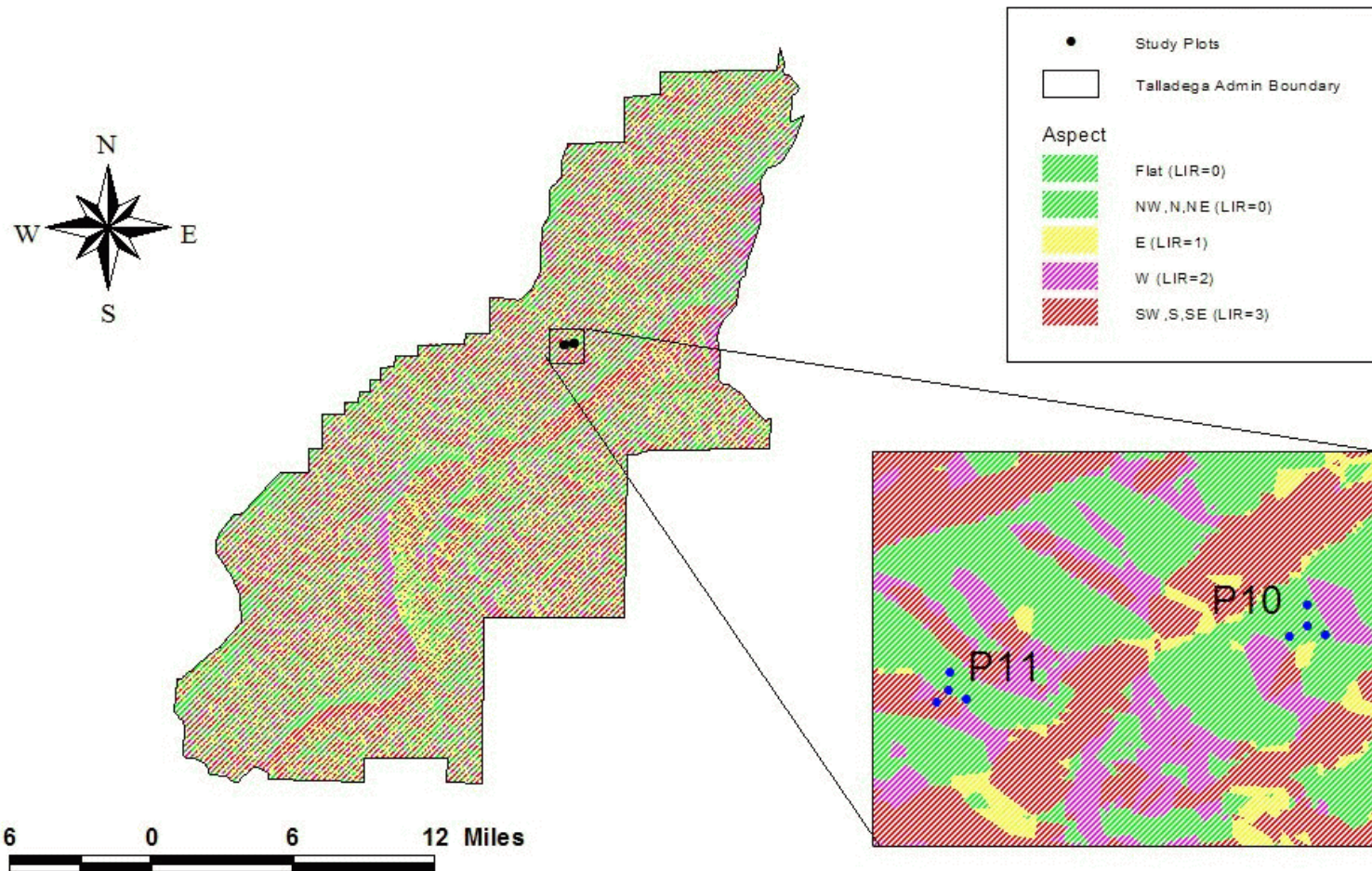


Figure 7.7. A spatial geographic layer representing LIR by aspect of the Talladega Ranger District, Talladega National Forest. P10 and P11 are decline plots rated LIR = 2. While these plots have a favorable aspect, they have a slope greater than 15% and are convex and are in decline.

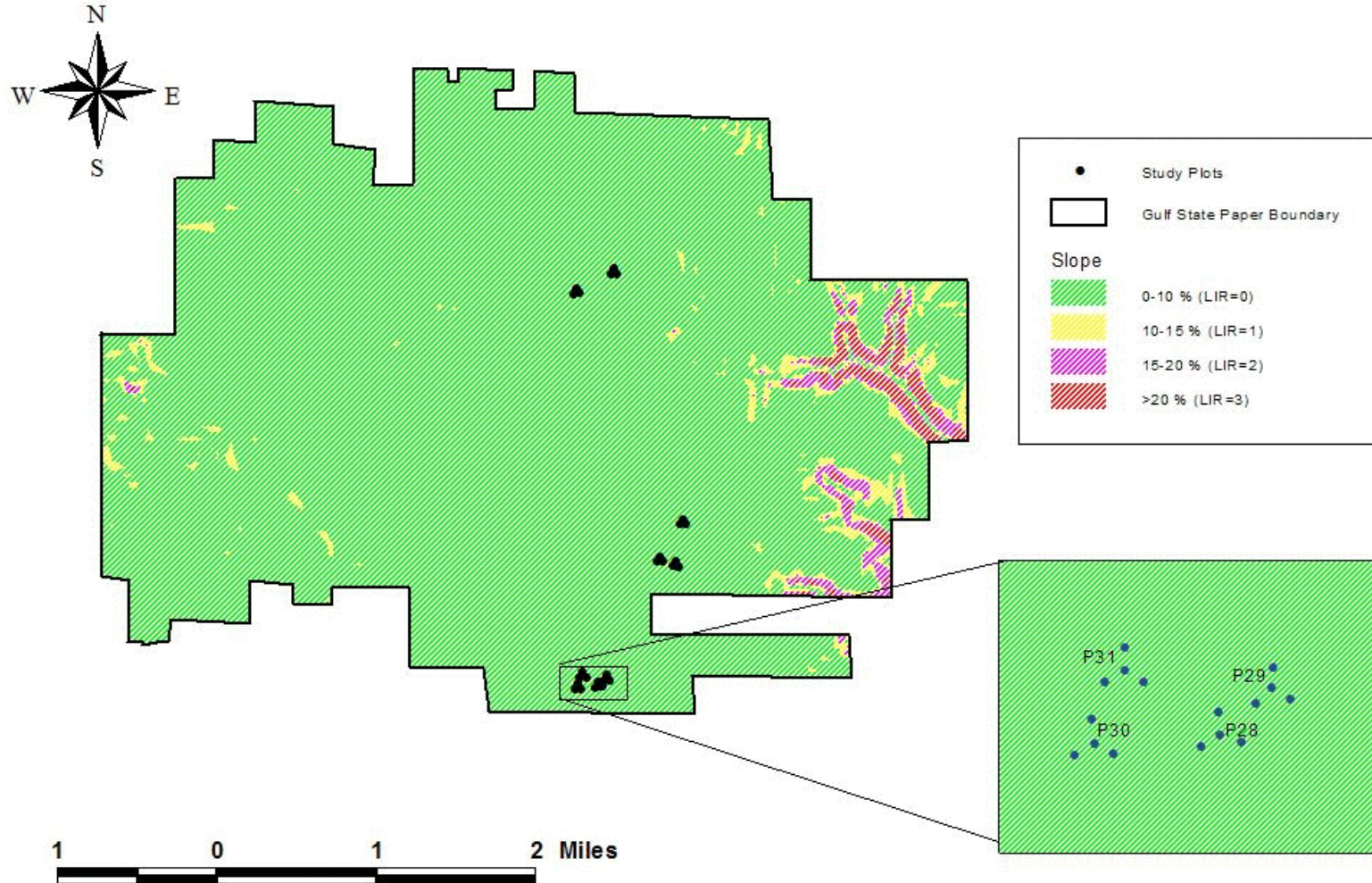


Figure 7.8. A spatial geographic layer representing LIR by slope of Gulf State Paper lands. P28, P30, and P31 are decline plots rated at LIR = 0 and P29 rated at LIR = 1. P29 was adjacent to a southern pine beetle spot that was clearcut and therefore *Leptographium* vectors were present.

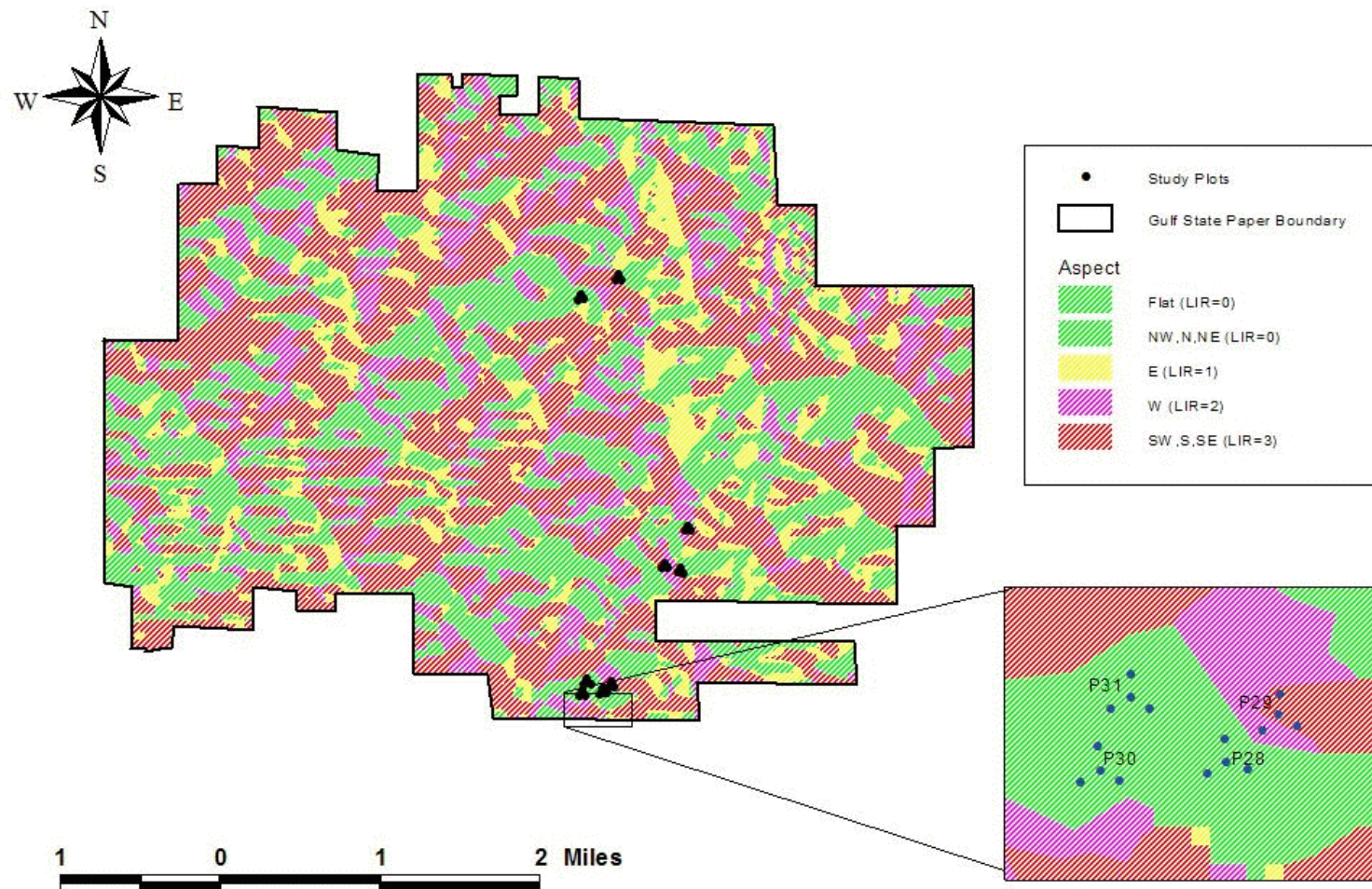


Figure 7.9. A spatial geographic layer representing LIR by aspect of Gulf State Paper lands. P28, P30, and P31 are decline plots rated at LIR = 0 and P29 rated LIR = 1. P29 with a slope of 5%, the aspect is not that influential.

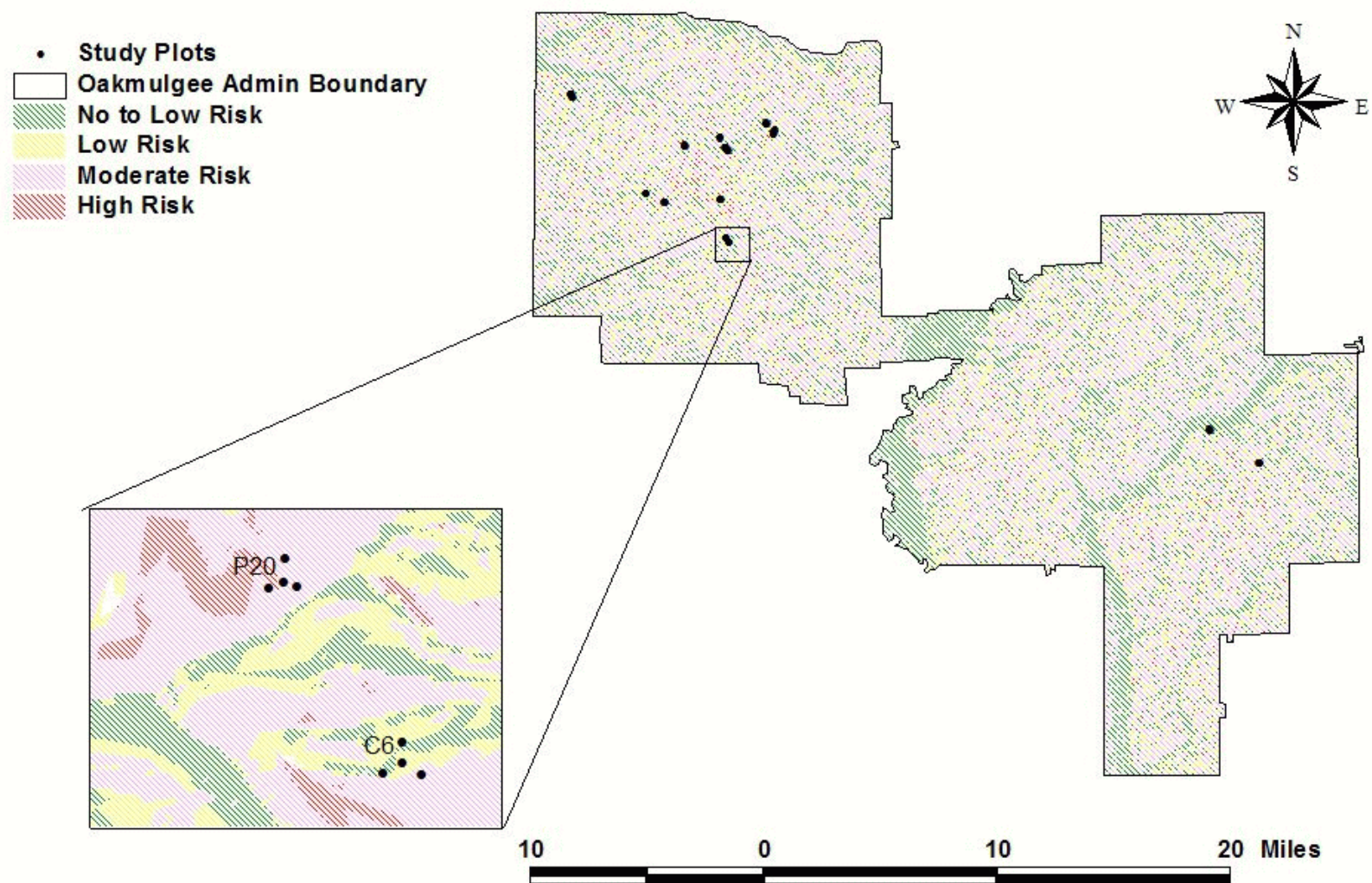


Figure 7.10. A predictive risk map for loblolly pine decline on the Oakmulgee Ranger District, Talladega National Forest produced by generating a geographic representation of the intersection of aspect and slope.

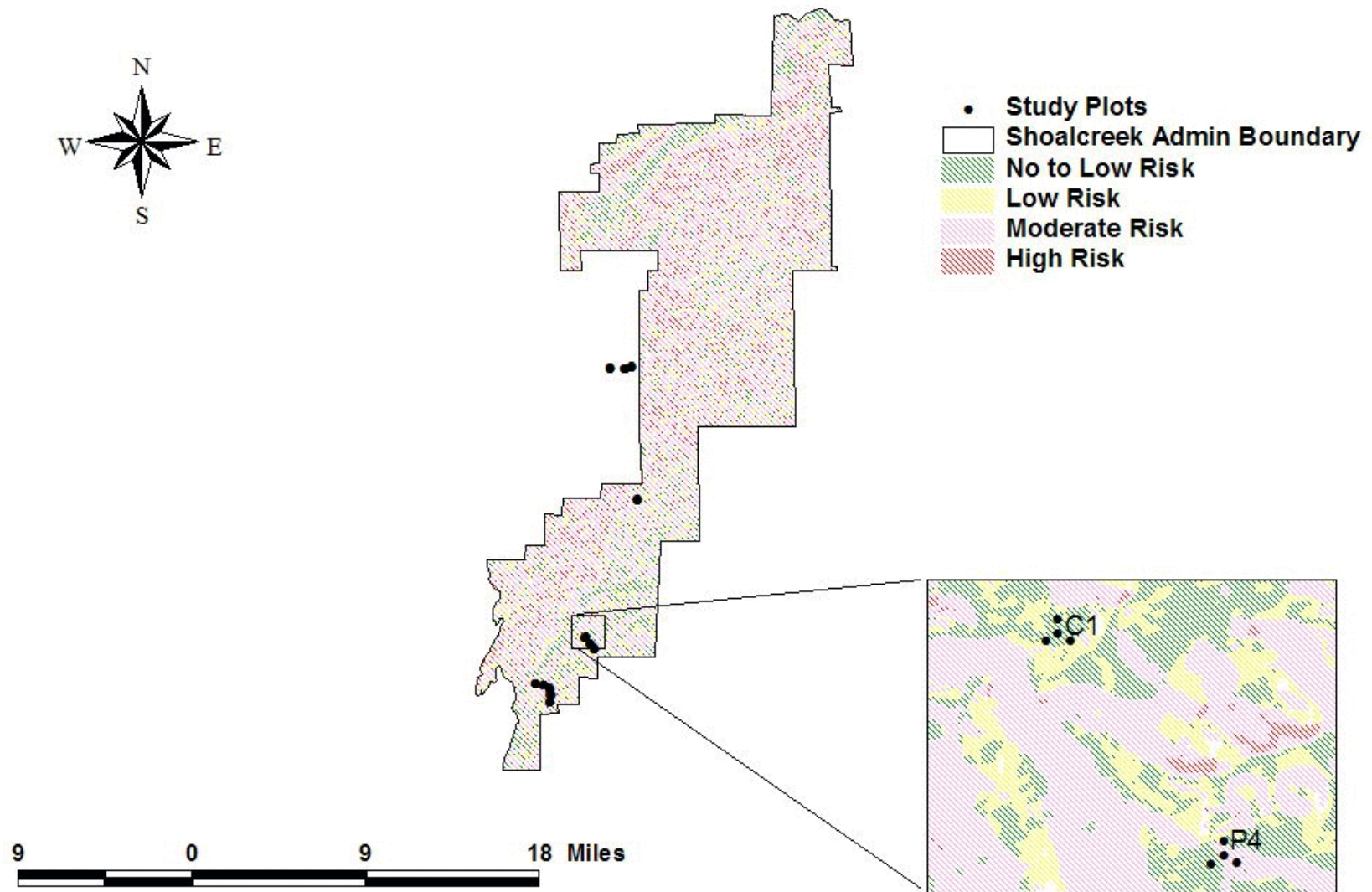


Figure 7.11. A predictive risk map for loblolly pine decline on the Shoal Creek District, Talladega National Forest produced by generating a geographic representation of the intersection of aspect and slope.

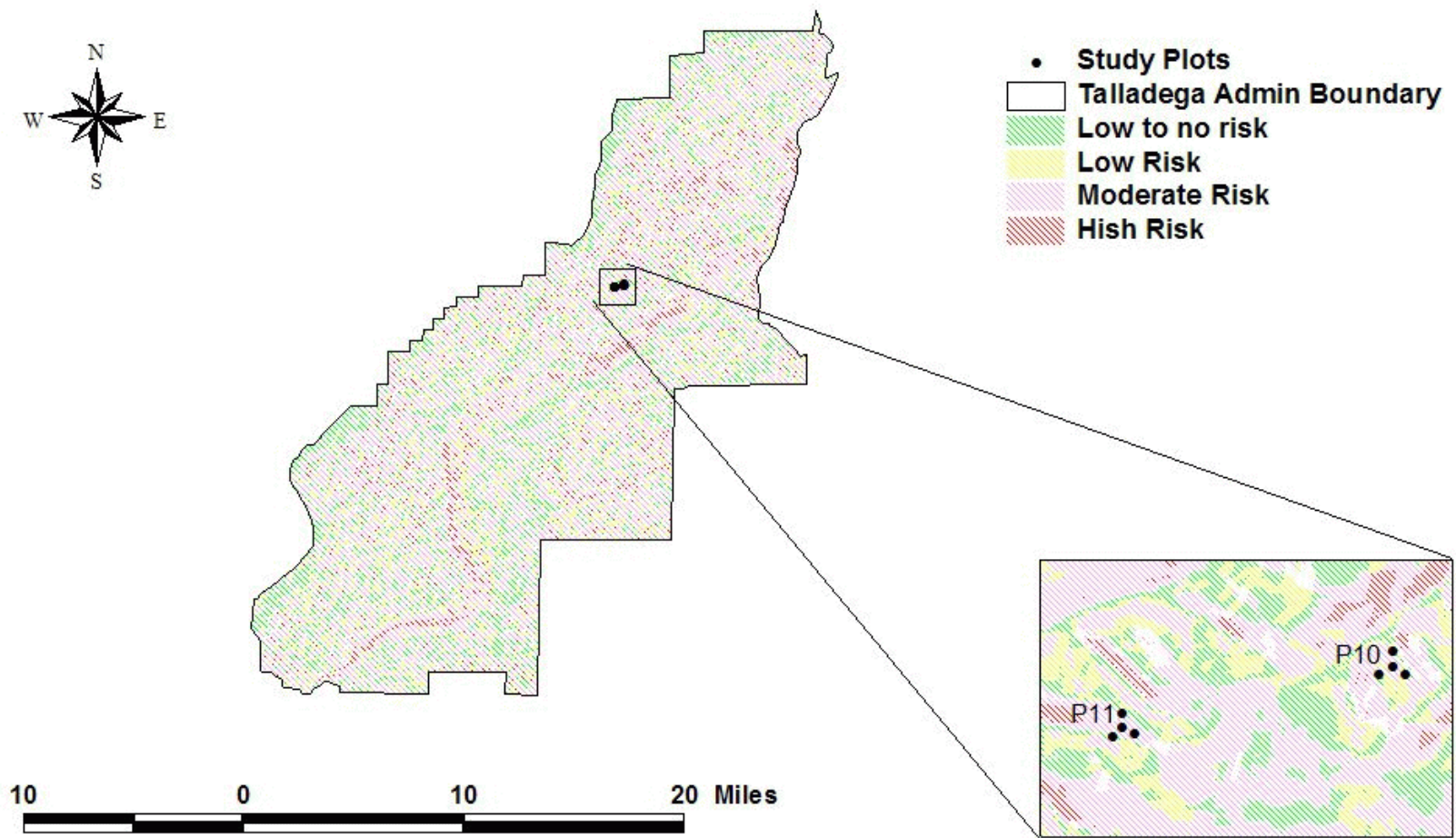


Figure 7.12. A predictive risk map for loblolly pine decline on the Talladega Ranger District, Talladega National Forest produced by generating a geographic representation of the intersection of aspect and slope.

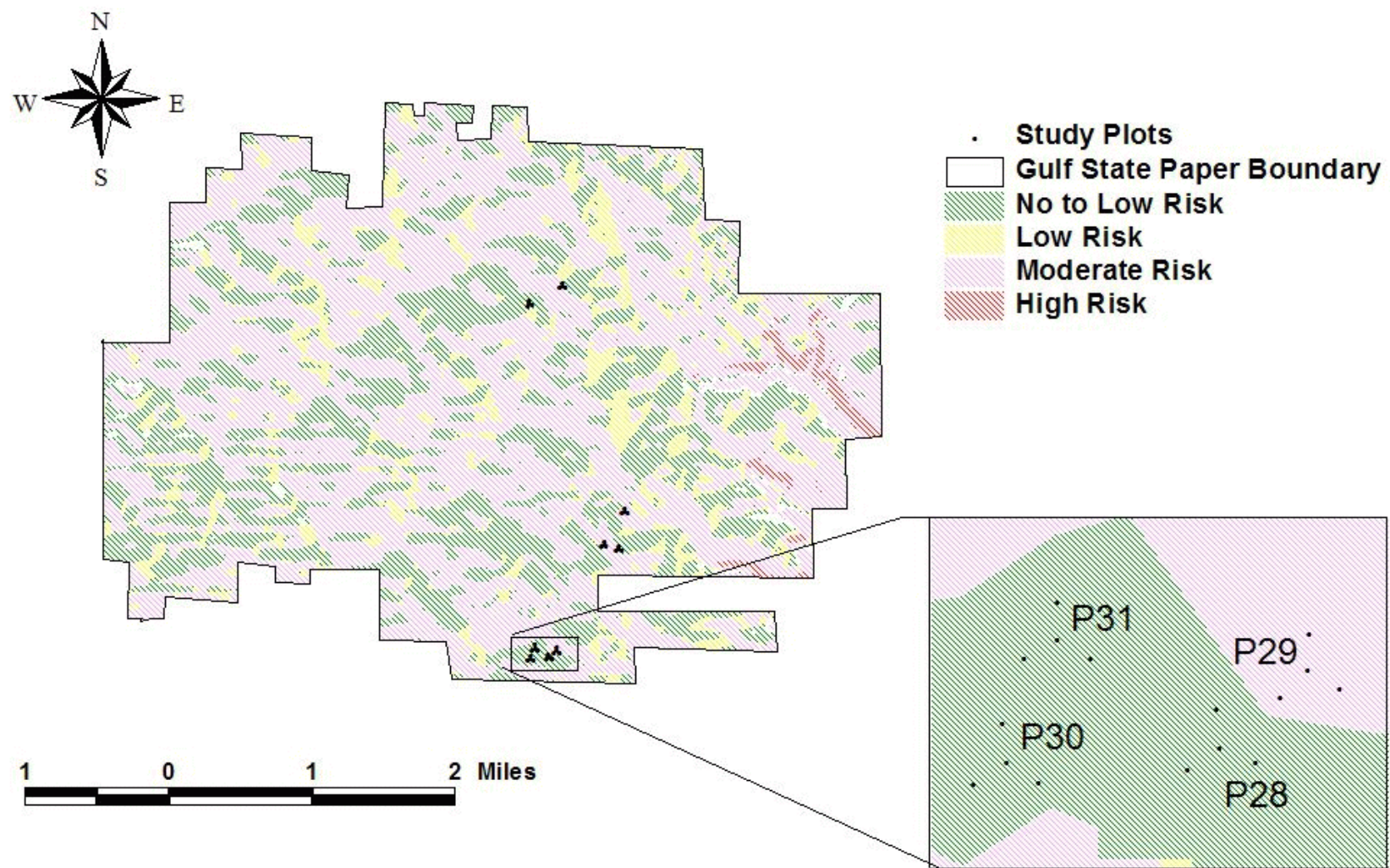


Figure 7.13. A predictive risk map for loblolly pine decline on Gulf State Paper lands produced by generating a geographic representation of the intersection of aspect and slope.

abundant precipitation. Most healthy plots with low or null gradient were located at footslope positions, which would tend to increase the water saturation of the soils. Healthy areas found on ridgetops were located in convex landform that holds water.

Aspect was also statistically significant in the model. For example, plots with SE/S/SW aspects were more likely to be in decline than areas with NE/N/NW aspects. It has been suggested that the aspect markedly affects soil temperature (Marshall and Holmes, 1988) and soil water balance (Hanna *et al.*, 1982) in high latitude regions. However, it was also observed that when soil water outputs exceed rainfall, soil water was not significantly correlated with aspect, probably because of the influence of the shading of trees on soil microclimate (Hanna *et al.*, 1982). In the case of this study, the effect of aspect (slope orientation) on decline was significant.

The complex relationship of these physiographic factors in combination produces varying levels of decline. Combinations of more influential factors such as steep slopes and south/southwest aspects produce the most severe decline symptoms, across all age classes studied. Low to moderate slope ratings (10-20 %), in combination with a severe aspect rating (south/southwest), produced an increased LIR. An increase in symptom severity was also seen when there was a combination of slope and convexity, or slope and elevation.

The potential relationship between site and stand characteristics that have been identified in this analysis represent a “snapshot” in time for the current stage of the developing of LPD epidemic in this study area. These relationships help identify areas where LPD will likely spread and/or intensify first. Further study in this area could also help researchers predict future LPD areas in a region. The ability to identify areas of LPD over time would help land managers direct

mitigation efforts. The implication is that these factors could be developed into a predictive model for LPD in similar geographic locations, providing a powerful management tool for species restoration, wildlife habitat management or other land management objectives.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

The essential elements for LPD consist of hosts, pathogens, insects, and site factors. I, and others, have established (Chapter III; Harrington and Cobb, 1983; Lackner and Alexander, 1983; Wingfield, 1983; Cook and Hain, 1985; Cook and Hain, 1988; Raffa and Smalley, 1988; Nevill *et al.*, 1995) that *Leptographium* spp. can cause staining and other symptoms in pine, and that various insects can vector these pathogens into roots (Chapter V; Caird, 1935; Bramble and Holst, 1940; Reid *et al.*, 1967; Herbert *et al.*, 1975; Goheen and Cobb, 1980; Harrington, 1983; Livingston *et al.*, 1983; Ferrell and Parmeter, 1989; Klepzig *et al.*, 1991; Ferrell *et al.*, 1994; Nevill *et al.*, 1995; Otrosina *et al.*, 1997). This is not definitive proof of a cause and effect relationship between *Leptographium* and LPD, but it does provide strong evidence for the involvement of these fungi in this decline.

I believe that a variety of necessary, interacting, factors lead to LPD. Specifically, I propose that LPD results from the debilitation of root systems of stressed trees by *Leptographium* spp., which are vectored by various insects whose populations and behavior are mediated by a number of environmental factors.

Significant numbers of loblolly trees were reported to be declining and dying in central Alabama (Hess *et al.*, 2002, 2001, 1999). Their symptoms resembled those for littleleaf disease of shortleaf pine, which is associated with *Phytophthora cinnamomi*, although the sites were atypical for development of LLD. An earlier study (Otrosina *et al.*, 1997), however, had indicated that there was a statistical correlation between the presence of species of the fungal

genus *Leptographium*, which can cause blue-stain on the roots of southern pine, and the presence of southern pine beetle . The above statements summarize the only information available at the beginning of this study for the formation of hypotheses regarding the etiology of loblolly decline. It was, therefore, decided to investigate the roles of insects, *Leptographium* spp., *P. cinnamomi*, and site factors and their associations with LPD syndrome.

To investigate this syndrome 39 plots spanning nine counties and four physioregions in central Alabama were established. Soil and root samples were taken from each plot, and insect traps which targeted root attacking insects, were placed on each plot. Cultures were then made from the samples and trapped insects to determine the presence of *Leptographium* species. Root health was assessed by visually estimating the amount of blue-stain, insect and fire damage. The understory was rated for vegetation density, and tree vigor was assessed by resin sampling, foliage transparency, and crown density. Soil structure and mineral content were analyzed by Dr. Emily Carter at the Southern Research Center in Auburn, AL.; rainfall and temperature data were obtained from National Oceanic and Atmosphere Administration (NOAA).

Four species of *Leptographium* were found in the roots, but only one of these was also isolated from the soil. Two species, *L. serpens* and *L. lundbergii*, had not been previously reported from the United States. All four *Leptographium* spp. were isolated from trapped insects and were consistently associated with declining stands. Sixteen species of insects, all of which attack pine roots, trapped on these sites yielded *Leptographium* species upon culturing. Eleven of these insect-*Leptographium* associations represent new reports. Symptomatic plots had lower vegetation densities and higher insect numbers than did asymptomatic plots. Plots with low vegetation densities and higher pestiferous insect numbers had higher *Leptographium* incidence

ratings, which were also correlated to prescribed burning. Prescribed fire over time leads to multiple attacks by root feeding insects that repeatedly vector *Leptographium* spp. into the host root system. High *Leptographium* incidence was also statistically related to decreased resin flow, poor root condition, radial growth reduction, foliar transparency and crown density. High vegetation and low pestiferous insect numbers were correlated to higher levels of beneficial arthropods. Greater abundance of beneficial arthropods and carabid beetles in the moderate to high vegetation density plots suggested that increased vegetation encourages greater abundance of these organisms or that fire factors that lead to low vegetation densities also lead to lower arthropod diversity.

The presence of *P. cinnamomi* was inversely correlated with LPD. It could be isolated from soils near healthy trees, but was never isolated from roots of either healthy or declining loblolly trees (Ann Weber, personal communication, unpublished data). Because *P. cinnamomi* could not be associated with the occurrence of LPD symptoms and does not appear to be a factor in this disease, a preliminary survey was initiated to determine if the presence of hardwoods on the plots could account for the presence of *P. cinnamomi*. A positive correlation between the presence of *P. cinnamomi* in soils near loblolly pine and the distance of a hardwood infected with *P. cinnamomi* was found (see Appendix A).

Pathogenicity studies were initiated to determine the potential pathogenicity of the *Leptographium* spp. isolated from the soil and/or roots of trees with LPD symptoms in central Alabama. Freshly lifted seedlings and 21 year-old loblolly pines were wound-inoculated with these *Leptographium* spp. Seedlings inoculated with *L. procerum* in the greenhouse produced significantly fewer root initials, leading to a smaller root mass with a higher rate of infection than

did uninoculated seedlings. Vertical lesions produced in seedlings by *L. serpens* and *L. terebrantis* were significantly longer than control inoculations. Lesions produced in mature trees by *L. serpens* and *L. lundbergii* were significantly longer than control inoculations. Of the fungi tested, *L. serpens*, *L. terebrantis* and *L. lundbergii* were the most virulent and these three fungi may pose the greatest threat to loblolly pine. The most virulent species are most commonly associated with xylem feeding insects, as opposed to those that feed on phloem.

A study was conducted to assess the efficiency of two insect vectors and the effect of the *Leptographium* on that vector. Field collected adult *H. salebrosus* and *H. tenuis* were surface-sterilized and inoculated with *L. terebrantis* and *L. serpens*, and then confined with *Leptographium* and insect-free loblolly roots. One hundred percent of roots confined with inoculated insects developed stain and yielded *Leptographium* upon culturing. None of the roots confined with non-inoculated *H. salebrosus* or *H. tenuis* developed stain, nor yielded *Leptographium* upon culturing. More *H. salebrosus* and *H. tenuis* brood emerged from roots inoculated with *Leptographium* spp. than from controls, indicating that the *Leptographium* spp. enhanced *Hylastes* reproduction or development. The extensive staining seen in these root sections, the extensive growth of *L. terebrantis* and *L. serpens* around the entrance and exit holes, and within galleries and pupal chambers demonstrates the effectiveness of these insects as vectors of these fungi.

Geographic information system (GIS) analysis also showed that decline was significantly correlated to site and stand physical factors. Slope and aspect were determined to be the predominant “predictive” variables when considering loblolly decline in central Alabama. Convexity and elevation were predictive only when in combination with other topographical

factors. There was a significant effect of tree age upon development of decline, but trees of all ages (29-62) studied, showed all levels of decline, with severity increasing with tree age. There were significant decline differences among the physioregions also, but decline was found in all of them. These data indicate that geographic and topological factors may be very useful to resource managers for predicting where loblolly decline will occur.

The work performed in this dissertation forms the foundation to begin to understand the process leading to a major decline problem with loblolly pine. Since no work with this problem had been done before the question was simple: What causes loblolly decline? The answers presented in this dissertation inevitably lead to a bewildering array of new questions. A new project is presently being developed to explore and develop more completely the predictive value of geographic and topographical databases for this disease. The model presented here is more of an indicator of the potential for this approach than a developed system.

The decline of loblolly pine in Alabama fits the definition of a decline disease suggested by Manion (1991) and Houston (1992); a syndrome of canopy-dominant trees characterized by a gradual deterioration in health and vigor that frequently ends in death. Decline diseases seem to result from complex interactions of abiotic and biotic factors that predispose or weaken trees, followed by inciting or triggering events that result in dieback and mortality. The work performed during the last three years has led to a solid understanding of the biological components of the disease, and a basic understanding of the disease cycle (Fig. 8.1). However, the biological and ecological questions generated by this work are numerous. The exact nature of the relationship between *Leptographium* and the insects deserves investigation. For example,

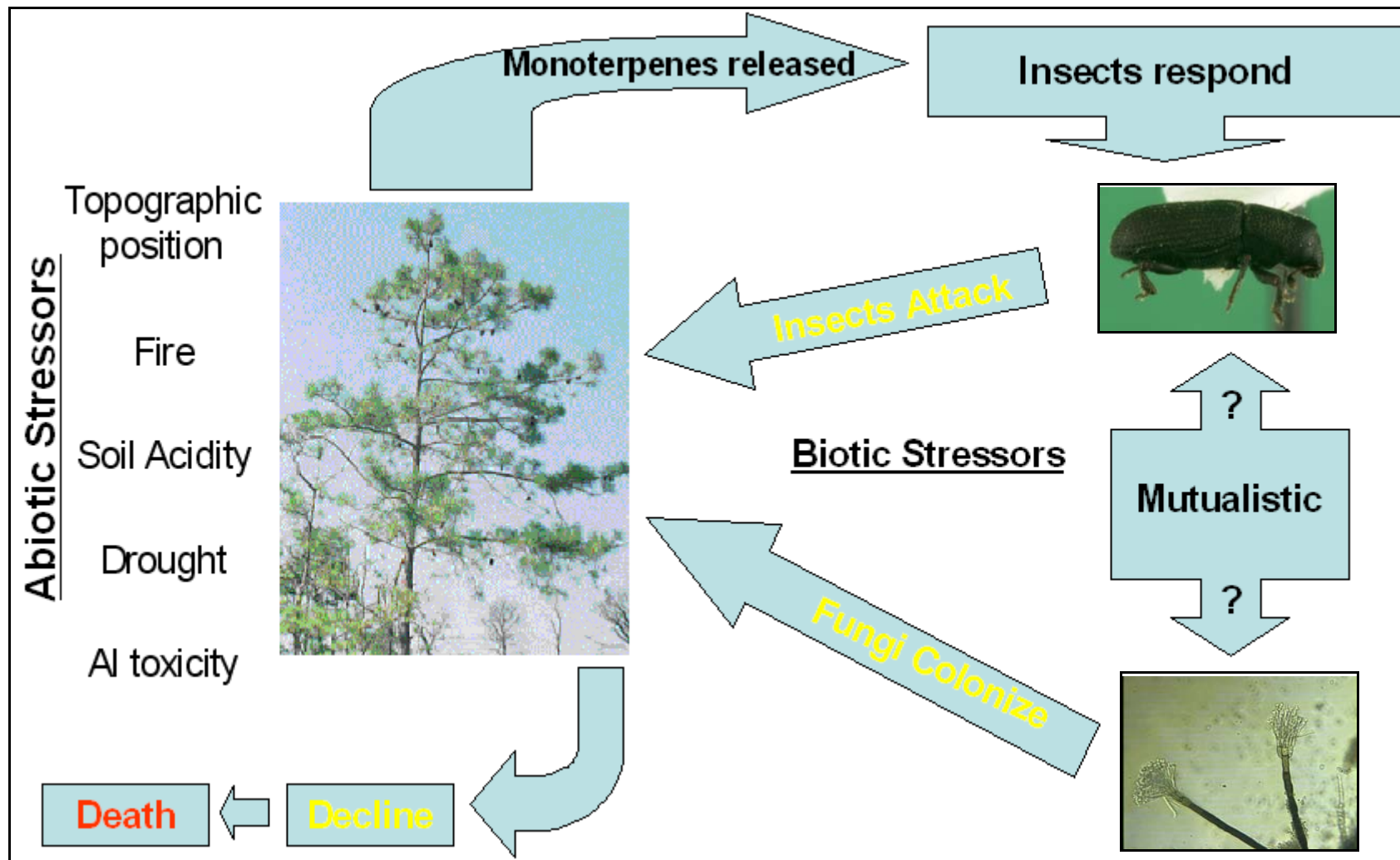


Figure 8.1. Proposed diagram of Loblolly Decline Cycle.

how does *Leptographium* affect the reproductive capacity of the insect? The interactions of the various *Leptographium* spp. are totally unknown and would probably be a fruitful area of research.

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APPENDIX

DO HARDWOOD SPECIES ACCOUNT FOR THE PRESENCE OF *P. CINNAMOMI* IN DECLINING LOBLOLLY PINE STANDS?

Introduction

Loblolly pine decline (LPD) is a syndrome associated with loblolly pine (*Pinus taeda* L.) in the southeastern United States and it is similar to littleleaf disease (Campbell and Copeland, 1954) in shortleaf pine (*Pinus echinata* Mill). Littleleaf disease (LLD) was described as resulting primarily from site conditions, and was secondarily associated with *P. cinnamomi*. Affected trees declined slowly and died prematurely, fine roots deteriorated, needles were short and chlorotic, crowns were sparse and tufted, radial growth declined, and large cone crops and mortality occurred in the 35-yr age class. These symptoms are also commonly associated with some forms of malnutrition. The role of *P. cinnamomi* in littleleaf disease was attributed to its ability to kill feeder roots (Campbell and Copeland, 1954) and, thus, inhibit root growth and nutrient absorption. Littleleaf, in extreme conditions, can affect loblolly pine, but the data do not support the involvement of *P. cinnamomi* with loblolly pine decline. The similarity in symptoms did, however, lead to the initiation of a preliminary investigation of a possible role of *P. cinnamomi* LPD. No *P. cinnamomi* was found in the roots of sampled loblolly pines, but it was recovered from the surrounding soils. Therefore, a survey was conducted to determine if there was a reservoir of *P. cinnamomi* in the hardwoods on the plots where it was recovered from the soils and to see if there was a spatial relationship between infected soils and hardwoods infected with *P. cinnamomi*. Methods of regressing spatially correlated variables, including geostatistical analysis (Besag, 1972; Cliff and Ord, 1981; Haining, 1990; Stein and Corsten, 1991; Gotway

and Stroup, 1997;) and multivariate statistical procedures (Johnson and Wichern, 1988), were used to describe the correlations among soil variables, pathogen propagules, and disease incidence. These techniques can be used to gain a better understanding of the spatial heterogeneity of *P. cinnamomi* in soil and the relationship of initial inoculum and movement to subsequent disease.

Materials and Methods

Hardwood trees were selected based on their proximity to loblolly pine trees growing in soil from which *P. cinnamomi* had been isolated. Root and soil samples were collected as described in chapter II to determine their potential as reservoirs for *P. cinnamomi*. These samples were also evaluated to determine if there were any *Leptographium* species present. All trees sampled were located on the Talladega National Forest Oakmulgee Ranger District.

Spatial Mapping

Spatial maps of plots were created by determining azimuths and distances of all trees on plots from originally sampled loblolly pine trees. All tree distances were measured with a loggers tape, and azimuths determined with a compass from a center stake. Distances between trees were measured using scale drawings of plots. Hardwoods outside of the plot circumference, but within 4.6 m of a sampled loblolly pine were included in these maps. These maps were then created in ArcviewTM (ESRI, 1996) to determine surface water flow that would provide movement for *P. cinnamomi*.

Tree Selection

Sample trees were selected as either being positive or negative for *P. cinnamomi* using the spatial maps created to locate hardwoods. Positive trees were trees which were < 4.6 m from

a previously sampled loblolly tree where *P. cinnamomi* was found in the surrounding soil (Weber *et al.*, 2001). Negative trees were selected using two methods: (1) the hardwood was >4.6 m from a loblolly pine from which *P. cinnamomi* was isolated from the soil near its roots or (2) the hardwood was <4.6 m from a loblolly pine from which no *P. cinnamomi* was isolated from the soil near its roots. The species of tree sampled, global positioning satellite (GPS) coordinates, and distance from *P. cinnamomi* infected or uninfected soils were recorded. Twelve hardwoods [five *Cornus florida* L., one *Oxydendrum arboreum* (L.) DC., three *Liquidambar styraciflua* L., and three *Quercus flacata* Michx.] were selected from positive sites. Ten hardwoods [four *C. florida*, one *Sassafras albidum* (Nutt) Nees, one *Nyssa sylvatica* Marsh, one *Q. stellata* Wangenh., one *Carya* sp., one *Q. incana* Bartr., and one *Liriodendron tulipifera* L.] were selective from sites negative for *P. cinnamomi*.

Root Sampling

Roots were collected from 13 research plots in April of 2002. Roots were sampled in the same manner as pine roots and *Leptographium* spp. isolations performed using the same procedures as described in Chapter II.

To determine the presence of *P. cinnamomi*, the samples were processed by baiting (Jeffers, 2000) and direct plating. Roots were surface sterilized and plated directly onto a *Phytophthora* selective media (PARPH, Jeffers 2000). *Phytophthora*-like colonies were transferred to clarified V-8 juice media (CVA), adapted from Jeffers (2000). Roots were also placed in sterile containers held down with netting and flooded with 300 ml of water. Ten pieces each of juniper and camellia leaves were floated on the waters surface to attract zoospores. After 24-72 h, the leaf pieces were placed on PARPH plates and incubated in the dark. *Phytophthora*-

like isolates were subcultured onto CVA plates, and then pure cultures were placed on CVA agar slants and stored at room temperature.

Soil Sampling

Soils samples were collected from 13 plots in 2002. Soil samples were collected and *Leptographium* spp. isolations performed using the same procedures as described in Chapter II. The same baiting method (Jeffers, 2000) described above for roots was also used to determine the presence of *P. cinnamomi* in soil (isolations made by Ann Weber).

Results

Tree root examination prior to sampling revealed little damage, lesion or resinous areas for most of the hardwoods sampled. The yellow poplar (*Liriodendron tulipifera* L.), however, had a large resinous lesion (17.8 x 7.6 cm) characterized by black exudate and resinous tissue. There were also dead primary and fine roots.

There was a positive correlation ($r=0.9421$, $p<0.0001$) between the presence of *P. cinnamomi* in soils near loblolly pine and the proximity of a *P. cinnamomi* infected hardwood. *Phytophthora cinnamomi* was isolated from all hardwood roots sampled within 4.6 m of infested *P. cinnamomi* soil, except for the sourwood (*Oxydendrum arboreum* (L.) DC.) (Fig. A1, Fig. A2, Fig. A3). *Phytophthora cinnamomi* was also recovered from the soils surrounding 13 of the hardwoods (9 positive and 4 negative). All negative tree roots were free of *P. cinnamomi* except for the post oak (*Quercus stellata* Wangenh.), dogwood (*Cornus florida* L.) and the southern red oak (*Quercus flacata* Michx.) (Fig. A2, Fig. A3, Fig. A4). Because these negative trees were within the 4.6 m distance of a sampled loblolly where no *P. cinnamomi* was found, I did not expect to find *P. cinnamomi*. However, these control trees were down slope from or on the same

plane as the sampled loblolly, providing no surface water movement toward the sampled loblolly where no *P. cinnamomi* was found. All loblolly pines that had *P. cinnamomi* in the soil surrounding their roots were in the surface water flow pattern of the landscape (Fig. A1, Fig. A2, Fig. A3). Loblolly pines falling outside of these water flow patterns and/or more than 4.6 m from an infected hardwood were free of *P. cinnamomi* in their soil areas (Fig. A2, Fig. A3, Fig. A4).

An undescribed *Leptographium* species was isolated from several of the hardwood species sampled: american sweetgum (*Liquidambar styraciflua* L), dogwood (*Cornus florida* L.), black gum (*Nyssa sylvatica* Marsh.), and hickory (*Carya* spp.). Pine-associated *Leptographium* spp. were an earlier study (Chapter II). This undescribed *Leptographium* species was also found to be associated with *Colopterus unicolor* (Say) (Coleoptera: Nitidulidae) (see Chapter III).

Discussion

Phytophthora cinnamomi has been implicated in LPD because of the common symptom set it shares with LLD. I, therefore, conducted this preliminary investigation to determine if there was a possibility of its involvement. *Phytophthora cinnamomi* was never isolated from the roots of loblolly pine, although it could sometimes be found in the soil. It was not statistically associated with the occurrence of loblolly decline symptoms and does not appear to be a factor in this disease. Its presence can be accounted for by its occurrence in the roots of the hardwood trees sampled and water surface flow patterns. Sampling was limited to the roots of hardwood trees, but *P. cinnamomi* is also commonly associated with the root systems of perennial woody bushes, which were common on the site.

Movement of *Phytophthora* species in surface water in forest ecosystems has been well documented (Dawson and Weste 1985; Kinal *et al.* 1993; Shea *et al.* 1983). Uphill disease

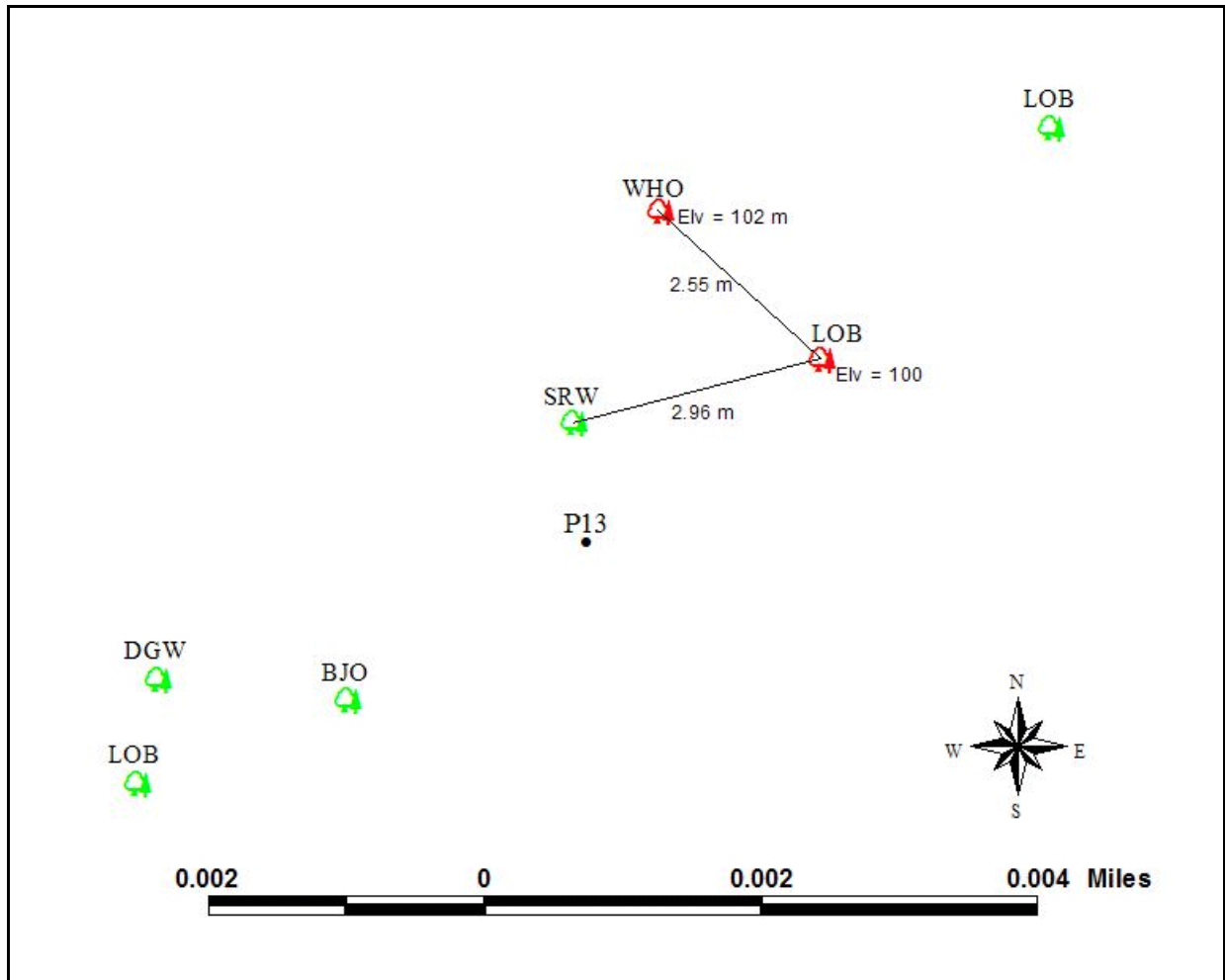


Figure A1. Plot 13 was sampled as a positive plot. *Phytophthora cinnamomi* (Pc) was isolated from soil near the loblolly (LOB) roots, colored in red. Pc was isolated from the roots and soil surrounding the roots of the white oak (WHO) that is uphill from the LOB. The sourwood (SRW) was free from Pc.

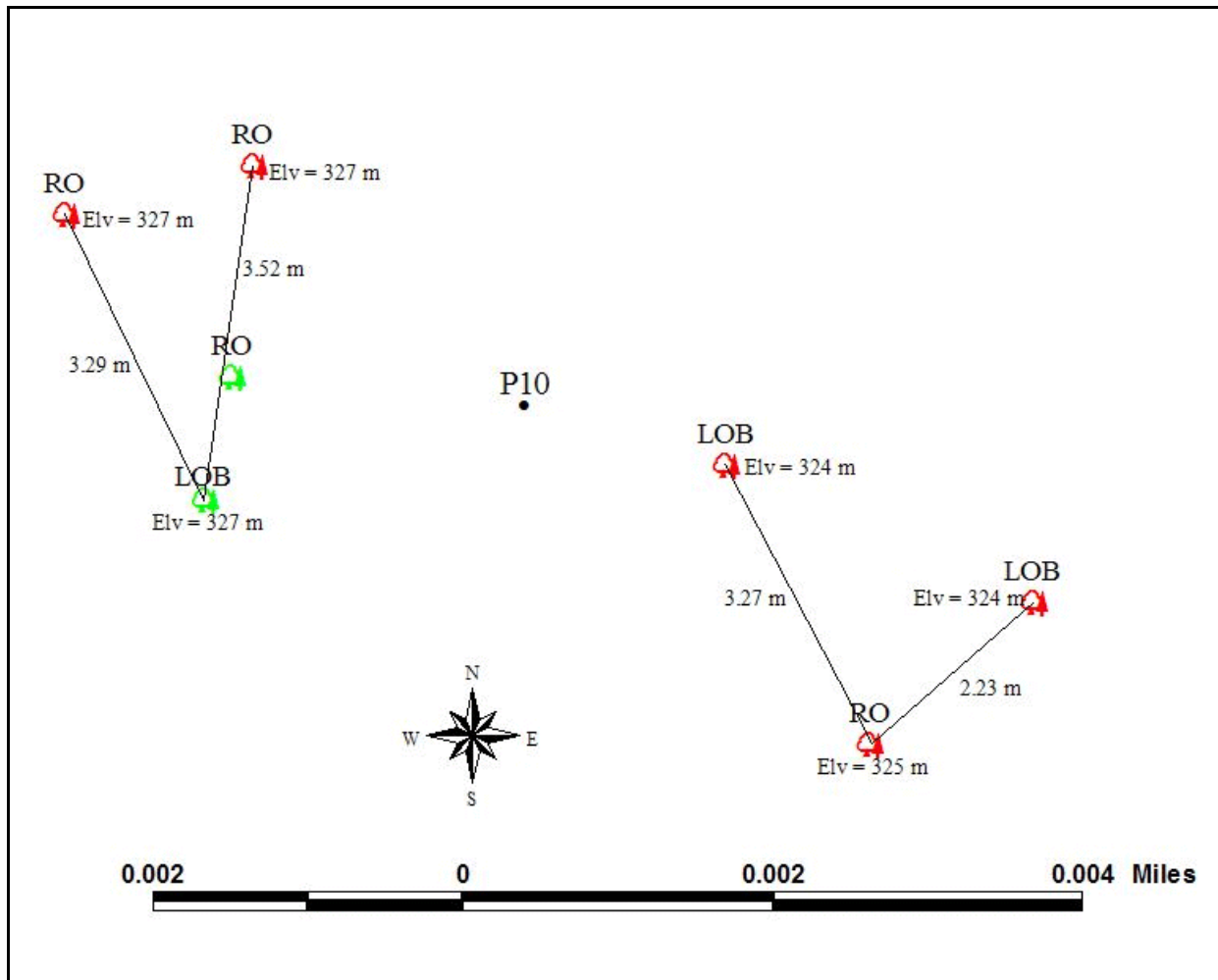


Figure A2. Plot 10 has both situations, positive and negative. On the left, there is a negative example, with the LOB being on the same plane as and < 4.6 m from the red oaks (RO). On the right, there is an positive example, with the LOBs being downhill from and < 4.6 m from the RO.

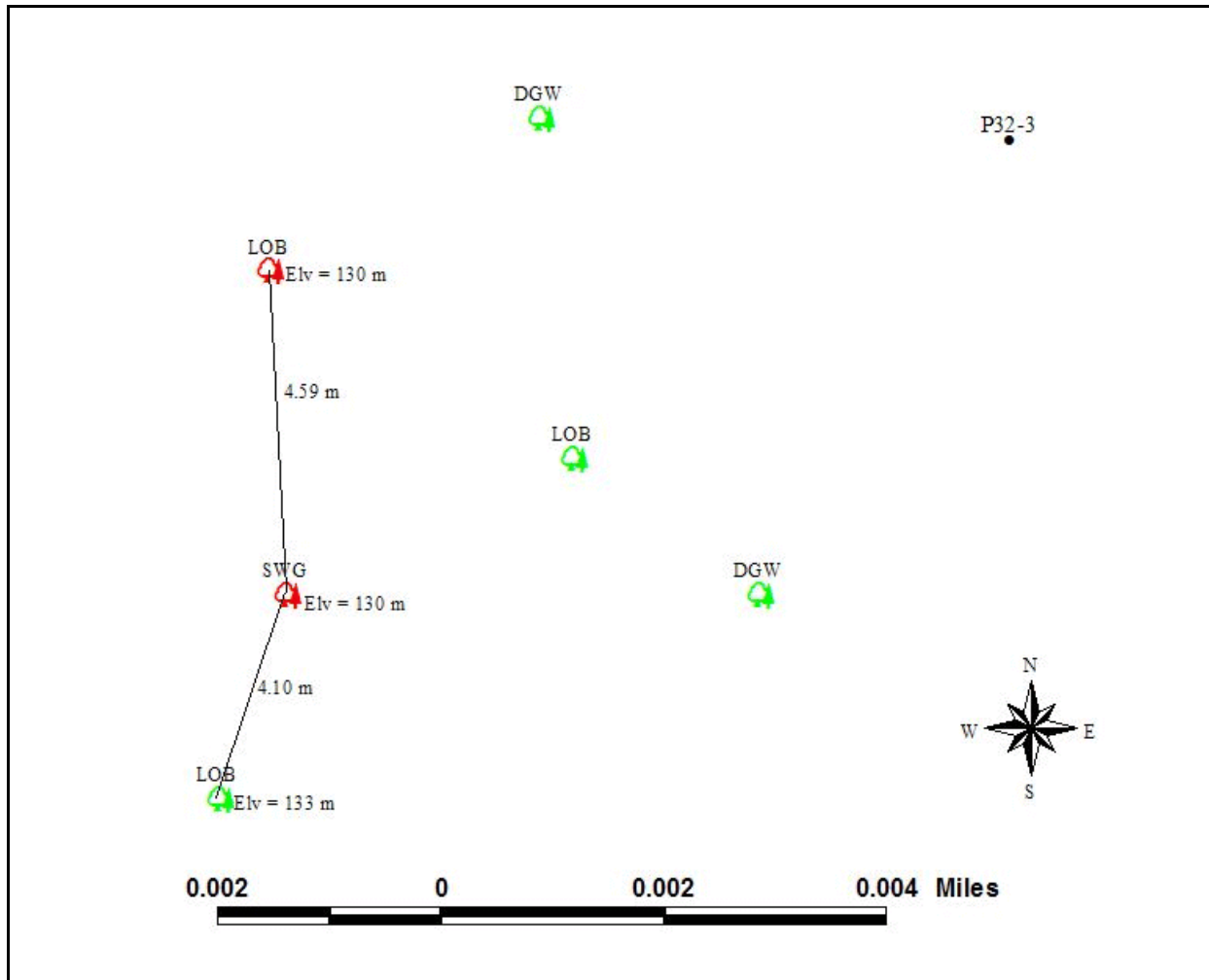


Figure A3. Plot 32-3 has both situations. *Phytophthora cinnamomi* (Pc) was isolated from the soil surrounding the roots of the LOB (colored red) and it is on the same plane and <4.6 m from the infected sweetgum (SWG). While the other LOB is <4.6 m from the infected SWG, it is also up slope and outside of water flow.

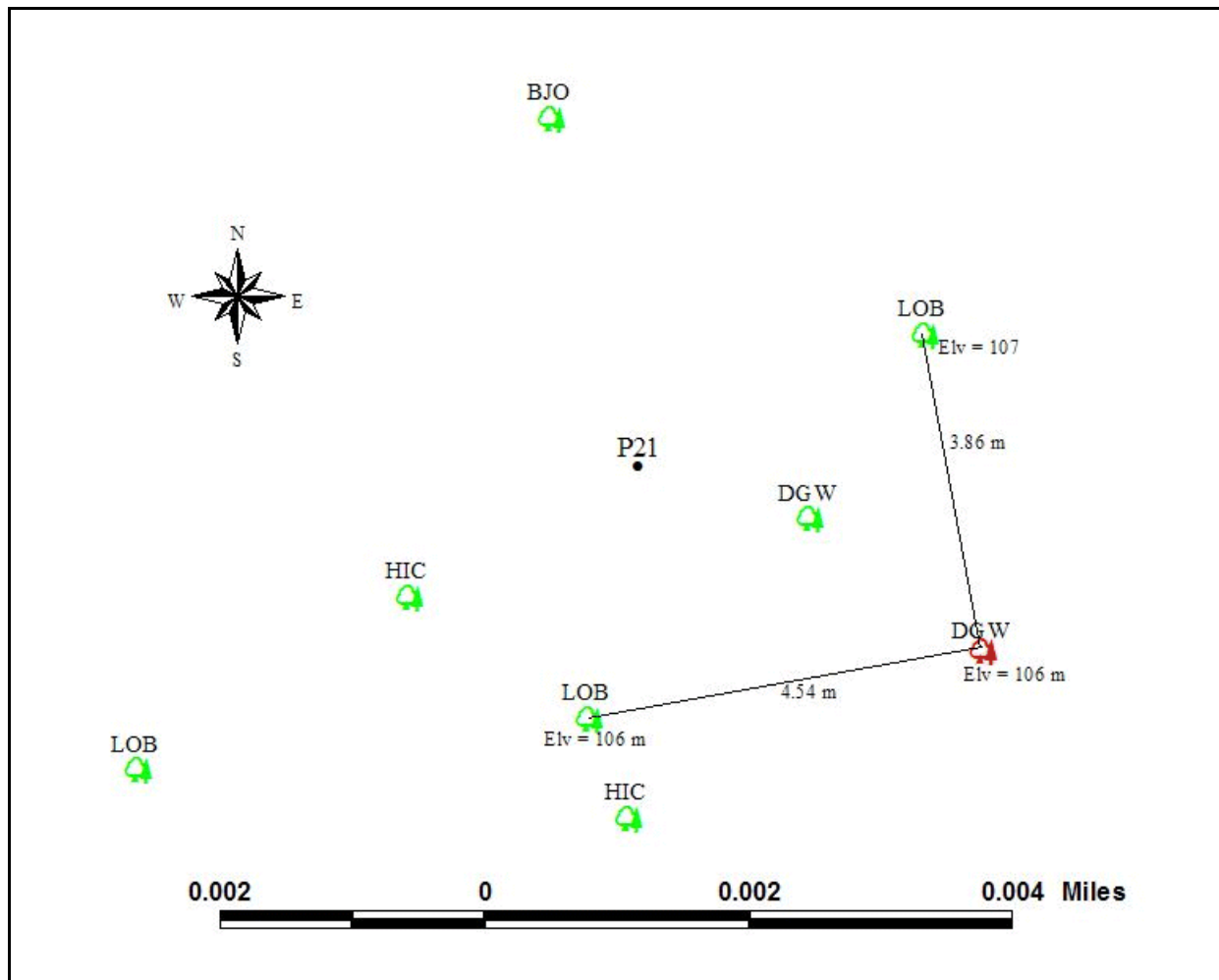


Figure A4. Plot 21 was sampled as a negative control. No *P. cinnamomi* (Pc) was expected on this plot, since none was isolated from soil near loblolly (LOB) roots. Although Pc was found in the DGW, water flow was away from sampled loblolly (LOB uphill or on same plane of slightly convex landform from DGW) and they were > 4.6 m from the hardwood.

spread has been attributed to inoculum movement from root to root in soil, whereas downhill disease spread has been attributed to drainage of surface and subsurface water (Hill *et al.* 1994; Weste *et al.* 1976). Aerial photography and quadrat mapping have been used to delineate differences in the boundary margins between diseased and healthy trees (Dawson and Weste 1985). Surface drainage water movement on logging roads, and movement of soil from logging and recreational vehicle traffic, have also been associated with pathogen spread in this system (Dawson and Weste 1985).

Population densities of *Phytophthora* species in soil are dynamic and can be influenced by soil physical and chemical factors in addition to the presence of actively growing, susceptible roots. Soil physical and chemical characteristics are spatially heterogeneous within soils (Entz and Chang 1991; Trangmar *et al.* 1985; Warrick *et al.* 1986). There is little published information relating the heterogeneity of physical and chemical factors of soil with the heterogeneity of initial spatial patterns of propagules of *Phytophthora* spp. in soil. Soil clay content, sodium, and copper concentrations were useful in explaining the spatial variation among densities of several plant parasitic nematodes in field soils (Noe and Barker 1985). Soil chemical factors are known to affect diseases caused by soilborne pathogens including *Phytophthora* spp. (MacDonald 1982; Meyer and Shew 1991; Muchovej *et al.* 1980). For example, soil salinity may affect the spatial pattern of propagules and dispersal of *Phytophthora* spp. in soil, since high concentrations of soluble salts can predispose plant roots to more severe *Phytophthora* root rot (MacDonald 1982).

VITA

Lori Eckhardt was born in Riverside, California, on October 5, 1964. In 1966 her family moved to Memphis, Tennessee, where she spent most of her secondary school years. She joined the Navy in 1984 and currently still serves as a reserve naval aircrewman. She received her Associate of Arts degree in 1989 in merchandise marketing and management from the Fashion Institute of Design and Merchandising in Los Angeles, California. In 1988 she married a Navy man and had a lovely daughter, and spent the next few years moving from base to base and working as a flight attendant for Northwest Airlinck. She returned to school in 1995 and completed a Bachelor of Science Degree in cell molecular biology and genetics at the University of Maryland in College Park in 1997. She was a Research Associate at the Center for Agriculture Biotechnology in College Park from 1996 to 1999, working in molecular virology. Her husband was transferred to New Orleans, Louisiana, and in August 1999 she enrolled at Louisiana State University to pursue a doctoral degree with an emphasis in forest pathology and entomology under the guidance of Dr. Jones and Dr. Goyer. She is a member of the American Phytopathological Society, Mycological Society of America, Entomological Society of America, Canadian Entomological Society, and the Society of American Foresters. She is now a candidate for the degree of Doctor of Philosophy in plant health, which will be granted in August, 2003.