Insect-Fungal Complex Associated with Loblolly Pine Decline in Central Alabama

Lori G. Eckhardt, Ann M. Weber, Roger D. Menard, John P. Jones, and Nolan J. Hess

Abstract: Loblolly pine decline, characterized by an expanding area of declining and dead trees, is becoming increasingly prevalent in loblolly pine (*Pinus taeda* L.) forests in central Alabama. A 3-year study was conducted to determine the fungal, root, and lower stem-infesting insect, and/or soil parameters associated with this decline. *Hylastes salebrosus*, *Hylastes tenuis*, *Pachylobius picivorus*, and *Hylobius pales* were significantly more abundant in declining plots than in asymptomatic plots. Root- and lower stem-infesting insects consistently carried *Leptographium terebrantis*, *L. procerum*, and *L. serpens.* Sampled roots had high levels of root damage, mortality, and staining typically associated with *Leptographium* species. Root damage and mortality preceded aboveground symptoms of short chlorotic needles, sparse crowns, reduced radial growth, and tree mortality. A sequence of biotic and abiotic factors is proposed as the cause of loblolly pine decline complex. FOR. SCI. 53(1):84–92.

Keywords: bark beetles, forest decline, *Leptographium*, root disease, weevils

FOREST DECLINE AND MORTALITY syndromes have been increasingly reported in the past 20 years in many areas in the southeastern United States, including central Alabama. Several biotic and abiotic factors, both natural and anthropogenic, have been proposed as causal factors (Manion 1991). Some of these syndromes involve complexes of closely associated, interdependent, pathogenic organisms. In the southern United States littleleaf disease of shortleaf pine (LLD), for example, is characterized by a strong relationship between a specific soil/site profile and the symptom development of *Phytophthora cinnamomi* Rands (Campbell and Copeland 1954, Oak and Tainter 1988).

Loblolly pine is the most extensively planted pine species in the southeastern United States because of its ability to grow rapidly on diverse sites (Schultz 1997). Over the past 30 years loblolly pine decline has been reported in areas of central Alabama. Loblolly pine decline (LPD) in the southeastern United States has similar symptomatology to LLD of shortleaf pine (Campbell and Copeland 1954) with symptoms including short chlorotic needles, sparse crowns, and reduced radial growth followed by mortality (Lorio 1966, Hess et al. 1999), but LPD is typically found on well-drained sites, whereas LLD is associated with poorly drained sites. The onset of symptoms in both LLD and LPD is associated with older trees that decline slowly and die prematurely, typically accompanied by symptoms associated with several insect and fungal species. Loblolly pine decline is potentially more damaging to age classes > 40 years, because weakened trees lead to an increased incidence of southern pine beetle (Otrosina et al. 1997).

Possible explanations for the observed decline in loblolly pine include *P. cinnamomi* (soil-borne fungi), *Heterobasidion annosum* (Fr.) Bref. (air-borne fungi), or *Leptographium* spp. (insect-vectored fungi). However, sites associated with the LPD tend to be well-drained soils that are typically not conducive to *P. cinnamomi*, and while *H. annosum* tends to favor well drained soils, the harvesting and thinning operations favoring fungal establishment are missing from decline sites.

Leptographium species have been associated with pine decline and mortality, primarily as associates of root-colonizing bark beetles and weevils that attack living trees (Harrington 1983, Klepzig et al. 1991, Otrosina et al. 1997, Eckhardt et al. 2004a). In the southeastern United States, loblolly pine stands showing decline symptoms are more susceptible to attack by southern pine beetle and more likely to contain *Leptographium* spp. within their root systems than asymptomatic stands (Otrosina et al. 1997, Hess et al. 1999). In white pines, the disease white pine root decline or procerum root disease is associated with *Leptographium procerum*. Symptoms include decreased shoot growth, delayed bud break, needle wilt, exudation of resin from the

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root collar area, and resin soaking of affected wood tissue (Sinclair and Hudler 1980, Swai and Hindal 1981).

Leptographium procerum has also been isolated from dying roots of other conifer species in the United States (Livingston and Wingfield 1982, Wingfield 1983, Alexander et al. 1988, Eckhardt et al. 2004a), Canada and Sweden (Kendrick 1962), and was recently implicated as the main cause of seedling mortality in shortleaf pine (Eckhardt et al. 2004b). The purpose of this study was to determine which insect and fungal species were associated in asymptomatic and symptomatic loblolly pine stands to help assess their relationship with loblolly pine decline.

Materials and Methods

Plot Descriptions

Thirty-nine sites were established in central Alabama in nine counties located in Choccolocco State Forest, the Talladega National Forest, (Oakmulgee and Shoal Creek Ranger Districts), and on Gulf State Paper properties (Table 1). Sites were established using visual crown symptomatology. Loblolly pine on 32 sites exhibited decline symptoms (trees with sparse, thinning crowns), and 7 sites were asymptomatic (trees with thick, full crowns). It was later determined through topographic data collection that symptomatic sites had slopes greater than 5% and southerly slope aspects, while asymptomatic sites had slopes equal to or less than 5% and aspects of north and/or northeast. At each site, one central plot and three subplots were established. Subplots were located 120 m from the central plot at bearings of 120, 240, and 360 degrees (Dunn 1999). Research sites were established in 1999 and monitored through September, 2002.

Insect Activity

Pitfall traps (adapted from Klepzig et al. 1991) were used to capture crawling insects continuously for an 8-week period on 15 sites (10 symptomatic and 5 asymptomatic) of the 39 sites during the spring (March–May) of 2000, 2001, and 2002. One trap was placed at the center of each subplot for each of the 15 sites (3 traps per site). Traps were baited with two 8 ml glass vials, one containing 95% ethanol and one containing steam-distilled southern pine turpentine (Hercules, Wilmington, DE), and two cut pine stems approximately 5 cm long by 2 cm diameter. Insects were collected weekly, placed in sterile polyethylene specimen cups, and refrigerated at 4°C.

Data were analyzed using generalized linear models with repeated measures analysis in Proc GLM (SAS Institute, Inc. 2001). The model was $Y = m +$ treatment, where m is the mean, treatment the treatment effect. When significant treatment differences were indicated, means were separated by Fisher's Protected LSD test ($P = 0.05$).

Fungal Associations

Within 3 days of capture all field-collected insects were rolled nondestructively on MEA (2% malt extract agar) and CSMA (MEA, containing 800 mg/l of cycloheximide and 200 mg/l of streptomycin sulfate) for fungal isolation (Hicks et al. 1980). Plates were incubated at 25°C under fluorescent lighting (460 μmol $m^{-2} s^{-1}$) for 2 weeks, examined for fungal growth, and single-spore isolations onto MEA under a 12-hour photoperiod (460 μmol $m^{-2} s^{-1}$). Plates were also placed on silica gel (Dhingra and Sinclair 1995) for long-term storage at 4°C. For identification, cultures were plated on MEA and grown in the dark for comparison to species in Jacobs and Wingfield (2001).

Root Condition

Root samples were collected from all 39 sites during April, May, and June of 2000. A two-root excavation method modified from Otrosina et al. (1997) was used. Three dominant/codominant trees nearest to the plot center were selected for root excavation. Two lateral root segments $>$ 3 cm diameter from each of the three trees were excavated with hand tools from opposite sides of the tree to the approximate crown drip line. Root depth was recorded and lateral roots were examined visually for primary root damage, fine root presence, absence, or damage. Primary roots were defined as the major lateral roots extending from the base of the tree to the drip line. All remaining roots were categorized as fine roots. Logistic regression methods using PROLOGIST (SAS Institute, Inc. 2001) were used to analyze the incidence of staining fungi in symptomatic versus asymptomatic plots.

Twenty-cm-long sections of each root were cut, starting 16 cm from the root collar of each tree, and placed in plastic bags. All fine and feeder roots were cut from lateral roots and placed in plastic bags. All root samples were chilled for transport to the laboratory. Roots were stored at 4°C (about 2–3 days) and then examined for the presence of insect damage, fungi, and classified as alive, dead, or stained (Klepzig et al. 1991).

To determine the presence of *Leptographium* spp. the root sections from each tree were processed similarly to Otrosina et al. (1999). Surface sterilization was modified to use commercial bleach, ethanol, and deionized water (10:10:80 v/v/v). One hundred and sixty root pieces were plated (4 per plate, 40 plates per sample) from each tree on MEA and CSMA and incubated at 25°C under fluorescent lighting (460 μmol $m^{-2} s^{-1}$). After 2 weeks the plates were examined for fungal growth and putative *Leptographium* isolates were processed as above.

To determine the presence of *P. cinnamomi*, the feeder root samples were processed by direct plating and baiting (Jeffers 2000). 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 also were placed in sterile containers bound with netting and flooded with 300 ml of water. Ten pieces each of juniper and camellia leaves were floated on the water surface to attract zoospores. After 24 to 72 hours, the leaf pieces were placed on PARPH plates and incubated in the dark. Putative *Phytophthora* isolates were subcultured onto CVA plates, and then pure cultures were placed on CVA agar slants and stored at room temperature for subsequent identification.

Soil samples were collected at the same time as root excavation from around the lateral roots of the three

Table 1. Locations and characteristics of sites used in the study of loblolly pine decline in central Alabama

Site	County	Location	Physioregion	Stand age	Forest type	Stand type
1	Clay	N 33 28.485 W 85 44.315	Piedmont	40	Mono	Symptomatic
2	Clay	N 33 28.194 W 85 44.276	Piedmont	42	Mono	Symptomatic
3	Clay	N 33 27.870 W 85 44.325	Piedmont	35	Mono	Symptomatic
4	Cleburne	N 33 30.444 W 85 42.114	Piedmont	35	Mixed	Symptomatic
5	Cleburne	N 33 30.212 W 85 41.895	Piedmont	35	Mixed	Symptomatic
6	Calhoun	N 33 42.826 W 85 40.920	Ridge & valley	41	Mono	Symptomatic
7	Calhoun	N 33 42.797 W 85 40.100	Ridge & valley	53	Mono	Symptomatic
8	Clay	N 33 28.660 W 85 44.663	Piedmont	39	Mixed	Symptomatic
9	Clay	N 33 28.722 W 85 45.094	Piedmont	45	Mono	Symptomatic
10	Talladega	N 33 23.384 W 85 56.898	Piedmont	35	Mixed	Symptomatic
11	Talladega	N 33 23.316 W 85 57.303	Piedmont	54	Mono	Symptomatic
12	Bibb	N 32 58.802 W 87 27.123	Coastal plain	54	Mixed	Symptomatic
13	Bibb	N 32 58.245 W 87 23.170	Coastal plain	59	Mono	Symptomatic
14	Bibb	N 32 57.928 W 87 24.715	Coastal plain	62	Mono	Symptomatic
15	Hale	N 32 59.835 W 87 29.780	Coastal plain	59	Mono	Symptomatic
16	Hale	N 32 59.741 W 87 29.733	Coastal plain	59	Mono	Symptomatic
17	Bibb	N 32 57.850 W 87 22.933	Coastal plain	57	Mono	Symptomatic
18	Bibb	N 32 57.767 W 87 22.805	Coastal plain	65	Mixed	Symptomatic
19	Bibb	N 32 55.911 W 87 23.147	Coastal plain	57	Mono	Symptomatic
20	Bibb	N 32 54.484 W 87 22.836	Coastal plain	64	Mixed	Symptomatic
21	Hale	N 32 55.818 W 87 25.595	Coastal plain	56	Mixed	Symptomatic
22	Tuscaloosa	N 32 56.113 W 87 26.420	Coastal plain	56	Mono	Symptomatic
23	Tuscaloosa	N 33 24.806 W 87 26.657	Cumberland Plateau	36	Mono	Symptomatic
24	Tuscaloosa	N 33 24.697 W 87 26.900	Cumberland Plateau	45	Mono	Symptomatic
25	Tuscaloosa	N 33 23.436 W 87 26.202	Cumberland Plateau	29	Mono	Symptomatic
26	Tuscaloosa	N 32 23.206 W 87 26.245	Cumberland Plateau	31	Mono	Symptomatic
27	Tuscaloosa	N 33 23.229 W 87 26.356	Cumberland Plateau	34	Mono	Symptomatic
28	Tuscaloosa	N 33 22.545 W 87 26.748	Cumberland Plateau	38	Mono	Symptomatic
29	Tuscaloosa	N 33 22.585 W 87 26.694	Cumberland Plateau	39	Mono	Symptomatic
30	Tuscaloosa	N 33 22.537 W 87 26.882	Cumberland Plateau	41	Mono	Symptomatic
31	Tuscaloosa	N 33 22.603 W 87 26.853	Cumberland Plateau	35	Mono	Symptomatic
32	Perry	N 32 47.302 W 87 01.456	Coastal plain	60	Mixed	Symptomatic
C1	Cleburne	N 33 28.696 W 85 45.673	Piedmont	32	Mono	Asymptomatic
C2	Calhoun	N 33 42.889 W 85 34.725	Ridge & valley	43	Mono	Asymptomatic

Continued

Table 1. (Continued)

Site	County	Location	Physioregion	Stand age	Forest type	Stand type
C3	Cleburne	N3336.917 W8539.513	Piedmont	34	Mono	Asymptomatic
C4	Bibb	N3258.511 W8720.751	Coastal plain	60	Mixed	Asymptomatic
C5	Bibb	N3258.392 W8720.811	Coastal plain	60	Mixed	Asymptomatic
C6	Bibb	N3254.307 W8722.788	Coastal plain	64	Mono	Asymptomatic
C7	Chilton	N3246.117 W8659.283	Coastal plain	59	Mixed	

dominant/co-dominant trees (Lewis et al. 1987). Soil samples for each root were placed in plastic bags, kept on ice, transported to the laboratory, and stored a 4°C.

To determine the presence of *Leptographium* spp., a 10 g subsample was taken from each soil sample and passed through a #12 sieve (1.7 mm) to remove fragments and suspended in 40 ml of sterile 0.5% water agar. One-ml aliquots were spot inoculated onto each of five CSMA plates. Five additional plates were swirl-inoculated with 1-ml aliquots (adapted from Johnson and Curl 1972). Plates were incubated at 25°C under fluorescent lighting (460 μmol $m^{-2} s^{-1}$) for 2 weeks and examined for fungal growth. Putative *Leptographium* isolates were processed as described above.

The bating method of Jeffers (2000) was used to determine the presence of *P. cinnamomi* in the soil. A representative 100 g subsample was sifted through a #12 sieve (1.7 mm) to remove fragments. Soil was placed in sterile containers and flooded with 300 ml of water. Ten pieces each of juniper and camellia leaves were floated on the water surface to attract zoospores. After 24 to 72 hours leaf pieces were placed on PARPH plates and incubated in the dark. Putative *Phytophthora* isolates were transferred onto CVA plates, and then pure cultures were placed on CVA agar slants and stored at room temperature for identification.

Aboveground Symptomatology

Crown condition, level of insect damage, age, and radial growth rates were measured at all 39 sites on central and subplots. Live crown ratio, crown light exposure, crown position, crown density, crown dieback, and foliage transparency were visually measured and recorded using FHM protocols (USDA 2001) for all loblolly pine with dbh $\geq$ 12.7 cm or greater. Increment cores were collected at 1.5 m height and 5- and 10-year radial growth calculated by determining the last 5 and 10 years' growth and measuring it in millimeters. A correlation analysis using PROC CORR options in SAS (2001) was performed to determine which of the above variables were significant and relevant to LPD. Because there was little variation between symptomatic and asymptomatic plots with regard to live crown ratio, crown light exposure, crown position, and crown dieback, they were not included in the analysis, leaving age, radial growth (5- and 10-year), dbh, crown density, and foliage transparency.

Insect damage was determined by direct observation at the time of root sampling. Black turpentine beetle, *Dendroctonus terebrans* Olivier, activity was determined by counting the number of pitch tubes on each tree in the lower 1 m of the trunk and 12.7 cm below the soil line. Infestations and damage caused by bark beetles, *Hylastes salebrosus* Eichoff and *Hylastes tenuis* Eichoff, and root weevils, *Hylobius pales* Herbst. and *Pachylobius picivorus* (Germar), were determined by counting entrance/exit holes and pitching on bark. Insect damage on roots was determined in the laboratory by removing bark and counting the galleries present.

Results

Insect Activity

A total of 5,783 beetles from 17 species in two families were captured. The phloeophagous herbivores included two bark beetles (*Ips* and *D. frontalis*) associated with the main stem, one *Orthotomicus* (Scolytidae) associated with the upper stem and branches, one species (*D. terebrans*) associated with the lower stem, and two bark beetles (*Hylastes salebrosus* and *H. tenuis*) and two weevils (*P. picivorus* and *H. pales*) associated with the roots.

Sites with LPD symptoms were associated with increased numbers of insect species (Fig. 1). All 17 insect species monitored were relatively more abundant in symptomatic sites, but four species (*H. salebrosus*, *H. tenuis*, *P. picivorus*, and *H. pales*) of root and lower-stem feeding insects were significantly more abundant than in asymptomatic sites (Fig. 1).

Root and lower-stem feeding insects showed strong treatment effects (*H. salebrosus* $F_{1,13} = 13.35$, $P = 0.0029$; *H. tenuis* $F_{1,13} = 26.02$, $P = 0.0002$; *P. picivorus* $F_{1,13} = 18.19$, $P = 0.0009$; *H. pales* $F_{1,13} = 20.70$, $P = 0.0005$). There were marginal to no year (*H. salebrosus* $F_{2,12} = 0.29$, $P = 0.7515$; *H. tenuis* $F_{2,12} = 0.48$, $P = 0.6322$; *P. picivorus* $F_{2,12} = 1.25$, $P = 0.3223$; *H. pales* $F_{2,12} = 4.23$, $P = 0.0408$) or treatment *x* year (*H. salebrosus* $F_{2,12} = 4.64$, $P = 0.0322$; *H. tenuis* $F_{2,12} = 0.81$, $P = 0.4671$; *P. picivorus* $F_{2,12} = 4.11$, $P = 0.0438$; *H. pales* $F_{2,12} = 0.60$, $P = 0.5629$) interactions for these species.

Fungal Associates

Among 17 insect species trapped, three of them carried all 4 species of *Leptographium*. From the remaining species, five of them carried more than 2 species of *Leptographium*

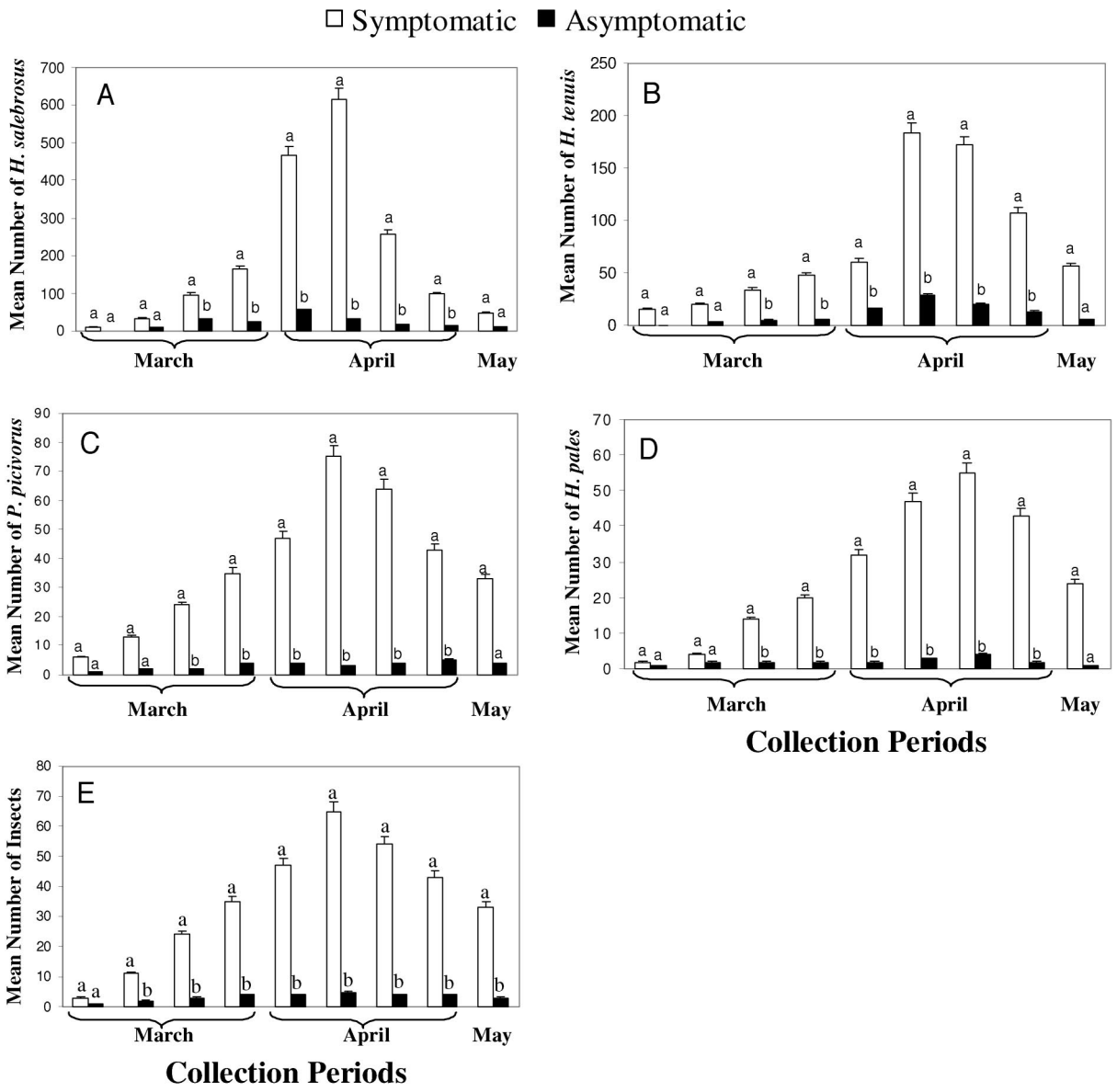

Figure 1. Temporal distributions of bark beetles and weevils in loblolly pine stands of various conditions. (A) *H. salebrosus*, (B) *H. tenus*, (C) *P. picivorus*, (D) *H. pales*, and (E) the remaining insects [*C. unicolor*, *X. saxesini*, *D. frontalis*, *I. pini*, *X. crassiusculus*, *P. cephalotes*, *M. mali*, *X. compactus*, *C. punctatissimus*, *D. terebrans*, *O. caelatus*, and *G. materiorius*]. Data show mean numbers of insects caught per plot per 7-day sample period, over 2000 to 2002. Bars with the same letter at each collection period are not significantly different ($P > 0.05$). Proc Mixed and Tukey's Protected LSD test.

and 9 species carried a single *Leptographium* sp. Three *Leptographium* spp. were consistently isolated from four of the most abundant insects trapped from LPD sites (Table 2). Only insects trapped at the Choccolocco State Forest were consistently associated with *Leptographium truncatum*. *Leptographium procerum* was isolated from *H. pales* (66%) and occasionally from *H. salebrosus* (30%), *H. tenuis* (25%), and *P. picivorus* (14%). *Leptographium terebrantis* was isolated from *P. picivorus* (92%), *H. pales* (78%), *H. salebrosus* (25%), and *H. tenuis* (20%). *Leptographium serpens* was isolated from *H. salebrosus* (57%) and *H. tenuis* (51%), but never from *P. picivorus*, and *H. pales.* While isolations occasionally yielded *Graphium* spp. and nonstaining fungi such as *Aspergillus* spp., *Aureobasidium* spp., *Gliocladium* spp., *Penicillium* spp., and *Trichoderma* spp., these fungi are considered saprophytic or gut symbionts of the insects (Klepzig et al. 1991).

Root Condition

Trees from sites with LPD had higher root mortality and root staining compared to trees from asymptomatic sites (Table 3). Symptomatic trees had fewer fine roots, more fire and insect damage, and staining of the primary root system than trees from asymptomatic sites (Table 4). Root system damage in these sites was positively correlated with the number of insects trapped ($r^2 = 0.89$, $P < 0.0002$).

Leptographium procerum, *L. terebrantis*, and *L. serpens* were consistently isolated from the primary roots of symptomatic trees. *Leptographium procerum* was consistently isolated from the secondary roots of symptomatic trees. *Leptographium serpens* was isolated only from sites with the most severe pine decline symptoms and pine mortality. These sites had 30 to 50% mortality during the 3-year study. Fungi occasionally isolated from roots included *Graphium*

Table 2. Percentage of insects from which *Leptographium* spp. were isolated in 2000–2002

Number of insects trapped (2000, 2001, 2002)	*Lt* [1,2]	*Lp*	*Ls*	*Ltr*
Hylastes salebrosus[3] (22, 2013, 1322)	25	30	57	2
Hylastes tenuis[3] (12, 801, 424)	20	25	51	1
Pachylobius picivorus (7, 327, 148)	92	14	—	—
Colopterus unicolor[3,5] (0, 0, 279)	17	15	33	4
Hylobius pales (11, 2, 143)	78	66	—	—
Xyleborinus saxesini[3] (0, 29, 98)	—	—	70	—
Dendroctonus frontalis[4] (8, 33, 0)	17	—	—	—
Ips spp.[4] (3, 24, 0)	95	—	—	—
Xylosandrus crassiusculus[3,6] (0, 9, 12)	—	—	62	—
Pityophagus cephalotes[3] (0, 10, 5)	40	20	55	—
Monarthrum mali[3] (0, 14, 0)	—	—	64	—
Xylosandrus compactus[3,6] (0, 0, 9)	—	—	77	—
Corthylus punctatissimus[3] (0, 7, 0)	—	29	14	—
Dendroctonus terebrans (0, 0, 4)	90	—	—	—
Orthotomicus caelatus[3] (0, 0, 4)	—	—	50	—
Gnathotrichous materiorius[3] (0, 0, 3)	—	—	67	—

[1] *Lt* = *L. terebrantis*, *Lp* = *L. procerum*, *Ls* = *L. serpens*, *Ltr* = *L. truncatum.*
[2] Some insects had associations with more than one species of *Leptographium.*
[3] Not previously associated with a *Leptographium* species.
[4] Only 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 wks before trapping in 2002.
[5] Seventy-eight percent of these insects also carried an undescribed *Leptographium* species that was recovered from hardwood roots.
[6] Not known or reported to utilize conifers as a primary host.

Table 3. Incidence of staining fungi in relation to stand conditions of loblolly pine

	(All trees) % fungal isolation		(Insect-damaged trees only) % fungal isolation	
Fungal species	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
Leptographium procerum	43.0	87.8	33.0	89.2
Leptographium lundbergii	14.0	14.0	0	23.0
Leptographium serpens	14.0	42.8	0	71.4
Leptographium terebrantis	40.1	82.5	33.0	85.7
Pooled *Leptographium* species[1]	41.5	86.5	66.0	94.6
Graphium species	53.5	71.0	33.0	80.3
Pooled species[1]	53.6	94.8	99.0	96.4

[1] Since some samples from the same root contained more than one species of *Leptographium* and/or *Graphium* spp., total pooled species is smaller than their sum.

spp., *Ophiostoma* spp., *Aspergillus* spp., *Aureobasidium* spp., *Cladosporium* spp., *Curvularia* spp., *Gliocladium* spp., *Mortierella* spp., *Mucor* spp., *Penicillium* spp., and *Trichoderma* spp. The root decay fungi *Heterobasidium annosum* was not recovered in either the symptomatic or asymptomatic stands. Although all stained roots were dead, not all dead roots were stained. Some roots that were alive exhibited extensive resinosis but no staining. *Leptographium procerum* was the only species isolated from the soil samples, and was more common in soils from symptomatic (57%) than asymptomatic (2%) sites. *Phytophthora cinnamomi* was recovered from the soil in only 5 of the 39 plots (4 asymptomatic, 1 symptomatic), and never from root samples.

Aboveground Symptomatology

Increased tree age ($r^2 = 0.92$, $P < 0.0001$) and reduced 10-year radial growth ($r^2 = 0.91$, $P < 0.0001$), were positively correlated with the incidence of *Leptographium*. Trees in symptomatic sites had a reduced radial growth. Trees with lower growth rates and high root mortality and staining were positively correlated to low crown density ($r^2 = 0.89$, $P < 0.001$), high foliage transparency ($r^2 = 0.91$, $P < 0.0001$), increased insect populations ($r^2 = 0.93$, $P < 0.0001$), and increased incidence of *Leptographium* spp. ($r^2 = 0.91$, $P < 0.0001$).

Hylastes spp. damage was evident on roots of living trees sampled on symptomatic sites, as was *Dendroctonus terebrans*. Trees on six symptomatic plots with decline symptoms and damage from both *Hylastes* and *Dendroctonus* spp. eventually became infested with *Ips* spp. and woodborers (Cerambycidae), and died.

Discussion

Loblolly pine decline was found to be a complex association of host, insect, pathogen, and site interactions. The main factor in this association centers on the host vigor condition and the influence of biotic and abiotic elements, the result of which is exhibited in a definable etiology. Symptomatic sites positively correlated to high numbers of root-feeding insects. The four main root-feeding insects (*H. salebrosus*, *H. tenuis*, *H. pales*, and *P. picivorus*) had significantly higher numbers and increased root-feeding activity in symptomatic sites when compared to asymptomatic.

These four insects have also been demonstrated to be associated with *L. procerum*, *L. terebrantis*, and *L. serpens*, and may be serving as vectors (Eckhardt et al. 2004b). These insects have been reported to carry similar fungi in other disease complexes (Lackner and Alexander 1982, Wingfield 1983, Raffa and Smalley 1988, Klepzig et al. 1991). Increased vector activity within stands is associated with the incidence of black stain root disease caused by *L. wageneri* (Hansen 1978, Harrington et al. 1985) and with red pine decline associated with *L. procerum* and *L. terebrantis* (Klepzig et al. 1991). The role of these insects in decline is evident by the increased root damage from feeding, root disease with consistent *Leptographium* isolations, reductions in growth, and symptomatic crowns. *Leptographium procerum*, *L. terebrantis*, and *L. serpens* were consistently associated with declining trees in symptomatic, but not asymptomatic, sites and may be acting as primary pathogens. This study also provides the first report of an association between *H. salebrosus* and *Leptographium* spp.

The attraction of root-feeding insects by a reduced vigor host can begin as an abiotic environmental stress. Once insects are attracted, a cascade of effects is correlated to their increasing numbers. Root disease symptoms develop and root infection, deterioration, and mortality increase on symptomatic sites. The increased root disease progression resulting from the biotic stress of root-feeding insects vectoring *Leptographium* spp. is reflected by reduced growth rates and thinning crowns. The decline trees continue to be attractive to root-feeding insects, further increasing their activity as well as other stem-infesting bark beetles.

The role of root- and lower-stem feeding insects in the overall declining process is supported by the higher population densities of these insects in symptomatic sites and infected trees, the levels of *Leptographium* in these sites, and the consistent isolation of *Leptographium* from these insects and their galleries. *Hylastes salebrosus* and *H. tenuis* damage was frequently observed in both asymptomatic and symptomatic sites, whereas *H. pales* and *P. picivorus* were seldom observed in asymptomatic sites. The different insect populations could indicate that *H. salebrosus* and *H. tenuis* are either relatively more aggressive in attacking healthy trees or the abundance of these insects is affected by site and environmental conditions. Although a consistent sequence of infestation remains to be established in loblolly stands, the current study suggests a possible pathogen/vector relationship.

The importance of root infection and root deterioration and the subsequent weakening loblolly pine is supported by the prior appearance of root staining to the onset of aboveground symptoms and the overall higher rates of root injury than in asymptomatic sites. Aboveground symptoms of radial growth reduction and crown thinning, within declining stands as compared to asymptomatic, correspond to the belowground pattern. This hypothesis is in agreement with a reduction in radial growth before tree death (Wagener and Mielke 1961), thin crowns (Leaphart and Gill 1959, Otrosina et al. 1999) and association with *Leptographium* spp. in other pines.

Trees appear to increase in susceptibility to *H. salebrosus* and *H. tenuis* by their increased infestation rate in the root-infested living trees. That is, these insects may be both inducers and beneficiaries of a progressively deteriorating root system (Eckhardt et al. 2004b). More importantly, trees 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 disease progression is similar to red pine decline in Wisconsin reported by Klepzig et al. (1991). Root-infesting agents predisposing trees to fatal *Ips* attack has been observed among trees with thin crowns (Christiansen et al. 1987), low periodic growth ratios (Raffa and Berryman 1983), and stem infection (Raffa and Berryman 1982), all symptoms observed in symptomatic sites. Moreover, Otrosina et al. (1995, 1997), Goheen and Cobb (1980), and Wagener and Mielke (1961) observed higher frequencies of bark beetle infestation among pines with *Leptographium* root disease.

Hylobius pales and *P. picivorus* are known to be rapid colonizers of dead tree and stump roots. Although *Hylastes salebrosus* and *H. tenuis* may prefer and/or be more successful in stressed than in dead trees, because of competition with the secondary weevils the former two species can also colonize dead tissue. The increased breeding substrate for *H. pales* and *P. picivorus*, and their potential role as additional sources of fungal inoculum, are supported by the increased densities of these insects in declining sites (Figure 1) and their association with *L. procerum.* The biology and behavior of *H. salebrosus* and *H. tenuis* is still poorly understood, and therefore their exact role is unknown.

Contamination of insects with fungal spores could occur when different or the same species of bark beetles are captured in the same trap. At least one sample of each of the former four species was found to carry *Leptographium* when no other positive insect sample was present in the same trap. This indicates that cross-contamination in the trap from other insects is unlikely. This is consistent with other insect-fungal association trapping studies (Wright 1935, Viiri 1997, Schweigkofler et al. 2005).

Of the other insects recovered in the traps, *Colopterus unicolor* and *P. cephalotes* (sap beetles) are attracted to fungal mats and may inadvertently spread *Leptographium*. Both *C. punctatissimus* and *M. mali* are considered hardwood pests that breed in maple and dogwood (USDA 1985), and were abundant in the Alabama study sites, 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 use conifers as a primary host. The exact nature of the relationships of the various insects with *Leptographium* are unclear, although we have shown that *Hylastes* spp. may derive some benefit from the presence of *Leptographium* spp. in their galleries (Eckhardt et al. 2004b).

These studies show a strong association between LPD and the occurrence of high vector populations and the presence of *Leptographium* spp. Future research on LPD will concentrate on testing the individual components in the syndrome. A better understanding of the pathogenicity and taxonomy of the associated fungi, and the behavioral characteristics of the associated insects, should lead to a more complete understanding of root disease associated with pine

Table 4. Condition of primary and fine roots from asymptomatic and symptomatic loblolly pine

	% of roots		
	Asymptomatic	Symptomatic	*P*-value[1]
Fine roots			
Present (alive)	85	35	0.2660
Present (dead)	10	32.5	0.1246
Absent	5	32.5	0.4555
Primary roots			
Alive	100	100	—
Damaged[2]	15/10.5	58.5/48.5	0.0194/0.0046
Dead	0	0	—
Stained	15	74.5	0.0499

[1] *P*-values are for logistic regression comparisons of asymptomatic ($N = 7$) versus symptomatic ($N = 32$) sites for each category.
[2] The first value indicates insect damage, the value following the slash indicates fire damage.

declines. Current investigations also are focused on the prior disturbance history and site conditions of these sites, which appear to have significant effects on the expression of decline and the density of insect populations.

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