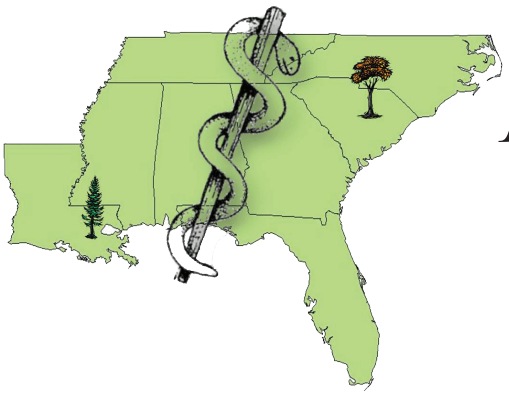

AUBURN UNIVERSITY
FOREST HEALTH COOPERATIVE

RESEARCH REPORT 2014-01

FACTORS ASSOCIATED WITH INCIDENCE OF OPHIOSTOMATOID FUNGAL SPECIES CONTRIBUTING TO SOUTHERN PINE DECLINE

by
Yuan Zeng and Lori Eckhardt
Forest Health Cooperative, Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences
Auburn University, AL

ABSTRACT
Ophiostomatoid fungi such as *Grosmannia* spp., *Ophiostoma* spp., and *Leptographium* spp. are known as contributing factors to Southern Pine Decline (SPD) in the southeastern United States. This study was developed to identify factors associated with ophiostomatoid fungi and quantify their fluctuations in response to mechanical thinning in *Pinus taeda* L. stands in central Alabama and Georgia. Nine research plots were established on five *P. taeda* plantations to quantify fungal incidence from pre- treatment root samples. Roots of *P. taeda* were excavated and assayed for ophiostomatoid fungal infections from both pre- and post-treatments. The dominant fungus recovered was Leptographium procerum followed by other species including *L .terebrantis*, *G. alacris*, *G. huntii* and *O. ips*. Roots of *P. taeda* older than 40 years had greater recovery rates of *O. ips*. Sites with steeper slopes increased incidence of *L. terebrantis* affecting *P. taeda* root systems. Sites with mechanical thinning increased the incidence of ophiostomatoid fungal species that may serve as a source to infest the remaining trees in the stand and predispose them to SPD.

INTRODUCTION
Southern Pine Decline (formerly Loblolly Pine Decline) was first reported on *P. taeda* stands in the southeastern United States in the Talladega National Forest in 1959 (Brown and McDowell 1968). Symptoms of SPD include thinning crowns, root deterioration, and reduced radial growth at the age of 40 to 50. In central Alabama, *P. taeda* were more prone to show decline symptoms with steeper slopes and southeast/ south/ southwest aspects (Eckhardt and Menard 2008). Root pathogens (*Leptographium* spp., *Grosmannia* spp., and *Ophiostoma* spp.) have been consistently found on sites suffering from SPD in central Alabama (Hess et al. 1999, Eckhardt et al. 2007).

Leptographium procerum, *L. terebrantis*, *G. alacris* (formerly *L. serpens*), *L. truncatum*, *G. huntii*, and *O. ips* have been recovered from roots and soil near *P. taeda* showing decline symptoms in the southern United States (Eckhardt 2003, Jacobs and Wingfield2001, Zanzot et al. 2010).

Leptographium procerum is associated with *P. strobus* root decline in the northeastern United States (Kendrick 1962, Wingfield et al. 1988) and has been isolated from declining loblolly pine roots (Eckhardt et al. 2007). The pathogenicity of *L. procerum* has been debated for many years. Lu et al. (2010) reported it pathogenic and could cause more disease on *P. tabuliformis* seedlings than other fungal isolates. However, *L. procerum* has also been reported to be unable to kill host species compared to *L. terebrantis* and *G. alacris* (Wingfield et al. 1988, Eckhardt et al. 2004b). Unlike *L. procerum*, *L. terebrantis* is highly pathogenic as inoculations with *L. terebrantis* causes larger lesion development and kills *P. strobus* and *P. taeda* seedlings (Wingfield 1986, Eckhardt et al. 2004b). In order to compare pathogenicites of *L. procerum*, *L. terebrantis*, *G. huntii*, and *G. alacris* on southern pine spp., research which inoculated four ophiostomatoid fungal species in root systems and reported that lesions and mortality caused by *G. alacris* on *P. taeda*, *P. palustris*, and *P. elliottii* were greater than lesions caused by *L. procerum* and *L. terebrantis* (Matusick et al. 2010, Matusic et al. 2011). With respect to *Grosmannia huntii*, much less *is* known when compared to the other three species of *Leptographium*. Inoculations using *G. huntii* resulted in lesions and occlusion length that were longest in *P. taeda* and *P. elliottii* seedlings when compared to *G. alacris*, *L. terebrantis* and *L. procerum* (Matusick and Eckhardt 2010). However, although *O. ips* caused longer lesions than *G.alacris* on *P. elliottii*, *P. caribaea* Morelet (Caribbean pine), and *P. radiata* in South Africa, it was suggested that *O. ips* should not be considered a serious pathogen (Zhou et al. 2002).

Several species of ophiostomatoid fungi can be carried in the mycangia, a specific organ of their associated insect vector (Barras and Perry 1971, Solheim 1995). Cobb et al. (1974) showed a high degree of association between root disease and species of *Dendroctonus* infesting conifers. *Hylastes* spp. which were considered as a nonaggressive species have been associated with ophiostomatoid fungi, such as *L. terebrantis*, *L. procerum*, *G. alacris*, and *G. huntii* (Klepzig et al. 1991, Jacobs and Wingfield 2001, Eckhardt and Menard 2005, Eckhardt et al. 2007, Zanzot 2009), because they can carry sticky spores on their body. The infestation of ophiostomatoid fungi would block water movement and nutrient avalabitity to decrease tree vigor, then lead secondary pest as *Hylastes* spp. to attack root systems. Regeneration weevils (*Pachylobius picivorus* and *Hylobius pales*) had a positive correlation with incidence of *Leptographium* spp. (Eckhardt et al. 2007). In addition, a variety of insect vectors have been found to transport *G. huntii* that include *D. ponderosae*, *H. ater*, *Ips pini* (Jacobs and Wingfield 2001) and *Hylastes* spp. (Zanzot et al. 2010).

In addition to biotic factors which can cause root diseases, abiotic factors include silvicultural disturbances could also incite root contamination. For example, thinning could damage residual trees, compact soil, increase windthrow, and provide infection courts for root pathogens (Ferrell 1996, Schwilk et al. 2006). Thinned plots exacerbated diseases such as *Armillaria gallica*, *Heterobasidion irregular*, and *Cronartium ribicola* compared with unthinned plots (Maloney et al. 2008). Therefore, stand management such as prescribed burns, agricultural practices, and lower vegetation density could affect the incidence and severity of SPD. Drought and storm damage are also factors to SPD (Gill 1992). Soil and root disturbance caused by silvicultural treatments can also incite decline. For example, thinning may either directly cause physical injury and stress of roots, or indirectly increase secondary pests such as root-feeding bark beetles (Eckhardt and Menard 2009).

Understanding factors which predispose, incite and contribute to SPD are necessary to develop planting and stand management options. This study will identify factors associated with the incidence of ophiostomatoid fungal species contributing to SPD, and examine effects of mechanical thinning on fluctuations in blue-stain fungi incidence in *P. taeda* stands.

METHODS AND MATERIALS

Study Sites

Five study sites (SS, RAY, WEY, WV and F&W) were established on property managed or owned by members of the Forest Health Cooperative in either central Alabama or Georgia. Within each of the study sites, 9 FHM plots were established per US Forest Service FHM guidelines (Dunn 1999) in January 2009. Four subplots were established with three subplots located 36.6 m away from a center subplot at a bearing of 120, 240, and 360 degree (Dunn 1999). Latitude and longitude coordinates of center subplots were measured by using a GPS unit (Garmin GPSMAP 76Cx, Garmin International Inc., Olathe, KS). The row thinning timeline for each site is presented in Table 3.1, and because of access problems, plot 2 at study site WEY was not thinned. Weather data was accessed from the National Climatic Data Center (http://www7.ncdc.noaa.gov/IPS/coop/coop.html). Data from the Bankhead L&D weather station (AL), Alexander city weather station (AL), Maion Junction 2 NE weather station (AL), Columbus #2 weather station (GA), and Cuthbert weather station (GA) were used.

Tree Vigor and Site Characteristic Measurements

All *P. taeda* with DBH greater than 10 cm within a 7.3 m radius on each subplot were rated for tree health based on FHM procedures (Dunn 1999). As crown condition is an indication of tree health, the live crown ratio (a percentage of the live crown length by the actual tree length), crown light exposure (the amount of crown quarters equal to or greater than 35% of live crown ratio and crown top receiving direct light; 0 - 5), live crown position (superstory, overstory, understory, open story), live crown density (the amount of crown branches, foliage, and reproductive structures that block light visibility through the crown) as well as crown dieback (a percentage of the dieback area by the live crown area) and live foliage transparency (the amount of light visible through the live foliated portion of the crown) were measured and recorded for each tree (Schomaker et al. 2007). In addition to crown conditions, DBH, tree height and radial growth increment were collected from six trees randomly selected at the center subplot. Increment cores were collected, and core samples were returned to the Forest Health Dynamics Laboratory where five-year and ten-year growth values were obtained with a Mitytoyo Digimatic (Mitutoyo Corporation, Maplewood, New Jersey) electronic ruler.

Plot conditions, including landform (convex, concave, flat), slope inclination (%), slope aspect (NW, NE, SE, SW, N, E, W, S, NA), and elevation of each plot were obtained in the center. Topographic position, e.g. side-slope, ridge-top, toe-slope was also recorded for each plot (Eckhardt 2003).

Insect Trapping

To determine the relationship between the percentage of ophiostomatoid fungi isolated from each plot and insect vector captures from pre-treatment collections within every plot, three types of insect traps such as pitfall trap, panel trap and flight intercept trap were placed in center subplot

to monitor bark beetle population dynamics over time. In this study, *H. salebrosus*, *H. porculus*, *H. tenuis*, *D. terebrans*, *P. picivorus*, and *Hb. pales* were considered as pathogen vectors of ophiostomatoid fungi.

The panel traps were installed 2 m above the ground with a plastic cup attached to the bottom that contained a 2:1 mixture of water and antifreeze to preserve captured insects. Pitfall traps were buried into the soil/litter layer so that the entrance holes around the circumference were slightly above the ground line. The interior of each trap was coated with a thin layer of liquid Teflon™ (Northern Products Woonsockets, RI) to prevent the escape of captured insects. Flight intercept traps were made from plastic 3785 ml containers fitted with a 120 ml collection cup attached at the bottom. It is 1 m far off the ground. Each container was cut open on three sides to expose the bait/attractants, with the fourth side attached to a metal pole. Two 8 ml glass vials, filled with southern pine turpentine (W.M. Barr & Co., Inc., Memphis, Tennessee) and 95% ethanol (1: 1) were installed in every trap as an insect attractant. Both vials and panel trap cups were refilled every two weeks during insect collections. Insects traps were monitord from March 2009 till thinning treatment occurred (Table 3.1). Captured insects were placed in sterile polyethylene cups transported back to the Forest Health Dynamics Laboratory at Auburn University (Auburn, AL, USA) for sorting and identification.

Root Sampling

Root samples were taken from pre-treatment plots and post-treatment plots. Roots from pre-treatment plots (45 plots in total) were sampled from October 2009 to March 2010. Post-treatment roots were only excavated and sampled in thinned and control plots (30 plots in total). For all treatments, lateral roots with a diameter greater than 2 cm from three dominant/co-dominant *P. taeda* per subplot were sampled using a method modified from Otrosina et al. (1997). From each tree, two lateral roots were excavated up to 1 m from the tree base. Three new trees were randomly selected using the same method during August 2011 to October 2011 as post-treatment root samples. In addition, remaining trees that were excavated in thinned plots and trees sampled for pre-treatment in control plots were re-sampled to observe if different ophiostomatoid fungal species would be isolated.

From every excavated root, three sample cores (0.5 cm x 2 cm) (six cores per tree) were collected using and increment hammer (Suunto USA, Inc., Ogden, UT). The hammer was sterilized with 95% ethanol after sampling each tree and allowed to air-dry to limit cross-contamination. Roots were then reburied with soil after the sample cores were collected. Root sample cores were placed in sterile plastic bags, transported back to the Forest Health Dynamics Laboratory at Auburn University (Auburn, AL, USA) in a cool ice chest and kept at 4 °C until processed. To determine the presence of ophiostomatoid species within the root samples, root sample were surface sterilized with a (10:10:80 v/v) mixture of commercial bleach, ethanol, and distilled water. Tissues were cultured in CSMA (MEA containing 800 mg/l Cycloheximide and 200 mg/l streptomycin sulfate) media (Hicks et al. 1980). After two weeks, the plates were examined for blue-stain fungal growth characteristic of Ophiostomatoid- like fungi. Suspect colonies were subcultured to sterile MEA plates for identification. Each isolated ophiostomatoid fungal species was marked as positive per sampling tree.

DATA ANALYSIS

The presence of each ophiostomatoid species per tree was counted as 1 (minimum = 0, and maximum = 12 per plot), and the percentage of each species recovered were calculated by plot. Since the variables were percents which did not distribute normally, original data were transformed in SAS [PROC RANK; BLOM versin; SAS 9.2; $y = {}^{\Phi-1}((r_i-3/80/(n+1/4))]$.

Same species isolated from pre-treatment samplings after transformation were compared among study sites to examine dominant ophiostomatoid species in the study area (ANOVA; Tukey's Studentized Range Test; PROC GLM; SAS 9.2). In order to observe if the percentage of each fungal isolation associated with site characteristics, dummy variables of stand age class (10- 19 yrs; 20- 29 yrs; 30- 40 yrs; > 40 yrs), slope class (minimum risk ≤ 5%; low risk = 6 to 10%; moderate risk = 11 to 15%; high risk > 15%), and aspect class (minimum risk = 337.5 to 67.5°; low risk = 67.6 to 112.5° and 292.6 to 337.4°; moderate risk = 247.6 to 292.5°; high risk = 112.6 to 247.5°) (modified Eckhardt 2003) were created in SAS 9.2. A one-way analysis of variance (ANOVA) test was used to examine if class variables had effects on isolations of blue-stain fungi species. Transformed means of the percentage of ophiostomatoid species isolated by plot from pre-treatment data were analyzed using Tukey's Studentized Range test (PROC GLM; SAS 9.2) to tell differences among classes. As crown conditions are indicators of declining symptoms, and root-feeding bark beetle (*Hylastes* spp. and *D. terebrans*) and regeneration weevils (*P. picivorus* and *Hb. pales*) are considered as vectors which carry spores of ophiostomatoid species, pre-treatment fungal isolation were also correlated with mean insect captures by species and crown conditions including the live crown ratio (%), crown exposure light, live crown density (%), and live crown transparency (%) (PROC CORR; SAS 9.2). Since crown exposure light is a catorgerical variable, according to its definition, 0%- 100% were used to describe crown light instead of 0- 5 when analyze their relationship in Pearson Correlation.

The responses of ophiostomatoid species to the thinning treatments were compared using a two-way analysis of variance (Two-Way ANOVA). Fungal isolations of both pre- and post-treatment data were pooled by treatment in each study site. *P*-values were produced using Tukey's Multiple Comparisons Procedure (PROC GLM; SAS 9.2). All statistics were analysized at the significant level of 0.05.

RESULTS

Description of Study Area

Forty-five plots were observed before the thinning treatments occurred. Plot conditions and average values of crown rating parameters are presented in Tables 3.2 and 3.3. Among those plots, the youngest was established in 1998 in WEY site and the oldest plot dates to 1959 in WV site. Plots were distributed across percent slopes from 0% to 30% with variable aspects. Elevation ranged from 93 to 265 m above sea level. The average biweekly temperature data for the five study sites are presented in Figure 3.1.

Captures of Insect Vectors

A total of 7,608 bark beetles and weevils were captured before thinning treatments occurred. They included *Dendroctonus terebrans* (n = 117), *H. porculus* (n = 2173), *H. salebrosus* (n =

2731), *H. tenuis* (n = 828), *P. picivorus* (n = 387), *Hb. pales* (n = 611), *D. frontalis* (n = 7), *I. avulses* (n = 107), *I. grandicollis* (n = 1477), *I. calligraphus* (n = 3), *Pissodes nemorensis* (n = 245), and *Orthotomicus caelatus* (n = 121). In addition, Plot SS7, SS9, WV6, WV7, and WV8 had greater captures of *Hylastes* spp. than other plots (Table 3.4).

Five ophiostomatoid species were isolated from the root samples: *L. procerum*, *L. terebrantis*, *G. alacris*, *G. huntii*, and *O. ips*. In general, isolations of *L. procerum* in all sites were consistently higher than other species among all study sites (Table 3.5). Incidence of *L. procerum*, *G. alacris*, and *G. huntii* had no differences ($F_{L.\ procerum} = 1.71$, $P_{L.\ procerum} = 0.1658$; $F_{G.\ alacris} = 2.19$, $P_{G.\ alacris} = 0.0881$; $F_{G.\ huntii} = 0.95$, $P_{G.\ huntii} = 0.4447$; df = 4, 40; ANOVA; Table 3.6); however, isolation of *L. terebrantis* and *O. ips* had the greatest frequency in WV site ($F_{L.\ terebrantis} = 3.02$, $P_{L.\ terebrantis} = 0.0287$; $F_{O.\ ips} = 3.40$, $P_{O.\ ips} = 0.0174$; df = 4, 40; ANOVA; Table 3.6). In addition, ophiostomatoid fungi isolations were greatest in WV site, and there were no observations of *O. ips* from root samples collected in RAY and FW study sites.

Potential Factors Associated with Incidence of Ophiostomatoid Fungi

Of the isolated fungal species, age category had a significant effect on incidence of *O.ips* (ANOVA; $F_{O.ips} = 5.15$, $P_{O.ips} = 0.0041$; df = 3, 41). Isolations of *O.ips* were significantly higher in plots older than 40 years when compare to the other age classes (Table 3.7). Plot slopes only affected isolations of *L. terebrantis* (ANOVA; $F_{L.\ terebrantis} = 2.89$, $P_{L.\ terebrantis} = 0.0467$, df = 3, 41) compared to other four species. Isolations of *L. terebrantis* in plots whose slope are greater than 15% was significantly higher than plots with slope class from 11% to 15% (Table 3.8). However, aspect did not show significant impacts on all those five blue-stain fungal species (ANOVA; $F_{L.\ procerum} = 0.59$, $P_{L.\ procerum} = 0.6220$; $F_{L.\ terebrantis} = 0.01$, $P_{L.\ terebrantis} = 0.9995$; $F_{G.alacris} = 0.25$, $P_{G.\ alacris} = 0.8615$; $F_{G.huntii} = 0.98$, $P_{G.huntii} = 0.4118$; $F_{O.ips} = 1.24$, $P_{O.\ ips} = 0.3089$; df = 3, 41; Table 3.9).

Most of the insect vector species did not show any relationships between fungi recovered collected prior to thinning. However, isolations of *O. ips* were positively correlated with captures of *H. porculus* and *H. salebrosus* (Pearson Correlation; $P_{H.\ Porculus} = 0.0013$; $P_{H.\ salebrosus} = 0.0080$; a = 0.05; Table 3.10), while isolations of *L. procerum* were negatively associated with numbers of *H. tenuis* trapped from study sites (Pearson Correlation; $P_{H.\ tenuis} = 0.0468$; a = 0.05; Table 3.10). Each plot crown condition was compared to fungal isolations, however, incidence of ophiostomatoid fungi was not correlated to any of the crown class conditions (Table 3.11).

Mechanical Thinning Treatments Effect on Incidence of Ophiostomatoid Fungal Species

After row thinning treatments, the incidence of blue-stain fungi increased significantly when compared to reisolations taken from the control plots (Table 3.12; Table 3.13). In addition, multiple ophiostomatoid species were isolated from remaining trees in thinned plots which were sampled before thinning treatment occurred, and *D. terebrans* infection were observed on lower *P. taeda* trunk in thinned plots.

DISCUSSION

Mechanical thinning increased the incidence of blue-stain fungi incidence in loblolly pine stands, which could further increase the possibility of SPD becoming established in those stands. Higher

populations of *Hylastes* spp. in thinned stands (see chapter two) could then lead to higher inoculations of ophiostomatoid fungi in *P. taeda* roots. Additionally, the use of heavy equipment on *P. taeda* stands may cause root and soil compaction (Eckhardt and Menard 2009). Thus, minimizing thinning acrivities to limit root compaction and logging damage to residual trees is important. If a pine stand contains a significant level of diseased trees, a landowner may decide to perform a light row thinning as fifth row thinning, or avoid thinning stands during wet season. Thinning treatments increased root infections of ophiostomatoid fungi in thinned plots, which has also been observed in other studies that reported an increase in bark beetle populations and further provide infection potential for root pathogens (Ferrell 1996, Schwilk et al. 2006). A three-year study showed that thinned plots exacerbated *A. gallica, H. irregular,* and *Cronartium ribicola* in mixed-conifer stands (Maloney et al. 2008), because freshly cut stumps can be easily colonized by *H. irregulare* and some *Armillaria* species (Harrington 1993). In addition, pitch tubes were observed in thinned *P.taeda* plots (*D. terebrans* infection), which will further lead to tree vigor loss, and predispose remaining trees to other secondary pests and disease infection.

Ophiostamatoid fungi, such as *L. procerum, L. terebrantis, G. alacris, G. huntii,* and *O. ips,* which contribute to SPD, were recovered from lateral roots collected from pre- thinned treatment *P. taeda* root samples. *Leptographium procerum* and *L. terebrantis* were consistently isolated at a greater frequency among different plots. Although *L. procerum* is the dominant species in this study and it was frequently isolated from root- feeding bark beetles and weevils (Klepzig et al. 1995, Eckhardt et al. 2007), most studies suggested that it is a mild pathogen (Klepzig et al. 1996, Nevill et al. 1995, Wingfield 1986), especially to mature *P. taeda* roots (Eckhard et al. 2004b). Previous studies have showed *L. terebrantis* to produce longer lesions on *P. taeda* than *L. procerum* (Nevil et al. 1995, Eckhardt et al. 2004b), so greater incidence of *L. terebrantis* could become a problem in WV plots in the future. *Grosmannia alacris* and *G. huntii* are non-native fungal species, and the pathogenicity of those two fungi on mature *P. taeda* trees or seedlings resulted in the larges lesions reported compared to other fungi tested (Eckhardt et al. 2004b, Matusick 2010).

Stands in the 40 + age class had significantly more *O.ips* recovered than the other age classes examined. In addition, slope over 15% had greater recovery rates of *L. terebrantis*. *Pinus taeda* on slopes greater than 10% had an increasing SPD incidence (Eckhardt and Menard 2008), thus the greater number of re- isolations of several ophiostomatoid species in these plots are in agreement with the SPD model (Eckhardt and Menard 2008). Hence, those high risk stands should be either clearcut or converted to appropriate species genetically resistant *P. taeda* or *P. palustris* to decrease SPD contamination and avoid losses. However, the S/ SW aspect did not increase the incidence of stain fungi as would be predicted by the SPD model. Similar recovery rates on the various aspects were also observed in longleaf pine *P. palustris* stands (Zanzot 2009).

Previous studies (Eckhardt et al. 2004b, Eckhardt et al., 2007, Zanzot et al. 2010) have reported that pine decline was found to be associated with interaction of factors such as tree host, insect, pathogen and site characteristics. According to the SPD theory (Eckhardt et al. 2007, Eckhardt and Menard 2008, Eckhardt and Menard 2009), crown class conditions were a good indication of disease severity. However, the recovery of ophiostomatoid fungi was not correlated to any of the crown conditions measured. It is possible that no symptoms would be found in a stand with

vigorous trees even though there is a presence of ophiostomatoid fungi in the root systems. Therefore, it would be difficult to predict stand infection prior to symptomology without using other methods.

Table 3.1 Mechanical thinning timeline in study sites.

Study Site	Mechanical Thinning
SS	20 November 2009-24 February 2010 (Plot 2) 9 October 2010-17 December 2010 (Plot 1&3)
RAY	19 November 2009-4 December 2009
FW	March 2011
WV	21 July 2010-5 August 2010
WEY	25 July 2010-10 August 2010 (Plot 1&3)

Table 3.2 Plot conditions and site characteristics in Alabama and Georgia.

Plot	Age	Elevation (m)	Slope (%)	Aspect (°)	Convexity	Topographic Position
WV1	16	121	22	350	Convex	Side-slope
WV2	16	100	18	270	Convex	Side-slope
WV3	16	124	16	0	Convex	Side-slope
WV4	19	107	14	315	Convex	Side-slope
WV5	18	106	8	315	Convex	Side-slope
WV6	18	101	26	80	Convex	Ridge-top
WV7	51	102	5	45	Convex	Ridge-top
WV8	52	114	9	75	Convex	Ridge-top
WV9	51	113	28	225	Convex	Side-slope
SS1	18	247	19	90	Convex	Toe-slope
SS2	18	210	4	315	Concave	Toe-slope
SS3	18	254	19	315	Convex	Nose-slope
SS4	26	253	3	135	Convex	Nose-slope
SS5	26	245	4	90	Convex	Toe-slope
SS6	26	239	3	315	Flat	Ridge-top
SS7	26	265	2	225	Flat	Toe-slope
SS8	26	258	5	45	Concave	Toe-slope
SS9	26	265	1	0	Flat	Side-slope
WEY1	13	94	13	298	Convex	Toe-slope
WEY2	13	116	2	0	Convex	Ridge-top
WEY3	13	93	13	245	Convex	Ridge-top
WEY4	28	121	30	225	Convex	Side-slope
WEY5	28	127	6	270	Convex	Side-slope
WEY6	13	131	3	0	Convex	Ridge-top
WEY7	30	106	6	248	Convex	Ridge-top
WEY8	30	130	18	340	Convex	Side-slope
WEY9	30	131	10	270	Convex	Side-slope
F&W1	17	128	25	205	Convex	Side-slope
F&W2	17	141	6	200	Convex	Side-slope
F&W3	17	132	8	320	Convex	Side-slope
F&W4	24	150	6	315	Convex	Ridge-top
F&W5	20	119	11	30	Convex	Toe-slope
F&W6	23	109	19	135	Convex	Side-slope
F&W7	32	94	1	0	Flat	Side-slope
F&W8	23	111	8	150	Convex	Side-slope
F&W9	32	104	1	0	Flat	Ridge-top
Ray 1	16	146	14	20	Convex	Side-slope
Ray 2	18	123	4	80	Convex	Ridge-top
Ray 3	16	180	0	0	Flat	Ridge-top
Ray 4	16	159	8	225	Concave	Side-slope
Ray 5	16	163	6	200	Flat	Side-slope
Ray 6	18	137	1	0	Flat	Ridge-top
Ray 7	22	111	2	315	Flat	Ridge-top
Ray 8	22	123	8	135	Convex	Side-slope
Ray 9	16	126	10	75	Convex	Side-slope

NA Indicates no aspect.

Table 3.3 Mean values of pre-thinning treatment data for growth and crown rating parameters.

Plot	DBH (in)	CR (%)	CL	CP	CDen (%)	CDie (%)	FT (%)	5-yr Growth (cm)	10-yr Growth (cm)
WV1	7.9	35	1	2	30	0	30	1.53	4.23
WV2	6.6	30	1	2	25	0	35	1.68	4.25
WV3	8.2	35	2	2	35	0	25	1.8	4.0
WV4	6.8	35	1	2	30	0	25	1.42	2.9
WV5	7.5	35	2	2	35	0	25	1.32	3.33
WV6	6.3	40	3	2	35	0	30	1.73	3.75
WEY1	8.4	35	1	2	35	0	30	2.12	5.57
WEY2	7.3	40	1	2	35	0	30	1.93	5.12
WEY3	7.4	35	1	2	40	0	30	2.03	5.77
WEY4	9.4	35	2	2	30	0	30	1.3	2.82
WEY5	12.1	40	3	2	35	0	25	1.65	4.33
WEY6	6.9	45	2	2	35	0	25	2.1	5.42
F&W1	8.3	30	1	2	35	0	25	1.23	3.47
F&W2	6.2	35	1	2	30	0	25	1.53	3.6
F&W3	5.6	30	1	2	30	0	25	1.33	3.23
F&W4	6.3	30	1	2	35	0	25	1.04	3.12
F&W5	6.9	30	2	2	30	0	35	0.9	2.82
F&W6	6.5	30	2	2	30	0	45	1.06	3.67
Ray1	6.5	35	1	2	30	0	330	1.76	4.64
Ray2	6.7	25	1	2	30	0	25	1.4	3.73
Ray3	6.2	30	1	2	30	0	30	1.47	1.63
Ray4	5.6	30	1	2	25	0	35	1.32	4.44
Ray5	5.8	25	1	2	25	0	25	1.52	4.7
Ray6	7.0	25	1	2	35	0	35	1.28	3.3
Ray7	6.7	25	1	2	35	0	25	NA	NA
Ray8	5.9	30	1	2	35	0	25	NA	NA
SS1	7.0	30	1	2	35	0	25	1.3	3.84
SS2	8.3	35	1	2	40	0	30	1.44	4.5
SS3	6.9	35	1	2	30	0	30	1.88	4.58
SS4	8.4	35	1	2	35	0	35	1.6	2.75
SS5	10.0	30	1	2	40	0	30	NA	NA
SS6	9.3	30	1	2	45	0	45	1.8	3.5
SS7	10.2	35	2	2	35	0	25	2.3	4.8
SS8	9.1	35	2	2	35	0	25	1.67	3.86
SS9	9.7	50	1	2	40	0	30	NA	NA

CR = crown ratio; CL = crown light; CP = crown position; CDen = Crown density; CDie = crown dieback; FT = foliage transparency; and NA = that growth measurements didn't record during the experiment period.

Table 3.4 Pre-treatment insect captures by plot among study sites.

Plots	*D. terebrans*	*H. porculus*	*H. salebrosus*	*H. tenuis*	*P. picivorus*	*Hb. pales*
F&W1	3	41	24	19	5	10
F&W2	5	30	58	16	8	6
F&W3	3	76	80	48	18	9
F&W4	4	72	77	31	15	20
F&W5	2	20	34	9	5	10
F&W6	0	35	17	14	3	8
F&W7	2	17	9	15	14	6
F&W8	0	29	6	8	9	15
F&W9	1	16	11	20	11	14
RAY1	1	12	12	11	10	4
RAY2	10	13	31	10	15	12
RAY3	2	12	26	3	35	9
RAY4	1	23	32	18	7	16
RAY5	1	8	29	6	5	2
RAY6	8	38	76	6	13	6
RAY7	1	11	16	6	15	9
RAY8	3	11	5	4	15	14
RAY9	8	25	43	8	21	5
SS1	3	60	77	24	18	46
SS2	0	49	30	24	7	26
SS3	4	38	34	22	12	30
SS4	9	98	93	50	5	39
SS5	0	55	40	27	3	25
SS6	2	66	72	24	14	45
SS7	2	108	111	27	4	20
SS8	3	53	24	48	3	29
SS9	12	289	530	66	6	18
WEY1	0	6	9	21	10	12
WEY2	0	7	3	9	2	2
WEY3	1	8	14	9	5	7
WEY4	3	39	38	17	5	4
WEY5	0	28	35	8	2	2
WEY6	0	31	19	7	7	19
WEY7	0	10	21	11	2	8
WEY8	1	58	40	21	3	4
WEY9	0	30	21	7	0	1
WV1	0	12	14	5	5	12
WV2	0	11	7	5	5	8
WV3	5	45	117	24	10	15
WV4	1	19	27	9	4	21
WV5	1	46	47	17	9	12
WV6	8	104	255	51	13	10
WV7	6	234	238	19	5	6
WV8	1	104	132	8	0	7
WV9	0	76	97	16	4	8

Table 3.5 Means of the percentage of fungal isolation from pre-treatment root samples per study sites.

Study Site	*L. procerum*	*L. terebrantis*	*G. alacris*	*G. huntii*	*O. ips*
SS	6	6	0	1	1
RAY	15	4	3	5	0
F&W	12	4	1	12	0
WEY	20	2	12	7	1
WV	24	15	5	6	6

Table 3.6 Tukey's Studentized Range (HSD) test for means of transformed percentage of fungal isolation from pre-thinning treatment root samples among study sites.

Study Site	*L. procerum*	*L. terebrantis*	*G. alacris*	*G. huntii*	*O. ips*
SS	-0.62a	-0.09ab	-0.41a	-0.43a	-0.06ab
RAY	0.09a	-0.18ab	-0.06a	-0.07a	-0.24b
F&W	-0.10a	-0.12ab	-0.26a	0.25a	-0.24b
WEY	0.19a	-0.39b	0.33a	0.15a	-0.06ab
WV	0.44a	0.78a	0.39a	0.12a	0.61a

Note: mean values with different letters within a column indicate significant difference within the species.

Table 3.7 Summary statistics for Tukey's Studentized Range (HSD) test for means of transformed percentage of ophiostomatoid fungal isolation among age class from pre- thinning treatment root samples.

	Age Class (yr)			
Fungi Species	**10-19**	**20-29**	**30-40**	**>40**
L. procerum	0.23a	-0.45a	-0.26a	0.80a
L. terebrantis	-0.04a	0.09a	-0.42a	0.59a
L. alacris	0.27a	-0.31a	-0.41a	0.04a
G. huntii	0.17a	-0.13a	-0.57a	0.23a
O. ips	0.06b	-0.24b	-0.24b	1.10a

Table 3.8 Summary statistics for Tukey's Studentized Range (HSD) test for means of transformed percentage of ophiostomatoid fungal isolation among slope class from pre- thinning treatment root samples.

	Slope Class (%)			
Fungi Species	**1-5**	**6-10**	**11-15**	**>15**
L. procerum	-0.11a	0.003a	0.38a	-0.01a
L. terebrantis	-0.24ab	-0.04ab	-0.42b	0.58a
L. alacris	-0.15a	0.004a	0.48a	0.002a
G. huntii	-0.22a	0.21a	0.32a	-0.07a
O. ips	0.01a	-0.12a	-0.24a	0.23a

Note: mean values with different letters within a row indicate significant difference within the species.

Table 3.9 Summary statistics for Tukey's Studentized Range (HSD) test for means of transformed percentage ophiostomatoid fungal isolation among aspect class from pre-thinning treatment root samples.

	Aspect Class (°)			
Fungi Species	**minimum**	**low**	**moderate**	**high**
L. procerum	0.19a	0.09a	-0.25a	-0.25a
L. terebrantis	0.01a	-0.004a	-0.05a	0.01a
L. alacris	0.11a	0.03a	-0.07a	-0.14a
G. huntii	0.07a	0.18a	-0.57a	-0.12a
O. ips	0.16a	0.11a	-0.24a	-0.24a

Note: mean values with different letters within a row indicate significant difference within the species.

Table 3.10 Pearson correlation between ophiostomatoid fungal isolation and mean insect captures per plot from pre-thinning treatment collections.

		BTB	**HPO**	**HS**	**HT**	**PP**	**HP**
L. procerum	r	-0.1731	0.0148	-0.0083	-0.2980	-0.1550	-0.2429
	P	0.2555	0.9238	0.9569	0.0468	0.3092	0.1080
L. tenuis	r	0.0694	0.1890	0.2250	-0.0016	0.0295	0.1425
	P	0.6506	0.2137	0.1372	0.9917	0.8477	0.3503
G. alacris	r	-0.1512	-0.2271	-0.1594	-0.2206	-0.0998	-0.2291
	P	0.3215	0.1335	0.2956	0.1454	0.5142	0.1301
G. huntii	r	-0.1104	-0.0383	-0.0801	-0.0418	0.0281	-0.0132
	P	0.4704	0.8029	0.6010	0.7850	0.8549	0.9317
O. ips	r	0.1839	0.4646	0.3907	0.1061	-0.0669	-0.0616
	P	0.2266	0.0013	0.0080	0.4880	0.6624	0.6879

$P \leq 0.05$ indicates significant correlation; n=45; BTB = *D.terebrans*; HPO = *H. porculus*; HS = *H. salebrosus*; HT = *H. tenuis*; PP = *P.picivorus*; HP = *Hb. pales*.

Table 3.11 Pearson correlation between the percentage of ophiostomatoid fungal isolation and mean crown variables per plot from pre-thinning treatment collections.

		CR	CL	CD	FT
L. procerum	r	0.0279	-0.0121	-0.0930	0.0102
	P	0.8734	0.9451	0.5954	0.9533
L. terebrantis	r	0.0062	0.1410	-0.0119	-0.0737
	P	0.9718	0.4193	0.9461	0.6995
G. alacris	r	0.1430	-0.1793	0.1034	0.0867
	P	0.4126	0.3027	0.5542	0.6205
G. huntii	r	0.0348	-0.0602	0.0022	0.0428
	P	0.8426	0.7314	0.9901	0.8070
O. ips	r	0.2363	0.2780	0.0447	-0.0166
	P	0.1717	0.1059	0.7986	0.9244

$P \leq 0.05$ indicates significant correlation; n=35; CR = crown ration; CL = crown light; CD = crown density; FT = foliage transparency.

Table 3.12 Interaction of treatment variable and time variable effects on ophiostomatoid species by Two-Way ANOVA.

Insect Species	Statistic results of treatment * time
WV	F = 6.07; P = 0.0185*
WEY	F = 14.33; P = 0.0014*
F&W	F = 7.38; P = 0.0108*
RAY	F = 7.50; P = 0.0104*
SS	F = 6.59; P = 0.0148*

Table 3.13 *P*-values produced from Tukey's Multiple Comparison test comparing treatment effects on means of ophiostomatoid fungal isolation from root samples.

	Treatment	
Study Sites	**Thinning**	**Control**
WV	0.0448 (+)	0.5319
WEY	0.0256 (+)	0.8385
F&W	0.0034 (+)	0.0742
RAY	0.0021 (+)	1.0000
SS	0.0451 (+)	0.8741

$P \leq 0.05$ indicates significant correlation; + indicates increasing response.

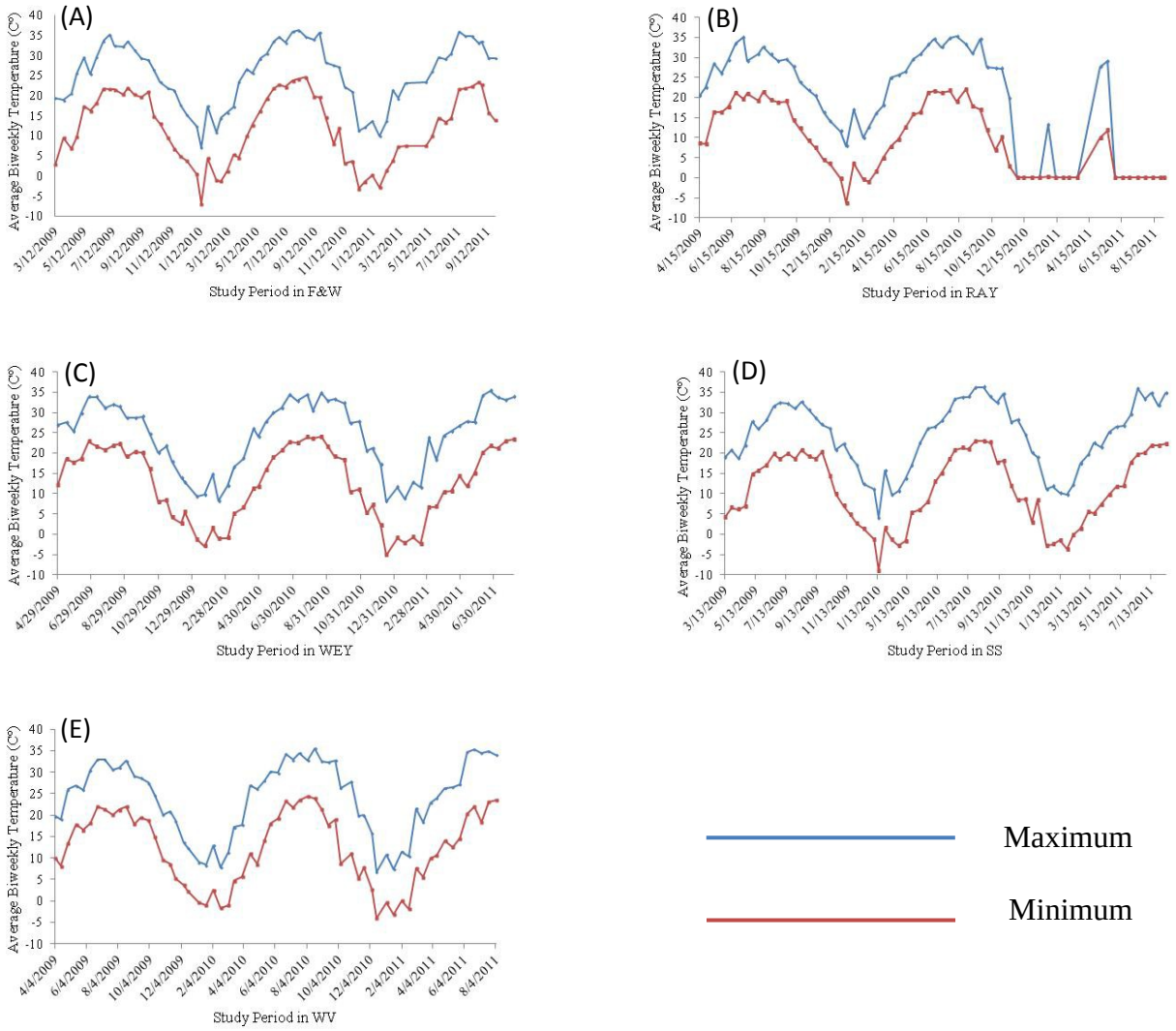

Fig. 3.1 Average biweekly maximum and minimum temperature in study sites. (A) Biweekly average temperature in F&W site. (B) Biweekly average temperature in RAY site. *Indicates no records from the weather station. (C) Biweekly average temperature in SS site. (D) Biweekly average temperature in WEY site. (E) Biweekly average temperature in WV site.

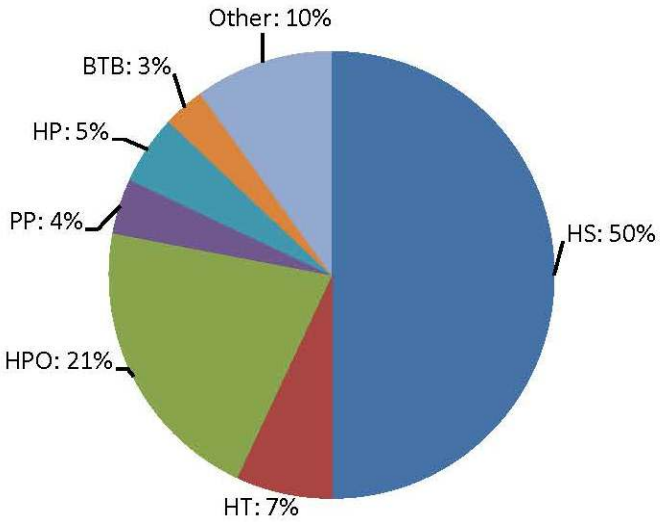

Fig. 3.2 Percentage of bark beetles and weevils captured in loblolly pine stands using pitfall, panel, and flight intercept traps in Alabama and Georgia (BTB-*D. terebrans*; HPO-*H. porculus*; HS-*H. salebrosus*; HT-*H. tenuis*; PP-*P. picivorus*; HP-*Hb. pales*. Other species included *D. frontalis*; *I. avulses*; *I. grandicollis*; *I. calligraphus*; *P. nemorensis*; *O. caelatus*).