Silvicultural Disturbances Affect on Root-feeding Bark Beetle Populations and the Incidence of Ophiostomatoid Fungal Species Contributing to Southern Pine Decline in *Pinus taeda* Stands

by

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Abstract

Root-feeding beetles and weevils are known to be vectors of ophiostomatoid fungi which contribute to Southern Pine Decline (SPD) in the southeastern United States. This study examined population changes of *Hylastes* spp. in response to either mechanical thinning or harvesting in *Pinus taeda* L. stands and the factors associated with the incidence of ophiostomatoid fungi. In addition, the study also quantified ophiostomatiod fungal response to mechanical thinning in central Alabama and Georgia. Three different insect traps were used during the two-and-half-year study. *Pinus taeda* roots were excavated and assayed for ophiostomatoid fungal infections from both pre- and post-treatments in thinned and control plots. Of the 46,865 total insects captured, 22,495 were *Hylastes* spp. Populations of the *Hylastes* spp. significantly increased after thinning treatments at study sites. Although *Hylastes* spp. decreased in response to harvesting in some plots, their populations recovered to pre-treatment levels and were stable over the study duration. The dominant fungus recovered was *Leptographium procerum* (Kendr.) Wingf. followed by other species including *L. terebrantis* Barras & Perry, *Grosmannia alacris* T.A. Doung, Z.W. de Beer & M.J. Wingf. sp. nov., *G. huntii* (Rob.-Jeffr.) Zipfel, Z.W. de Beer & M.J.Wingf., and *Ophiostoma ips* (Rumbold) Nannf. *Grosmannia alacris* and *O. ips* were recovered from tree roots in plots with severe decline symptoms. Sites with mechanical thinning had increased incidence of ophiostomatoid fungal species that may serve as a source to infest the remaining trees in the stand leading to SPD.

In general, thinning and harvesting are recommended as bark beetle management strategies. However, in the current study, recent mechanical thinning significantly increased pathogen-vectoring *Hylastes* spp. and ophiostomatoid fungi which contribute to SPD. Thus, future research should consider either how to thin or how to control the insect vectors to reduce possibility of SPD infestation in *P. taeda* stands.

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Chapter One

Introduction and Review of Literature

1.1 Tree and Forest Decline

"Decline" and "dieback" are terms used to describe a pathological symptom complex involving growth reductions, leaf size or number losses and twig and branch necrosis that sometimes leads to death of the entire trees (Manion and Lachance 1992). In the 1980's, more attention was gave to the status of forest health than ever before. This interest was fostered due to several dieback and decline situations in European and North American forests that were perceived as being unprecedented.

For example, in the Black Forest (Schwarzwald) of southern Germany, both Norway spruce [*Picea abies* (L.) H.Karst] and silver fir (*Abies alba* Mill.) displayed dramatic symptoms of crown thinning and needle yellowing because of drought and mineral nutrient deficiencies, as well as increasing air pollution (Bruck 1989, Krahl-Urban et al. 1988, Ke and Skelly 1990, Kandler and Miller 1991). In eastern North America, similar reports of declines emerged concerning high elevation spruce-fir [*P. rubens* Sarg. and *A. balsamea* (L.) Mill.] and sugar maple (*Acer saccharum* Marsh.), that was associated with road construction (Holmes 1961, Lacasse and Rich 1964), drought (Hibben 1962, Hibben 1966), and root freezing during winters with no snow cover (McLaughin et al.1985). Significant outbreaks of sugar maple decline and mortality have followed defoliation by a

variety of insects including a leafroller webworm complex in Wisconsin (Giese et al. 1964), the saddled prominent (*Heterocampa guttivitta* Walker) in New York and New England, and the forest tent caterpillar (*Malacosoma disstria* Hübner) in New York, New England and Canada (Allen 1987). Other examples include decline and mortality of northern red oak (*Quercus rubra* L.) that occurred on the Nantahala National Forest in North Carolina in the late 1970s on dry shaley soils (Tainter et al. 1984); several southern oak species (*Q. phellos* L., *Q.laurifolia* Michx., *Q. nigra* L. and *Q. falcata* Michx.) in South Carolina in 1980 and 1981 (Tainter et al. 1983); red pine (*Pinus resinosa* Ait.) decline associated with the root and lower stem infesting insects that vector *Leptographium terebrantis* Barras & Perry sp. nov. and *L. procerum* (Kendrick) M.J. Wingfield in Wisconsin, Michigan and Illinois in the 1970s (Klepzig et al. 1991); littleleaf disease (*Phytophthora cinnamomi* Rands) of shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) on poorly drained soils with clay hardpans; Eucalypts (*Eucalyptus* spp.) decline and dieback in the late 20th century throughout Australia (Day 1981, Wylie et al. 1993, Keane et al. 2000).

Manion (1991) reviewed tree decline in North America, and identified some indicators related to decline in the area. For example, site condition and climate are predisposing or inciting factors. Biotic factors include fungi and insects that usually contribute to decline. Additionally, he also reported that declines occur as trees approach maturity. Five theories have been proposed to explain tree and forest decline: germ theory,

climatic impacts, cohort senescence or natural succession, human impacts and complex interactions of factors (Manion 1991).

1.1.1 Germ Theory

According to the germ theory, dieback is caused by a single agent. A classical example to support germ theory is chestnut blight [*Cryphonectria parasitica* (Murrill) M.E. Barr]. It was an introduced canker disease from Asia to North America circa 1900 which eliminated most American chestnut [*Castanea dentata* (Marsh.) Borkh.] trees (Anagnostakis 1987). Most introduced pests or pathogens associated with tree dieback or decline can be explained by this theory. However, germ theory is sometimes controversial. For example, it is generally accepted that the native pathogen *P. cinnamomi* caused dieback of *Eucalyptus marginata* Donn *ex* Sm. in Western Australia, while factors as weather condition and site characters also first predisposed tree vigor and affected the distribution of the decline (Shear and Smith 2000). Therefore, trees are not susceptible to be damaged by native pathogens and pests unless they are stressed by an array of interacting factors such as drought, fire, fertilization, herbicides and competition with other plants (Manion 1991). *Armillaria mella* (Vahl: Fr.) Kummer is a root rot fungus found throughout the United States which has been involved in conifers and broad-leaved tree decline and dieback. Diebacks caused by *Armillaria* spp. are also attributed to competition, other pests, as well as climatic factors (Shaw and Roth 1978).

1.1.2 Climate and Weather Stress

The primary cause of diebacks and declines that have occurred throughout the World's forest's since the 1940s have been considered to be climate and weather stress induced (Hawboldt and Skolko 1948). Some researchers indicate that global climate change is the primary factor causing dieback and decline by inducing cavitations and reducing water potential in trees around the world (Wardlaw 1990, Auclair et al. 1990). For example, dieback and mortality of yellow birch (*Betula alleghaniensis* Britton), red spruce (*P. rubens*), European silver fir (*A. alba*), and Norway spruce [*P. abies* (L.) H. Karst.] have been linked to changes in climate (Becker et al. 1989, Johnson et al. 1986, Redmond 1955). A classic example for climate change theory is the severe dieback and mortality of balsam fir [*A. balsamea* (L.) Mill] which occurred in 1954 within the northern hardwoods. This case coincided with the extreme mean temperature in 1954 (Redmond and Reid 1961). Sudden freeze in cold weather blocked water transportation which further lead to chronic injury to the xylem and caused crown dieback in the dry years. When warmer weather coincides with low soil moisture availability, xylem tension may be exceeded. Then a vapor bubble containing air and water is formed in the xylem (canivation) and further blocks water movement upward from below.

1.1.3 Cohort senescence or natural succession

Mueller-Dombois (1982) and Wardle et al. (2004) proposed the “succession and cohort senescence” theory for explaining declining forests around the world. They reported that nutrition deficiency is an important predisposing factor. They also suggested that canopy decline is due to the interaction of aging and environmental disturbance. Mueller-Dombois’s nutrient study (1983) showed that nutrient imbalances are contributors to predispose *Metrosideros polymorpha* Gaudich. to be more stressful in the Montane rain forest ecosystem. The year-round high precipitation level promoted soil acidification, which leaded to alumimum and manganese and iron toxicities in poorly drained soils. In addition, the immobilization of phosphorus could also lead to productivity decline. In another study, wild fires which are part of natural disturbance regimes are considered to cause dead or dying trees in the boreal forest of North America (Heinselman 1981). However, natural succession does not occur in eucalypt forest because eucalypts always remain the dominant species in enviroment and their lifespan is measured in centuries although eucalypts may be affected by decline from the age of 20 to 30 years (Burrows et al. 1995, Hickey et al. 1999).

1.1.4 Human impacts and complex interactions of factors

Human activities such as construction, logging, recreation, and agricultural actions may be factors which impact forest decline. For example, intensive agricultural practices

have been shown to cause more severe Eucalyptus decline (Landsberg et al. 1990, Farrow 1999), and logging damage would result in birch dieback. When logging happens in birch stands, soil and air temperature are increased due to more open areas which incites birch dieback (Manion 1991). In addition, some declines of *E. obloqua* L'Hér. have been shown to be caused by nutrient depletion and soil erosion (Florence 1996). In the Central European forests, harvesting had severe impacts on forest ecosystems because stands were depleted of neutralizing capacity and nutrients (Gerhard 1991). He also noted that excessive biomass harvesting led to nitrogen and acid neutralizing capacity of the ecosystems to be depleted.

In general, all five decline theories have limitations and use different terms, limitations, key factors, models, and applications for their interpretations of forest decline, yet they all involve a number of interacting factors. In recent years, it is generally accepted that decline and dieback of trees can be attributed to the interactions of a number of abiotic and biotic factors. This theory is called the “theory of complex interactions of factors”, which causes stress within the individual tree over some indefinite period of time. Abiotic and biotic factors include pathogens, insects, climatic factors, agricultural and other human activities. Manion (1991) proposed a number of factors associated with tree declines in North America that predispose, incite or contribute to tree declines. His theory includes: (1) climate, air pollution, unsuitable soil and site conditions, tree age and the genetic potential as predisposing factors to tree

decline; (2) insect defoliators, frost, drought and air pollutants are incitants which have a short duration and can accentuate predisposed trees; (3) fungi, bark beetles, and viruses are considered as contributors which cause tree decline or death. In general, predisposing factors put permanent stress on trees and decrease tree vigor which in turn will attract incitants and contributing factors. Manion also identified some common denominators to describe tree decline: (1) at least one factor from each group (predisposing, inciting, and conributing) should be involved in a decline; (2) site and climate factors are always major predisposing or inciting factors; (3) fungi, insects, and viruses are often contributors; (4) feeder root and mycorrhizae degenerate before aboveground symptoms.

1.2 Loblolly Pine Decline and Associated Factors

1.2.1 Loblolly Pine (*P. taeda* L.)

Loblolly pine, also known as North Carolina pine, Bull pine and Old-field pine (Moore et al. 2008), is a native pine species to the southern United States. Its range extends through 14 states from southern New Jersey to central Florida and west to Texas. Loblolly pine responds well to different management treatments in even-aged and uneven-aged natural stands as well as plantations. Because loblolly pine is an adaptable species, it has been successfully introduced to other continents (Schultz 1997). The growth rate is fast and the yellowish, resinous wood is highly prized for lumber.

Fig.1.1. Loblolly Pine

Photo by Woodlot

1.2.2 Damaging Agents of Loblolly Pine

Agents which cause periodic damage to loblolly pine trees and stands include wind, lightning, extreme temperature, ice, drought, flooding, insects, and disease. Large dominant trees usually are more vulnerable to high winds compared to smaller ones, and windthrow is most common on shallow soils with coarse-textured profiles. Wind damage is also more likely to occur in recently thinned stands (Fowells 1965, Trousdell et al. 1965). Large, open-grown loblolly pine are generally the most vulnerable to lightning. Damage or seedling mortality often caused by drought and extremely high or low freezing temperatures, because heat and drought cause trees to lose vigor which can lead to more insect and disease infestations.

Insect pests cause a lot of losses of loblolly pine trees. For example, pine engraver beetles (*Ips* spp.) can cause death of trees; pine tip moths (*Rhyacionia* spp.) often attack

young trees; regeneration weevils (*Hylobius* spp. and *Pachylobius* spp.) contribute to girdling and death of young seedlings up to 13 mm in diameter. Bark beetles are the most serious insect pests to loblolly pine, particularly the southern pine beetle (SPB) (*Dendroctonus frontalis* Zimmermann) that is the most destructive pest of pines throughout the South (Thatcher et al. 1980). All species of southern pines are susceptible to attack during SPB outbreaks, but more loblolly pines were killed than any other species in its range. From 1999 to 2003, SPB caused unprecedented damage in several states including Alabama, Florida, Georgia, Kentucky, North Carolina, South Carolina and Tennessee. These attacks roughly coincide with the distribution of loblolly pine (Thatcher and Barry 1982). According to the SPB Prevention and Restoration Program that initiated by the USDA Forest Service and the Southern Group of State Foresters, more than 1 million acres on National Forests, private properties, industry, and state and other federal lands were affected by SPB from 1999 to 2003.

Diseases associated with loblolly pine include root rot (*Heterobasidion irregular* Otrosina & Garbelotto) [formerly *H. annosum* (Fr.) Bref.] and fusiform rust [*Cronartium quercuum f. sp. Fusiforme* (Hedgc. & N. Hunt) Burdsall & G. Snow]. Saplings and older trees, especially if planted, are attacked by *H. irregular* in some stands where cutting has taken place. Fusiform rust is the most serious stem disease, and it kills and disfigures loblolly and slash pines (*Pinus elliottii* Engelm.) throughout their range.

1.2.3 Loblolly Pine Decline

Loblolly pine decline (LPD) was first reported in the southeastern United States in the Oakmulgee Ranger District on the Talladega National Forest (TNF) in 1959 (Brown and McDowell 1968). Symptoms of LPD include thinning and yellowing crowns, fine root deterioration and reduced radial growth in the age class 40 to 50 years. Hess et al. (1999) reported that loblolly pine mortality would occur two to three years following decline symptoms. Other areas of central Alabama including National Forest lands in Anniston and Heflin, and Tuscaloosa and Bibb Counties have reported LPD (Hess 1997, Allen 1994). This problem also occurs from the Piney Woods of Texas, eastern Mississippi to central Alabama, and Georgia to South Carolina and North Carolina (Menard and Eckhardt, unpublished data).

Before the 1970s, the agents causing LPD were debatable. In order to determine the cause, decline rates, and degree of mortality of loblolly pine stands, a five-year study in the TNF was established on TNF in 1966 (Brown and Macdowell 1968). While, the results of this study did not find a specific pathogen causing the decline, it did indicate that lateral and fine root deterioration present in the stand occurred prior to the appearence of either *H. irregular* or *P. cinnamomi*. Although these two root diseases were observed in some plots, they were not considered to be the primary contributor to LPD. Symptoms of LPD appeared when pines reached 40-50 years. Further evaluations were concluded in 1976, and the results indicated reductions in loblolly pine growth by age 50.

Sites with sandy or moderately to well-drained soils and other interactions as soil chemical characteristics were the cause of the stand decline and tree mortality (Loomis 1976).

In the early 1990s, Ostrosina et al. (1997) initiated a forty-paired plot study to look at blue-stain fungi associated with SPB attack in southern pine stands from eastern Texas to Alabama. Plots were established in SPB-attacked pine stands and control plots (without SPB) located at the north edge of the SPB plot. The study showed that 50% of the SPB attacked trees had *L. terebrantis*, *L. procerum* and *Ophiostoma ips* (Rumb.) Nannf. contamination in their root systems and only 25% of the control trees (no SPB) had those three species ($P = 0.03$). Ostrosina's results suggested that *L. terebrantis*, *L. procerum* and *O. ips* were important pathogens in the dynamics of susceptibility of southern pines to SPB attack.

In 1998, a study was established on four compartments including five stands with loblolly pine decline or dieback symptoms in loblolly pine stands in the Oakmulgee Ranger District in Alabama. Hess et al. (1999) identified *Pythium spp.* and *P. cinnamomi* from each plot, and *Leptograpium* spp. were recovered from 7 of the 15 plots. They suggested that *Pythium spp.* and *P. cinnamomi* were the primary cause of loblolly decline symptoms and mortality in five stands even though the fungi was only recovered from the soil and not the roots.

In 1999, a similar study was installed as part of the Forest Health Monitoring

program to evaluate soil, insect, and fungal parameters associated with declining loblolly pine stands (Eckhardt et al. 2007). According to the results, *Leptograpium* spp. were recovered from lateral root and soil samples, while *P. cinnamomi* was not recovered from roots but a few were recovered from soil samples. Three species of *Leptographium* spp. were isolated. They were *L. procerum*, *L. terebrantis* and *Grosmannia alacris* T.A. Doung, Z.W. de Beer & M.J. Wingf. sp. nov. [formerly *L. serpens* (Goid.) Siemaszko]. Root feeders such as *Hylastes salebrosus* Eichhoff, *H. tenuis* Eichhoff, *Pachylobius picivorus* Germar and *Hylobius pales* Herbst were the dominant insect species. A positive relationship was shown between those insects and a higher incidence of *Leptographium* spp. (Eckhardt et al. 2007). A further study (Eckhardt and Menard 2008) was established to measure site topographic features with LPD in central Alabama. It was reported that loblolly pine were more prone to show decline symptoms on steeper slopes and in stands with SE/S/SW aspects.

Fig.1.2. Thinning and yellowing crowns of loblolly pine
Photo by James Johnson

1.2.3.1 Abiotic Factors

Although LPD occurs among all soil types, loblolly pine planted in predominately loam, sandy loam or sandy clay loam are quite susceptible (Eckhardt et al. 2007). In addition, trees older than 40 years, aspect and convexity, increased slope and organic matter content in the soil are also associated with pine decline. Eckhardt and Menard (2008) reported that symptoms of LPD were more often observed on areas which had greater slope with a southern aspect. Similar results have been reported in sugar maple decline (Horsley et al. 2000, Drohan et al. 2002) and Chilean cedar [*Austrocedrus chilensis* (D. Don) Pic. Serm. & M.P. Bizzarri] decline (Baccala et al. 1998). Declining plots of sugar maple were found more often at higher elevations and at S/SW and W/NW aspects. With increasing of slope, dead sugar maple basal area increased (Horsley et al. 2000, Drohan et al. 2002). Baccala et al. (1998) found that declining Chilean cedar stands were associated with sites having low precipitation and higher altitudes because slope and precipitation are important to determine the soil water availability.

Site management history is another contributor to the occurrence of LPD. Factors such as recent prescribed burns, past agricultural practices, and lower vegetation density correspond to pine decline. Drought or storm damage are also significant factors relating to pine decline (Gill 1992). Soil and root disturbance caused by silvicultural treatments can incite decline. For example, thinning effects may directly cause physical injury and

stress of roots, or indirectly increase attractions of secondary pests such as root-feeding bark beetles (Eckhardt and Menard 2009).

1.2.3.2 Biotic Factors

1.2.3.2.1 Insect Associations

Bark beetles (Coleoptera: Curculionidae, Scolytinae), a large group consisting of approximately 550 species in North America, are considered to be important mortality agents in conifers. Several beetles are commonly associated with ophiostomatoid fungi such as *Leptographium* spp. and *Ophiostoma* spp. (Kendrick 1962, Wingfield and Gibbs 1991). Two hypotheses are established to explain the relationship between ophiostomatoid fungi and insects. The first hypotheses is that ophiostomatoid fungi are transported as a benefit resource to the insects (Lewis and Alexander 1986), those fungi then serve as a food source (Hinds 1972, Brand et al. 1976) for the insects or play some role in the development of the brood (Leach et al. 1934). Several species of *Ophiostoma* and *Leptographium* can be carried in the mycangia, a specific organ of their associated insect such as *Dendroctonus* spp. or exoskeleton (Barras and Perry 1971, Solheim 1995). The removal of these fungi can lead to a reduction in the number and development of the pine beetle brood (Barras and Perry 1971). Eckhardt et al. (2004a) had similar reports about the presence of ophiostomatoid fungi which would increase reproduction rates for their vectors *H. salebrosus* and *H. tenuis*. The second hypothesis is that the association of

the insects and the fungi is coincidental. The ophiostomatoid fungi would be considered "weeds" in the habitat of beetles (Harrington 1993) because the conidia of *Leptographium* spp. are sticky and adhere easily to the body surfaces of insects and therefore can be transported by the insects. Bark beetles associated with *Leptographium* mostly occur on conifers. These insects can be primary pests to attack and kill unhealthy hosts, or secondary pests that rarely kill their host trees (Paine et al. 1990). Several studies indicate that blue-stain fungi predispose trees to further attack by bark beetles (Kullhavy et al. 1984, Lieutier et al. 1989, Otrosina et al. 1997). Cobb et al. (1974) showed a high degree of association between root disease and species of *Dendroctonus* that infest trees.

Hylastes spp. considered nonaggressive, have been associated with ophiostomatoid fungi, such as *L. terebrantis*, *L. procerum* and *G. alacris* (Klepzig et al. 1991, Jacobs and Wingfield 2001, Eckhardt and Menard 2005, Eckhardt et al. 2007). In the southeastern United States, the most abundant species are *H. salebrosus* and *H. tenuis* (Eckhardt et al. 2007). Another species in this genus is *H. porculus* (Miller and Rabaglia 2009, Eckhardt et al. 2007). Those three *Hylastes* spp. are root phloem-feeding bark beetles that typically attack stressed pines and breed in roots and lower stumps.

Hylastes salebrosus (Fig. 1.3A) is approximately 3.3-5.0 mm long, 2.4-2.5 times as long as wide in both sexes. The color for this species is black. It has been observed throughout Texas east to Florida and north to New Jersey (Wood 1982).

Hylastes tenuis (Fig. 1.3B) is approximately 2.1-2.7 mm long in both sexes, and is about 3.0 times as long as wide. It is dark brown to almost black. The range of this species extends from Hidalgo, Mexico, north and east to New York State, and with rare exceptions, it is found exclusively on pines in roots and stumps within the range (Wood 1982).

Hylastes porculus (Fig. 1.3C) is approximately 3.8-5.0 mm long, and about 2.7 times as long as wide in both sexes. The color is black. Its range extends from Manitoba and New Brunswick to Texas and Florida (Wood 1982).

Fig.1.3. *Hylastes* spp. (A) *Hylastes salebrosus*, photo by Jeffrey W. Lotz. (B) *Hylastes tenuis*, photo by J.R. Baker & S.B. Bambara. (C) *Hylastes porculus*, photo by David T. Almquist.

Large numbers of *Hylastes* spp. can carry spores of blue-stain fungi to pine roots which would significantly reduce host vigor (Christiansen et al. 1987). Mycellia of blue-stain fungi can block the movement of water and nutrients further weakening the tree. Thus mass root-feeding bark beetle attacks may predispose trees to other pine bark beetle attacks.

Otrosina et al. (1997) and Hess et al. (1999) found that declining loblolly pine appear to be more vulnerable to be attacked by SPB than healthy trees in the southeastern United States, because *L. terebrantis* and *L. procerum* may predispose trees to further beetle attacks by decreasing tree defenses.

Stressed pine trees usually release chemicals as alpha-pinene. Miller and Rabaglia (2009) reported that funnel traps baited with (-)-alpha-pinene lures were attractive to *H. porculus, H. salebrosus* and *H.tenuis*. Ethanol enhanced responses of those three *Hylastes* spp. have also been shown to be attracted to traps baited with (-)-alpha-pinene in some locations. Those species are attracted to trees that are under stress from natural and or anthropogenic causes (Eckhardt et al. 2007). In addition, stand treatments could impact their population levels. Sullivan et al. (2003) reported populations of *H. salebrosus* and *H. tenuis* were greater in the first year post-burn treatment than controls.

Hylastes salebrosus and *H. tenuis* were reported to vector *L. terebrantis, L. procerum*, and *Grosmannia huntii* (Rob.-Jeffr.) Zipfel, Z.W. Beer & M.J. Wingf. and are associated with longleaf pine (*Pinus palustris* Mill.) decline (Otrosina et al. 2002, Zanzot et al. 2010).

1.2.3.2.2 Ophiostomatoid Species Associations

Root pathogens (*Leptographium* spp., *Grosmannia* spp., and *Ophiostoma* spp.) have been consistently found on sites exhibiting LPD in central Alabama (Hess et al. 1999,

Eckhardt et al. 2007). During the past few decades, several *Leptographium* spp. have become recognized internationally as pathogens of conifers or as agents of blue-stain in timber. For example, the best known pathogenic species are the three varieties of *L. wageneri* (W.B. Kendr.) M.J. Wingf. which are responsible for black-stain root disease of conifers in the western Unites States (Wagener and Mielke 1961; Harrington 1993). *Leptographium procerum*, *L. terebrantis*, *G. alacris*, *L. truncatum* (M.J. Wingf. & Marasas) M.J. Wingf. (formerly as *L.lundbergii)*, and *G. huntii* have recently been isolated from roots and soil near loblolly pine trees that showed decline symptoms in the southern United States (Eckhardt et al. 2007, Jacobs and Wingfield 2001, Zanzot et al. 2010).

Leptographium procerum (Fig. 1.4A) can be recognized by its characteristic forming of dark concentric rings on the surface of agar where it has been cultured. It is consistently associated with white pine (*P. strobus* L.) root decline and with symptoms of decreased shoot growth, delayed bud break, and needle wilt in the northeastern United States (Kendrick 1962, Wingfield et al. 1988). Resin is observed at the root collar of infested white pine trees and *L. procerum* has the ability to colonize resin-soaked woody tissue (Horner and Alexander 1985). The fungus has been isolated from sand pine [*P. clausa* (Chapm. *ex* Engelm.) Vasey *ex* Sarg.], slash pine and declining loblolly pine (Barnard et al. 1985, Barnard et al. 1993, Eckhardt et al. 2007). The pathogenicity of *L. procerum* has been extensively debated for many years. Lu et al. (2010) have suggested

that this fungus is pathogenic and can cause severe disease. It has also been reported that *L. procerum* isolated from red turpentine beetle (RTB) (*Dendroctonus valens* LeConte) in China caused larger lesions and mortality on Chinese pine (*P. tabuliformis* Carrière) seedlings than other fungal isolates such as *L. terebrantis* and *L. procerum* from the United States. In other cases, *L. procerum* was found to be weakly pathogenic and unable to kill wounded or unwounded host trees compared to *L. terebrantis* and *G. alacris* (Wingfield et al. 1988, Eckhardt et al. 2004b, Matusick 2010). *Hylobious pales* and *Pissodes nemorensis* Germar were the main vectors of *L. procerum*. Both species were reported to transmit *L. procerum* to eastern white pine seedlings and 5-year-old eastern white pine (Nevill and Alexander 1992). In addition, transmission of *L. procerum* was observed to the next generation of *Hb. pales* and *P. nemorensis* during their ovipositions on white pine seedling. Another study showed that *L. procerum* was isolated from 30% of *H. salebrosus*, 25% of *H. tenuis*, and 14% of *P. picivorus* collected from loblolly pine decline stands (Eckhardt et al. 2007).

Conidiphore color of *L. terebrantis* (Fig. 1.4B) is yellow to light green. The fungus can cause phloem lesions and has induced resin-soaking of the xylem to wound-inoculated seedlings and mature trees (Harrington et al. 1983, Rane and Tattar 1987). Like *L. procerum*, infestations of *L. terebrantis* will increase crown symptom severity and resinous lesions in longleaf pine stands which exhibit various stages of decline in South Carolina. Although the fungus has never been considered a primary cause of tree

disease, it is moderate to highly pathogenic. Wingfield (1986) and Eckhardt et al. (2004b) showed that inoculation of *L. terebrantis* could kill both *P. strobus* and *P. taeda* seedlings and cause larger lesion development when compared to *L. procerum*. In addition, *L. terebrantis* is the only fungal species that is pathogenic to *P. thunbergiana* Mikawa and *P. sylvestris* L. seedlings compared to *L. procerum* and *O. ips* (Rane and Tattar 1987). *Leptographium terebrantis* is a common blue-stain fungus which is associated with a wide range of bark beetles, particularly black turpentine beetle (BTB) (*D. terebrans* Olivier*)* (Barras and Perry 1971), RTB (Wingfield 1983) and *Hylurgops porosus* LeConte (Harrington and Cobb 1983). Rane and Tattar (1987) reported *L. terebrantis* was responsible for the blue sapwood discoloration near *D. terebrans* galleries in *P. thunbergiana* and *P. sylvestris*. Two root-feeding bark beetles (*H. salebrosus* and *H. tenuis*) and regeneration weevils (*Hb. pales* and *P. picivorus*) were reported to be associated with this fungus and apparently act as vectors (Eckhardt et al. 2004a). It also has been found that *L. terebrantis* has the ability to block water movement through stems (Owen et al. 1987, Paine 1984).

Unlike *L. procerum* and *L. terebrantis*, *G. alacris* (formerly *L. serpens*) (Fig. 1.4C) often grow serpentine-like hyphae (Kendric 1962). This fungus has been associated with a root disease of stone pine (*P. pinea* L.) in Italy (Lorenzini and Gambogi 1976). reported In south Africa, *Grosmannia alacris* was isolated from roots of dying *Pinus* spp. in infection centers (Wingfield and Knnox-Davies 1980). Within the United States, *G.*

alacris has been found in Christmas tree plantations (Nevil and Alexander 1992) and on *P. strobus* stands (Lacker and Alexander 1981). *Grosmannia alacris* was isolated from 42% of loblolly pine roots with thinning crowns in Alabama (Eckhardt et al. 2007). Since limited pathogenicity tests have been undertaken before the 1990s, Wingfield et al. (1988) concluded that the pathogenicity of *G. alacris* has not been conclusively established. However, Wingfield et al. (1988) pointed out the combined feeding activity of the insects and the subsequent colonization by the fungus may result in tree death. Wingfield and Knox-Davies (1980) reported that *G. alacris* produced 20 cm lesions after inoculation on root systems after six months. In contrast, Zhou et al. (2002) found it to be nonpathogenic to *Pinus* spp. branches in South Africa after inoculation because it produced lesions only between 1.5 and 3.7 cm. A similar result to Wingfield's study was reported by Eckhardt et al. (2004b). This pathogenicity test found average 3.0 cm lesion length developed on loblolly pine seedling stems after inoculation of *G. alacris* four months later. Although the lengths of lesions were different, the results still suggest *G. alacris* can grow successfully in *Pinus* spp. roots and it is pathogenic to various *Pinus* species. Matusick and Eckhardt (2010) found that longleaf pine seedling lesions and mortality caused by *G. alacris* were greater in wounded seedlings. However, average lesion and occlusion length caused by *G. alacris* were smaller in the second trial year which could indicate a reduction in virulence, while the amount of mortality and average lesion length on adequately watered seedlings suggests *G. alacris* is a mild to moderate pathogen to healthy longleaf pine seedlings. In addition, *G. alacris* has been found to be vectored by

insects. It was found transported consistently by *H. angustatus* Herbst (Wingfield et al. 1988), *H. ater* Erichson (Wingfield and Gibbs 1991), *H. linearis* Erichson (Wingfield and Knox-Davis 1980), *H. tenuis*, and *H. salebrosus* (Eckhardt et al. 2007).

Grosmannia huntii (Fig. 1.4D) [formerly *O. huntii* (Robins-Jeff) DeHoog & Scheffe] is less known compared to the other three *Leptographium* spp. discussed previously. The fungus has been recovered in British Columbia, New Zealand, England, Australia, and other areas of the United States including New York, Colorado, Oregon, Washington, Arizona and Georgia (Davidson and Robinson-Jeffrey 1965, Gibbs and Inman 1991, Jacobs and Wingfield 2001, Reay et al. 2002, Zanzot et al. 2010). Hosts of *G. huntii* include *P. ponderosa* Laws. (Davidson and Robinson-Jeffrey 1965), *P. sylvestris* (Gibbs and Inman 1991), *P. palustris (*Zanzot 2009), and *P.taeda* (Menard 2007). A variety of insect vectors have been found to transport *G. huntii*. Vectors include *D. ponderosae* Hopkins., *H. ater* Erichson, *Ips pini* Say (Jacobs and Wingfield 2001) and *Hylastes* spp. (Zanzot et al. 2010). In the early 2000s, the pathogenicity of *G. huntii* is still unknown; however, Matusick (2010) reported that lesions and occlusion length associated with *G. huntii* were longest in loblolly pine and slash pine seedlings when compared to lesions produced by *G. alacris, L. terebrantis* and *L. procerum*. *Grosmannia huntii* and *G. alacris* lesions were not significantly different in longleaf pine which is considered more resistant to other insect pests and disease (Snow et al. 1990). In addition, both lesion length and lesion area developed by *G. huntii* on mature *P. palustris* roots were longer

and larger when compared to *L. procerum*, *G. alacris*, and *L. terebrantis* (Matusick et al. 2010).

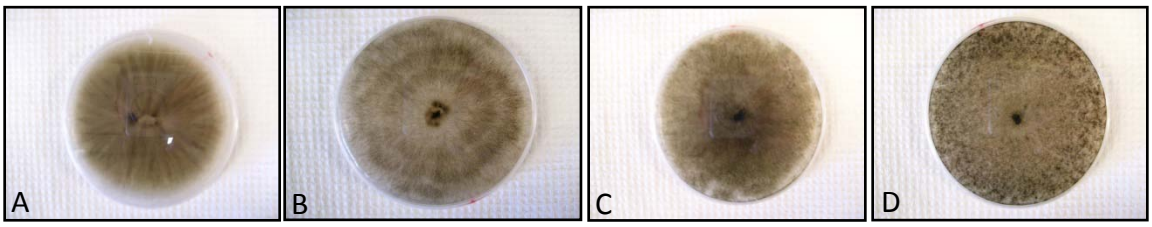

Fig.1.4. Ophiostomatoid fungi which contribute to SPD. (A) *Leptographium procerum* (B) *Leptographium terebrantis* (C) *Grosmannia alacris* (D) *Grosmannia huntii.*

1.3 Forest Managements's Affect on Insect and Fungus Populations

Forest management methods are used to enhance wildlife habitat, control disease and insects, and prepare sites for reforestation. Silvicultural treatments can result in changes in vegetation that can affect arthropod communities, stimulate water and nutrient fluxes, and increase tree growth (Wilson and Puettmann 2007, Thomas et al. 1999, Kremen et al. 1993, Schowalter 2006). Taki et al. (2010) reported that thinning positively affected some insect group species richness and abundance (Coleoptera, Diptera, Lepidoptera, and Hymenoptera) in a two year study period. Other studies reported the diversity and abundance of Coleoptera and Hymenoptera had increased in thinned Japanese cedar [*C. japonica* (L.f.) D. Don] stands compared to unthinned plots in central Japan (Maleque et al. 2007). Forest management methods also affect abundances of predators which can help control herbivore population (Schowater 2008).

Bark beetles are natural disturbance agents of conifer forests. For example, mountain pine beetle (*D. ponderosae* Hopkins) and SPB are two important species that cause a substantial loss in western and southern coniferous forests in the United States. Stand conditions have been consistently linked with bark beetle infestations in conifers (Fettig et al. 2007). Silviculture methods including thinning, prescribed burning, patch cutting, and stand regeneration are used to prevent bark beetle infestations (Fettig et al. 2007). Among those treatments, thinning, patch cutting, and prescribed burning are the primary methods to mitigate pest problems by reducing host density (Ferrell 1996). In order to reduce the susceptibility of mountain pine beetle in ponderosa pine plantations, Kolb et al. (2007) suggested that thinning would increase tree vigor of remaining ponderosa pine by reducing competition. Fettig et al. (2007) and Schmid and Mata (2005) reported that partial cutting has reduced mountain pine beetle damage in ponderosa pine stands compared with untreated stands. Sartwell (1971) concluded that thinning reduced competition and increased tree vigor which further reduced stand susceptibility to mountain pine beetle attack. Thinning or patch cutting is also a management strategy to control SPB. Schowalter et al. (1981) reported that the probability of pine hosts being colonized by *D. frontalis* decreased from 14-17% to less than 4%. Larsson et al. (1983) and Mitchell et al. (1983) reported that if thinning decreased basal area in the range of 348-436 m^2/ha, it could prevent bark beetle outbreaks in pine forests, because thinning enhanced tree vigor by increasing light, water and nutrient availability to remaining trees.

Increasing distance between potential hosts and elevating temperature beyond insect species tolerance threshold are reasons for decreasing bark beetle attack.

However, it is controversial to predict the positive effect of those strategies because thinning and other strategies often damage residual trees, cause soil compaction, increase rate of windthrow, and increase the buildup of root disease caused by *H. irregulare* and *Armillaria* spp. (Ferrell 1996). Mechanical thinning and prescribed fire can influence the amount and distribution of bark beetles as well as provide infection potential for root pathogens (Ferrell 1996, Schwilk et al. 2006). A three-year study after thin and burn treatment in mixed-conifer stands by Maloney et al. (2008) showed that the number of bark beetles attacking trees was greater in burn plots compared with no-burn plots. Thinned plots had increased root disease (*A. gallica* and *H. irregulare)* and white pine blister rust (*Cronartium ribicola* J.C. Fisch.). The occurrence of root pathogens is increased in thinned stands because freshly cut stumps can be colonized by *H. irregulare* and some *Armillaria* spp. (Harrington 1993). Fettig and McKelvey (2010) found higher tree mortality was attributed to western pine beetle (*D. brevicomis* LeConte) and *D. ponderosae* in ponderosa pine, and fir engraver (*Scolytus ventralis* LeConte) in white fir [*A. concolor* (Gordon) Lindley *ex* Hildebrand] in prescribed fire treatment stands in the Black Mountain Experimental Forest, California.

Clearcutting is used as a reproduction method to mimick disturbance and increase primary successional species. Clearcutting also has been proven to be effective in

improving food resources for wild animal habitat and increasing water yields. However, clearcutting has several major negative impacts. It can cause soil erosion, poor species regrowth, increase risk of pest epidemics, decrease biodiversity, and loss of economic sustainability. Duchesne et al. (1999) used the Shannon-Weaver index and reported that carabid (Coleoptera: Carabidae) species richness and diversity tended to be higher on recent clear-cut plots in a boreal mixed-wood ecosystem than in mature or undisturbed plots in Ontario.

Campbell et al. (2008) reported species richness of Scolytinae was higher following anthropological disturbances such as thin plus burn plots and thin only treatments when compared to untreated controls in longleaf pine stands on the Coastal Plain of Alabama. For instance, *D. terebrans*, *Xyleborinus saxeseni* Ratzeburg, *Xyleborus* spp., and *H. tenuis*, increased numbers to treatments. Sullivan et al. (2003) reported that populations of root-feeding bark beetles *H. salebrosus* and *H. tenuis*, the ambrosia beetles *Xyleborus pubescens* Zimmermann, and the reproduction weevil *P. picivorus* were positively correlated with burn severity. Also, the study showed that *Hylastes* spp. and *P. picivorus* were found to be carrying spores of *Leptographium* spp. in or near the burned sites. Harrington et al. (1985) and Schweigkofler et al. (2005) reported that populations of *Hylastes* spp. and weevils which vector black-stain root disease, *L. wageneri* (W.B. Kendr.) M.J. Wingf., increased immediately following thinning.

1.4 Central Theme

The central theme of this thesis is to understand the response of root-feeding *Hylastes* spp. which vector ophiostomatoid fungi to forest trees in response to forest management. It is as important as the main stem beetles *D. frontalis* and *D. ponderosae* that cause significant tree mortality throughout the United States. Examing factors which predispose, incite and contribute to pine decline are necessary to develop planting and stand management options. These studies will examine the effects of standard pine management practices on the population levels of bark beetles that are known to carry root pathogens and on fluctuations in blue-stain fungi in an attempt to understand their role in loblolly pine decline.

Chapter Two

Thinning and Harvesting Effects on Root-feeding Bark Beetle Population Dynamics in *Pinus taeda* L. Plantations in Central Alabama and Georgia

2.1 Abstract

Root-feeding beetles, particularly *Hylastes* spp., *Hylobius pales* Herbst and *Pachylobius picivorus* Germar, are known to be vectors of *Grosmannia* spp. and *Leptographium* spp. which contribute to Southern Pine Decline (SPD) in the southeastern United States. This study examined population changes of root-feeding beetle in response to either mechanical thinning or harvesting in *P. taeda* stands in central Alabama and Georgia. Plots were established on five loblolly pine stands that were either thinned, harvested or control stands. Three different insect traps were used during the two-and-half-year study. All root-feeding bark beetles collected in the traps were identified. The most abundant root-feeding bark beetles were *Hylastes salebrosus* Eichhoff, *H. porculus* Erichson and *H. tenuis* Eichhoff. The number of *H. salebrosus* and *H. porculus* captured had peaks either in spring or fall, while the population of *H. tenuis* captured was erratic throughout the collection periods. Population of the *Hylastes* spp. significantly increased after thinning treatments at all five sites. Although *Hylastes* spp. decreased in

response to harvesting in some plots, their populations recovered and were stable over the studies duration.

2.2 Introduction

Bark beetles, such as the southern pine beetle (*Dendroctonus frontalis* Zimmerman), mountain pine beetle (*Dendroctonus ponderosae* Hopkins), and the European spruce bark beetle (*Ips typographus* Linnaeus) are major conifer pests in North America and Europe. Most bark beetles attack weakened or dying trees, but *D. ponerosae* and *D. frontalis* can attack and kill healthy hosts (Amman and Baker 1972, Hofstetter et al. 2006, Wermelinger 2004). Bark beetle species that result in significant economic losses to forest landowners tend to be studied more thoroughly. However, there are many forest pests that are poorly understood. For example, the root-feeding *Hylastes* spp. are bark beetles reported to typically attack weakened pines and vector ophiostomatoid fungi, such as *Grosmannia alacris* T.A. Doung, Z.W. de Beer & M.J. Wingf.. sp. nov., *Leptographium procerum* (Kendr.) Wingf. and *Leptographium terebrantis* Barras & Perry which contribute to southern pine decline (Klepzig et al. 1991, Jacobs and Wingfield 2001, Eckhardt and Menard 2005, Eckhardt et al. 2007).

In order to prevent bark beetle infestations and mitigate pest problems, silviculture treatments such as thinning, prescribed burning, and partial cutting are recommended to reduce insect populations (Ferrell 1996, Fettig et al. 2007). Most research exploring the relationship between management practices and insect infestations have only considered

the impact on a few important insect species such as *Dendroctonus* and *Ips*. For example, numerous studies suggest that thinning and partial cutting will reduce tree competition and accelerate growth rate of ponderosa pines, which reduced stand susceptibility to *D. ponderosae* attack compared to untreated stands in the western United States (Sartwell 1971, Schmid and Mata 2005, Fettig et al. 2007, Kolb et al. 2007). In the southeastern United States, thinning is also a management strategy to control *D. frontalis* outbreaks by maintaining pine basal area to 34 m^2/ha (Larsson et al. 1983, Mitchell et al. 1983). However, stand management practices can also increase beetle populations. Campbell et al. (2008) reported that species richness of Scolytinae (Coleoptera: Curculionidae) in longleaf pine (*Pinus palustris* Mill.) stands on the Coastal Plain of Alabama was higher following a thin plus burn when compared to untreated controls. Harvesting a forest stand is an effective method to create animal habitat and browsing areas. However, stand disturbance can have negative impacts such as soil erosion, poor quality re-growth, increased risk of pests, loss of biodiversity and economic sustainability. For example, species richness and diversity of carabid beetles (Coleoptera: Carabidae) was greater on recently clear-cut plots in a boreal mixed-wood ecosystem than in mature or undisturbed plots (Duchesne et al. 1999). Because bark beetle population responses' to common silvicultural disturbances is controversial, forest stand treatment consequences should be well understood prior to forest management implementation.

Southern pine forests were historically dominated by longleaf pine (*P. palustris*), a tree species which is tolerant to fire and resistant to bark beetles. However, forest stand

composition and densities of southern pine forests have changed primarily to loblolly pine (*P. taeda*), which is faster growing and more vulnerable to bark beetles (Baker 1972; Thatcher et al. 1980). In recent years, forest stands have begun to show decline symptoms from age 25, especially at sites with steeper slope and south/ southwest aspects (Eckhardt and Menard 2008). Once thought to be only associated with loblolly pine, other southern pine species have shown similar symptoms (Zanzot 2010, Matusick 2010). Through this association, loblolly pine decline is now refered as Southern Pine Decline (SPD). Management history is considered as an inciting factor in the occurrence of SPD (Menard et al. 2006, Menard 2007) because stand disturbance may be either directly responsible such as causing physical injury and stress, or indirectly resulting in the attraction of, or increasing the susceptibility to insects such as root-feeding bark beetles and weevils (*Hylastes* spp., *Hb. pales* and *P. picivorus)*. In this case, forest managers need a better understanding of the short- and long-term impacts of forestry practices on pine ecosystems.

Understanding the response of root-feeding bark beetles to forest management is just as important as the main stem beetles *D. frontalis* and *D. ponderosae* that cause significant tree mortality throughout the United States. An awareness of the biological relationships that predispose loblolly pine stands to stress and potential root-feeding beetle outbreaks are essential to develop preventative stand management options. These studies will examine the effects of standard pine management practices on the population levels of bark beetles that are known to carry root pathogens in an attempt to understand

their role in SPD.

2.3 Methods and Materials

2.3.1 Study Site and Plot Measurements

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 (Fig. 2.1). SS sites located in Tallapoosa County, AL with an area of 106 ha. RAY sites were established in Stewart County, GA with an area of 16 ha. WEY sites were chosen from loblolly pine plantations in Perry County, AL with an area of 71 ha. WV sites are in Pickens County, AL with an area of 39 ha. FW sites located in Cusseta County, GA with area of 19 ha. Within each of the study sites, 9 monitoring plots were established per US Forest Service, Forest Health Monitoring (FHM) guidelines (Dunn 1999) in January 2009. Plots were evenly divided among the three treatments: 1) thinned, 2) harvested, and 3) control (no stand activity). Within each treatment, 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) (Fig. 2.2). Latitude and longitude coordinates of center subplots were measured by using a GPS unit (Garmin GPSMAP 76Cx, Garmin International Inc., Olathe, KS). Plot conditions, including pine and hardwood basal area, slope inclination, slope aspect, and convexity of each plot were recorded from the center subplot before treatments occurred.

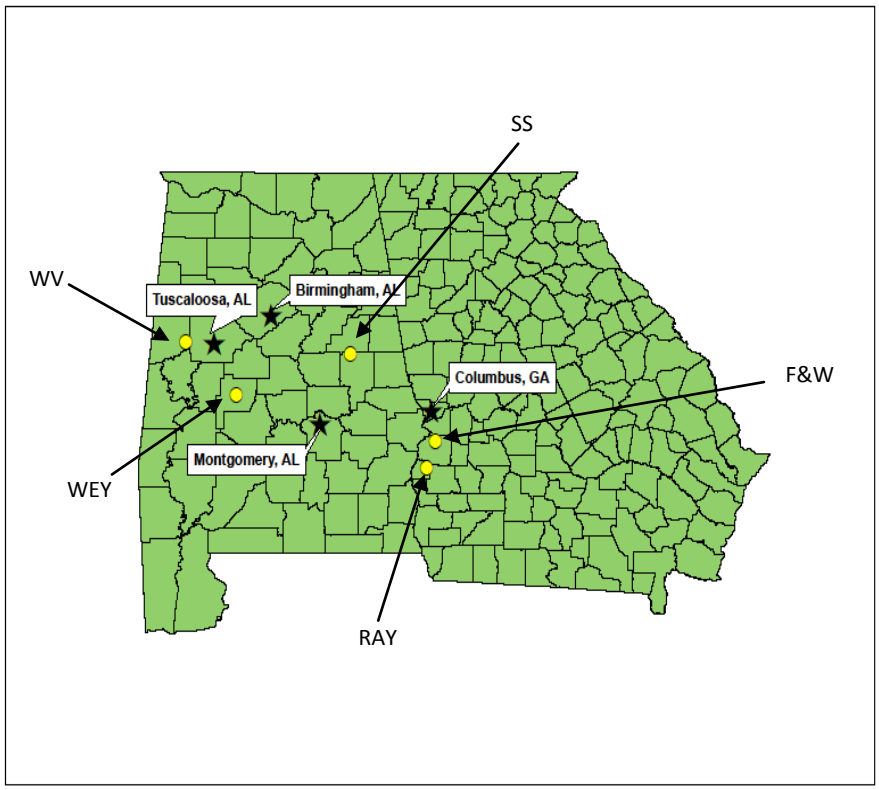

Fig. 2.1. Study locations in Alabama and Georgia.

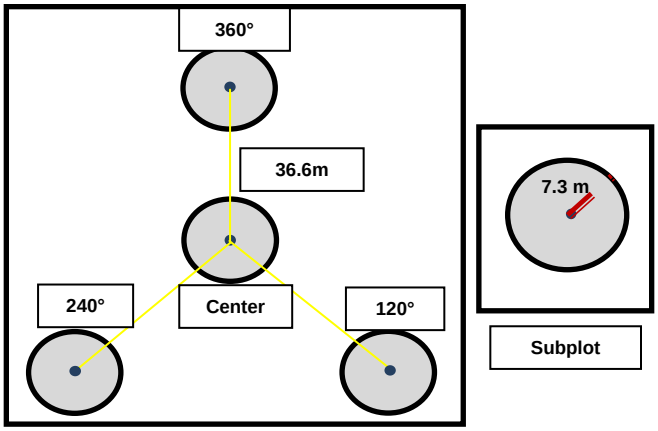

Fig. 2.2. Subplot layout at each treatment site.

The treatment timeline for each plot is presented in Table 2.1. The thinning method used in these studies was row thinning, which removes trees by row. Because of poor road conditions and access problems, plot 2 at study site WEY was not thinned. Plot 7 and plot 8 in SS study site were not harvested as planned.

Weather data was obtained 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. The average bi-weekly maximum and minimum was calculated from daily record.

Table 2.1. Treatment timeline in study sites.

Study Site	Thinning	Harvesting
SS	20 Nov 2009-24 Feb 2010 (Plot 2) 9 Oct 2010-17 Dec 2010 (Plot 1&3)	Febr 2010 (Plot 9)
RAY	19 Nov 2009-4 Dec 2009	19 Nov 2009-4 Dec 2009
F&W	NA	19 Nov 2009-29 Jan 2010
WV	21 Jul 2010-5 Aug 2010	9 Dec 2009- 22 Jan 2010
WEY	25 Jul 2010-10 Aug 2010 (Plot 1&3)	16 Dec 2009-28 Feb 2010

NA Indicates no treatment during collection years.

2.3.2 Insect Trapping

To monitor bark beetle population dynamics in the plots over time, three types of insect traps (pitfall trap, panel trap, and flight intercept trap) were placed in every center

subplot. Panel traps (APTIV Company, Portland, Oregon) (Fig. 2.3A) are made of black corrugated plastic, and designed to capture flying beetles. 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 (Fig. 2.3B) consisted of a 20-cm length of a 10-cm-diameter polyvinyl chloride plastic pipe with eight holes spaced equally around the circumference (Klepzig et al. 1991). Both ends of the pipe were capped with removable lids, and two holes were drilled in the bottom lid for drainage. The traps were buried into the soil/litter layer so that the entrance holes were slightly above the ground line. The interior of each trap was coated with a thin layer of liquid Teflon^{TM} (Northern Products Woonsockets, RI) to prevent the escape of insects captured between each collection period. Each pitfall trap was baited with two 3 cm long by 1 cm diameter loblolly pine twigs placed in the base of interior trap. Flight intercept traps (Fig. 2.3C) were made from plastic 3785 ml containers fitted with a 120 ml collection cup attached at the bottom. The trap was 1 m 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. Like that of the pitfall trap, two 3 cm long by 1 cm diameter loblolly pine twigs were placed in the collection cup. In addition to the pine twigs, 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.

Insect collection traps were monitored and sampled every 2 wk from March 2009 to September 2011. The traps were set in each of the plots and insects were collected one year prior to treatments to determine pre-treatment populations within each stand. During the thinning and harvesting periods, the insect traps were removed from the plots and then reinstalled upon completion. 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.

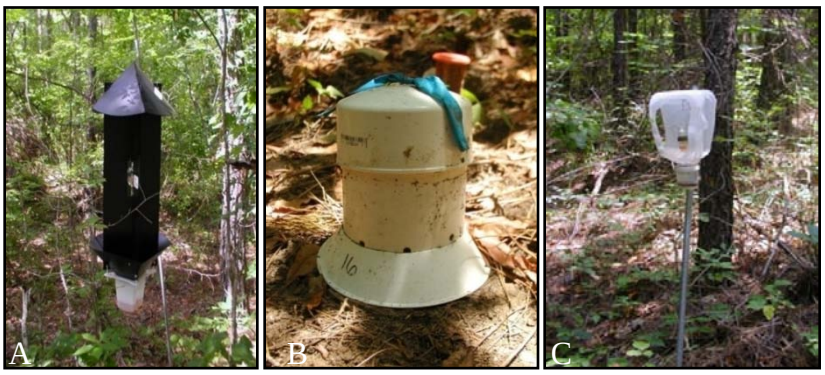

Fig. 2.3. (A) Panel trap (B) pitfall trap and (C) flight intercept trap placed at the center subplot to capture ground and flying insects.

2.3.3 Tree Measurements

All loblolly pine with DBH greater than 10 cm within a 7.3 m radius on each subplot were tagged and rated for tree health based on Forest Health Monitoring (FHM) procedures (Dunn 1999). Since 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; understoyry; 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.

In addition to crown condition, tree height and radial growth increment were collected from six trees randomly selected at center subplot. Increment cores were collected and returned to the Forest Health Dynamics Laboratory where 5-year and 10-year growth values were obtained using a digital (Mitutoyo Corporation, Maplewood, NJ) electronic ruler.

2.3.4 Stump Sampling

To assess insect gallery formation, brood levels and fungal populations and viability in roots on harvested trees, two lateral roots, greater than 2 cm dia, were collected from three stumps in harvested center plots. Roots were sampled every 3 months for one year post-treatment from September 2010 to October 2010 (stump samples in SS9 were collected in September 2011). Root sections from each stump were severed from the root system, labeled by site and treatment and then transported back to the laboratory for

measurements. After peeling root bark, *Hylastes* spp. feeding galleries, larvae, pupae, and adult of each species observed were record.

2.4 Data Analysis

Insects captured were identified and recorded by species bi-weekly over two and half years. Bi-weekly totals of *H. salebrosus, H. porculus,* and *H. tenuis* of pre-treatment (plots before thinning and harvesting, and plots for the first year control treatment were considered as pre-treatment plots) data were pooled by plot per site. In order to determine what variables had effects on root-feeding *Hylastes* spp., dummy variables of stand age class, live crown ratio class, live crown density class, crown sunlight exposure class, and season were created in SAS 9.2. Effects of those dummy variables on population of *Hylastes* spp. were analyzed using analysis of variance (ANOVA). Means of *Hylastes* spp. captured by plot weekly from pre-treatment data were analyzed using Tukey's Studentized Range test (PROC GLM; SAS 9.2) to compare means among classes. Four seasons were defined according to average temperature during the pre-treatment year, captures of *Hylastes* spp. were also compared among four season. In addition, Pearson Correlation Coefficients were used to determine the relationships among *Hylastes* spp. and *D. terebrans* and *Ips grandicollis*. The response of *Hylastes* spp. to the thinning and harvesting treatments were compared using ANOVA. Bi-weekly totals of *H. salebrosus, H. porculus,* and *H. tenuis* of both pre- and post-treatment data were pooled by treatment in each study site. Significant was determined using Tukey's Multiple Comparisons

Procedure (PROC GLM; SAS 9.2). All tests were analysized at the significant level of 0.05. Bi-weekly insect data from pre-treatment plots were pooled as well as data from post-treatment plots, the number were used to calculate diversity index (Shannon-Weaver Index; H' = - $\sum_{i=1}^{R} p_i \log p_i$) in Excel 2010.

2.5 Results

2.5.1 Description of Study Area

The plot conditions and crown rating parameters for all study plots are presented in Tables 2.1 and 2.2. The youngest plot was planted in 1998 and the oldest plot was 1959. Plots were distributed across percent slopes from 0% to 28% with variable aspects. Elevation ranged from 94 to 265 m above sea level. Pine basal area ranged from 4 to 16 m^2ha^{-1} (Table 2.2). Pre-treatment data of crown conditions (Table 2.3) showed that loblolly pine at SS plot 9 appeared to be more vigorous than other plots (Avg. DBH=9.7 in, Crown ratio=50, Crown density=40, Foliage transparency=30).

2.5.2 Relationship of *Hylastes* spp. and Stand Age and Crown Parameters

There was no correlation between the number of *Hylastes* spp. collected during the study and live foliage transparency within the stand (ANOVA; $F_{H.\ salebrosus} = 0.26$, $P_{H.\ salebrosus} = 0.7678$; $F_{H.\ porculus} = 0.26$, $P_{H.\ porculus} = 0.7709$; $F_{H.\ tenuis} = 0.36$, $P_{H.\ tenuis} = 0.6975$; df = 6, 28; Table 2.4). Even though there were no significant age effects on population of *H. salebrosus* (ANOVA; $F_{H.\ salebrosus} = 2.83$, $P_{H.\ salebrosus} = 0.0504$, df = 3, 41), *P. taeda*

stands in the 30-40 and >40 year age classes attracted more *H. salebrosus* than age classes of 10-19 and 20-29 years (Tukey's Studentized Range (HSD) test; Table 2.5). Stands in the >40 year age class had significantly higher numbers of *H. porculus* than all other stand ages examined. Stand age had no effect on the number of *H. tenuis* collected (ANOVA; $F_{H.\ tenuis} = 0.52$, $P_{H.\ tenuis} = 0.6677$, df = 3, 41; Table 2.5).

Table 2.2. Plot locations and pre-treatment site characteristics in Alabama and Georgia.

Plot	Location	Age	PBA (m^2ha^{-1})	TBA (m^2ha^{-1})	Elev (m)	SL (%)	Asp	LF	TP
WV 1	N 33.217 W87.891	16	16	17	121	22	N/NW	v	Ss
WV 2	N 33.214 W87.893	16	17	18	100	18	W	v	Ss
WV 3	N 33.211 W 87.895	16	15	15	124	16	N	v	Ss
WV 4	N 33.2057 W 87.949	19	14	16	107	14	NW	v	Ss
WV 5	N 33.2058 W 87.948	18	15	17	106	8	NW	c	Ss
WV 6	N 33.206 W 87.949	18	11	11	101	26	E/NE	v	Rt
WV 7	N 33.181 W 87.928	51	4	4	102	5	NE	v	Rt
WV 8	N 33.1814 W 87.927	52	4	4	114	9	E/NE	v	Rt
WV 9	N 33.191 W 87.904	51	7	10	113	28	SW	v	Ss
SS 1	N 33.087 W 85.879	18	15	16	247	19	E	v	Ts
SS 2	N 33.090 W 85.884	18	16	16	210	4	NW	c	Ts
SS 3	N 33.085 W 85.880	18	13	13	254	19	NW	v	Ns
SS 4	N 32.913 W 85.709	26	10	10	253	3	SE	v	Ns
SS 5	N 32.9126 W 85.699	26	12	13	245	4	E	v	Ts
SS 6	N 32.9119 W 85.695	26	12	14	239	3	NW	f	Rt
SS 7	N 32.9110 W 85.714	26	7	8	265	2	SW	f	Ts
SS 8	N 32.913 W 85.715	26	11	13	258	5	NE	c	Ts
SS 9	N 32.916 W 85.713	26	10	10	265	1	NW	f	Ss

(Continued)

Plot	Location	Age	PBA (m^2ha^{-1})	TBA (m^2ha^{-1})	Elev (m)	SL (%)	Asp	LF	TP
WEY 1	N 32.755 W 87.413	13	13	13	94	13	NW	v	Ts
WEY 2	N 32.750 W 87.4128	13	13	13	116	2	N	v	Rt
WEY 3	N 32.759 W 87.4121	13	14	15	93	13	W/SW	v	Rt
WEY 4	N 32.796 W 87.4357	28	9	10	121	30	SW	v	Ss
WEY 5	N 32.794 W 87.4353	28	7	10	127	6	W	v	Ss
WEY 6	N 32.743 W 87.401	13	13	14	131	3	N	v	Rt
WEY 7	N 32.655 W 87.280	30	7	8	106	6	W/SW	v	Rt
WEY 8	N 32.658 W 87.277	30	7	10	130	18	N/NW	v	Ss
WEY 9	N 32.661 W 87.276	30	9	10	131	10	N	v	Ss
FW 1	N32.1892 W 84.853	17	8	9	128	25	S/SW	v	Ss
FW 2	N 32.189 W 84.858	17	14	14	141	6	S/SW	v	Ss
FW 3	N 32.185 W 84.860	17	16	16	132	8	N/NW	v	Ss
FW 4	N 32.191 W 84.859	24	13	15	150	6	NW	v	Rt
FW 5	N 32.174 W 84.839	20	14	17	119	11	N/NE	v	Ts
FW 6	N 32.156 W 84.942	23	9	12	109	19	SE	v	Ss
FW 7	N 32.150 W 84.934	32	11	15	94	1	NA	f	Ss
FW 8	N 32.154 W 84.932	23	8	13	111	8	S/SE	v	Ss
FW 9	N 32.152 W 84.930	32	7	11	104	1	NA	f	Rt

(Continued)

Plot	Location	Age	PBA (m^2ha^{-1})	TBA (m^2ha^{-1})	Elev (m)	SL (%)	Asp	LF	TP
Ray 1	N 32.002 W 84.977	16	10	10	146	14	N/NW	v	Ss
Ray 2	N 31.997 W 84.860	18	13	15	123	4	E/NE	v	Rt
Ray 3	N 31.992 W 84.904	16	20	20	180	0	NA	f	Rt
Ray 4	N 32.014 W 84.970	16	9	9	159	8	SW	c	Ss
Ray 5	N 32.009 W 84.969	16	9	9	163	6	S/SW	f	Ss
Ray 6	N 31.992 W 84.866	18	19	19	137	1	NA	f	Rt
Ray 7	N 31.890 W 84.956	22	13	14	111	2	NW	f	Rt
Ray 8	N 31.893 W 84.950	22	13	14	123	8	SE	v	Ss
Ray 9	N 32.003 W 84.981	16	11	12	126	10	E/NE	v	Ss

PBA = pine basal area; TBA = total basal area; Elev = elevation; SL = slope; Asp = aspect; LF = ; v= convex; c = concave; f = flat; TP = topographic position; NA = no aspect; Ss = side-slope; Rt = ridge-top; and Ts = toe-slope.

Table 2.3. Mean values of pre-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
FW1	8.3	30	1	2	35	0	25	1.23	3.47
FW2	6.2	35	1	2	30	0	25	1.53	3.6
FW3	5.6	30	1	2	30	0	25	1.33	3.23
FW4	6.3	30	1	2	35	0	25	1.04	3.12
FW5	6.9	30	2	2	30	0	35	0.9	2.82
FW6	6.5	30	2	2	30	0	45	1.06	3.67
Ray1	6.5	35	1	2	30	0	30	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 = growth measurements didn't record during the experiment periods.

There was a significant correlation between live crown ratio and the population of *H. salebrosus* (ANOVA; $F_{H.\ salebrosus} = 7.47$, $P_{H.\ salebrosus} = 0.0025$, df = 6, 28). Stands that contained >35% live crown ratio had significantly more *H. salebrosus* captured than the other live crown ratio classes. Live crown ratio did not have a significant effect on the population of *H. porculus* and *H. tenuis* (ANOVA; $F_{H.porculus} = 2.39$, $P_{H.\ porculus} = 0.1102$; $F_{H.tenuis} = 0.27$, $P_{H.\ tenuis} = 0.7678$; df = 6, 28). However, live crown ratio lower than 30% had fewer *H. porculus* than live crown ratio >30%. Although there was no significant difference of *H. porculus* and *H. tenuis* captured among the live crown ratio classes examined, the mean numbers of *H. porculus* and *H. tenuis* were higher in stands with higher live crown ratios (Table 2.6). Loblolly pine stands with higher live crown density had more *H. porculus* captured than lower live crown density class (Table 2.7). There were no significant differences among live crown light class and populations of *Hylastes* spp.

Table 2.4 Summary statistics for Tukey's Studentized Range (HSD) test for means of *Hylastes* spp. captured among live crown transparency class in central Alabama and Georgia, March 2009 to March 2010.

Insect Species	**Live crown transparency class (%)**		
	<= 25	30-35	>35
H. salebrosus	4.0 a	3.2 a	2.0a
H. porculus	2.7 a	2.6 a	2.3 a
H. tenuis	1.0 a	0.9 a	0.9 a

Mean values with different letters within a row indicate significant difference within the species.

Table 2.5 Summary statistics for Tukey's Studentized Range (HSD) test for means of *Hylastes* spp. captured among stand age class in central Alabama and Georgia, March 2009 to March 2010.

Insect Species	Age class (yr)			
	10-19	20-29	30-40	>40
H. salebrosus	3.0 ab	4.0 ab	1.2 a	8.2 b
H. porculus	1.7 b	3.6 b	1.5 b	7.3 a
H. tenuis	0.8 a	1.0 a	0.8 a	0.7 a

Mean values with different letters within a row indicate significant difference within the species.

Table 2.6 Summary statistics for Tukey's Studentized Range (HSD) test for means of *Hylastes* spp. captured among live crown ratio class on *Hylastes* spp. in central Alabama and Georgia, March 2009 to March 2010.

Insect Species	Live crown ratio class (%)		
	<30	30-35	>35
H. salebrosus	2.4 b	2.7 b	8.5 a
H. porculus	1.1 b	2.3 ab	4.0 a
H. tenuis	0.5 a	0.9 a	1.2 a

Mean values with different letters within a row indicate significant difference within the species.

Table 2.7 Summary statistics for Tukey's Studentized Range (HSD) test for means of *Hylastes* spp. captured among live crown density class in central Alabama and Georgia, March 2009 to March 2010.

Insect Species	Live crown density class (%)		
	<30	30-39	40-45
H. salebrosus	1.5 a	3.3 a	5.7 a
H. porculus	0.9 b	2.2 ab	4.7 a
H. tenuis	0.6 a	0.9 a	1.1 a

Mean values with different letters within a row indicate significant difference within the species.

2.5.3 Insect Activity

A total of 46,865 beetles and weevils comprising 25 different insect species in 15 genera were captured from March 2009 to September 2011 (Fig. 2.4, 2.5). The most frequently captured insects were four species of scolytine bark beetles (*H. porculus*, *H. salebrosus*, *H. tenuis*, and *Ips grandicollis*), two species of molytine weevils (*Hb. pales* and *Pb. picivorus*) and four scolytine ambrosia beetles (*Gnathotrichus materiarius* Fitch, *Xyleborus pubescens* Zimmerman, *Xyleborinus saxesenii* Ratzeburg, *Xylosandrus crassiusculus* Motschulsky). Of all the insects collected, 48% were the root-feeding *Hylastes* spp. Other scolytines and curculionidae captured included *Dendroctonus terebrans* Oliver (n=799), *D. frontalis* (n=9), *I. avulsus* Eichhoff (n=195), *I. calligraphus* Germar (n=50), *Xylosandrus compactus* Eichhoff (n=212), *Monarthrum mali* Fitch (n=230), *M. fasciatum* Say (n=387), *Xyleborus atratus* Ecihhoff (n=230), *Xylosandrus germanus* Blandford (n=136), *Pissodes nemorensis* Germar (n=292), *Orthotomicus caelatus* Eichhoff (n=252), *Cnestus mutilatus* Blandford (formerly *Xylosandrus mutilatus*) Blandford (n=1518), *Xyleborus ferrugineus* Fabricius (n=134), *Trypodendron scabricollis* LeConte (n=221), *Pityborus comatus* Zimmerman (n=289), and *Dryoxylon onoharaensum* Murayama (n=196).

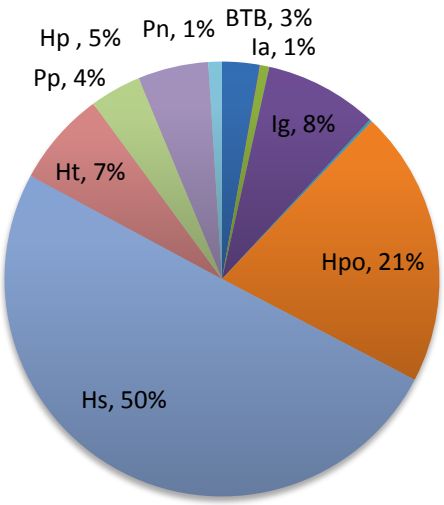

Fig. 2.4. Percentage of bark beetles and weevils captured in loblolly pine stands using pitfall, panel, and flight intercept traps, from 13 March 2009 to 29 September 2011 in Alabama and Georgia (BTB-*D. terebrans*; SPB-*D. frontalis*; Ia-*I. avulses*; Ig-*I. grandicollis*; Ic-*I. calligraphus*; Hpo-*H. porculus*; Hs-*H. salebrosus*; Ht-*H.tenuis*; Pp-*Pb. picivorus*; Hp-*Hb. pales*; Pn-*Pissodes nemorensis*; Oc-*O. caelatus*).

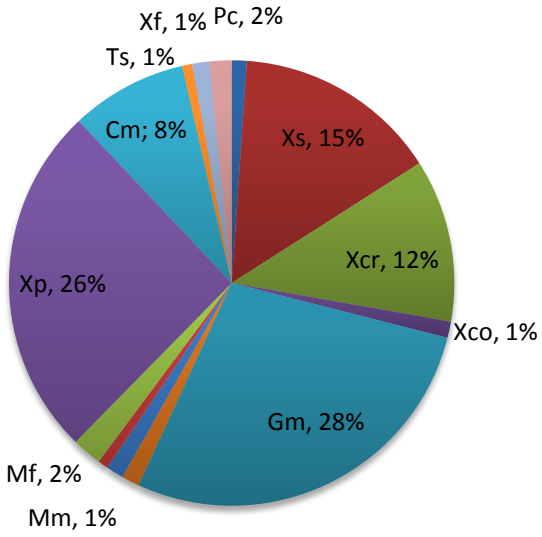

Fig. 2.5. Percentage of ambrosia beetles captured in loblolly pine stands using pitfall, panel and flight intercept traps, from 13 March 2009 to 29 September 2011 in Alabama and Georgia (Do- *Dryoxylon onoharaensum*; Xs- *Xyleborinus saxesenii*; Xcr- *Xylosandrus crassiusculus*; Xco- *Xylosandrus compactus*; Gm- *G.s materiarius*; Mm- *M. mali*; Xa- *Xyleborus atratus*; Xg- *Xylosandrus germanus*; Mf- *M. fasciatum*; Xp- *Xyleborus pubescens*; Cm- *C. mutilatus*; Xf- *Xyleborus ferrugineus*; Ts- *T. scabricollis*; Pc- *Pityborus comatus*).

2.5.3.1 Population Trends of *Hylastes* spp. and Seasonal Effects on Populations

During the two and a half year collection period, *H. salebrosus* was the most frequently captured insect (Fig. 2.4). Even though numbers of *Hylastes* spp. captures were different among sites (Table 2.8), the *Hylastes* spp. (Fig. 2.6) in SS site was representative of the insect populations captured at the other 4 study sites in Alabama and Georgia when looking at overall insect population trends. Season had a significant effect on the *Hylastes* spp. activity (ANOVA, $F_{H.\ salebrosus} = 10.68$, $P < 0.0001$; $F_{H.porculus} = 8.49$, $P < 0.0001$; $F_{H.tenuis} = 7.63$, $P < 0.0001$; df = 3, 133). Both *H. salebrosus* and *H. porculus*

peaked in spring, while only *H. porculus* had an additional peak in the fall. Unlike *H. salebrosus* and *H. porculus*, *H. tenuis* population fluctuated frequently over the growing season (Tukey's Studentized Range (HSD) test; Table 2.10). Fewer *Hylastes* spp. were captured during the winter and several collections of *H. salebrosus* and *H. tenuis* dropped to zero corresponding to a period of low temperature (Table 2.9).

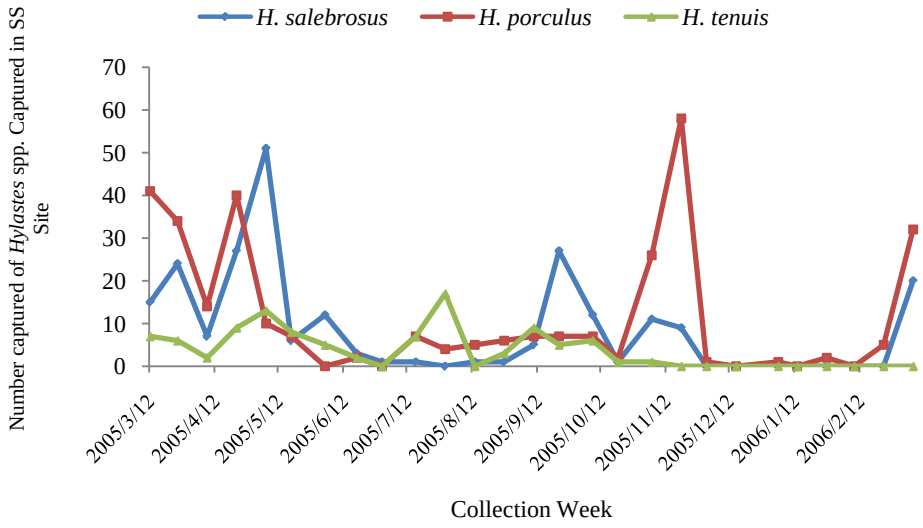

Fig. 2.6. Biweekly Captures of *Hylastes* spp. in baited pitfall, panel, and flight intercept traps on SS Site, from 13 March 2009 to 10 March 2010.

Table 2.8. Mean ± SE captures of *Hylastes* spp. per collection among sites.

Site	*H. salebrosus*	*H. porculus*	*H. tenuis*
SS	13.4 ± 2.7	13.0 ± 2.2	3.9 ± 0.6
RAY	5.3 ± 0.7	3.2 ± 0.7	1.4 ± 0.3
FW	4.3 ± 1.0	4.9 ± 1.2	2.6 ± 0.4
WEY	9.7 ± 3.4	4.2 ± 1.0	1.9 ± 0.2
WV	16.0 ± 3.2	9.0 ± 2.7	2.0 ± 0.4

Table 2.9. Average air temperature among season during pre-treatment sampling year.

Season	Air Temperature (°C)		
	Minimum	Maximum	Average
Spring	-1.6-18.5	11.4-29.4	15.3
Summer	17.0-22.8	28.1-35	33.2
Fall	4.8-20.9	17.8-29.6	19.1
Winter	-6.9-5.5	4-17.6	6.1

Table 2.10. Summary statistics for Tukey's Studentized Range (HSD) test for seasonal effects on *Hylastes* spp. in central Alabama and Georgia, March 2009 to March 2010.

Insect Species	Means captured by season			
	Spring	Summer	Fall	Winter
H. salebrosus	29.3 a	10.6 b	8.7 b	0.9 b
H. porculus	13.1 a	4.8 b	10.0 a	1.6 b
H. tenuis	3.3 a	4.5 a	2.2 ab	0.4 b

Different letters within a row indicate significant difference within the species.

2.5.3.2 Correlations among *Hylastes* spp., *D. terebrans* and *I. grandicollis*

Populations of *H. salebrosus*, *H. porculus* and *H. tenuis* were correlated to each other. ($r_{H.\ salebrosus\sim H.\ porculus}$ = 0.9177, $P < 0.0001$; $r_{H.\ salebrosus\sim H.\ tenuis}$ = 0.6689, $P < 0.0001$; $r_{H.\ porculus\sim H.\ tenuiss}$ = 0.96504, $P < 0.0001$; Pearson correlation and Scatter plot matrix; Fig. 2.7). Additionally, plots with higher captures of *D. terebrans* had higher populations of *Hylastes* spp. (Table 2.11).

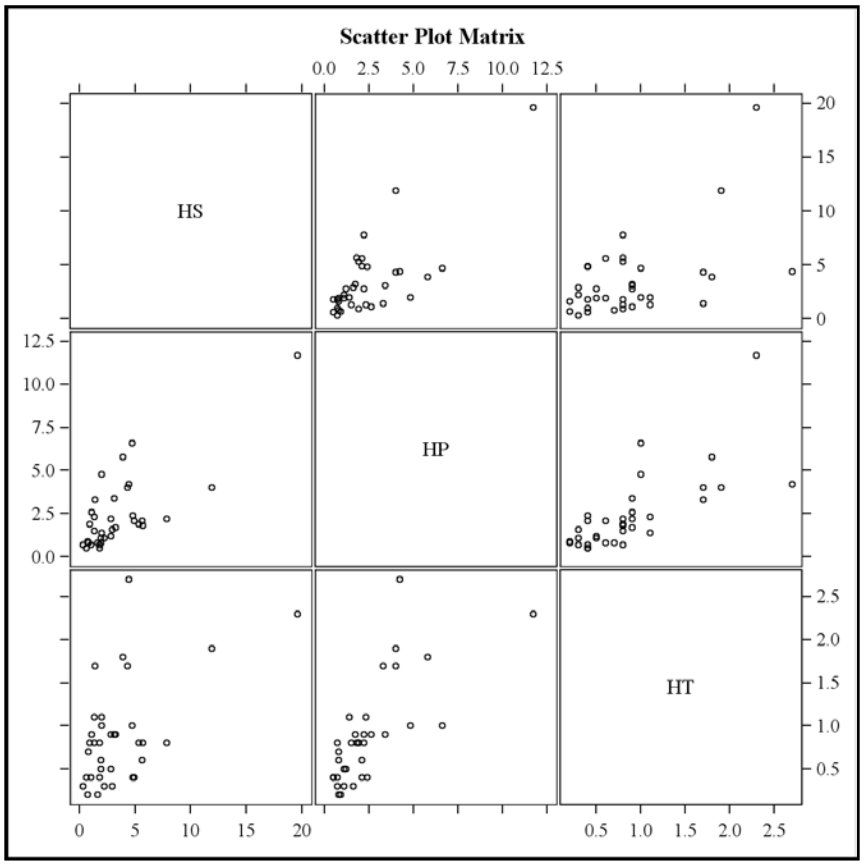

Fig. 2.7. Scatter Plot Matrix showed the correlations among *Hylastes* spp. captured from 13 March 2009 to 10 March 2010.

Table 2.11. Pearson correlation results between root-feeding *Hylastes* spp. (captured from March 2009 to March 2010), *D. terebrans* and *I. grandicollis*.

Insect species	*D. terebrans*		*I. grandicollis*	
	r	*P*	r	*P*
H. salebrosus	0.6628	<.0001	0.1493	0.3275
H. porculus	0.5580	<.0001	-0.0629	0.6817
H. tenuis	0.4763	0.0009	-0.0002	0.9991

$P < 0.05$ indicates correlations between variables are different.

2.5.3.3 *Hylastes* spp. Response to Thinning Treatment

The interaction effects of treatment and time on *Hylastes* spp. populations were significant (Table 2.12). Two-year insect collection data indicates a significant increase in captures of *H. salebrosus* and *H. porculus* after thinning treatments when compared to insect captures in the control plots (Tukey's Multiple Comparison; Fig. 2.8 & 2.9; Fig. 2.10 & 2.11; Fig. 2.12 & 2.13; Table 2.13). In addition, both *H. salebrosus* and *H. porculus* were active the first winter season after thinning. More *H. tenuis* were captured in thinned plots in WEY, RAY, and SS sites than captures in control plots. The second year collections of *H. tenuis* in control plots in WV and SS sites were less than the first year (P_{WV}=0.0217, P_{SS}=0.0174; a=0.05).

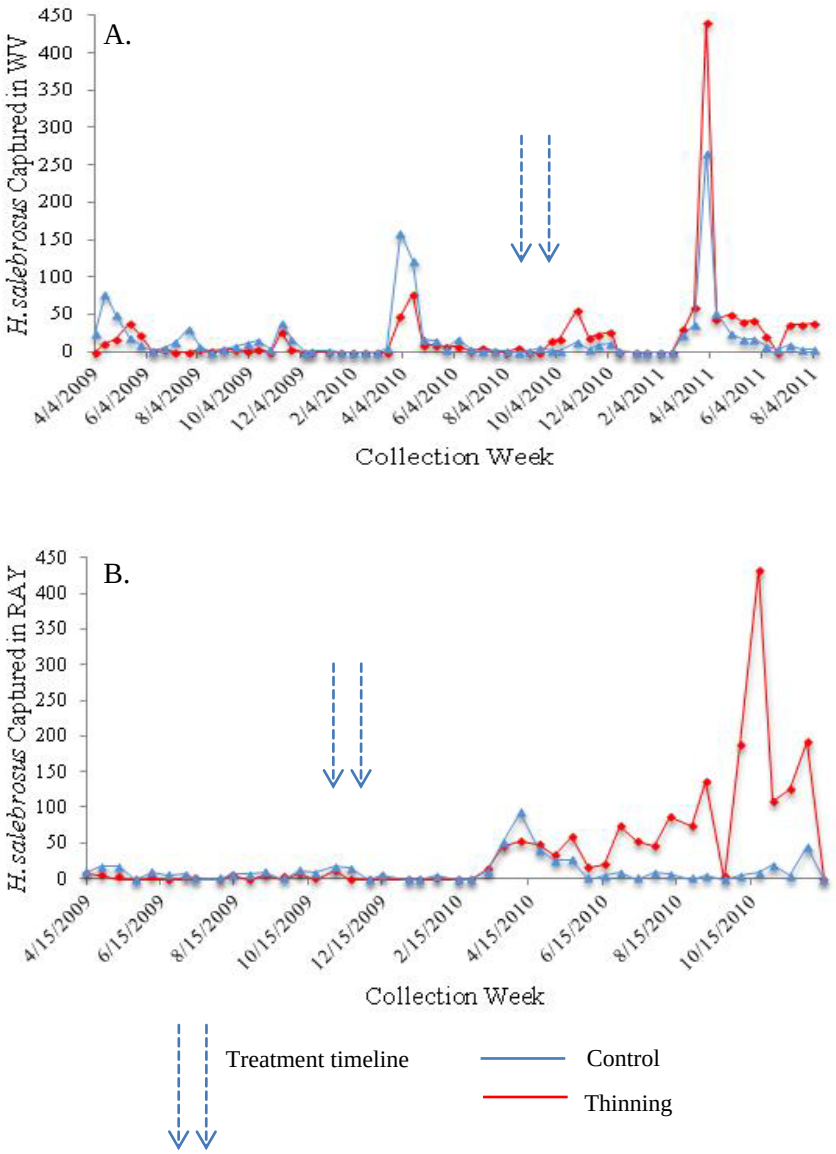

Fig. 2.8. Bi-weekly captured *Hylastes salebrosus* in thinning treatment plots and control plots, showing both pre- and post-treatment data. (A) *H. salebrosus* captured in WV site from April 2009 to August 2011. (B) *H. salebrosus* captured in RAY site from April 2009 to December 2010.

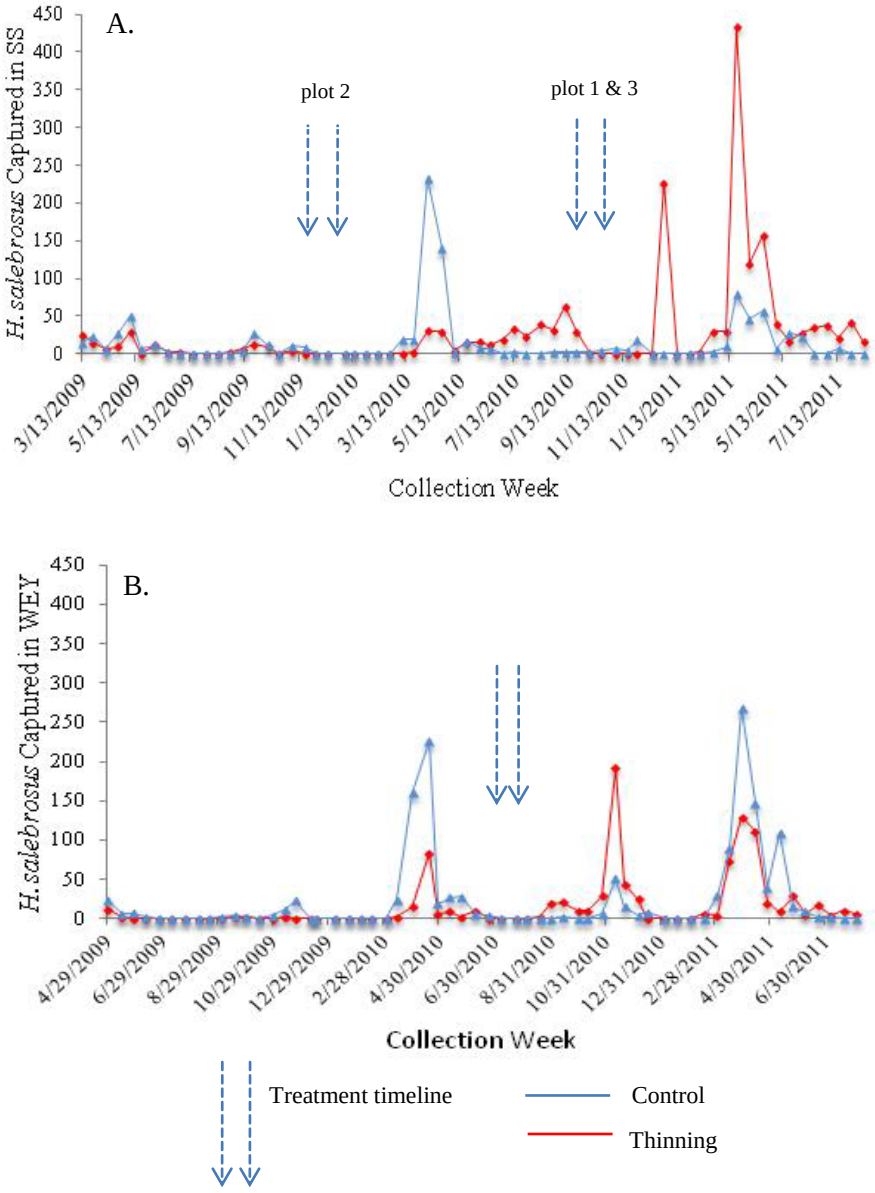

Fig. 2.9. Bi-weekly captured *Hylastes salebrosus* in thinning treatment plots and control plots, showing both pre- and post-treatment data. (A) *H. salebrosus* captured in SS site from March 2009 to August 2011. (B) *H. salebrosus* captured in WEY site from April 2009 to August 2011.

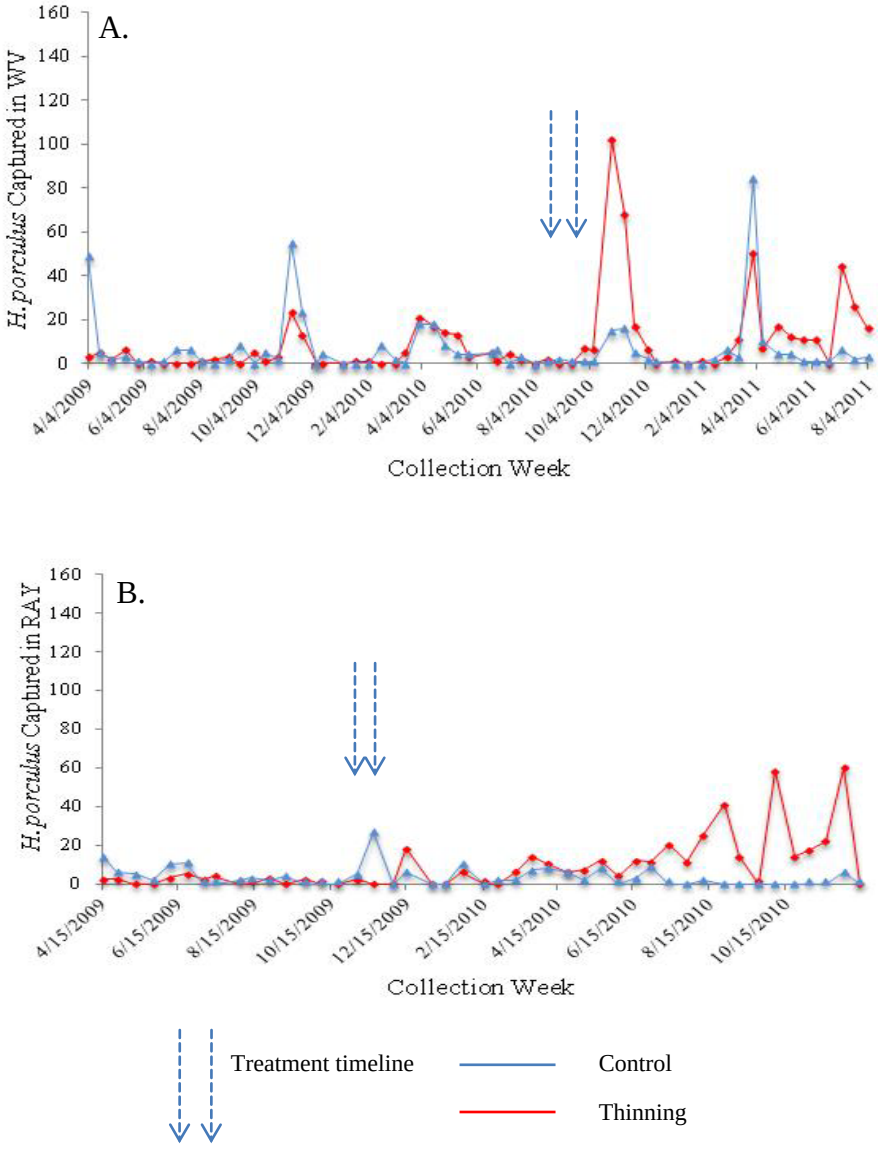

Fig. 2.10. Bi-weekly captured *Hylastes porculus* in thinning treatment plots and control plots, showing both pre- and post-treatment data. (A) *H. porculus* captured in WV site from April 2009 to August 2011. (B) *H. porculus* captured in RAY site from April 2009 to December 2010.

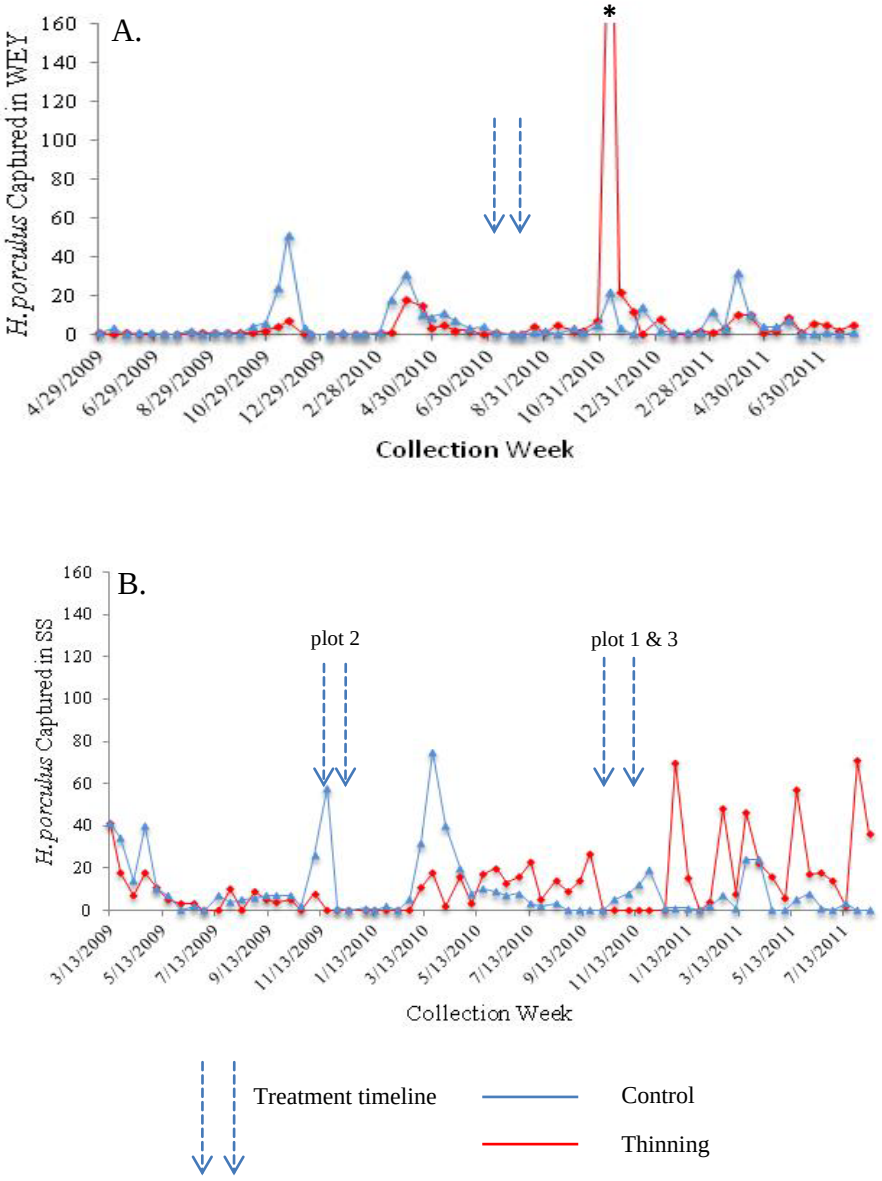

Fig. 2.11. Bi-weekly captured *Hylastes porculus* in thinning treatment plots and control plots, showing both pre- and post-treatment data. (A) *H. porculus* captured in WEY site from April 2009 to August 2011, and * indicated that 254 *H. porculus* were captured. (B) *H. porculus* captured in SS site from March 2009 to August 2011.

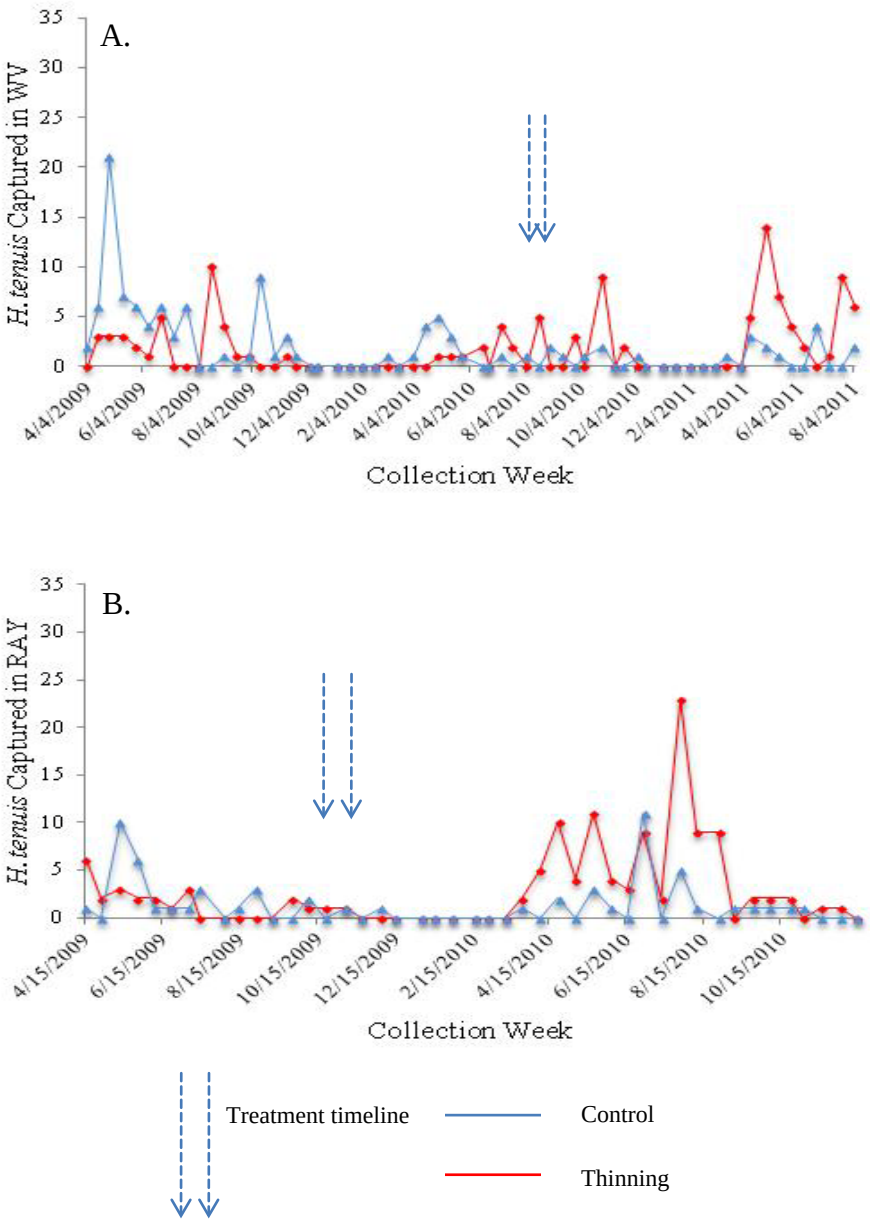

Fig. 2.12. Bi-weekly captured *Hylastes tenuis* in thinning treatment plots and control plots, showing both pre- and post-treatment data. (A) *H. tenuis* captured in WV site from April 2009 to August 2011. (B) *H. tenuis* captured in RAY site from April 2009 to December 2010.

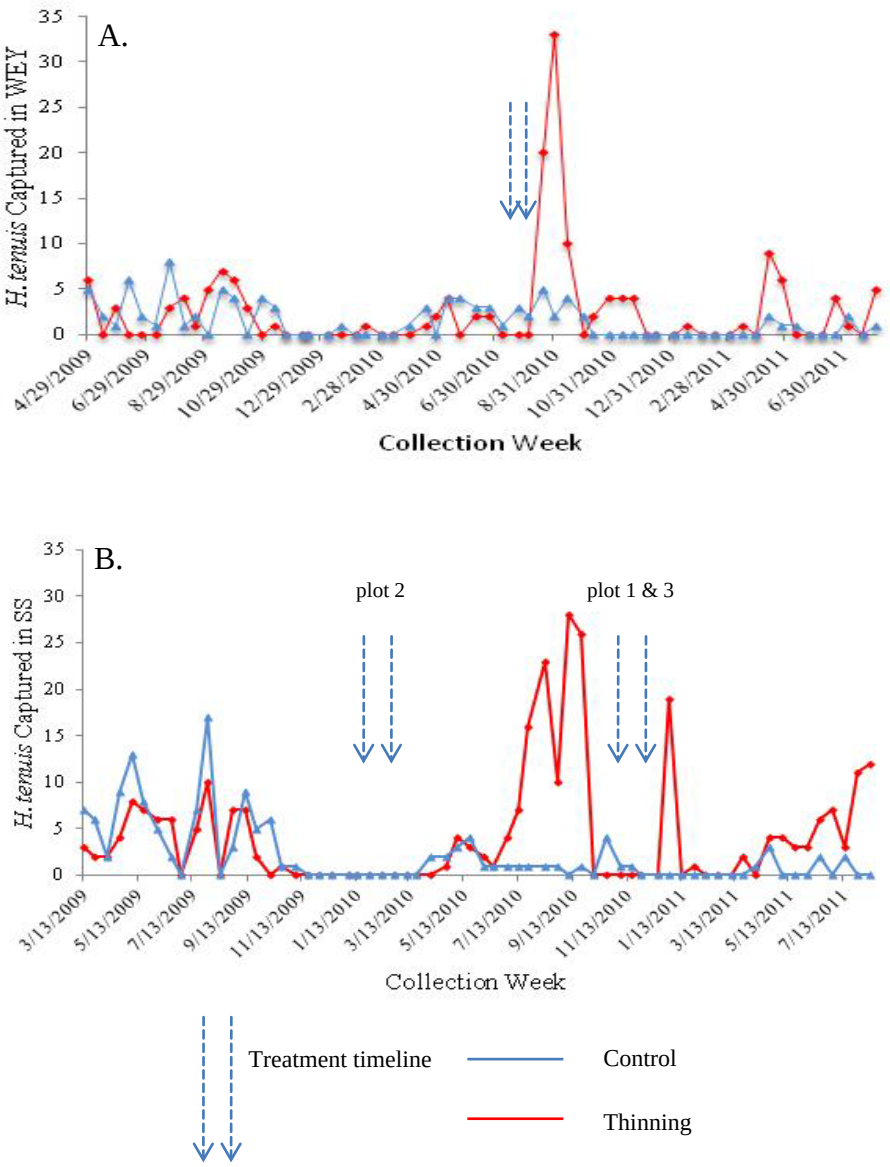

Fig. 2.13. Bi-weekly captured *Hylastes tenuis* in thinning treatment plots and control plots, showing both pre- and post-treatment data. (A) *H. tenuis* captured in WEY site from April 2009 to August 2011. (B) *H. tenuis* captured in SS site from March 2009 to August 2011.

Table 2.12. Interaction of treatment variable and time variable effects on *Hylastes* spp. by ANOVA.

Insect Species		Statistic results of treatment * time
H. salebrosus	WV	$F = 1.88$; $P = 0.1374$; df = 3, 120
	WEY	$F = 2.36$; $P = 0.0748$; df = 3, 116
	RAY	$F = 8.08$; $P < 0.0001$*; df = 3, 86
	SS	$F = 3.58$; $P = 0.0158$*; df = 3, 124
H. porculus	WV	$F = 3.22$; $P = 0.0251$*; df = 3, 120
	WEY	$F = 1.39$; $P = 0.2497$; df = 3, 124
	RAY	$F = 9.55$; $P < 0.0001$*; df = 3, 86
	SS	$F = 3.45$; $P = 0.0188$*; df = 3, 124
H. tenuis	WV	$F = 2.77$; $P = 0.0448$*; df = 3, 120
	WEY	$F = 3.42$; $P = 0.0197$*; df = 3, 124
	RAY	$F = 3.06$; $P = 0.0326$*; df = 3, 86
	SS	$F = 5.33$; $P = 0.0017$*; df = 3, 124

* Indicates significant difference at a = 0.05.

Table 2.13. Tukey's Multiple Comparison of pre-treatment data and post-treatment data.

Insect Species		***P*-values**	
		Thinning Treatment	Control Treatment
H. salebrosus	WV	0.0199 * (+)	0.9484
	WEY	0.0299* (+)	0.2426
	RAY	<0.0001* (+)	0.6517
	SS	0.0051* (+)	0.3624
H. porculus	WV	0.0035 * (+)	0.9394
	WEY	0.0493* (+)	0.9098
	RAY	<0.0001* (+)	0.2296
	SS	0.0032* (+)	0.6074
H. tenuis	WV	0.0915	0.0217 * (-)
	WEY	0.0140* (+)	0.2111
	RAY	0.0022* (+)	0.6257
	SS	0.0421* (+)	0.0174 * (-)

* Indicates significant difference between pre- and post- treatment at a = 0.05;
+ Indicates increasing captures; - Indicates decreasing captures.

2.5.3.4 Insect Diversity Response to Thinning Treatment

Captures of most bark beetle and weevil species increased after thinning treatment (Table 2.14). Although some species were trapped after thinning treatment compared to pre-thinning captures, the Shannon-Weaver index of bark beetle and weevils decreased in all study sites (Table 2.16). However, the diversity change of ambrosia beetle is not consistent. In RAY and WEY site, ambrosia beetle diversity decreased after thinning while it increased in SS and WV site post-thinning treatment (Table 2.15 & 2.16).

Table 2.14. Number of bark beetle and weevil species captured pre-thinning and post-thinning among study sites

Study Sites	Insect Species	Pre-thinning Captures	Post-thinning Captures
RAY	*D. terebrans*	13	63
	D. frontalis	0	2
	I. avulses	5	18
	I. gradicollis	71	142
	I. calligraphus	0	0
	H. porculus	37	415
	H. salebrosus	69	1735
	H. tenuis	24	99
	Pb. picivorus	60	74
	Hb. pales	25	51
	P. nemorensis	4	23
	O. caelatus	0	50
SS	*D. terebrans*	7	26
	D. frontalis	0	0
	I. avulses	6	4
	I. gradicollis	25	76
	I. calligraphus	0	1
	H. porculus	147	268
	H. salebrosus	141	415
	H. tenuis	70	116
	Pb. picivorus	37	12
	Hb. pales	102	27
	P. nemorensis	22	11
	O. caelatus	6	7
WEY	*D. terebrans*	1	60
	D. frontalis	0	0
	I. avulses	0	8
	I. gradicollis	10	55
	I. calligraphus	0	0
	H. porculus	71	373
	H. salebrosus	156	780
	H. tenuis	51	104
	Pb. picivorus	20	13
	Hb. pales	25	36

	P. nemorensis	35	1
	O. caelatus	2	1
WV	*D. terebrans*	11	68
	D. frontalis	0	0
	I. avulses	8	11
	I. gradicollis	110	29
	I. calligraphus	0	0
	H. porculus	155	322
	H. salebrosus	304	942
	H. tenuis	45	61
	Pb. picivorus	23	21
	Hb. pales	42	60
	P. nemorensis	30	5
	O. caelatus	5	5

Table 2.15. Number of ambrosia species captured pre-thinning and post-thinning among study sites

Study Sites	Insect Species	Pre-thinning Captures	Post-thinning Captures
RAY	*D.onoharaensum*	5	10
	X. saxesenii	55	224
	X. crassiusculus	52	179
	X. compactus	3	2
	G. materiarius	94	134
	M. mali	6	8
	X. atratus	6	22
	X. germanus	2	6
	M. fasciatum	1	18
	X. pubescens	79	536
	C. mutilatus	41	33
	X. ferrugineus	0	7
	T. scabricollis	0	9
	P. comatus	9	33
SS	*D.onoharaensum*	2	6
	X. saxesenii	95	55
	X. crassiusculus	17	59
	X. compactus	3	5
	G. materiarius	181	165
	M. mali	16	14
	X. atratus	10	3
	X. germanus	3	6
	M. fasciatum	0	51
	X. pubescens	56	124
	C. mutilatus	27	40
	X. ferrugineus	0	11
	T. scabricollis	2	26
	P. comatus	6	29
WEY	*D.onoharaensum*	3	0
	X. saxesenii	45	11
	X. crassiusculus	89	50
	X. compactus	10	0
	G. materiarius	126	33
	M. mali	8	0

	X. atratus	4	1
	X. germanus	6	3
	M. fasciatum	3	7
	X. pubescens	40	70
	C. mutilatus	77	25
	X. ferrugineus	2	6
	T. scabricollis	5	11
	P. comatus	27	1
WV	*D.onoharaensum*	3	2
	X. saxesenii	57	13
	X. crassiusculus	28	79
	X. compactus	21	1
	G. materiarius	396	67
	M. mali	10	1
	X. atratus	0	2
	X. germanus	6	3
	M. fasciatum	7	5
	X. pubescens	99	150
	C. mutilatus	38	33
	X. ferrugineus	6	2
	T. scabricollis	10	6
	P. comatus	2	2

Table 2.16. Shannon-Weaver Index for pre- and post-treatment captures among study sites

Study Sites	**Insect Catergory**	**Pre-thinning Index**	**Post-thinning Index**
RAY	Bark beetles & Weevils	1.91	1.26
	Ambrosia beetles	1.89	1.70
SS	Bark beetles & Weevils	1.86	1.54
	Ambrosia beetles	1.67	2.13
WEY	Bark beetles & Weevils	1.65	1.30
	Ambrosia beetles	2.00	1.85
WV	Bark beetles & Weevils	1.70	1.23
	Ambrosia beetles	1.50	1.65

2.5.3.5 *Hylastes spp.* Response to Harvesting Treatment

Unlike the thinning treatment, harvesting seemed to have no effect on *Hylastes* populations. Only *H. porculus* in WV and SS sites and *H. tenuis* in SS site decreased after harvesting treatment (Fig.2.17A & 2.19; Fig.2.22; Table 2.15). Populations of *H. porculus* and *H. tenuis* captured in control plots at SS site were reduced compared to year one data (Fig.2.19 & 2.22; Table 2.15). Significantly fewer *H. salebrosus* at the WV site and *H. porculus* in WV and F&W were captured when post-harvested numbers were compared to pre-harvesting captures. However, *H. salebrosus* captured in WV site returned to levels in the second year after harvesting. The population of *H. tenuis* did not respond to the harvesting treatment, but the number of *H. tenuis* caught in F&W site decreased in the second year after harvesting. More *H. tenuis* were captured in WEY site after harvesting, but the population dropped in the second year after harvesting.

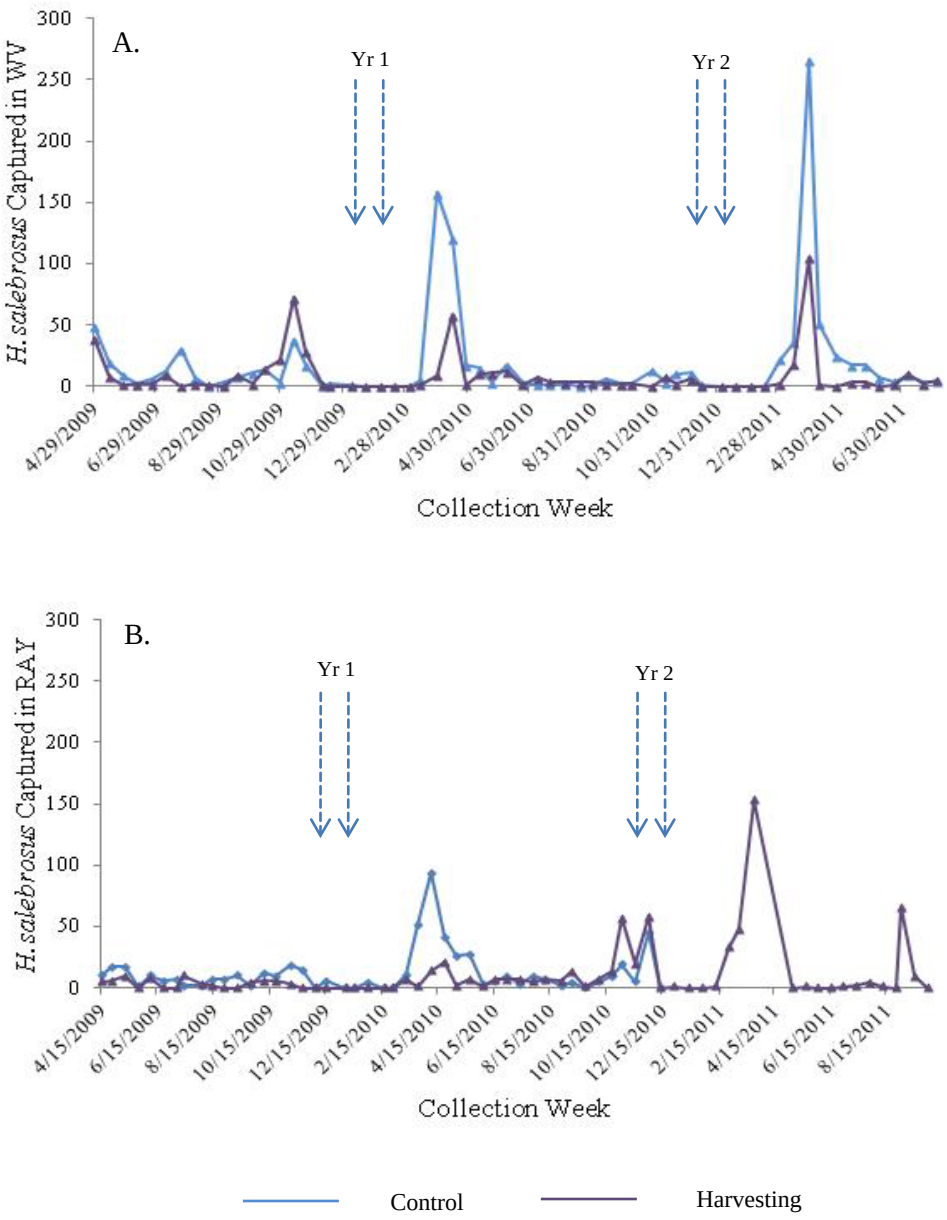

Fig. 2.14. Bi-weekly captured *Hylastes salebrosus* in harvesting and control plots, showing both pre- and post-treatment data. (A) *H. salebrosus* captured in WV site from April 2009 to August 2011. (B) *H. salebrosus* captured in RAY site from April 2009 to September 2011.

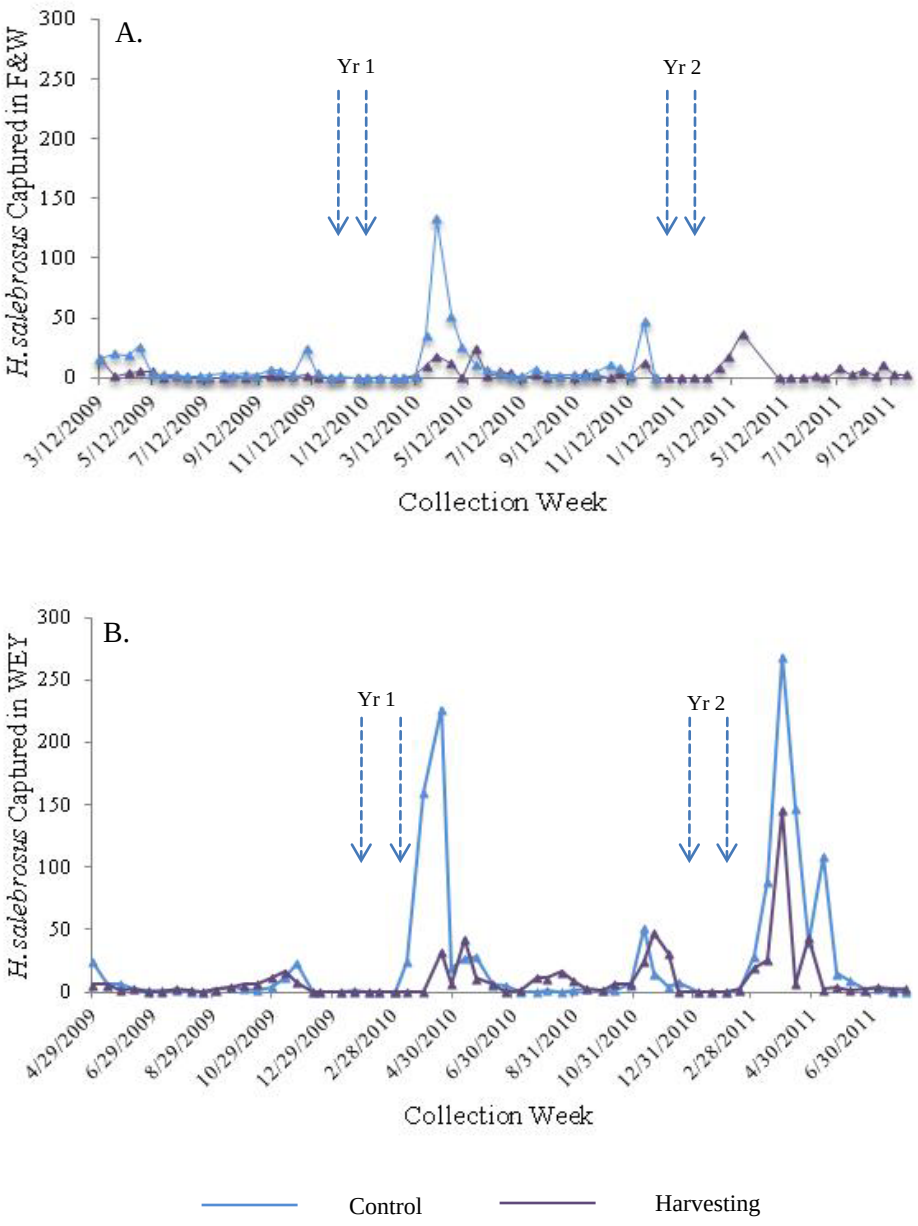

Fig. 2.15. Bi-weekly captured *Hylastes salebrosus* in harvesting and control plots, showing both pre- and post-treatment data. (A) *H. salebrosus* captured in F&W site from March 2009 to September 2011. (B) *H. salebrosus* captured in WEY site from April 2009 to August 2011.

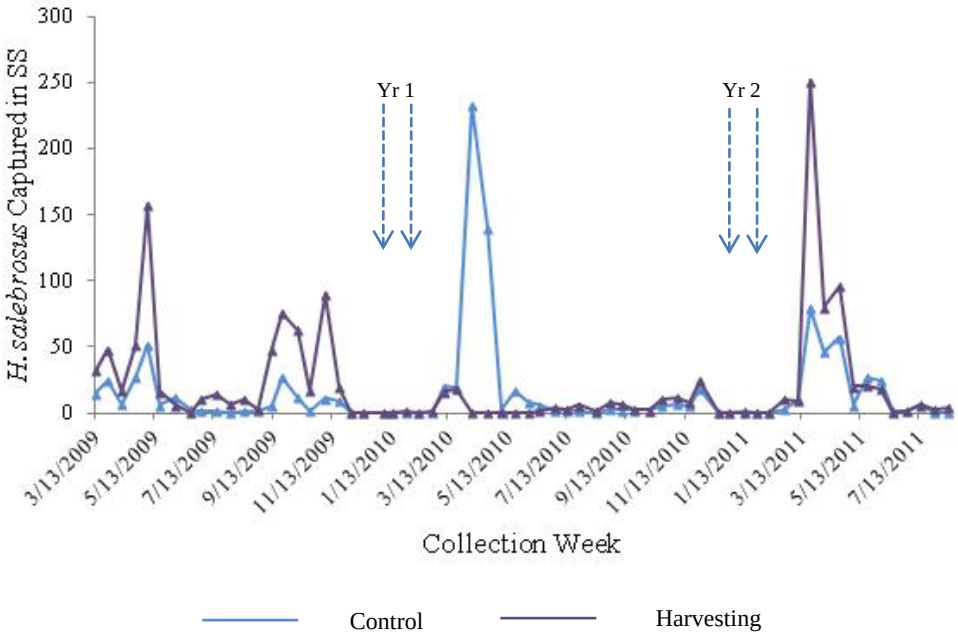

Fig. 2.16. Bi-weekly captured *Hylastes salebrosus* in harvesting and control plots in SS site from March 2009 to August 2011, showing both pre- and post-treatment data.

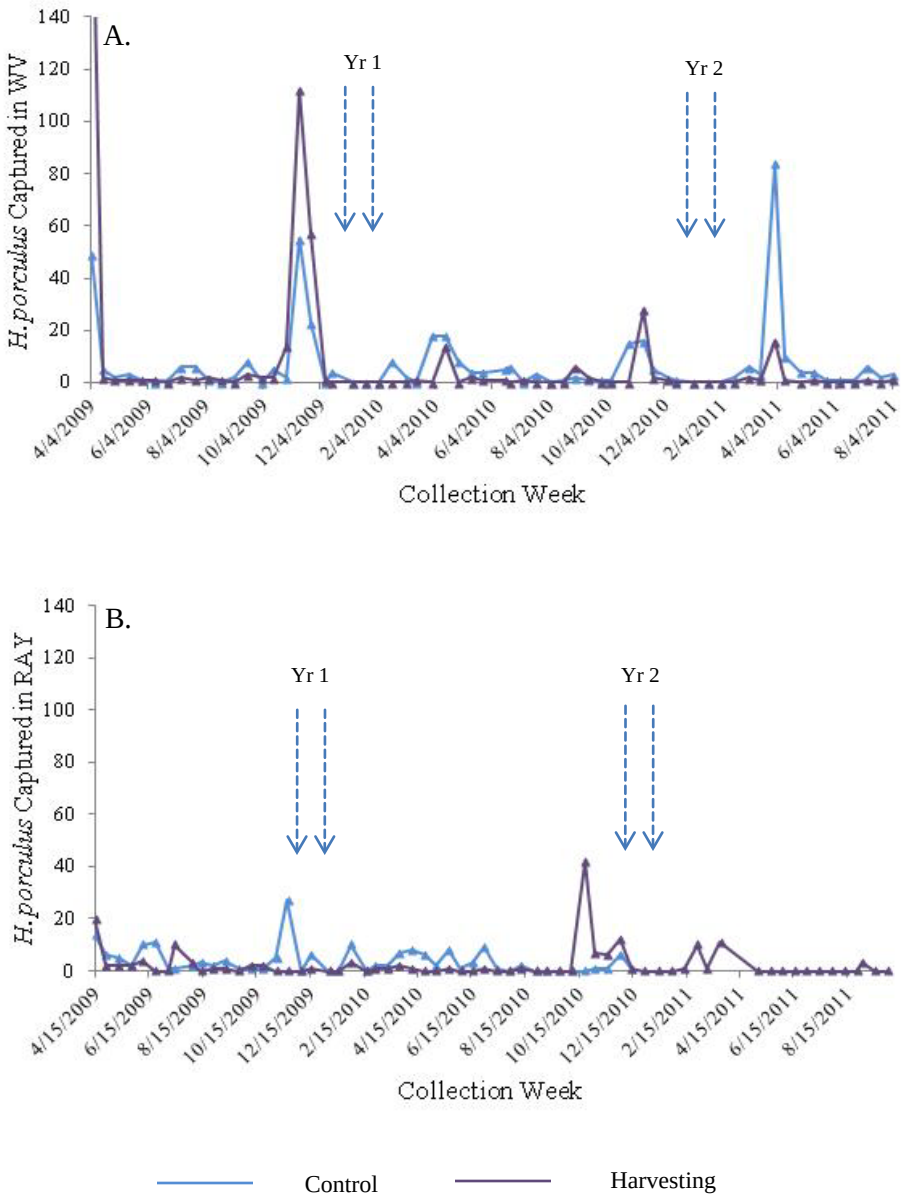

Fig. 2.17. Bi-weekly captured *Hylastes porculus* in harvesting and control plots, showing both pre- and post-treatment data. (A) *H. porculus* captured in WV site from April 2009 to August 2011. (B) *H. porculus* captured in RAY site from April 2009 to September 2011.

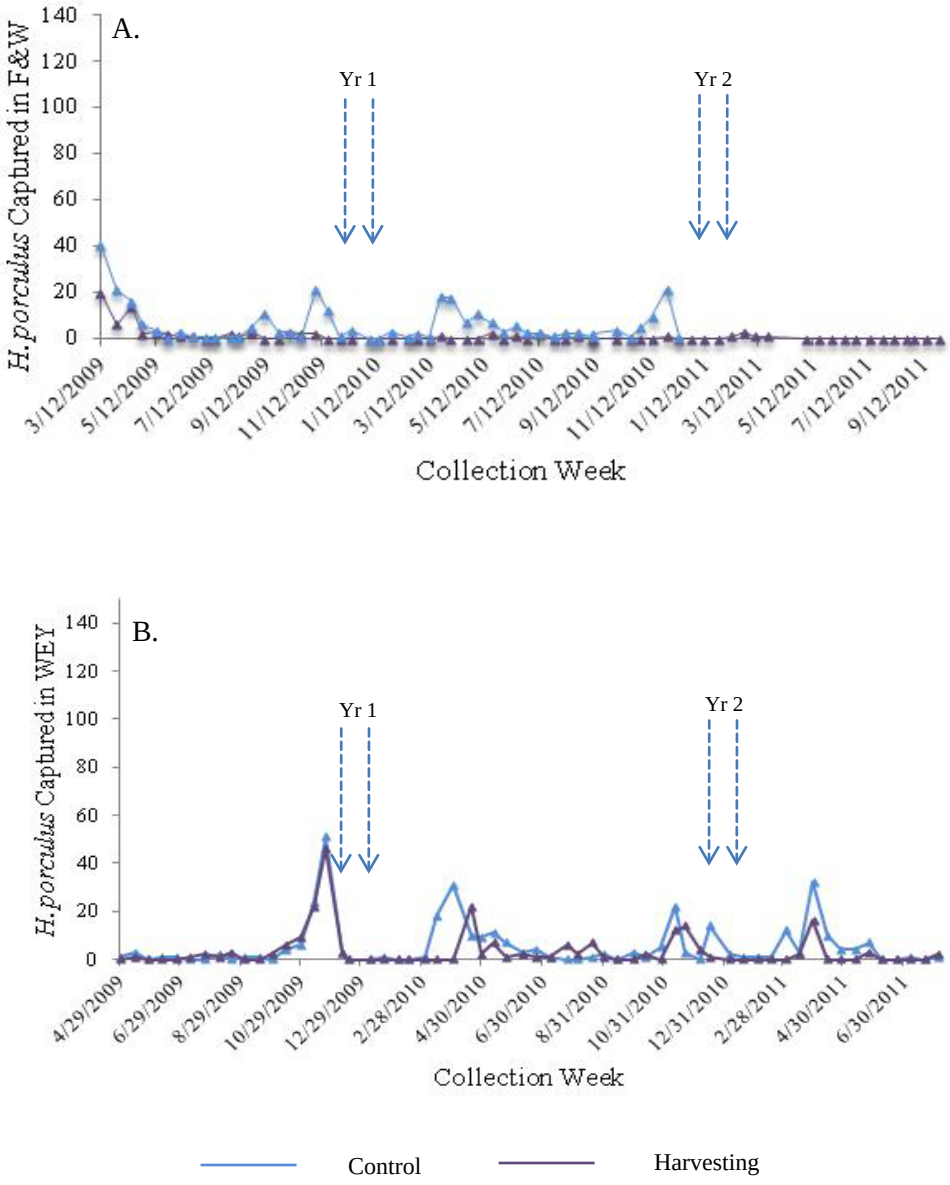

Fig. 2.18. Bi-weekly captured *Hylastes porculus* in harvesting and control plots, showing both pre- and post-treatment data. (A) *H. porculus* captured in F&W site from March 2009 to September 2011. (B) *H. porculus* captured in WEY site from April 2009 to August 2011.

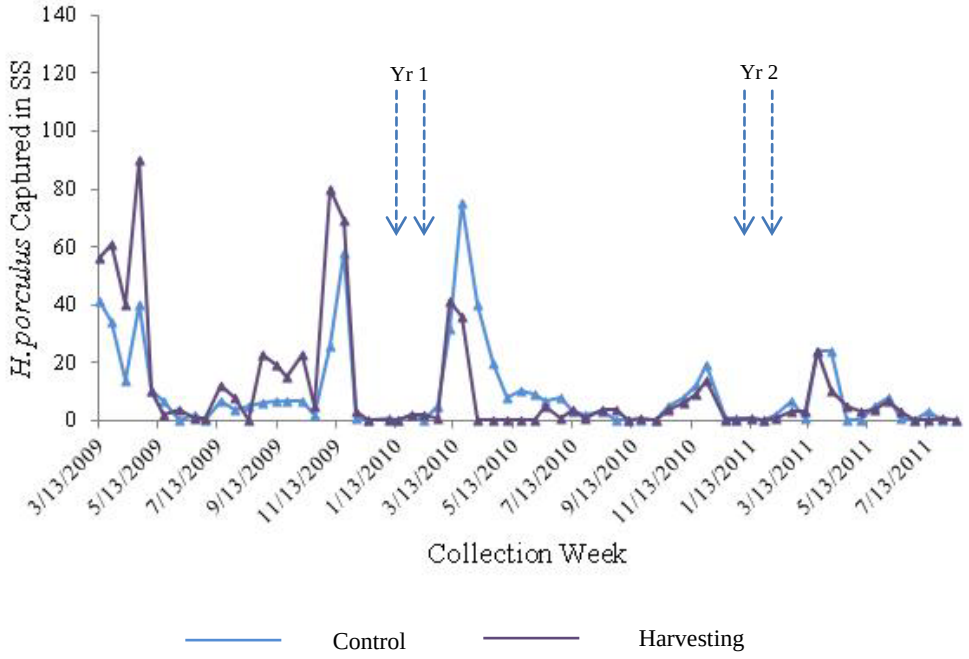

Fig. 2.19. Bi-weekly captured *Hylastes porculus* in harvesting and control plots in SS site from March 2009 to August 2011, showing both pre- and post-treatment data.

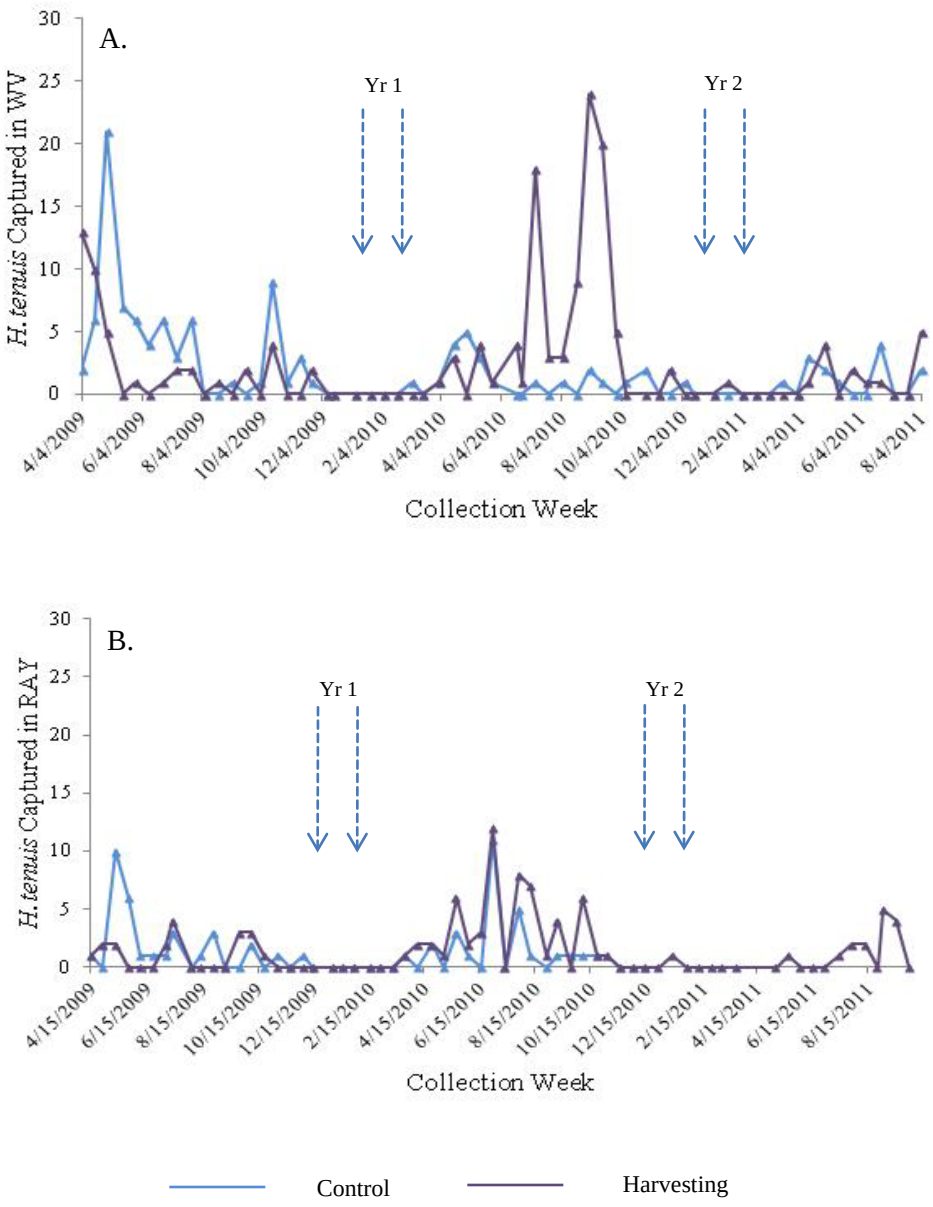

Fig. 2.20. Bi-weekly captured *Hylastes tenuis* in harvesting and control plots, showing both pre- and post-treatment data. (A) *H. tenuis* captured in WV site from April 2009 to August 2011. (B) *H. tenuis* captured in RAY site from April 2009 to September 2011.

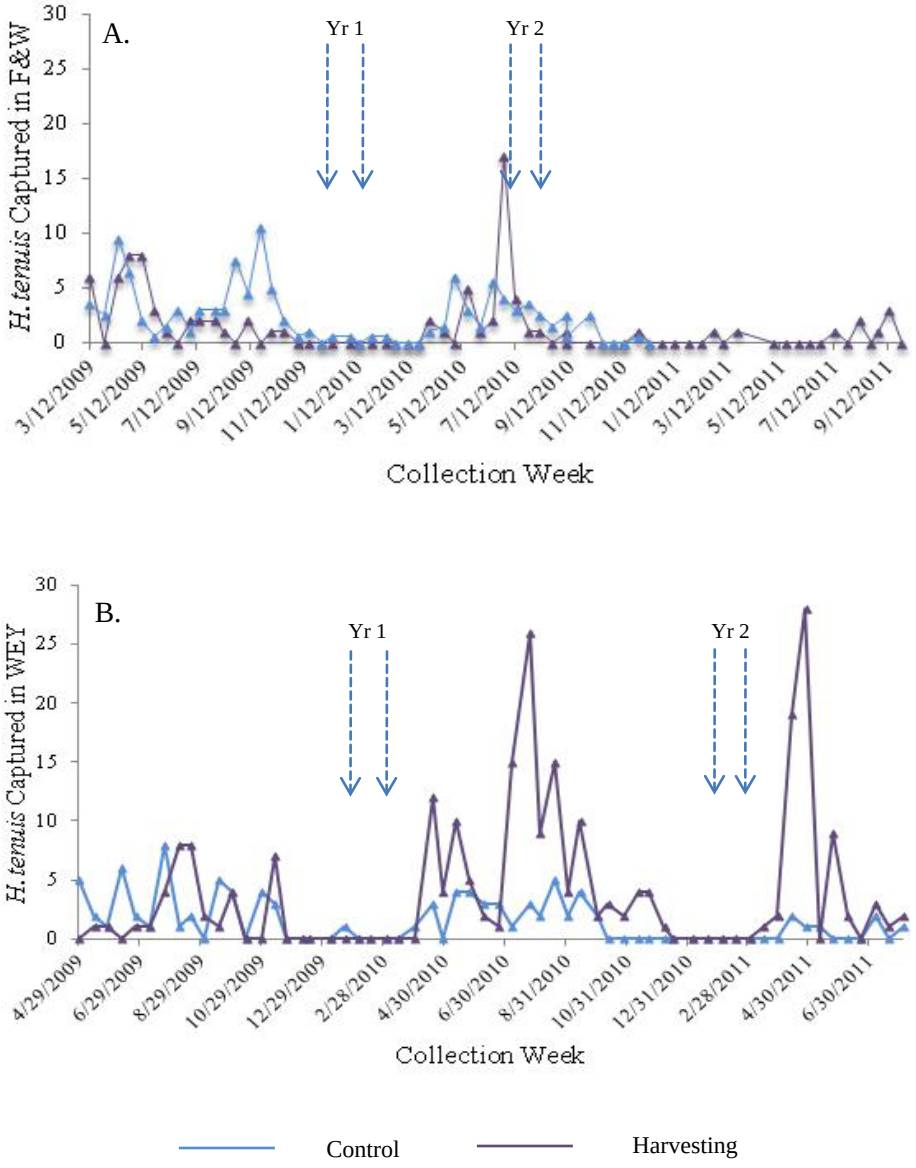

Fig. 2.21. Bi-weekly captured *Hylastes tenuis* in harvesting and control plots, showing both pre- and post-treatment data. (A) *H. tenuis* captured in F&W site from March 2009 to September 2011. (B) *H. tenuis* captured in WEY site from April 2009 to August 2011.

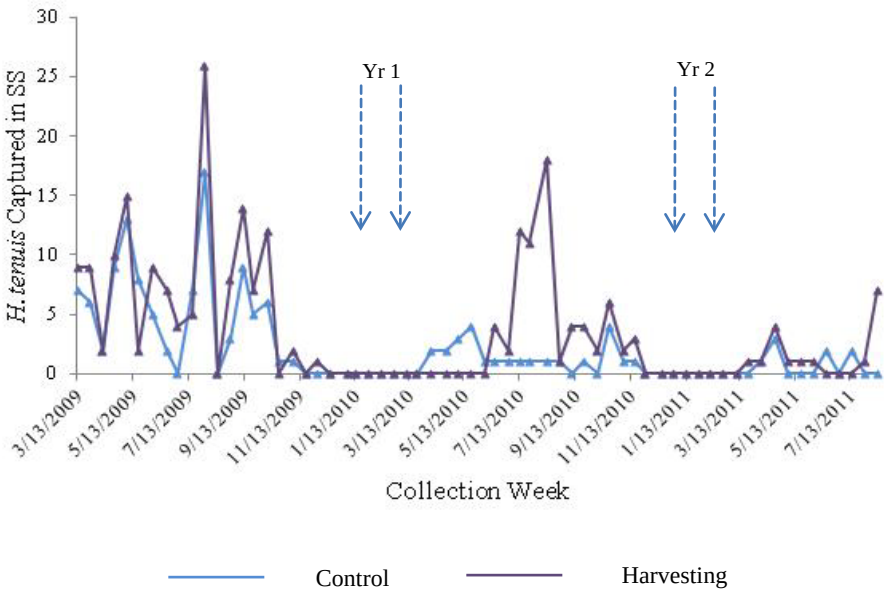

Fig. 2.22. Bi-weekly captured *Hylastes tenuis* in harvesting and control plots in SS site from March 2009 to August 2011, showing both pre- and post-treatment data.

Table 2.17. Interaction of treatment variable and time variable effects on *Hylastes* spp. by ANOVA.

Insect Species	Treatment * Time interaction	
H. salebrosus	WV	$F = 1.46$; $P = 0.2284$; df = 3, 117
	WEY	$F = 1.56$; $P = 0.2042$; df = 3, 107
	RAY	$F = 1.19$; $P = 0.3184$*; df = 3, 106
	SS	$F = 0.83$; $P = 0.4790$; df = 3, 121
	F&W	$F = 4.61$; $P = 0.0045$*; df = 3, 103
H. porculus	WV	$F = 3.10$; $P = 0.0293$*; df = 3, 117
	WEY	$F = 0.6$; $P = 0.6193$; df = 3, 107
	RAY	$F = 1.4$; $P = 0.2474$; df = 3, 106
	SS	$F = 8.07$; $P < 0.0001$*; df = 3, 121
	F&W	$F = 7.04$; $P = 0.0002$*; df = 3, 103
H. tenuis	WV	$F = 2.36$; $P = 0.0749$; df = 3, 117
	WEY	$F = 6.5$; $P = 0.0004$*; df = 3, 107
	RAY	$F = 0.55$; $P = 0.6487$; df = 3, 106
	SS	$F = 5.34$; $P = 0.0017$*; df = 3, 121
	F&W	$F = 8.50$; $P = 0.0001$*; df = 3, 103

* Indicates significant difference at a = 0.05.

Table 2.18. Tukey's Multiple Comparison of mean *Hylastes* spp. captured pre- and post-treatment.

Insect Species		***P*-values**	
		Harvesting Treatment	Control Treatment
H. salebrosus	WV	0.0798	0.5172
	WEY	0.2966	0.1322
	RAY	0.4464	0.6496
	F&W	0.5449	0.0058* (+)
	SS	0.4661	0.3070
H. porculus	WV	0.0031* (-)	0.7878
	WEY	0.3281	0.8408
	RAY	0.8506	0.2269
	F&W	0.0606	0.9079
	SS	<0.0001* (-)	0.0188* (-)
H. tenuis	WV	0.6257	0.0191* (-)
	WEY	0.0122* (+)	0.6019
	RAY	0.4928	0.6242
	F&W	0.2081	0.0020* (+)
	SS	0.0220* (-)	0.0133* (-)

* Indicates significant response at a=0.05.

+ Indicates increasing captures; - Indicates decreasing captures.

Table 2.19. Tukey's Multiple Comparison of mean *Hylastes* spp. captured pre-treatment with year one post-treatment data, and pre-treatment with year two post-treatment data in harvesting plots.

Insect Species		***P*-values**	
		Yr1-Post	Yr2-Post
H. salebrosus	WV	0.0365* (-)	0.0903
	WEY	0.1967	0.1280
	RAY	0.3925	0.0780* (+)
	F&W	0.6508	0.5473
H. porculus	WV	0.0472* (-)	0.0478* (-)
	WEY	0.6584	0.1325
	RAY	0.8998	0.5130
	F&W	0.0093* (-)	0.0071* (-)
H. tenuis	WV	0.2304	0.4276
	WEY	0.0218* (+)	0.4230
	RAY	0.1509	0.2907
	F&W	0.4394	0.0279* (-)

* Indicates significant response at a=0.05.
+ Indicates increasing capture; - Indicates decreasing capture.

2.5.3.6 Insect Diversity Response to Harvesting Treatment

Captures of insect species respond different among study sites after harvesting treatment (Table 2.20 & 2.21). The diversity of bark beetle and weevils decreased in RAY and F&W sites compared to the diversity change in SS, WEY, and WV sites. However, the diversity of ambrosia beetles decreased in RAY, F&W, WEY, and WV sites except the captures in SS site (Table 2.22).

Table 2.20. Number of bark beetle and weevil species captured pre-harvest and post-harvest among study sites

Study Sites	Insect Species	Pre-harvest Captures	Post-harvest Captures
RAY	*D. terebrans*	12	8
	D. frontalis	0	0
	I. avulses	3	5
	I. gradicollis	58	77
	I. calligraphus	0	0
	H. porculus	47	12
	H. salebrosus	64	106
	H. tenuis	18	49
	Pb. picivorus	51	42
	Hb. pales	28	107
	P. nemorensis	9	14
	O. caelatus	2	20
FW	*D. terebrans*	3	9
	D. frontalis	0	0
	I. avulses	8	2
	I. gradicollis	45	74
	I. calligraphus	0	0
	H. porculus	62	8
	H. salebrosus	26	105
	H. tenuis	43	35
	Pb. picivorus	34	103
	Hb. pales	35	102
	P. nemorensis	5	12
	O. caelatus	1	17
SS	*D. terebrans*	20	47
	D. frontalis	0	0
	I. avulses	3	5
	I. gradicollis	19	41
	I. calligraphus	0	0
	H. porculus	604	116
	H. salebrosus	720	576
	H. tenuis	142	71
	Pb. picivorus	13	49
	Hb. pales	67	54
	P. nemorensis	12	0
	O. caelatus	3	10

WEY	*D. terebrans*	1	22
	D. frontalis	0	1
	I. avulses	0	6
	I. gradicollis	13	97
	I. calligraphus	0	0
	H. porculus	98	86
	H. salebrosus	82	268
	H. tenuis	39	138
	Pb. picivorus	5	43
	Hb. pales	13	58
	P. nemorensis	0	8
	O. caelatus	1	12
WV	*D. terebrans*	7	29
	D. frontalis	0	0
	I. avulses	0	3
	I. gradicollis	28	90
	I. calligraphus	0	1
	H. porculus	414	59
	H. salebrosus	467	145
	H. tenuis	43	99
	Pb. picivorus	9	95
	Hb. pales	21	120
	P. nemorensis	1	29
	O. caelatus	3	17

Table 2.21. Number of ambrosia species captured pre-harvest and post-harvest among study sites

Study Sites	Insect Species	Pre-harvest Captures	Post-harvest Captures
RAY	*D.onoharaensum*	8	5
	X. saxesenii	48	144
	X. crassiusculus	67	23
	X. compactus	12	1
	G. materiarius	80	38
	M. mali	3	1
	X. atratus	9	16
	X. germanus	6	1
	M. fasciatum	0	0
	X. pubescens	88	310
	C. mutilatus	25	23
	X. ferrugineus	0	9
	T. scabricollis	0	6
	P. comatus	1	4
FW	*D.onoharaensum*	9	1
	X. saxesenii	61	200
	X. crassiusculus	52	53
	X. compactus	10	2
	G. materiarius	160	11
	M. mali	17	0
	X. atratus	7	3
	X. germanus	3	4
	M. fasciatum	1	4
	X. pubescens	26	129
	C. mutilatus	17	11
	X. ferrugineus	0	7
	T. scabricollis	0	15
	P. comatus	1	0
SS	*T. scabricollis*	53	2
	P. comatus	236	44
	X. crassiusculus	100	83

	X. compactus	5	2
	G. materiarius	690	95
	M. mali	40	0
	X. atratus	17	2
	X. germanus	6	3
	M. fasciatum	40	7
	X. pubescens	236	128
	C. mutilatus	77	99
	X. ferrugineus	0	2
	T. scabricollis	1	5
	P. comatus	9	3
WEY	*D.onoharaensum*	1	5
	X. saxesenii	49	88
	X. crassiusculus	29	72
	X. compactus	22	2
	G. materiarius	137	27
	M. mali	2	1
	X. atratus	1	12
	X. germanus	2	1
	M. fasciatum	1	1
	X. pubescens	68	343
	C. mutilatus	72	43
	X. ferrugineus	0	9
	T. scabricollis	0	2
	P. comatus	3	1
WV	*D.onoharaensum*	4	0
	X. saxesenii	98	236
	X. crassiusculus	17	35
	X. compactus	2	1
	G. materiarius	170	95
	M. mali	5	2
	X. atratus	4	3
	X. germanus	2	0
	M. fasciatum	1	63
	X. pubescens	224	489
	C. mutilatus	106	38
	X. ferrugineus	1	16
	T. scabricollis	0	9
	P. comatus	2	7

Table 2.22. Shannon-Weaver Index for pre- and post-treatment captures among study sites

Study Sites	Insect Catergory	Pre-harvest Index	Post-harvest Index
RAY	Bark beetles & Weevils	1.97	1.93
	Ambrosia beetles	1.89	1.43
FW	Bark beetles & Weevils	1.96	1.87
	Ambrosia beetles	1.75	1.50
SS	Bark beetles & Weevils	1.28	1.42
	Ambrosia beetles	1.71	1.79
WEY	Bark beetles & Weevils	1.45	1.83
	Ambrosia beetles	1.74	1.44
WV	Bark beetles & Weevils	1.14	2.05
	Ambrosia beetles	1.58	1.51

2.5.3.7 Stump Observations

The most commonly collected insect from loblolly pine root sections was *H. tenuis* followed by *H. salebrosus, Hb. pales, Pb. picivorus, O. caelatus,* and termites (species not identified). Galleries of *H. tenuis* and tunnels of regeneration weevils were frequently found on root samples (Fig. 2.23). Xylem and phloem tissues collected from root sections were discolored and *L. procerum* and *L. terebrantis* was recovered from those tissues (Table 2.17).

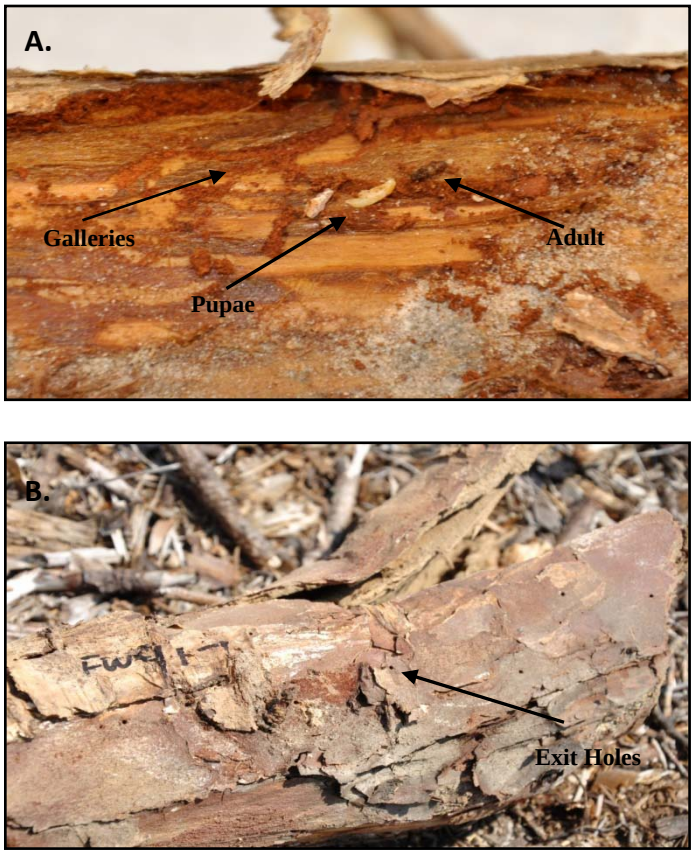

Fig. 2.23. (A) *P. taeda* root sections from stump sampling infested with *H. tenuis* root beetle, showing galleries, pupae and adult of *H. tenuis*. (B) Root section showing exit holes of *H. tenuis*.

Table 2.23. Characteristics of stump samples collected from center subplot in harvested plots.

Plot	Mean ± SE of root length (cm)	Mean ± SE of root diameter (cm)	Roots with galleries (%)	Range of numbers of exit holes	Roots with insects present (%)	Roots with stain fungus (%)
WV7	35.05± 2.81	6.22±1.26	50%	0-7	33%	17%
WV8	28.19±2.54	5.55±1.23	33%	0-7	17%	0
WV9	31.24±1.10	5.63±0.43	67%	2-11	33%	0
WEY7	42.32±4.01	4.47±0.58	33%	0-4	33%	17%
WEY8	32.82±1.39	4.48±0.60	50%	0-8	50%	50%
WEY9	34.24±3.05	5.04±0.33	83%	0-4	50%	50%
F&W7	27.05±1.65	4.06±1.78	50%	0-22	50%	0
F&W8	16.34±5.80	3.89±1.03	33%	0-23	17%	17%
F&W9	18.72±3.32	2.14±0.18	67%	0-28	33%	17%
Ray7	32.92±1.14	3.71±0.56	50%	0-5	33%	0
Ray8	32.41±3.34	3.56±0.66	17%	0-2	0	0
Ray9	32.26±2.88	6.31±1.00	50%	0-19	50%	17%
SS9	30.87±2.62	4.32±0.78	60%	0-10	33%	40%

2.6 Discussion

This study is the first report of population responses of pathogen-vectoring root-feeding beetles (*H. salebrosus*, *H. porculus* and *H. tenuis*) to a thinning treatment in loblolly pine stands. Summer and winter thinning may significantly increase populations of *Hylastes* spp. which have been shown to vector *Leptographium* spp. involved with Southern Pine Decline by releasing plant volatile compunds. Generally, thinning is recommended as a bark beetle management strategy because it maintains higher vigor of remaining trees, removes trees which are susceptible to diseases and pests, and decrease

infestation rates of remaining trees. Thinning also can keep residual trees alive and increase light around the entire crown (Werner 2002). In recent years, row thinning operations have become preferred in pine plantations because it is a quick and economical method. However, a row-thinning considers little about the crown conditions of either the removed or residual trees. Thinning may cause either visible damage to residual trees or invisible damage to root systems. In these current trials, the more recent thinning damaged some of the remaining trees. For example, large branches were broken, and the bark of remaining trees was damaged. Wounded trees exposed xylem tissue and the cut stumps released plant volatiles such as turpentine and alpha-pinene (USDA guidelines 2011) that attract root-feeding *Hylastes* spp. High populations of *Hylastes* spp. in thinned stands may cause higher infestation of ophiostomatoid fungi in root systems and could further predispose the remaining trees to other secondary pests such as *Dendroctonus* spp. and *Ips* spp. In order to reduce losses, a landowner could treat damaged trees and remaining stumps with preventative chemicals to decrease host volatiles release, and minimize logging damage to residual trees during thinning. Feduccia and Mann (1976) found in a previous study that spraying injured trees with preventative chemicals immediately after thinning in *P. taeda* stands prevented *D. terebrans* from attacking damaged trees. In addition, 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 instead of third row thinning because only 40% of remaining trees are impacted compared with third row thining, or perform thinning treatment during fall

season because trees are more susceptible to be damaged in spring and summer when they are growing. In a high risk stands, to avoid SPD infestation, a landower should either plant resistant species or plant loblolly pine in wider space.

Previous studies reported that *H. salebrosus* and *H. porculus* were usually captured in panel traps while *H. tenuis* was often traped in pitfall traps (Thompson 2011). Therefore, *H. salebrosus* and *H. porculus* may establish their colonies in the root systems and in the upper stumps of cut trees. Following harvesting, temperature of the air near the ground and within upper soil horizons may be increased, and the humidity near the surface may be decreased (Nyland 2002), thus higher air temperature would dry remaining stumps and roots close to soil surface. Because of habitat removal, lack of food sourses and temperature limitations, it is hypothesized that harvesting would reduce populations of *Hylastes* spp.when compared to control treatments. In the present study, however, the harvesting treatment did not affect captures of *H. salebrosus* and *H. tenuis*, although fewer *H. porculus* were trapped in some harvested sites. *Hylastes porculus* is reported to have a more northern range (Wood 1982), so its activity would be expected to be reduced in higher temperatures. However, *H. tenuis* galleries and different stage of *H. tenuis* were observed very often one year after harvesting in root samples, which may explain why the populations of *H. tenuis* were more stable in response to harvesting comparing to *H. salebrosus* and *H. porculus*. The number of *Hylastes* spp. in harvested stands returned to pre-treatment capture levels in the second year following harvesting. Since the harvesting effects on insect populations are inconsistent, it is difficult to

summarize conclusions on how harvesting affected root-feeding bark beetles. However, there were no reports of these *Hylastes* spp. attacking pine seedlings in the United States as reported in New Zealand with *H. ater* (Reay et al. 2012). Hence, it will not be an issue if landowners replant pine seedlines in those harvested plots.

Seasonal data prior to stand treatment (Table 2.3; Fig. 2.6) indicates that root-feeding *Hylastes* beetles are active throughout most of the year which is in agreement with the previous work (Zanzot et al. 2010, Thompson 2011). Thus it is necessary to monitor insect population peaks using the year-round sampling method as insect activity does not always overlap the traditional spring trapping period for southern pine beetle (Thatcher et al. 1980, Gardner 2011). Numbers of *H. salebrosus* captured were greater than the other two *Hylastes* spp., which is unlike previous work (Zanzot 2009) showing *H. tenuis* as the dominant species in longleaf pine stands. However, *H. porculus* and *H. salebrosus* were dominant species in other studies (Bauman 2003, Eckhardt et al. 2007, Sullivan et al. 2003).

Hylastes spp. are less active in summer and winter than in spring and fall (Table 2.3), however, captures of *H. porculus* were less than *H. salebrosus* and *H. tenuis* in summer while greater in winter. Although little is known about the biology and physiology of *Hylastes* spp., it is possible that both the maximum and minimum temperature threshold of *H. porculus* is lower than other two species because *H. porculus* is a northern species (Wood 1982). During the survey period, most of the *H. salebrosus* and *H. porculus* were

consistently collected from panel and flight intercept trap, while *H. tenuis* was captured frequently from pitfall trap. Numbers of *Hylastes* spp. captured is positively correlated with captures of *D. terebrans* which also showed spring and fall peaks in this study. Therefore, *D. terebrans* might be a good indicator of *Leptographium* root infection (Fatzinger 1985).

Hylastes spp. are vectors of ophiostomatoid fungi which contribute to SPD. In this study, more *Hylastes* spp. captured in older stands (Zanzot et al. 2010) provided additional evidence that loblolly pines at age class 40-50 years were more apt to show decline symptoms than younger trees. Futher research on isolating blue-stain fungi from root-feeding *Hylastes* spp. should be considered in those stands in order to better prove loblolly pines are more prone to infest SPD disease although previous studies (Eckhardt et al. 2007, Zanzot et al. 2010) reported that ophiostomatoid fungi were recovered from exoskeletons of *H. salebrosus* and *H. tenuis*.

Crown conditions such as live crown ratio, live crown density, and crown light were associated with higher captures of *Hylastes* spp. However, live foliage transparency had no correlation with collections of *Hylastes* spp., which is in contrast with the study conducted by Menard (2007) and Thompson (2011). Higher percentage of live crown ratio, crown density and foliage exposure to light generally indicates vigorous loblolly pines (Schomaker et al. 2007). Thus, crown variables may not be a good indicator to

estimate initial populations of root-feeding bark beetle species as no symptoms are present until significant root damage occurs.

Chapter Three

Factors Associated with Incidence of Ophiostomatoid Fungal Species Contributing to Southern Pine Decline

3.1 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.

3.2 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 Wingfield 2001, 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.

3.3 Methods and Materials

3.3.1 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.

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)

3.3.2 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).

3.3.3 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.

3.3.4 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 × 2 cm) (six cores per tree) were collected using an 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.

3.4 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; $y = {}^{\Phi-1}((r_i\text{-}3/80/ (n+1/4))]$.

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 $\leq$ 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.

3.5 Results

3.5.1 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.

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

Plot	Age	Elevation (m)	Slope (%)	Aspect (°)	Convexity	Topographic position
WV 1	16	121	22	350	Convex	Side-slope
WV 2	16	100	18	270	Convex	Side-slope
WV 3	16	124	16	0	Convex	Side-slope
WV 4	19	107	14	315	Convex	Side-slope
WV 5	18	106	8	315	Convex	Side-slope
WV 6	18	101	26	80	Convex	Ridge-top
WV 7	51	102	5	45	Convex	Ridge-top
WV 8	52	114	9	75	Convex	Ridge-top
WV 9	51	113	28	225	Convex	Side-slope
SS 1	18	247	19	90	Convex	Toe-slope
SS 2	18	210	4	315	Concave	Toe-slope
SS 3	18	254	19	315	Convex	Nose-slope
SS 4	26	253	3	135	Convex	Nose-slope
SS 5	26	245	4	90	Convex	Toe-slope
SS 6	26	239	3	315	Flat	Ridge-top
SS 7	26	265	2	225	Flat	Toe-slope
SS 8	26	258	5	45	Concave	Toe-slope
SS 9	26	265	1	0	Flat	Side-slope
WEY 1	13	94	13	298	Convex	Toe-slope
WEY 2	13	116	2	0	Convex	Ridge-top
WEY 3	13	93	13	245	Convex	Ridge-top
WEY 4	28	121	30	225	Convex	Side-slope
WEY 5	28	127	6	270	Convex	Side-slope
WEY 6	13	131	3	0	Convex	Ridge-top
WEY 7	30	106	6	248	Convex	Ridge-top
WEY 8	30	130	18	340	Convex	Side-slope
WEY 9	30	131	10	270	Convex	Side-slope
F&W 1	17	128	25	205	Convex	Side-slope
F&W 2	17	141	6	200	Convex	Side-slope
F&W 3	17	132	8	320	Convex	Side-slope
F&W 4	24	150	6	315	Convex	Ridge-top
F&W 5	20	119	11	30	Convex	Toe-slope
F&W 6	23	109	19	135	Convex	Side-slope
F&W 7	32	94	1	0	Flat	Side-slope
F&W 8	23	111	8	150	Convex	Side-slope
F&W 9	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	30	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.

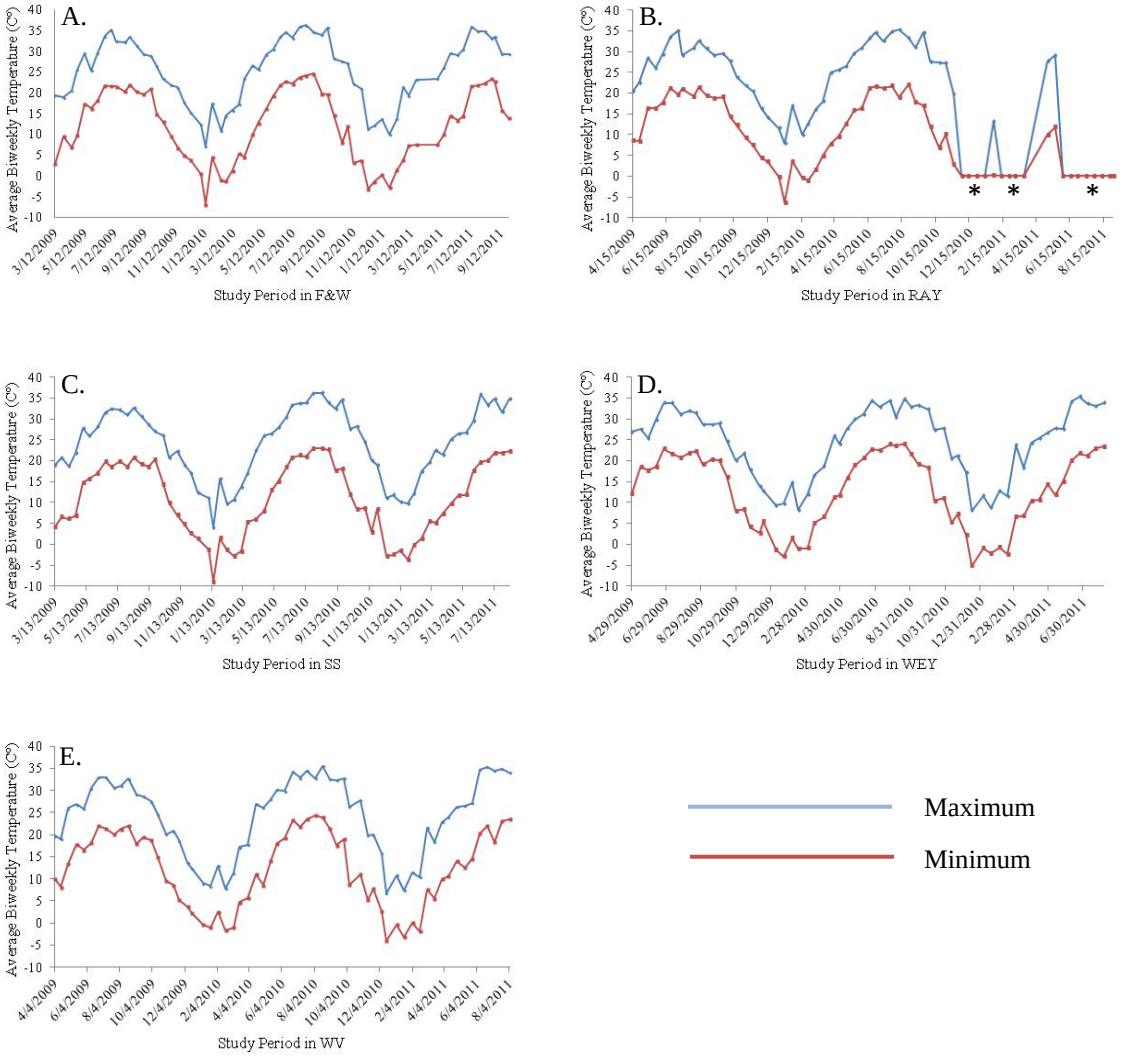

Fig. 3.1. Average biweekly maximum and minimum temperature in study sites. (A) Biweekly average temperature in F&W site. (B) Biweekly average temperature in RAY site. * Indicates no records from the weather station. (C) Biweekly average temperature in SS site. (D) Biweekly average temperature in WEY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

3.5.2 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).

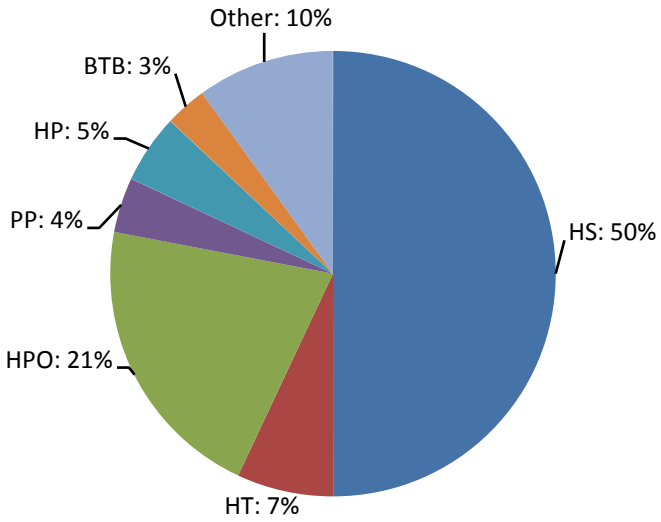

Fig. 3.2. Percentage of bark beetles and weevils captured in loblolly pine stands using pitfall, panel, and flight intercept traps in Alabama and Georgia (BTB-*D. terebrans*; Hpo-*H. porculus*; Hs-*H. salebrosus*; Ht-*H.tenuis*; PP-*P. picivorus*; Hp-*Hb. pales*. Other species included *D. frontalis*;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

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

(Continued)

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

3.5.3 Fungal Isolations among Sites

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.

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
FW	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.

3.5.4 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).

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.

Fungi Species	Age Class (yr)			
	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

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

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.

Fungi Species	Slope Class (%)			
	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.

Fungi Species	Aspect Class (°)			
	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 ratio; CL = crown light; CD = crown density; FT = foliage transparency.

3.5.5 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.

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*

df = 3, 8.

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

Study Sites	Treatment	
	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.

3.6 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.

Chapter Four

Conclusions

4.1 Pathogen-vectoring Root-feeding *Hylastes* Species

Root-feeding *Hylastes* spp. are active throughout most of the year but are less active in the summer and winter than in they are in the spring and fall. In the current trails, *H. salebrosus* were captured in higher numbers than the other two important root feedling *Hylastes* spp. These root-feeding *Hylastes* speices are vectors of ophiostomatoid fungi which have been shown to contribute to SPD. In this study, more *Hylastes* spp. were captured in older stands (Zanzot et al. 2010) and provided additional evidence that *P. taeda* at age class 40-50 years were more apt to show decline symptoms than younger trees. Crown conditions such as live crown ratio, live crown density, and crown light were associated with higher captures of *Hylastes* spp. However, the other crown variables did not correlate with any insect species captured. Therefore, crown variables are not a good indicator to estimate initial populations of root-feeding bark beetle species as no above-ground symptoms are present until significant root damage occurs. This may be years after a stand management treatment, thus an early warning system is still needed to rand stand risk to SPD.

Pathogen-vectoring root-feeding beetles (*H. salebrosus*, *H. porculus* and *H. tenuis*) were captured in higher numbers in recently thinned *P. taeda* stands than unthinned stands which could further increase innoculations of ophiostomatoid fungal species involved with SPD. Mechanical thinning in forest stands causes both visible damage to residual trees and invisible damage to root systems. For example, large branches were broken, and some bark of remaining trees was removed. In this case, semio-chemicals such as turpentine and alpha-pinene released from wounds and stumps attract more root-feeding *Hylastes* spp. Unlike the thinning treatments, the harvesting treatment did not significantly increase captures of the root-feeding *Hylastes* spp. Since there are no reports concerning *Hylastes* spp. attacking pine seedlings in the United States, survival of seedlings after ouplanting not be an issue if landowners choose to reforest the curover site.

4.2 The Incidence of Ophiostomatoid Species in *P. taeda* stands

Mechanical thinning increased blue-stain fungi incidence in loblolly pine stands, which could futher increase the occurance of SPD becoming a stand management issue in those stands. Higher populations of *Hylastes* spp. captured in thinned stands may lead to more inoculations or introductions of ophiostomatoid fungi into loblolly pine roots. The 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 in central Alabama and Georgia. *Leptographium procerum* and *L. terebrantis* were consistently isolated at a greater frequency in stands

when compared to recovery of *G. alacris*, *G. huntii*, and *O. ips*. However, stands in the 40 + age class had significantly more *O.ips* recovered than the other age classes which correlated with more insect captures in older stands. In addition, slope class greater than 15% had greater recovery rates of *L. terebrantis*. However, the S/ SW aspect did not have an increase in incidence of stain fungi as would be predicted by the SPD model.

There was no correlation between the incidence of ophiostomatoid species recovered from root systems and any of the crown conditions measured in any of the treated stands. It is possible that symptoms would not be observed in stands even though there is a presence of ophiostomatoid fungi in the root systems. Therefore, it would be difficult to predict stand infection until declining symptoms are observed.

4.3 Potential Future Research

Although mechanical thinning did have an effect on the number of insect captures, future study may focus on how to thin to minimize *Hylastes* infestation that results in the development of SPD over time. For example, plant less dense and reduce thinning in high hazard areas. Because larvae of *Hylastes ater* Paykull could take up to 300 days to develop to maturity in the log sections, and adult beetles merge to and feed on seedlings which are planted immediately (Reay et al. 2012). To date, there have been no reports and this study does not indicate the ability of either *H. salebrosus*, *H. porculus*, or *H. tenuis* to attack pine seedlings after planting. However, therefore, future research

monitoring Hylastes populations should consider setting up thermometers in study sites, and study beetles in the lab to better understanding their biology and physiology to help predict the population changes.

Mechanical thinning increased blue-stain fungi incidence in *P. taeda* stands, however, further research on isolating blue-stain fungi from root-feeding *Hylastes* spp. should be considered in thinned stands in order to better show *P. taeda* are more prone to infestation of SPD disease after thinning treatments. Additionally, although there were no declining symptoms observed immediately after recent thinning, future research may keep focusing on crown class changes related to the time lag between thinning, insect vector, and fungi recovery rate over time.

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