

Impact of *Imperata cylindrica* on Populations of Root-feeding Bark Beetle Populations and Factors Associated with Loblolly Pine Decline in a *Pinus taeda* Stand

by

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Abstract

The non-native, invasive plant, cogongrass (*Imperata cylindrica* (L.) Beauv) is a threat to the diversity of native plant species of the southeastern United States. Another issue facing landowners of southeastern forests is Loblolly Pine Decline (LPD). The factors associated with LPD include a complex of abiotic and biotic stressors that cause economically significant premature mortality in pine forests. A suite of root-feeding bark beetles associated with LPD could potentially have higher populations in areas containing cogongrass due to additional stresses attracting them. This is focused on determining if cogongrass is causing an increase in populations of root-feeding bark beetle populations, altering soil properties that could potentially be contributing to LPD, and the affects cogongrass is having on the tree vigor of loblolly pine (*Pinus taeda* L.). Twenty plots were established in loblolly pine stands in southeastern Mississippi with ten plots located in areas with cogongrass infestation (CO plots) and ten located in areas without cogongrass infestation (NCO plots). Comparisons of insect populations, soil properties, and tree vigor measurements between CO and NCO plots were made. Results suggest soil conditions were more conducive to fine root growth for loblolly pine but significantly less pine fine roots were observed in CO plots suggesting that cogongrass is outcompeting the pine roots through its extensive rhizome system. Cogongrass could be causing stress to establish pine stands through competition with the pine root system and increased stress could possibly lead to an increase in LPD associated bark beetle species populations. Continued research should be performed to see if cogongrass' impact is compounding as stand age increases.

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Chapter One

Introduction and Review of Literature

1.1 Loblolly Pine

Loblolly pine (*Pinus taeda* L.) (Figure 1.1) was once just a minor pine species native to the southeastern United States prior to European settlement. Now loblolly pine is the major timber species planted in the southern United States, where it predominated on 45% (13.4 million ha) of commercial forest land in 1989 (Shultz, 1999) and covers approximately 80% of southern U.S. commercial timberlands according to (Smith et al., 2007). In the late 1800's and early 1900's, native longleaf pine was harvested for conversion to agriculture for production consisting mainly of cotton. The migrations of the boll weevil (*Anthonomous grandis* Boheman) to the United States lead to severe crop damage and abandonment of piedmont and coastal plain regions of the South (Shultz, 1997). Most of these lands had been farmed for extensive periods without the use of chemical fertilizers and implementation of erosion control (Trimble, 1974), which contributed to the land becoming highly depleted of nutrients and eroded. Early nursery operations and outplantings demonstrated that loblolly pine could be grown rapidly on many diverse sites and artificially regenerated on a large scale (Shultz, 1999). Loblolly pine volume increased dramatically from roughly 0.6 billion m³ in the late 1940's (Wahlenburg, 1960) to 1.4 billion m³ in 1989 (Shultz, 1999). Loblolly pine now encompasses over 50% of the dominant and co-dominant growth-stock in the southeastern United States (Shultz, 1997), where it mostly grows on poor and eroded sites.



Figure 1.1. *Pinus taeda* L.

1.2. Forest Decline

1.2.1. General Concepts

Paul Manion defines decline as it pertains to forests as “an interaction of interchangeable, specifically ordered abiotic and biotic factors to produce a gradual general deterioration, often ending in the death of trees” (Manion, 1991). Unlike many diseases, declines are less understood and difficult to explain. Many theories have been developed since the 1960’s to help understand and explain forest declines. One well-known theory is the concept of predisposing, inciting, and contributing factors theory developed by Wayne Sinclair (Sinclair and Hudler, 1988).

Predisposing factors impose an underlying, constant stress, inciting factors act as a secondary stressor that can force the tree into a state of poor health, and contributing factors ultimately cause premature mortality of the tree. Declines are typically apparent as a series of symptoms beginning with foliage chlorosis, followed by thinning crowns, branch dieback, reduced annual increment, and ultimately premature mortality (Marion, 1991).

1.2.2. World Declines

Declines affecting many tree species have been observed and studied throughout the world. For example, both silver fir (*Abies alba* Mill.) and Norway spruce [*Picea abies* (L.)] exhibited needle yellowing because of drought and mineral nutrient deficiencies, as well as increasing air pollution (; Krahl-Urban et al., 1988; Bruck, 1989; Kandler and Miller, 1991; Ke and Skelly, 1990). Ciesla and Donaubaue (1994) reported signs of decline on *Eucalyptus* spp. in Brazil. Several important hardwood species in North America have been found to have declining symptoms including ash (*Fraxinus* spp.), birch (*Betula* spp.), maple (*Acer* spp.), and oak (*Quercus* spp.)(Tainter et al., 1983; Castello et al., 1985; Becker et al., 1989). Also, declines have been studied on several coniferous species in North America including pole blight of western white pine (*Pinus monticola* Dougl.)(Leaphart and Copeland, 1957), ponderosa pine decline (*Pinus ponderosa* Laws.)(Livingston and Mangini, 1981), littleleaf disease of shortleaf pine (*Pinus echinata* Mill.)(Campbell and Copeland, 1959), and loblolly pine decline of loblolly pine (*Pinus taeda* L.)(Eckhardt et al., 2007).

1.2.3. Loblolly Pine Decline

1.2.3.1. History of Loblolly Pine Decline (LPD)

Loblolly pine decline (Figure 1.2) was reported as loblolly pine die-off by Brown and McDowell (1968) in the southeastern United States in the Oakmulgee Ranger District on the Talladega National Forest in 1959. Loblolly Pine Decline(LPDP) was first diagnosed as littleleaf disease due to similar symptomology (Campbell and Copeland, 1954). Symptoms were found to be sparse crowns, reduced radial growth, deterioration of fine roots, decline, and mortality by age 50 (Lorio 1966; Hess et al.1999, 2001, 2002; Eckhardt et al., 2007). *Phytophthora cinnamomi* Rands was discovered to be consistently associated with roots of declining shortleaf pine which

lead to the description of littleleaf disease in shortleaf pine (Campbell and Copeland, 1954). After early studies by Brown and McDowell (1968) of “loblolly pine die-off”, *P. cinnamomi* was not associated with the roots of declining loblolly. Root deterioration of declining loblolly pine was found to be more severe than observed in littleleaf disease (Roth, 1954) but observers were unable to determine the causal factor. Littleleaf disease is found mostly on sites that have heavy, poorly drained soils, where *P. cinnamomi* is known to have better growth and reproduction. These soils were mostly associated with the Piedmont Plateau region so in order to more decisively determine the causal factor in “loblolly pine die-off” Eckhardt et al. (2007) plots were established in central Alabama in three physiographic regions. Root-inhabiting ophiostomatoid fungi and their insect vectors were found to correlate with the symptomology of declining loblolly pine across all physiographic regions (Eckhardt et al., 2007). Predisposing factors that were found to be associated with declining stands of loblolly pine included increased slope and south to southwest facing aspects (Eckhardt and Menard, 2008). The soil types found to be associated with increased susceptibility to loblolly pine decline were sandy loam or sandy clay loam soils (Eckhardt et al., 2007) but, tree age, topography, and organic matter content were found to be more associated with loblolly pine decline than soil type.



Figure 1.2. Loblolly pine showing symptomology of Loblolly Pine Decline
(Andrew J. Boone, South Carolina Forestry Commission, Bugwood.org)

1.2.3.2. Insect Vectors

Two bark beetles species (Coleoptera: Curculionidae: Scolytinae) *Hylastes salebrosus* Eichhoff (Figure 1.3) and *Hylastes tenuis* Eichhoff (Figure 1.4) , as well as two weevil species (Coleoptera: Curculionidae: Molytinae) *Hylobius pales* Herbst (Figure 1.5) and *Pachylobius picivorus* Germar (Figure 1.6), were found to be associated with LPD (Eckhardt et al., 2007). Miller and Rabaglia (2009) found that baiting traps with ethanol and (-)- α -Pinene attracted *Hylastes salebrosus* and *Hylastes tenuis*, as well as *Hylastes porculus* Erichson and *Dendroctonus terebrans* Olivier. *Hylastes* spp. are known root-feeding bark beetles that attack unhealthy, declining, and wounded pines (Wood, 1982; Klepzig et al., 1991,1995; Jacobs and Wingfield, 2001) and create galleries and oviposition in roots (Matusick et al., 2013). The subfamily Molytinae are a group of weevils described by Carl Johan Schönherr (Arnett Jr. et al., 2002).

Species in this subfamily share a large, hook-like apical tooth on the hind tibia, or have various modifications to the apex of the hind tibia. Together, *Pachylobius picivorus* and *Hylobius pales* are commonly known as pine regeneration weevils. They often cause extensive mortality of artificial seedling regeneration of pines (Edmonds et al., 2000) and also are known to oviposition in roots (Drooz, 1985). Each of these insects found to be associated with LPD also frequently vector root-inhabiting ophiostomatoid fungal associates with anamorphs in the genus *Leptographium* (Eckhardt et al., 2007).

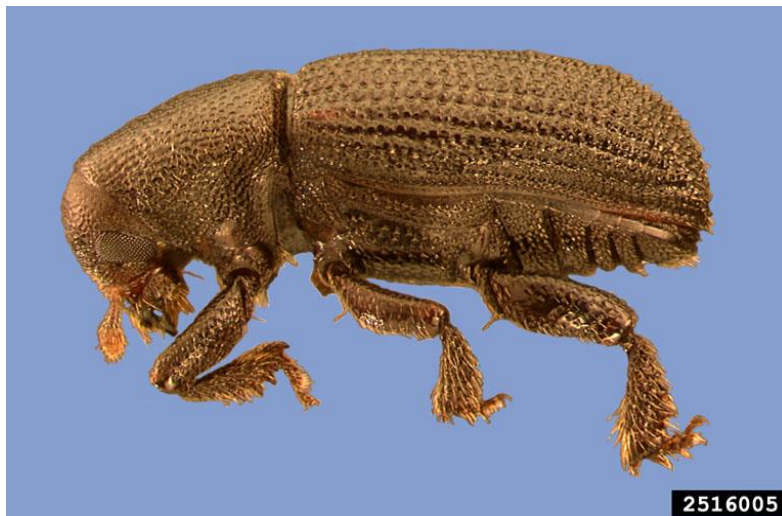


Figure 1.3- *Hylastes salebrosus*
(David T. Almquist, University of Florida , forestryimages.org)



Figure 1.4- *Hylastes tenuis*

(J.R. Baker & S.B. Bambara, North Carolina State University, Bugwood.org)



Figure 1.5- *Hylobius pales*

(Wayne N. Dixon, Florida Department of Agriculture and Consumer Services, Bugwood.org)



Figure 1.6. *Pachylobius picivorus*
(Jennifer C. Giron Duque, University of Puerto Rico, Bugwood.org)

1.2.3.3. Fungal Associations

Bark beetles are well known vectors of ophiostomatoid fungi including species of *Ophiostoma*, *Grosmannia*, and *Ceratocystis* (Six et al., 2011). *Leptographium* species mainly associated with root-colonizing bark beetles and weevils that attack living trees have been found to be associated with decline and mortality of pine (Harrington, 1983; Klepzig et al., 1991; Ostrosina et al., 1997; Eckhardt et al., 2004). Ostrosina et al. (1997) and Hess et al. (1999) reported that symptomatic loblolly pine stands in the southeastern United States are more susceptible to attack by southern pine beetle and more likely to contain *Leptographium* spp. within their root systems than asymptomatic stands. *Leptographium procerum* (W.B. Kendr.) M.J. Wingf was found to be the associated fungus of white pine root decline that has symptoms of decreased shoot growth, delayed bud break, needle wilt, exudation of resin from the root collar area, and resin soaking of affected wood tissue (Sinclair and Hudler, 1980; Swai and Hindal, 1981). Distribution of *Leptographium* species are distributed worldwide where appropriate hosts are found (Jacobs and Wingfield, 2001) and most are found to inhabit conifer

species, with a few known to colonize hardwood species (Jacobs et al., 2006). In the southeastern United States, *Leptographium procerum*, *L. terebrantis* Barras and Perry, *Grosmannia alacris* T.A. Duong, Z.M. de Beer & M.J. Wingf. sp. nov. (formerly *L. serpens* (Goid.) Siemaszko), *L. truncatum* M.J. Wingf. & Marasas) M.J. Wingf, and *G. huntii* (R.C. Rob. Jeffer.) Zipfel, Z.W. de Beer & M.J. Wingf (formerly *L. huntii* M.J. Wingf). have been isolated from the roots and soil associated with *P. taeda* that exhibited decline symptoms (Eckhardt et al., 2007; Jacobs and Wingfield, 2001; Zanzot et al., 2010).

1.3 Cogongrass

1.3.1. Background

Cogongrass (*Imperata cylindrica* (L.) P. Beauv) (Figure 1.7) is a grass species native to Asia (Dozier et al. 1998) and was accidentally introduced near Grand Bay, Alabama circa 1911 via packing material of satsumas from Japan (Dickens, 1974; Tabor, 1952). Cogongrass was also introduced intentionally in 1921 as potential forage material in Mississippi (Hubbard, 1944; Dickens and Buchanan, 1975), Alabama, Florida, and Texas (Hubbard, 1944; Dickens and Moore, 1974). Current estimates suggest that more than 500,000 hectares are infested by cogongrass in Florida, Alabama, and Mississippi (MacDonald, 2004) and over 500 million hectares worldwide (Holm et al., 1977; Dozier et al. 1998).



Figure 1.7. *Imperata cylindrica* in a loblolly pine stand

1.3.2. Taxonomy

Cogongrass is found in the grass family Poaceae, tribe Andropogoneae, subtribe Saccharine. The genus *Imperata* has nine species worldwide and is considered to be of major importance due to the species *I. cylindrica* (Gabel, 1982). Other species in this genus includes *I. conferta* (J. Presl) Ohwi, *I. contracta* (Kunth) Kuhlm., *I. brevifolia* Vasey, *I. brasiliensis* Trin., *I. tenius* Hack., *I. cheesemanii* Hack., *I. condensate* Steud., and *I. minutiflora* Hack. Both *I. brasiliensis* and *I. brevifolia* are found within the United States along with *I. cylindrica*. *Imperata brevifolia* (California satintail) is native to California and its range extends east to Texas. This species is considered a Class B¹ noxious weed in California. *Imperata brasiliensis* (Brazilian satintail) is native to southwestern North America, Central America, and South

¹ Any pest of known economic or environmental detriment and, if present in California, it is of limited distribution. B-rated pests are eligible to enter the state if the receiving county has agreed to accept them. If found in the state, they are subject to state endorsed holding action and eradication only to provide for containment, as when found in a nursery. At the discretion of the individual county agricultural commissioner they are subject to eradication, containment, suppression, control, or other holding action.
http://www.cdfa.ca.gov/plant/ipc/encycloweedia/winfo_weedratings.htm

America. Brazilian satintail can also be found in the southeastern United States and can often be confused with cogongrass. Although Brazilian satintail is not considered as serious of a threat as cogongrass, it is still considered a Class A noxious weed in Alabama², North Carolina², and Vermont², as well as, a noxious weed in Florida and Mississippi. Brazilian satintail has also been found to hybridize with cogongrass but, the extent of that hybridization is unknown. Cogongrass is characterized by having two anthers, whereas other *Imperata* species only have one (Gabel, 1982; Hitchcock, 1951). Hall (1978) does not separate *Imperata cylindrica* and *Imperata brasiliensis*, reporting single populations with both one and two anthers.

Within the species *I. cylindrica*, there are five varieties as reported by Hubbard et al., (1944) and Santiago (1980) to include *I. cylindrica* var. *europaea* (Andersson) Asch. and Graebn., *I. cylindrica* var. *major* (Nees) C. E. Hubb, *I. cylindrica* var. *africana* (Andersson) C.E. Hubb., *I. cylindrica* var. *latifolia* (Hook. f.) C.E. Hubb, and *I. cylindrica* var. *condensate* (Steud.) Hack. These different varieties were separated based on growth, geographic origin, and morphological characteristics. According to King and Grace (2000), *Imperata cylindrica* var. *major* is the variety of cogongrass that was introduced to the United States.

1.3.3. Biology

Cogongrass is a rhizomatous, perennial, warm season grass species found throughout subtropical and tropical regions that range in annual rainfall from 750 to 5000 mm (Holm et al., 1977; Bryson and Carter, 1993). Cogongrass is characterized by having a dense rhizome system which allows the plant to spread asexually. Sajise (1976) found that cogongrass rhizomes

² Class A - Any noxious weed on the Federal Noxious Weed List, or any noxious weed that is not native to the State, not currently known to occur in the State, and poses a serious threat to the State.
<http://www.alabamaadministrativecode.state.al.us/docs/agr/McWord10AGR14.pdf>
<http://www.eddmaps.org/ipane/ipanespecies/noxleg.htm>
http://www.watershedmanagement.vt.gov/lakes/docs/ans/lp_weedquarantine.pdf

comprise greater than 60% of the total plant biomass. This results in a high root to shoot ratio which allows for the survival of the plant following a disturbance such as fire or harvest.

Cogongrass is also a prolific seed producer, producing as many as 3000 seeds per plant (Holm et al., 1977). Seed germination has been found to be 95% (Santiago, 1965) and 98% (Shilling et al., 1997) when spiklet fill was adequate but have low survival (about 20%) one year after germination (MacDonald, 2004). Studies show that rhizome fragments are the main form of local spread but seed contributed more long distance spread to isolated areas (Patterson et al., 1980; Patterson and McWhorter, 1980, Willard et al., 1990).

Another identifying feature of cogongrass is the offset-midrib of its slender, flat, and linear-lanceolate leaves (Holm et al., 1977; Hall, 1978; Terry et al., 1997). Holm et al., (1977) reported that amphistomatus cogongrass leaves under favorable conditions of moisture and fertility can reach heights of 1.5 meters. Cogongrass is noticeably a stemless plant apart from the flowering stalk and grows in loose-to-compact tufts from the underground rhizomes (MacDonald, 2004).

1.3.4. Impact

Cogongrass is considered one of the top ten worst weeds of the world and over 73 countries consider cogongrass a nuisance (MacDonald, 2004). In Asia, cogongrass is a major weed to tea, rubber, pineapple, coconut, oil palm, and other perennial plantation crops in Asia, while a major pest in agronomic production in Africa (Ivens, 1980). Cogongrass has been found to be a problem when trying to establish crops, including trees, in many countries by directly competing with the crop for light, water, and nutrients (MacDonald, 2004). In a study by Daneshgar et al. (2008), cogongrass was found to significantly affect the survival and growth of establishing loblolly pine in cogongrass infested plots versus native competition and vegetation-free plots. Cogongrass has several survival mechanisms that make the plant a successful invader:

an extensive rhizome system, tolerance to drought, ability to grow on a wide range of soils, prolific seed production, adaptation to fire, and high genetic plasticity (Hubbard et al., 1944; Holm et al., 1977; Brook, 1989; Dozier et al., 1998).

In some areas the densities of cogongrass rhizomes have been estimated to be over 4.5 million shoots per hectare (Soerjani, 1970), this high density of rhizomes makes cogongrass a mechanical interference of native plant growth (Daneshgar et al., 2008). In addition, the sharp rhizome tips, may penetrate the root system of other established plant species, causing infection or mortality (Eussen and Soerjani, 1975). Cogongrass has been found to be allelopathic in tests on tomatoes (*Solanum lycopersicum* [L.]) and cucumbers (*Cucumis sativus* [L.]) particularly at low pH (Eussen and Wirjahardja, 1973). *Cynodon dactylon* (L.) and *Lolium multiflorum* (Lam.) germination was found to be inhibited by up to 62% by concentrations of cogongrass extract as low as 0.5% in a study by Koger and Bryson (2003).

Cogongrass has been found to occupy a wide range of soils ranging from coarse sands of shorelines to greater than 80% clay soils but, has been found to grow best in acidic soils with low fertility and organic matter (MacDonald, 2004). Cogongrass has also been found to exhibit extreme efficiency in nutrient uptake (Saxena and Ramakrishnan, 1983). Cogongrass was found to hold more nitrogen per hectare in its rhizome system than native vegetation (Daneshgar and Jose, 2009), leading to a shift in the nitrogen pool from above ground to below ground. Brook (1989) found that cogongrass has a mycorrhiza association which can assist the cogongrass in invading unfertile sites. Brewer and Cralle (2003) also suggested that cogongrass better competes for phosphorus than native vegetation in pine- savanna species in the southern United States.

Cogongrass is able to form large monotypic expanses which can alter fire regimes of forests, produce hotter temperatures and higher flame lengths than native vegetation (Holzmueller and Jose, 2011, Lippincott, 2000). Lippincott (2000) observed that swards of cogongrass could reach greater heights and higher temperatures (e.g., 450°C) than those of native vegetation. Lippincott (2000) also suggested that frequent intense fires could convert a pine savanna into cogongrass-dominated grassland since cogongrass allocates a significant amount of carbon to its rhizome system, enabling it to recover quickly after fires. A study by King and Grace (2000) showed that cogongrass was able to germinate, survive, and grow regardless of disturbance type (e.g., burning, mowing, vegetation removal, and tilling) and gap size in wet pine savanna of Florida.

1.3.5. Control

Successful control of cogongrass has been documented through the use of herbicides (Willard et al., 1996, 1997) but Shilling et al. (1995) found that without establishment of desirable and competitive species, remnant rhizomes will eventually allow cogongrass to re-infest treated areas. Willard et al. (1996) also found that disking twice with no other CO reduced rhizome biomass by 66% but, Gaffney (1996) found that rhizomes with the shoot apices removed still produced 31% more shoots along the length of the rhizome weeks after cutting. Due to its extensive rhizome biomass, cogongrass can easily recover from control attempts and multiple control applications are necessary in order to successfully eradicate cogongrass from an area. Ramsey et al. (2003) concurred with previous research that suggests that multiple herbicide applications are needed annually in order to have complete control over a cogongrass infestation. Tillage or disking can be effective in open pastures or crop areas if repeated multiple times during the growing season but this practice may not be suitable for areas with established crops

or trees e.g. pine plantation. Glyphosate and imazapyr have been found to be the only two active herbicide ingredients that are consistently effective on cogongrass (Miller and Enloe, 2009).

Correct application of a mixture containing water and one or a combination of the two active ingredients just mentioned can effectively control cogongrass infestations in open areas as well as areas where mechanical control is limited (Miller and Enloe, 2009; Shilling et al., 1997).

Several insects have been reported to cause damage to cogongrass throughout the world. Vayssiere (1957) reported several species in Malaysia and Bryson (1985, 1987) noted three species of the North American skipper butterfly (Lepidoptera: Hesperidae) that consumed cogongrass but were not considered biocontrol agents due to their lack of host specificity. The gall midge (*Orseolia javanica* Kieffer and van Leeuwen-Reijnvaan) is reportedly host specific to cogongrass (Mangoendihardjo, 1980; Soenarjo, 1986) but are known to be highly parasitized (Van Loan et al., 2002) which limits their effectiveness as biocontrol agents. Other known potential agents for biological control of cogongrass have included several fungi, nematodes, mites, elaterid and scarab beetles, skipper butterfly and moth caterpillars, and grasshoppers found in Florida (Minno and Minno, 1998).

Chapter Two

Impact of Cogongrass (*Imperata cylindrica*) on Populations of Root-feeding Bark Beetle Species Associated with Loblolly Pine Decline

2.1 Abstract

The non-native, invasive grass, *Imperata cylindrica* (L.) Beauv, commonly known as cogongrass, is an increasing threat to the diversity of native plant and wildlife species of the southeastern United States. Another issue facing landowners of southeastern forests is loblolly pine decline. The factors associated with loblolly pine decline include a complex of abiotic and biotic stressors that cause economically significant premature mortality in pine forests. A suite of root-feeding bark beetles, which vector pathogenic ophiostomatoid fungi, are attracted to stressed pines. *Imperata cylindrica* could be inducing additional stresses, leading to higher infestations of root-feeding bark beetles and contributing to loblolly pine decline. To determine the effects of cogongrass on root-feeding bark beetles, 20 plots were established in a loblolly pine plantation located in southeastern Mississippi (10 with *I. cylindrica*/10 without *I. cylindrica*). Insect traps on each plot were checked bi-weekly for 24 months to observe insect populations over time. Insect collections indicated that *Hylastes salebrosus* is the most abundant species and had consistently higher populations in *I. cylindrica* plots, but were not significantly different between plots with and without cogongrass. *Dendroctonus terebrans* had higher populations in *I. cylindrica* plots. *Hylastes porculus* and *Hylobius pales* had similar trends but

plots with and without were not different. *Pachylobius picivorus* had significantly higher populations in plots without *I. cylindrica*. *Hylastes tenuis* had a similar trend to *P. picivorus* but cogongrass had no effect on populations. Based on these trapping studies it appears that cogongrass does not increase insect populations associated with loblolly pine decline.

2.2. Introduction

Loblolly pine decline (LPD) is a stress-induced decline complex that involves root-feeding bark beetle and weevil species that vector root-inhabiting ophiostomatoid fungal species (Eckhardt et al., 2007). Eckhardt et al. (2004) found that these root-inhabiting fungi and their insect vectors correlated with the symptomology of LPD across multiple physiographic regions in central Alabama. Eckhardt and Menard (2008) found that increased slope and south to southwest facing aspect were predisposing stress factors associated with symptomology of LPD. Sandy loam or sandy clay loam soils were also found to be associated with increased susceptibility to LPD but tree age, topography, and organic matter content had a higher association to LPD than soil type (Eckhardt *et al.*, 2007). Some abiotic factors associated with LPD include environmental stressors such as drought and wind damage as well as mechanical damage from management activities such as thinning operations or prescribed burning.

The non-native, invasive grass *Imperata cylindrica* (L.) Beauv. , more commonly known as cogongrass, is becoming an increasing threat to the diversity of native plant and wildlife species of the southeastern United States. Cogongrass is estimated to infest between 500,000 and 1,000,000 acres in Florida, Alabama, and Mississippi (Faircloth et al., 2005). Due to rapid expansion through rhizomatous reproduction and fire adapted physiology, *I. cylindrica* is becoming an important threat to pine plantations of the southeastern United States (Daneshgar et al., 2008). Studies have found that *I. cylindrica* drastically reduced loblolly pine (*Pinus taeda* L.)

and longleaf pine (*Pinus palustris* Mill.) regeneration in infested sites. Daneshgar et al. (2008) found that *I. cylindrica* competition resulted in only 26% survivability of loblolly pine regeneration as compared to 52% survivability in native vegetation competition. Seedlings growing in *I. cylindrica* competing vegetation had significantly smaller root collar diameters after the first growing season. Lippincott (2000) found that fire induced mortality in juvenile longleaf pine was higher in *I. cylindrica* invaded sites as compared to sites containing native vegetation.

Due to the high invasiveness of *I. cylindrica*, many studies such as the ones previously mentioned have documented the impact(s) that this weed species places upon plant species throughout the world. Since its introduction to the southeastern United States and subsequent spread into forested areas, research has evaluated the impact *I. cylindrica* has on the regeneration of southern pine species, but few have looked at its impact that *I. cylindrica* is having to on established, more mature pine. There are even fewer studies looking at the impact of *I. cylindrica* on populations of insects, more specifically, bark beetle populations. *Imperata cylindrica* could be causing additional stresses to loblolly pine roots and consequently increasing root-feeding bark beetle populations to infested sites. This study was instigated to test the hypothesis that *Imperata cylindrica* is increasing the population of root-feeding bark beetle species associated with LPD.

2.3. Materials and Methods

The research site was located on The Westervelt Co. land east of Leakesville, Greene County, Mississippi (N 31.147755, W -88.458567). Twenty plots were established by visually assessing *I. cylindrica* presence and density with 10 plots established in areas containing heavy infestation of *I. cylindrica* (Figure 2.1) and the remaining ten plots were established in areas

containing no *I. cylindrica* (Figure 2.2). Plots without *I. cylindrica* competition mainly consisted of an open understory containing sparse patches of native grasses and scattered woody plants with a few patches *Rubus* spp. being dense. Some species found in the understory of these plots included American beautyberry (*Callicarpa americana* L.), blackberry (*Rubus* spp.), green briar (*Smilax rotundifolia* L.), yaupon (*Ilex vomitoria* Aiton) and winged sumac (*Rhus copallinum* L.). Plot boundaries were marked by placing metal poles at each corner of a square with each side being 15.24 m (50 ft.) in length. Center plot was determined by placing a metal pole half the distance diagonally across the plot. A metal tag was attached to each center pole denoting the plot identification. CO plots were labeled as C1- C10 while control plots were labeled NC1- NC10. The location (Table 2.1) and elevation of each center pole was mapped using a handheld geographical positioning system device (Garmin GPSMAP 76Cx, Garmin International Inc., Olathe, KS). Each tree within each plot was assigned a number and a metal tag with this number was attached to the tree at breast height (137 cm from ground level).



Figure 2.1. *Imperata cylindrica* infested plot.



Figure 2.2. Plot containing no infestation of *Imperata cylindrica*.

Table 2.1. Plot locations in Greene County, Mississippi

Plot	Location	
C1	N 31.14768	W 88.48222
C2	N 31.14788	W 88.48177
C3	N 31.14896	W 88.48191
C4	N 31.14804	W 88.47518
C5	N 31.14785	W 88.48038
C6	N 31.14884	W 88.48322
C7	N 31.14684	W 88.47682
C8	N 31.14850	W 88.47382
C9	N 31.14952	W 88.47441
C10	N 31.14819	W 88.47145
NC1	N 31.15198	W 88.48292
NC2	N 31.15067	W 88.48293
NC3	N 31.14714	W 88.47630
NC4	N 31.14825	W 88.48052
NC5	N 31.15009	W 88.48226
NC6	N 31.14842	W 88.48200
NC7	N 31.14861	W 88.48422
NC8	N 31.14839	W 88.48536
NC9	N 31.14896	W 88.48483
NC10	N 31.14935	W 88.48349

Traps for monitoring bark beetles were installed on each plot to trap and collect bark and ambrosia beetles as well as weevils. The traps used included one panel trap (Figure 2.3) placed one meter north of plot center and one pitfall trap (Figure 2.4) placed one meter south of plot center. The contents of each trap were collected on a bi-weekly basis for two years starting March 2010. Bait for each trap was replenished at the time of each collection.



Figure 2.3. Panel trap



Figure 2.4. Pitfall trap

Panel traps (APTIV Company, Portland, Oregon) are made of black, corrugated plastic panels with a top hood, bottom collecting funnel, collection cup, and a hanging lure opening. Panel traps were suspended from a metal pole approximately two meters from ground level. Two 8-ml glass vials, one filled with turpentine the other filled with 95% ethanol, were used as attractant for the bark beetle and weevil species. A 3-to-1 water to commercial anti-freeze (ethylene glycol) mix was placed in the collection cup to preserve captured insects. During a collection, the contents of the collection cup were poured through a strainer and emptied into a sterile 120 ml polyethylene specimen cup for transporting back to the lab. Collection cups were replaced, refilled with 3-to-1 water to antifreeze mix, and the bait vials refilled after each collection. Pitfall traps were made up of a 20 cm section of 10 cm diameter polyvinyl chloride (PVC) drain pipe with eight equally spaced holes drilled along the circumference (Klepzig et al., 1991). Both ends were capped with a removable PVC end cap. Two drain holes were drilled into the bottom of one end to allow excess moisture to drain. Traps were buried so that the eight holes were positioned just above ground level. Each pitfall trap was baited with an 8 ml vial of

turpentine and an 8 ml vial of 95% ethanol. Two 3 cm long by 1 cm in diameter loblolly pine twigs were also placed into each pitfall trap. A coating of liquid TeflonTM (Northern Products Woonsocket, RI) was applied to the inside of each pitfall trap to ensure captured insects remain inside the trap. Captured insects were transferred to a sterile 120 ml polyethylene specimen cup for transporting back to the lab. At the time of collection, bait vials were refilled, two cut loblolly twigs were replaced, and another coat of liquid Teflon applied to the trap. All collected insects were brought back to the Forest Health Dynamics Laboratory (Auburn University, Auburn, AL) to be identified and enumerated by species. Approximately 10% of each species collected in the pitfall traps were further non-destructively rolled across CSMA (cycloheximide-streptomycin-malt extract agar) and MEA (2% malt extract agar) media to check for vectoring of fungi. Plates were incubated under florescent lights ($460 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 25°C and inspected for fungal growth. Ophiostomatoid-like fungal growth was subcultured to sterile CSMA plates via transferring of hyphal tips. This process was repeated till pure hyphal growth was observed and then subcultured to MEA plates. Hyphal growth was then transferred to MEA slants for identification of the fungal species.

Pine basal area was determined using a twenty factor prism. Diameter at breast height (approximately 137 cm from soil surface) was measured on each tree with a diameter of 11.43 cm or greater within each plot using a logger's tape and recorded to the nearest 0.25 cm. Percent slope was measured using an electronic clinometer (HEC, Haglof Sweden, Inc.) by targeting an object at equal height of my eye at the point of greatest slope along the aspect of the plot. Elevation was determined by GPS (Garmin GPSmap 76S) at the center of each plot. Forest Health Monitoring (FHM) crown/damage measurements were taken on each pine tree within each plot. These measurements include crown light exposure, live crown ratio, crown density,

and foliar transparency. Crown light exposure equals the total number of 1/4 crown areas plus tree top (5 parts in total) receiving full light with a minimum of 35% live crown ratio. Crown dieback is the percentage of live crown dead: the code 0 =no dieback through 100 =total dead crown. Live crown ratio is the percent of live crown length divided by the actual tree length. Crown density is the amount of crown branches, foliage, and reproductive structures that blocks light visibility through the crown (includes missing spaces and dead foliage of live crown ratio). The code 00 =no crown, 05 =1-5%, through 99 =96-100%. Foliage transparency is the percentage of skylight visible through the live, normally foliated portion of the crown. The code 0 =no skylight visible through 99= complete visibility of skylight, the lower the percentage the less light visible through the crown. Tree increment cores were taken from the same six study trees per plot to determine tree age, five year annual growth, and ten year annual growth. Increment cores were removed from each tree at breast height (approximately 137 cm from the ground) using increment borers. The number of annual rings was counted from the pith of the core to the inside of the bark to determine age. Five year annual growth was determined by measuring the length of the most recent five annual rings with a metric ruler beginning from the early-wood of fifth annual ring from the bark to the end of the late-wood of the most recent annual ring. Ten year annual growth was determined using same procedure as for the five year annual growth except measuring the most recent ten annual rings. Five and ten year annual growth was measured and recorded to the nearest tenth of a centimeter. Soil bulk density and soil gravimetric moisture content were calculated from three 60 cm soil cores taken at each plot. Soil strength was measured using a Rimik CP20 recording cone penetrometer by recording 3 insertions per soil sample site (9 total for each plot). Coarse and fine root weights were determined by excavating two 0.25 m² squares of soil to a depth of 23 cm per plot. One square

was excavated at the drip line and one halfway between the drip line and the trunk of the tree. Excavated soil was placed in a wheel barrow and the pine roots were separated from other roots found within that soil. Pine roots were separated by coarse and fine roots, washed, dried in an oven for 48 hours at 70°C. Once dried, roots were weighed and the weight recorded to the nearest tenth of a gram. Fine roots were classified as any pine root ≤ 2 mm in diameter, roots greater than 2 mm in diameter were classified as coarse roots. Rhizome density was determined by weighing the amount of rhizomes present in the same pits where pine roots were sampled. Rhizomes were washed, oven dried for 48 hours at 70°C, then weighed to the nearest tenth of a gram. Thatch cover was determined by graphing each plot on paper and drawing the areas that didn't contain *I. cylindrica* on the graphing paper. The area that wasn't drawn out was then determined by subtracting the drawn out area from the total square area of the plot.

Analysis of the differences between root-feeding bark beetle populations from CO plots and NCO plots for each bark beetle species were completed using PROC GLM with Tukey's Studentized Range (HSD) Test in Statistical Analysis Software (SAS Institute, 9.2., Cary, NC). Using the distribution chart produced by SAS, individual outliers were observed and removed from the data to observe the effects that one observation had on the significance and to determine if or how it could impact this study. All p-values in this study reported with no outliers removed.

2.4. Results

2.4.1. Population comparisons between treatment and control plots

Twenty-three species of bark and ambrosia beetles as well as three species of weevils were identified and populations recorded from panel and pitfall traps (Table 2.2). Of these 26 species, four species of root- and lower bole-feeding bark beetles, as well as two root-feeding weevil species, were considered study species due to their relevance to LPD. These six species

include *Hylastes salebrosus*, *Hylastes porculus*, *Hylastes tenuis*, *Dendroctonus terebrans*, *Hylobius pales* (weevil), and *Pachylobius picivorus* (weevil). *Hylastes salebrosus* was found to be the most abundant species collected in this study with >16,000 captures, approximately 51% of the total insects captured (Table 2.1). *Hylastes salebrosus*, *H. porculus*, *D. terebrans*, and *Hs. pales* had consistently higher populations in plots containing cogongrass throughout the collection period but no significance difference was found for these species (Table 2.3). *Pachylobius picivorus* and *H. tenuis* had opposite trends and populations of *P. picivorus* were found to be significantly higher in control plots ($P=0.0299$, $F=5.56$) (Table 2.3).

Table 2.2. Total insect captures by species

Species	Number Caught
<i>Hylastes salebrosus</i>	16404
<i>Hylastes porculus</i>	1653
<i>Hylastes tenuis</i>	1462
<i>Dendroctonus terebrans</i>	1218
<i>Pachylobius picivorus</i>	624
<i>Hylobius pales</i>	507
<i>Xyleborinus saxesenii</i>	1635
<i>Xylosandrus crassiusculus</i>	2695
<i>Gnathotrichus materiarius</i>	540
<i>Xyleborus pubescens</i>	3288
<i>Dendroctonus frontalis</i>	0
<i>Ips grandicollis</i>	1087
Other	1201

Note: Other includes *Ips avulsus*, *Ips calligraphus*, *Hylastes opacus*, *Xylosandrus compactus*, *Monarthrum mali*, *Pissodes nemorensis*, *Monarthrum fasciatum*, *Xyleborus ferrugineus*, *Pityoborus comatus*, *Trypodendron scabricollis*, *Dryoxylon onoharaensum*, *Xyleborus atratus*, *Xylosandrus germanus*, and *Orthotomicus caelatus*

Table 2.3. Mean population comparison between CO plots and NCO plots by study species for panel and pitfall traps combined.

Species	CO	NCO	P-value
<i>Dendroctonus terebrans</i>	64	46	0.1009 ¹
<i>Hylastes porculus</i>	102	91	0.5907 ²
<i>Hylastes salebrosus</i>	1036	878	0.3505
<i>Hylastes tenuis</i>	91	105	0.4312 ³
<i>Pachylobius picivorus</i>	32	68	0.0299 [†]
<i>Hylobius pales</i>	29	22	0.4797 ⁴

*Significantly higher population in CO plots

[†]Significantly higher population in NCO plots

¹Significance determined after removal of outliers C8 and NC2 population data ($P=0.0124$, $F=7.92$)

²C9 and NC2 were outliers but removal of those plots was not significant

³C9, C10, and C7 were outliers but removal of those plots was not significant

⁴C8 found to be an outlier but removal of that plot was not significant

Significance tested at $P<0.05$

Even though panel trap captures greatly exceeded pitfall captures, analysis of population comparisons among CO and NCO plots for just pitfalls showed a different trend than panel traps. All species except *D. terebrans* had higher populations in the NCO plots with *P. picivorus* being the only one that had a significant difference ($P=0.0153$, $F=7.27$) (Table 2.4).

Table 2.4. Tukey comparisons of average pitfall populations between CO and NCO plots

Species	CO Plot Mean	NCO Plot Mean	P-value
<i>Dendroctonus terebrans</i>	3	2	0.2905
<i>Hylastes porculus</i>	11	17	0.4320
<i>Hylastes salebrosus</i>	121	153	0.5852
<i>Hylastes tenuis</i>	20	30	0.2475
<i>Pachylobius picivorus</i>	9	21	0.0153 [†]
<i>Hylobius pales</i>	4	6	0.1002

[†]Significantly higher population in NCO plots

Significance tested at $P<0.05$

2.4.2. Population comparisons to field data

Populations of bark beetles were compared to tree vigor covariables to assess if any interactions exist. Covariables include basal area of pine, diameter at breast height (DBH) of pine, five- and ten-year annual growth of measured pine, coarse and fine root weight, elevation, slope, soil moisture levels, soil bulk density, soil strength, crown density, crown ratio, foliar transparency, and crown light. Cogongrass plots were also analyzed with covariables *I. cylindrica* rhizome density and thatch cover area. Populations of *D. terebrans* was found to be significantly higher in plots with larger DBH ($P=0.0077$, $F=8.98$). Lower populations of *P. picivorus* ($P=0.0397$, $F=4.92$) were correlated with plots of higher mean DBH (Table 2.5). Higher populations of *D. terebrans* ($P=0.0118$, $F=7.86$) and *H. salebrosus* ($P=0.0078$, $F=8.95$) was found to be correlated with plots at higher elevation (range 37 to 87.5 m) (Table 2.6). Lower populations of *P. picivorus* were also found to be correlated with plots of higher levels of percent moisture of total sample weight ($P=0.0314$, $F=5.45$) and gravimetric water content ($P=0.0409$, $F=4.85$) (Table 2.7). Populations of *H. porculus* ($P=0.0485$, $F=0.0311$) and *H. salebrosus* ($P=0.0311$, $F=6.81$) were negatively correlated with increased *I. cylindrica* thatch cover area while *P. picivorus* populations were positively correlated with increased thatch cover area ($P=0.0120$, $F=10.45$) (Figure 2.8). Lower *P. picivorus* populations were found to be correlated with higher crown density percentages ($P=3.93$, $F=4.94$) (Table 2.9).

Table 2.5. P-values of correlations between insect species populations and tree vigor measurements

Species	(Range of Data)	Basal Area	DBH	5-year Annual Growth	10-year Annual Growth	Pine Coarse Root Wt.	Pine Fine Root Wt.
		(4.4-10.5m ²)	(16.5-25 cm)	(18-30mm)	(35-61mm)	(8.5-120g)	(11.5-42g)
<i>Dendroctonus terebrans</i>							
		0.3745	0.0077* ¹	0.9897	0.4717	0.1085	0.4794
<i>Hylastes porculus</i>							
		0.3440	0.3369	0.8349	0.3993	0.4312	0.9748
<i>Hylastes salebrosus</i>							
		0.8037	0.1475	0.6284	0.5144	0.0813	0.7300
<i>Hylastes tenuis</i>							
		0.4579	0.9495	0.3068	0.3736	0.9998	0.8360
<i>Pachylobius picivorus</i>							
		0.3780	0.0397 [†]	0.0592 ³	0.0893 ⁴	0.8681	0.3158
<i>Hylobius pales</i>							
		0.1782	0.4188 ²	0.7004	0.5681	0.0346 [†]	0.7434

* significantly higher population with increase in the corresponding vigor measurement

[†] significantly lower population with increase in the corresponding vigor measurement

¹ P-value after removal of C8 as outlier (0.2765, F= 6.04)

² P-value after removal of C8 and C7 as outliers (0.0089, F=0.8.87)

³ P-value after removal of NC5 as outlier (0.0138, F=7.54)

⁴ P-value after removal of NC5 as outlier (0.0110, F= 8.13)

Significance tested at P<0.05

Table 2.6. P-values of correlations between insect species populations and topography measurements

Species	(Range of Data)	Elevation	Slope
		(37-88m)	(2-11%)
<i>Dendroctonus terebrans</i>			
		0.0118*	0.4193
<i>Hylastes porculus</i>			
		0.4166	0.8708
<i>Hylastes salebrosus</i>			
		0.0078*	0.5841
<i>Hylastes tenuis</i>			
		0.6580	0.1387
<i>Pachylobius picivorus</i>			
		0.0074 [†]	0.9995
<i>Hylobius pales</i>			
		0.2645	0.0856

*significantly higher population with increase in the corresponding topography measurement

[†] significantly lower population with increase in the corresponding topography measurement

¹ P-value after removal of C8 data (0.0869, F=7.86)

Significance tested at P<0.05

Table 2.7. *P*-values of correlations between insect species populations and soil moisture measurements

Species	(Range of Data)	Bulk Density	% Moisture of Section	% Moisture of Total Sample Wt.	Gravimetric Water Content	Soil strength
		(1.36-1.65g/cm ³)	(11-24%)	(14-28%)	(20-35g)	(1.7-2.7mPa)
<i>Dendroctonus terebrans</i>		0.3977	0.2397	0.4560	0.4028	0.6549
<i>Hylastes porculus</i>		0.2842	0.4753	0.2926	0.2337	0.4068
<i>Hylastes salebrosus</i>		0.0930	0.5805	0.3912	0.2580	0.8577
<i>Hylastes tenuis</i>		0.9627	0.1262	0.0757	0.1235	0.1643
<i>Pachylobius picivorus</i>		0.3260	0.0807	0.0409 [†]	0.0314 [†]	0.7772
<i>Hylobius pales</i>		0.7187	0.0804	0.2735	0.2735	0.1579

* significantly higher population with increase in the corresponding vigor measurement

[†] significantly lower population with increase in the corresponding vigor measurement

Significance tested at *P*<0.05

Table 2.8. *P*-values of correlations between insect species populations and *Imperata cylindrica* data

Species	(Range of Data)	Rhizome Density	Thatch cover
		(50-180g)	(149-223m ²)
<i>Dendroctonus terebrans</i>		0.5832	0.9935
<i>Hylastes porculus</i>		0.4617	0.0485 [†]
<i>Hylastes salebrosus</i>		0.6212	0.0311 [†]
<i>Hylastes tenuis</i>		0.3975	0.3674
<i>Pachylobius picivorus</i>		0.5634	0.0120*
<i>Hylobius pales</i>		0.2261	0.6139

* significantly higher population with increase in the corresponding vigor measurement

[†] significantly lower population with increase in the corresponding vigor measurement

Significance tested at *P*<0.05

Table 2.9. *P*-values of correlations of insect species populations and crown data

Insect species	(Range of Data)	Crown	Crown Ratio	Foliar	Crown Light
		Density		Transparency	
		(27-33%)	(38-51%)	(24-24%)	(1.5- 4)
<i>Dendroctonus terebrans</i>		0.1807	0.3058	0.8847	0.8034
<i>Hylastes porculus</i>		0.8020	0.4560	0.5792	0.6481
<i>Hylastes salebrosus</i>		0.9508	0.7684	0.3213	0.6780
<i>Hylastes tenuis</i>		0.5753	0.4249	0.9613	0.9772
<i>Pachylobius picivorus</i>		0.0393 ^{†1}	0.5426	0.8020	0.7627
<i>Hylobius pales</i>		0.9135	0.9419 ²	0.7414	0.5100

[†] significantly lower population with increasing crown measurement

¹ *P*-value after removal of NC5 data (0.1465, *F*-2.32)

² *P*-value after removal of C8 data (0.0271, *F*=5.85)

Significance tested at *P*<0.05

2.5 Discussion

Hylastes salebrosus was the most abundant species captured throughout the entire collection period and is consistent with other studies that captured root-feeding bark beetle species associated with LPD (Eckhardt et al., 2007). Temporal trends found in this study for each bark beetle species were also similar to other population studies for these species. The six insect species combined were approximately 68% of the total insect population. Populations of *D. terebrans*, *H. porculus*, *H. salebrosus*, and *Hy. pales* all showed consistently higher numbers in cogongrass plots than non-cogongrass plots. However, the lack of significance indicates that the presence of *I. cylindrica* in forest plots is not affecting populations of these species. In addition, *Pachylobius picivorus* and *H. tenuis* had fewer numbers in cogongrass plots than in non-cogongrass plots. The lack of significant bark beetle population differences between CO and NCO plots occurred throughout the study period. Therefore, it appears that the presence of

cogongrass in forest plots does not result in stand stress such that insects associated with LPD are increased by *I. cylindrica*.

Pitfall insect trap collections showed that except for *D. terebrans*, insect captures were higher in NCO plots than CO plots, even though there were higher mean captures for most of these species in the panel traps in the CO plots. These trends may suggest that the *I. cylindrica* thatch cover is having an effect on the attractiveness of the baited pitfall trap by dampening the effects of the ethanol and turpentine used as attractants. This could possibly infer that *I. cylindrica* thatch is having a “trapping” or “suppressing” effect on the chemicals released by the pine roots themselves therefore reducing the attraction of the root-feeding bark beetle species to roots under heavy infestation of *I. cylindrica*.

Symptoms of LPD have been found to include fine root deterioration, short chlorotic needles, sparse crowns, and reduced radial growth (Eckhardt et al., 2007). Populations of root-feeding bark beetle species were found to be correlated with plots exhibiting these symptoms (Eckhardt et al., 2007). Tree vigor measurements were made to assess whether *I. cylindrica* is having negative effects on loblolly pine and subsequently increasing the populations of root-feeding bark beetle species. Insect populations were analyzed to assess if correlation exist between the tree vigor measurements and increased bark beetle populations.

Results shown in Table 2.5 illustrate that insect populations did not correlate with reduced tree vigor measurements. Lower populations of *P. picivorus* were found to be associated with plots with higher DBH and lower populations of *P. picivorus* were associated with increase in soil moisture. Although these were opposite trends than we anticipated, lower *Hy. pales* populations were found to be correlated with increase pine coarse root weight which was expected. Average DBH was found to be significantly higher in CO plots versus NCO plots

($P=0.0306$, $F=5.51$) which is mostly likely due to the fact that the majority of CO plots are located in an 18-year old stand while the majority of NCO plots are located in a 13-year old stand. This difference in age probably accounts for the significance in higher average DBH for CO plots.

Topographical effects have been found to correlate with stands containing declining loblolly pine (Eckhardt and Menard, 2008) in which increased slopes greater than 5% and SE/S/SW aspect were found to be pre-disposing factors to decline. There was no correlation between insect populations and increased slope (range 2-11%) but plots located at higher elevations (range 37-87.5 ft) correlated with increased populations of *D. terebrans* and *H. salebrosus*. CO plots were found to be located at significantly higher elevations than NCO plots ($P=0.0006$, $F=16.94$). The fact that CO plots were located at higher elevations and the fact that there were higher insect populations associated with those higher elevations did not support our hypothesis. Other factors, including soil moisture, composition, and nutrient content, can vary along an elevation gradient and maybe what is actually contributing to the increase in insect populations with response to elevation. We did observe a high concentration of pitch tubes in the larger diameter trees on plot C8, which are indicative of *D. terebrans* attacks.

Lower populations of *P. picivorus* being associated with plots with higher soil moisture levels are also not suggestive of our hypothesis because increased soil moisture is not typically associated with stress to loblolly pine except in cases where there is prolonged saturation (i.e. flooding). Albaugh et al. (2004) showed a growth increase in loblolly pine, although relatively small, in response to irrigation.

The thatch cover area and rhizome density of each of the CO plots was analyzed against each of the insect populations to see if higher populations were associated with increased density

of *I. cylindrica*. We found the opposite effect occurred to *H. salebrosus* and *H. porculus* populations, which had lower populations associated with increase thatch cover area which supports the idea of cogongrass suppressing the effects of chemicals given off by the roots or being a physical barrier to the insects attracted to the roots. A surprising correlation between higher populations of *P. picivorus* and increased thatch cover was also observed. It was surprising to see this result because *P. picivorus* has shown to have trends toward lower populations with more healthy parameters (i.e. increased DBH and crown density) and lower populations with increase soil moisture. Having a significantly higher population in NCO plots also makes this result surprising. The variability in these results also lends to the idea that infestations of *I. cylindrica* are not increasing root-feeding bark beetle populations.

Forest Health Monitoring (FHM) crown/damage measurements were compared to beetle populations to see if there was a correlation between increased insect activity and reduced crown density, crown ratio, and light availability as well as high foliage transparency. Eckhardt et al. (2007) found trees with lower growth rates and high root mortality and staining were positively correlated to low crown density, high foliage transparency, and increased insect populations. Populations of *P. picivorus* were found to be lower in plots that had higher crown density. This result also suggests that the trees are not enduring additional stress from the *I. cylindrica* infestation. According to these results, it can be suggested that trees in CO plots are not being stressed more by the *I. cylindrica* than trees in NCO plots.

Overall, all study species except *P. picivorus* and *H. tenuis* had higher populations in plots containing *I. cylindrica* infestations. Pitfall trap collection showed an opposite trend in all species but *D. terebrans* which could suggest that *I. cylindrica* is influencing the attractiveness of the baited trap to the root-feeding bark beetle species. Population comparisons to tree vigor and

plot measurements did not have suggestive trends that tree conditions in areas containing *I. cylindrica* were attracting higher populations of root-feeding bark beetle species. These insect populations should be reexamined in the future to see if prolonged infestations to cogongrass may eventually lead to the significantly higher populations of root-feeding bark beetle species due to increase age of the trees and compounding stresses to the trees.

Chapter 3

Assessing Soil Dynamics Associated with *Imperata cylindrica* in its relation to Loblolly Pine Decline

3.1. Abstract

Loblolly pine decline is a decline complex associated with root-feeding bark beetle populations that vector ophiostomatoid fungi. These bark beetle populations are attracted to stressed pine trees and can contribute to premature mortality. The water and nutrient content of the soil as well as the availability of water and nutrients to a tree can greatly influence the health and vigor of that tree. In this study, the soil dynamics of a loblolly pine stand in southeastern Mississippi infested with *Imperata cylindrica* was assessed to determine the impact *I. cylindrica* is having on soil chemical and physical properties. Soil nutrient levels were found to be consistently higher in the *I. cylindrica* plots as compared to NCO plots with significantly higher levels for some of the nutrients at some or all of the depths tested. The exact affect that *I. cylindrica* is having on these nutrient concentrations cannot be derived from this study but, it seems that *I. cylindrica* is having an effect on soil physical properties. Soil bulk density, gravimetric water content, and soil strength all are more conducive to root growth in *I. cylindrica* plots which may be due to the constant growth of rhizomes and the slow decomposition of *I. cylindrica* leaf litter.

3.2. Introduction

Exotic species are commonly recognized as one of the major threats to biodiversity and ecosystem stability (Wilcove et al., 1998, Mack et al., 2000) but little focus has been made on the potential impacts by these species to soil processes (Ehrenfeld, 2003). The effects of invasive plants on soil properties can be potentially important to invasion processes, their ecological impacts, and strategies to restore the desired species in those infested areas (Hook et al., 2004). The success that invasive exotic plants have can be improved by their negative or positive effects on the availability of nutrients in the soil (Vinton and Burke, 1995, Sagggar et al., 1999, Ehrenfeld et al., 2001). Exotic invasive plants have the potential to alter soil processes including carbon, nitrogen, and water (Duda et al., 2003, Ehrenfeld, 2003). Invasive species can affect the availability of nutrients through their litter inputs and accelerated rates of decomposition and uptake can increase nutrient cycling (Allison and Viousek 2004, Ashton et al., 2005). Increased uptake of nutrients could be driving the higher photosynthetic rates and specific leaf area exhibited by some exotics (Daneshgar and Jose, 2009). Exotic plants alter soil nutrient dynamics by differing from native plant species in biomass and productivity, morphology, phenology, and tissue chemistry (Ehrenfeld, 2003). Available data suggest that exotic invasive plants can exhibit a wide range of effects on soil nutrients even within a single species. Increases (Rutherford et al., 1986; Witkowski, 1991), decreases (Feller, 1983; Versfeld, 1986), or no change in soil nitrogen (Belnap et al., 2001) have been documented to occur due to invasions by plants. Plant invasions by exotics have been shown to affect other soil elements as well, including P, K, Ca, and Mg. A strong positive relationship was found by Howard et al. (2004) between soil carbon (C), phosphorus (P), and magnesium (Mg) and degree of invasiveness for a variety of invading species in New York. Yonekura et al. (2010) reported that soil carbon stock increased by 23% as

primary forestland was converted to *I. cylindrica* dominated grassland and this was attributed largely to the organic matter supply by grass roots, rhizomes, and charred materials from wildfires. Invasive plants have also been found to change soil pH levels. Soil pH in invaded areas of New Jersey by *Berberis thunbergii* DC and *Microstegium vimineum* (Trin.) A. Camus was found to be significantly higher than in areas not invaded by these species (Kourtev et al., 1998). In contrast, a study by Scott et al. (2001) found that a *Hieracium* species invading areas in New Zealand, lowered soil pH and mineral nitrogen while increasing total soil carbon and nitrogen. Since grasses generally have shallow root systems, they can reduce the nutrient availability of the uppermost soil layers and when they form dense root systems with significant belowground biomass, they can restrict nutrients to other plants by retaining increased amounts of available nutrients (Daneshgar and Jose, 2009). Fagan and Peart (2004) found the shrub *Rhamnus frangula* (L.) reduced the growth and survival of *Acer rubrum* (L.), *A. saccharum* Marsh., *Fraxinus Americana* (L.), and *Pinus strobus* (L.) in New Hampshire, which the authors attribute to its extensive shallow root system.

Imperata cylindrica (L.) Beauv. is one of the major species impacting the southeastern forest ecosystem currently. *Imperata cylindrica* can invade a wide range of soil types with a wide range of nutrient concentrations as shown in a study in Mississippi by Bryson et al. (2010). *Imperata cylindrica* appears to grow best in soils with low pH, low fertility, and low organic matter but can range from the coarse sands of shorelines to soils containing > 80% clay of reclaimed phosphate settling ponds (MacDonald, 2004). *Imperata cylindrica* is extremely efficient in nutrient uptake as reported by Saxena and Ramakrishnan (1983). Lippencott (1997) determined that *I. cylindrica* is capable of altering community function of the native Florida sandhill areas by changing vegetation structure, soil processes, resource availability, fire regime,

and native seedling recruitment. Daneshgar and Jose (2009) found that *I. cylindrica* alters nitrogen availability to *P. taeda* seedlings and found that the pine seedlings had significantly less nitrogen in their foliage and roots as compared to native competition. Brewer and Cralle (2003) suggested that *I. cylindrica* was a better competitor of phosphorus than native pine savanna plants. A study by Hartemink and O'Sullivan (2001) comparing leaf litter decomposition of *Piper aduncu* (L.), *Gliricida sepium*,(Jacq.) Kunth ex Walp, and *I. cylindrica*, found that *I. cylindrica* leaves had much slower decomposition rates and the soil contained significantly more water, as well as significantly reduced soil nitrogen levels. Daneshgar et al. (2008) found that *I. cylindrica* significantly reduced productivity and growth of an established *P. taeda* stand with only 26% survival in plots with *I. cylindrica* competition; pine seedlings were observed to have significantly smaller root collar diameters. The authors suggest that these results may be explained by reduced amounts of foliar nitrogen and water stress to the pine seedlings due to competition of *I. cylindrica*. Several studies have focused on the impact of *I. cylindrica* on pine regeneration but little is known about the impact *I. cylindrica* has to an established pine plantation. *Imperata cylindrica* could be significantly limiting nutrient availability and altering soil physical properties to established pine and consequently reducing the productivity of the pine. The purpose of this study was to compare the impacts *I. cylindrica* is having on soil chemical and physical properties of areas with and without *I. cylindrica* within an established pine plantation to determine if *I. cylindrica* could potentially be a stressor associated with Loblolly Pine Decline.

3.3. Materials and Methods

3.3.1. Site Description

The research site was located on The Westervelt Co. land east of Leakesville, Mississippi. Twenty plots were established by visually assessing the presence and density of *I. cylindrica* leaf area. Ten plots were established in areas containing heavy infestation of *I. cylindrica* (CO plots) while the remaining ten plots were established in areas containing no infestation of *I. cylindrica* (NCO plots). Plot dimensions were 15.24 m² (50 ft.²) in length and the center of the plot located half the distance diagonally across the plot. A metal tag was attached to each center pole denoting the plot identification. *Imperata cylindrica* plots were labeled as C1- C10 while plots without *I. cylindrica* were labeled NC1- NC10. The location (Chapter 2 Table 2.1) and elevation of each center pole was mapped using a handheld geographical positioning system device (Garmin GPSMAP 76Cx, Garmin International Inc., Olathe, KS). Each tree within each plot was assigned a number and a metal tag with this number was attached to the tree at breast height (approximately 137 cm from ground level).

Plot NC4 and all of the plots east of there (C4, C5, C7, C9, C8, C10, and NC3) are located on 18-year old plantation while the remaining plots (C1, C2, C3, C6, NC1, NC2, NC5, NC7, NC8, NC9, and NC10) are located on a 13-year old plantation (Figure 3.1). Both plantations were managed similarly with both receiving fertilization treatments of 36.7 kg/ha of urea (46-0-0 NPK) with the 18-year old stand receiving this in 2007 and the 13-year old stand in 2011 (Ken James, personal communication).

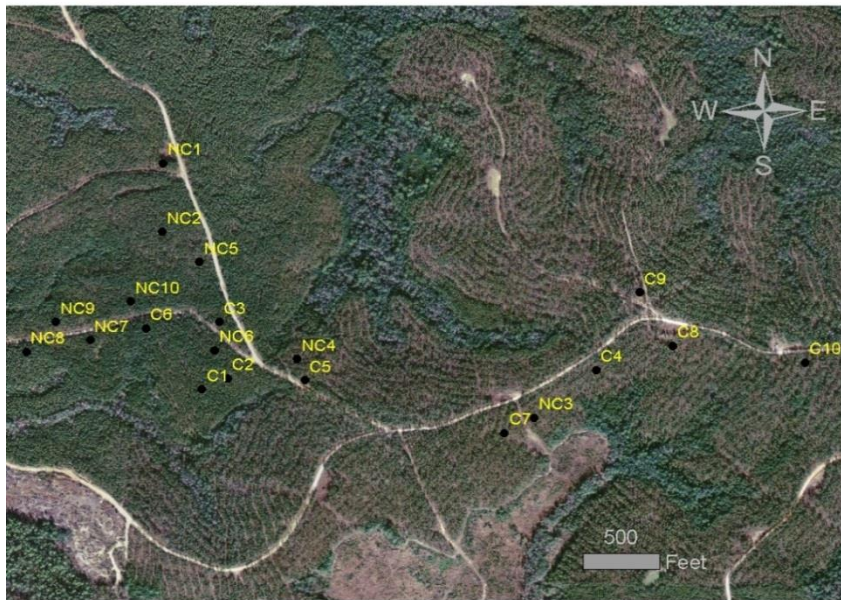


Figure 3.1. Map of plot distribution. C1-C10 are *Imperata cylindrica* (CO) plots, and NC1-NC10 are plot without *Imperata cylindrica* (NCO)

3.3.2. Soil Sampling

The soil series was identified as Benndale sandy loam on 8 to 15% slopes, and Benndale sandy loam or McLaurin sandy loam on 2 to 5% slopes. Benndale soils are classified as a coarse-loamy, siliceous, semiactive, thermic Typic Paleudults and McLaurin soils are classified as a coarse-loamy, siliceous, subactive, thermic Typic Paleudult. These are well drained soils and well drained soils are generally nutrient poor soils due to having low amounts of organic matter and clay which help maintain a higher CEC and slow water infiltration thus reducing leaching of the nutrients (Soil Survey Staff, 2012).

Three- 60 cm long by 5 cm diameter soil cores were sampled on each plot. Each sample location was also plotted on a handheld GPS device (Garmin GPSMAP 76Cx). Each soil sample was divided into 10 cm sections and then further divided into halves. One half of each 10 cm section was weighed and oven dried at 105°C for 48 hours, while the remaining half of each

section was weighed and was allowed to air dry. Bulk density and gravimetric soil moisture were calculated in the Forest Health Dynamics Lab as described in Soil Survey Laboratory Staff, 2004, while nutrient analyses were performed by the Soil Characterization Lab at University of Missouri. Samples from each depth segment were composited for each plot, sifted through a 2 mm sieve, and processed for pH via 1:1 water plus 1:2 0.1 M CaCl₂ method (McLean, 1982) and % total nitrogen (N_{tot}), % organic carbon (C_{org}), and % inorganic carbon (C_{inorg}) and % total carbon (C_{tot}) via combustion analyzer (Bremner, 1996). The Mehlich-3 procedure (Mehlich, 1984) was also used to determine the quantity of available Phosphorus (P), Potassium (K), Aluminum (Al), Magnesium (Mg), Manganese (Ma), Calcium (Ca), Zinc (Zn), Molybdenum (Mo) and Iron (Fe). Three penetrometer insertions were also taken using a Rimik CP20 cone penetrometer around each soil sample site (9 total for each plot) to measure soil strength levels (Mulqueen et al., 1977).

3.3.3. Statistical Analysis

Soil nutrient levels were averaged within each plot by soil depth segment (0-10 cm, 11-20 cm, and 21-30 cm) and the means of CO plots and NCO plots compared to each other using Tukey's Studentized Range test (PROC GLM; SAS 9.2). Since nitrogen fertilization was performed at different years between the two stands, the nitrogen means were analyzed by stand age and by treatment with a stand age co-variable to see if there was an effect of the different fertilization times to the current nitrogen levels. Soil moisture and bulk density levels were also averaged for each plot by soil depth segment (0-10 cm, 11-20 cm, etc.) and CO and NCO plot means were compared using Tukey's Studentized Range test (PROC GLM; SAS 9.2). Soil strength data were averaged per plot by soil depth (2.5, 5, 7.5,...30cm) and CO and NCO plot means were plotted using Microsoft Excel. The slope and intercept of each linear regression line

for each plot were determined and the means CO and NCO plots were compared using Tukey's Studentized Range test (PROC GLM; SAS 9.2).

3.4. Results

A comparison of means between CO plots and NCO plots of C_{tot} , N_{tot} , C:N ratio, and pH by water and salt are included in Table 3.2. There was no C_{inorg} found so C_{org} and C_{tot} are equal. There were significantly higher C_{tot} levels throughout the top 30 cm of soil in CO whereas N_{tot} was not different in the top 10 cm but was significantly different in the CO plots from 11 to 30 cm of soil. There was no difference found in the C:N ratio throughout the 30 cm of soil tested. The pH_{H_2O} and pH_{Na} both showed a trend of having higher values in the CO plots but pH_{H_2O} was only significantly higher at 21-30 cm of soil.

Mean comparisons of base cations as well as the total of base cations, Al, and effective cation exchange capacity (ECEC) between CO and NCO plots are shown in Table 3.3. All base cations (Ca, K, Mg, and Na) had higher quantities in the CO plots compared with NCO plots. Ca was significantly higher in the CO plots throughout the entire profile, K was higher in the upper 20 cm, and Mg and Na were higher in the upper 10 cm. Overall, total base cations were found to be significantly higher in the lower 20 cm. Aluminum was not found to be significantly different between CO and NCO plots but had higher means in the top 10 cm in the CO plots. The ECEC also showed a similar trend as there was no significant difference in the entire 30 cm of soil.

Comparison between CO and NCO plots of the means of other available nutrients including P, B, Cu, Mn, Zn, and Mo are shown in Table 3.4. Mean quantities of P were found to be higher in the CO plots but only significant in the 11-20 cm soil depth. Boron levels in the NCO plots were found to be below detection levels (<0.01 ppm) so a comparison was not performed. Elevated levels of Cu in the CO plots did not result in significant differences but Mn level were found to

be significantly higher in the CO plots in the upper and lower 10 cm segments. Zinc was found to be significantly higher in CO plots in the upper 10 cm of soil and Mo levels were found to be significantly higher in the middle 10 cm of soil only.

Figure 3.2 depicts mean BD of soils in CO and NCO plots through the top 60 cm. Bulk density was significantly higher in the NCO plots for the top 10 cm of soil but the BD tended to become higher in CO plots deeper into the soil profile. Figure 3.3 shows the comparison of the GMC at each 10 cm segment depth between the CO and NCO plots. The GMC had an opposite trend as the bulk density in which it was found to be significantly higher in the top 20 cm of the CO plot versus the NCO but tended to become more prevalent in the lower portions of the NCO soil profile. Figure 3.4 shows the soil strength linear slope comparison between CO and NCO means through the upper 60 cm of soil. There was a significant difference found between the slopes of the linear regression lines and the graph shows that the soil strength was consistently higher in the NCO plots through the upper 20 cm.

Table 3.1. Comparison of CO and NCO soil chemical content means by soil depth.

Soil Depth (cm)	Total Carbon (%)		Total Nitrogen (%)		C:N Ratio		pH _{H2O}		pH _{Salt}	
	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO
0-10	1.61** (18.85)	1.16	0.058	0.044	27.99	26.67	5.02	4.88	4.34	4.18
11-20	0.766** (36.3)	0.539	0.0322* (27.1)	0.025	23.28	22.03	5.21	5.06	4.40	4.34
21-30	0.362** (33.4)	0.0262	0.0193** (22)	0.015	18.59	16.62	5.27* (2.9)	5.05	4.45	4.26

Mean (coefficient of variation) presented

* significant at P<0.10 level

** significant at P<0.05 level

*** significant at P<0.0001 level

Table 3.2. Comparison of CO and NCO soil cation means by soil depth

Soil Depth (cm)	cmol(+)/kg													
	Calcium		Potassium		Magnesium		Sodium		Total Base Cations		Aluminum		ECEC	
	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO
0-10	0.68** (65.4)	0.29	0.05** (31.7)	0.03	0.21* (55)	0.13	0.09** (28.1)	0.06	1.00	0.48	4.54	4.48	5.53	4.98
11-20	0.48** (81.8)	0.18	0.03* (37.1)	0.02	0.10	0.07	0.08	0.06	0.69** (48.8)	0.33	4.94	5.00	5.63	5.34
21-30	0.37** (69)	0.14	0.02	0.02	0.10	0.08	0.07	0.07	0.51** (43.7)	0.33	4.71	5.23	5.22	5.56

Mean (coefficient of variation) presented

* significant at P<0.10 level

** significant at P<0.05 level

*** significant at P<0.0001 level

Table 3.3. Comparison of CO versus NCO soil available nutrient means by soil depth

Soil Depth (cm)	Available nutrients (mg/kg)											
	Phosphorus		Boron		Copper		Manganese		Zinc		Molybdenum	
	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO
0-10	17.99	14.85	0.56	BDL	0.80	0.51	64.1** (82)	53.2	0.94** (56)	0.38	2.22	1.75
11-20	10.09** (55.9)	2.72	0.43	BDL	0.55	0.44	56.46	49.52	0.36	0.22	2.60* (44)	1.59
21-30	8.07	5.56	0.3	BDL	0.38	0.35	46.51* (105.8)	42.07	0.36	BDL	2.05	1.38

Mean (coefficient of variation) presented

* significant at P<0.10 level

** significant at P<0.05 level

*** significant at P<0.0001 level

BDL-Below Detection Levels

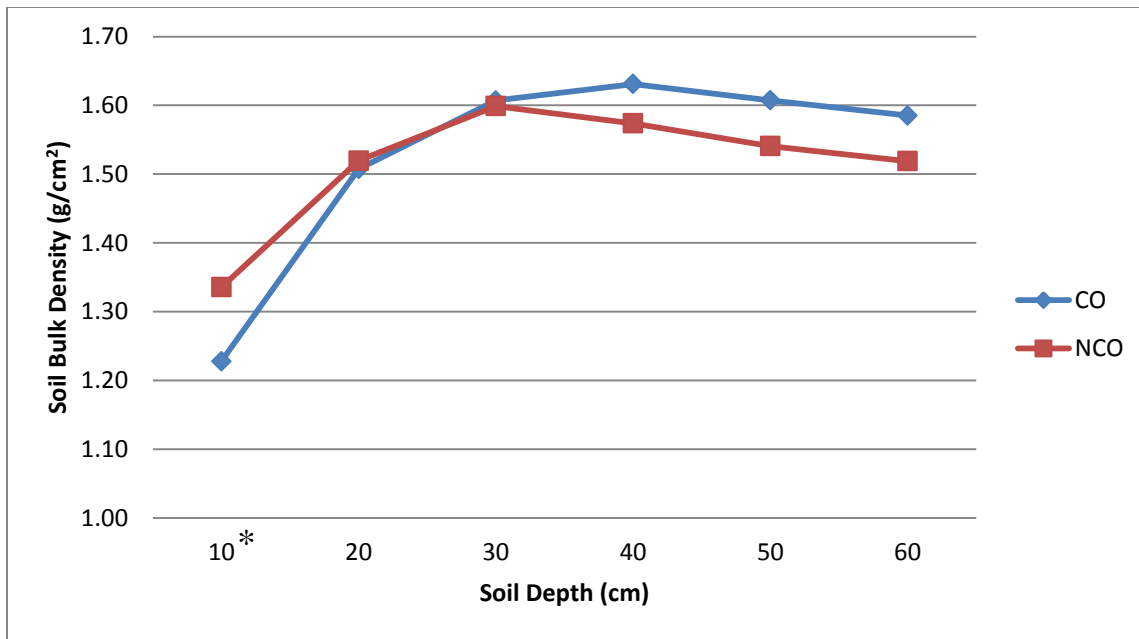


Figure 3.2. Soil bulk density comparison between CO and NCO plots
 *NCO bulk density mean significantly greater than CO mean ($p < 0.1$)
 $C_v = 9.3$

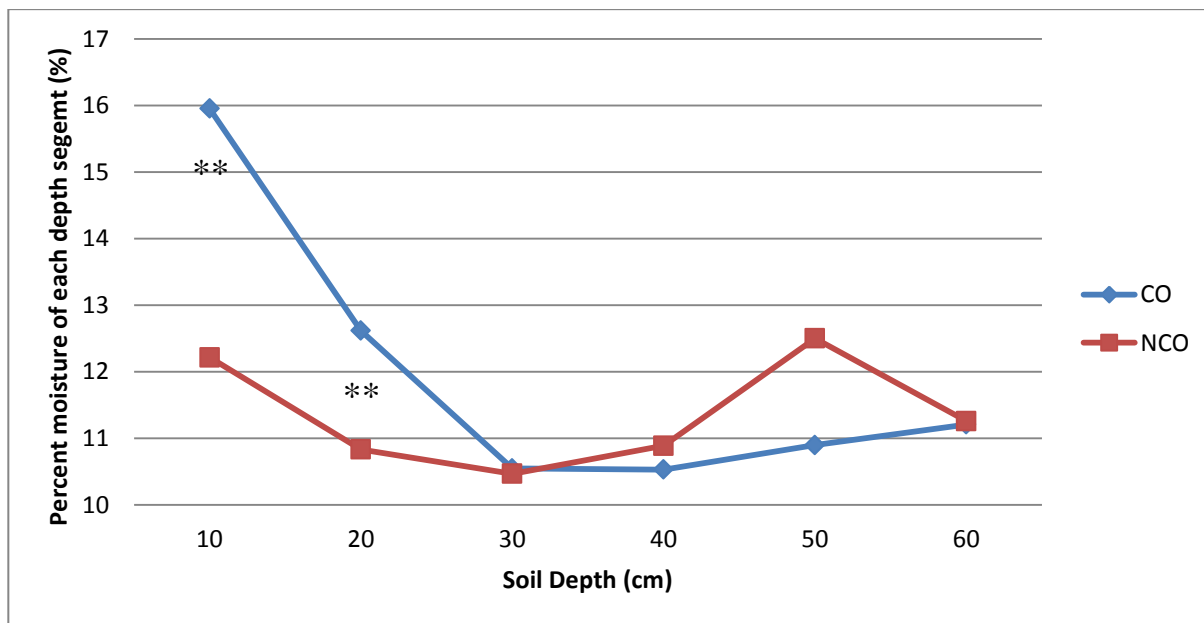


Figure 3.3. Gravimetric water content in the top 60 cm of soil for CO and NCO plots
 **CO mean significantly greater than NCO mean $p < 0.05$

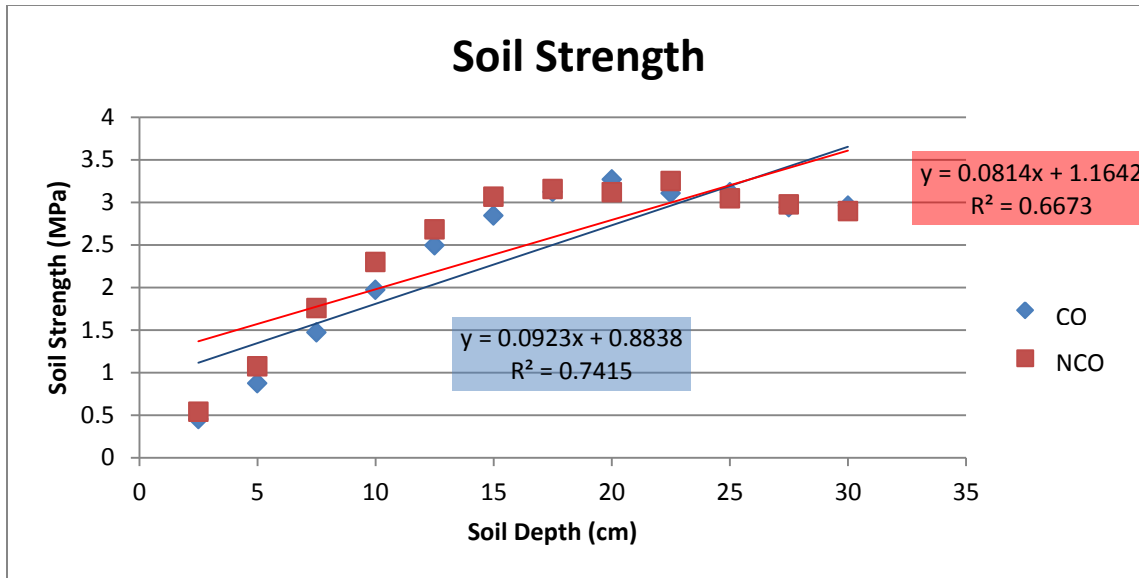


Figure 3.4. Soil strength mean slope and intercept comparison between CO and NCO means

3.5. Discussion

No stand or stand x treatment effect on N levels suggests that the fertilization application time differences had no effect on nutrient differences between CO and NCO plots. Since the 13 year old stand had been fertilized more recently we expected to see higher N content in the soil of the NC plots but actually found the opposite with N levels being higher in the CO plots which suggests that other factors are causing the difference. Higher total soil N levels in CO plots at all depths would typically be beneficial to the standing loblolly pine growth but significantly less N was found in the foliage (Chapter 4) of these plots which may indicate that the *I. cylindrica* is limiting the available N to the pine in infested areas. Daneshgar and Jose (2009) also found a similar result in which pine seedlings grown in *I. cylindrica* competition had significantly less N in their foliage as compared to seedlings grown in area of native competition and vegetation free areas. Yonekura et al. (2010) found that after conversion of forestland to *Imperata cylindrica*, soil C stock increased by 23% due to increased grass roots, rhizomes, and charred material from

wildfires. Collins and Jose (2008) reported lower soil pH levels in *I. cylindrica* infested stands compared to non-infested stands in longleaf pine ecosystem which is the opposite than found in this study as pH was significantly higher at 21-30 cm soil depth. Ehrenfeld (2003) reviewed the literature looking at the effect of 53 species of exotic plant invasions on soil nutrient cycling processes and found that increases and decrease in soil pH by exotic invasions occurred in equal numbers. The author also suggests that increases in pH may be due to the preferred uptake of nitrate as N source or increased base cation concentrations in the litter. The higher CEC levels we observed in the CO plots may suggest that *I. cylindrica* litter decomposition is leading to slightly higher pH levels in the soil due to the decrease in available cations because of fewer H⁺ ions to push cations into the soil solution. The consistently higher base cation levels found in the CO plots explain why the CEC is significantly higher in CO versus NCO plots. This was mostly attributable to the high levels of Ca found in the CO plots with respect to the other cations. There is little research that has focused on the impact *I. cylindrica* has on levels of soil P, B, Cu, and Mn. Research by Brewer and Cralle (2003) suggests that *I. cylindrica* maybe a better competitor for phosphorus than are native pine-savanna plants. Phosphorus is most limiting on very poorly to poorly drained sites and applications of P early in the rotation can increase growth of pine throughout the entire rotation

Plants tend to decrease the bulk density of the soil through root channeling and litter deposition, making water and root penetration easier (Tiedmann and Klemmedson, 1986, Callaway et al., 1991, Joffre and Rambal 1993). The extreme growth of the rhizome network that *I. cylindrica* exhibits could be disturbing the soil at a much higher rate than native plant species and therefore decreasing soil bulk density in the top 10 cm of the soil profile. The higher litter deposition of *I. cylindrica* leaves may be contributing to this lower bulk density in CO plots. This

could be an explanation of why gravimetric soil moisture content was higher in the top 20 cm of soil. Hartemink and O'Sullivan (2001) found significantly more water under *I. cylindrica* leaf litter due to the relatively slow decomposition of the leaves. The trends found in this study for soil strength in the upper 18 cm suggest that root growth shouldn't be limited. A study by Zou et al. (2001) found that root elongation rate of Radiata pine decreased exponentially with increasing soil strength and decreased by half its maximum rate at a penetrometer resistance of 1.3 MPa. Even though there was a lower soil strength value throughout the root growth zone of the CO plots, which means a more conducive environment for root growth, we found that fine root weight of loblolly pine in the CO plots was significantly lower in these plots. The fact that the soil nutrient contents are higher in cogon plots, soil moisture content is higher in cogon plots, soil strength is lower in cogon plots, and bulk density is lower in cogon plots all are more conducive values for fine root growth and yet we found fewer fine roots suggesting that there is a significant amount of mechanical hindrance exerted by the *I. cylindrica* rhizomes on the growth of pine roots.

Chapter 4

Assessing Tree Vigor Impact of *Imperata cylindrica* to *Pinus taeda* in southeastern Mississippi

4.1 Abstract

The nonnative, invasive weed *Imperata cylindrica* (L.) Beauv has been found to greatly reduce survival and productivity of many native plant species throughout the United States and the world. The establishment of pine seedlings in areas infested by *I. cylindrica* has been found to be difficult to near impossible. This is the case for the regeneration of most plant species in the path of *I. cylindrica*. The effects *I. cylindrica* has on the establishment of pine species in the southeastern United States has been well documented but the effects it has on established pine has not been researched thoroughly. In this study, we compared tree vigor of established loblolly pine between areas infested by *I. cylindrica* and areas that are free of *I. cylindrica*. We found that pine fine root weight was significantly reduced in the infested plots. Foliar nitrogen levels were also found to be lower in infested plots.

4.2 Introduction

The rate of tree growth in general is closely associated with the genetic potential of a tree and the environmental conditions influencing that tree. Environmental and genetic factors influence the physiological processes that determine the productivity or growth of a tree (Teskey et al., 1987). Environmental factors can include water and nutrient availability, topographic features, and soil and air temperatures. Optimum levels of these abiotic factors can greatly influence positive tree productivity (Albaugh et al., 2004) but, can also be the limiting factor in

the growth of a tree species. In the case of LPD, several abiotic factors have been found to be related to reduced growth and premature death of loblolly pine. Greater slopes, south to southwest facing aspect, tree age, topography, and organic matter content were found to be predisposing abiotic factors associated with declining loblolly pine (Eckhardt and Menard, 2008) with increased susceptibility found on sandy loam and sandy clay loam soils (Eckhardt et al., 2007). These predisposing stresses can lead to the attraction of root-feeding bark beetle species to loblolly pine. Root-feeding bark beetles can vector several species of ophiostomatoid fungi. Additional stresses, whether natural or anthropogenic, can possibly increase the populations of these bark beetles, expediting the decline symptomology. Symptomology found to correlate with declining stands were sparse crowns, reduced radial growth, deterioration of fine roots, decline, and mortality by the age of 50 (Lorio 1966; Hess et al. 1999, 2001, 2002; Eckhardt et al., 2007). Infestations by exotic invasive plants have been found to affect the growth and productivity of desired native species of herbaceous and woody plants. Walker and Vitousek (1991) found that the invasive nitrogen-fixing *Myrica faya* (Ait.) prevented the establishment of the dominant tree species *Metrosideros polymorpha* (Gaud.) in Hawaii. Survival and biomass of *Acer saccharum* (Marsh.) was reduced by the invasive *Lonicera maackii* (Rupr.) in Ohio (Gorchov and Trisel, 2003). Fagan and Peart (2004) found that growth and survival of *A. saccharum*, as well as *Acer rubrum* (L.), *Fraxinus Americana* (L.), and *Pinus strobus* (L.) in New Hampshire was limited by competition from *Rhamnus frangula* (L.) but they were unable to determine whether or not above- or below- ground competition was the mechanism for the reduced survival and growth.

One invasive species becoming an increasing threat to the establishment and growth of native pine and other plant species in the southeastern United States is the exotic grass species *Imperata cylindrica* (Cogongrass). Daneshgar et al. (2008) found that *I. cylindrica* significantly

reduced survival of *Pinus taeda* seedlings as well as significantly smaller root collar diameter in seedlings growing in areas of *I. cylindrica* competition as compared to *P. taeda* seedlings subject to native competition and vegetation free areas. *Imperata cylindrica* has been found to negatively affect the growth and survival of many species worldwide. In a study by Otsamo et al. (1997) examining capabilities of 83 exotic and native tree species to reforest *I. cylindrica* grasslands in Indonesia, several of the study species exhibited increased mortality by the age of 7 to 8 despite high initial survival of some of the species. Coster (1932, 1939) reports that *I. cylindrica* retarded the growth of teak trees (*Tectona grandis* L.) in the first year of establishment by more than 85 percent. *Imperata cylindrica* has also been found to be a major hindrance to the establishment of rubber, pineapple, tea, banana, citrus, and coconut plantations (Soerianegara, 1980; Dela Cruz, 1986; Ohta, 1990).

Imperata cylindrica can cause mechanical hindrance to root growth of native species due to the extensive belowground rhizome network it produces (Daneshgar et al., 2008). The sharp rhizome tips of *I. cylindrica* can penetrate the roots of native plants, contributing to damage or mortality by infection (Eussen and Soerjani, 1975). *Imperata cylindrica* has also been found to negatively impact forest ecosystems by altering nutrient availability (Daneshgar and Jose, 2009) and normal fire cycles and intensity (Bryson and Carter, 1993; Byrd and Bryson, 1999; Lippincott, 2000). Although many studies have focused on the impact *I. cylindrica* has on growth and survival of desired tree species, they have mainly been limited to seedling survival and growth. There is little research focused on the effect *I. cylindrica* is having on the productivity of an established forest. The intent of this study is to assess the impact *I. cylindrica* is having on an establish *Pinus taeda* plantations and to determine if *I. cylindrica* could be a possible biotic stressor to *P. taeda*, potentially leading to the onset of Loblolly Pine Decline.

4.3 Materials and Methods

4.3.1. Plot Description

The site of research was located on The Westervelt Co. land east of Leakesville, Mississippi (Fig. 4.1). Twenty plots were established by visually assessing *I. cylindrica* presence and density. Ten plots were established in areas containing heavy infestation of *I. cylindrica* (CO plots). The remaining ten plots were established in areas containing no infestation of *I. cylindrica* (NCO plots). Plot boundaries were marked by placing metal poles at each corner of a square with each side being 15.24 m (50 ft.) in length. Center plot was determined by placing a metal pole half the distance diagonally across the plot. A metal tag was attached to each center pole denoting the plot identification. Plots containing *I. cylindrica* were labeled as C1- C10 while plots without *I. cylindrica* were labeled NC1- NC10. The location (Chapter 2 Table 2.1.) and elevation of each center pole was mapped using a handheld geographical positioning system device (Garmin GPSMAP 76Cx, Garmin International Inc., Olathe, KS). Each tree within each plot was assigned a number and a metal tag with this number was attached to the tree at breast height (137 cm from ground level).



Figure 4.1. Plot location (green dot) in Greene County, Mississippi

4.3.2. Tree Vigor Measurements

Several measurements were taken to assess the vigor of the pine trees within each plot. The measurements were made to compare differing conditions of the pine tree vigor versus cogon grass presence/absence and insect populations. Diameter at breast height (dbh, approximately 137 cm from soil surface) was measured on each tree with a diameter of 11.43 cm or greater within each plot using a logger's tape and recorded to the nearest 0.254 cm. Pine basal area and total tree basal area were determined for each plot. Percent slope was measured using an electronic clinometer by targeting an object at equal height of my eye at the point of greatest slope along the aspect of the plot. Elevation was determined by GPS unit (Garmin GPSmap 76S) at the center of each plot.

Forest Health Monitoring (FHM) crown/damage measurements were taken on each pine tree within each plot. These measurements include crown light exposure, crown dieback, live crown ratio, crown position, crown density, and foliar transparency. Crown light exposure total number of 1/4 crown areas plus tree top (5 parts in total) receiving full light with a minimum of 35% live crown ratio. Crown dieback is the percentage of live crown dead. The code 0 =no dieback through 100 =total dead crown. Live crown ratio is the percent of live crown length divided by the actual tree length. Crown position is the relative position of each tree in relation to the overstory canopy zone. The code 1 =Superstory, 2= Overstory, 3= Understory, and 4= Open Canopy. Crown density is the amount of crown branches, foliage, and reproductive structures that blocks light visibility through the crown (Includes missing spaces and dead foliage of live crown ratio). The code 00 =no crown, 05 =1-5%, through 99 =96-100%. Foliage transparency is the percentage of skylight visible through the live, normally foliated portion of the crown. The code 0 =no skylight visible through 99= complete visibility of skylight. The lower the percentage the less light visible through the crown.

Resin was sampled from six randomly selected study trees within each of the plots for a total of 120 tubes. Fifteen ml resin collection tubes were pre-weighed, recorded to the nearest tenth of a gram, and labeled each with a reference number (1-120) for the weight. Once in the field, a hole was cut to the cambium at breast height (approximately 137 cm from the ground) using a punch and a rubber hammer. The removed portion of the tree was saved in a Ziploc bag. A spout was applied to the tree at the location of the cut with two wood screws. One of the resin collection tubes was inserted each of the spouts. These tubes were allowed to collect resin for a 24 hour period. Once removed, the corresponding tree number was recorded for each of the resin tube numbers, the spout was then removed, and one of the saved pieces from the tree was applied

back to the cut area of the tree. The resin tubes were brought back to the lab where they were each post-weighed and the weight recorded to the nearest tenth of a gram. The resin tubes were also placed in tube stands to allow resin content to settle. The resin volume was then visually assessed and recorded to the nearest milliliter.

Tree increment cores were taken from the same six study trees per plot. To determine tree age, five year annual growth, and ten year annual growth was measured increment cores were removed from each tree at approximately breast height (137 cm from the ground) using increment borers. Each core was placed in a core holder and labeled by plot number and tree number. The number of annual rings was counted from the pith of the core to the inside of the bark to determine age. Five year annual growth was determined by measuring the length of the most recent five annual rings with a metric ruler beginning from the early-wood of fifth annual ring from the bark to the end of the late-wood of the most recent annual ring. Ten year annual growth was determined using same procedure as for the five year annual growth except measuring the most recent ten annual rings. Five and ten year annual growth was measured and recorded to the nearest ten of a centimeter.

4.3.3. Foliage Nutrients

Foliage samples were taken from the same six selected trees per plot. A foliage sample from the top and bottom half of each crown was removed by shooting a 0.22 caliber rifle bullet at the branch of a targeted sample till the sample was recovered on the ground. Each sample was labeled by plot number, tree number, and whether it was from the top or bottom of the tree. Samples were then placed in coolers with ice to ensure retention of moisture. The needles of each top and bottom were then separated by first and second year needles. The first year needles of each top and bottom were combined to make one sample per tree and the second year needles

of each top and bottom were combined to make another sample per tree. Each of these samples was then oven dried for 72 hours at 70°C. Once dried, each sample was then ground using a foliage grinder with a blank sized sieve and placed in 20 ml scintillation vials. Two grams of each of the six first year samples per plot were then combined to form one first year sample per plot. Two grams of each of the six second year samples per plot were also combined to form one second year sample per plot. A total of 40 (20 first year and 20 second year) samples were sent to A&L Plains Laboratories, Inc., Lubbox, Texas for nutrient analysis. The nutrients analyzed for were Nitrogen (N), Sulfur (S), Phosphorus (P), Potassium (K), Magnesium (Mg), Calcium (Ca), Sodium (Na), Boron (B), Zinc (Zn), Manganese (Mn), Iron (Fe), Copper (Cu), and Aluminum (Al).

4.3.4. Root Fungal Sampling

Each of the six selected sample trees per plot were sampled for the containment of several ophiostomatoid fungal species associated with loblolly pine decline. Three of the six were sampled in the first year of the study, while the remaining three were sampled in the second year. Using the two-root excavation method (Ostrosina et al., 1997), modified by Eckhardt et al., (2007), two main lateral roots with a diameter of 3 cm or greater were excavated using a pulaski axe. Using an increment hammer, three cores per root (six per tree) were removed and placed into a Ziploc bag and labeled by plot number and tree number. Samples were then placed in a bleach/ethanol solution containing 20% bleach, 20% 190 proof ethanol, and 60% distilled water to surface sterilize each core for approximately three seconds. Each sample was then placed in 100% distilled water to dilute any remaining bleach/ethanol solution and then placed on a paper towel for approximately one minute to allow drying. The six samples were then placed on two plates (three samples per plate) containing a selective media [2% malt extract agar (MEA)

containing 800 µg/ml of cycloheximide and 200 µg/ml of streptomycin sulfate] (Hicks et al., 1980). This media, cycloheximide-streptomycin-malt extract agar (CSMA) is excellent for growth of ophiostomatoid fungi and suppressive of growth of undesirable fungi. Plates were labeled by plot number, tree number, and date plated. Any ophiostomatoid growth was further subcultured by means of hyphal tip transfer to sterile plates of CSMA until pure growth of that particular ophiostomatoid species was present. Hyphal tips were then transferred to a sterile MEA plate before finally subculturing to MEA slant to be stored for identification. All isolates of ophiostomatoid fungi were identified based on morphological structures.

Analysis of the differences between each tree vigor measurement from CO plots and NCO plots were completed using PROC GLM with Tukey's Studentized Range (HSD) Test in Statistical Analysis Software (SAS Institute, 9.2., Cary, NC). Using the distribution chart produced by SAS, individual outliers were observed and significance was retested without the outlier data and determined if the outlier is relevant to the interpretation of the results. Ten-year annual radial growth data and fine pine root data were the only data in this study in which outliers were reported to be relevant to the interpretation.

4.4 Results

A Tukey's comparison between the means of diameter at breast height of CO plots and NCO plots showed that loblolly pine in CO plots were significantly larger in diameter than in NCO plots ($P=0.0306$, $F=5.51$)(Fig. 4.2). Pine basal area was found to be similar between CO and NCO plots. Assessment of the mean 5- and 10- year annual radial growth of the loblolly pine on CO plots versus NCO plots showed that there was no significant difference in 5-year annual growth but 10-year annual growth was significantly greater in NCO plots ($P=0.0161$) when plots NC3 and NC4 were removed from being outliers (Figure 4.3). These outliers were removed

because these are the two NCO plots within the 18-year old stand. Significance gained from removal of these two NCO plots lead me to do an analysis of radial means again with the addition of a stand covariable which resulted in a significant treatment and stand interaction ($P < 0.0001$ for 10 year annual growth and $P < 0.0004$ for 5 year annual growth). Fine root weight was found to be significantly greater in NCO plots ($P = 0.0162$, $F = 7.12$) after plot C3 mean was removed (Figure 4.4). Plot C3 was removed because it had a mean of 41.7 g while the average of all the other CO plots combined was only 17.5 g. Pine coarse root weight was not found to be different between CO and NCO plots but had a slightly higher mean in NCO plots (Figure 4.4). Figure 4.5 shows observations made during root sampling that shows the rhizomes of *I. cylindrica* competing with the fine and coarse roots of loblolly. Elevation was found to be significantly higher in CO plots ($P = 0.0006$) but slope was not a significant factor. None of the FHM Crown measurements were found to be significantly different between CO and NCO plots (Table 4.2).

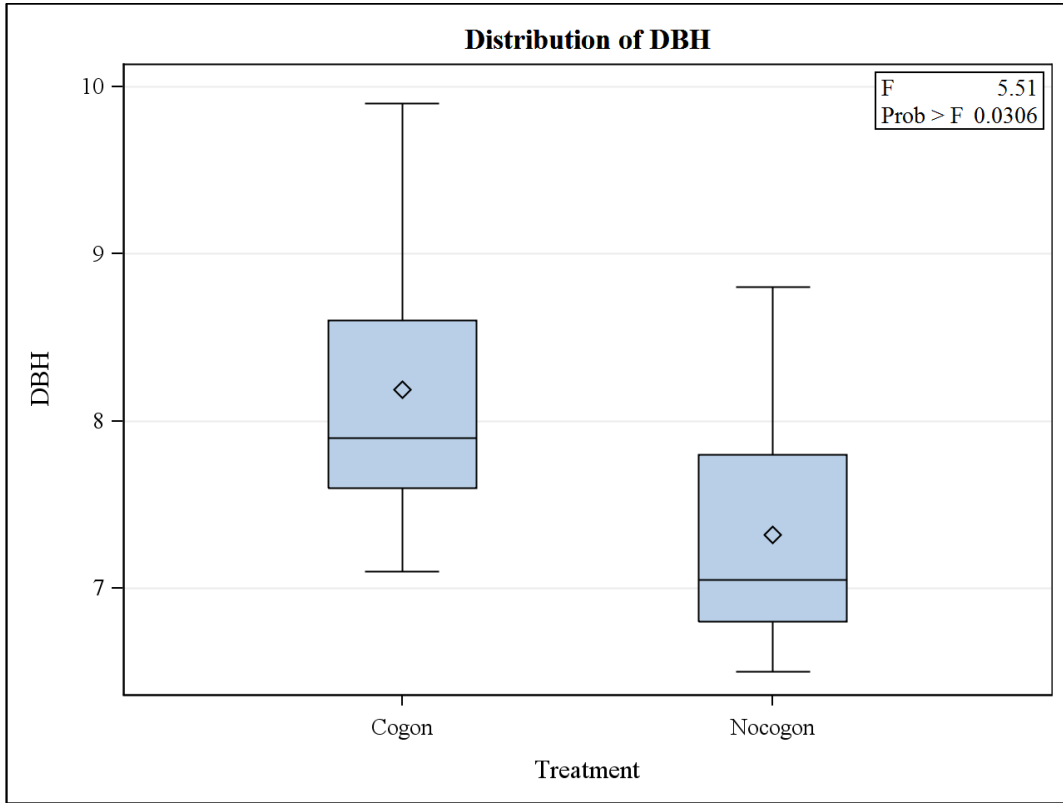


Figure 4.2. Mean diameter at breast height of *Pinus taeda* comparison between CO and NCO plots

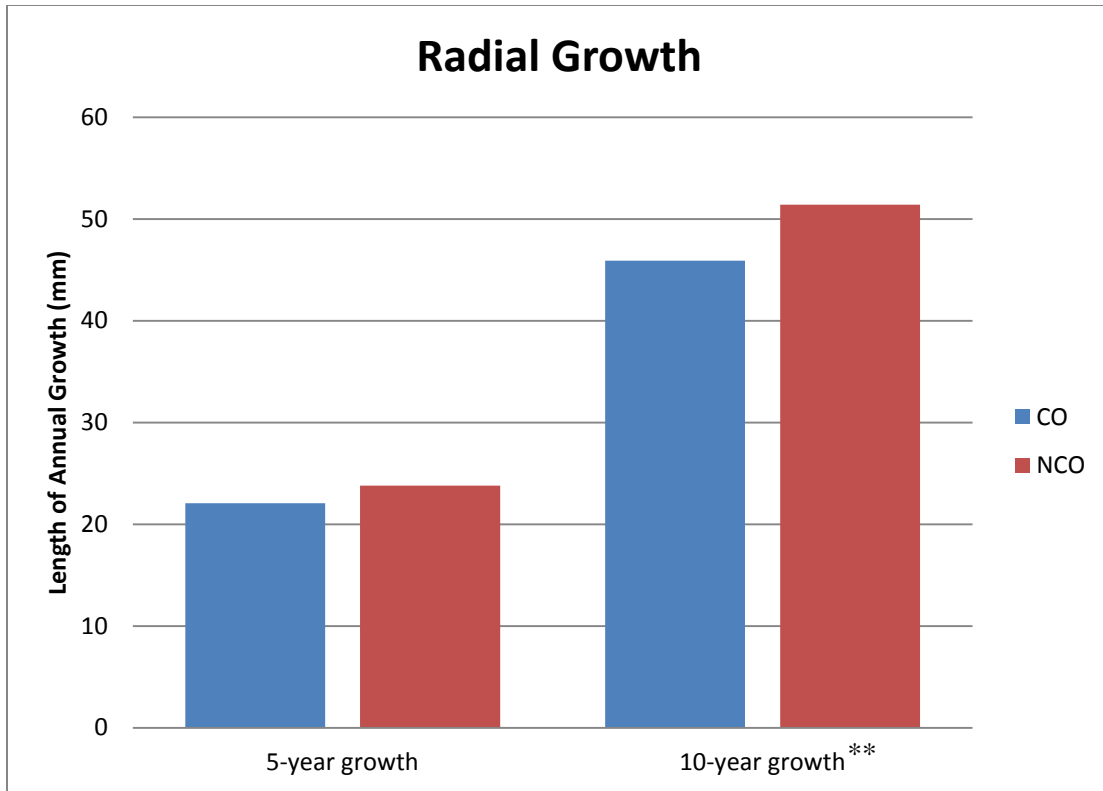


Figure 4.3. Mean 5-year and 10-year annual growth comparison of *Pinus taeda* between CO and NCO plots ($P=0.2074, F=1.71$ of 10-year data prior to NC3 and NC4 removal)

**significant at $P<0.05$ level

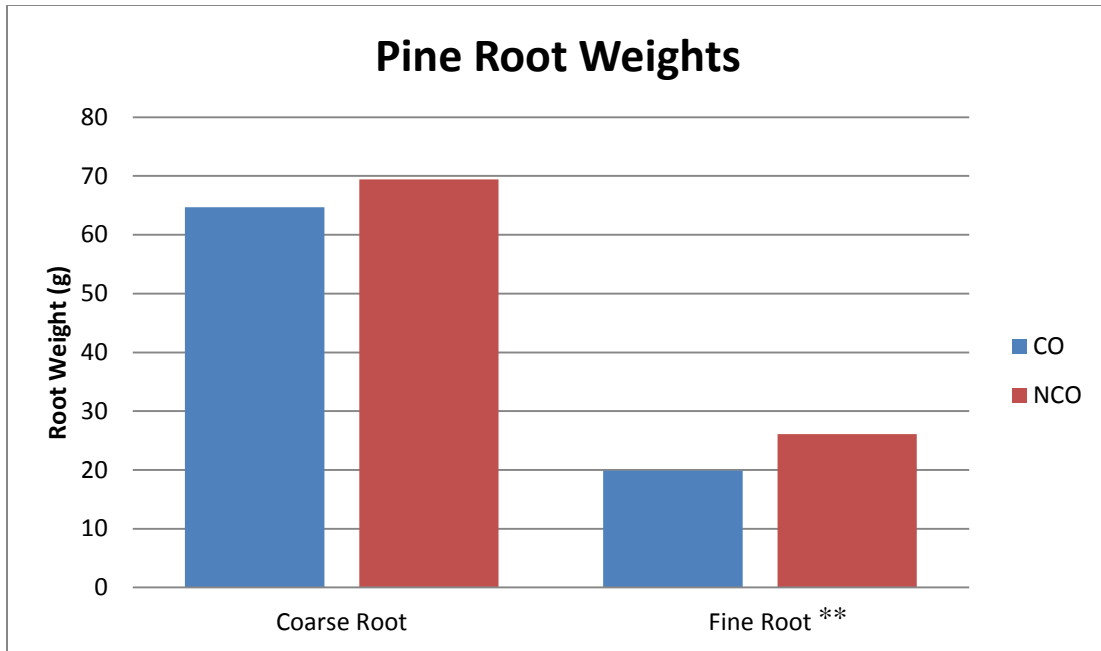


Figure 4.4. Mean coarse and fine root weight comparison between CO and NCO plots ($P=0.1300, F=2.52$ for fine root data prior to removal of C3 data)
**significant at $P<0.05$ level



Figure 4.5. Rhizome ball surrounding pine roots (A) Pine root pierced by *I. cylindrica* rhizome (B) (field observations)
Photos by Dr. Nancy Loewenstien, Auburn University

Table 4.1. Comparison of FHM crown rating measurement means between CO and NCO plots

	Crown Density	Crown Light	Crown Ratio	Foliar Transparency
CO	30.8	2.819	43.6	25.72
NCO	29.4	2.79	42	25.44
P-value	0.1235	0.8922	0.4003	0.4426
F-value	2.61	0.02	0.74	0.62

First and second year foliage nutrient analysis comparison between CO and NCO plot means showed that Zinc ($P_{\text{First}}=0.0002$, $F_{\text{First}}=22.01$, $P_{\text{Second}}=0.254$, $F_{\text{Second}}=5.94$) and Manganese ($P_{\text{First}}=0.0003$, $F_{\text{First}}=19.72$, $P_{\text{Second}}=0.0051$, $F_{\text{Second}}=10.14$) levels were all significantly higher in CO plots (Table 4.3). Magnesium ($P=0.0186$, $F=6.69$), Calcium ($P=0.0195$, $F=6.57$), Iron ($P=0.0113$, $F=7.97$), Copper ($P=0.0005$, $F=17.57$), and Aluminum ($P=0.0129$, $F=7.62$) levels were all found to be significantly higher in CO plots for the first year foliage only (Table 4.4). Boron was found to be higher in CO plots for the second year only ($P=0.022$, $F=6.28$) Table 4.3. Nitrogen concentration was significantly higher in NCO plots versus CO plots for second year foliage ($P=0.0277$, $F=5.73$) (Table 4.5). Significantly higher levels of sulfur were found in NCO plots for first year foliage ($P=0.0054$, $F=10.00$) (Table 4.5).

Table 4.2. Mean B, Zn, and Mn nutrient concentration comparison between CO and NCO plots

Needle Year	mg/kg					
	Boron		Zinc		Manganese	
	CO	NCO	CO	NCO	CO	NCO
First	21.1	15.2	22.92** (16.55)	18.58	466.44** (28.11)	269.85
Second	23.1** (11.89)	13.3	16.14** (13.63)	16.01	262.83** (26.72)	247.27

Mean (coefficient of variation) presented

**significant at P<0.05 level

Table 4.3. Mean foliage Mg, Ca, Fe, and Cu concentration comparison between CO and NCO plots

Needle Year	mg/kg									
	Magnesium		Calcium		Iron		Copper		Aluminum	
	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO
First	0.084** (9.79)	0.075	0.202** (13.99)	0.144	50.89** (18.71)	27.27	2.74** (13.86)	2.42	67.5** (19.64)	33.7
Second	0.092	0.086	0.172	0.132	40.14	31.79	2.11	2.34	52.9	39.9

Mean (coefficient of variation) presented

**significant at P<0.05 level

Table 4.4. Mean foliage N and S concentration comparison between CO and NCO plots

Needle Year	mg/kg									
	Nitrogen		Sulfur		Phosphorus		Potassium		Sodium	
	CO	NCO	CO	NCO	CO	NCO	CO	NCO	CO	NCO
First	1.16	1.19	0.025	0.035** (23.57)	0.084	0.087	0.337	0.315	0.015	0.015
Second	1.26	1.33** (5.62)	0.035	0.03	0.088	0.087	0.353	0.358	0.01	0.01

Mean (coefficient of variation) presented

**significant at P<0.05 level

The root fungal sampling resulted in only three trees infected with ophiostomatoid fungi. All three trees were located in NCO plots; one tree on NC4 contained *Grosmannia alacris* T.A. Duong, Z.W. de Beer & M.J. Wingf., while *Grosmannia huntii* (R.C. Rob. Jeffr.) Zipfel, Z.W. de Beer & M.J. Wingf. was found in the roots of one tree in plot NC5 and one tree in NC10.

Analysis comparing the weight of resin produced from the resin sampling showed that there was a similar amount produced between the CO and NCO plots although NCO plots had a slightly higher mean. NCO plot mean was 2.08 grams while cogon plot mean was 1.87 grams ($P=0.6149$).

4.5. Discussion

In this study it was found that there was significantly less pine fine root weight in the CO plots as compared to the NCO plots. This could be the result of the extensive *I. cylindrica* rhizome system hindering the growth of fine roots and/or the penetration of the pine roots by the sharp rhizome tips leading to the reduction of these pine fine roots (Eussen and Soerjani, 1975). The fact that the CO plots were mainly concentrated in the 18 year old plantation (Chapter 3) and that CO plots had a significantly higher DBH but had a significant reduction in pine fine root weight exemplifies the idea that *I. cylindrica* is having a physical impact on root growth and survival of established loblolly pine. There is a strong correlation with the extent of the loblolly pine root system and tree age (Shultz, 1997). Makkonen and Helmisaari (2001) found that Scots pine fine root biomass increased with stand age. It would seem that the plots in the 18 year old stand would have greater fine root biomass than the 13 year old stand but *I. cylindrica* may be decreasing the fine root biomass through physical competition. Very little information can be found on the impact that *I. cylindrica* has on mature pine roots but Danseshgar et al. (2008) found that there was significantly less root biomass in pine seedlings growing in *I. cylindrica*

competition as compared to native competition and vegetation free treatments after 3 growing seasons.

Another interesting find is that there was a significant reduction in 10-year annual radial growth of pine in CO plots. This result could be additional evidence that *I. cylindrica* is reducing the productivity of the established pine but radial growth means for plots NC3 and NC4, which are NCO plots located in the 18-year old stand, being significantly lower than the rest of the NCO plots could mean that the result is more related to the stand effect of age and not the presence of *I. cylindrica*. Since there was a significant stand x treatment interaction with 5 and 10- year radial growth, age of the tree had more of a direct effect on radial growth differences than CO versus NCO effect.

It was interesting to see higher levels of foliage nutrients of CO plots since we observed the opposite trend in the soil nutrient analysis (Chapter 3). Nitrogen levels were lower in CO plots which may be an indication that *I. cylindrica* is limiting the uptake of this nutrient. Limiting N could affect the growth potential of the *P. taeda* on those sites. Daneshar and Jose (2009) found similar results with *P. taeda* seedlings in *I. cylindrica* competition where *I. cylindrica* was maintaining nitrogen availability due to its extensive belowground biomass.

Chapter 5

Summary and Conclusions

Although there were no significant differences between the CO and NCO bark beetle populations, the fact that four of the six species including *H. salebrosus* had consistently higher populations in the CO plots throughout the study period suggests that there is additional attraction of these bark beetles in the CO plots. It is hypothesized that, if revisited, these study plots would yield significant results in population differences in the future because of compounding stresses from the *I. cylindrica* competition and increased stand age. This is the first study to assess the impact of *I. cylindrica* on root-feeding bark beetle populations and if studied further could give us further insight on the impact *I. cylindrica* can and does have on mature loblolly pines susceptibility to these insects and associated ophiostomatiod fungi. The fact that there were higher populations of root-feeding bark beetles in the pitfall traps of NCO versus CO plots suggests that *I. cylindrica* may be having a “suppression” effect on the ethanol and turpentine used for attracting the bark beetles to the trap. This should be studied closer as this may affect the root-feeding bark beetles ability to find stressed and therefore impact the level of beetle infestation and inoculation of pine roots with ophiostomatiod fungi that leads to the onset of LPD. Additionally, if *I. cylindrica* is having a “suppression” or “dilution” effect of the stress chemicals (i.e. alpha and beta-pinene) released by the roots and it is allowed to persist in a stand for an extended period of time, then if eradicated prior to harvest, stressed residual trees could be

more susceptible to attack by root-feeding bark beetles, fungal infections, the onset of LPD, and pre-mature mortality.

Results of this study suggests that *I. cylindrica* affects belowground activities through altering nutrient availability, soil bulk density, soil strength, water content, and root growth.. The lower bulk density and soil strength of the soil in CO plots could be more conducive for root-feeding bark beetles to penetrate the soil to get to the stressed root system of loblolly pine. The increase gravimetric water content of the soil in CO plots could also make penetration by root-feeding bark beetles to such as stressed roots easier and may prove to be more suitable habitat for water-active fungi such as *Phytophthora cinnamomi*. A more in-depth analysis of this hypothesis should be considered as this could be another means that *I. cylindrica* could increase the susceptibility of loblolly pine to LPD. Physical interaction between rhizomes and pine roots also could be a significant factor in the attraction of root-feeding bark beetles, whether from physically piercing the pine roots with rhizomes or mechanically hindering pine root growth within the root zone (both observed in this study, Ch. 4 Fig. 4.5). The piercing of the pine roots can cause open wounds and make the pine root more venerable to attack by root-feeding bark beetles and inoculation by ophiostomatoid fungi as well as other soil borne pathogens such as *Phytophthora cinnamomi*.

Imperata cylindrica could limit the fine root production and mycorrhizae associations which affect uptake of essential nutrients by loblolly pine to the point that it can significantly stress the pine and be a contributing factor of LPD. With increasing stand age and prolonged infestation of *I. cylindrica*, increased populations of root-feeding bark beetles will persist and the mature pine can become more susceptible to LPD through increased attack on stressed pine roots. Increased root-feeding bark beetle populations and their ability to easily penetrate the soil

layer to reach stressed root systems would mean higher amounts of ophiostomatoid fungi being inoculated into the root system and a higher probability of pre-mature mortality to these pines in *I. cylindrica* infested areas.

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