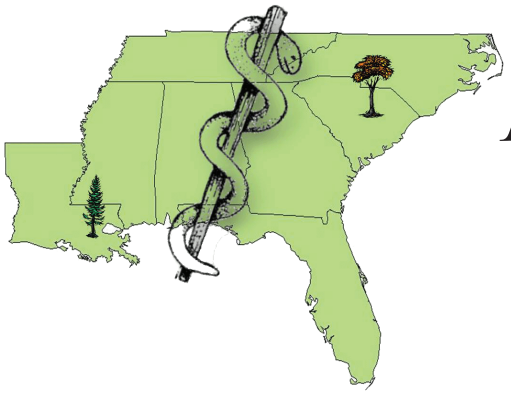

AUBURN UNIVERSITY
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ASSESSING TREE VIGOR IMPACT OF *IMPERATA CYLINDRICA* TO *PINUS TAEDA* IN SOUTHEASTERN MISSISSIPPI

by
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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.

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. saccharam,* 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.

MATERIALS AND METHODS

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).

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.

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).

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.

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).

First and second year foliage nutrient analysis comparison between CO and NCO plot means showed that Zinc (P_{First}=0.0002, F_{First}=22.01, P_{Second}=0.254, F_{Second}=5.94) and Manganese (P_{First}=0.0003, F_{First}=19.72, P_{Second}=0.0051, F_{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).

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).

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.

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

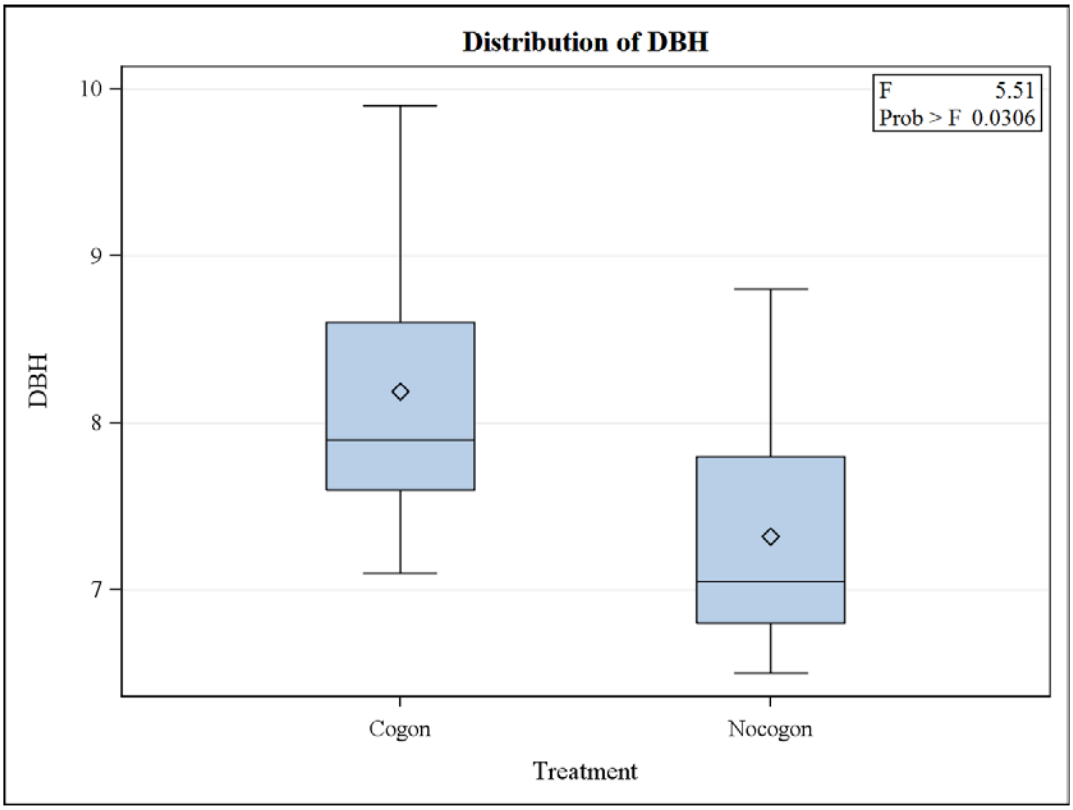

Figure 4.2. Mean diameter at breast height of *Pinus taeda* comparison between CO and NCO plots

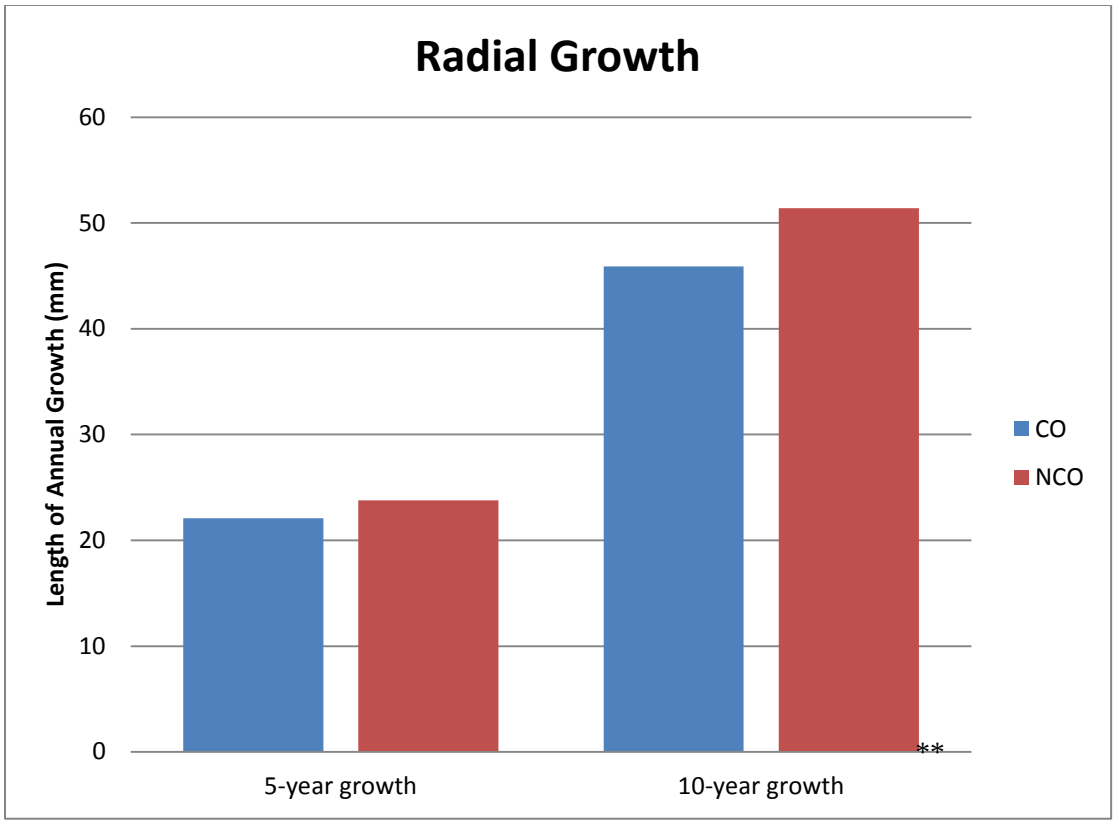

Figure 4.3. Mean 5-year and 10-year annual growth comparison of *Pinus taeda* between CO and NCO plots (P=0.2074,F=1.71 of 10-year data prior to NC3 and NC4 removal)
**significant at P<0.05 level

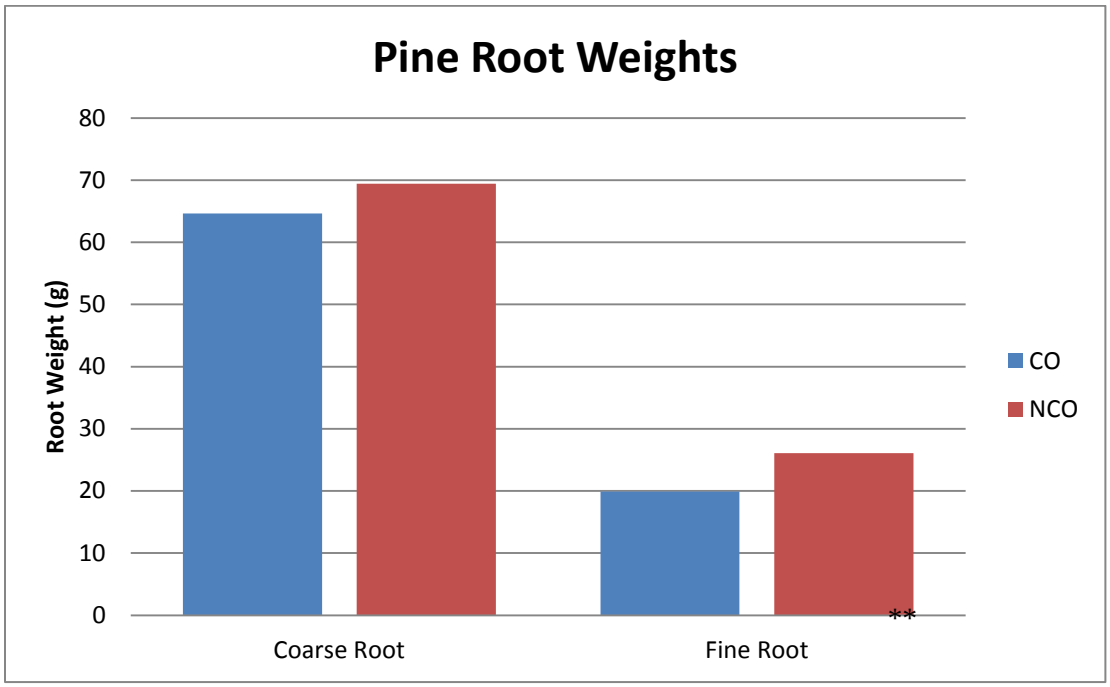

Figure 4.4. Mean coarse and fine root weight comparison between CO and NCO plots (*P*=0.1300,*F*=2.52 for fine root data prior to removal of C3 data)
**significant at P<0.05 level

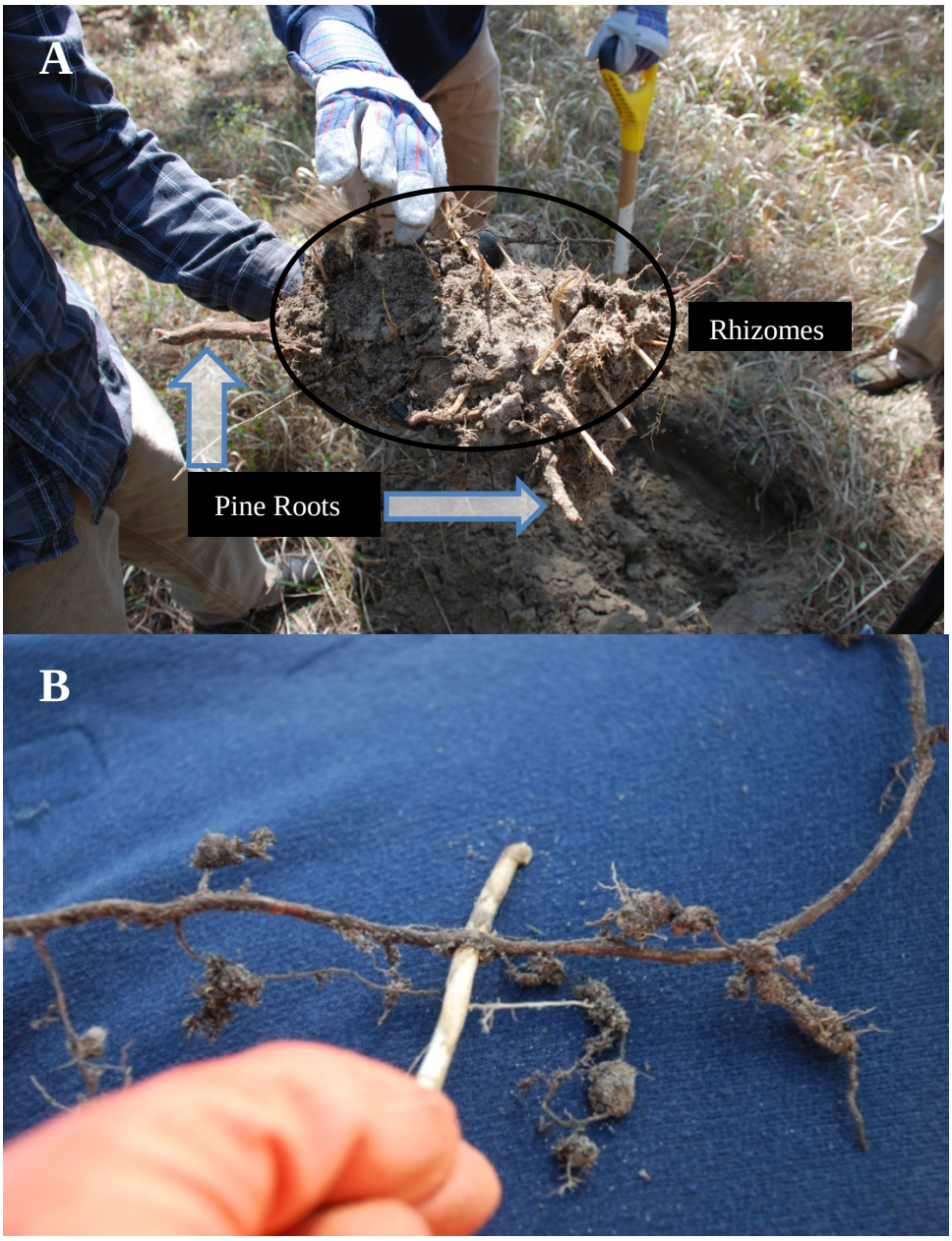

Figure 4.5. Rhizome ball surrounding pine roots (A) Pine root pierced by *I. cylindrica* rhizome (B) (field observations). Photos by Dr. Nancy Loewenstein, Auburn University.

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

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