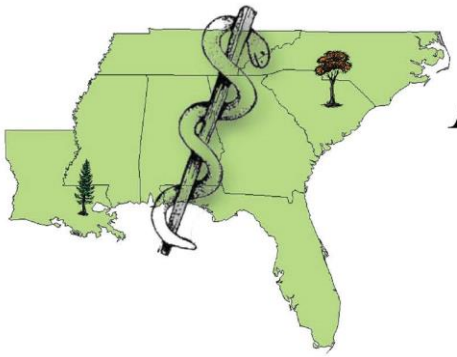

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LEPTOGRAPHIUM TEREBRANTIS INOCULATION AND ASSOCIATED CROWN SYMPTOMS AND TREE MORTALITY IN *PINUS TAEDA*

by
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6.1. ABSTRACT

Leptographium terebrantis S.J. Barras and T.J. Perry, is a bark beetle vectored fungus, commonly isolated from roots of *Pinus taeda* L. undergoing tree decline. Over the past several decades, the root pathogen has been implicated as a contributing factor of *P. taeda* decline and mortality. We examined the potential of *L. terebrantis* to cause decline symptoms and determine the relationship between pathogen spread and the formation new sapwood growth. The study was undertaken in 13-year-old *P. taeda* plantation near Eufaula, Alabama, U.S.A. using artificial inoculations of fungal-colonized, sterilized toothpicks. We found that *L. terebrantis* was not only associated with dying trees but caused decline symptomology and mortality when trees were inoculated at a high density. The highest of three inoculum densities inoculated at a rate of one *L. terebrantis* colonized toothpick per 1.2 cm of bark circumference was the threshold of inoculum density required to produce decline symptomology 19 months following inoculation. It was found that 20% mortality and severe growth loss among surviving trees occurred with *L. terebrantis* infection at the high inoculum density. At the two lower inoculum densities, despite pathogen spread, trees were capable of producing a complete ring of new sapwood that sustained physiological activities. The results of this study suggest that forest management practices which minimize bark beetle infestation in *P. taeda* plantations will result in reduced pathogen inoculum densities.

6.2. INTRODUCTION

Pinus taeda L. (loblolly pine) is the dominant tree species in commercial forest plantations across the southeastern United States. In some settings this tree species may be susceptible to several fungal root pathogens that reduce tree growth and result in mortality (Hansen and

Goheen, 2000; Chavarriaga et al., 2007; Gori et al., 2013). The majority of fungal tree root pathogens are members of the Basidiomycetes in the genera: *Armillaria*, *Heterobasidion*, *Phaeolus*, and *Phellinus* (Shaw and Kile, 1991; Worall and Harrington, 1992). Other tree root pathogens, namely *Leptographium* and *Phytophthora* belong to the Ascomycetes and Oomycetes, respectively, and are known to infect *P. taeda* roots and result in tree decline symptomology (Harrington and Cobb, 1988; Hansen, 2015).

Southern pine beetle (SPB) (*Dendroctonus frontalis* Zimm.), is the most destructive pest of *P. taeda*, and accounts for over 80 percent of the insect-related economic loss of these forests estimated at $1.5 billion (Price et al.,1998). Nonetheless, the ability of SPB to kill trees is partly attributed to symbiotic fungal associates that are vectored during insect infestation (Schultz, 1999; Repe and Jurc, 2010). The beetles carry fungi in mycangia or on the exoskeleton (Six, 2003) and infect host trees through feeding activities. *Leptographium* species are among the root pathogens vectored by bark beetles (Harrington and Cobb, 1988).

Fungi associated with bark beetles play a key role in the interaction between the host and infesting beetles (Berryman, 1972; Goheen and Hansen, 1993; Six and Wingfield, 2011). For instance, once established, these fungi alter tree carbon metabolism by stimulating host tree defenses (Six and Wingfield, 2011). Also, as the pathogen spreads, it may provide supplemental nutrition for the developing bark beetle larvae (Paine et al., 1997). The fungi vectored by bark beetles, however, are not noted for killing host trees (Horntvedt et al., 1983; Lieutier et al., 2009; Six andWingfield, 2011; Krokene, 2015), but are mostly regarded as a contributory factor to tree mortality caused by bark beetle damage to the vascular cambium and phloem (Berryman, 1972; Six and Wingfield, 2011). For example, Horntvedt et al., (1983) described the potential of *Ceratocystis polonica* (Siem.) C. Moreau, an ophiostomatoid fungus vectored by *Ips typographus* L., to kill mature Norway spruce trees (*Picea abies* L. Karsten), but did not observe mortality in artificially inoculated trees. They also noted that a large portion of the sapwood must be infected to inhibit water transport and alter tree physiological processes. In mature lodgepole pines (*Pinus contorta* Dougl. ex Loud. var *latifolia* Engelm. ex S. Wats), Lee et al., (2006) observed the development of chlorotic crowns but no mortality when trees were inoculated with *Leptographium longiclavatum* Lee, Kim & Brueuil. These symptoms only became apparent nine months following artificial inoculation for trees inoculated with a high inoculum density (800 points/m^2) (Lee et al., 2006).

Despite the low incidence of tree mortality caused by *Leptographium* species, in localized settings, bark beetle-associated *Leptographium* fungi have been reported to contribute to mortality in *Pinus* species. For example, *Leptographium wageneri* Kendrick is known to cause considerable mortality in Douglas fir (*Pseudotsuga menziesii* Mirb. Franco), lodgepole pine (*Pinus contorta* Dougl. ex Loud.), and ponderosa pine (*Pinus ponderosa* Dougl. ex P. & C. Laws.), in the U.S. and Canada (Jacobs and Wingfield, 2001). This fungus is vectored by *Hylastes* and *Pissodes* species and once the tree is infected by the pathogen, mortality is attributed to sapwood occlusion caused by the fungus. Other *Leptographium* species are regarded as either weak pathogens or saprophytes and thus are considered unlikely to either cause disease or mortality (Hansen, 1997).

In the southeastern U.S., several *Leptographium* species such as *L. terebrantis* Barras & Perry, *L. serpens* (Goid.), and *L. procereum* (Kendrick) Wingfield have frequently been isolated from woody roots of declining *P. taeda* trees (Klepzig et al., 1991, Eckhardt et al., 2004; 2007). Particularly, in localized areas across several counties in central Alabama and Georgia in the United States, *Leptographium* species have been isolated from *P. taeda* plantations exhibiting symptoms of decline (Eckhardt et al., 2007; 2010). These fungi are vectored by root-feeding bark beetles such as *Hylastes salebrosus* Eichhoff and *H. tenuis* Eichhoff, the weevil species, *Hylobius pales (*Herbst) and *Pachylobius picivorus* (Germar) (Eckhardt et al., 2004), and lower stem bark beetles such as *Dendroctonus* species (Barras and Perry, 1971; Klepzig et al., 1991). Characteristically, trees attacked by the beetle-fungal complex develop sparse and chlorotic crowns, short needles, reduced radial growth and deterioration of fine roots (Ostrosina et al., 1999; Eckhardt et al., 2007; 2010).

Although *L. terebrantis* infection of woody roots has been associated with declining, mature pine trees, the ability of this fungus to independently cause tree growth decline and/or mortality has not yet been established. To date, studies on the pathogenicity of *L. terebrantis* have primarily focused on the virulence of various isolates at different stages of host development (Devkota and Eckhardt, 2018). Several species of *Leptographium* are pathogenic to seedlings, saplings, and the woody roots of mature pines (Wingfield, 1986; Matusick et al., 2016; Devkota and Eckhardt, 2018; Devkota et al., 2019). Mortality, however, has only been reported for eastern white pine (*Pinus strobus* L.) seedlings following artificial inoculation (Wingfield, 1986; Rane and Tattar, 1987).

Complete sapwood colonization by *L. terebrantis* can occur within weeks after infection of pine seedlings (Devkota and Eckhardt, 2018). However, for mature trees, the period for complete sapwood colonization may take several months to years (Horntvedt et al., 1983; Lee et al., 2006). In a stand of young *P. taeda* trees, Mensah et al., (2020) noted thorough occlusion of sapwood that existed at the time of stem inoculation with *L. terebrantis*. Despite high sapwood occlusion, neither foliage symptoms nor mortality occurred by 24 weeks after inoculation. Furthermore, occluded sapwood-imposed limitations to hydraulic conductivity but the current-year sapwood produced after inoculation remained uninfected with *L. terebrantis* and sustained water transport (Mensah et al., 2020). The study was conducted to assess the potential of *L. terebrantis* to cause crown symptomology and mortality in plantation *P. taeda* trees and determine the relationship between pathogen lesion, occlusion and post-inoculation sapwood growth. We hypothesized that *L. terebrantis* infection will cause sapwood occlusion and loss of hydraulic function and induce formation of sparse crown. Furthermore, crown thinning would limit carbon fixation and allocation for new sapwood growth and cause tree mortality.

6.3. MATERIALS AND METHODS

6.3.1. Study organism

This study was undertaken on mature loblolly pine trees (*Pinus taeda* L.) using the fungus *Leptographium terebrantis* S.J. Barras and T.J. Perry. The fungal isolate (LOB-R-00-805) used for the study was originally isolated from woody roots of declining *P. taeda* trees at Talladega National Forest, Oakmulgee Ranger District, AL, U.S.A. (Eckhardt et al., 2007; Devkota and Eckhardt, 2018) and cultured on sterile toothpicks as described by Devkota et al., (2019).

Previous studies found this fungal isolate to be the most virulent among 42 *L. terebrantis* isolates (Devkota and Eckhardt, 2018).

6.3.2. Study site and experimental design

The study was located in a loblolly pine plantation near Eufaula, Alabama, U.S. in Barbour County (32°1'13.10"N, 85°12'31.76"W). The plantation was situated on the East Gulf Coastal Plain physiographic region and the humid subtropical climatic zone. Soil series identified within the study area included Annemaine and Wahee. Their taxonomic classification is a fine, mixed, semi-active, thermic Aquic Hapludult and fine, mixed, semi-active, thermic Aeric Endoaquult, repectively. Annemaine is the predominant soil series, consisting of a fine sandy loam surface and clayey subsoil, and moderately well drained. Wahee contains a clay loam subsoil overlain by fine sandy loam surface and poorly drained (Trayvick, 2005; Ditzler et al., 2017). Average annual precipitation and air temperature of the area are 1407 mm and 18.1 ^{o}C, respectively (NOAA, 2020). The plantation was established in 2003 at1.2 m x 3.0 m spacing using open-pollinated seedlings and third-row thin at 12 years age in 2014. The study site received nitrogen and phosphorus fertilization at planting but no herbicide or pesticide control after planting and has a site index of 22 m at 25 years.

Fifteen plots containing two rows, 3.0 m apart, of 10 trees per each row were established in the plantation at age 13 years in December 2015 in a completely random experimental design with three replications and five inoculation treatments. All plot trees were permanently identified by numbered metal tags and outfitted with a manual dendrometer band (D1, UMS GmbH, Munich, Germany) installed at 1.4 m above the ground line (DBH) on five randomly chosen trees in each row per plot. A weather station (WatchDog 2000, Spectrum Technologies Inc., Aurora, IL, U.S.) was installed adjacent to the study site to monitor local precipitation, air temperature, solar radiation, relative humidity, and wind speed.

Inoculation treatments were applied to the five randomly chosen measurement trees that were fitted with dendrometer bands in one of the two rows per plot. Treatments of the study included a no inoculation or wounding (control), no inoculation but sterile toothpick wounding (wound), and three levels of increasing fungal inoculum density (low, medium, high). Inoculum densities were selected based on earlier studies that established the relationship between number of *L. terrebrantis* toothpick inoculum points, occluded radial area of the stem (Devkota et al., 2019), and stem hydraulic conductivity in loblolly pine (Mensah et al., 2020). The treatments were applied by a procedure similar to that described by Devkota et al., (2019) with modification due to differences in tree size. For each tree, the number of inoculation points was marked on a stencil sheet adhered to the inoculation zone to ensure proper inoculum placement around the stem circumference. Three series of inoculation points were identified at 1.2 cm, 2.4 cm and 3.6 cm below the initial inoculum point (Devkota et al., 2019). The low, medium, and high inoculum densities received three series of 5-8, 20-28, or 40-58 *L. terebrantis*-colonized toothpicks, respectively, around the circumference of the lower stem in March 2017. The wound treatment was applied similar to the high inoculum treatment.

6.3.3. Inoculation method

Prior to treatment application, the dead cork of the bark was scraped around the circumference of the lower stem between 20 cm and 30 cm above the ground line with a 20.3 cm long ironton straight draw shave (Northern Tool + Equipment, Burnsville, MN, USA). The inoculation points,

approximately 1.2 mm in diameter and 5 mm deep, were drilled into the trees stems through the identified points on the stencil sheet placed between 23 cm and 27 cm above ground level.

To prepare for treatment application, wooden toothpicks, sterilized at 121 °C for 30 min and soaked overnight in malt extract broth (MEB) (BD Bacto ™ Malt Extract, BD Biosciences, San Jose, CA), were inoculated with *L. terrebranits* or not inoculated and incubated in the dark at 23 ^{o}C for 24 days as described by Devkota et al., (2019). Trees were inoculated in March 2017 by inserting toothpicks containing *L. terebrantis* inoculum (mycelium and spores) into the holes within 5 min of drilling. After inoculation, the protruding ends of the toothpicks were cut, and the inoculation zone of the stem was sealed with duct tape to prevent contamination (Devkota et al., 2019; Mensah et al., 2020).

6.3.4. Post fungal inoculation monitoring and measurements

Post-treatment observation of the inoculation zone and tree appearance were undertaken monthly from April 2017 to December 2019. Observations of host response to treatments included oleoresin exudates near the edge of the inoculation zone, presence of chlorotic foliage, and sparse crowns. In January and February 2020 which was 34 months post-inoculation, treated trees were cut at ground-level with a chainsaw and examined for insect attack along the stem of the tree; although none was found. For each tree, the stem section between ground level and 100 cm above ground level that contained the inoculation zone was extracted by a chain saw and transported to the laboratory for lesion and occlusions assessments. One stem disc, 5 cm wide, was extracted above and below 1.3 m (DBH) above ground level. The two stem discs from each tree were sealed in a plastic bag and transported to the laboratory where moisture content was determined by disc weights before and after discs were oven-dried at 70^{o}C to a constant weight. Disc moisture content was expressed as a percentage of oven-dried weight, and stem moisture content was expressed as the mean of stem disc moisture content above and below DBH.

6.3.5. Vertical stem lesion and occlusion assessment

The extent of lesion spread was determined for each tree by assessing the inoculation zone of the extracted 100 cm long stem sections. Duct tape was removed and bark above and below the inoculation zone was gently shaved with an ironton straight draw shave to expose the phloem-cambium interface. Discoloration due to resinosis in the phloem indicated the vertical extent of the lesion caused by pathogen spread. Shaving continued in both upward and downward directions, and around the entire stem section until the distal end of lesions was detected. Lesion length by tree was calculated as the mean of the four values.

In order to assess the extent of stem sapwood occlusion, a chain saw was used to sequentially cut stem discs, 5.0 cm wide, from terminal and basal ends of the extracted stem sections until occluded sapwood was observed. Occlusion was identified by a darkened sapwood appearance (Solheim and Krokene 1998; Lee et al., 2006). Occluded stem length by tree was calculated as the mean of two values on opposite sides of the stem segment.

6.3.6. Radial stem occlusion and sapwood assessment

Areas of occluded sapwood and sapwood growth after inoculation were measured on the basal side of each disc cut from the occluded length of the stem sections. These areas were traced onto a transparent plastic sheet and their areas were determined by a planimeter (Lasico®, Los Angeles, CA, USA). Areas of total sapwood, occluded sapwood, and sapwood growth since

inoculation among all 5 cm wide stem discs per 100 cm long stem section were determined by tree. Subsequently, areas of sapwood occlusion and new sapwood growth after inoculation by tree were expressed as a percentage of total sapwood area in 100 cm stem sections.

6.3.7. Classification of sapwood at the inoculation zone

Sapwood infection by *L. terebrantis* was used to classify the transverse sections that were taken through the inoculated zone of the trees. Sapwood at the inoculation zone was classified as continuous new sapwood but non-uniform in width, discontinuous new sapwood around the circumference of the tree, or absence of new sapwood growth. *Leptographium terebrantis* was re-isolated from each transverse section of the harvested trees. From each section a 5 mm of the stem tissue around the inoculation point was plated on selective media (MEA containing 800 mg L^{-1} of cycloheximide and 200 mg L^{-1} of streptomycin sulphate) to confirm the re-isolation of the inoculated fungus from the host tissue. Plates were incubated at 23°C for 14 days and cultures resulting from plating were morphologically identified.

6.3.8. Data analysis

The main effect of *L. terebrantis* inoculum density on stem lesions, sapwood occlusion and new sapwood area formed were analyzed by one-way analysis of variance (Proc GLM, SAS Inc., Cary, NC, USA). The relationship among occluded sapwood, lesions and new sapwood area was analyzed using Proc Reg (SAS Inc., Cary, NC, USA). Prior to analysis, each dependent variable was checked for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests respectively. The treatment effects were considered significant at α=0.05 and Tukey's adjustment for differences in least square means. Linear (occlusion vs lesions) and non-linear (new sapwood area vs occlusion area) relationships were also examined. The control treatment was not included in the analysis because of a consistent absence of lesions and occlusions.

6.4. RESULTS

Visibly, a high volume of oleoresins exuded from the inoculation zone of the high inoculum treatment trees with less oleoresins visibly produced by the low and medium inoculation treatment trees by five months after inoculation with *L. terebrantis* (Figure 6.1). The wound treatment trees did not visibly produce oleoresins during the study period. Oleoresin exudation from the high inoculation treatment trees continued but no crown symptoms were evident 12 months post-inoculation. Foliage chlorosis was initially detected among some high inoculum treatment trees 19 months (Figure 6.2) following inoculation after a summer drought in 2018. Foliage chlorosis and the development of sparse crowns continued and were associated with 20% mortality among the high inoculum treatment trees. Lateral roots of some high inoculum treatment trees showed resinosis (Figures 6.3a and 6.3c).

Inoculation treatment significantly affected occlusion lesion length (P<0.0001). Lesion length among the low, medium and high treatments were significantly different at 16.0, 21.5 and 27.6 cm, respectively (Figure 6.6a). A significant (P<.0001, r^2 =0.95) linear positive correlation was found between lesion and occlusion lengths caused by the pathogen.

Inoculation treatment significantly affected occlusion length (P<0.0001). The trend in occlusion length in response to inoculum density was similar to, but less distinct than that of occluded sapwood area. Occlusion lengths of the high and medium inoculation densities were not

significantly different and averaged 38.4 cm (Figure 6.6b). Occlusion lengths associated with both high and medium inoculation densities were significantly greater than that of the low inoculation density (21.7%) and control wound treatment (1.2%). Occluded length of the low inoculation density was significantly greater than that of the control wound treatment.

Inoculation treatment effect significantly (P<0.0001) affected sapwood occlusion area (Table 6.1). The high (83.1%) and medium (36.2%) inoculum treatments were significantly different from each other and from the low and control wound treatments. The low and wound treatments were not significantly different and averaged 8.5% (Figure 6.6c).

Fungal inoculation treatment had no significant effect on stem moisture content (MC) at breast height (P=0.2862) (Table 6.1). However, a non-significant trend in tissue MC was evident as MC decreased between the wound control (89.2%) and inoculum density (low: 86.9%, medium: 84.7%, high: 77.8%) treatments. The pathogen was re-isolated from 100% of the sapwood disks of harvested trees that were inoculated at the low, medium or high inoculum densities.

Post-treatment sapwood growth was not significantly different among the wound control, and low and medium inoculum densities and averaged 31.5%. Post-treatment sapwood growth of the high inoculum density (15.2%) was significantly less than those of the wound control, and low and medium inoculum densities. The pattern of post-treatment sapwood growth in response to an increase in inoculum density is opposite to that of lesion and occlusion lengths, and occluded sapwood cross-section area (Figure 6. 4).

A significant (P<0.0001, r^2=0.89) but non-linear relationship was observed between post-treatment sapwood growth and occlusion area (Figure 6.7). The inflection point of this function (40, 33.4) represents the threshold of occluded sapwood area that was tolerated while post-inoculation sapwood growth continued at a normal level. The low and medium inoculum treatments were 97.5% and 9.2% below the inflection point, respectively and high inoculum treatment was 107.5% above the point. The post-inoculation sapwood of the low and medium inoculum and control wound trees appeared as a uniform and continuous ring (Figure 6.4a, b, c). The post-inoculation sapwood of the high inoculum density trees was, however, non-uniform in width (Figure 6.5a), discontinuous around the circumference of the tree (Figure 6.5b and 5c), or absent (Figure 6.5d). Among trees treated with the high inoculum density, 27% formed a continuous ring of sapwood and 53% formed a discontinuous ring of sapwood around the periphery of occluded sapwood, and 20% failed to produce sapwood after stem inoculation with *L. terebrantis*.

6.5. DISCUSSION

We assessed the potential of the root pathogen *L. terebrantis* to contribute to loblolly pine crown symptomology and tree mortality. Using artificial stem inoculation as a surrogate for woody roots to study the mechanism and process of *L. terebrantis* infection in pine decline, we found that stem infection at high fungal inoculum density caused the formation of sparse crown. The crown deterioration and associated tree mortality, however, occurred during moderate drought at the study site. Nevertheless, below this threshold of fungal infection of one colonized toothpick per 1.2 cm over the bark circumference, there was no manifestation of crown symptomology. It

is noteworthy that this threshold applies to artificially inoculated stems, and that the threshold of inoculum density may differ for woody roots of loblolly pine undergoing growth decline.

Fungal inoculum density must reach a critical threshold to cause symptoms development in the host. Among the three *L. terebrantis* inoculum densities, only the high inoculum density contributed to tree mortality. Previously, pine seedling mortality involving *L. terebrantis* has been reported (Wingfield, 1986) but mortality in mature trees was unknown. Other *Leptographium* species have been shown to cause tree mortality at low inoculum levels when additional tree stresses was imposed. For instance, Solheim et al., (1993) found that *Leptographium wingfieldii* Morelet and *Ophiostoma minus* (Hedge.) H. et P. Syd. fungi killed *Pinus sylvestris* L (Scots pine) when inoculated at 800 points/m^2. Additionally, they noted that both fungi also cause mortality at lower inoculum density when the tree vigor was reduced through pruning. Lee et al., (2006) found that in mature lodgepole pine (*Pinus contorta* Dougl. ex Loud. var *latifolia* Engelm. ex S. Wats), artificial inoculations with *Leptographium longiclavatum* sp. nov., vectored by the mountain pine beetle caused the formation of chlorotic crowns. However, symptoms were only apparent 9 months post-inoculation and only occurred at a high inoculum density (800 points/m^2) without mortality.

The damage caused by *L. terebrantis* infection occurred in two phases. The first phase was direct damage to sapwood caused by *L. terebrantis* which includes lesions and occlusions. Transverse sections of the high inoculum trees at the inoculation zone indicate that over 80% of the sapwood was occluded. The occlusion was due to the deposition of oleoresins and other secondary metabolites in response to pathogen spread (Viiri et al., 2001; Arango-Velez et al., 2018). The comparatively higher lesions and sapwood occlusions produced in the high inoculum treatment trees may explain the apparent whole-tree physiological disorders associated with the high inoculum trees. Additionally, the lesions, a direct cause of pathogen invasion, block phloem tissues and inhibit translocation of photosynthates from the crown to below ground tissues. The growth of new fine roots is largely dependent on new photosynthates (Lynch et al., 2013), which affect fine root turnover.

The second phase of damage was the loss of sapwood and whole-tree hydraulic function when significant *L. terebrantis* spread as a contributing factor, coincided with drought. This indirect response to *L. terebrantis* infection occurred when together, reduced sapwood function caused by the high inoculum density and drought as an inciting factor created water limitations to C-fixation. Moisture stress due to xylem tissue occlusion compromises stomatal conductance leading to a decrease in photosynthesis rate and whole-crown carbon fixation (Wertin et al., 2010; Oliva et al., 2014). By the time trees were destructively harvested, it was evident that post-inoculation sapwood growth was C-limited among trees treated with the high inoculum density. This reiterate the assertion of *Leptographium* sp. being a weak pathogen (Hansen, 1997) and unlikely to independently cause crown symptoms. Specifically, as long as predisposing and inciting factors are not present, the mechanism of *L. terebrantis* in loblolly pine decline may not be manifested.

Stem moisture content was affected beyond the lesion and occlusion areas suggesting a similar effect on foliage water stress. The relatively low MC of the high treatment at DBH suggests that the effect of the sapwood occlusion transcend the inoculation zone and can have whole-tree effects, including foliage MC (Mensah et al., unpublished). The reduction in MC content of the

high treatment trees relative to the low and medium treatments, perhaps imposed water and nutrient stress on the foliage, leading to chlorosis, needle shedding, and lack of needle replacement (i.e. thinning crowns) (Butnor, et al., 1999; Lee et al., 2006; Mensah et al., 2020). Collectively, the pathogen inoculum density, moisture stress due to pathogen spread and the drought condition play a crucial role in *L. terebrantis* and *P. taeda* interactions, leading to whole-tree symptomology. Under drought conditions or moisture stress, tree vigor is reduced and pathogen-induced sapwood occlusion and lesions may hasten crown symptoms and tree mortality, as has been observed in natural pine stands with high pathogen infection (Eckhardt et al., 2007; Kolb et al., 2019). Elsewhere, reductions in tree vigor increases pathogen activity at lower inoculum levels in ophiostomatoid fungi (Solheim et al., 1993).

Tree response to *L. terebrantis* inoculum density could be classified into three levels, continuous new sapwood but non-uniform in width, discontinuous new sapwood around the circumference of the tree, or lack of new sapwood. Continuous new sapwood growth after inoculation was observed in trees that were wounded or received the low or medium inoculum density. In addition, four of the 15 trees receiving the high inoculum density grew a continuous but non-uniform band of sapwood around their stem circumference. Despite the greater occluded sapwood area compared to the wound, low, and medium trees, the "superior" high inoculum trees sustained growth after inoculation. This suggests that the physiological conditions required for adequate C-fixation and allocation to the stem were met in these four "superior" trees. Again, a positive genetic effect on hydraulic function perhaps sustained whole-crown gas exchange at higher rate during the day instead of shutting down. More than half (8 trees) of the high inoculated trees formed a discontinuous sapwood growth around the circumference of the trees. The absence of continuous sapwood growth perhaps may be attributed partly to pathogen damage to, or occlusion of vascular cambium and/or phloem cells. Without active phloem in the vicinity of the vascular cambium, transport to the vascular cambium cells would die.

Three high inoculum trees failed to produce new sapwood growth around the occluded sapwood. Absence of new sapwood formation could be explained by the tree's genetics which did not allow production of oleoresin or other defenses to isolate the pathogen. Again, it may also be attributed to a low ratio of root to shoot, and under the stress of massive sapwood occlusion and a smallish root system. These trees could not keep water supply for C-fixation to grow adequate sapwood after inoculation to compensate for the massive occlusion of older sapwood. Devkota et al., (2018) demonstrated intraspecies variation in relative susceptibility of *P. taeda* trees to *L. terebrantis* and *Grosmannia huntii* after 8 weeks of infestation. The variation in susceptibility of loblolly pine to the pathogen may be due to the seed source used for the establishment of the stand plantation. Open pollinated stands carry different male genotypes and the differences in genes perhaps may explain the difference in susceptibility of loblolly pine to *L. terebrantis* infection.

Loblolly pine trees and saplings differ in their post-inoculation sapwood response to similar high inoculum *L. terebrantis* inoculation. Susceptibility to decline (inability to sustain sapwood growth) might be greater for older trees compared to younger trees at the same inoculum level. Larger trees have higher maintenance respiration costs and thus will have less fixed C available for growth or even defense at the vascular cambium/phloem location. Again large trees in plantation settings near or at crown closure may have less leaf area/stem basal area and lower light levels than open-grown young trees. Therefore the larger trees may not be able to supply

the C as readily for post-inoculation sapwood growth and defense compared to the younger trees. Finally, the hydraulic conductivity of inner sapwood is lower than that of outer sapwood and larger trees may not be able to sustain water supply to their crowns as easily as younger trees. This limits whole-crown C-fixation in larger trees compared to younger trees. For example, Mensah et al., (2020) found that young *P. taeda* trees inoculated at similar inoculation densities could tolerate *L. terebrantis* in naturally regenerated stands approximately 5-7 years old. The young trees produced a complete ring of new growth around the occluded sapwood regardless of the inoculum density (Mensah et al., 2020). In contrast, three high inoculum trees in this study failed to produce new sapwood to sustain hydraulic function culminating in mortality. This suggests that the chances of *P. taeda* trees survival following attack by bark beetle associated *L. terebrantis* may be determined by the size of new sapwood growth formed.

6.6. CONCLUSIONS

The study shows that *L. terebrantis* can contribute to the formation of sparse crown symptomology and tree mortality in plantation *P. taeda*. Crown deterioration occurred at high fungal inoculum density coupled with drought during the study period. Below this inoculum threshold of one colonized toothpick per 1.2 cm over the stem circumference, the trees survived by forming post-inoculation sapwood devoid of fungal infection to sustain hydraulic function. Post inoculation sapwood formation among the high inoculum treatment was either continuous with non-uniform width or discontinuous around the occluded sapwood. High inoculum trees that failed to produce sapwood around the occluded sapwood culminated in tree mortality. This indicates that the chances of *P. taeda* trees survival following attack by bark beetle associated *L. terebrantis* may be determined by the new sapwood growth formed.

Table 6.1. Probabilities of a greater F value (*P>F*) for lesion, occlusion length, occlusion area, new growth and stem moisture content of *P.taeda* inoculated with *L. terebrantis*.

Variable	**Df**	**F value**	***P > F***
Lesion	3	64.29	<0.0001
Occlusion length	3	39.31	<0.0001
Occlusion area	3	146.18	<0.0001
New growth	3	14.16	<0.0001
Moisture content	3	1.31	0.2862

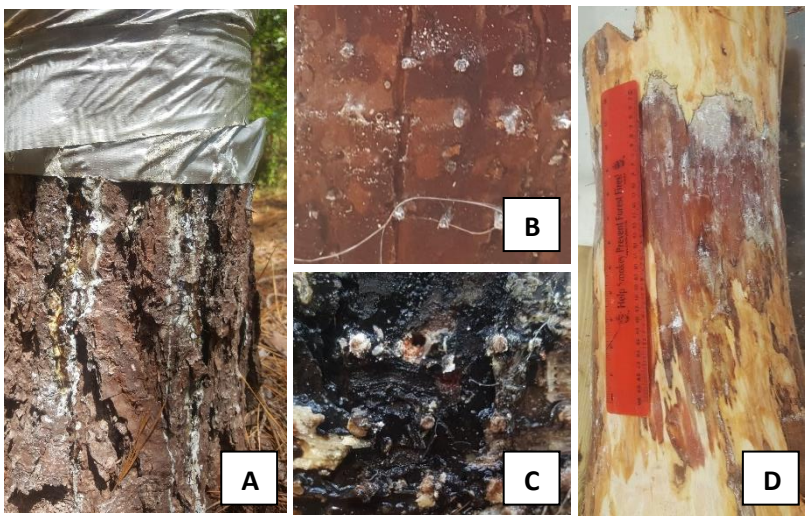

Figure 6.1. Inoculation zone symptoms of high inoculum treatment trees of *P. taeda* (a) oleoresin exudates (b) wound treatment without oleoresin exudates (c) black stains (d) occlusions extending from the inoculation zone.

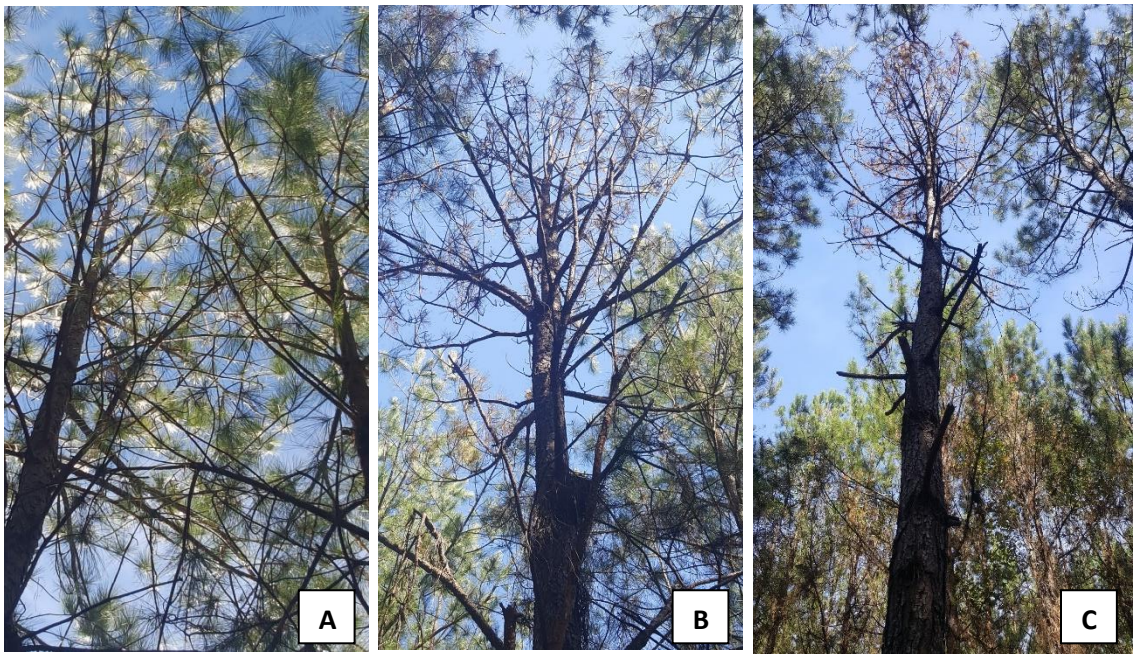

Figure 6.2. *P. taeda* showing crown symptoms after inoculation with *L. terebrantis* at high inoculum density (a) two trees showing symptomatic crown (b) chlorotic and thin crown (c) crown of dying tree.

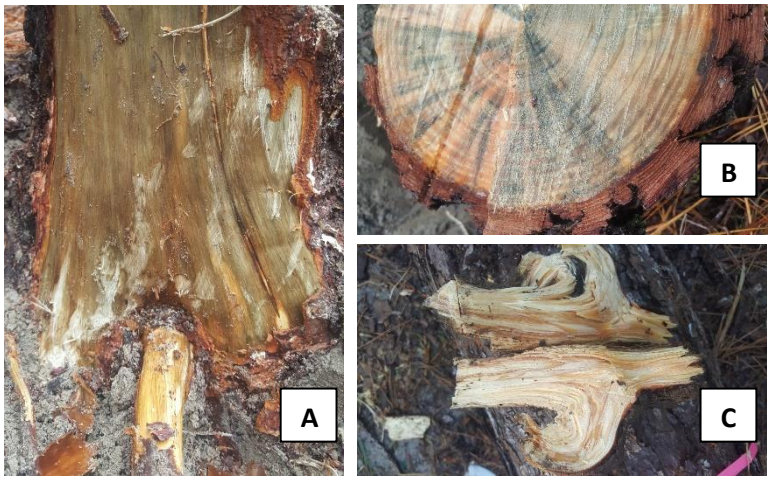

Figure 6.3. Below ground and stump surface symptoms of high inoculum treatment trees of *P. taeda* (a) lateral root resinosis (b) blue black stains at the surface of a stump (c) split lateral root showing resinosis.

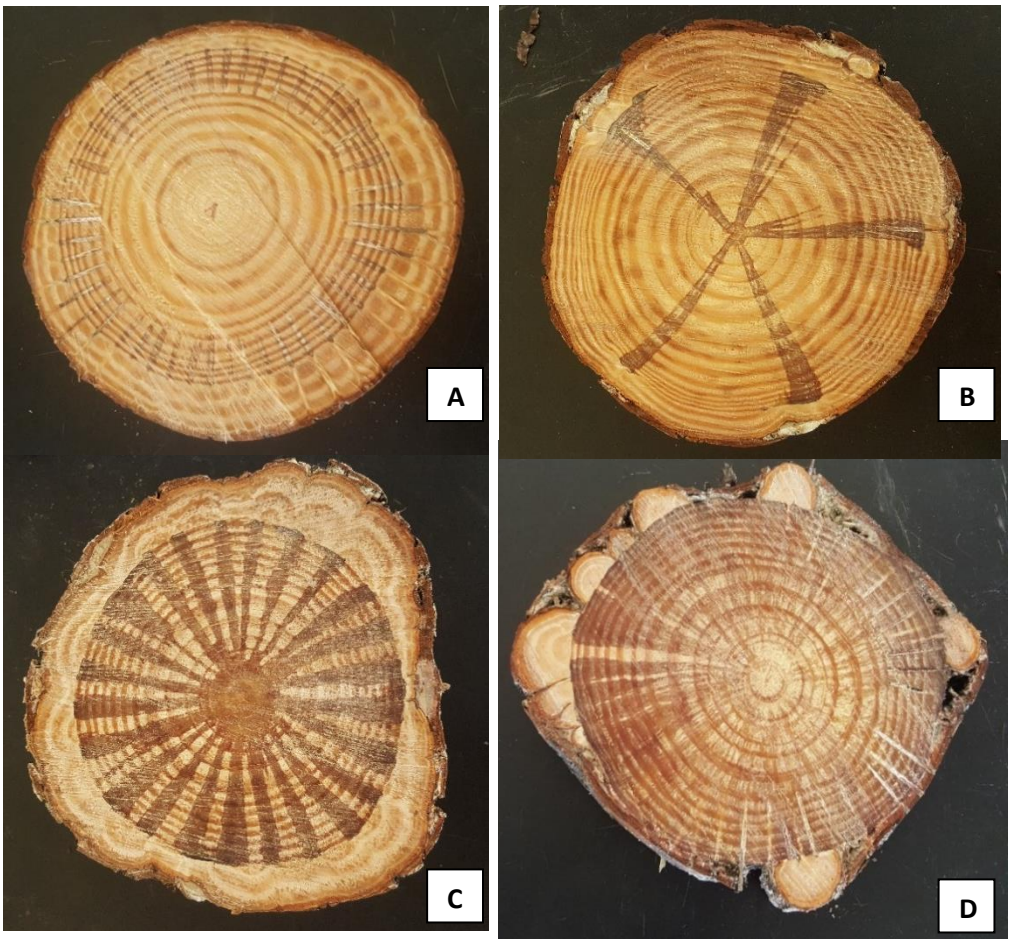

Figure 6.4. Cross-sections at the inoculation zone of *P. taeda* trees showing infection levels of the different treatment (a) wound treatment with sterile toothpicks (b) occlusions at low inoculum density (c) occlusions at medium inoculum density (d) occlusions at high inoculum density.

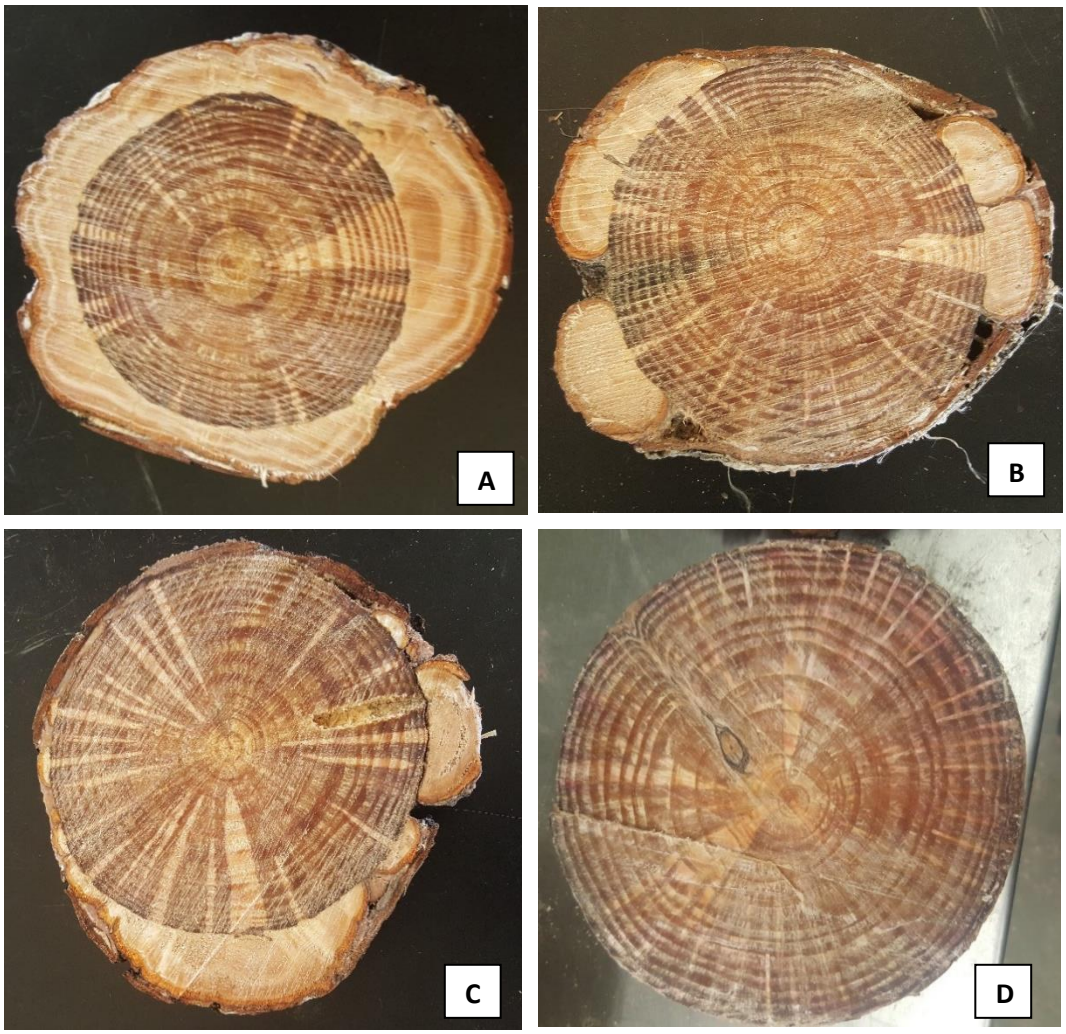

Figure 6.5. Cross-sections of high inoculum *P. taeda* trees showing new sapwood (a) trees with continuous new sapwood growth (b) discontinuous new sapwood with approximately 50% of new growth ring (c) discontinuous new sapwood with less than 50% of new growth ring (d) trees without new sapwood around occluded sapwood.

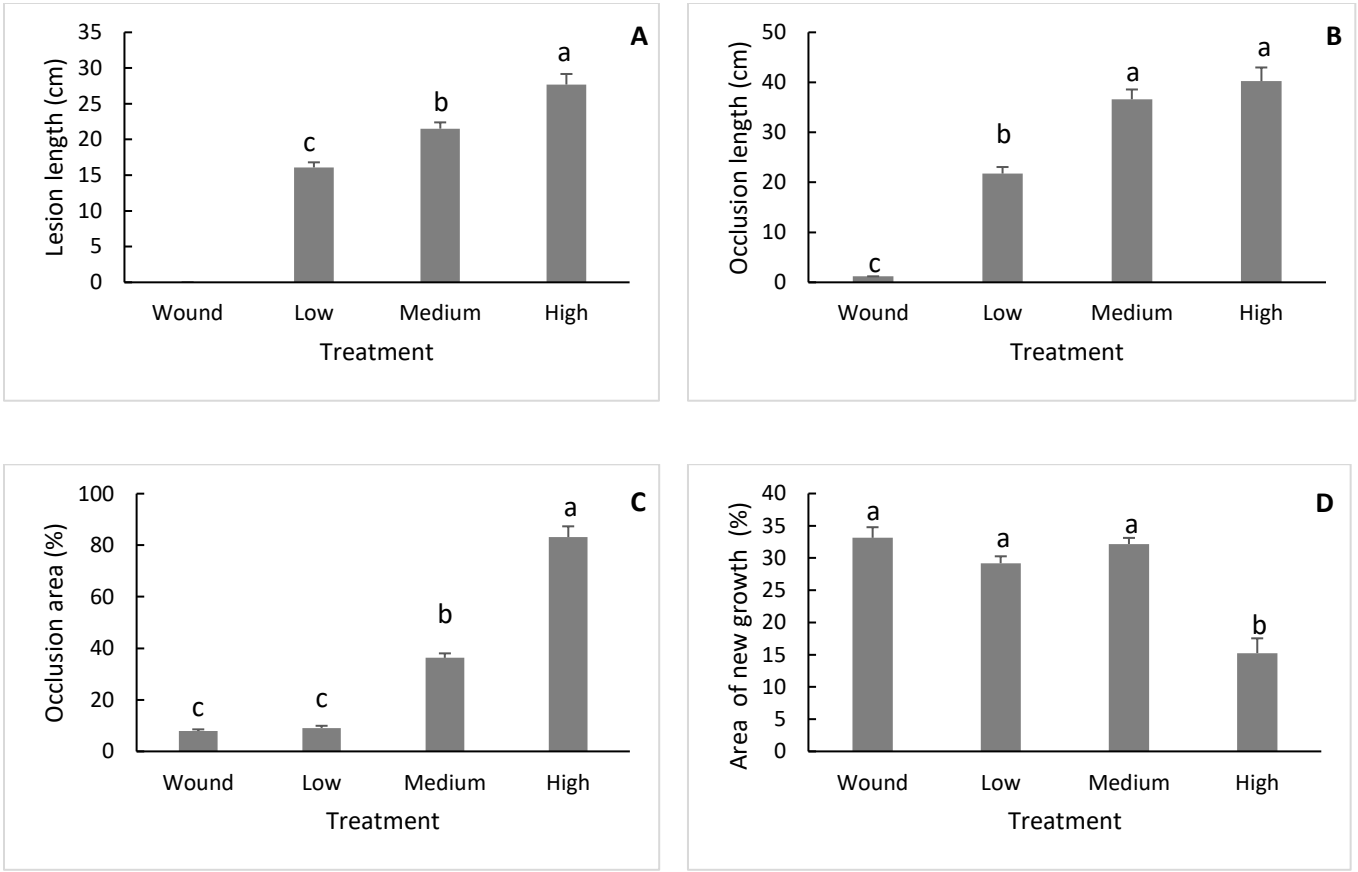

Figure 6.6. *Pinus taeda* response to *L. terebrantis* infestation following lower stem inoculation: (a) lesions produced in phloem (b) occlusion length (c) occlusion area) and (d) area of new sapwood growth formed following destructive sampling in January and February 2020.

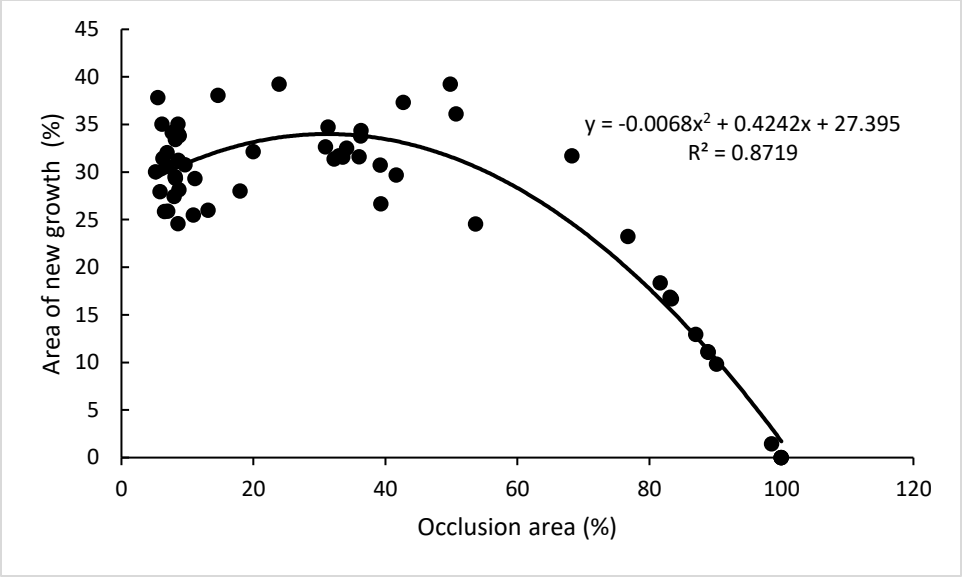

Figure 6.7. A significant non-linear relationship between area of new sapwood growth devoid of infestation and sapwood occlusion area caused by *L. terebrantis* infestation.