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EFFECT OF *LEPTOGRAPHIUM TEREBRANTIS* AND DROUGHT ON FOLIAGE, NEW ROOT DYNAMICS AND STEMWOOD GROWTH IN PLANTATION *PINUS TAEDA*

by
John Mensah and Lori Eckhardt

4.1. ABSTRACT

The course of the bark beetle-vectored fungus, *Leptographium terebrantis* S. J. Barras and T. J. Perry, in stemwood growth losses of declining pines of the southeastern U.S. was assessed. The study was installed in a 13-year-old loblolly pine (*Pinus taeda* L.) plantation near Eufaula, Alabama, U.S. Artificial inoculation with sterile toothpicks colonized by *L. terebrantis* at varying inoculum densities was used to elicit host growth responses. The root pathogen compromised xylem function and caused a reduction in foliage moisture content, leaf area (A_L) and the ratio of A_L to tree sapwood area (A_S). Decreases in relative stemwood growth were more pronounced in trees receiving the high inoculum treatment relative to those receiving the low, medium, or wound-control treatments. This decline in stemwood growth was associated with 7-months of water deficit suggesting that in the loblolly pine and *L. terebrantis* pathosystem an additional factor of water deficit is required to enable the pathogen's role in stemwood growth loss. Thus, presence of *L. terebrantis* in pine forests of the southeastern U.S. which are vulnerable to water deficit has the potential to widen the gap between predicted and actual stemwood production.

4.2. INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is the principal tree species grown in forest plantations across the southeastern U.S. (Schultz, 1997). To sustain economic and ecological contributions of this tree species in the region, about one billion loblolly pine seedlings are planted each year (McNabb and Enebak, 2008). Dominance of this tree species in the region is projected to increase for decades (Huggett et al., 2013) as a result of improved genetics and silvilcultural treatments that enhance plantation productivity. Over the past six decades, loblolly pine growth loss and mortality (declines) have been reported in localized areas of central Alabama and Georgia (Eckhardt et al 2010; Forest Health Cooperative, 2017).

Forest declines are caused by complex interactions between pests and abiotic factors (Manion, 1991; Manion and Lachance, 1992), and several theories have been formulated to explain the forest decline concept (Sinclair and Hudler, 1988; Manion, 1991; Auclair et al., 1992; Houston, 1992; Mueller-Dombois, 1992). Common among these theories is the supposition that no single factor causes forest decline but it is a multiplicity of factors that act in a sequential order for a decline outcome. The decline spiral model (DSM) proposed by Manion (1991) is the most common among the forest decline theories. The DSM reclassified the cause of decline from the chain reaction theory (Sinclair and Hudler, 1988) to the systematic occurrence of predisposing, inciting, and contributing factors.

According to the DSM, predisposing factors are long-term, underlying conditions such as genetic potential, climate, and soil quality that put permanent physiological stress on trees. Inciting factors are relatively short-term conditions such as drought or insect attack including defoliation that worsen the stress imposed by predisposing factors (Manion, 1991; Bigler et al., 2006; Liu et al., 2013; Williams et al., 2013). For example, Bigler et al., (2006) noted that the high mortality of Scots pine (*Pinus silvestris* L.) in the Rhône Valley and other dry areas of the European Alps during the early 20th century was incited by several years of drought.

Predisposing and inciting factors establish favorable conditions for contributing factors of decline which are biotic in nature such as population increases in stem- and root-feeding bark beetles and the subsequent spread of their associated fungi. These factors worsen physiological stress (Manion, 1991; Jurskis, 2005) and may accelerate growth loss and mortality. For example insects such as lesser pine shoot beetle (*Tomicus piniperda* L., *T. minor* Hart.), the pine processionary moth (*Thaumetopoea pityocampa*, Denis and Schiff.) and the six toothed bark beetle (*Ips sexdentatus*, Boern.) contributed to the Scots pine mortality in the Rhône valley (Rigling and Cherubini, 1999; Bigler et al., 2006)

In North America, forest declines threaten sustainable timber production across thousands of acres. Over 300,000 acres (approximately 121,405 hectares) of *Quercus* spp. (oak) were negatively affected by decline in the Ozark Mountains of northern Arkansas and southern Missouri in 1999 and 2000 (Starkey et al., 2000; Heitzman, 2003). Declines reported in these locations were attributed to interacting factors that included drought and outbreaks of red oak borer (*Enaphalodes rufulus* Haldeman) that led to stand mortality and modification of the landscape. Other forest declines in North America include aspen (*Populus tremuloides* Michx) in the western U.S. and Canada (Worrall et al., 2013), whitebark pine (*Pinus albicaulis* Engelm.) in western North America (Wong and Daniels, 2017), pinyon pine (*Pinus edulis* Engelm.) in the western U.S. (Gaylord et al., 2015), and loblolly pine (Brown and McDowell, 1968; Eckhardt et al., 2007) decline in the southern U.S.

Since the 1950s, loblolly pine decline (LPD) has been reported in several Alabama counties (Brown and McDowell, 1968; Eckhardt et al., 2007; Eckhardt et al., 2010). Stands of loblolly pine exhibiting decline symptoms are characterized by sparse crowns, short and chlorotic needles, reduced radial growth, woody roots with resinous bark wounds and stained sapwood, and tree mortality (Hess et al., 1999; Eckhardt et al., 2007). Root-feeding bark beetles that vector *Leptographium* spp. are commonly found in declining loblolly pines (Eckhardt et al., 2004; Eckhardt et al., 2007).

In accordance with the DSM (Manion, 1991), the bark beetle-fungal complex of LPD acts as a contributing factor to decline after trees have been predisposed. The role of stem-feeding bark beetles in pine mortality is well known and attributed to the tunneling beneath the bark and subsequent larvae production which girdles the tree and disrupts the transport of photosynthates from the foliage to the roots (Millar et al., 2012; Hicke et al., 2016; Berner et al., 2017). Similarly, root-feeding bark beetles damage the vascular cambium and conducting tissues near the point of woody root entry (Paine et al., 1997). Further disruption of xylem and phloem occurs by the spread of vectored fungal associates and the net effect may lead to stemwood growth loss and tree mortality. These fungal associates are not considered to be aggressive pathogens unless they overcome host defenses leading to an advancement of sapwood occlusion (Six, 2003; Six and Wingfield, 2011). *Leptographium terebrantis* is one of several bark beetle-vectored fungi commonly isolated from woody roots of loblolly pine exhibiting symptoms of LPD.

Several studies have shown that *L. terebrantis* may be pathogenic by inducing sapwood occlusion in loblolly pine seedlings, saplings, and mature trees under greenhouse or field conditions (Matusick et al., 2016; Devkota and Eckhardt, 2018; Mensah et al., 2020). At the same time, tree growth and physiological responses to *L. terrebrantis* are variable despite distinct signs of pathogenesis (Mensah et al., 2020). Thus, the contribution of *L. terebrantis* infection to LPD when vectored by root-feeding bark beetles is unknown. *Leptographium terebrantis* grows into xylem tissues and disrupts water and mineral nutrient transport from the soil to the crown (Oliva et al., 2014; Mensah et al., 2020). This compromises xylem function by limiting water conductance and reduces both stomatal function and carbon fixation. Poor xylem function risks inadequate carbon for normal foliage, stem, and root system growth and constitutive chemical defense production. For example, Viiri et al., (2001) noted the reduction in total soluble carbohydrates near the site of fungal infection when Norway spruce (*Picea albeis* L.) was inoculated with the bark beetle-associated fungus, *Ceratocystis polonica* (Siemaszko) C. Moreau**.** Disruption of xylem can lead to mortality in mature trees (Tyree and Zimmermann, 2002).

In this study, we assessed the annual stemwood growth of loblolly pine trees grown in commercial plantation. These trees were either non-inoculated or inoculated at one of three densities with *L. terebrantis*, to determine the potential for this pathogen to affect stemwood growth. In addition, seasonal assessments of new root growth, and destructive measurements of leaf area and stem sapwood area 34 months after inoculation provide knowledge about the effect of *L. terebrantis* infection during the progression of LPD. We hypothesized that *L. terebrantis* infection has the potential to impair whole-crown carbon fixation such that tree leaf area, new root production, and stemwood growth become carbon-limited. We further hypothesize that this response is not apparent until together, site conditions and a threshold of *L. terrebrantis* infection cause carbon limitations that cannot be tolerated by the tree.

4.3. METHODS

4.3.1. 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.

4.3.2. Minirhizotron tube installation

Four clear acrylic tubes, 82 cm in length and 3.81 cm inner diameter, were installed around each of two trees of comparable DBH per row and plot (two treated and two untreated trees). Each tube was installed at a 152.4 cm distance from the base of the bole and at a 45^{o} angle toward the tree with two tubes installed on each side of the original planting row. The circumference of the tubes was scored at eight 10 cm increments corresponding to 0, 7.1, 14.1, 21.2, 28.3, 35.4, 42.4

and 49.5 cm below ground level as described by Duwadi (2019). The 10 cm length of the upper end of the tube was covered with a black tape to prevent light from entering the tube and plugged with a #7 rubber stopper to prevent invasion of the tube by insects or water. The tubes were then covered with small plastic pot as described by Duwadi (2019).

4.3.3. 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 °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).

4.4. Measurements

4.4.1. Stem growth

Tree diameter at breast height (DBH) expressed as cm was determined from measurement tree dendrometer bands every month from January 2016 to February 2017 before inoculation treatments were applied. These measurements continued between March 2017 and December 2019 after inoculation treatments were applied. Total measurement tree height was quantified and expressed as m by a TruPulse 200 Rangefinder-Hypsometer (Laser Technology Inc, Centennial, CO, USA) in January each year between 2016 and 2020. Measurement tree basal area (BA) was determined by equation (1),

$$BA= (\pi D^2)/4 \qquad (1)$$

where D is DBH expressed as cm. Annual tree basal area increment (BAI) expressed as cm^2, was calculated in 2016 through 2019 as the difference between current year (BA_2) and previous year (BA_1) tree basal areas in January.

Relative stem radial growth (RG) of the measurement trees was determined as the ratio of BAI and BA_1 from 2016 to 2019 (Johnson and Abrams, 2009). Annual outside-bark stem volume of the measurement trees was estimated as described by Burkhart (1977) for loblolly pine and expressed as m^3.

4.4.2. Root growth

New root growth was assessed and counted with the aid of an optical root periscope (JRD Merrill Speciality Equipment, Logan, UT) that had a fiber optic light powered by a battery. New root (< 2 mm diameter) growth measurements started 6 months after minirhizotron root tube installation and one-month post-inoculation in April 2017 and were repeated at 3-month intervals until trees were harvested in January-February 2020. During new root assessments, the number of pine roots that intersected the seven scored lines below ground level of each tube were visually identified and counted. New roots were identified by color and diameter (initially white/translucent and turning reddish-brown with time). New root growth, calculated as root length density (RLD) and expressed as cm cm^{-2} according to Newman (1996), was determined by equation (2),

$$\text{RLD} = R/A \qquad (2)$$

where R is total root length (cm) expressed by the equation, $R = (\pi * N * A)/(2 * H)$, N is the number of roots intersecting scored lines, A is the area of the minirhizotron tube between two scored lines, and H is the length of a scored line. Cumulative RLD (CRLD) by tube was calculated as the sum of seven RLD values between ground level and the 49.5 cm depth and tree CRLD was calculated as the average of four CRLD values by tree.

4.4.3. Leaf area, sapwood area and tissue moisture content

In January and February 2020, the five treated measurement trees in one of two rows per plot were felled at the ground line with a chainsaw. Green foliage was removed from branches and weighed within 2 to 4 h after tree harvest to determine live foliage fresh weight per tree (W_1). Green foliage was transported to the laboratory. Three subsamples, each containing between 15 and 25 three-needle fascicles were randomly sampled by tree to estimate the ratio of total leaf area and foliage dry mass per tree. Subsequently, the remaining fresh foliage was oven-dried at 70 ^{o}C to a constant weight (W_2). The moisture content (MC) of the foliage at the time of tree harvest was expressed as percentage of oven-dried weight by the equation, $MC= [(W_1-W_2)/W_2]*100$.

The total leaf area of each subsample of fascicles was determined by volume displacement of bundles of two fascicles as described by Johnson (1984) using equation (3),

$$A = 2L\left[1 + \frac{\pi}{n}\right]\sqrt{(Vn/\pi L)} \qquad (3)$$

where A is total surface area (cm^2), L is cumulative green needle length (cm), V is volume displaced by the fascicle bundle (cm^3), and n is the number of needles per fascicle bundle. The total leaf area of fascicle subsamples was calculated as the sum of A among fascicle bundles and projected leaf area by fascicle subsample was expressed as A divided by 3.142 (Grace 1987). Fascicle subsamples were oven-dried at 70^{o}C to a constant weight and their specific leaf area (SLA) was calculated as the ratio of projected leaf area and dry weight (cm^2 g^{-1}). Tree SLA was estimated as the mean of three SLA values by tree and projected leaf area by tree (A_L) was calculated as the product of tree SLA and W_2.

A wood disc, approximately 5 cm in thickness, was cut with a chainsaw at DBH. The circumference of sapwood area was traced on a transparent sheet and sapwood area (A_S) was determined with a planimeter (Lasico®, Los Angeles, CA, USA). The ratio of A_L and A_S was calculated by tree. Tree growth efficiency (GE) at the time of tree harvest was determined as the BAI in 2019 divided by leaf area (A_L) and expressed as cm^2 m^{-2}

4.5. Data analysis

The main effect of *L. terebrantis* inoculum density, treatment duration and their interaction on growth parameters was analyzed. Values of DBH, total tree height, stemwood volume, relative radial stem growth (RG), tree leaf area (A_L), foliage moisture content (MC), tree growth efficiency (GE), A_L-to- sapwood area (A_S) ratio, and cumulative root length density (CRLD) were assessed for normality and equal variance assumptions. Values of DBH, total tree height, stemwood volume, and RG were evaluated by a completely randomized experimental design using two-way repeated measures analyses of variance and the Mixed procedures of SAS statistical software (SAS Institute, Version 9.4, Cary, NC, USA) with compound symmetry as the covariance structure.

Similarly, tree A_L, MC, GE, A_L:A_S, and CRLD were analyzed by one-way analyses of variance and the GLM procedures of SAS statistical software (SAS Institute, Version 9.4, Cary, NC, USA). Treatments were control, wound, and low, medium, and high inoculum densities. Significant main and interaction effects were further evaluated by a pair-wise comparison among means using the post-hoc Tukey's Honest Significance Difference Test (HSD) for multiple comparisons. Linear relationships between A_L, and DBH were assessed by regression. Regression parameters of pairs of significant A_L and DBH lines were compared by the general linear test using the REG procedure of SAS statistical software (Neter and Wasserman 1974). Probabilities of a greater *F*-value and mean comparisons were considered marginally significant at an α-level of 0.10 when a biologically related difference was significant elsewhere in the data at an α-level of 0.05.

4.6. RESULTS

4.6.1. Temperature and precipitation at the study area

Annual precipitation during the four-year period was 992.1, 1311.4, 1259.8 and 954.5 mm in 2016, 2017, 2018 and 2019 respectively (Figure 4.1). Prior to tree inoculation in 2017, severe drought occurred in the dormant season of 2016 and in October there was no precipitation. Moderate drought also occurred in 2019 after inoculation and the site received 7 months of less precipitation from March to September with 45% lower than the 30-year average for the area. Overall average monthly air temperature for the measurement period was 18.2, 18.0, 18.1 and 18.4^{o}C in 2016, 2017, 2018 and 2019, respectively. The average monthly air temperature during the 7- month drought period is 22.8 ^{o}C relative to the 30-year average of 22.4 ^{o}C within the same period (Figure 4.1).

4.6.2. DBH, tree height and stemwood volume

At the time of plot establishment in December 2015, mean values of DBH, total height, and stemwood volume by measurement tree were 16.88 ± 0.50 cm, 14.02 ± 0.20 m, and 0.3209 ± 0.023 m^3 respectively. Tree growth was monitored for 14 months prior to treatment application and during this period, no symptoms or signs of disease were observed among the study trees. At the time of treatment application in March 2017, mean plot values of DBH, total height, and stemwood volume by measurement tree were not significantly ($P>0.05$) different among the treatments. Mean values of DBH, total height, and stermwood volume for the treated plot trees are shown in table 4.1. Stem inoculation of loblolly pine trees with *L. terebrantis* did not significantly ($P>0.05$) affect DBH, total height or stemwood volume between treatment application in March 2017 and tree harvest in December 2019. Over the 3-year period since treatment application, DBH, total height, and stemwood volume were significantly affected by year, but not treatment as a main effect. Interaction between treatment and year did not significantly affect DBH but significantly affected total height and had a marginally significant effect on stemwood volume (Table 4.2). Mean total height was 14.8 ± 0.2 m (Figure 4.2a, b and c).

Relative growth (RG) was significantly affected by the main effects of inoculation treatment and year as well as their interaction (Table 4.2). The low and high treatments had the highest (18.5 m) and lowest (16.7 m) height growth, respectively, at the end of 2019. Relative growth peaked in 2017 and declined in the subsequent years and attained minimum value in 2019. The control and medium treatments had highest and lowest RG growth respectively in 2017. Although trend of RG growth declined among the treatments in 2018 and 2019, the high treatment trees had the highest rate of decline (47%) (Figure 4.2d).

At the end of 2017, decline symptoms such as chlorotic and thin crown were not observed among the treatments. In 2018, several trees treated with the medium and high inoculum densities exhibited resinosis above and below the inoculation zone which was sealed with duct tape. In late 2018 and throughout 2019, decline symptoms including chlorotic and thin crowns were manifested in trees treated with the high inoculum density.

4.6.3. Leaf and sapwood areas, foliage moisture content, and tree growth efficiency

Estimates of whole crown projected leaf area (A_L) were significantly affected by inoculation treatment at the end of the study (Table 4.3). Values of A_L were significantly lower among trees receiving the high inoculum density treatment compared to those receiving the wound or control treatments (Figure 4.3). Variables derived from A_L were also significantly affected by inoculation treatment at the end of the study. Specifically, A_L:A_S was significantly greater for the control and wound treatments compared to the high inoculum density treatment and GE was significantly greater for the control treatment compared to the low and high inoculum density treatments (Table 4.4). A marginally significant effect of inoculation treatment on MC was observed at the end of the study ($P = 0.0904$) with 13.7% higher MC among control trees compared to those treated with the high inoculum densities. A significant ($P < 0.0001$) positive linear relationship was found between A_L and DBH for all inoculation treatments except the high inoculum treatment (control: $P = 0.0005$, $r^2 = 0.84$; wound: $P = 0.0013$, $r^2 = 0.67$; low: $P = 0.0001$, $r^2 = 0.87$; medium: $P = 0.0011$, $r^2 = 0.57$; high: $P = 0.5125$, $r^2 = 0.04$). Slope and y-intercepts associated with the control, wound, and low and medium inoculum density treatments were not significantly different from each other. However, the slopes and y-intercepts of the control, wound, and low and medium inoculum density treatments were significantly different from those of the high inoculum density treatment. The slope of this relationship for trees receiving the high inoculum density treatment was significantly lower than those of trees receiving the control ($P = 0.0029$), wound ($P = 0.0030$), or low ($P = 0.0155$), or medium ($P = 0.0414$) inoculum density treatments. An inverse but similar treatment response was observed for y-intercept with significantly lower values among the control ($P = 0.0119$), wound ($P = 0.0131$), and low ($P = 0.0004$), and medium ($P = 0.0162$) inoculum density treatments compared to the high inoculum density treatment.

4.6.4. Root growth

Cumulative root length density (CRLD) was not significantly affected by inoculation treatment in 2017, 2018, or February 2019 (Duwadi 2019). A trend of reduced CRLD was seen with increasing inoculum density in July 2019, but a significant treatment effect was not observed. Three months later in October 2019, CRLD was significantly affected by inoculation treatment ($P = 0.0361$). Mean CRLD for trees treated with the high inoculum density was 60% lower than that among trees treated with the control, wound, or low or medium inoculum density treatments (Figure 4.5).

4.7. DISCUSSION

We investigated the potential of *L. terebrantis* to affect *P. taeda* growth and hypothesized that the pathogen infection will impair whole-crown carbon fixation such that tree leaf area, new root production, and stemwood growth become carbon-limited. We further hypothesize that this response is not apparent until together, site conditions and a threshold of *L. terrebrantis* infection can cause carbon limitations that cannot be tolerated by the tree. *Leptographium terebrantis* adversely affected *P. taeda* growth and caused growth decline by compromising xylem function. Decline symptoms however, occur at a critical threshold of pathogen spread and coincided with a seven-months growing season moderate drought. Hydraulic malfunction due to sapwood occlusion caused by the pathogen induced premature foliage senescence and decline in stem growth of *P. taeda* trees.

Leptographium terebrantis infestation caused a reduction in A_L and was most severe at high inoculum density relative to the low, medium, wound and control treatments. Loss of A_L reduces tree water use, light interception and carbon fixation. Tree growth is a function of A_L and factors that limit A_L reduces tree productivity (Vose and Allen, 1988; Albaugh et al., 1998; Jokela and Martin, 2000). Albaugh et al., (1998) found that in 8-yr-old loblolly pine stand in Scotland County, North Carolina; stem volume increased by 152% with an increase in peak leaf area index (LAI) of 101% after four years of fertilization. Additionally, they established that, irrigation treatment also increased stem volume by 25% with increase in LAI by 16%. The loss of A_L may be attributed to two conditions, drought and poor hydraulic function. First, prolonged drought between March and September of 2019 may have caused premature senescence of older foliage that would normally have senesced only in fall 2019. Premature foliage senescence would have been driven by several factors including drought (Hennessey et al. 1992; Naidu et al., 1993; Warren et al., 2011). Hennessey et al., (1992) noted that the variation in needle-fall patterns in loblolly pine correlates with the droughtiness of the growing season. They found that maximum monthly needle fall occurred earlier in dry years than in wet years when 10-year-old loblolly pine stand was monitored for 5 years after thinning. Second, poor hydraulic function of the stem due to the spread of *L. terebrantis* in the xylem tissues perhaps created water and mineral nutrient limitations to foliage growth which were exacerbated by growing season drought. The pathogen spread must, however, extend over a large cross-sectional area of the conducting sapwood before significant disruption in water transport occurs (Joseph et al., 1998; Liu et al., 2018; Mensah et al., 2020,).

The leaf area of a stem or branch is proportional to cross-sectional sapwood area (A_S) that sustains it (Tyree and Ewers, 1991) and the ratio of A_L:A_S is a key parameter for understanding tree water relations (Whitehead et al.,1984). In this study, the ratio of A_L:A_S was inversely related to *L. terebrantis* inoculum density, thus, trees inoculated at high inoculum density had the lowest A_L:A_S ratio compared to the low, medium, wound and control trees. As observed with A_L, reduced water availability that was simultaneously due to *L. terebrantis* spread in the sapwood and prolonged growing season drought may explain the lower A_L:A_S ratio in the high inoculum treatment trees. Togashi et al., (2015) noted that in several evergreen species, a positive correlation exist between moisture and A_L:A_S. In a Scots pine stand (*Pinus sylvestris* L), trees growing under moisture stress produce less leaf area per unit conducting sapwood area relative to tree growth in wetter areas (Mencuccini and Grace, 1995). The ratio of A_L to A_S is proportional to hydraulic conductivity (Whitehead et al., 1984), thus it can be inferred that the high inoculum treatment had a lower hydraulic conductivity (HC) relative to the low, medium, wound and control treatments.

A reduction in water transport was manifested in the trend of foliage MC, as it was lowest at high inoculum density. A loss in MC reduces tree vigor as stomatal conductance and photosynthesis are reduced (Drake et al., 2010; Wertin et al., 2010). Mensah et al., (2020) showed that *L. terebrantis* occlusions in young *P. taeda* trees significantly reduced sapwood moisture content in the inoculation zone. Reduction in water transport could adversely affect tree growth and cause branch or crown death during periods of water deficit (Klos et al., 2009; Ganey and Vojta, 2011; Anderegg et al., 2013). Naturally, reduction in resource availability such as moisture stress or drought reduces tree vigor and predisposes tree to attack by bark beetles and their associated fungal pathogens (Lorio Jr et al., 1995; Negron, 2009; Ganey and Vojta, 2011; Hart et al., 2014). It is therefore not surprising that Mensah et al. (Unpublished), found chlorotic crown symptoms and mortality when *P. taeda* trees were inoculated at high *L. terebrantis* inoculum density.

Stem relative growth (RG) correlates with precipitation and in 2017, RG peaked and declined in 2018 and 2019. Among the treated trees, RG was lowest in the high treatment trees prior to pathogen inoculation. But 9 months after inoculation, the high treatment trees had the highest increase (59%) in RG growth compared to less than 30% of the control, wound, low and medium at the end of 2017 (Figure 4.2d). The higher RG growth in 2017 also coincided with maximum precipitation (1311.4 mm) within the study period. This suggests that the contributory role of *L. terebrantis* to *P. taeda* growth decline may not be manifested when precipitation at the study site is similar to the mean annual precipitation of the area.

On the contrary, RG declined in 2018 and 2019 among the treated trees irrespective of the inoculum density or control treatment. This period of growth decline also corresponds with lower precipitation levels/drought in 2018 and 2019. For instance, in 2019, the localized precipitation was approximately 27.2% lower than 2017 and about 30% less the 30 year mean annual precipitation of the area (Figure 4.1). Within this period of drought, growth decline was most severe, 47.5% among the high treatment trees. Furthermore, comparable RG decline was observed for control, wound, and low treatment trees in 2018 and 2019. This suggests that the drought of 2019 was not bad enough to affect RG under minimal (low) *L. terebrantis* infection. This indicates that RG decline will only occur when drought interacts/combined with a critical threshold of *L. terebrantis* inoculum density.

The decline in 2019 RG among trees treated with the high inoculum density coincided with a gradual reduction in cumulative root length density at the 0 to 50 cm soil depth that became significant by October 2019. This loss of new root growth among trees treated with the high inoculum density may have contributed to the loss of RG as a result of compromised function of the tree's absorbing root network. The amount of fine root and ectomycorrhizal growth is influenced by resource availability (King et al., 2002; Jones et al., 2003; Sayer and Haywood, 2006; Coleman and Aubrey, 2018). King et al., (2002) found that fertilization increased net production of fine and ectomycorrhizal roots in 8-yr-old loblolly pine plantation after three years. In longleaf pine (*Pinus palustris* P. Mill), Sayer and Haywood (2006) observed that severe water limitation during growing season was associated with a delay in peak root growth, and prolonged drought also coincided with a reduction in root starch storage. Similarly, insufficient carbon allocation to the root system in the present study may have reduced new root growth and accelerated fine root and ectomycorrhizae mortality.

In addition to the simultaneous occurrence of poor hydraulic function caused by *L. terebrantis* and growing season drought, reallocation of carbon from stemwood growth to the synthesis of defense chemical compounds may have contributed to RG loss (Klepzig et al., 1995; Schultz et al., 2013; Sampedro, 2014; Villari et al., 2014). Production of defensive compounds increases in response to attack resulting in an increase demand for carbon (Schultz et al., 2013). Carbohydrates support the biosynthesis of plant phenolics and terpenes which are essential in defense against invading pest. Klepzig et al., (1995) found that both phenolics and monoterpenes production in the phloem increased following inoculation of 25-year-old red pine (*Pinus resinosa* Aiton) with *L. terebrantis* relative to non-inoculated trees. In the present study, copious oleoresins were exuded from the inoculation zone of trees treated with the high inoculum density about 5 months after inoculation and this continued till the end of 2019 when a reduction in RG was apparent.

As with tree declines in general, no single factor is known to cause growth loss and according to Manions's model (1991), growth decline occurs after predisposition, inciting, and contributing factors have acted upon the tree sequentially. However, from this study, it is obvious that notwithstanding the predisposition/inciting of the trees by the 2016 drought prior to inoculation with *L. terebrantis* as a contributing agent, infection require an additional factor of drought before the pathogen can significantly contribute to growth decline. This additional factor for successful interaction between *P. taeda* and *L. terebrantis* pathosystem is suggested as an activating agent (Figure 4.6) to enable the occurrence of tree decline.

4.8. CONCLUSIONS

The potential of *L. terebrantis*, a weak wilt pathogen commonly associated with declining pines in the southeastern U.S., to cause growth loss and tree decline was assessed using artificial stem inoculation of plantation *P. taeda* with toothpicks colonized by *L. terebrantis*. The study showed that together with the occurrence of rainfall deficit over a 7-month period during the growing season, this pathogen caused a reduction in foliage moisture content, tree leaf area, and the ratio of tree leaf area and sapwood area. These losses contributed either directly or indirectly to the stemwood growth loss and decline of *P. taeda* trees. This decline was more pronounced in trees treated with the high inoculum density relative to trees treated at lower inoculum densities. A prolonged period of reduced precipitation/drought appeared to act as an activating agent. Thus, in the presence of *L. terebrantis* as a contributing factor for LPD, an additional factor of drought stress (activating agent) is necessary for successful host pathogen interaction. Additionally, *L.*

terebrantis must reach critical inoculum threshold before growth decline symptoms can be manifested in loblolly pine trees.

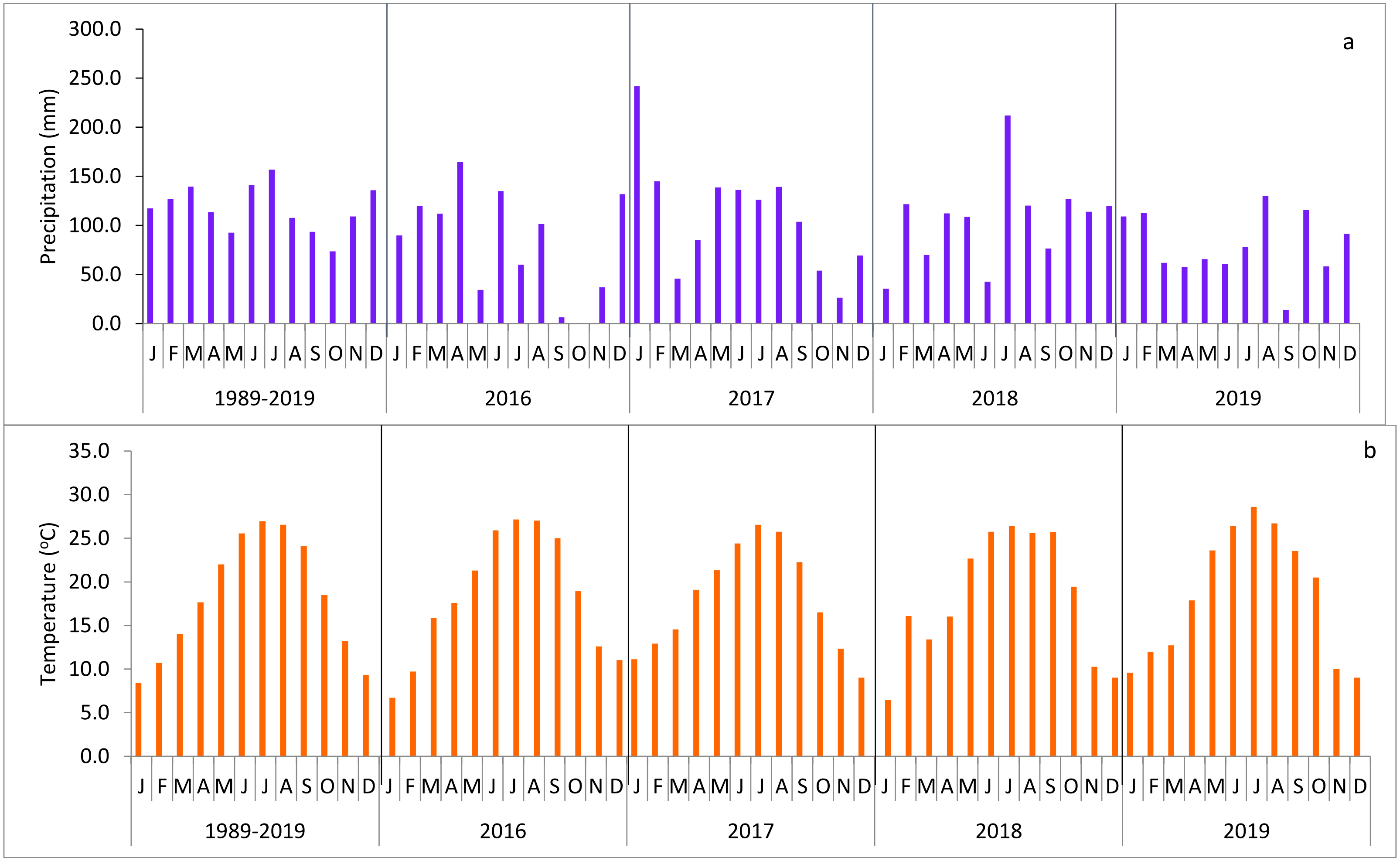

Figure 4.1. (a) Precipitation at the study site from 2016 to 2019 and 30 year average between 1989 and 2019 (b) daily air temperatures at the study site from 2016 to 2019 and the 30 year average.

Table 4.1. Mean DBH, total height, and stemwood volume of *P. taeda* measurement trees after plot establishment and 14 months before inoculation treatments were applied.

Treatment	DBH (cm)	Height (m)	Volume (m^3)
Control	17.38±0.34	14.68±0.18	0.3696±0.02
Wound	17.49±0.51	14.58±0.19	0.3564±0.02
Low	17.62±0.85	15.20±0.25	0.3874±0.04
Medium	17.67±0.46	15.10±0.22	0.3755±0.02
High	16.83±0.43	14.53±0.22	0.3300±0.02

Table 4.2. Probabilities of a greater *F*-value (*P* > *F*) from two-way analyses of variance of DBH, total height, stemwood volume and stem relative growth (RG) thirty-four months (3 years) following stem inoculation of *P. taeda* trees with *L. terebrantis* near Eufaula, AL.

Variable	Source of variation	Df[1]	Mean square	*P* > *F*
DBH	Treatment (T)	4	0.10	0.9826
	Year (Y)	2	283.07	**<0.0001**
	T x Y	8	1.12	0.3515
Tree height	T	4	2.22	0.0697
	Y	2	443.26	**<0.0001**
	T x Y	8	2.75	**0.0062**
Stemwood volume	T	4	0.99	0.4150
	Y	2	227.63	**<0.0001**
	T x Y	8	1.73	0.0915
RG	T	4	2.82	**0.0277**
	Y	2	84.02	**<0.0001**
	T x Y	8	4.94	**<0.0001**

[1] Df, degrees of freedom; *P* > *F*, probability of a greater *F*-value.

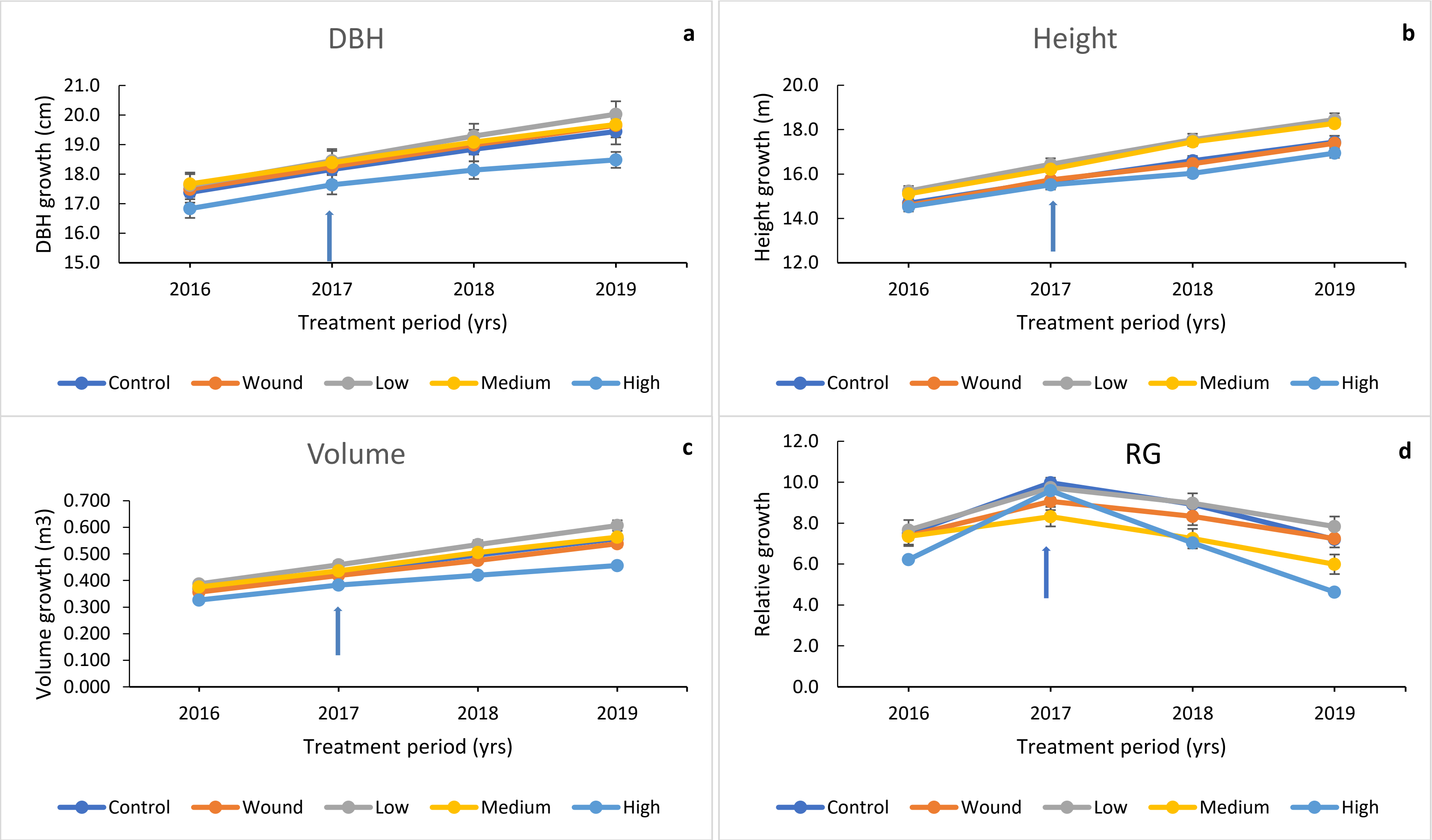

Figure 4.2. Infuence of *L. terebrantis* on *P. taeda* growth (a) DBH (B) Height (C) Volume and (D) Relative growth. The arrow indicates the period at which pathogen inoculation occurred in March 2017.

Table 4.3. Probabilities of a greater *F*-value from one-way analyses of variance for tree projected leaf area (A_L), the ratio of A_L to sapwood area (A_L:A_S), tree growth efficiency (GE), and foliage moisture content thirty-four months following stem inoculation of *P. taeda* trees with *L. terebrantis* near Eufaula, AL.

Variable	Df[1]	*F*-value	*P* > *F*
A_L	4	3.07	<0.0001
A_L:A_s	4	2.81	<0.0001
GE[2]	4	4.27	0.0328
MC	4	2.11	0.0904

[1] Df: degrees of freedom; *P* > *F*, probability of a greater *F*-value.

[2] GE is calculated as tree annual increment of basal area divided by tree A_L.

Table 4.4. Mean ± standard errors of the ratio of tree leaf area to sapwood area (A_L:A_S), tree growth efficiency (GE), and foliage moisture content (MC) thirty-four months following stem inoculation of *P. taeda* trees with *L. terebrantis* near Eufaula, AL.

Treatment	A_L:A_S	GE[1]	MC (%)
Control	0.72±0.06**a**	0.12±0.01**a**	116.3±3.4**ab**
Wound	0.68±0.06**a**	0.09±0.02**ab**	117.8±5.8**a**
Low	0.64±0.05**ab**	0.08±0.01**b**	114.0±3.6**ab**
Medium	0.63±0.04**ab**	0.09±0.02**ab**	110.3±3.0**ab**
High	0.39±0.04**b**	0.09±0.01**b**	100.5±3.5**b**

[1] GE is calculated as tree annual increment of basal area divided by tree A_L.

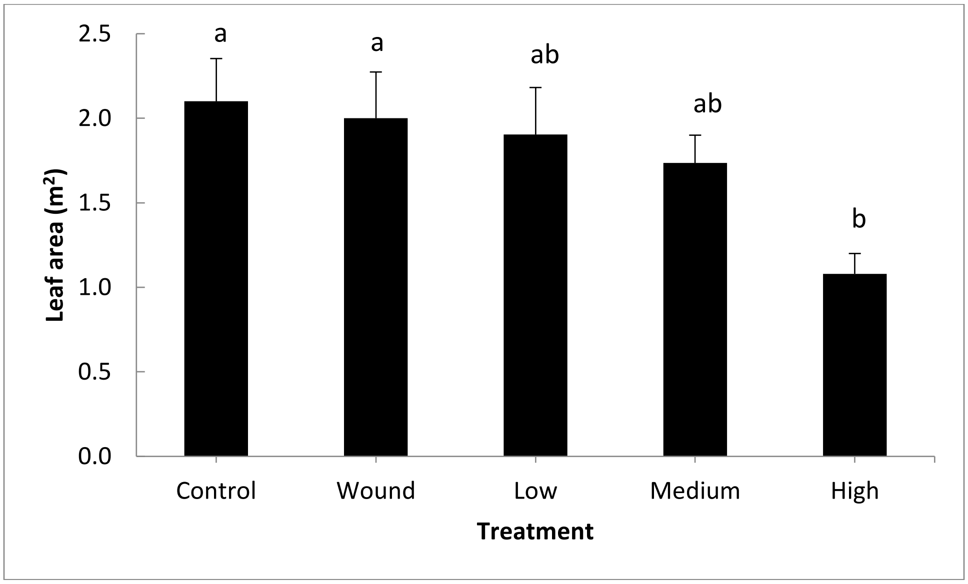

Figure 4.3. Mean leaf area of *P. taeda* trees inoculated with *L. terebrantis* after destructive sampling in January to February 2020.

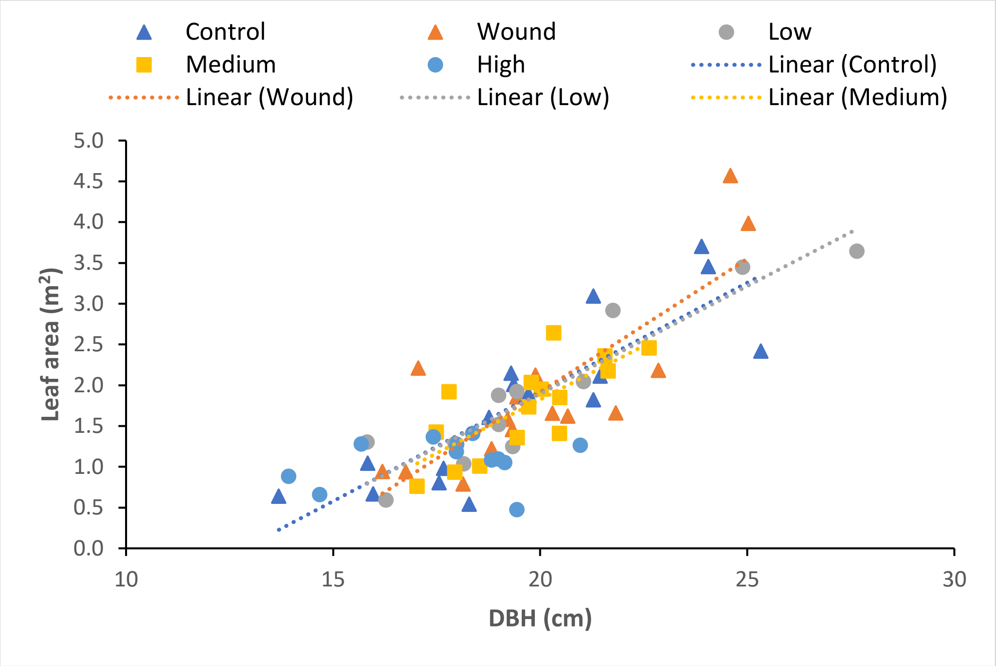

Figure 4.4. Relationship between leaf area and DBH growth of *P. taeda* following lower stem inoculation with toothpick colonized *L. terebrantis*. Note the non-significant relationship for the high inoculum treatment but the slope of the control, wound, low and medium were significantly higher than the high inoculum treatment.

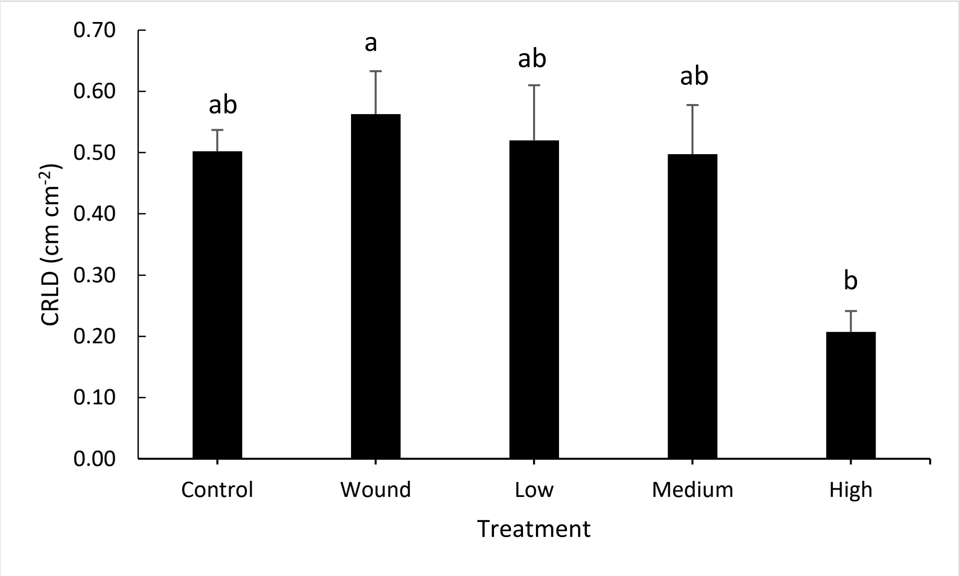

Figure 4.5. Cumulative root length density (CRLD) in October 2019 from 0 to 50 cm depth following stem inoculation of *P. taeda* with *L. terebrantis.*

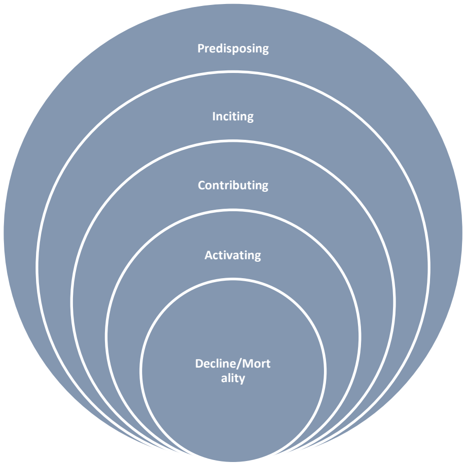

Figure 4.6. Proposed modified Manion's (1991) model of factors for growth decline in *P. taeda* and *L. terebrantis* pathosystem.