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NEEDLE PATHOGEN, *LECANOSTICTA ACICOLA*, EFFECTS ON *PINUS TAEDA* SHOOT AND NEEDLE LENGTHS

by
Debit Datta and Lori Eckhardt

ABSTRACT

Loblolly pine is a crucial economic component of plantation forestry in the southeastern United States. Loblolly pine has been experiencing repeated defoliation due to brown-spot needle blight fungus, *L. acicola* in Alabama. The study objectives were to assess the repeated infection of *L. acicola* on loblolly pine (1) needle growth and (2) shoot growth as well as (3) to monitor and record the annual progression of this disease in loblolly pine stands. Results indicate that high incidence trees produced shorter shoots and needles compared to low incidence trees.

Shoot length was reduced by 3.42 cm and 1.61 cm at lower whorl and upper whorl heights respectively of high incidence trees than low incidence trees. Moreover, high incidence trees were found to have 3.16 cm and 2.59 cm (lower whorl and upper whorl, respectively) shorter needles compared to low incidence trees. High incidence and low incidence trees were increasingly chlorotic and defoliated from 2019 to 2021 indicating that disease is progressing both temporally and spatially. Apart from needle necrosis and premature defoliation, *L. acicola* can additionally reduce the tree's photosynthetic ability through the reduction of needle area. The pathogen is likely to spread in the coming years and cause significant volume loss of infected loblolly pine stands.

3.1. INTRODUCTION

Brown spot needle blight is a conifer disease caused by the fungus *Lecanosticta acicola* (*Mycosphaerella dearnessii* (syn. *Scirrhia acicola*)). This fungal pathogen was first reported by Thümen in South Carolina in 1878 and has been found to be a destructive pathogen of over 50 *Pinus* species in North and Central America, Eastern Asia and the Central European and

Mediterranean Plant Protection Organization (EPPO) region (Quaedvlieg et al., 2012). Genetic analyses of this pathogen suggests that *L. acicola* is a native pathogen of Central America specifically in the mountain areas of Guatemala and Honduras (Bednářová et al., 2013). This pathogen is well-known for its potential of killing foliage and retarding the growth of trees, most notably, longleaf pine seedlings in the southern United States (Siggers, 1932, 1944).

The first symptoms of this disease appear as small yellow, sometimes reddish-brown, or grey-green, irregular circular spots which turn brown over time and are often surrounded by a yellow halo at the point of infection (Hedgcock 1929; Skilling & Nicholls, 1974). Infection is usually more severe on the lower crown and moves upward as the disease progresses (Sinclair & Lyon, 2005). Depending on the host and fungal prevalence, infections can occur on several parts of the needles leading to rapid necrosis resulting in severe defoliation which may lead to the death of branches and whole trees (Kais, 1975). Generally, second and third-year needles are infected only leaving new growth needles at the tip of the branches. Current year needles become infected in the next year because of conidia spreading from infected old needles (Siggers, 1944; Skilling & Nicholls, 1974). Development of disease symptoms and damage depend on the fungal strain and favorable climatic conditions (Kais, 1975).

Lecanosticta acicola overwinters as vegetative mycelium or asexual acervuli or sexual ascostromata in the infected needles (Siggers, 1974). When light, temperature, and humidity arefavorable, *L. acicola* can spread through its mucilaginous conidia or sexual ascospores (Kais, 1975; Tainter & Baker, 1996). Conidial dispersal to adjacent trees is mostly triggered by rainfall patterns and contributes to rapid disease build-up in pine stands. Conidia germinate on the needle surfaces, penetrate germ tubes, enter stomatal antechamber, and then increase in diameter and become thick-walled and melanized (Van der Nest et al., 2019; Kais, 1975). Light indirectly plays a role as it stimulates the opening of the guard cell of the stomata. Similarly, the wound helps conidia to penetrate needles (Kais, 1975). Asci form on necrotic parts of the infected living needles or dead needles, and ascospores are forcibly expelled and disseminated by wind currents and/or by rain (Henry, 1974). Based on the strain of *L. acicola*, conidia and ascospores both can survive, spread, and germinate between -5oC to 35oC. High levels of infection occur with warm temperatures and high rainfall after a long period of dryness. Conidial dispersals were recorded the highest in the United States between late spring and summer. Abundant ascospores were recorded between late summer to autumn in the United States when the temperature is above 15oC (Kais, 1975). Insects, animal movement, and anthropogenic movement of infected materials could be other mechanisms of conidial spread and dispersal (Skilling & Nicholls, 1974; Tainter & Baker, 1996; Wingfield et al., 2015). However, these are deemed to be unlikely based on the biology of the pathogen, *L. acicola*.

Loblolly pine is a commercial timber species native to the southeastern United States. According to Boyce (1958), all ages of loblolly pines affected by *L. acicola* which caused die- back of the needles. However, no needle casting resulted in defoliation leading to reduced growth and tree mortality was documented for loblolly pine because of brown spot needle blight infection.

Siggers (1932, 1944) stated that loblolly pine is a common associate of longleaf pine and thus a frequent host of brown spot needle blight fungus, *L. acicola*. Histological analyses of symptomatic loblolly pine needles indicated that mesophyll tissues were collapsed, and resin ducts exhibited cellular necrosis and dissolution as a result of *L. aciola* infection (Jewel, 1993).

Lecanosticta acicola is the causal agent of loblolly pine defoliation and tree mortality in the southeastern United States. Based on chapter II, this is the first study that reported defoliation causing loblolly pine mortality in the region due to this *L. acicola* pathogen (see Chapter I). As a primary infection, symptoms appear as chlorosis, needle necrosis, and premature defoliation (Figure 3.1) resulting in a thin sparse crown. Anecdotal evidence suggests that loblolly pine with high levels of infection have shorter needles and shoots compared to trees with low or no levels of infection. An epidemiology study of *Dothistroma septosporum*, a fungus mimicking a similar life cycle and biology as *L. acicola* was found to affect needle and shoot growth of *Pinus nigra* subsp. *laricio* (Corsican pine) trees in Britain. *Dothistroma septosporum* was able to cause shorter needles and shoots in the years following infection which resulted in the reduction of photosynthetic ability and growth of the trees (Mullet, 2014). However, additional influence on needle and shoot growth due to repeated infection by *L. acicola* remains unknown. The study objectives were to assess the repeated infection of *L. acicola* on loblolly pine (1) needle growth and (2) shoot growth as well as (3) to monitor and record the annual progression of this disease in loblolly pine stands.

3.2. MATERIALS AND METHODS

3.2.1. Stand characteristics and symptomology of trees

In the permanent study plots, plots were composed of 16 years, 11 years, 10 years and 8 years old plantations. Symptoms were showing yellowing to progressively browning needles, defoliation, thinned crown, and tree mortality across stands (Figure 3.1). The disease was first expressed in 2004 plot and eventually discovered in all other adjacent stands. Only 2004 plot received fertilization after three years of plantation establishment. Other plots didn’t receive any silvicultural treatments. Soils consisted of Tibbie soils (40%), Pinebarren soils (35%) and 5% of other minor components. (Web Soil Survey).

3.2.2. Study design and data collection

The study was conducted in one of the *Pinus taeda* infected permanent plots in Chatom, Alabama, Washington County (-88.31687 N 31.26497 W). This plantation plot was established in 2012 and no silvicultural treatments were applied after establishment. To analyze *L. acicola* disease severity on shoot and needle length, *L. acicola* infected trees were assessed at the end of the growing season to ensure maximum retention of needle and shoot growth. Two infection levels [low infection level (10-20% crown infection) and high infection level (>50% crown infection)] were chosen to evaluate comparative impacts in needle and shoot lengths. Since whorl height has effects on the shoot and needle lengths due to light availability, two whorl heights were chosen. From the base of the tree, the first whorl height at 2-5 m and the second whorl height at 5-8 m were chosen. A total of twenty-eight low infection trees and thirty-three high infection trees were measured in 2019 and 2020. From each tree, nine to eleven side apical shoots were assessed at each whorl height. Shoot length and needle length were measured in centimeters. A total of ten random fascicles were chosen per shoot to obtain the average needle length. The experiment was conducted in October 2019 and November 2020. 2018 growth was measured for 2018 in 2019 and 2019 growth was measured for 2019 in 2020 (Mullett, 2014).

3.2.2. Three-year loblolly pine health monitoring

To monitor loblolly pine health, seven permanent monitoring plots were established at Chatom, Alabama, Washington County in summer 2019. Those plots were of eight- to sixteen- year-old plantations and were suffering from needle damage at least since 2018. At each experimental plot, 6-8 high incidence trees (>1/3 tree crown is affected), and 2-4 low incidence trees (<1/3 tree crown are infected) were tagged in summer 2019. It was very difficult to find a completely healthy tree in a diseased stand; therefore, trees with less than 1/3 crown infections were chosen and considered as low incidence trees. Detailed information of tagged trees such as height (m), DBH (cm), GPS coordinates (latitude and longitude), location of damage (upper, middle, or lower canopy) was collected.

Crown rating conditions of trees were ranked and recorded in the summer of 2019, 2020, and 2021. The crown rating was based on visual inspection of a proportion of tree crowns affected (Figure 3.2) and categorized as (1) less than one-third of tree crown infected (<1/3), (2) one-third to two-third of crown infected (1/3 to 2/3), and (3) more than two-thirds crown infected (>2/3) followed by Broders et al., (2015). Crown ratings were recorded as “chlorosis per tree” and “defoliation per tree”.

3.2.3. Statistical analyses

To evaluate the effect of “infection level” and “whorl height” on the shoot and needle lengths, a restricted maximum likelihood model was fitted. Statistical software SAS version 9.4 was used to conduct data analysis. Growth year and individual trees were considered as random effects whereas infection level and whorl height were considered as fixed effects.

Loblolly pine health was analyzed using linear mixed-effect models where the “stand” was considered as a random effect and “year” and “initial health status” were treated as fixed effects. In both cases, normality and homogeneity of data were checked. No transformations were required. Goodness fit test was performed, and no assumptions of the linear regression model were violated.

3.3. RESULTS

3.3.1. Measurement of shoot and needle lengths

High incidence trees produced significantly shorter shoots than low incidence trees. Both infection level and whorl height had impacts on shoot length (F statistics = 27.31; d.f.4,115, $P<0.0001$; F statistics = 33.70; d.f.4,115, $P<0.0001$ respectively) as shown in Table 3.1. Infection level and whorl height independently influenced the tree shoot length and therefore, did not have any additive effects on the model (F statistics = 3.51; d.f.4,115, $P<0.06$). High incidence trees had 3.42 cm and 1.61 cm short shoots compared to low incidence trees at their lower whorl and upper whorls respectively (Figure 3.3).

Needle length was affected by *L. acicola* infection. High incidence trees produced on average 3.16 cm and 2.59 cm (lower whorl and upper whorl, respectively) than low incidence trees (Figure 3.3). There were nearly 24.8% and 17.7% of needle length reduction of lower branches and upper branches, respectively. Infection level (F statistics = 73.07; d.f.4,115, $P<.0001$) and whorl height (F statistics = 38.06; d.f.4,115, $P<.0001$) were found inversely related to needle length (Table 3.1). The study found that shoot length and needle length were positively correlated (Pearson's correlation coefficient = 0.312277, p = 0.0005).

The total number of needles per shoot and fascicle density were positively correlated to shoot length (Pearson's correlation coefficient = 0.6243, $p<0.05$). However, no significant interactions between infection level and whorl height was detected for total number of needles (F statistics = 38.79; d.f. 1, 118, $P<0.05$; F statistics = 6.48; d.f. 1, 118, $P=0.01$; F statistics = 0.31; d.f. 1, 118, $P=0.58$;) and fascicle density (F statistics = 42.19; d.f. 1, 118, $P<0.001$; F statistics = 8.87; d.f. 1, 118, $P=0.04$; F statistics = 3.98; d.f. 1, 118, $P=0.05$;) as shown in Table 3.1.

3.3.2. Three-year loblolly pine health monitoring in Chatom, Alabama

Seventy trees were tagged and assessed for chlorosis and defoliation rating. Based on visual observation, the crown rating was conducted in the summer of 2019, 2020, and 2021. Trees displayed chlorosis and defoliation in the summer. Initially considered low incidence trees became significantly chlorotic and defoliated from 2019 to 2021 (Figure 3.4 & Figure 3.5). High incidence trees did not recover from crown damage. Furthermore, the proportion of tree crowns ranked chlorotic and defoliated in 2019 considerably increased in 2021 for both low incidence and high incidence trees. Crown damage progressed from bottom to top of the tree crown and high incidence trees to low incidence trees.

3.4. DISCUSSION

These studies demonstrate that *L. acicola* had substantial impacts on tree shoot and needle lengths. Infection level contributed to shoot and needle lengths reduction. In the pathosystem, needle size was reduced due to a needle pathogen *D. septosporum* infection in *P. nigra* subsp. *laricio* (Corsican pine) infected stands in Britain (Mullett, 2014). Needle length reduction could be related to the previous year needle damage such as chlorosis, necrosis, and premature defoliation caused by *L. acicola*. Needle damage resulted in (1) a reduced photosynthesizing area, (2) a reduction of the photosynthesizing ability, and (3) fewer carbohydrate reserves that feed new shoot and needle growth. Consequently, needle damage in 2018 reduced needle growth in 2019 which further cumulatively led to fewer energy sources for trees for shoot and needle growth in 2020.

Needle size was positively correlated with tree whorl height indicating that the lower crown of infected trees was more impacted compared to the upper crown and experienced greater needle length reduction. Other studies supported the findings that brown spot needle blight disease is more detected in the lower crown in comparison to the upper crown (Siggers, 1944; Skilling & Nicholls, 1974; van der Nest et al., 2019). Needle pathogen, *L. acicola* usually starts infection in the lower crown and stay there until get favorable conditions because of high moisture availability which resulted in greater needle damage in the lower crown. Pallardy (2008) stated that the growth and development of needles are influenced by shoot locations on the stems. In the healthy tree, larger and heavier fascicles, longer flushes of shoots, and more leaf area are positively correlated to crown position i.e., vertical light gradient within the canopy (Pensa & Jalkanen, 2005). A study by Wang et al., (2019) found that loblolly pine needle length is positively correlated to leaf mechanical support and physiological capacity. Therefore, reduction of needle length significantly diminished leaf mechanical support and physiological functions indicating that repeated *L. acicola* infection on tree foliage such as chlorosis, needle necrosis, premature defoliation, and needle length reduction supported tree mortality and substantial volume loss in loblolly pine infected stands.

Shoot length also similarly reduced at lower whorl and upper whorl heights of high incidence tees compared to low incidence trees. Tanga et al., (1998) found that upper crown shoots were significantly longer than lower crown shoots as the effect of thinning and fertilizer application. Since no silvicultural treatments were applied on the experiment plot, the study safely excluded fertilizer and thinning effects on the seasonal shoot and needle growth. Shoot length of irrigated trees versus drought trees are also varied almost double based on water availability (Garrett & Zahner, 1973). Since the study plot didn’t vary significantly from tree to tree, other factors such as water availability, temperature variation and light accessibility were not related to making such a difference. This supported that comparative effects of *L. acicola* caused significant differences in shoot length variations based on infection severity.

Long-term monitoring of loblolly pine health confirmed that *L. acicola* was progressing both temporally and spatially on every experimental plot. Within three years of observation, both infection and mortality increased. Only the 2010 experimental plot showed little variation. This variation may result from either genetic variability or soil conditions (Eldridge & Lambert, 1980). Disease progression indicates that either air currents or rain-splash spores of *L. acicola* were spreading from high incidence to low incidence trees and lower crown to upper crown (Siggers, 1944) and causing chlorosis, needle necrosis, repeated defoliation, needle length reduction leading to rapid death of the infected trees (Broders et al., 2015). Tree mortality is likely to increase in the coming years, which in turn, may cause large-scale volume loss in loblolly pine stands.

3.5. CONCLUSION

The current emergence of brown spot needle blight in loblolly pine stands has impacted both shoot and needle lengths. The study found that infection level is inversely related to tree shoot and needle lengths. High incidence trees significantly reduced shoot and needle lengths compared to low incidence trees. High levels of infection caused 3.16 cm and 2.59 cm of lower whorl and upper whorl needle growth reduction compared to low levels of infection. Similarly, shoot length was reduced by 3.42 cm and 1.61 cm at lower whorl and upper whorl height of high incidence trees respectively. Whorl height was positively correlated with the shoot and needle lengths. During the study, low incidence and high incidence tagged trees were ranked as increasingly chlorotic and defoliated indicating that *L. acicola* is progressing from lower crown to upper crown and trees to trees. To conclude, *L. acicola* can reduce shoot and needle lengths which can result in an additional reduction of the photosynthetic ability of infected trees leading to carbon deficit and rapid death of the infected trees.

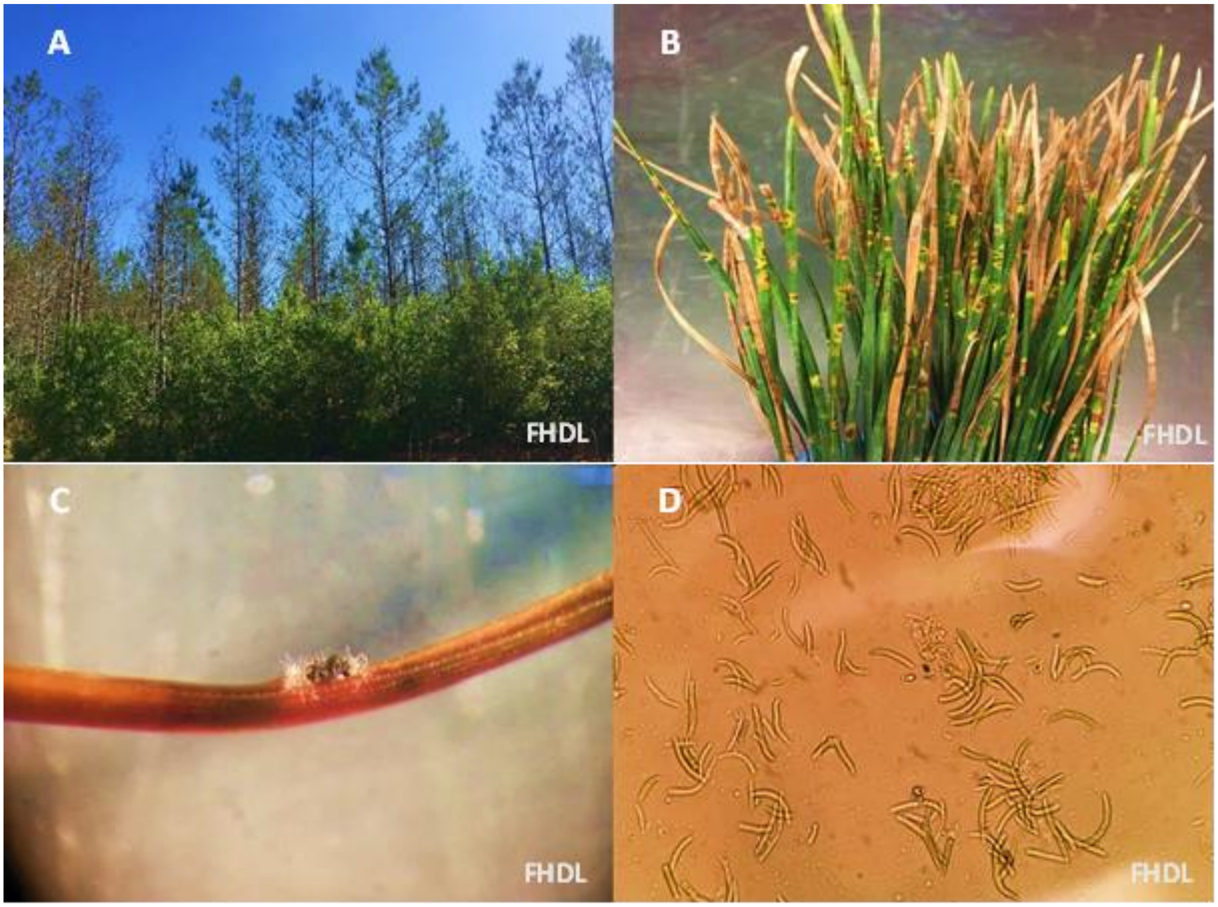

Figure 3.1. Disease symptoms and reproductive structures showing (A) stands infected by brown-spot needle blight, note the thinning canopies (B) irregular frequent brown-spots surrounded by a yellow halo (C) black shiny fruiting body protruding needles & (D) microscopic banana-shaped septate conidia.

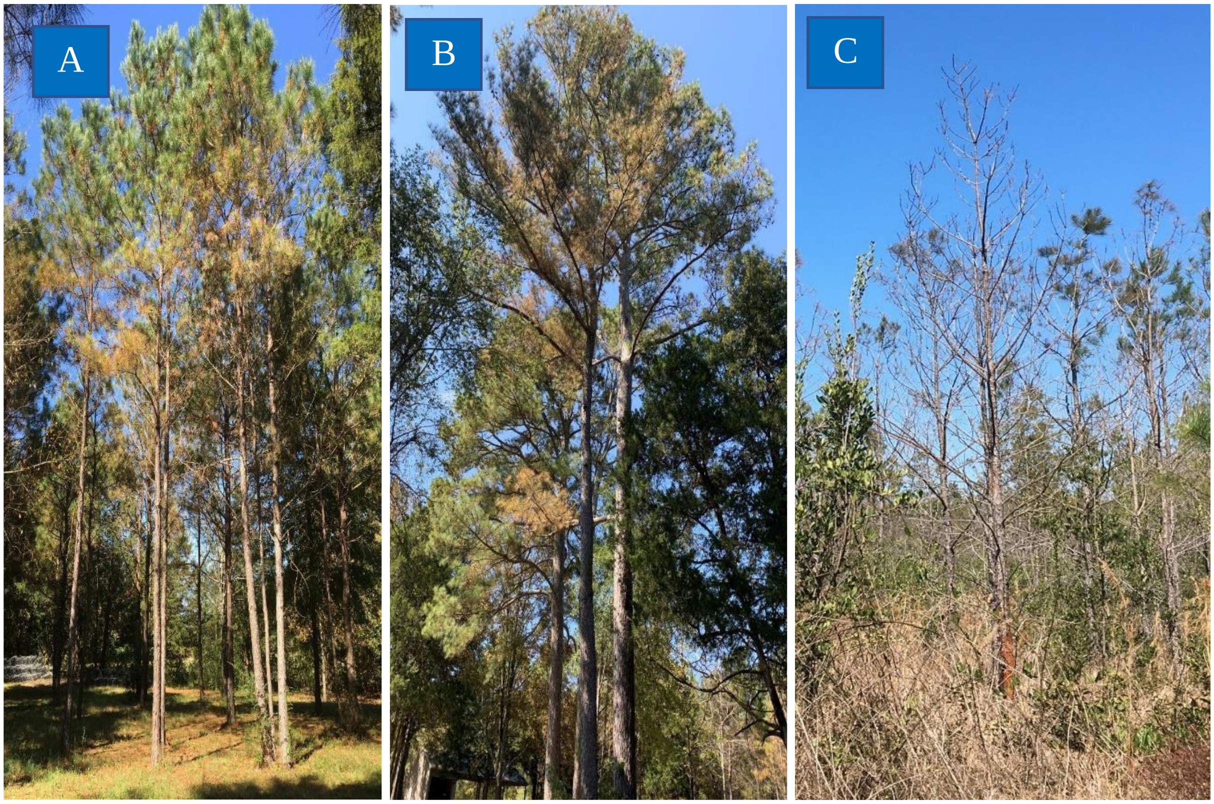

Figure 3.2. Crown severity rating such as (A) One-third of crown infected (B) One-third to two-third of crown defoliated/infected (C) More than two-third to full crown defoliated/infected.

Table 3.1. Probabilities of a greater *F*-value (*P* > *F*) of *P. taeda* trees to *L. acicola* infection on needle length, shoot length, and fascicle density.

Effect	**aDF**	**bDen DF**	**Infection Level (IL)**		**Whorl Height (WH)**		**IL*WH**	
			F Value	**P>F**	**F Value**	**P>F**	**F Value**	**P>F**
Shoot Length	4	115	27.31	<.0001	33.70	<.0001	3.51	0.06
Needle Length	4	115	73.07	<.0001	38.06	<.0001	0.00	0.96
Fascicle Density	4	115	42.19	<.0001	8.87	.004	3.98	0.05

[a]DF, numerator degrees of freedom; [b]Den DF, denominator degrees of freedom; *P* > *F*, probability of a greater *F*-value

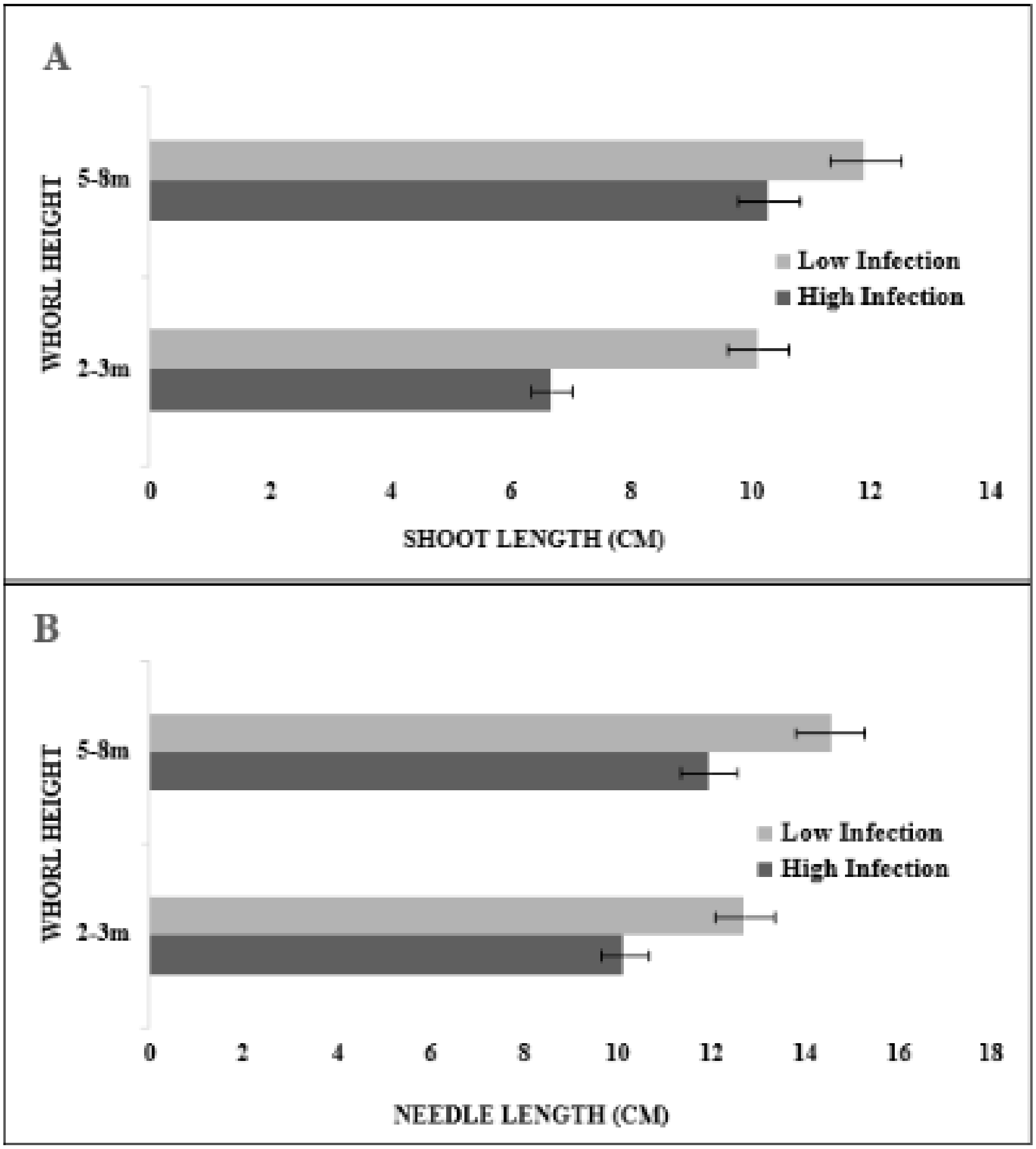

Figure 3.3. Observed means and standard errors of (A) shoot length and **(B)** needle length.

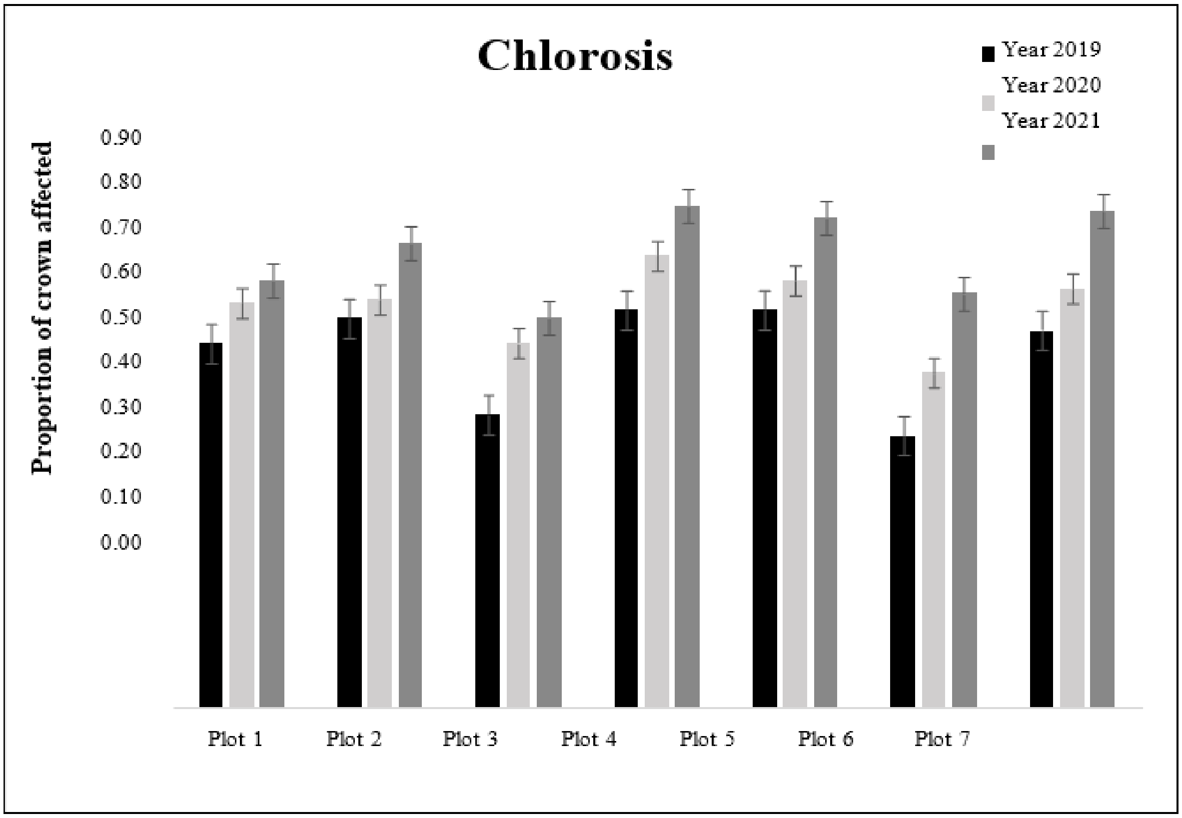

Figure 3.4. Bar represents the mean chlorosis of seventy tagged trees at seven long-term monitoring plots in Chatom, Washington County, Alabama in the summer of 2019, 2020& 2021.

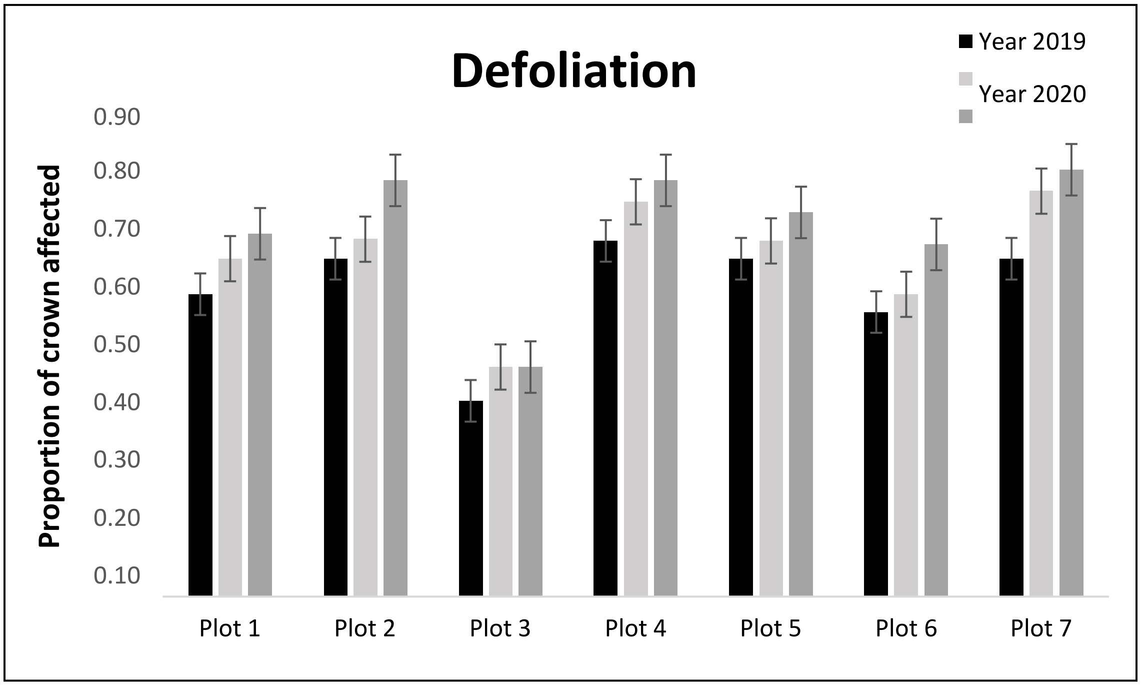

Figure 3.5 Bar represents the mean defoliation of seventy tagged trees at seven long-term monitoring plots in Chatom, Washington County, Alabama in the summer of2019, 2020, & 2021.