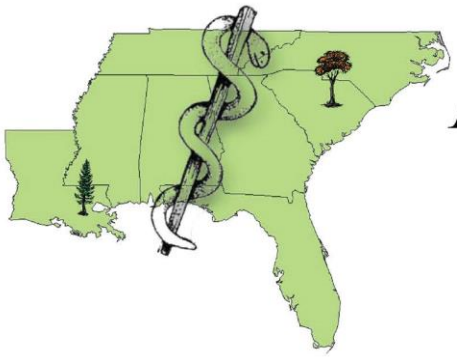

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FOLIAR NUTRIENTS RESPONSE OF *PINUS TAEDA* TO *LEPTOGRAPHIUM TEREBRANTIS* INFECTION

by
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5.1. ABSTRACT

Leptographium terebrantis is a root pathogen commonly associated with declining *Pinus taeda* L. (loblolly pine) in the southeastern U.S. and has been hypothesized to alter *Pinus* foliar nutrition. The influence of *L. terebrantis* inoculation treatments on loblolly pine foliar nutrition and shoot morphology were examined. We found that *L. terebrantis* inoculation significantly affected foliar concentrations of N, Mn, and Fe. Nitrogen concentrations decreased below an adequate level and this reduction was most severe in the high inoculation treatment compared to the control treatment. In contrast, foliar Mn concentration increased within the treatment period and was most elevated in the high inoculation treatment. Shoot length, fascicle number and fascicle density were not affected by inoculation treatment, but mean fascicle length was significantly reduced by the high inoculation treatment.

5.2. INTRODUCTION

Mineral nutrient availability is essential for tree growth and productivity. Low soil fertility has been identified as a limiting factor in *Pinus* wood production (Fox et al., 2007a; Chapin et al., 1986). Among the southern pines, insufficient mineral nutrition causes reduced whole-crown leaf area development and hence, low stem growth (Fox et al., 2007). Several studies have shown increases in the radial growth of loblolly pine grown on nutrient-poor sites following fertilization (Albaugh et al., 2004; Jokela et al., 2004; Sayer et al., 2004). Growth losses are attributed to nutrition levels below or above optimum levels that create insufficiencies or imbalances of essential mineral nutrients. In turn, abnormal nutrition may interfere with physiological processes leading to growth loss.

Nutrient-deficient soil is one of several factors that may contribute to growth decline and mortality. Allen et al., (1990) indicated that nutritional limitations occur when nutrients demanded by plants cannot be supplied by the soil or remobilized internally. At the initial stages of tree growth, nutrient demand is low; however, as growth accelerates and tree size increases, the demand for nutrients increases (Miller, 1981). The high requirement and use of nutrients continue until canopy closure or when environmental conditions cannot sustain a high supply of nutrients (Miller, 1981; Piatek and Allen, 1999). Irrespective of the role of nutrients in pine growth and productivity, some available nutrients have greater impacts whilst others have less or variable impacts. For instance, among the macronutrients, nitrogen (N) and phosphorus (P) have been shown to have the greatest influence on loblolly pine growth (Jokela and Martin, 2000; Albaugh et al., 2004). Other macronutrients such as potassium (K) may also improve growth in combination with N and P depending on the site or location (Carlson et al., 2013). In the southern U.S. macronutrients such as N and P may become deficient at crown closure (Fox et al., 2007).

In order to meet nutrient requirements for growth, some mobile nutrients such as N and P are retranslocated from senescing foliage to active photosynthetic foliage to partially sustain tree growth (Wells and Jorgensen, 1975). But this reallocation may be inadequate to meet the demands of photosynthetically active foliage. Other silvicultural interventions such as multiple fertilizer applications are used by silviculturists for sustainable tree growth (Allen, 2001). Studies have shown that over the years, the acreage of pine forests under fertilization continue to increase. For instance, in 1990 about 200,000 acres of pine plantations were fertilized, and nearly a decade later, the area under fertilization increased 7-fold to about 1.6 million acres (Forest Nutrition Cooperative, 2005).

Regardless of efforts to maximize pine productivity, the presence of root pathogens hinders the ability of pine roots to assimilate and translocate nutrients. The activity of the pathogen within the root sapwood interferes with tree physiology by reducing water and nutrient translocation to foliage. This causes a reduction in the amount of carbon fixed by the needles and allocated to growing shoots leading to a reduction in leaf area (Jokela and Martin, 2000; Albaugh et al., 2004). Ultimately, poor leaf area development risks inadequate carbon allocation to fine root and mycorrhizae maintenance and growth. As a result, root system acquisition of soil resources becomes compromised by both root pathogens and carbon limitations.

The nutritient content of foliage is usually used in assessing the nutritional status of forest stands (Adams and Allen, 1985). It provides an index of the soil supply of, and stand demand for nutrients (Jokela et al., 1991), and may serve as an indicator of nutrient availability in the soil (Brockley, 2001). Additionally, foliar nutrient ratios have shown promise in identifying nutrient deficient stands (Hockman and Allen, 1990). These ratios are particularly important when either one or both chemical elements are at a deficient level (Marschner, 2011).

Pathogen colonization of the host plant may lead to changes host nutrient status. Sayer et al., (2009) assessed foliar nutrients concentrations in *Pinus palustris* Mill. (longleaf pine) stands that were normal in appearance or exhibited symptoms of low vigor and decline at two military locations, Fort Benning, Georgia and Eglin Air Force Base, Florida. They reported that under both conditions, most macro- and micronutrients were at sufficiency levels. However, foliar Mn

concentration was elevated at both sites. Elevated foliar Mn concentration has the potential to disrupt physiological processes tied to carbon fixation and allocation (Mehne-Jakobs, 985; Cakmak and Kirby 2008). Furthermore, Mn has been shown to be toxic to *Pinus elliottii* Engelm. (slash pine) seedlings at 300 mg/kg (Van Lear and Smith, 1990).

Other studies have found changes in nutrient metabolism following pathogen infestation. For instance, host plant N metabolism and tissue concentration may be altered following pathogen infestation (Singh and Bhure, 1974; Walters and Bingham, 2007). Singh and Bhure (1974) found that *Armillaria mellea* (Vahl ex Fr.) Kummer infestation induced reduction in foliar nutrient concentrations of N, P, K, Mg, and Na in coniferous species but caused an increase in foliar Mn, Ca, Fe, and Zn concentrations. In addition, Singh and Bhure (1974) also noted that changes in foliar nutrient levels caused a reduction in the height growth of infected trees. In this study, we assessed the influence of stem inoculation with *L. terebrantis* on *P. taeda* foliar macro- and micro-nutrient concentrations over a 2-year period and subsequent responses of shoot morphology. We hypothesize that stem infestation with *L. terebrantis* will indirectly change foliar nutrient concentrations and shoot morphology as a result of compromised sapwood function.

5.3. METHODS

5.3.1. 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.

5.3.2. Soil sampling and chemical analysis

Soil samples for chemical analysis were collected on March 2, 8 and 9, 2017 prior to treatment application. Four soil cores of approximately 6 × 50.8 cm were removed from each plot, capped and transported in a cooler to the USDA Forest Service lab in Auburn, Alabama. Soil samples were returned within 3 hours of collection and kept at 4°C until processed. Processing consisted of sectioned cores into 10 cm increments and weighed, then sectioned longitudinally and one half dried at 105ºC for 72 hours and the remainder air dried until no change in weight. Coarse materials including stones, root pieces, pine needles and other plant parts were manually removed during processing.

The air-dried soil samples were composited by plot and depth then sieved through a 2 mm opening sieve (No.10). Soil samples were then sent to the Soil Health Assessment Center, University of Missouri, Columbia, Missouri where pH and nutrients were analyzed. Soil pH was determined by water (pH_{water}) (Kalra and Maynard, 1991). Percentage (%) total nitrogen (N_T), and % total sulfur (S_T) were analyzed via combustion analyzer (Kowalenko, 2006). Soil aluminum (Al) and manganese (Mn) (Kachurina et. al, 2008) were extracted with 1M KCl. The quantity of available phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn), copper (Cu), and iron (Fe) were determined according to the Mehlich-3 procedure (Mehlich, 1984) and expressed in mg kg^{-1}.

5.3.3. Inoculation method

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 used to culture the fungus in the dark at 23 ºC for 24 days as described by Devkota et al., (2019). Prior to treatment application, the dead outer bark was removed between 20 cm and 30 cm above the ground line with 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 at 27 cm from the ground level. Trees were inoculated 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).

5.3.4. Needle Sampling, processing and Nutritional Analysis

Pre-treatment foliage was collected in July 2016 from four randomly chosen measurement trees per plot (60). An upper-crown shoot was shot from each chosen tree with a 0.22 caliber rifle and the first flush foliage of 2016 was removed from the internodes. From each foliage sample, approximately 25 3-needle fascicles of the first flush were placed in a paper bag and oven-dried at 70^{o}C to equilibrium (i.e., 72 h). The samples were ground in a Wiley mill to pass through a 0.5 mm mesh screen before being sent to Waypoint Analytical Laboratory (Memphis, TN, U.S.A.) for chemical analysis. Phosphorus (P) concentration was analyzed by combustion (Bryson et al., 2014) whilst nitrogen (N), potassium (K), magnesium (Mg), calcium (Ca), sulphur (S), sodium (Na), boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), and aluminium (Al) were determined by a wet digestion standard procedure (Bryson et al., 2014).

Post-treatment sampling and mineral nutrient assessments were done in July 2018 and 2019 as described above with foliage sampled from the first flush of 2018 and 2019, in July 2018 2019, respectively. However, following destructive sampling of the five measurement trees per plot in January and February 2020, the most recently matured flush of 2019 (second flush produced in 2019) was assessed for internode length, number of fascicles, mean fascicle length, and fascicle density (number of fascicles per shoot length).

5.3.5. Analysis

Pre-treatment soil chemistry, and foliar nutrient concentrations, and post-treatment second flush internode length, fascicle number, mean fascicle length, and fascicle density at the time of tree harvest were analyzed by one-way ANOVA with inoculation treatment as the main effect (PROC GLM, SAS Inc., Cary, NC, USA). The main effects of inoculation treatment and treatment duration on post-treatment foliar nutrient concentrations were analyzed by repeated measures ANOVA (Proc Mixed, SAS Inc., Cary, NC, USA) with compound symmetry as the covariance structure. Prior to analysis, each dependent variable was checked for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests respectively. Main and interaction effects were considered significant at $P > F$ values less than 0.05 and significant effects were further evaluated by a pair-wise comparison among means using the post-hoc Tukey honest significant difference (HSD) test for multiple comparisons at $\alpha = 0.05$.

5.4. RESULTS

Pre-treatment soil pH_{water} differed significantly ($P<0.0001$) among the treatment plots (Table 5.1) and was significantly higher (5.4) on the medium inoculum density plot compared to low (5.0) and wound (5.1) and control (5.2) treatment plots. Pre-treatment soil macro- and micro- nutrient levels did not differ significantly by treatment (Table 5.2).

Among the mineral nutrients assessed, pre-treatment foliar concentrations of N, P, and K exhibited the smallest coefficients of variation (CV) which were less than 12% compared to CV values for foliar concentrations of Ca and Na which were greater than 20% (Table 5.3). Prior to

treatment, CV values associated with foliar micronutrient concentrations were greater than 20% with those for Mn and B exhibiting the highest CV values of more than 30%.

In response to inoculation treatment and treatment duration, a repeated-measure of analysis variance showed that treatment and its duration but not their interaction significantly affected foliar N concentration (Table 5.4). The foliar concentration of N was highest (1.17%) and lowest (1.04%) for wound and high treatment respectively a year after treatment application (2018). During the second year, the concentration of N reduced to 1.04%, 1.02% and 0.92% and among the wound, control and high, treatments respectively (Table 5.5).

Foliar Mn and Fe concentrations were also significantly affected by inoculation treatment and treatment duration but not their interaction (Table 5.4). Foliar B, Cu, and Al concentrations were significantly affected by treatment duration but not inoculation treatment. Foliar Mn concentration was significantly higher, 417.8 mg kg^{-1} in the high inoculum treatment than the low inoculum treatment, 203 mg kg^{-1} in 2018. The mean Mn concentration in 2019, 360.2 mg kg^{-1} was significantly higher in Mn concentration, 276.7 mg kg^{-1} in 2018. The medium inoculum treatment had a significantly higher Fe concentration, 30.8 mg kg^{-1} in 2019 than the control, 16.4 mg kg^{-1} and wound, 14.3 mg kg^{-1} treatments in 2018. Iron concentration was significantly lower 17.7 mg kg^{-1} in 2018 than 25.6 mg kg^{-1} in 2019. Similar to significant treatment duration effects on foliar Mn concentrations, treatment duration had a marginally significant effect on foliar Ca concentration (*P* = 0.0688*)* with a 5.3% higher foliar Ca concentration in 2019 compared to 2018.

Inoculation treatment significantly affected mean fascicle length but did not significantly affect internode length, fascicle number, or fascicle density of the second flush of 2019 (Table 5.6). Mean fascicle length of the wound inoculum density treatment was significantly greater than that of the high inoculum density treatment (Figure 5.1). Mean fascicle lengths of the control, wound, low, and medium inoculum density treatments were statistically similar. Mean internode length, fascicle number, and fascicle density across treatments at the time of tree harvest were 9.8 ± 0.4 cm, 68 ± 3, and 6.9 ± 0.3 fascicles cm^{-1}, respectively.

5.5. DISCUSSION

Foliar nutrient responses of mature loblolly pine trees to stem inoculation with *L. terebrantis* were assessed over a 2-year period. The pathogen affected foliar N, Mn and Fe concentrations and reduced mean fascicle length. The foliar nutrients and fascicle length changes were most severe in the high inoculum density treatment which also showed symptoms of pine decline.

Soil pH is an important factor for optimum tree growth as it affects the solubility of mineral nutrients in the soil, and their availability to, and uptake by trees. Although soil pH differed significantly before treatments were applied, the range of plot soil pH was 5.0 - 5.4 which is within the optimum pH range (4.5- 6) for loblolly pine production (Schultz, 1997). Therefore, it is unlikely that soil pH influenced inoculation treatment effects in this study. Available soil P in the treatment plots was similar to the critical threshold of 3-5 mg kg^{-1} in loblolly pine stands (NCFS, 2012), thus, available soil P at the study site was at sufficient level for loblolly pine.

Foliar nutrient concentration is an indication of mineral nutrient availability in the soil and foliar macronutrients concentrations prior to treatment application showed less variability compared to foliar micronutrient concentrations. The high variability among foliar micronutrient levels may be due to high spatial variability within the plots. Similarly, Albaugh et al., (2010) noted higher variability among micronutrient levels relative to macronutrient levels when foliar nutrient concentrations were characterized in loblolly pine plantations across the southeastern U.S. The mean initial foliar macronutrient concentrations of N, P, K, S, Ca, and Mg are comparable to critical or adequate levels in loblolly pines (Albaugh et al., 2010; Jokela, 2004). Similarly, concentrations of the foliar micronutrients, Mn, B, and Cu were comparable to earlier findings (Jokela, 2004; FNC, 2009). This indicates that, soil and foliar nutrient concentrations were present at sufficient levels prior to treatment application.

Leptographium terebrantis inoculation affected foliar N concentration and this effect was evident by 12 months after inoculation and continued until trees were harvested at 34 months post-inoculation. In both years foliar N concentration among the low, medium, wound and control treatments were higher than that of the high inoculum treatment. Nitrogen is one of the essential mineral nutrients for plant growth and its concentration in foliage is closely related to maximum net photosynthesis rate (Evans, 1989). The amount of N uptake from the soil is largely determined by plant growth rate (Gastal and Lemaire, 2002). Mensah et al. (Unpublished) found a decline in relative radial growth among the treatment trees which correlate with a reduction in foliar N concentration. Across treatments, the trajectory of foliar N concentration reduced by 7.3 % between 2018 and 2019.

The reduction in foliar N concentration could be ascribed to water deficit during the 2019 growth season (moderate drought). The water deficit caused less root uptake of N in 2019 compared to 2018 due to low soil water content and earlier fascicle senescence in 2019 than 2018. However, the level of N reduction was most severe in high inoculum treatment (12.5%) as compared to control (4.6%). Foliar N concentrations also suggest that the high inoculum density treatment was subjected to additional water stress not experienced by the other treatments. This additional moisture stress experienced by the high inoculum treatment was due to a decrease in stem hydraulic conductance (HC) caused by the pathogen occlusion. Moreover, a significant reduction in the cumulative root length density (CRLD) of the high inoculum treatment reduced the absorbing root surface area. Collectively, the lower HC and CRLD among the high inoculum treatment may explain the lower foliar N concentration.

Foliar P concentration was significantly affected by treatment period. Across treatments, foliar P concentration was 22% less in 2019 compared to 2018. However, foliar P concentration at pre- and post-treatment with *L. terebrantis* was below sufficiency level of 0.12% (Albaugh et al. 2010; Jokela 2004). This is a direct contrast to soil P concentration which was at sufficiency level. The limited P concentrations in the foliage perhaps may be due to several factors including moisture deficit, soil compaction and soil pH. Soil pH values below 5.5 and between 7.5 and 8.5 have been shown to limits P availability to plants (Penn and Camberato, 2019). The average pH across treatments plot was 5.2, which is below 5.5 and may possibly explain the limited P uptake from the soil.

While N and P are mobile and exhibited a decrease in foliar concentration between 2018 and 2019, Mn, Fe, Cu, and Al which are plant-immobile, increased in foliar concentration between the same period. A similar, marginally significant trend (P= 0.0688) was observed for Ca which is also plant immobile. Across 2018 and 2019, the high inoculum density treatment yielded higher foliar Mn and Fe concentrations compared to those of the control, wound and low treatments. By 2019, the high inoculum density treatment caused greater sapwood occlusion compared to other treatments (Chapter 6). Sapwood occlusion results in the loss of stem hydraulic conductivity (Butnor et al., 1999; Lee et al., 2006; Mensah et al., 2020). Thus, compromised sapwood function as well as a decrease in new root growth (Chapter 4) suggests that the high treatment trees experienced greater water deficit compared to trees receiving other treatments. A concomitant water stress-induced decrease in crown leaf area by premature senescence (Chapter 4), and concentration of plant-immobile mineral nutrients in transpiring foliage is one explanation for elevated foliar Mn and Fe levels in the high treatment trees.

Although the observed levels of foliar Mn are within the realistic range observed by Albaugh et al. 2010 of 84 to 916 mg kg^{-1}, they are greater than the recommended level of 200 – 400 mg kg^{-1} (Jokela, 2004). Furthermore, elevation of Mn concentration in plant tissue has the potential to negatively alter physiological processes tied to growth. Van Lear and Smith (1990) showed that Mn was toxic to *Pinus elliottii* Engelm. (slash pine) seedlings when foliar levels reached 300mg kg^{-1}. Perhaps foliar Mn in the high treatment trees accumulated to a level that was detrimental to fascicle physiological processes. For example, Kitao et al., (1997) found that the net photosynthetic rate at saturating light and ambient CO_2 decreased with increasing foliar Mn concentrations in white birch (*Betula platyphylla* var. *japonica*). They noted that the carboxylation efficiency, decreased with greater leaf Mn accumulation.

Iron is an essential nutrient for plants but can be toxic when it accumulates at high levels (Connolly and Guerinot, 2002; Santana et al. 2014). For example, Santana et al., (2014) noted anatomical damage, such as protoplast retraction, changes in cell volume, and cell collapse, when *Setaria parviflora* (Poir.) Kerguélen and *Paspalum urvillei* Steudel plants were exposed to excess iron.

Observations of deficient foliar N, elevated foliar Mn and Fe levels were an indirect response to water limitation caused by the simultaneous occurrence of moderate drought and pathogen interference in sapwood function at the high inoculation level. As pathogen spread worsened water stress, carbon limitations led to a drop in new root growth and a loss of whole-tree leaf area (Chapter 4). This leaf area response is attributed, in part, to a 11% decrease in mean fascicle length in response to the high inoculation treatment. It is hypothesized that foliar N deficiency represents an additional compromise to carbon fixation caused by the pathogen. Similarly, elevated foliar Mn levels that adversely affect photosynthesis challenge the recovery of whole-tree carbon dynamics for normal growth.

5.4. CONCLUSION

Stem inoculation of *Pinus taeda* trees with colonized toothpicks of *L. terebrantis* affected foliar nutrient concentrations and mean fascicle length which was most severe at high inoculum density treatment. Pre-treatment foliar nutrient concentrations were at sufficiency level, but

inoculation with the pathogen affected foliar N, Mn and Fe concentrations. The deficient foliar N, elevated foliar Mn and Fe levels particularly in the high inoculum treatment were an indirect response to water limitation caused by the simultaneous occurrence of moderate drought and *L. terebrantis* interference in sapwood function. As pathogen spread worsened water stress, carbon limitations led to a reduction in fascicle length.

Table 5.1. Probabilities of a greater *F*-value **(*P* > *F*)** of pre-treatment soil chemistry variables in March 2017.

Variable[1]	Df[2]	F value	*P* > *F*
pH_{water}	4	9.30	<.0001
N_T	4	0.39	0.8159
S_T	4	1.22	0.3078
P	4	1.18	0.3275
K	4	0.87	0.4845
Mg	4	0.54	0.7099
Ca	4	0.76	0.5567
Na	4	1.25	0.2974
Zn	4	0.93	0.4530
Mn	4	0.56	0.6912
Fe	4	0.27	0.8966
Cu	4	1.24	0.2996
Al	4	1.92	0.1151

[1] pH_{water}, soil pH in water solution; N_T, total combustible nitrogen; total combustible sulphur; P, exchangeable P; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium; Zn, zinc ; Mn, manganese; Fe, iron; Cu, copper; and Al, aluminum.
[2] Df, degrees of freedom; *P* > *F*, probability of a greater *F*-value.

Table 5.2. Mean values of soil nutrients concentration in *P. taeda* tree stand prior to treatment application.

	Inoculation treatment[1]				
Variable	Control	Wound	Low	Medium	High
pH_{water}[2]	5.2 ± 0.1bc[3]	5.1 ± 0.1bc	5.0 ± 0.1c	5.4 ± 0.1a	5.3 ± 0.1ab
N_T (%)	0.04±0.01	0.04±0.01	0.05±0.01	0.03±0.01	0.04±0.01
S_T (%)	0.005±0.001	0.038±0.006	0.005±0.001	0.004±0.001	0.006±0.001
Nutrient (mg kg^{-1})					
P	3.3±0.8	3.3±0.7	5.6±1.2	4.1±0.8	3.5±1.0
K	10.6±2.3	12.5±1.5	10.5±2.4	11.1±2.4	15.5±2.5
Mg	12.6±2.6	12.0±1.6	10.0±2.6	11.3±1.6	14.5±1.6
Ca	36.5±7.7	43.1±8.2	46.9±13.0	40.4±7.2	64.1±9.8
Na	11.1±1.1	9.4±0.5	11.0±0.8	9.4±0.6	11.6±1.3
Zn	0.72±0.06	0.58±0.06	0.63±0.08	0.76±0.08	0.74±0.11
Mn	34.2±6.7	35.6±6.6	24.8±6.4	35.4±6.3	29.0±5.5
Fe	88.5±9.6	85.7±8.5	98.1±11.6	86.5±8.7	88.5±9.1
Cu	0.41±0.08	0.35±0.03	0.47±0.06	0.35±0.03	0.53±0.12
Al	561.7±41.6	560.6±26.3	610.3±42.5	485.8±15.5	605.8±45.5

[1] Control, not wounded; Wound, wounded control; Low, Medium and High are increasing levels of *L. terebrantis* inoculum density assigned for treatment.

[2] pH_{water}, soil pH in water solution; N_T, percentage of dry weight; S_T, percentage of dry weight; P, exchangeable P; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium; Zn, zinc ; Mn, manganese; Fe, iron; Cu, copper; and Al, aluminum in mg kg^{-1}.
[3] Means followed by the same lower-case letter are not significantly different by Tukey's Honest Significant Difference test at $\alpha = 0.05$.

Table 5.3. Descriptive statistics of the foliar mineral nutrition of *P. taeda* in July 2016 prior to stem inoculation with *L. terebrantis*. Foliage was from the first flush produced in 2016.

Nutrient element	N[1]	Mean	SD	CV (%)	Minimum	Maximum
Macronutrients (%)						
N[2]	60	1.09	0.12	11.28	0.86	1.39
S	60	0.10	0.02	17.26	0.05	0.14
P	60	0.10	0.01	8.64	0.09	0.13
K	60	0.42	0.04	9.07	0.31	0.53
Mg	60	0.12	0.02	14.93	0.08	0.16
Ca	60	0.26	0.05	20.52	0.16	0.41
Na	60	0.03	0.01	21.36	0.02	0.04
Micronutrients (mg/Kg)						
B	60	16.98	7.15	42.12	8.00	44.00
Zn	60	27.16	6.05	22.30	15.00	43.00
Mn	60	392.93	127.00	32.32	220.00	766.00
Fe	60	56.93	16.90	29.68	31.00	125.00
Cu	60	4.36	1.04	23.79	3.00	8.00
Al	60	531.79	104.34	19.62	329.00	789.00

[1] N, number of samples; Mean, mean among samples; SD, standard deviation; CV, coefficient of variation; Minimum, minimum value among samples; Maximum, maximum value among samples.
[2]N, nitrogen; S, sulphur; P, phosphorus; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium; B, boron; Zn, zinc; Mn, manganese; Fe, iron; Cu, copper; Al, aluminum.

Table 5.4. Probabilities of a greater *F*-value (*P* > *F*) of foliar nutrient concentrations after stem inoculation of *P. taeda* trees with *L. terebrantis* in March 2017**.** Foliage was sampled in July from the first flush produced in 2018 or 2019.

Effect	Num DF[1]	Den DF[2]	Treatment (T)		Time (Y)		T X Y	
			F Value	***P* > F**	**F value**	***P* > F**	**F Value**	***P* > F**
Macronutrients								
N[3]	4	55	3.18	0.0199	12.21	0.0009	0.67	0.6145
S	4	55	1.61	0.1843	7.49	0.0083	1.32	0.2746
P	4	55	1.26	0.2948	17.3	0.0001	1.37	0.2546
K	4	55	1.16	0.3396	0.24	0.6272	0.8	0.5274
Mg	4	55	1.18	0.3276	0.08	0.7773	0.62	0.6517
Ca	4	55	0.45	0.7715	3.45	0.0688	0.22	0.9274
Na	4	55	1.23	0.3082	0.66	0.4198	1.71	0.16
Micronutrients								
B	4	55	0.79	0.5384	12.45	0.0009	0.17	0.9513
Zn	4	55	0.59	0.6711	0.1	0.7515	0.09	0.9851
Mn	4	55	4.09	0.0055	5.63	0.0212	0.25	0.9073
Fe	4	55	3.64	0.0104	25.14	<.0001	0.48	0.7477
Cu	4	55	1.04	0.3965	400.33	<.0001	0.48	0.7468
Al	4	55	1.28	0.2894	15.44	0.0002	0.74	0.5697

[1]Num DF, degrees of freedom of the numerator; [2]Den DF, degrees of freedom of denominator
[3]N, nitrogen; S, sulphur; P, phosphorus; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium; B, boron; Zn, zinc; Mn, manganese; Fe, iron; Cu, copper; Al, aluminum. *P* > *F*, probability of a greater *F*-value

Table 5.5. Mean foliar nutrient concentrations in July, approximately one (2018) and two (2019) years after stem inoculation of *P. taeda* trees with *L. terebrantis* in March 2017. Foliage was sampled in July from the first flush produced in 2018 or 2019.

Year	Macronutrient (%)	Inoculation treatment[1]					Mean
		Control	Wound	Low	Medium	High	
2018	N[2]	1.07±0.02	1.17±0.05	1.14±0.06	1.12±0.04	1.04±0.03	1.09±0.02
	S	0.06±0.001	0.06±0.004	0.06±0.005	0.07±0.003	0.07±0.006	0.06±0.002
	P	0.09±0.002	0.09±0.004	0.09±0.003	0.08±0.005	0.09±0.004	0.09±0.001
	K	0.41±0.015	0.40±0.017	0.37±0.041	0.37±0.042	0.39±0.017	0.40±0.01
	Mg	0.09±0.002	0.09±0.003	0.10±0.002	0.09±0.008	0.09±0.006	0.09±0.002
	Ca	0.20±0.006	0.19±0.015	0.20±0.021	0.18±0.025	0.18±0.012	0.19±0.005
	Na	0.01±0.001	0.01±0.002	0.01±0.004	0.01±0.003	0.01±0.001	0.01±0.001
	Micronutrient (mg/Kg)						
	B	17.1±0.95	18.2±3.28	16.7±1.45	18.5±1.64	17.2±1.77	17.3±0.70
	Zn	16.2±0.962	16.7±2.512	16.0±3.474	16.7±2.348	17.5±1.190	16.3±0.76
	Mn	252.8±17.1	249.3±43.5	203.0±27.2	380.0±87.3	417.8±63.2	276.7±17.1
	Fe	16.4±0.72	14.3±1.60	22.5±3.79	22.7±2.59	20.2±2.88	17.7±0.76
	Cu	1.1±0.073	1.5±0.224	1.2±0.167	1.2±0.200	1.5±0.224	1.2±0.06
	Al	350.1±15.2	336.5±43.6	393.6±47.9	373.2±19.4	350.5±39.5	354.5±11.7
2019	Macronutrient (%)						
	N	1.02±0.03	1.05±0.05	1.03±0.04	1.08±0.05	0.91±0.03	1.01±0.01
	S	0.07±0.002	0.06±0.005	0.07±0.003	0.08±0.005	0.06±0.003	0.07±0.001
	P	0.07±0.002	0.07±0.003	0.07±0.002	0.08±0.004	0.08±0.002	0.07±0.001
	K	0.40±0.015	0.37±0.050	0.36±0.016	0.38±0.031	0.36±0.022	0.38±0.01
	Mg	0.09±0.003	0.09±0.007	0.09±0.008	0.09±0.012	0.09±0.009	0.09±0.003
	Ca	0.19±0.010	0.21±0.024	0.18±0.030	0.21±0.028	0.25±0.026	0.20±0.009
	Na	0.02±0.003	0.01±0.002	0.01±0.002	0.01±0.002	0.01±0.001	0.01±0.002
	Micronutrient (mg/Kg)						

B	12.4±0.60	13.3±1.17	13.5±1.23	15.8±1.57	15.2±2.08	13.2±0.48
Zn	15.9±0.77	16.2±0.80	16.0±1.09	17.0±2.32	17.0±1.15	16.2±0.54
Mn	341.9±27.3	367.3±12.5	311.3±50.7	402.3±66.3	469.8±98.6	360.2±20.9
Fe	24.8±0.94	22.5±1.7	26.0±1.9	30.8±4.9	26.9±3.8	25.6±0.91
Cu	5.7±0.15	5.3±0.21	5.0±0.09	5.8±0.307	5.8±0.36	5.6±0.11
Al	450.9±23.3	443.0±21.7	476.2±42.0	464.7±38.7	432.5±43.3	451.8±15.6

Foliar nutrient sufficiency threshold for *Pinus taeda* according to Albaugh et al. (2010) and references cited therein: N: 1.2%, S: 0.10-0.12%, P: 0.12%, K: 0.35-0.40, Mg: 0.08%, Ca: 0.15%, B: 4-8mgkg^{-1}, Zn: 10-20 mgkg^{-1}, Mn: 20-40 mgkg^{-1}, Cu: 2-3 mgkg^{-1}. Na, Fe and Al sufficiency threshold not determined for *P. taeda*. Note that these values are for samples collected during the dormant season from December to February and cannot be directly compared to growing season data.

[1] Control, not wounded; Wound, wounded control; Low, Medium and High are increasing levels of *L. terebrantis* inoculum density assigned for treatment. [2] N, nitrogen; S, sulphur; P, phosphorus; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium; B, boron; Zn, zinc; Mn, manganese; Fe, iron; Cu, copper; Al, aluminum.

Table 5.6. Probabilities of a greater *F*-value (*P* > *F*) of four shoot morphological variables approximately three years (34 months) after stem inoculation of *P. taeda* with *L. terebrantis*. Shoots were the second flush produced in 2019 and were measured in January -February 2020.

Variable	Df[1]	F value	*P* > F
Shoot length	4	0.55	0.7010
Fascicle number	4	1.25	0.2971
Fascicle length	4	2.80	0.0327
Density	4	0.56	0.6895

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

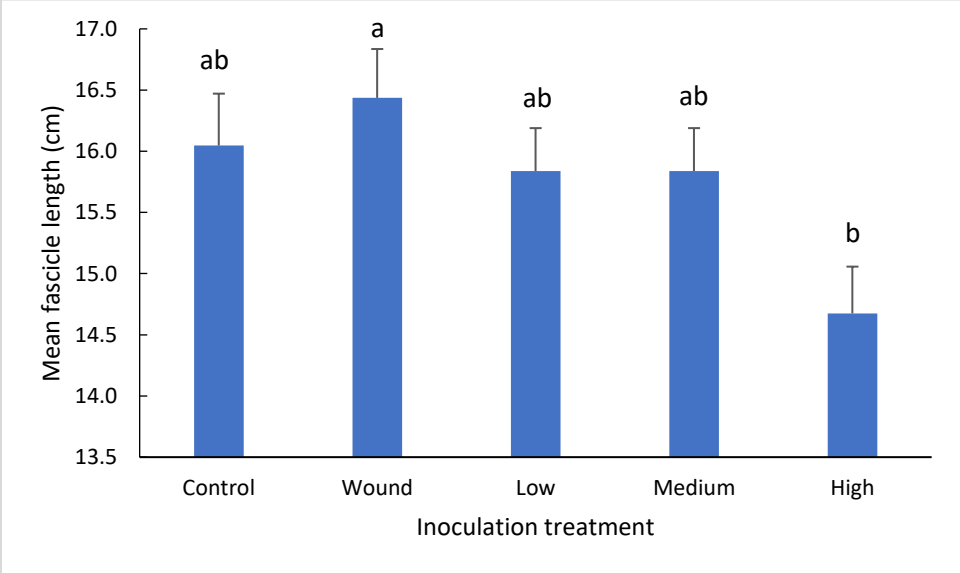

Figure 5.1. Mean fascicle length of *P. taeda* approximately three years (34 months) after stem inoculation with *L. terebrantis*. Bars represent one standard error of the mean. Means associated with the same lower case letter are not significantly different by Tukey's Honest Significant Difference test at $\alpha = 0.05$.