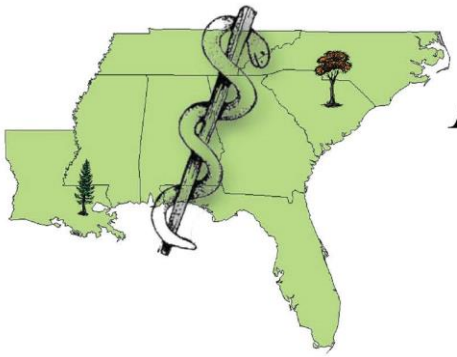

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LECANOSTICTA ACICOLA IMPACTS ON FOLIAR NUTRIENT CONTENTS AND TOTAL PHENOLICS IN *P. TAEDA* NEEDLES

by
Debit Datta and Lori Eckhardt

ABSTRACT
Brown-spot needle blight fungus, *L. acicola*, has been reported from loblolly pine stands in Alabama. Needle sampling to measure macro and micro-nutrient contents and total phenolics was conducted at seven permanent plots in Chatom, Alabama, Washington County. Infection of *L. acicola* increased the total phenolics concentrations (($p < 0.005$). Higher concentrations were positively correlated with disease severity. Foliar concentrations of N, Na, S and B ($p \leq 0.05$) were also correlated with *L. acicola* infection severity. Higher concentrations of foliar nutrients could be a result of leaf area loss and concentration effects in those remaining needles.

4.1. INTRODUCTION

Pinus taeda is an economically valuable timber species native to the southeastern United States. Commercially managed plantations account for nearly half of the pine growing stock in the south (McKeand et al., 2003) as intensive management practices (mechanical and chemical site preparation, fertilizer application) and improved genetics have increased stand productivity (Wear & Gries, 2002; McKeand et al., 2003).

Nutrient availability is essential to determine tree growth and productivity (Fox et al., 2007). Tree growth is affected if the nutrient level is below or above the threshold level. Sayer et al., (2009) confirmed that elevated Mn concentrations contributed to reduced vigor of longleaf (*P. palustris*) plantations in Georgia and Florida. However, growth is often limited by soil nutrient availability in southern pine plantations. Soil nutrients such as nitrogen (N) and phosphorus (P) promote the expansion of leaf area, which in turn, contributes to wood production. Therefore,

nutrient-deficient stands respond quickly following N and P fertilization and increase their radial growth (Fox et al., 2007).

Foliar nutrient contents can be used as an indirect indicator of soil nutritional status.

Content and relative abundance of macronutrients in the needles can be used to determine the nutritional status of pine forests across varied habitats (Ballard and Carter, 1986; Braeke and Sahin, 2002). Nutrient elements are also interrelated as excess or deficiency of one mineral can affect the function of other elements and alter normal physiological processes. Nutrient deficiency of an element can indicate available soil supply and stands demand for nutrients (Marschner, 2011).

Host-pathogen interactions have been reported to cause changes in foliage nutrition status in many conifers (Singh & Bhure, 1974; Lambert et al., 1986; Lan et al., 2019). Infetstation of *Armillaria mellea* (Vahl ex Fr.) Kummer, a root pathogen on coniferous species induced reduction of foliar N, P, K, Mg, and Na concentrations and caused an increase in Mn, Ca, Fe, and Zn concentrations (Singh & Bhure, 1974). Disease severity and nutrient relations were examined on Douglas fir infected with *Pseudotsuga menziesii* (Mirb.) Franco, Swiss Needle Cast. Disease severity correlated with an increase of C, N, Na, K and S concentrations and a reduction of P and Mn (Lan et al., 2019). In addition, correlation was observed between sulfur decrease and infection level of Dothistroma needle cast fungus on *Pinus radiata* species (Lambert et al., 1896).

Many conifers including loblolly pine develop carbon-based soluble compounds such as total phenolics in their foliage as defensive compounds (Aspinwall et al., 2011). These phenolics play a role in herbivore deterrence, nutrient cycling, litter decomposition, and soil carbon sequestration (Chung & Barnes, 1977). Increased total phenolic response has been observed in response to insects (Holopainen et al., 2006; Williams et al., 1994), or fungal infestation (Bahnweg et al., 2006) and foliar injury (Booker et al., 1996). Needles high in phenolic compounds have a reduce rate of litter decomposition that results in nutrients accumulating and decreasing the tree productivity. Therefore, it may be possible by assessing total phenolics can shed a light the overall tree productivity.

Brown spot needle blight (BSNB) has been observed in loblolly pine plantations in Alabama, causing repeated needle defoliation and tree mortality. Base on needle cast disease, it is possible that *L. acicola* would decrease loblolly pine foliar nutrient contents and increase defensive chemicals such as total phenolics response as a defensive mechanism against *L. acicola* infection and severity. To determine the effects of *L. acicola* on tree growth and physiological functions, this study examined the available nutrient contents and total phenolics in the foliage at BSNB infected plots.

4.2. MATERIALS AND METHODS

4.2.1. Study area and sample collection

The study was conducted at seven permanent infected plots located in Chatom, Alabama, Washington County (31°27′42″N 88°14′53″W). Infected plots were of 2004, 2009, 2010, and 2012 plantations. Those plots were categorized as low, moderate, high or very high incidence stands based on the scoring of visual crown severity of infected trees and scoring of needle chlorosis and necrosis. Only 2004 plot received fertilizer treatment within three years of planting. No silvicultural treatments were applied on other experimental stands. A 0.22 mag caliber rifle was used to collect symptomatic foliage from the trees (Lan et al., 2019). To determine baseline nutrient nutrients, a healthy plot with no symptomology was chosen nearly

1.5 km away from the infected sites and used to collect needles. Approximately 50 fascicles were collected per tree with 50 trees sampled in the study. Needle samples were oven-dried at 70oC for up to three days and then passed through a 0.5 mm mesh screen in a Wiley mill. Nutritional analyses were conducted by Waypoint Analytical Laboratory in Memphis, Tennessee, USA. Phosphorus (P) concentration was analyzed by combustion (Bryson et al., 2014) while nitrogen (N), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), sodium (Na), boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), and aluminum (Al) were determined by a wet digestion standard procedure (Bryson et al., 2014).

Total phenolic were determined from 40 needle samples collected from high incidence and low incidence plots as discussed above. Symptomatic needle samples were collected from both the upper and lower crown of sampled trees. Tree age, DBH and height were collected. Trees were ranked visually based on their severity of chlorosis and necrosis. Needle samples were kept in dry ice during field collection and stored at -200C when brought back in the laboratory.

Samples were placed in a mortar along with liquid nitrogen and ground up with a pestle. Ground samples were then placed in a desiccator (Aspinwall et al., 2011) and total phenolic extractions were followed by 4.2.2.

4.2.2. Determination of soluble components

Two extraction procedures were used to ensure total phenolics recovery. The first extraction used 70% acetone and the second extraction used 250 ml sodium citrate in combination with 0.04% sodium bisulfite (pH 7) (Blum, 1997). Needle tissue was extracted 4 times with each 1ml solvent, then vortexed and incubated for 5 minutes at room temperature. Total phenolic extractions following both procedures were examined and compared. It was observed that 70% acetone was 100% efficient compared to 250 ml sodium citrate with 0.04% sodium bisulfite which was 97-98% efficient.

Two 50 mg needle tissue per sample by whorl height were extracted 3 times with 70% acetone. The mixture was vortexed for 5 minutes at room temperature and centrifuged (max 16,000g) for another 5 minutes to pellet insoluble materials. Both sample supernatants were combined and the Folin-Ciocalteu method was used to determine total phenolics concentrations in the foliage (Booker and Maier, 2001). Supernatants were diluted at 1:10 with 70% acetone and duplicate 10

µl aliquots were mixed with 495µl of 0.25 N Folin-Ciocalteu reagent and 495µl of 1 M Na2CO3. With two aliquots and two 50 mg samples extracted, a total of four samples were analyzed for each sampled tree. Samples were vortexed and incubated at 250C for 30 min. The solution absorbance wavelength was recorded at 724 nm on spectrometer. Absorbance was based on a calculated catechin concentration equivalent (Booker & Maier, 2001) for which a standard curve was produced. Lastly, total phenolic concentrations were determined for the foliage and expressed as catechin equivalents i.e., mg g-1 dry needle mass.

4.2.4. Statistical analyses

Normality and homogeneity of data were verified using Shapiro-Wilk and Levene's tests. Two sample welch t-tests were performed to evaluate total phenolics differences between low and high infection needles. Simple linear regression models were performed to determine relationships of total phenolic concentrations with infection level, disease severity, tree age, whorl height, DBH and whorl height. Similarly, needle nutrients were analyzed using the ANOVA test. Data analyses were conducted by statistical R version 4.0.3 software. A simple linear regression was performed to determine the relationship between infection level and needle nutrient concentrations.

4.3. RESULTS

Foliar Ca was the greatest (coefficient of variance [CV] > 38%) and Na was the least variable (CV < 0%) macronutrients in the asymptomatic trees (Table 4.1). Macronutrients in asymptomatic trees were ranked from most to least variable as follows Ca > N > S > Mg > P > K > Na. Conversely, foliar Na was the greatest (CV >70%) and foliar N was the least variable (CV<15%) macronutrients in the symptomatic trees. Macronutrient ranking from most to least variable as follows Na > K > Ca > P > Mg > S > N in the symptomatic trees (Table 4.2).

Nutrient content Al (CV > 130%) and Fe (CV > 68%) were the greatest variable micronutrients in the asymptomatic and symptomatic trees, respectively. However, asymptomatic and symptomatic trees showed Zn was the least variable macronutrient in their needles. Ranking of micronutrients followed as Al > Mn > Fe > Cu > B > Zn in the asymptomatic trees and Fe > Mn > B > Al > Cu> Zn in the symptomatic trees (Table 4.2).

Nitrogen content showed significant positive correlation (*p*-value=0.02; R2=31%) with infection level. Marginal relationship was obtained for S concentration (*p*-value=0.05; R2=14%) and *L. acicola* infection. A statistically significantly increased amount of Na (*p*-value=0.004; R2=27%) concentration was detected for the infected trees when compared to asymptomatic trees (Figure 4.1). No statistically significant relationship was observed for other macronutrients such as S, K, Ca, and Mg contents with *L. acicola* infection severity. Macronutrients such as Al, Cu, Fe, Mn, and Zn were not correlated with *L. acicola* infection (Table 4.1 & Table 4.2). Only macronutrient B showed positive correlation (*p*-value=0.009; R2=28%) with infection level (Figure 4.1).

No relationships were detected for nutrient ratios such as N/S, N/K, P/S, P/Zn, K/Mg, K/Mn, Ca/K, Fe/Mn, and Ca/Mg with the infection level. Only Ca/B showed significant negative correlation (*p*-value = 0.002; R^2=41%) with infection level (Figure 4.2). The foliar ratios showed a wide range of variation in the foliage that were not statistically significantly affected by the brown spot needle blight fungus, *L. acicola*. Thus, the range is not due to the presence of the fungus, *L. acicola*.

Total phenolic concentrations in the needles were measured as a catechin equivalent (mg/ml) (Figure 4.3). There was a significant increase in total phenolic concentrations (*p*-value 0.006) on high incidence trees when compared to low incidence trees. Similarly, total phenolics increased in response to disease severity (*p*-value 0.002). Tree whorl height, height, and age were negatively associated with total phenolics. However, tree DBH was not significantly correlated with infection severity (*P*-value 0.218) (Figure 4.4).

4.4. DISCUSSION

Needle nutrient contents of N, Na, B and P were positively correlated to infection severity. Previous studies examined responses of foliar nutrient concentrations either with fertilization application or as an effect of thinning treatment (Fox et al., 2007; Gurlevik et al., 2003). In the study, one experimental plot was fertilized which was planted in 2004 and fertilized after three years of planting. Other six experimental plots didn't receive any fertilization or silvicultural treatments. Therefore, it is difficult to extrapolate fertilizer application relation to BSNB disease severity. However, needle disease impacts are varied with plantation attributes such as foliar nutrient application (Lan et al., 2019). Therefore, foliar nutrients may influence BSNB severity and interacts with *L. acicola* severity which requires further investigation.

Other nutrient content P, K, Mg, Ca, Zn, Mn, Fe, Cu and Al showed variations among infection levels. Foliar nutrient deficiency or abundance are related to soil nutrient availability (Pietrzykowski et al., 2013). Therefore, those variations of nutrient contents might be related to spatial variations of the plots. Albaugh et al., (2010) reported that loblolly plantations showed highly variable foliar micronutrients comparative to foliar macronutrients across the southeastern United States. Variations also could be due to the severity of infection or interaction between severity of the infection and some other variable related to tree vigor (Vose et al., 1994; Sayer et al., 2009). For example, crown class (dominant/codominant vs intermediate/suppressed) could affect light availability to foliage, C-fixation, and C allocation to defense (Niinemets et al., 2002; Vose et al., 1994). Variability of N might be due to one of these factors or a combination of these factors. A high amount of infection is going to be more detrimental under poor light conditions because the defense would be compromised by available C. In a similar manner, tree leaf area, live crown ratio, and crown condition are other variables that could interact with infection severity to affect vigor. Higher concentrations could be driven by a loss of leaf area before sampling that resulted in a concentration effect in the foliage. High variability of the "affected" trees suggests that these trees might be in varying stages of vigor loss.

Some of the variability could be attributed to the age class of foliage sampled- especially for immobile nutrients like Ca, B, and Mn. In loblolly pine trees, variations of needle nutrient contents are driven by season, needle age, soil, and crown position. Older needles contain more nutrients than young needles. Seasonal variations such as spring and winter also contribute to nutrient variations. Soil pH also changes the form of nutrients in the soil and affects their nutrient availability to, and uptake by the trees (Schultz, 1997). Percentage of N, Ca and Mg are greater in upper crown foliage and percentage of P and K are greater in lower crown foliage (Wells & Metz, 1963). Apart from that, needle morphology (needle weight and length), and growth of tree stands (average height and DBH of the stands) are related to needle nutrient variability. Since the study has found lots of variation of foliar nutrition data in loblolly pine needles, the study requires further investigation to make a conclusion about foliar nutrients and BSNB severity.

The study detected a positive correlation of total phenolic in loblolly pine foliage with infection level and disease severity. Total soluble phenolics in the needles are linked to plant defense capacity against fungi, insects, or microbial infestation. It is expected that infected trees could increase defensive chemicals in the foliage to defense against *L. acicola*. Total phenolic decreased with increasing tree height, whorl height, and age. Although *L. acicola* infection progressed from lower canopy to higher canopy (see chapter III), needle pathogens are likely to stay more on the lower canopy due to high relative moisture availability. Higher concentrations of total phenolics in the lower canopy needles might be in response to severe *L. acicola* infection in the lower crown.

4.5. CONCLUSION

Foliar concentrations such as N, B, Na and S and nutrient ratios such as Ca/B were affected by *L. acicola* infection. High incidence trees had higher total phenolic when compared to low incidence trees perhaps as a mechanism to defend against *L. acicola* infection. The higher concentration of foliar nutrients in samples are most probably a function of loss of leaf area due to the defoliation. Variability among foliar concentrations could also be influenced by other factors such as soil pH, tree vigor and crown condition.

Table 4.1. Descriptive statistics of foliar nutrition contents in loblolly pine needles(asymptomatic trees) collected in spring 2020.

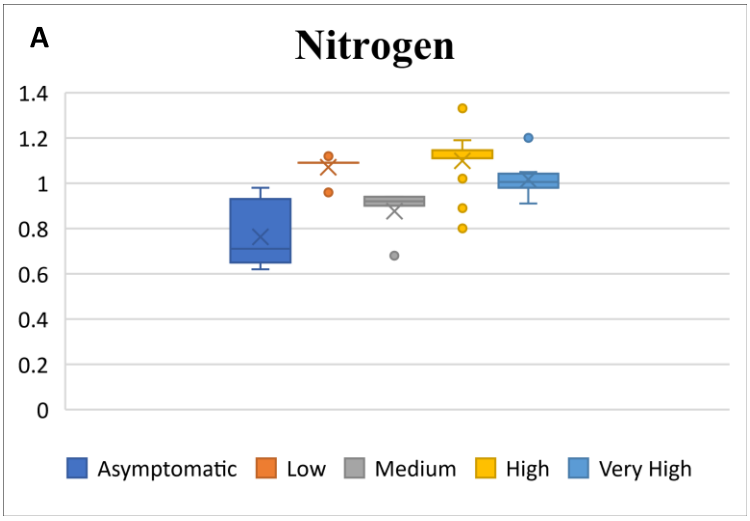
Nutrient element	1_N	Mean	SD	CV (%)	Minimum	Maximum
Macronutrients (%)						
2_N	15	0.77	0.15	19.97	0.65	0.94
S	15	0.09	0.01	13.32	0.08	0.10
P	15	0.08	0.01	6.93	0.08	0.09
K	15	0.40	0.02	3.85	0.38	0.41
Mg	15	0.16	0.02	10.83	0.15	0.18
Ca	15	0.32	0.13	39.28	0.25	0.47
Na	15	0.01	0.00	0.00	0.01	0.01
Micronutrients (mg/Kg)						
B	15	7.00	1.73	24.74	6.00	9.00
Zn	15	49.33	5.51	11.16	44.00	55.00
Mn	15	108.67	58.07	53.44	67.00	175.00
Fe	15	68.00	30.79	45.28	42.00	102.00
Cu	15	4.67	1.53	32.73	3.00	6.00
Al	15	97.67	132.00	135.15	17.00	250.00

[1]N, number of samples; Mean, an average of samples; SD, standard deviation; CV, coefficient ofvariation; Minimum, minimum value of samples; Maximum, maximum value of samples. [2]N, nitrogen; S, sulfur; P, phosphorus; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium; B, boron; Zn, zinc; Mn, manganese; Fe, iron; Cu, copper; Al, aluminum. Needle samples were collected from both lower branches and upper branches of control and infected trees.

Table 4.2. Descriptive statistics of the foliar nutrition contents in loblolly pine needles(symptomatic trees) collected in spring 2020.

Nutrient element	a_N	Mean	SD	CV (%)	Minimum	Maximum
Macronutrients (%)						
b_N	35	1.03	0.15	14.18	0.68	1.33
S	35	0.10	0.01	14.39	0.08	0.13
P	35	0.08	0.01	18.74	0.04	0.09
K	35	0.47	0.18	38.14	0.28	1.01
Mg	35	0.14	0.02	16.64	0.10	0.20
Ca	35	0.40	0.13	32.04	0.20	0.81
Na	35	0.03	0.02	72.07	0.01	0.09
Micronutrients (mg/Kg)						
B	35	15.59	6.05	38.82	6.00	32.00
Zn	35	38.50	10.42	27.07	23.00	61.00
Mn	35	242.36	138.53	57.16	57.00	576.00
Fe	35	73.18	51.09	69.81	35.00	255.00
Cu	35	4.45	1.26	28.33	3.00	9.00
Al	35	354.86	134.93	38.02	151.00	733.00

[a]N, number of samples; Mean, an average of samples; SD, standard deviation; CV, coefficient ofvariation; Minimum, minimum value of samples; Maximum, maximum value of samples. [b]N, nitrogen; S, sulfur; P, phosphorus; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium; B, boron; Zn, zinc; Mn, manganese; Fe, iron; Cu, copper; Al, aluminum. Needle samples were collected from both lower branches and upper branches of control and infected trees.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1.2
1
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0.6
0.4
0.2
0
Asymptomatic
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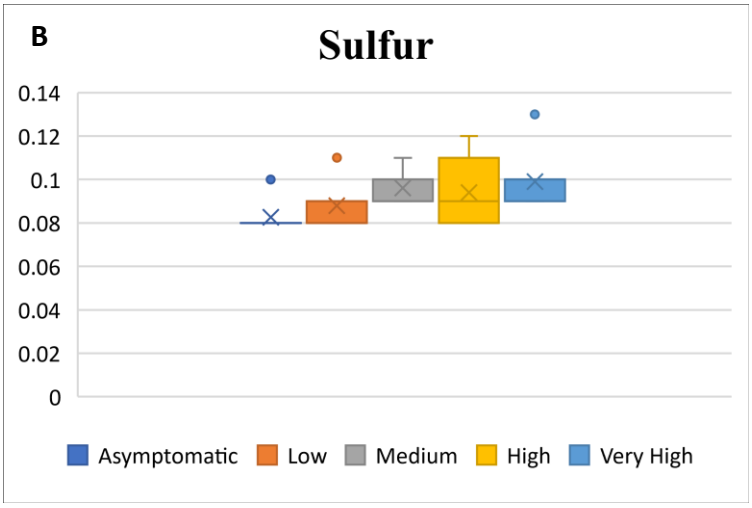
Medium
High
Very High


B
Sulfur
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Asymptomatic
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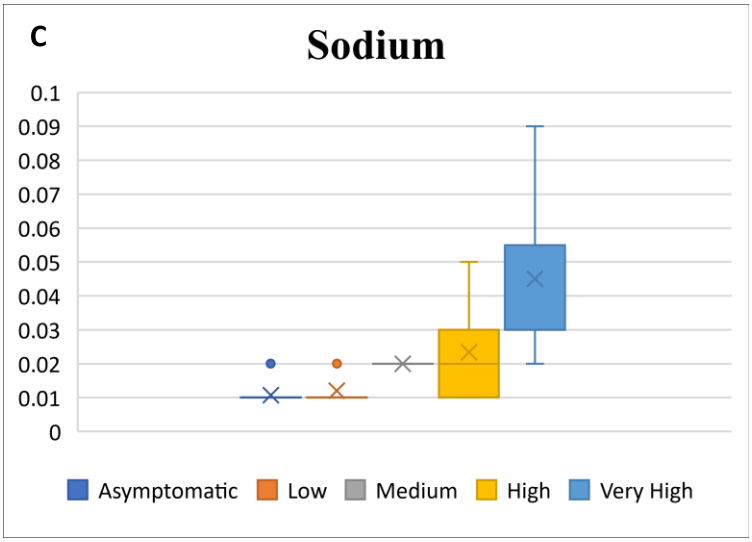
Very High


C
Sodium
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Asymptomatic
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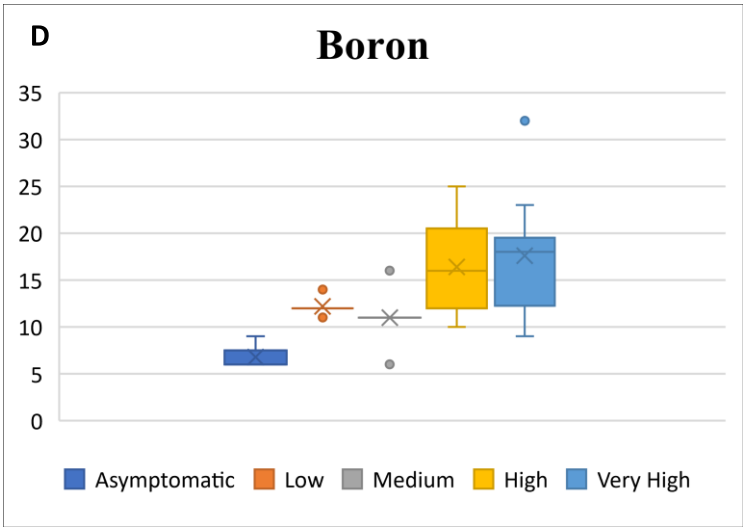

Figure 4.1. Relationships of foliar macronutrients(A) N (*p*-value=0.02; R^2=31%)
(B) S (*p*-value=0.05; R^2=14%)
(B) Na (*p*-value=0.004; R^2=27%) and micronutrient
(C) B (*p*-value=0.009; R^2=28%) with BSNB severity.

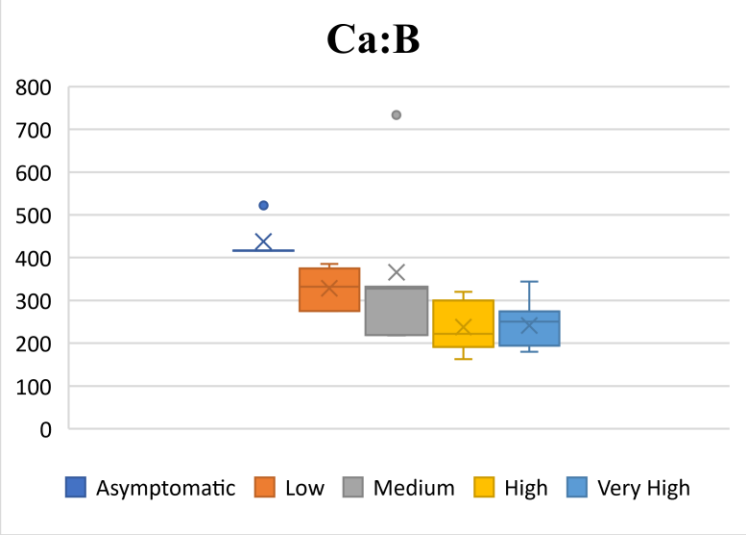

Figure 4.2. Relationship of foliar nutrient ratio Ca/Bwith BSNB severity.

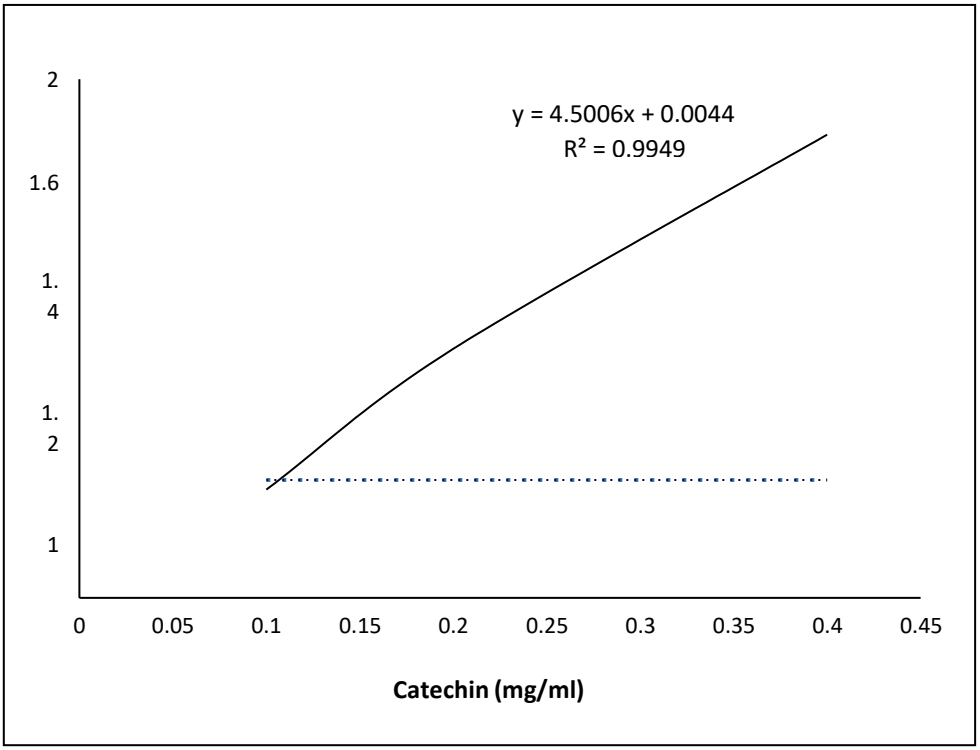

Figure 4.3. Catechin calibration curve for total phenolics contents

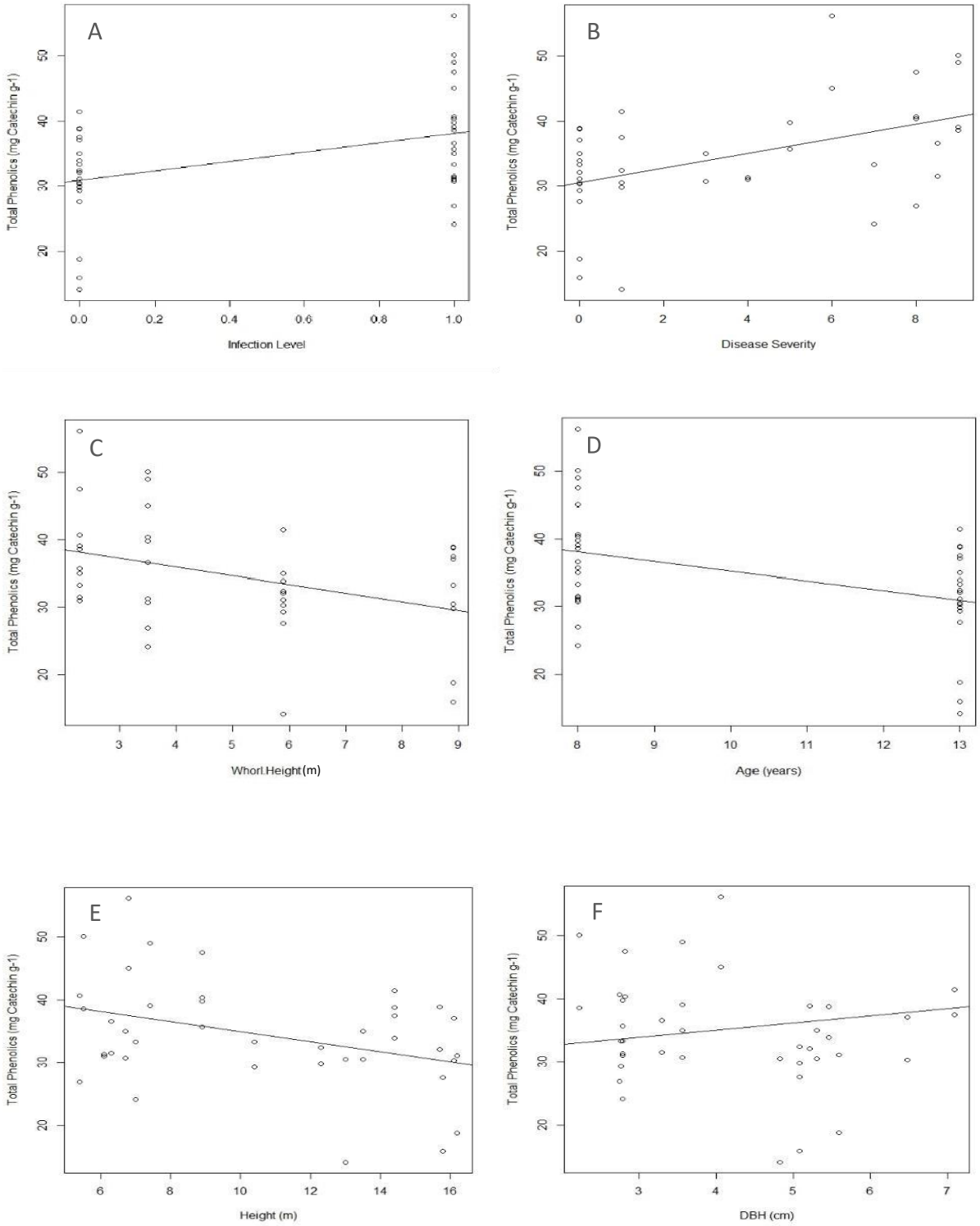

Figure 4.4. Relationships between total phenolics concentration and (A) infection level (*p*-value:0.005954) (B) disease severity (*p*-value: 0.00208) (C) whorl height (*p*-value: 0.01414) (D) age (*p*-value: 0.005954) (E) height (*p*-value: 0.01759) and(F) dbh (*p*-value: 0.218).