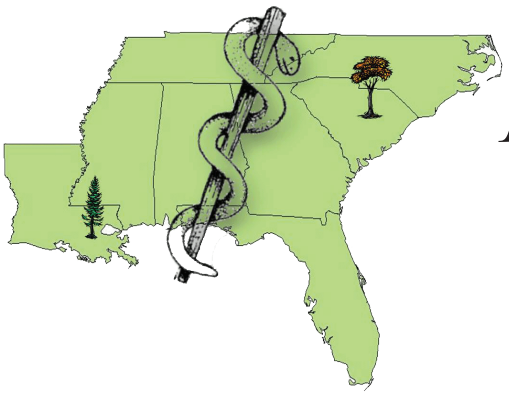

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ASSESSMENT OF NITROGEN NUTRITION AND FAMILY EFFECTS ON *GROSMANNIA HUNTII* INFECTION TO IDENTIFY A SEEDLING INDICATOR OF DEFENSE CAPACITY

by
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ABSTRACT

The southeastern United States is experiencing isolated cases of tree mortality associated with a pine decline disease complex. Biotic and abiotic factors such as the activity of root-feeding bark beetles, root disease caused by *Leptographium* and *Grosmannia* fungal species, topography, and silvicultural practices have been studied as causal factors, but the role of physiological processes that sustain tree growth in pine decline is still being evaluated. Genotypes of loblolly pine vary in their carbon allocation patterns to various plant parts. Pine families that have carbon allocation patterns favoring root system growth and defense chemicals may perform better against infection to pathogens such as *Leptographium* and *Grosmannia*. If so, an indicator variable that reflects these genetically controlled carbon allocation patterns could be identified to aid land managers in identifying the best loblolly pine genotype to plant where the risk of pine decline is high. This study was conducted to assess the effect of nitrogen nutrition on *Grosmannia huntii* infection, patterns of carbon allocation to above and below ground plant parts, and stem total phenolic concentration of 15 loblolly pine families currently utilized by the timber industry. Relationships between pattern of carbon allocation, concentration of total phenolics in stem tissue, and infection by *G. huntii* were evaluated and the resilience of these relationships under added nitrogen was assessed. Results indicated that *G. huntii* successfully produced lesions on inoculated seedlings but that nitrogen concentration had no effect on lesion length, lesion width and stem total phenol concentration and lesion dimensions did not vary notably by family. Pathogen virulence under the study conditions may have interfered with lesion response to the treatments. However, family differences in stem total phenolic concentration indicate that there may be an opportunity to select genotypes with superior preformed defense capacity. Also, while carbon allocation pattern did not prove to be an effective predictor of disease resistance, total seedling size and foliage mass relative to the mass of other tissues were positively related to stem total phenolic concentration for several families. With continued research, therefore, these morphological variables show promise as indicators of preformed defense potential.

INTRODUCTION

A large area of the southeastern United States is managed for timberland with loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) the dominate tree species on 55 million acres; approximately one-fourth of all southern forests (Smith et al. 2007). While shortleaf pine is more widely distributed than

loblolly pine, loblolly pine is the backbone of the southern pine industry, covering 80% of commercial forest area in the South (Smith et al. 2007) with geographical range extending across 15 states in the south and mid-Atlantic region (Baker and Langdon 1990). Mortality following poor growth rate, the appearance of an unhealthy crown, and an overall decline in tree vigor has been seen in loblolly pine over a 50 year period particularly in saw timber sized trees (Brown and McDowell 1986; Hess et al. 2002). Field observations have shown that ophiostomatoid fungi, with root feeding bark beetles as their vectors, are an important component of pine decline (Otrosina et al. 1999; Eckhardt et al. 2007).

Declining loblolly pine exhibits symptoms such as sparse and chlorotic foliage, fine and lateral root deterioration, large cone crops, and poor radial growth (Brown and McDowell 1968; Eckhardt et al. 2007). Premature mortality of loblolly pine has been well documented as a decline disease syndrome that involves interactions of biotic and abiotic factors (Eckhardt et al. 2004a). Root feeding bark beetles are attracted to forest stands due to stress induced by abiotic factors such as topography, increased slope and southwest facing aspects (Eckhardt et al. 2007; Eckhardt and Menard 2009). The beetle attack introduces the fungus to the trees and help in transporting the fungus from infected tree to healthy trees (Paine et al. 1997). The fungus, in turn, aids to enhance the beetle brood by providing food for the developing larvae and by creating favorable conditions such as decreased host resistance to the beetles due to changes in host chemistry or formation of nutritive metabolic products for the beetles (Eckhardt et al. 2004b). This insect-fungus interaction increases the expansion of the decline disease is understood as a mutualism in which the fungi and the beetles benefit each other (Paine et al. 1997). However, in certain conditions the fungus alone can contribute to decline by blocking the vascular tissues and hence preventing translocation of water and nutrients (Eckhardt et al. 2004a).

Resource deficiencies within a tree may accelerate pine decline by interfering with natural stress avoidance mechanisms (Eckhardt et al. 2010). Trees respond to and sustain the resource stress by a variety of changes in physiology such as dynamic changes in crown leaf area which helps the plants sustain foliar physiology under low soil fertility and water availability (Pallardy et al. 1995). A subtle decline in leaf area that concentrates limiting mineral nutrients in the existing foliage may be favorable, for example, to achieve critical levels of the essential elements required by photosynthesis. At the same time, the loss of some leaf area may also aid photosynthesis by increasing water availability within the crown. If the carbon allocation pattern favors the root system, resource stress is also reduced as demonstrated for loblolly pine in different soil types and moisture regimes in North Carolina (Hacke et al. 2000) and longleaf pine in Georgia (Addington et al. 2006). The ratio of root surface area to leaf area was greater on the sites with low soil water availability. This pattern of carbon allocation led to more water acquisition from the soil which helped to maintain stomatal conductance and evade water stress on the drier sites.

Trees are able to tolerate resource stress as long as whole-tree carbon demand is met by the leaf area, thus, providing sufficient energy to continue the cellular processes. Severe stress, that causes a drop in leaf area below critical levels, however may jeopardize tree performance (Eckhardt et al. 2010). In addition to leaf area adjustment and a carbon allocation pattern that favors the root system, a deep root system may help to meet the demand for water by accessing

deep water that is either immediately translocated to the crown or is hydraulically redistributed to the surface soil horizons (Warren et al. 2007). In addition, any action that helps sustain the vascular transport of mineral nutrients and other solutes aids tree response to resource stress. Because many essential mineral nutrients (e.g., nitrogen, potassium, phosphorus) are dynamic and can be remobilized within the plant (Marschner 2012), new tissues rely on this source of some essential elements. Among the pines, nutrient remobilization from naturally senescing foliage is an important means of nutrient supply to new tissues in spring regardless soil fertility (Dalla-Tea and Jokela 1994). Nutrients can also be mobilized from older foliage before natural senescence to meet the nutritional demands of younger foliage (Nambiar and Fife 1991). In doing so, the nutrient-demanding physiological processes of older and younger foliage persist during periods of nutritional resource stress as long as foliar nutrient concentrations are maintained at or above critical levels.

Nitrogen availability affects the seedling and tree growth rate, biomass partitioning to plant parts, defense chemicals synthesis, and susceptibility to the pathogens (Holopainen et al. 1995; Samuelson 2000; Rühmann et al. 2002; King et al. 2008). In a study done on Scots pine seedlings, for example, elevated nitrogen concentration resulted in increased biomass of above ground plant parts and decreased root biomass allocation (Holopainen et al. 1995). Specifically, a 36 to 60% increase in foliar nitrogen concentration above 1.4% caused decreases in starch, total monoterpene, and total phenolic concentrations of shoot and root tissues. At the same time, this increase in nitrogen concentration caused increases in tissue concentrations of eight amino acids. Increases in oviposition and growth of *Lygus rugulipennis* Popp. accompanied these responses suggesting that elevated nitrogen nutrition decreases resistance to herbivory (Holopainen et al. 1995). Others have also reported that conifer seedlings and trees have a lower concentration of defense chemicals under elevated nitrogen environment indicating that nitrogen fertilization may play a role in plant pathogen interactions (Muzika 1993; Gebauer et al. 1998; Booker and Maier 2001). For example, Gebauer et al. (1998) reported lower concentrations of condensed tannins and total phenolics in the needles, stem, and lateral roots of loblolly pine seedlings as nitrogen availability increased.

The effects of genotype on loblolly pine carbon allocation patterns have been observed in previous studies (Bongarten and Teskey 1987; Li et al. 1991; Stovall et al. 2012). For example, Li et al. (1991) reported that biomass allocation among the stem, needles, and roots of loblolly pine differed among 23 families. Stovall et al. (2012) found that divergence in carbon allocation between two loblolly pine clones occurred in the taproot and foliage. A carbon allocation patterns that support the energy needs of a vigorous root system help to maintain tree vigor by evading water and mineral nutrient stress when availability of water and nutrients is low. Also, genotypes that allocate more carbon to defense chemicals in response to insect infestation may counteract insect vectored pathogens. Genotypes exhibiting small, rather than large nitrogen-induced decreases in the synthesis of defense chemicals could further benefit insect and disease resistance. It is hypothesized that under normal levels of nitrogen, genetically controlled carbon allocation patterns are indicative of pathogenic infection and defense chemical production, and under elevated levels of nitrogen nutrition, these relationships are negated for most families. The objectives of this study are first to identify the family morphological traits of loblolly pine that correlate to *G. huntii* infection after artificial inoculation, and the concentration of defense chemicals near the inoculation site. The second objective of the study is to evaluate the effect of

nitrogen nutrition on relationships between loblolly pine family morphology, *G. huntii* infection, and defense chemical concentration. Results will be used to identify family morphological traits indicative of resistance to *G. huntii* and defense chemical production, and identify families exhibiting only a minimum effect of nitrogen nutrition on these relationships.

MATERIAL AND METHODS

Twenty seedlings per family from 15 loblolly pine families (300 seedlings) were planted in trade gallon pots filled with ProMix BX® (Premier Tech, Quebec, and Canada) peat based potting mix and placed into a greenhouse. One- half of the seedlings received 100 ml of either normal (NN) or high nitrogen (HN) nutrient solution twice a week. A target foliar nitrogen (N) concentration of 1.0% was established for the NN treatment level because this is only slightly below the minimum foliar N concentration of 1.2% recommended for the normal physiological function of loblolly pine (Albaugh et al. 2010), and would likely accompany a carbon allocation shift toward the production of secondary metabolites such as defense compounds (Gebauer et al. 1998). The target concentration of foliar N for the HN treatment level was set at 1.5-2.5% because this represents the optimum foliar N concentration for planted loblolly pine seedlings (Dumroese 2003), and also would likely result in reduced rates of defense chemical production (Gebauer et al. 1998).

Information from three sources was synthesized to determine appropriate NH_4NO_3 application rates to achieve these target concentrations of foliar N. Specifically, Gebauer et al. (1998) quantified defense chemical concentrations of loblolly pine seedlings potted in sand and receiving daily applications of ammonium nitrate (NH_4NO_3) for 17 weeks. Distinct decreases in total phenolics and condensed tannins were observed by the daily application of 500 ml of 3.5 mmol/l (i.e., 280 mg/l) NH_4NO_3. Using sand culture, Dewald et al. (1992) achieved a slash pine foliar N concentration of 2 to 2.5% by the application of between 114 mg/l (40 mg/l N) and 857 mg/l (300 mg/l N) NH_4NO_3 two to three times per week. For loblolly pine seedlings potted in a mixture of sand-perlite-vermiculite (5:4:1), Samuelson (2000) obtained a foliar N concentration of 1.8% by the weekly application of 754 mg/l NH_4NO_3 (264 mg/l N) over a 21-week period. Thus, NN and HN levels were defined as the application of 100 ml of 280 mg/l (98 mg/l N) and 784 mg/l of NH_4NO_3 (274 mg/l N), respectively, to each potted seedling two times per week.

The NN and HN nutrient solutions provided equal amount of all other plant-essential mineral nutrients besides N (Table 1). Potted seedlings were watered to the point of saturation at least once per week as needed. Foliar nutrition was evaluated 14 weeks after the start of the study by pooling one fully elongated fascicle that grew during the study period per seedling among four families. Foliage was oven-dried (70°C) to equilibrium, ground to pass a 0.85 mm^2 screen, and evaluated for macro- and micronutrient concentrations (Waters Agricultural Laboratories, Inc., Camilla, GA). Results from this analysis indicated that a difference in foliar N concentration between the NN and HN levels had not been achieved. Therefore, the HN level was amended by increasing its NH_4NO_3 concentration from 280mg/l to 350 mg/l. At this time, foliar concentrations of phosphorus, potassium, calcium, and magnesium (i.e., (n=8, mean ± standard deviation) P: 0.21±0.02; K: 1.7±0.18; Ca: 0.90±0.84; Mg: 0.20±0.01%), were 75, 325, 500, 150% higher, respectively, than those recommended for loblolly pine (Albaugh et al. 2010). As a result, calcium and magnesium sources were removed from the nutrient solutions and

phosphorus and potassium additions were reduced. Amounts of chemicals in the amended nutrient solutions are shown in Table 2.

Before potting, 10 seedlings were randomly sub-sampled per family (i.e. 150 seedlings) to measure family morphological variables before the start of the study. For each seedling, root collar diameter (RCD) and total height (TH) were measured and seedlings were separated into fine roots (i.e. ≤ 2 mm diameter), coarse roots (i.e., > 2 mm diameter), stem, and foliage. Tissues were oven dried at 70°C to equilibrium. Root to shoot ratio (R:S) was calculated from the dry weight measurements. Seedlings were stem-inoculated with *G. huntii*, 12 weeks after potting. Two weeks before inoculations, the fungus was cultured on 2% malt extract agar (MEA) from single spore isolates of the fungus maintained in the laboratory. The wound inoculation method was followed which required making a vertical cut with a sterile razor blade in the lower stem of the seedling about 2 cm from the surface of potting mix (Nevill et al. 1995; Eckhardt et al. 2004a). After placing 3 mm diameter plug of medium colonized by mycelium in the cut, the wound was wrapped with sterile moist cotton, and sealed with Parafilm®.

Seedlings were placed on greenhouse benches in a randomized complete block (RCB), split plot design with 10 blocks (Steel and Torrie 1980). In the design, the two levels of nitrogen (NN and HN) were randomly assigned to two whole plots in each block. Subplots were one potted loblolly pine seedling from 15 families randomly placed in each whole plot.

Twenty-seven weeks after potting, one fascicle was pooled by family and nutrition treatment (i.e. 30 samples). Foliage was oven-dried (70°C) to equilibrium, ground to pass a 0.85 mm^2 screen, and evaluated for macro-and micronutrient concentrations (Waters Agricultural Laboratories, Inc., Camilla, GA). Twenty-eight weeks after potting, seedlings were harvested by gently shaking potting mix from root systems, washing root systems with tap water, and storing seedlings in plastic bags in the refrigerator. The presence or absence of a lesion, and lesion length, width, and depth, were measured by seedling. Lesion length was the length of dark brown tissue that occurred along, or extended from the vertical cut that was made during inoculation. After all lesion measurements were completed, shoots were separated from root systems, foliage was separated from the stem, and fine roots were separated from coarse roots. The foliage, fine roots, and coarse roots were placed in separate bags and oven-dried at 70°C to equilibrium.

From each stem, a 2 cm section approximately 0.5 cm above the point of inoculation was cut, freeze-dried, weighed, and ground to pass a 0.50 mm^2 screen. Ground stem tissues were analyzed for total phenolic concentration at the U.S. Forest Service Southern Research Station laboratory in Pineville, LA by a modification of the Folin-Ciocalteu method originally described by Singleton and Rossi (1965) and modified by Booker and Maier (2001) for loblolly pine. To confirm infection, re-isolation of associated fungi was conducted by cutting a 1 cm length of stem that was 0.5 cm above and below the lesion, and culturing it on CSMA (malt extract agar with 800 mg/l of cycloheximide and 200 mg/l of streptomycin sulfate). Cultures were incubated at room temperature for two weeks, and re-isolation success was determined visually. Oven-dry weights (70°C to equilibrium) of stem sections used for re-isolations were measured. Dry weights of the foliage, stem, fine roots, coarse roots and total seedling were quantified, fractions of total seedling dry weight as foliage, stem, fine roots, and coarse roots, and R:S values were calculated.

Ten seedling response variables (lesion length and width, final TH, final RCD, total seedling dry weight, fraction of total seedling dry weight as foliage, stem, fine roots, and coarse roots, and stem total phenolic concentration) were analyzed in SAS statistical software (SAS Institute, 9.2 ed., Cary, NC) using the generalized linear model (GLM) procedure. In the RCB split plot design, nutrition treatments levels (NN and HN) were assigned to the whole plots and fifteen loblolly pine families were assigned to the subplots within each whole plot, and there were 10 blocks. Means by nutrition treatments and among families were evaluated by the Tukey pairwise comparison test. Multiple regression analyses were conducted with SAS statistical software (SAS Institute, 9.2 ed., Cary, NC) using the generalized linear model (GLM) procedure. Relationship between lesion length and width and three independent variables (foliar nitrogen concentration, total stem phenolics, R:S) were assessed. Regressions were also conducted to determine the relationship between the variables representing virulence/defense (i.e. lesion length, lesion width, stem total phenolic concentration) and independent variables representing seedling growth (i.e. total seedling dry weight) and carbon allocation among plant components (i.e. R:S, and fraction of dry weight as foliage, stem, fine roots and coarse roots).

RESULTS

Pre-pot analyses indicated that the mean RCD of all families except L-4 met the minimum RCD (5 mm), and had TH (15-25 cm) values greater than the minimum recommended for planted loblolly pine (Johnson and Cline 1991). Mean R:S values of 13 families were less than 0.4 suggesting that root system size was sub-optimal at the time of potting for all but two families (Johnson and Cline 1991). Means and standard deviations of TH, RCD, foliage dry weight, stem dry weight, fine root dry weight, and coarse root dry weight, and R:S for all the families are presented (Table 3). One week before the end of the study, the NN and HN seedlings had achieved target N levels (NN~1.2%; HN ~1.6%) and foliar concentrations of other plant-essential macro- and micronutrients were sufficient for loblolly pine according to Albaugh et al. (2010) (Table 4).

Inoculations with *G. huntii* did not cause significant seedling mortality in the inoculated seedlings. Fourteen weeks after the seedlings were inoculated, brown colored lesions were observed on the seedlings stems. Although lesions were present on the inoculated seedlings, there were no differences in lesion length ($F = 0.10$, $P = 0.7569$) or lesion width ($F = 0.15$, $P = 0.7040$) between NN and HN treatments levels (Table 5). In addition, while family had no effect on lesion length ($F = 0.61$, $P = 0.8591$), differences in lesion width were observed among the 15 families tested ($F = 3.11$, $P = 0.0002$). The T x F interaction was significant for lesion length ($F = 1.90$, $P = 0.0275$). Further analysis compared treatment means among the families within each nitrogen treatment and between nitrogen treatments within each family. In doing so, families L-1 and L-6 differed in lesion length between the NN and HN treatments levels. The T x F interactions for lesion width and stem total phenolic concentrations were non-significant and hence the means were compared only among the families. Family L-1 had significantly different mean lesion width from families L-2 and L-13. Similarly, mean total phenol concentration was found to be significant between several family pairs (Table 6). Minor differences in mean lesion length and lesion width for each family under NN and HN treatments levels were seen with no significant differences such as family L-2 had similar mean lesion length and lesion width under both the treatment levels (Table 7 and 8). Means and standard deviations of stem total phenolic concentration among the families are presented in Figure 1. The fungus was consistently re-

isolated from the seedlings with 95% or greater re-isolation from all the families except L-1 and L-2 (85% and 65% respectively).

Total height did not differ between NN and HN treatment levels ($F = 0.60$, $P = 0.4568$). However, TH was significantly different among the families ($F = 3.26$, $P = 0.0001$). Values of RCD differed significantly between NN and HN treatment levels ($F= 10.71$, $P = <0.0096$) and it was also found to be significantly different among the families (and $F = 4.36$, $P = <0.0001$). Nitrogen nutrition treatments affected total seedling dry weight significantly ($F = 8.39$, $P = 0.0177$), and total seedling dry weight differed significantly among the families as well ($F = 3.16$, $P = 0.0001$).

Fractions of total dry weight as foliage and fine root dry weights were significantly affected by nitrogen nutrition treatment, while the stem and coarse roots dry weight did not differ between NN and HN treatment levels (Table 9). By the Tukey pairwise comparison test, however, only the mean differences between NN and HN treatment levels for fine root dry weight were significant. Seedling dry weight allocation to the foliage, stem, fine roots, and coarse roots differed significantly among the families (Table 9). Further analyses of TH, RCD, total seedling dry weight, and fraction of total seedling dry weight as foliage, stem, fine roots, and coarse roots was done by comparing the means among the families. Significant differences among family means for each of these variables were seen such as family L-13 had significantly different mean dry weight from families L-1, L-5, L-6 and L-12 (Table 10 and 11). Means values of seedling morphological variables at harvest are presented (Table 12).

Among the families, regressions between lesion dimensions and independent variables among the families including foliar nitrogen concentration, R:S and total stem phenolic concentration did not indicate significant correlation (lesion length: $P = 0.9263$, $R^2 = 0.0174$; lesion width: $P = 0.0698$, $R^2 = 0.2342$). Because nitrogen nutrition treatment did not significantly affect lesion dimensions or stem total phenolic concentrations, and foliar nitrogen concentration was not a significant predictor of lesion dimensions, data associated with the NN and HN treatment levels were combined. With the combined data, regressions were conducted for each family between the dependent variables of lesion length, lesion width, and stem total phenolic concentration, and the independent variables of seedling morphological traits to determine the role of seedling growth and carbon allocation pattern in defense against *G. huntii*. Regressions between lesion dimensions and the fraction of total seedling dry weight as stem, foliage, fine roots, and coarse roots indicated that families L-1, L-23, L-6, L-7 and L-9 had a significant, negative relationship between lesion width and the fraction of total seedling dry weight allocated to the stem. No consistent, significant relationship was found between lesion length and any family morphological trait. However, family L-12 had a significant, negative relationship between lesion length and the fraction of total seedling dry weight allocated to the stem ($P = 0.0211$, $R^2 = 0.2752$), family L-13 had a significant, positive relationship between lesion length and the fraction of total seedling dry weight allocated to the coarse roots ($P = 0.3015$, $R^2 = 0.3015$), and family L-18 had a significant negative relationship between lesion length and the fraction of total seedling dry weight allocated to the stem ($P = 0.0274$, $R^2 = 0.2424$). For families L-1, L-12, L-13, L-18, L-22, L-5, lesion width was significantly and positively correlated to total seedling dry weight (Table 13). Families L-10 and L-9 showed significant positive correlation between lesion width and R:S (L-10: $P = 0.0327$, $R^2 = 0.2863$; L-9: $P = 0.0032$, $R^2 = 0.3910$). Lesion width was

also significantly and positively correlated to the fraction of total seedling dry weight allocated to coarse roots for family L-13 ($P = 0.0089$, $R^2 = 0.3085$) but was significantly and negatively correlated to the fraction of total seedling dry weight allocated to coarse roots for family L-22 ($P = 0.0034$, $R^2 = 0.4248$).

Stem total phenolic concentration was significantly and positively related to the fraction of total seedling dry weight allocated to foliage for families L-1, L-10, and L-9 and was significantly and positively correlated to fraction of coarse roots for family L-11 ($P = 0.0184$, $R^2 = 0.2718$). Stem total phenolic concentration was significantly and negatively correlated to fraction of dry weight as stem for family L-18 ($P = 0.0421$, $R^2 = 0.2337$). Stem total phenolic concentration was significantly and positively correlated to total seedling dry weight for families L-10, L-12, L-13, L-16, L-18 (Table 13) and to fraction of coarse root dry weight for family L-11 ($P = 0.0271$, $R^2 = 0.2432$).

DISCUSSION

The overall results from this study showed that the nitrogen nutrition did not affect the susceptibility to *G. huntii* as indicated by the non-significant differences in lesion parameters between the two treatment levels (NN and HN). This is inconsistent with previous studies done with other pathogens such as nitrogen rich environment increased susceptibility of scots pine to tarnished plant bug *Lygus rugulipennis* Popp. (Holopainen et al. 1994). Also, longer lesions and excessive sporulation in apple trees was developed showing increased susceptibility to *Venturia inaequalis* (Cooke) G. Wint. under high nitrogen cultures as compared to low nitrogen cultures (Rühmann et al. 2001).

Grosmannia huntii, relatively less known compared to other *Leptographium* species involved in southern pine decline, was selected for the inoculations in this experiment based on virulence trials where it caused significantly more damage to pine seedlings compared to that caused by the other *Leptographium* species tested (Matusick and Eckhardt 2010). *Grosmannia huntii* successfully infected all the loblolly pine seedlings in the study with dark brown and sunken lesions seen on the seedling stems 14 weeks following inoculations. Previous studies have shown that in response to inoculations, dark brown lesions are seen on the seedling stems which normally extend vertically on either side of the length of the wound (Eckhardt et al. 2004a; Matusick and Eckhardt 2010). However, the lesions associated with *G. huntii* in this study commonly showed minimal extension above and below the point of inoculation with poor radial movement. Radial movement is associated with highly virulent ophiostomatoid fungi such as *L. wageneri* (Cobb 1988). Not only was lesion extension minimal, but lesion length was not significantly different among 15 families. Consistent re-isolation from the stem tissue confirms infection and rejects the contention that inoculation was unsuccessful. Furthermore, a simultaneous study with *Leptographium* and *Grosmannia* fungi including *G. huntii* artificial stem inoculation of loblolly pine families resulted in high degree of virulence (RR2014-06, RR2014-07).

While increased virulence of *G. huntii* was anticipated with added nitrogen (Huber and Thompson 2007), absence of a distinct lesion dimension response to nitrogen nutrition treatment also suggests that the spread of *G. huntii* was limited by unknown factors. The seedlings in the present study were housed in a greenhouse, and those exhibiting a high degree of *G. huntii*

virulence were housed outdoors. It is hypothesized, therefore, that greenhouse environmental conditions played a negative role in *G. huntii* virulence and lesion development. An understanding of the environmental factors that control *G. huntii* infection is warranted before additional greenhouse experiments containing a stem inoculation component are attempted.

A negative relationship between lesion width and the fraction of dry weight allocated to seedling stems for five families (L-1, L-23, L-6, L-7, L-9), and a positive relationship between lesion width and total seedling dry weight for seven families (L-1, L-12, L-13, L-13, L-18, L-22, L-5) illustrates the difficulty of using lesion width to assess infection. The horizontal length of the wound needed for inoculation was a function of initial seedling size, and stem diameter growth essentially diluted the effect of the inoculum on vascular tissues that grow radially. Therefore, it was not possible to assess virulence by lesion width without first removing the influence of seedling size and stem growth rate. Nitrogen nutrition affected several seedling morphological traits. Root collar diameter which is the most valuable indicator of seedling quality and field performance (Johnson and Cline 1991), was greater with an increase in nitrogen availability from a sufficient to a high level. Total height was not significantly different between the NN and HN treatment levels. However, this is consistent with the finding of Dewald et al. (1992) who reported that among slash pine seedlings receiving optimal but not low, and high amounts of nitrogen, final height was not affected by high nitrogen concentration. Alternatively, comparison between low and high nitrogen levels, demonstrated distinct benefits of excess nitrogen to loblolly and slash pine seedling TH and RCD (Samuelson 2000).

Total dry weight was also significantly different between the NN and HN treatment levels with elevated nitrogen leading to larger seedlings. Carbon allocation to foliage was greater, while that to fine roots was lower, under HN treatment level; whereas, carbon allocation to fine roots was greater, and that to foliage was lower, under the NN treatment level. These results support the assertion that leaf area and fine root biomass are dynamic in response to environmental conditions (Gower et al. 1995; Stovall et al. 2012). While others have found significant responses of loblolly pine seedling carbon allocation to coarse root and stem biomass in response to added nitrogen (Samuelson 2000; Stovall et al. 2012), the fraction of dry weight as coarse roots and stem did not differ significantly in this study. Absence of a nitrogen effect on woody tissues may be attributed to the inability to resolve these effects because the potted seedlings were variable in size at the start of the study (Table 3), the NN treatment level represented N-sufficiency rather than N-deficiency, and the nitrogen nutrition treatment was applied for only 27 weeks.

Simple and complex plant phenolic compounds serve as preformed (i.e., constitutive) and inducible means of resistance to insect attack and disease (Lattanzio et al. 2006). Past research has shown that elevated nitrogen levels depress the production of simple (e.g., caffeic acid) and complex (e.g., tannin) plant phenolic compounds (Ibrahim et al. 2011; Kováčik and Bačkor 2007; Muzika 1993). For example, Gebauer et al. (1998) found that total phenolic compound and condensed tannin concentrations in the foliage and lateral roots of loblolly pine seedlings were reduced as nitrogen availability increased. Booker and Maier (2001) reported a similar trend for loblolly pine receiving two levels each of nitrogen and carbon dioxide treatment. Elevated carbon dioxide not only boosted phenolic synthesis but it also increased whole-plant carbon fixation which indirectly decreased tissue nitrogen concentration. It was suggested that in

accordance with the growth differentiation balance hypothesis (Herms and Mattson 1992), the negative relationship between plant phenolic concentration and nitrogen addition was secondary in nature, arising in response to the ratio between carbohydrate production and utilization (i.e., source:sink ratio). Because carbohydrate utilization in the form of cell division and growth is nitrogen demanding, nitrogen amendments are diluted (i.e., the dilution effect) in plant tissues by accelerated growth (Jarrell and Beverly 1981). Booker and Maier (2001) suggested that phenolic compound synthesis serves as a mechanism to conserve fixed carbon that is not utilized for growth when nitrogen is limiting.

In the present study, sufficient nitrogen (i.e., NN) and excess nitrogen (i.e., HN) did not affect the stem total phenolic concentration. Based on the information presented by Booker and Maier (2001), lack of a nitrogen nutrition effect on total phenolic concentration may be explained by absence of different source:sink ratios between the NN and HN seedlings. While the HN seedlings exhibited more foliage and less fine root growth than the NN seedlings, stem and coarse root growth were unaffected by nitrogen nutrition treatment, and total seedling dry weight was only increased 8 percent by the HN treatment level. Perhaps the NN and HN treatment levels did not create a wide enough difference in carbon gain and seedling growth to diverge the source:sink ratios of the two treatment levels and as a result, change their stem total phenolic concentrations.

Naturally occurring phenolic compounds represent a source of preformed or constitutive defense (Lattanzio et al. 2006), and family differences in stem total phenolic concentration may provide guidance regarding a genotype's potential for resistance before insect attack or pathogenic infection. Family L-1 produced the highest, and families L-8 and L-9 produced the lowest concentrations of stem phenolic compounds in this study. Seedlings from family L-1 were 15 percent smaller in total dry weight than those from family L-9. Thus, families L-1 and L-9 may have had different amounts of carbohydrate that could be shunted to defense compound synthesis accounting for differences in stem total phenolic concentration. However, families L-1 and L-8 were similar in total seedling dry weight, differing by only 3 percent. Similar seedling size indicates that these families may have been similar in their amounts of carbohydrate that could be shunted to defense compound synthesis, dissimilar stem total phenolic concentration between families L-1 and L-8 point toward the possibility that ideal genetic sources of loblolly pine could be selected for planting where the risk of pathogenic attack is high. However, a great deal of additional research is needed before this concept can be effectively transferred to the field.

Regression analyses between stem total phenolic concentrations and total seedling dry weights were significant for 33 percent of the families tested (L-10, L-12, L-13, L-16, L-18). If stem total phenolic concentration is representative of constitutive defense potential, then contrary to the growth differentiation balance hypothesis (Herms and Mattson 1992), growth and total phenolic concentration may be positively correlated as observed by Aspinwall et al. (2011). Thus it is possible that families with strong positive relationships between growth and total phenolic concentration are more effective at constitutive defense than families with a poor relationship between these variables.

Among fractions of dry weight allocated to the foliage, stem, fine roots, and coarse roots, the fraction of dry weight allocated to foliage was the strongest independent predictor of stem total

phenolic concentration. Specifically, three families showed significant regressions between stem total phenolic concentration and fraction of dry weight allocated to foliage. This information supports the notion that for some loblolly pine families, there is a positive relationship between growth and constitutive defense potential (Aspinwall et al. 2011) because foliage production is closely tied to the growth of loblolly pine (Vose and Allen 1988; Luxmoore et al. 1995).

The hierarchy of plant carbon allocation prioritizes foliage and stem growth over root system growth and the production of nonstructural carbon containing compounds (e.g., phenolics, sugars, etc.) (Dickson 1991). This concept led to the hypothesis in the present study that a carbon allocation pattern favoring root system growth rather than foliage and stem growth would benefit defense chemical synthesis. Therefore, genetic control of R:S would parallel genetic control of natural levels of phenolic compound synthesis and constitutive defense potential. Correlation between stem total phenolic concentration and R:S was only significant for one family. Although R:S was not an acceptable predictor of constitutive defense potential, the results of this study indicate that there may be other seedling morphological variables that reflect defense capacity. Specifically, once loblolly pine seedlings meet established values of TH, RCD, and R:S that indicate superior quality, seedling size and/or foliage mass may gauge constitutive defense potential.

REFERENCES

Addington, R.N., Donovan, L.A., Mitchell, R.A., Vose, J.M., Pecot, S.D., Jack, S.B., Hacke, U.G., Sperry, J.S., and Oren, R. 2006. Adjustments in hydraulic architecture of *Pinus palustris* maintain similar stomatal conductance in xeric and mesic habitats. Plant Cell Environ. 29: 535-545.

Albaugh, J.M., Blevins, L. Allen, H.L., Albaugh, T.J. Fox, T.R., Staple, J.L., and Rubilar, R.A. 2010. Characterization of foliar macro- and micronutrient concentrations and ratios in loblolly pine plantations in the southeastern United States. South. J. Appl. For. 34(2): 53-64.

Aspinwall, M.J., King, J.S., Booker, F.L., and McKeand, S.E. 2011. Genetic effects on total phenolics, condensed tannins and nonstructural carbohydrates in loblolly pine (*Pinus taeda* L.) needles. Tree Physiol. 31: 831-842.

Baker, J.B., and Langdon, O.G. 1990. *Pinus taeda* L., Loblolly Pine. *In* Silvics of North America, Vol. 1: Conifers. *Edited by* R.M. Burns, and B.H. Honkala. U.S. Dep. Agric. Agric. Handb. 654, Washington, D.C. pp. 497-512.

Bongarten, B.C., and Teskey, R.O. 1987. Dry weight partitioning and its relationship to productivity in loblolly pine seedlings from seven sources. Forest Sci. 33(2): 255-267.

Booker, F.L., and Maier, C.A. 2001. Atmospheric carbon dioxide, irrigation, and fertilization effects on phenolic and nitrogen concentrations in loblolly pine (*Pinus taeda*) needles. Tree Physiol. 21: 609-616.

Brown, H.D., and McDowell, W.E. 1968. Status of loblolly pine die-off on the Oakmulgee District, Talladega National Forest, Alabama - 1968. U.S. Dep. Agric. For. Serv. Rept. No. 69-2-28.

Cobb, F.W. 1988. *Leptographium wageneri*, cause of black-stain root disease: a review of its discovery, occurrence and biology with emphasis on pinyon and ponderosa pine. *In* *Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb. American Phytopathological Society, St. Paul, MN.

Dalla-tea, F., and Jokela, E.J. 1994. Needlefall returns and resorption rates of nutrients in young intensively managed slash and loblolly pine stands. Forest Sci. 40(4): 650-662.

Dewald, L., White, T.L., and Duryea M.L. 1992. Growth and phenology of seedlings of four contrasting slash pine families in ten nitrogen regimes. Tree Physiol. 11: 255-269.

Dickson, R.E. 1991. Assimilate distribution and storage. *In* Physiology of Trees. *Edited by* A.S. Raghavendra. John Wiley & Sons, New York. pp. 51-85.

Dumroese, R.K. 2003. Hardening fertilization and nutrient loading of conifer seedlings. *In* National Proceedings: Forest and Conservation Nursery Associations—2002. *Edited by* L.E. Riley, R.K. Dumroese, and T.D. Landis. RMRS-P 28. Forest Service Rocky Mountain Research Station, Ogden, UT. pp. 31-36.

Eckhardt, L.G., and Menard, R.D. 2009**.** Declining loblolly pine stands: symptoms, causes and management options. AL Treasured Forest Magazine Volume XXV111, No. 2, pp. 10-12.

Eckhardt, L G., Jones, J.P., and Klepzig, K.D. 2004a. Pathogenicity of *Leptographium* species associated with loblolly pine decline. Plant Dis. 88: 1174-1178.

Eckhardt, L.G., Sword-Sayer, M.A., and Imm, D. 2010. State of pine decline in the southeastern United States. South J. Appl. For. 34: 138-141.

Eckhardt, L.G., Goyer, R.A., Klepzig, K.D., and Jones, J.P. 2004b. Interaction of *Hylastes* species (Coleoptera: Scolytidae) with *Leptographium* species associated with loblolly pine decline. J. Econ. Entomol. 97(2): 468-474.

Eckhardt, L.G., Weber, A.M., Menard, R.D., Jones, J.P., and Hess, N.J. 2007. Insect-fungal complex associated with loblolly pine decline in central Alabama. Forest Sci. 53(1): 84-92.

Gebauer, R.L.E., Strain, B.R., and Reynolds, J.F. 1998. The effect of elevated CO2 and N availability on tissue concentrations and whole plant pools of carbon-based secondary compounds in loblolly pine (*Pinus taeda*). Oecologia 113: 29-36.

Gower, S.T., Isebrands, J.G., and Sheriff, D.W. 1995. Carbon allocation and accumulation in conifers. *In* Resource Physiology of Conifers, Acquisition, Allocation, and Utilization. *Editied by* W.K. Smith and T.M. Hinckley. Academic Press, New York. pp. 217-254.

Hacke, U.G., Sperry, J.S., Ewers, B.E., Ellsworth, D.S., Schafer, K.V.R., and Oren, R. 2000. Influence of soil porosity on water use in *Pinus taeda*. Oecologia. 124: 495-505.

Herms, D.A., and Mattson, W.J. 1992. The dilemma of plants: to grow of defend. Q. Rev. Biol. 67: 283-335.

Hess, N.J., Otrosina, W.J., Carter, E.A., Steinman, J.R., Jones, J.P., Eckhardt, L.G., Weber,A.M., and Walkinshaw, C.H. 2002. Assessment of loblolly pine decline in central Alabama. Proceedings of the eleventh biennial southern silvicultural research conference. Gen. Tech. Rep. SRS–48.: U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, NC. pp. 622.

Holopainen, K.J., Rikala, R., Kainulainen, P., and Oksanen, J. 1995. Resource partitioning to growth, storage and defense in nitrogen – fertilized Scots pine and susceptibility of the seedlings to the tarnished plant bug *Lygus rugulipennis.* New Phytol. 131: 521- 532.

Huber, D.M., and Thompson, I.A. 2007. Nitrogen and plant disease. *In* Mineral Nutrition and Plant Disease. *Edited by* L.E. Datnoff, W.H. Elmer, and D.M. Huber. The American Phytopathological Society Press, St. Paul, MN. pp. 31-44.

Ibrahim, M.H., Jaafar, H.Z.E., Rahmat, A., and Rahman, Z.A. 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of kacip Fatimah (*Labisia pumila* Blume). Int. J. Mol. Sci. 12: 5238-5254.

Jarrell, W.M., and Beverly, R.B. 1981. The dilution effect in plant nutrition studies. Adv. Agron. 34: 197-224.

Johnson, J.J., and Cline, M.L. 1991. Seedling quality of southern pines. *In* Forest Regeneration Manual, *Edited by* M.L. Duryea, and P.M. Dougherty. Klewer Academic Publishers, The Netherlands. pp. 143-159.

King, N.T., Seiler, J.R., Fox, T.R., and Johnsen K.H. 2008. Post-fertilization physiology and growth performance of loblolly pine clones. Tree Physiol. 28: 703-711.

Kováčik, J., and Bačkor, M. 2007. Changes of phenolic metabolism and oxidative status in nitrogen-deficient *Matricaria chamomilla* plants. Plant Soil 297: 255-265.

Lattanzio, V., Lattanzio, V.M.T., and Cardinali, A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *In* Phytochemistry: Advances in Research. Research Signpost, Kerala, India. pp. 23-67.

Li, B., McKeand, S.E., and Allen, H.L. 1991. Genetic variation in nitrogen use efficiency of loblolly pine seedlings. Forest Sci. 37(2): 613-626.

Luxmoore, R.J., Oren, R, Sheriff, D.W., and Thomas, R.B. 1995. Source-sink-storage relationships of conifers. *In* Resource Physiology of Conifers, Acquisition, Allocation, and Utilization. *Edited by* W.K. Smith and T.M. Hinckley. Academic Press, New York. pp. 179-216.

Marschner, P. 2012. Marschner's Mineral Nutrition of Higher Plants, third edition. Academic Press, New York. pp. 651.

Matusick, G., and Eckhardt, L.G. 2010a. Variation in virulence among four root-inhabiting ophiostomatoid fungi on *Pinus taeda* L., *P. palustris* Mill., and *P. elliottii* Englem. seedlings. Can. J. Plant Path. 32: 361-367.

Muzika, R.M. 1993. Terpenes and phenolics in response to nitrogen fertilization: a test of the carbon/nutrient balance hypothesis. Chemoecology 4(1): 3-7.

Nambiar, E.K., and Fife, D.N. 1991. Nutrient retranslocation in temperate conifers. Tree Physiol. 9: 185-207.

Nevill, R.J., Kelley, W.D., Hess, N.J., and Perry, T.J. 1995. Pathogenicity to loblolly pines of fungi recovered from trees attacked by southern pine beetles. South. J. Appl. For. 19(2): 78-83.

Otrosina, W.J., Bannwart, D., and Roncadori, R.W. 1999. Root-infecting fungi associated with a decline of longleaf pine in the southeastern United States. Plant Soil 217: 145-150.

Paine, T. D., Raffa, K. F., and Harrington, T. C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu. Rev. Entomol. 42: 179-206.

Pallardy, S.G., Cermak, J., Ewers, F.W., Kaufmann, M.R., Parker, W.C., and Sperry, J.S. 1995. Water transport dynamics in trees and stands. *In* Resource physiology of conifers: Acquisition, allocation, and utilization. *Edited by* W.K. Smith, and T.M. Hinckley. Academic Press, Inc., New York. pp. 301–389.

Rühmann, S. Leser, C., Bannert, M., and Treutter, D. 2002. Relationship between growth, secondary metabolism and resistance of apple. Plant Biol. 4: 137-143.

Samuelson, E.J. 2000. Effects of nitrogen on leaf physiology and growth of different families of loblolly and slash pine. New Forest. 19**:** 95-107.

Singleton, V.L., and Rossi, Jr., J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16: 144-158.

Smith, W.B., Patrik, D.M., Charles, H.P., and Scott, A.P. 2007. Forest Resources of the United States. A technical Document Supporting the Forest Service 2010 RPA Assessment.

Steele, R.G.D., and Torrie, J.H. 1980. Principles and Procedures of Statistics, A Biometrical Approach, second edition. McGraw-Hill Book Company, New York. pp. 633.

Stovall, J.P., Fox, T.R., and Seiler, J.R. 2012. Short-term changes in biomass partitioning of two full-sib clones of *Pinus taeda* L. under differing fertilizer regimes over 4 months. Trees-Struct. Funct. 26(3) 951:961.

Vose, J.M., and Allen, H.L. 1988. Leaf area, stemwood, and nutrition relationships in loblolly pine. Forest Sci. 34: 547-563.

Warren, J.M., Meinzer, F.C., Brooks, J.R., Domec, J.C., and Coulombe, R. 2007. Hydraulic redistribution of soil water in two old-growth coniferous forests: Quantifying patterns and controls. New Phytol. 173: 753-765.

Table 1. Concentration of chemicals in NN and HN nutrient solutions and amount of plant-essential elements applied per seedling during the first 14 weeks of the study.

Chemical Name	Chemical Formula	Concentration (mg/l)		Total amount of compound added in 35 applications (mg)		Element
		NN	HN	NN	HN	
Ammonium nitrate	NH_4NO_3	280	784	343.0	960.4	N
Potassium phosphate	KH_2PO_4	132	132	105.3	105.3	P
				132.6	132.6	K
Potassium chloride	KCl	71	71	130.2	130.2	K
Calcium sulphate	$CaSO_4.2H2O$	155	155	126.4	126.4	Ca
				100.9	100.9	S
Magnesium sulphate	$MgSO_4.7H2O$	182	182	82.8	82.8	Mg
				63.1	63.1	S
Iron chelate	Ferric EDTA[1]	26	26	13.8	13.8	Fe
				6.92	6.92	N
Boric acid	H_3BO_3	3	3	1.84	1.84	B
Zinc sulphate	$ZnSO_4.7H_2O$	1.4	1.4	1.11	1.11	Zn
				0.54	0.54	S
Copper chloride	$CuCl_2.2H_2O$	0.40	0.40	0.52	0.52	Cu
Sodium molybdate	$MoNa_2O_4.2H_2O$	0.05	0.05	0.07	0.07	Mo
Manganese sulphate	$MnSO_4.H_2O$	1.5	1.5	1.71	1.71	Mn
				1.00	1.00	S

[1]ethylenediaminetetraacetic acid

Table 2. Amended amounts of nutrient solutions added to normal (NN) and high (HN) nitrogen treatments.

Chemical Name	Chemical Formula	Concentration (Mg/L)		Total amount of compound added in 19 applications (g)		Element
		NN	HN	NN	HN	
Ammonium nitrate	NH_4NO_3	280.0	994.0	186.2	661.01	N
Potassium phosphate	KH_2PO_4	79.0	79.0	34.2	34.2	P
				43.1	43.1	K
Iron chelate	Ferric EDTA[1]	26.0	26.0	7.51	7.51	Fe
				3.75	3.75	N
Boric acid	H_3BO_3	3.0	3.0	1.00	1.00	B
Zinc sulphate	$ZnSO_4.7H_2O$	1.4	1.4	0.60	0.60	Zn
				0.30	0.30	S
Copper chloride	$CuCl_2.2H_2O$	0.4	0.4	0.28	0.28	Cu
Sodium molybdate	$MoNa_2O_4.2H_2O$	0.05	0.05	0.04	0.04	Mo
Manganese sulphate	$MnSO_4.H_2O$	1.5	1.5	0.93	0.93	Mn
				0.54	0.54	S

[1]ethylenediaminetetraacetic acid

Table 3. Means followed by standard deviations (SD) of seedling total height, root collar diameter (RCD), foliage dry weight, stem dry weight, fine root dry weight, coarse root dry weight, and root to shoot ratio (R:S) from pre-pot measurements.

Family	RCD		Height		Foliage Dry Weight		Stem Dry Weight		Fine Root Dry Weight		Coarse Root Dry Weight		R:S	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
L-1	6.19	0.96	37.6	2.99	4.33	1.27	2.79	0.86	0.54	0.26	0.80	0.30	0.19	0.05
L-2	6.59	0.83	33.4	4.33	4.31	1.81	2.59	1.55	0.55	0.30	1.08	0.52	0.24	0.08
L-5	7.69	1.34	37.6	4.01	6.90	2.29	3.81	1.42	0.70	0.32	2.00	1.20	0.23	0.08
L-6	4.22	0.64	31.1	1.27	2.21	1.15	1.28	0.51	0.31	0.15	0.31	0.15	0.18	0.04
L-7	5.30	0.77	32.5	2.27	2.83	0.94	1.43	0.42	0.63	0.31	0.63	0.55	0.28	0.11
L-8	5.91	1.51	36.4	4.86	4.11	1.62	2.01	1.12	0.50	0.34	0.75	0.64	0.18	0.08
L-9	7.61	1.00	34.8	2.49	7.23	1.52	3.16	0.71	1.25	0.49	2.01	0.53	0.32	0.10
L-10	5.62	1.79	32.7	4.06	4.06	2.31	1.80	1.05	0.95	0.87	1.48	1.74	0.44	0.49
L-11	7.97	1.33	31.1	3.54	5.76	1.75	2.66	0.95	1.47	0.59	2.46	1.07	0.50	0.22
L-12	7.43	0.49	35.5	4.72	5.06	0.93	2.90	0.51	0.80	0.37	1.23	0.45	0.25	0.06
L-13	7.63	1.16	34.2	3.43	5.87	1.18	3.20	0.88	0.99	0.50	1.42	0.69	0.26	0.11
L-16	9.02	1.32	34.6	3.81	7.85	2.31	4.91	2.02	1.07	0.75	3.26	1.27	0.35	0.12
L-18	6.80	1.49	37.6	5.04	5.16	2.66	3.27	1.52	0.76	0.39	0.99	0.64	0.21	0.05
L-22	6.36	1.75	32.5	4.77	4.54	2.41	2.30	1.50	1.28	2.09	1.18	0.95	0.34	0.21
L-23	6.54	0.93	34.6	5.46	4.91	1.34	2.58	0.79	1.42	1.45	1.58	1.02	0.39	0.26

Table 4. Concentrations of plant-essential macro- and micronutrients in loblolly pine foliage one week before the end of the study in response to normal (NN) and high (HN) levels of nitrogen nutrition.

Family	Nitrogen Nutrition Treatment Level	N[1]	P	K	Ca	Mg	S	B	Zn	Mn	Fe[2]	Cu
		(%)						(ppm)				
L-1	HN	1.47	0.17	1.62	0.44	0.17	0.11	42	52	62	19	2
L-2	HN	1.53	0.19	1.71	0.46	0.19	0.13	39	51	60	20	3
L-5	HN	1.76	0.26	2.25	0.47	0.17	0.14	43	50	51	20	3
L-6	HN	1.67	0.20	1.61	0.39	0.16	0.13	38	52	66	26	3
L-7	HN	1.61	0.22	1.61	0.38	0.16	0.12	38	53	56	24	3
L-8	HN	1.58	0.19	1.75	0.48	0.18	0.13	39	46	69	23	3
L-9	HN	1.77	0.22	1.76	0.48	0.19	0.15	43	60	80	49	5
L-10	HN	1.54	0.20	1.67	0.44	0.16	0.12	44	47	69	22	2
L-11	HN	1.95	0.23	1.82	0.47	0.18	0.15	45	52	75	24	4
L-12	HN	1.71	0.23	1.76	0.31	0.14	0.13	45	50	62	22	4
L-13	HN	1.69	0.18	1.46	0.38	0.16	0.13	34	45	55	24	2
L-16	HN	1.61	0.19	1.63	0.34	0.15	0.12	36	43	51	26	2
L-18	HN	1.79	0.24	1.98	0.54	0.19	0.14	52	54	90	23	3
L-22	HN	1.86	0.25	2.05	0.45	0.18	0.14	51	57	55	22	3
L-23	HN	1.55	0.20	1.61	0.44	0.17	0.11	37	42	71	23	2
L-1	LN	1.28	0.20	2.16	0.59	0.26	0.11	56	53	52	22	3
L-2	LN	1.03	0.15	1.87	0.68	0.26	0.11	49	51	67	28	1
L-5	LN	1.00	0.14	1.66	0.67	0.26	0.11	54	46	68	32	2
L-6	LN	1.05	0.19	2.04	0.63	0.26	0.11	45	51	64	31	2
L-7	LN	1.23	0.19	1.99	0.6	0.24	0.12	43	51	67	28	2
L-8	LN	1.16	0.19	1.96	0.49	0.22	0.11	33	41	58	24	1
L-9	LN	1.05	0.21	2.15	0.53	0.23	0.13	34	55	55	29	3
L-10	LN	1.23	0.22	1.99	0.40	0.19	0.12	39	48	78	27	2
L-11	LN	1.29	0.23	2.23	0.55	0.25	0.14	38	56	72	27	3
L-12	LN	1.55	0.24	2.44	0.43	0.23	0.15	46	50	60	26	4
L-13	LN	1.12	0.19	1.73	0.41	0.21	0.12	29	49	48	27	3
L-16	LN	1.05	0.18	2.03	0.45	0.22	0.12	36	47	55	26	3
L-18	LN	1.29	0.20	2.08	0.60	0.23	0.14	54	54	92	28	3
L-22	LN	1.17	0.19	1.86	0.49	0.22	0.11	38	44	47	28	2
L-23	LN	0.98	0.18	1.73	0.52	0.20	0.10	33	46	64	29	2

[1]Foliar concentrations indicating sufficiency for loblolly pine according to Albaugh et al. (2010) and referenced cited therein: N: 1.2%, P: 0.12%, K: 0.35-0.40%, Ca: 0.15%, Mg: 0.08%, S: 0.10-0.12%, B: 4-8 ppm, Zn: 10-20 ppm, Mn: 20-40 ppm, Cu: 2-3 ppm.

[2]The foliar concentration of iron indicating sufficiency is 72 ppm for plants in general according to Marscher (2012).

Table 5. Probability of greater F-value lesion length, lesion width and stem total phenolic concentration in response to normal (NN) and high (HN) nitrogen nutrition treatment levels for 15 loblolly pine families.

Source of Variation	DF[1]	Lesion Length	Lesion Width	Stem Total Phenolic Concentrations
Block (B)	9	0.0534	0.6736	0.0298
Treatment (T)	1	0.7569	0.7040	0.5065
BxT	9	0.2832	0.0534	0.7785
Family (F)	14	0.8708	0.0002	<0.0001
TxF	14	0.0272	0.1268	0.3641

[1]DF: degrees of freedom

Table 6. Differences between means for stem total phenolic concentration and lesion length among 15 loblolly pine families tested.

Stem Total Phenolic Concentrations		Lesion Width	
Family comparison	Mean differences	Family comparison	Mean differences
L-1- L-7	7.942	L-1 - L-13	2.0500
L-1 - L-8	9.850	L-1 - L-2	20.500
L-1 - L-9	11.492		
L-1 - L-11	5.908		
L-1 - L-18	6.035		
L-2 - L-8	6.386		
L-2 - L-9	8.028		
L-5 - L-8	5.745		
L-5 - L-9	7.387		
L-6 - L-9	5.970		
L-7 - L-10	-6.233		
L-7 - L-16	-6.946		
L-8 - L-10	-8.141		
L-8 - L-13	-7.119		
L-8 - L-16	-8.855		
L-8 - L-23	-5.929		
L-9 - L-10	-9.783		
L-9 - L-12	-7.061		
L-9 - L-13	-8.761		
L-9 - L-16	-10.496		
L-9 - L-22	-6.996		
L-9 - L-23	-7.571		

Note: Only significant differences are presented.

Table 7. Means and standard deviations (SD) of lesion length for each loblolly pine family under normal (NN) and high (HN) nitrogen treatment levels.

Family	Trt	Lesion Length		Family	Trt	Lesion Length	
		Mean	SD			Mean	SD
L-1	HN	13.6	1.58	L-1	LN	16.2	3.26
L-2	HN	15.4	1.65	L-2	LN	15.4	2.13
L-5	HN	15.8	2.09	L-5	LN	15.7	2.24
L-6	HN	17.2	3.05	L-6	LN	14.1	1.73
L-7	HN	14.8	3.57	L-7	LN	14.8	2.44
L-8	HN	14.7	1.89	L-8	LN	14.9	2.18
L-9	HN	15.0	3.06	L-9	LN	14.8	2.10
L-10	HN	13.9	2.36	L-10	LN	15.6	2.13
L-11	HN	14.8	1.32	L-11	LN	15.0	1.94
L-12	HN	16.1	1.76	L-12	LN	15.7	2.16
L-13	HN	16.7	3.43	L-13	LN	14.9	2.74
L-16	HN	15.8	2.52	L-16	LN	16.8	2.92
L-18	HN	15.9	2.56	L-18	LN	14.8	1.89
L-22	HN	14.8	2.54	L-22	LN	15.3	3.50
L-23	HN	14.5	3.78	L-23	LN	16.8	3.68

Table 8. Means and standard deviations (SD) of lesion width for each loblolly pine family under normal (NN) and high (HN) nitrogen treatment levels.

Family	Trt	Lesion Width		Family	Trt	Lesion Width	
		Mean	SD			Mean	SD
L-1	HN	9.3	1.49	L-1	LN	8.6	1.17
L-2	HN	11.0	1.83	L-2	LN	11.0	1.85
L-5	HN	9.3	1.16	L-5	LN	9.6	1.23
L-6	HN	10.5	1.17	L-6	LN	10.5	2.01
L-7	HN	10.7	3.37	L-7	LN	10.1	1.86
L-8	HN	11.0	3.29	L-8	LN	9.9	1.20
L-9	HN	9.5	1.62	L-9	LN	10.8	1.85
L-10	HN	9.8	1.25	L-10	LN	9.6	1.50
L-11	HN	9.7	1.81	L-11	LN	11.2	1.94
L-12	HN	9.9	2.05	L-12	LN	9.4	1.85
L-13	HN	11.5	1.25	L-13	LN	10.6	1.57
L-16	HN	9.6	1.65	L-16	LN	10.5	1.27
L-18	HN	10.2	1.49	L-18	LN	9.9	2.02
L-22	HN	9.4	1.89	L-22	LN	10.4	1.91
L-23	HN	9.7	2.17	L-23	LN	10.9	0.92

Table 9. Probabilities of a greater F-value for total height, RCD, total seedling dry weight, and fraction of total dry weight as foliage, stem, fine roots, and coarse roots in response to normal (NN) and high (HN) nitrogen treatment levels for 15 loblolly pine families.

Source of Variation	DF[1]	Total Height	RCD	Total Dry Weight	Foliage	Stem	Fine Root	Coarse Root
					-------fraction of total dry weight-----			
Block (B)	9	0.0557	0.2619	0.0866	0.0772	0.1102	0.0175	0.4274
Trt (T)	1	0.4568	0.0096	0.0177	0.0014	0.5165	<0.0001	0.7388
BxT	9	0.7841	0.2009	0.7432	0.8823	0.7753	0.9151	0.3874
Family (F)	14	<0.0001	<0.0001	0.0001	0.0338	0.0168	<0.0001	0.0002
TxF	14	0.0575	0.5452	0.0508	0.4008	0.2594	0.0565	0.0819

[1]DF: degrees of freedom.

Table 10. Differences among the means of root collar diameter (RCD), total height, and total seedling dry weight for 15 loblolly pine families.

RCD		Total Height		Total Seedling Dry Weight	
Family Comparison	Mean Differences	Family Comparison	Mean Differences	Family Comparison	Mean Differences
L-1 - L-2	-1.9631	L-1 - L-7	-16.682	L-1 - L-13	-10.878
L-1 - L-13	-2.3520	L-1 - L-16	-15.400	L-5 - L-13	-11.024
L-1 - L-16	-3.1580	L-6 - L-11	15.350	L-6 - L-13	-11.563
L-2 - L-5	2.1459	L-7 - L-10	18.707	L-12 - L-13	-12.002
L-5 - L-13	-2.5348	L-7 - L-11	20.132		
L-5 - L-16	-3.3408	L-7 - L-23	14.879		
L-6 - L-13	-1.9630	L-10 - L-16	-17.425		
L-6 - L-16	-2.7690	L-11 - L-16	-18.850		
L-7 - L-16	-2.2179				
L-8 - L-13	-1.9520				
L-8 - L-16	-2.7580				
L-9 - L-16	-2.2215				
L-10 - L-16	-1.7014				
L-11 - L-16	-1.8245				
L-12 - L-13	-1.6035				
L-12 - L-16	-2.4095				
L-13 - L-22	1.9826				
L-16 - L-18	2.3165				
L-16 - L-22	2.7887				
L-16 - L-23	2.1046				

Note: Only significant differences are presented.

Table 11. Differences among mean fraction of total seedling dry weight as fine roots, woody roots, and stem for 15 loblolly pine families.

Fine Roots Dry Weight		**Coarse Roots Dry Weight**		**Stem Dry Weight**	
Family Comparison	Mean Differences	Family Comparison	Mean Differences	Family Comparison	Mean Differences
L-2 - L-5	0.07339	L-1 - L-2	-0.04531	L-2 - L-5	-0.13465
L-2 - L-9	0.07664	L-1 - L-16	-0.04905		
L-2 - L-22	0.05828				
L-5 - L-7	-0.05434				
L-5 - L-8	-0.06657				
L-5 - L-18	-0.05577				
L-7 - L-9	0.05758				
L-8 - L-9	0.06982				
L-9 - L-11	-0.05626				
L-9 - L-13	-0.05518				
L-9 - L-18	-0.05902				

Note: Only significant differences are presented.

Table 12. Means followed by standard deviations (SD) of seedling total height, root collar diameter (RCD), foliage dry weight, stem dry weight, fine root dry weight, and coarse root dry weight, and root to shoot ratio (R:S) from final measurements.

Family	Total Height		RCD		Total Dry Weight		Foliage		Stem		Fine Root		Coarse Root	
							----------fraction of total dry weight----------							
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
L-1	74.2	14.73	9.9	1.62	33.4	12.16	0.8	0.10	0.6	0.12	0.4	0.05	0.3	0.03
L-2	82.2	11.98	11.9	1.48	41.1	7.20	0.8	0.07	0.5	0.09	0.4	0.04	0.3	0.03
L-5	81.1	15.19	9.7	1.20	33.2	9.82	0.7	0.09	0.7	0.12	0.3	0.05	0.3	0.04
L-6	86.1	17.12	10.3	1.51	32.7	9.96	0.8	0.07	0.6	0.09	0.4	0.06	0.3	0.03
L-7	90.8	22.54	10.8	1.85	37.8	7.67	0.7	0.05	0.6	0.07	0.4	0.04	0.3	0.03
L-8	81.5	19.87	10.3	1.80	41.2	5.55	0.7	0.03	0.6	0.02	0.4	0.01	0.3	0.02
L-9	77.1	11.48	10.8	1.57	39.2	8.69	0.7	0.09	0.6	0.13	0.3	0.07	0.3	0.05
L-10	72.1	10.69	11.4	1.55	36.5	8.05	0.7	0.10	0.6	0.15	0.4	0.08	0.3	0.05
L-11	70.7	10.52	11.2	1.87	35.4	9.06	0.7	0.10	0.6	0.14	0.4	0.05	0.3	0.04
L-12	80.5	16.71	10.6	2.03	32.2	12.31	0.8	0.06	0.6	0.10	0.4	0.04	0.3	0.04
L-13	84.6	21.40	12.3	1.40	44.2	8.85	0.7	0.06	0.6	0.10	0.4	0.05	0.3	0.05
L-16	89.6	15.17	13.1	4.69	41.5	12.70	0.8	0.08	0.6	0.11	0.4	0.05	0.3	0.04
L-18	79.8	16.78	10.7	2.28	34.6	14.06	0.7	0.11	0.6	0.15	0.4	0.08	0.3	0.03
L-22	79.9	13.05	10.3	1.05	33.8	7.94	0.7	0.10	0.6	0.12	0.4	0.07	0.3	0.06
L-23	76.0	13.07	11.0	1.70	39.4	11.90	0.8	0.08	0.6	0.10	0.4	0.07	0.3	0.04

Table 13. Probabilities of greater F-value and regression coefficients for families having significant relationships between lesion width and independent variables (stem dry weight, total seedling dry weight, foliage dry weight).

Dependent Variable	Family	Morphological Variable	
		Stem Dry Weight	
Lesion Width	L-1	0.0288	0.2388
	L-6	0.0123	0.3006
	L-7	0.0049	0.3331
	L-9	0.0028	0.4001
	L-23	0.0208	0.2506
		Total Seedling Dry Weight	
Lesion Width	L-1	0.0054	0.3564
	L-5	0.0012	0.4323
	L-12	0.0048	0.3823
	L-13	0.0252	0.2371
	L-18	<0.0001	0.7303
	L-22	0.0057	0.3885
		Total Seedling Dry Weight	
Stem Total Phenolics	L-10	0.0309	0.2915
	L-12	<0.0001	0.6438
	L-13	0.0069	0.3567
	L-16	<0.0001	0.7389
	L-18	0.0099	0.3488
		Foliage Dry Weight	
Stem Total Phenolics	L-1	0.0130	0.3339
	L-10	0.0080	0.4056
	L-9	0.0326	0.2294

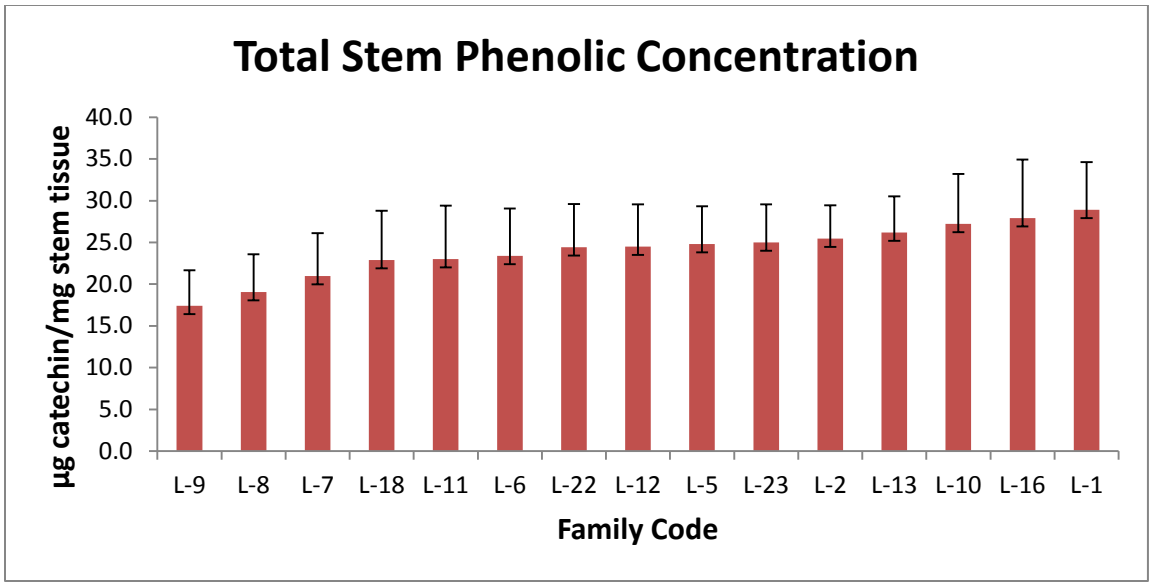

Figure 1. means and standard deviations of stem total phenolic concentration among 15 loblolly pine families.