ABSTRACT

ESPINOZA, JESUS ALBERTO. Genetic and Nutritional Effects on Stem Sinuosity in Loblolly Pine. (Under the direction of Drs. H. L. Allen and S. E. McKeand).

Genetically improved loblolly pine *(Pinus taeda* L.) trees under intensive silviculture have demonstrated dramatic increases in wood production. However, increased input intensity has been associated with a higher propensity for certain genotypes to increase stem and branch deformities, as well as reduced disease resistance. The responses of several genotypes of loblolly pine to nutrient additions on stem sinuosity were assessed at three and twelve years of age in two different tests located in South Carolina and North Carolina, respectively.

The objectives of the studies were to assess the effect of nutrient additions and genetics on stem form, particularly stem sinuosity, in loblolly pine. Tissue samples from of newly expanding shoots at the beginning of growing season were taken and analyzed to assess the association between the nutrient concentrations on the tissues and stem sinuosity in five families from two provenances (Atlantic Coastal Plain "ACP" and Lost Pine Texas "LPT"). The second study was established at the ArborGen research facility near Summerville, South Carolina, with six different genotypes of loblolly pine to determine whether high N and low Ca availability caused sinuous growth. In early spring, eight blocks were fertilized with N as $(NH4)_2SO_4$ and Ca as $CaSO_4$. Nutrient concentrations from flushing shoot tissue were examined and then correlated with measures of stem sinuosity.

Results from the study of repeated nutrient additions in North Carolina showed that the addition of nutrients increased stem sinuosity, branch sinuosity, height, and the levels of N,

P, K, Mg, Zn, B and S in the woody tissue of newly expanding shoots and decreased the levels of Mn and Cu. Calcium levels were the same in both treatments. Stem sinuosity was positively correlated with tissue nitrogen (N) concentration, while negatively correlated with manganese (Mn) levels. Negative family-mean correlations between N and Mn were found in both treatments (control and fertilized). There was a negative family-mean correlation between Mn and the height of the trees in the control and fertilized treatments. Differences in stem sinuosity and nutrient uptake were found among families within provenance, indicating a potential to reduce sinuosity by using genetic selection and appropriate nutrient additions.

The study in South Carolina where only N and Ca were added showed that nitrogen additions caused significant increases in both stem sinuosity and N concentrations. Calcium additions reduced stem sinuosity only when N was added, and did not significantly change in Ca concentrations in the flushing shoot tissue. Manganese (Mn), Ca, N and phosphorus (P) concentrations were all positively correlated with stem sinuosity.

The study from North Carolina showed also that in contrast with sweep, stem straightness, forking, and ramicorn branching were all negatively impacted (became worse) by the nutrient addition, especially in the LPT provenance. The ACP provenance showed 34% more susceptibility to fusiform rust than the LPT provenance.

Based on our findings, nutrient additions, especially N, increased not only stem and branch sinuosity but also increased the deformations in other stem traits such as straightness, forking, and ramicorn branching, and it also increased the proportion of the trees infected with fusiform rust. However, Ca addition reduced the negative impact of N addition on stem sinuosity. Provenance and family differences were also found.

Stem form traits are clearly affected by environmental differences and by genetics. This highlights the importance of matching appropriate site and cultural treatments with suitable genotypes. The choice of these three components has a large impact on the productivity and quality of the plantation.

Genetic and Nutritional Effects on Stem Sinuosity in Loblolly Pine

by
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DEDICATION

To my children Laura Andrea and Jesús Alberto,

my wife Nila,

my parents, family and friends who encouraged me to fulfill this

wonderful dream,

and in memory of my sister Marisol and my friend Lendis

BIOGRAPHY

Jesús Alberto Espinoza was born on July 23, 1967 in Encontrados, Zulia State, Venezuela. He graduated from Benito Puche High School in 1984, and proceeded to begin his university studies in Industrial Engineering. Eighteen months later, due to lacking of funding, he gave up his collegiate work. However, six months later (January 1986), his friend Lendis Bravo and his wife Niria, encouraged him to finish his studies. They granted him economic support to pursue an Engineering Degree in Forestry at the Los Andes University in Merida, Venezuela.

Jesus Espinoza received his Forestry Engineering Degree at the Universidad de Los Andes in Mérida, Venezuela on October 23, 1992. He graduated Cum Laude, and received an academic award "La Excelencia" conjointly given by the Estate of Mérida and the Venezuelan Board of National Science and Technology.

In 1993, he started working as an Inventory Engineer for Smurfit Cartón of Venezuela, a forestry company that grows Eucalypts, Melina and Caribbean pine plantations in western Venezuela. In 1994, he was promoted to Area Manager. This position placed him responsible for all aspects related to site preparation, plantation, and weeding control in several farms of the Forestry Division of Smurfit Cartón Venezuela. In 1996, he married Nila Minerva and three years later (August 23, 1999), they welcomed their wonderful son, Jesus Alberto born.

In 2001, he earned a scholarship from Smurfit Venezuela to pursue a MS in Forestry under the direction of Dr. William Dvorak, who is the director of Camcore (Central America and Mexico Coniferous Resources Cooperative) at North Carolina State University.

Espinoza started his MS in August 2001. By the end of 2003, his beautiful daughter, Laura Andrea, had been born, and Espinoza had also completed his MS. He returned to Venezuela, and continued working for Smurfit as a Harvester Manager. His responsibilities included managing all aspects of harvesting and transportation of pines and tropical hardwood to the mill. He also was involved with the acquisition of approximately 170.000 TM/year of Caribbean pine from the Venezuelan government. In January 2006, Espinoza returned to North Carolina State University to pursue a Doctoral degree in Forestry under the direction of Drs. Steve McKeand and Lee Allen, who have become wonderful friends, as well as mentors.

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1. CHAPTER

Stem Sinuosity of Loblolly Pine under Repeated Nutrient Additions

Abstract

Stem sinuosity is a deformation problem that has been associated with previous land use, high soil nitrification, and deficiencies in nutrients such as copper (Cu), calcium (Ca), boron (B), and zinc (Zn). The objective of this study was to assess how nutrient additions and genetics affect stem form, particularly stem sinuosity, in loblolly pine. We also assessed relationships among nutrients levels of the woody tissue of newly expanding shoots closest to the apical meristem in five families from each of the two provenances of loblolly pine under repeated nutrient additions in 2007.

Nutrient additions increased stem sinuosity, branch sinuosity, height, and the levels of N, P, K, Mg, Zn, B and S of the woody tissue of newly expanding shoots and decreased the levels of Mn and Cu. Calcium levels were the same in both treatments. Stem sinuosity was positively correlated with tissue N concentration, while negatively correlated with Mn levels. Negative family-mean correlations between N and Mn were found in both treatments (control and fertilized). There was a negative family-mean correlation between Mn and the height of the trees in the control and fertilized treatments. Significant differences in stem sinuosity and nutrient uptake were found among families within provenance, indicating a potential to reduce sinuosity by using genetic selection and appropriate nutrient additions.

1.1. Introduction

Stem sinuosity is defined as "any stem crookedness occurring in the segment within two whorls" (Campbell 1965). Numerous studies have been conducted in Pinaceae species to determine the possible causes of this deformation, which involved slight to severe curvature that affects stem quality. Stem deformations are usually formed during the juvenile growth period, with the highest frequency of occurrence from 2 to 6 years of age (Bail and Pederick 1989, Carlyle et al. 1989, Vargas-Hernandez et al. 2003) in most of the species. Deformations are associated with compression wood, which is formed in response to gravity to return a leaning stem to an upright position. Compression wood is undesirable not only for pulp but also for solid wood due to its unfavorable wood properties, such as 15% to 40% higher specific gravity and higher lignin content. Compression wood also has shorter tracheids than normal wood, a flatter microfibril angle, MFA, $(30^{o} - 50^{o})$, and cells tend to have a rounded shape forming voids where cells are joined. This results in the fragmenting of the cell during manufacturing processes (Zobel and van Buijtenen, 1989).

The presence of sinuosity has been reported principally in radiata pine (*Pinus radiata* D. Don) (Bail and Pederick 1989, Carlyle et al. 1989, Downes et al. 1991, Downes and Turvey 1990, Hopmans 1990), loblolly pine *(Pinus taeda* L.)* (Harrington et al. 1999, Murphy and Harrington 2004), slash pine (*Pinus elliottii* Engelm) (Murphy and Harrington 2004), and Douglas-fir (*Pseudotsuga menziesii* Mirb.) (Littke and Zabowski 2007, Middleton et al. 1989, Spicer et al. 2000, Vargas-Hernandez et al. 2003). Causes of sinuosity have been associated with previous land use (mainly agriculture), high soil nitrate concentrations, and

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Cu, B and Zn deficiencies (Carlyle et al. 1989, Hopmans et al. 1995, Hopmans 1990). Cooper, Mn, and B were reported to have an influence on tree form and/or the lignification process in plants (Turvey et al. 1992). In addition, Birk (1991) found that stem deformity was positively correlated with high foliar Mn, aluminum (Al), and Ca levels. Sinuosity has also been associated with site preparation and planting method used. Harrington et al. (1999) and Balneaves and Mare (1989) observed that increasing the depth of subsoiling and root penetration, as well as straightening the taproot, decreased stem sinuosity in radiata pine.

Stem sinuosity has been reported to be influenced by the provenance and/or seed source for loblolly pine (McKeand and Jett 1993). In general, planting exotic or non-adapted seed sources or provenances of tree species can result in trees with poor form or significant levels of deformation. Stem sinuosity appears to be under strong genetic control (Bail and Pederick 1989, Littke and Zabowski 2007, McKeand and Jett 1993, Pederick et al. 1984). Furthermore, Spicer et al. (2000) found in Douglas-fir that trees noted as highly sinuous in one year were more likely to be sinuous in following years. This suggests a genetic predisposition for sinuous growth.

Although previous studies have demonstrated individually the effects of genetics and nutrition on stem sinuosity, our objectives were to assess how nutrient additions and genetics affect stem form, particularly stem sinuosity, in loblolly pine.

1.2. Materials and Methods

1.2.1 Site description

The study site was located in the Sandhills region of Scotland County, North Carolina, which lies adjacent to the U.S. Forest Service - N.C. State University SETRES (Southeastern Tree Research and Education Site) study ($34^o48^{'}$N, $79^o12^{'}$W). The soil is an infertile, well-drained, sandy, siliceous, thermic Psammentic Hapludult (Wakulla series) with a water-holding capacity of 12–14 cm in a 2 m profile. The mean annual precipitation at this site is 1220 mm, which is evenly distributed throughout the year. Long-term mean air temperatures are 26°C and 9°C in the summer and winter, respectively (Albaugh et al. 2004).

1.2.2 Experimental design and treatments

Open-pollinated families from two provenances (the Coastal Plain of North Carolina and South Carolina and the "Lost-Pines" area of Texas) were planted in November and December of 1993. Five families from each provenance with an average or slightly above average breeding value for volume production were established in a split-split-plot design, with two nutrient treatments (fertilized and not fertilized (control)) as main plots, provenances as sub-plots, and families within provenances as sub-sub-plots. Fertilizer was applied almost every year (Table 1-1) to maintain a balanced supply of all nutrients in the fertilized plots based on foliage nutrient analyses (McKeand et al. 2000). Each family plot consisted of 100 trees planted at 1.5 m by 2 m spacing. Only the interior 64 trees were measured at age twelve. Initially, the study was replicated across 10 blocks; however, just five blocks were used to assess stem sinuosity in the current study. The blocks used were selected based on low within

family-plot coefficient of variation for height and high survival. A total of 6,400 measurement trees were used.

1.2.3 Measurements, data sampling and nutrient analysis

Height and diameter of each of the 64 interior trees in the plots were measured at year 12. Stem sinuosity was assess for all of the inner 64 trees, using a scale of 1 to 3, with 1 being the straightest and 3 as the most sinuous trees. After all the trees were scored for sinuosity, two dominant or co-dominant trees with sinuosity and two trees without sinuosity were selected randomly from each subplot (family x treatment combination) within each block. From each tree, samples of the woody tissue of each newly expanding shoot closest to the leader or apical meristem were taken. These were collected at the beginning of the growing season, from March 27-29, 2006. Samples were oven-dried at $70^{o}C$ for 72 hours. After oven-drying, needles were removed from the woody shoot and the woody tissues were ground in a Wiley mill to pass a 0.25-mm mesh sieve. Each sample, which contained ~ 8 mg of tissue, was pulverized, encapsulated in an aluminum tin, and combusted to determine nitrogen (N) concentrations using the CHN elemental analyzer (CE Instruments-NC 2100, CE Elantech Inc., Lakewood, NJ) at $1000^{o}C$. Phosphorus (P), potassium (K), Ca, magnesium (Mg), Mn, sulfur (S), B, and Zn concentrations were determined by wet–digesting a 0.16 or 0.4 g sample (based on the tissue availability) with a mixture of nitric acid and hydrogen peroxide (Zarcinas et al. 1987). Next, spectrometry analysis was done using an inductively coupled plasma atomic emission spectrometer (IPS-AES, Varian ICP, Liberty Series 2, Varian analytical instruments, Walnut Creek, CA).

1.2.4 Statistical analysis

The effects of genetics, nutrient additions, and their interactions on stem sinuosity were examined with the plot-level data using GLM and Mixed procedures in SAS (SAS Institute Inc., 2003). Nutrient additions and provenances were considered fixed effects, and families were considered random effects nested within provenances. The significance level used for all traits was 0.05. The model used was:

$$y_{ijkl} = \mu + r_i + \tau_j + r\tau_{ij} + \rho_k + r\rho(\tau)_{ijk} + t\rho_{jk} + f(\rho)_{l(k)} + \tau f(\rho)_{jl(k)} + \varepsilon_{ijkl}$$

Where:

y_{ijkl} is a trait's value in the lth family within the kth provenance in the jth nutrient treatment in the ith block.

μ is the overall mean;

r_i is the random effect due to i^{th} block [$r_i \sim N(0, \sigma^2_r)$],

$r\tau_{ij}$ is the random interaction effect of the ith block with the jth nutrient treatment [$(r\tau_{ij}) \sim N(0, \sigma^2_{r\tau})$]

$r\rho(\tau)_{ijk}$ is the random interaction effect of the ith block with the jth nutrient treatment with the kth provenance [$r\rho(\tau)_{ijk} \sim N(0, \sigma^2_{r\rho(t)})$];

$f(p)_{l(k)}$ is the random effect due to l^{th} family within k^{th} provenance [$f(\rho)_{lk} \sim N(0, \sigma^2_{f(\rho)})$]; $rf(\rho)_{il(k)}$ is the random interaction effect of the lth family within k^{th} provenance with the ith block [$(rf_{lk}) \sim N(0, \sigma^2_{rf(\rho)})$];

$\tau f(\rho)_{jl(k)}$ is the random interaction effect of the lth family within k^{th} provenance with the jth nutrient treatment [$\tau f(\rho)_{jl(k)}) \sim N(0, \sigma^2_{\tau f(\rho)})$],

e_{ijkl} is the random error effect of the lth family within k^{th} provenance with j^{th} nutrient treatment in the ith block [$\varepsilon_{ijkl} \sim N(0, \sigma^2_\varepsilon)$];

t_j is the fixed effect for nutrient treatment ($\sum\tau_i=0$),

ρ_k is the fixed effect for provenances ($\sum\rho_k=0$).

Family-mean phenotypic correlations within provenances and nutrient treatments were undertaken to determine the relationships between height and stem sinuosity, height and branch sinuosity, and between stem and branch sinuosity using the correlation procedure in SAS.

The relationships among nutrient concentrations were examined using principal components analysis (Proc Princomp) (SAS 9.1.3, 2003). The principal components analysis reduced the dimensionality of the nutrient data set. The eigenvalues for each of the principal components represent a partitioning of the total variation in the nutrient data. Principal components analysis seeks to maximize the variance of the linear combinations of the nutrient variables.

Principal components analyses were carried out at family mean level by treatment within each provenance to assess the relationship among nutrient variables. In addition, because principal component analysis investigates the relationships among variables without designating some as independent and others as dependent, the family-mean correlation matrices were used to determine the relationship between the nutrient variables, stem sinuosity, and height. These assessments were done by treatment within each provenance to

avoid correlations being driven by the treatment effects and to assess if there is any difference or trend in nutrient levels from the woody tissue between the two provenances.

1.3. Results and Discussion

1.3.1 Nutrient addition effects on sinuosity

Stem and branch sinuosity were slightly but significantly ($P < 0.05$) increased (1.13 vs. 1.32 and 0.16 vs. 0.41) by nutrient additions (Table 1-2, Figures 1-1 and 1-2). These results are similar to those from other studies in radiata pine in Australia (Bail and Pederick 1989, Birk 1991, Hopmans et al. 1995) and Douglas-fir in the US (Littke and Zabowski 2007) where sinuosity has been associated with nutrition, especially with N. Nevertheless, only some of those studies (Littke and Zabowski 2007 and Hopmans et al. 1995) had nutrient additions experimentally manipulated as in this study. Without experimental manipulation of the nutrient(s) it is very difficult to determine whether one specific nutrient or the deficiency or excess of other nutrients is the cause of the malformation or abnormal growth. Application of urea increases the concentrations of extractable NH_4^+ and NO_3^- in the soil (Gurlevik et al. 2004), and it is known that NH_4^+ has an antagonistic effect on the uptake of most cations that are essential to plants (Husted et al. 2004). The amount of N added caused significant changes (nutrient imbalances) in some nutrients such as K, Ca, Mg, Mn, B, and S (from other nutrients/N ratios analyses, Table 1-4), and contributed to increase stem deformations.

Nutrient additions increased stem sinuosity 20% with respect to control. The amount of wood affected by stem sinuosity could have significant commercial importance, especially

given that the sinuosity assessment was done at age twelve years where stem sinuosity in mildly affected trees could become disguised by subsequent diameter growth.

The levels of stem deformation found in this study were relatively low compared with those reported in several studies in radiata pine. Hopmans et al. (1995) reported that average potential loss in merchantable volume due to the severely deformed trees was estimated at 36% (range 11% to 63%), and the high rate of N (200 and 600 kg ha^{-1}) increased the level of stem deformity from 12% to 56%. Similarly, Birk (1991) found that up to 44% of the trees in an old pasture exhibited severe deformations and less than 50 stems ha^{-1} were considered as sawlog quality trees; while that only 12% of the trees in the native forest exhibited severe defects. Bail and Pederick (1989) found significant genetic variation in the degree of stem sinuosity on the highly fertile sites for 3-year-old progeny of 18 selected parents of radiata pine.

Based on the results found in our study and those reported on radiata pine, we infer that significant variation in stem deformation exits in these two species. Apparently, radiata pine shows higher susceptibility to severe stem sinuosity than loblolly pine. However, the establishment of other studies that include more genotypes, nutrient addition treatments, and ages could be useful to assess those patterns in loblolly pine.

1.3.2 *Provenance and family effects on sinuosity*

Provenances did not differ significantly in their stem and branch sinuosity, but families within provenances and family by nutrient treatment interactions did contribute significant variation (Table 1-2). Families from the ACP provenance showed no differences in sinuosity

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in the control plots. Families from LPT provenance were significant different in both treatments. Both provenances showed a very weak family by treatment interaction for stem sinuosity (Figure 1-1).

Stem sinuosity has been previously reported to have moderate individual-tree heritability (0.20 - 0.45) (Spicer et al. 2000, Bail and Pederick 1989, McKeand and Jett 1993). Our results and the heritability estimates in other studies suggest that some genetic improvement in stem sinuosity can be expected for the selection and breeding of straight families.

1.3.3 *Nutrient addition and genetic effects on shoot nutrient concentrations*

For shoot nutrient concentrations, N, P, Mg, Zn, and B were significantly increased by nutrient addition (Tables 1-2 and 1-3). At the provenance level, Mn was the only nutrient that was significantly different, having higher concentration in the LPT provenance than the ACP provenance. Magnesium, B, and S were the only elements that showed significant differences at family within provenance levels. No significant interactions were found.

1.3.4 *Family-mean phenotypic correlations*

Weak correlations between sinuosity and height were found within provenances and treatments. The family-mean correlations between height and stem sinuosity within provenance and treatment were r = 0.65 for the control and r = 0.27 for the fertilized treatments in the ACP provenance. In the LPT provenance, the correlations were r = 0.59 and r = - 0.19 for the control and fertilized treatments, respectively. These results from the family-mean correlations within provenance and treatment were similar to those reported by McKeand and Jett (1993). While there was a relatively strong correlation between stem

sinuosity and height across provenances, within provenances, the genetic correlations were not significant.

Stem sinuosity and branch sinuosity were highly correlated with a family-mean correlation of r = 0.99 across both provenances (Figure 1-3). This high family-mean correlation indicates that a family that tends to display sinuosity in the stem will most likely display sinuosity in the branches. Depending on the assessment age, stem sinuosity in mildly affected trees could become disguised by subsequent diameter growth, so the branch sinuosity assessment may be very helpful for identifying families that might be prone to have sinuous stems under the appropriate nutritional or environmental conditions. McKeand and Jett (1993) found similar results and made a similar suggestion.

1.3.5 Relationships among nutrients

The first two principal components accounted for 71%, and the first three explained 92%, of the total nutrient variance in the control treatment in the ACP provenance (Table 1-5).

When the nutrient loadings on the first and second principal components were plotted in the control treatment, the nutrients clearly cluster into two groups. The first group contains P, K, Ca, B, and Mg, and these have relatively high positive weights or coefficients (from 0.3 to 0.4) on the first principal component and relatively low or negative weights (from 0.16 to -0.25) on the second principal component. In contrast, the other nutrients (N, Mn, S, and Zn) tended to have low weights on the first principal component and high weights on the second principal component (Figure 1-4).

Principal components analysis of nutrients showed similar patterns under the nutrient addition treatment. In this case, the first two principal components accounted for 77% of the total nutrient variance, and the first three explained 91% (Table 1-5). The variations among nutrients were greater in this treatment than in the control. In addition, when the relationships among nutrient were compared between the two treatments, K, Zn, Mn exhibited greater variation due to nutrient additions, with K exhibiting the highest variation (Figure 1-4). Plotting nutrient loadings on the second principal component vs. third principal component highlighted the significant variations of the Mn, Zn and Cu caused by nutrient additions compared to the control (Figure 1-5).

In the LPT provenance, the first two principal components accounted for 71% of the total nutrient variance, and the first three explained 88% in the control treatment (Table 1-6). When nutrient loadings on the first principal component vs. second principal component were plotted in the control treatment, greater variation among nutrients on the first principal component were observed than in the control treatment in the ACP provenance. Again, the nutrients could be classified clearly in two groups, but the groupings differed in the LPT provenance compared to the ACP provenance. The first group included Mn, Mg, Ca, P, Cu, and Zn, and was characterized by relatively high positive weights (from 0.25 to 0.43) on the first principal component and low to high weights on the second principal component. The second group (S, B, K, and N) was characterized by nutrients with low weights (-0.1 to -0.3) on the first principal component and low to high weights on the second principal component (Figure 1-6).

Nutrient addition treatment showed a similar pattern to the control. The first two principal components accounted for 74% of the total nutrient variance, and the first three explained 94% in the nutrient addition treatment in the LPT provenance (Table 1-6). In this treatment, the nutrients could again be classified in two different groups but with lower variation between nutrients groups than in the control treatment. The first group was characterized by the nutrients P, Mg, Mn, Ca, Zn, and N with high positive weights (0.24 to 0.42) on the first principal component and low to moderate weights on the second principal component (0.15 to -0.15, except N with -0.34). The second group corresponded to the nutrients B, K, Cu, and S, with low weights (0.02 to 0.2) on the first principal component and high weights (0.27 to 0.50) on the second principal component. In addition, when the relationships among nutrient were compared between the two treatments, it was observed that nutrient additions reduced the variation among nutrients, particularly for N, Ca, K, B, and S (Figure 1-6). Plotting loadings on the second principal component vs. third principal component highlighted the significant variation in Ca, K, N, Zn and S caused by nutrient additions compared to the control (Figure 1-7).

These results imply that when the demands for some nutrients are satisfied by nutrient additions, other deficiencies or excesses of other nutrients could result. This is supported by Binkley and Högberg (1997) who pointed out that nutrient imbalances may occur in soils when one or more nutrients become greatly available, while the trees cannot use them owing to other limitations such as other nutrients. When comparing the plots of the principal components, it appears that the addition of several nutrients (Table 1-1) could have caused deficiencies or changes in the rations between nutrients such as the micronutrients Mn, Cu,

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and Zn in the ACP provenance and the macronutrients Ca, K, and N in the LPT provenance. This suggests the possibility of differences in nutrient uptake among families from these two provenances.

1.3.6 *Correlations among sinuosity, height, and nutrient concentrations*

For the ACP provenance in the control treatment there were negative family mean correlations between height and all nutrients except N and S. Furthermore, stem sinuosity was positive correlated with all nutrients, except K and S. Stem sinuosity and N showed the strongest positive correlation (0.91). No correlation was found between stem sinuosity and Mn (Table 1-7). Similarly, the data from the fertilized treatment showed that height was negatively correlated with all nutrients, except K. Stem sinuosity was positively correlated with all nutrients, except K and Mn (Table 1-8). Therefore, these results show that N has a negative effect on stem sinuosity, and low levels of Mn and K could therefore affect stem deformation.

When the LPT provenance was analyzed by nutrient treatment, it was found that in the control treatment the height was negative correlated (family-mean basis) with all nutrients, except B, K, and S. Stem sinuosity was positively correlated with all nutrients, except Mg and Mn (Table 1-9). In contrast, the data from the fertilized treatment showed that height was negatively correlated with all nutrients except B and S. Stem sinuosity was positively correlated with S only. The smallest negative correlation was found between stem sinuosity and N (-0.13) (Table 1-10). Therefore, these results also show that N may have a negative effect on stem sinuosity; low levels of other nutrients, especially, Mg, Mn, Ca, and Cu, could

then affect stem deformation. The results also suggest stem sinuosity and height for LPT provenance families could respond to nutrient additions differently.

Our results contrast with those reported by Pederick et al. (1984) and Turvey et al. (1992) where Cu deficiency was associated with stem deformation in radiata pine. We found that it is unlikely that Cu causes stem sinuosity in this study because tissue concentrations of this element were adequate (4 ppm) in trees that showed sinuosity in both treatments in each provenance. Boron and Zn are other nutrients that have been associated with stem deformation. However, our results indicate that it is unlikely that sinuosity was due to deficiencies of B and Zn, because tissue concentrations of these two elements were adequate in trees that exhibited sinuosity. These results are consistent with those reported by Carlyle et al. (1989) and Turvey et al. (1992), showing no observed effects of B or Zn on stem deformations.

In contrast with the nutrients discussed above, N concentration was moderately correlated with stem sinuosity in both provenances and treatments except the fertilized treatment in the LPT provenance. These results are consistent with other studies where high levels of nitrogen were associated with stem deformation (Birk 1991, Carlyle et al. 1989, Hopmans et al. 1995). Even though N concentration was positively correlated with Mn level in both treatments in the ACP provenance, the Mn levels were higher in the control treatment than fertilized treatment (Table 1-3). This could be due to the accelerated growth of the trees resulting from N additions, which apparently diluted in greater proportion the manganese in the woody tissue. Our results from correlation analyses suggest that low levels of Mn could be associated with stem deformation (Figure 1-8). This suggestion is opposite the results

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reported by Turvey et al. (1992) in radiata pine from a study where experimental treatments with Mn and Cu were applied. Turvey et al. (1992) observed a three-way interaction between Cu, Mn and families on stem sinuosity, where the addition of Mn without Cu showed the greatest stem sinuosity in one family, compared to instances when Cu had been added. Nevertheless, in our results, Mn levels were negatively correlated with height (Figure 1-9), supporting the results found by Turvey et al. (1992).

1.4. Conclusions

Results from stem sinuosity assessments and nutrient analysis at age twelve at SETRES-2 indicated that stem and branch sinuosity were negatively affected by nutrient additions. High nitrogen levels were associated with the presence and/or formation of stem sinuosity, and negatively correlated with manganese. The negative effect of low manganese on stem sinuosity could be magnified by the high level of nitrogen from nutrient additions. Additionally, we found a negative correlation between manganese and tree height. Results from the woody tissue analyses showed that the micronutrients Mn, Cu, and Zn and the macronutrients Ca, K, and N exhibited the greatest variations among nutrients and between treatments indicating that the addition of some nutrients could change the levels of other nutrients and cause an imbalance of nutrients in the trees.

The results of this study suggest that there is no a single factor that causes stem sinuosity. Stem sinuosity appears to be a complicated deformation of the stem, caused by a combination of different effects such as growth and unbalanced nutrient ratios as the found in this study (Ca/N, Mg/N, Mn/N, B/N, and S/N), as well as genetic and physiological factors.

1.5. Acknowledgements

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Table 1-1. Type and amount of fertilizer applied from 19994 to 2006.

Year	Fertilizer	Nutrient additions (Kg ha^{-1})					
		N	P	K	Ca	Mg	S
1994	10/10/10	52	22	43	0	0	<0.9
1995	12-6-6 + Micros[1]	41	9	17			
1996	Urea, TSP, KMagS	56	6	28	3	17	40
1997	Urea, TSP, Mg	90	9			18	11
1998	Urea, Mg	90				17	11
1999	Urea, DAP, B[2]	95	9				12
2000	Ammonium Sulfate	91	10		6		103
2001	Urea, DAP	90	9				
2002	Urea, DAP	90	9				
2003	Urea	123					
2004	Urea, TSP, KMagS	86	11	38		24	46
2005	Urea (estimate)	112					
2006	None						
Total		**1015**	**94**	**126**	**9**	**75**	**224**

[1]Micros 0.5 B, 2.0 Cu, 5.0 Fe, 5.0 Mn, 2.0 Zn, [2] 1.2 B

Table 1-2. P-values for stem and branch sinuosity assessment and of the nutrient concentrations from woody tissue of newly expanding shoots at SETRES-2.

Source	Height	Stem Sinuosity	Branch Sinuosity	Nutrient									
				N	P	K	Ca	Mg	Mn	Zn	B	Cu	S
Treatment	<.01	<.01	<.01	<.01	<.01	0.10	0.52	<.01	0.18	<.01	<.01	0.84	0.26
Provenace	0.03	0.15	0.13	0.12	0.36	0.13	0.06	0.07	0.01	0.42	0.12	0.86	0.34
Treatment * Provenance	0.39	0.27	0.87	0.53	0.92	0.97	0.18	0.38	0.19	0.33	0.12	0.26	0.98
Family(Provenance)	0.07	0.03	0.01	0.80	0.21	0.15	0.07	0.04	0.06	0.18	<.01	0.56	<.01
Treatment *Family(Provenace)	0.45	<.01	<.01	0.07	0.12	0.38	0.22	0.27	0.26	0.12	0.06	0.61	0.13

Table 1-3. LS means of nutrient concentrations from woody tissue of newly expanding shoots for treatments and provenances at SETRES-2. Means with * and shading differ between sources.

Source	Woody shoot nutrient concentrations									
	N	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**B**	**Cu**	**S**
	%	%	%	%	%	ppm	ppm	ppm	ppm	%
Treatment										
Control	1.35*	0.21*	0.67*	0.10	0.13*	356	42.9*	39.9*	7.25	0.15*
Fertilized	1.58	0.24	0.70	0.10	0.16	271	50.2	48.2	6.91	0.16
Provenance										
Atlantic Costal Plain	1.49*	0.22	0.70*	0.09*	0.14*	267*	45.8	35.4*	7.22	0.16*
Lost Pine Texas	1.44	0.23	0.66	0.11	0.15	359	47.3	52.7	6.94	0.15

Table 1-4. P-values for nutrient concentration/N ratios from woody tissue of newly expanding shoots at SETRES-2. Bold and shading number indicate significant difference at P-value <0.05

Source	Woody shoot nutrient ratios								
	P/N	K/N	Ca/N	Mg/N	Mn/N	Zn/N	B/N	Cu/N	S/N
Treatment	0.13	**0.02**	0.09	0.40	**0.03**	0.68	0.55	0.33	0.41
Provenace	0.56	0.36	**0.03**	**0.02**	**<.01**	0.09	0.10	0.92	0.64
Treatment * Provenance	0.87	0.92	0.85	0.38	0.08	0.32	0.06	0.30	0.66
Family(Provenance)	0.33	0.21	**0.03**	0.19	**0.02**	0.13	**<.01**	0.53	**<.01**
Treatment *Family(Provenace)	0.08	0.12	0.63	0.22	0.68	0.39	0.35	0.49	0.76

Table 1-5. Eigenvalues (principal components) of the family-mean correlation matrix of nutrients from woody tissue of newly expanding shoots for the 12th growing season by treatment for ACP provenance.

A. Control treatment

	Eigenvalue	Difference	Proportion	Cumulative
1	5.10	1.65	0.42	0.42
2	3.45	0.97	0.29	0.71
3	2.48	1.50	0.21	0.92
4	0.98	0.98	0.08	1.00

B. Fertilized treatment

	Eigenvalue	Difference	Proportion	Cumulative
1	6.58	3.87	0.55	0.55
2	2.70	1.03	0.23	0.77
3	1.67	0.61	0.14	0.91
4	1.05	1.05	0.09	1.00

Table 1-6. Eigenvalues (principal components) of the family-mean correlation matrix of nutrients from woody tissue of newly expanding shoots for the 12th growing season by treatment for LPT provenance.

A. Control treatment				
	Eigenvalue	**Difference**	**Proportion**	**Cumulative**
1	5.13	1.70	0.43	0.43
2	3.44	1.39	0.29	0.71
3	2.05	0.66	0.17	0.88
4	1.38	1.38	0.12	1.00

B. Fertilized treatment				
	Eigenvalue	**Difference**	**Proportion**	**Cumulative**
1	5.65	2.46	0.47	0.47
2	3.19	0.73	0.27	0.74
3	2.46	1.75	0.21	0.94
4	0.71	0.71	0.06	1.00

Table 1-7. Family-mean correlation matrix of nutrient concentrations in woody tissue of newly expanding shoots, stem sinuosity, and height at 12th growing season for ACP provenance in the control treatment.

	N	Ca	B	Cu	K	Mg	Mn	P	S	Zn	Stem sinuosity	Height
N	1	0.37	0.17	0.64	-0.36	0.11	0.14	-0.06	-0.09	0.23	0.91	0.26
Ca		1	0.61	0.72	0.49	0.69	0.37	0.89	0.02	0.18	0.62	-0.60
B			1	0.52	0.80	0.82	0.23	0.69	0.73	-0.64	0.21	-0.26
Cu				1	0.34	0.22	0.78	0.56	-0.14	0.22	0.58	-0.52
K					1	0.59	0.43	0.80	0.49	-0.49	-0.28	-0.65
Mg						1	-0.20	0.70	0.69	-0.50	0.35	-0.12
Mn							1	0.44	-0.38	0.30	0.00	-0.77
P								1	0.16	-0.03	0.19	-0.78
S									1	-0.96	-0.11	0.34
Zn										1	0.32	-0.33
Stem sinuosity											1	0.13

Table 1-8. Family-mean correlation matrix of nutrient concentrations in woody tissue of newly expanding shoots, stem sinuosity, and height for the 12th growing season for ACP provenance in the fertilized treatment.

	N	Ca	B	Cu	K	Mg	Mn	P	S	Zn	Stem sinuosity	Height
N	1	0.71	0.50	-0.27	-0.77	0.69	0.40	0.53	0.97	0.22	0.57	-0.45
Ca		1	0.82	0.32	-0.68	0.88	0.54	0.92	0.70	0.37	0.78	-0.75
B			1	0.68	-0.73	0.85	0.07	0.60	0.47	-0.19	0.86	-0.40
Cu				1	-0.25	0.29	-0.18	0.24	-0.32	-0.44	0.40	-0.14
K					1	-0.54	-0.36	-0.50	-0.61	0.24	-0.47	0.54
Mg						1	0.11	0.66	0.77	0.20	0.98	-0.34
Mn							1	0.78	0.30	0.67	-0.09	-0.94
P								1	0.51	0.60	0.53	-0.91
S									1	0.32	0.66	-0.34
Zn										1	0.05	-0.52
Stem sinuosity											1	-0.18

Table 1-9. Family-mean correlation matrix of nutrients from woody tissue of newly expanding shoots, stem sinuosity, and height for the 12th growing season for LPT provenance in the control treatment.

	N	Ca	B	Cu	K	Mg	Mn	P	S	Zn	Stem sinuosity	Height
N	1	-0.26	-0.23	-0.53	0.11	-0.44	-0.28	0.06	-0.30	0.17	0.47	-0.07
Ca		1	0.09	0.87	-0.39	0.85	0.75	0.89	-0.02	0.60	0.02	-0.19
B			1	0.30	0.25	-0.32	-0.55	0.14	0.89	-0.20	0.48	0.94
Cu				1	-0.01	0.77	0.61	0.60	0.37	0.63	0.17	-0.03
K					1	-0.40	-0.41	-0.58	0.63	0.32	0.72	0.20
Mg						1	0.97	0.60	-0.29	0.58	-0.32	-0.59
Mn							1	0.53	-0.50	0.60	-0.37	-0.77
P								1	-0.14	0.39	0.05	-0.03
S									1	0.03	0.62	0.78
Zn										1	0.51	-0.46
Stem sinuosity											1	0.40

Table 1-10. Family-mean correlation matrix of nutrients from woody tissue of newly expanding shoots, stem sinuosity, and height for the 12th growing season for LPT provenance in the fertilized treatment.

	N	Ca	B	Cu	K	Mg	Mn	P	S	Zn	Stem sinuosity	Height
N	1	0.70	-0.25	-0.42	-0.61	0.46	-0.39	0.30	0.15	0.83	-0.13	0.02
Ca		1	-0.04	0.28	-0.06	0.93	0.99	0.79	0.03	0.93	-0.68	-0.39
B			1	0.58	0.62	0.28	-0.10	0.31	0.76	-0.03	-0.17	0.67
Cu				1	0.68	0.58	0.21	0.72	0.28	0.14	-0.50	-0.16
K					1	0.25	-0.20	0.13	-0.03	-0.33	-0.62	-0.04
Mg						1	0.89	0.89	0.20	0.82	-0.78	-0.27
Mn							1	0.79	0.08	0.97	-0.56	-0.36
P								1	0.39	0.78	-0.49	-0.20
S									1	0.27	0.28	0.81
Zn										1	-0.39	-0.16
Stem sinuosity											1	0.48

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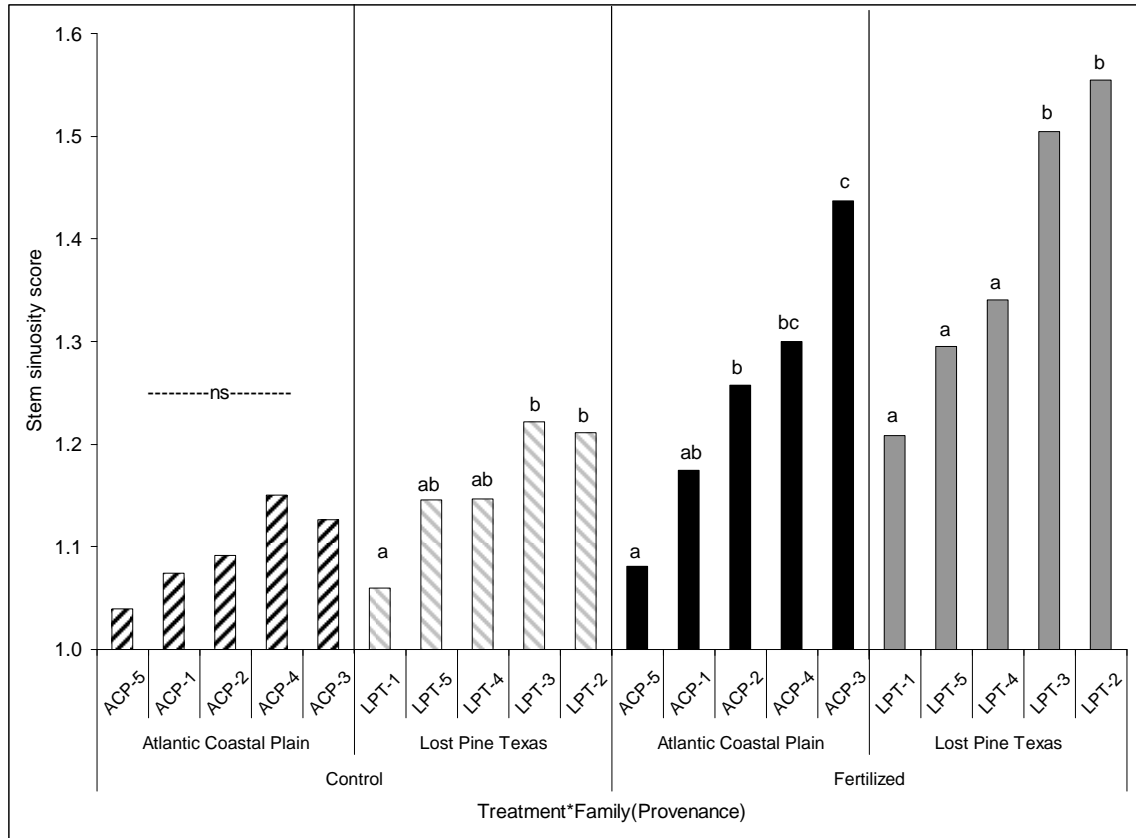
Figure 1-1. Mean of stem sinuosity scores for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots. Treatment and Family within Provenance effects were highly significant. Provenance effect was not significant. Even though Treatment by family within Provenance interaction effects was significant, families appear to rank very similarly in the two treatments; it could be due to a scale effect. Treatment by Provenance was not significant (Table 1-2). Families within provenance are ordered by their sinuosity score in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination were not significantly (P<0.05) different.

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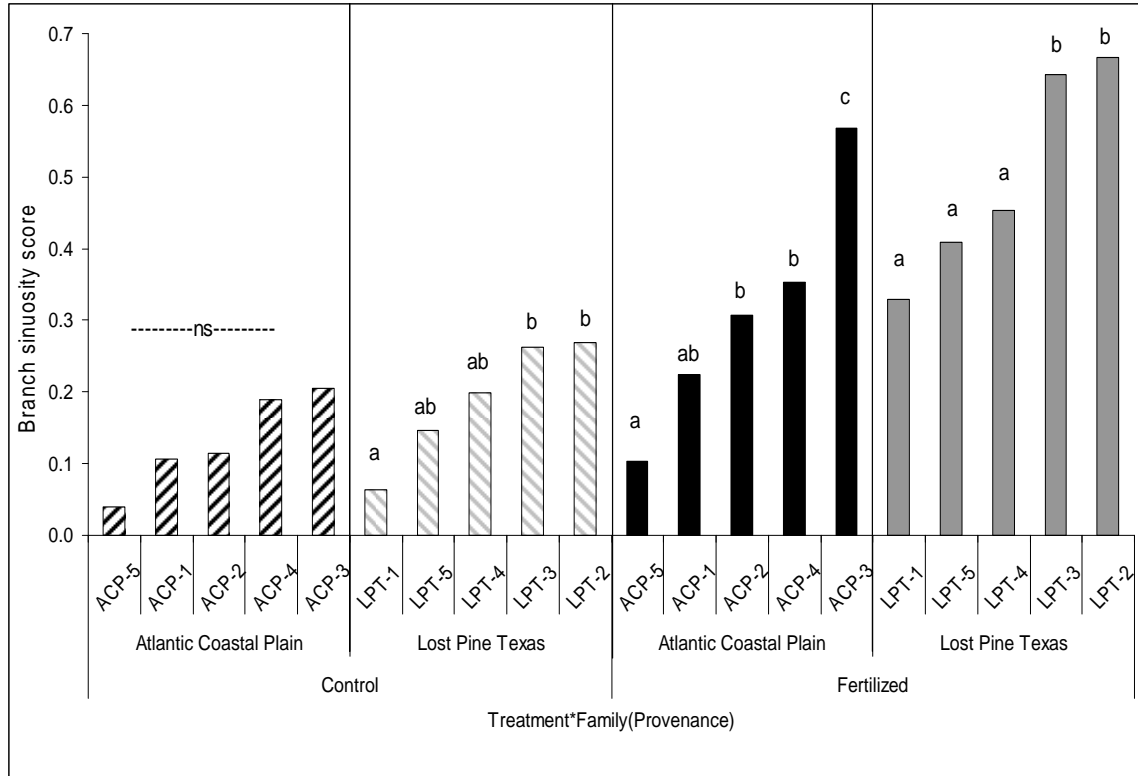
Figure 1-2. Mean of branch sinuosity scores for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots. Treatment and Family within Provenance effects were highly significant. Provenance effect was not significant. Significant Treatment by family within Provenance interaction must be due to scale effect, since families rank the same in the two treatments. Treatment by Provenance was not (Table 1-2). Families within provenance are ordered by their branch sinuosity score in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination are not significantly (P<0.05) different.
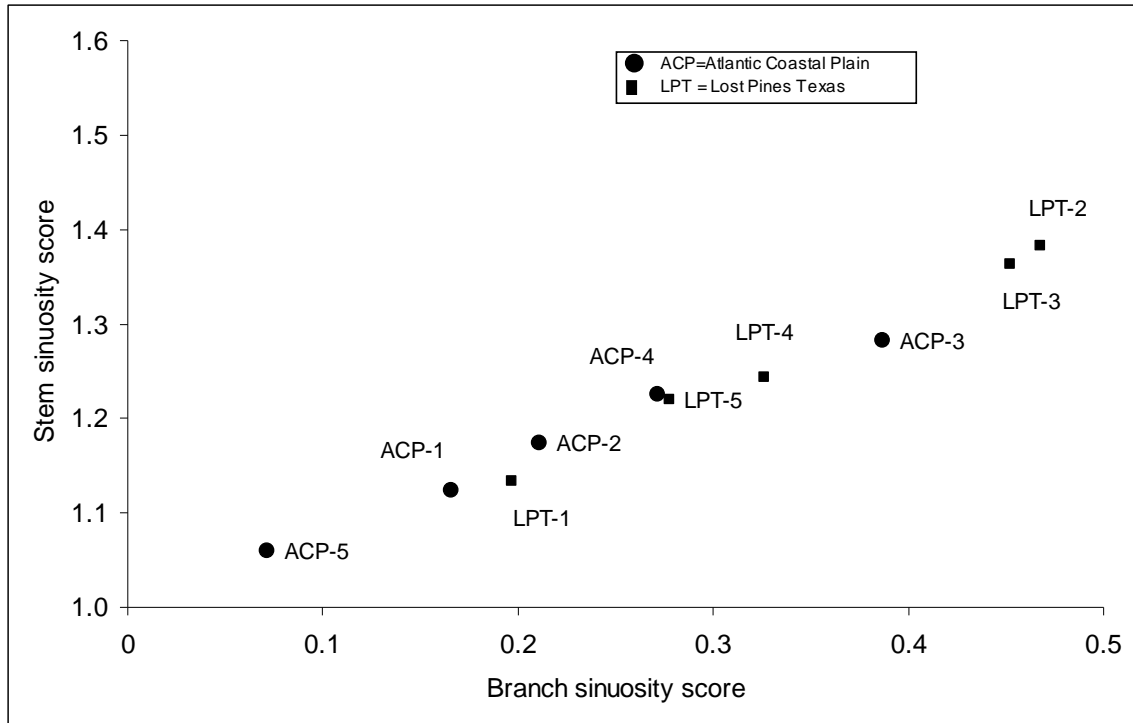
Figure 1-3. Family-mean correlation ($R^2 = 0.98$) between stem sinuosity and branch sinuosity scores across provenances.

34
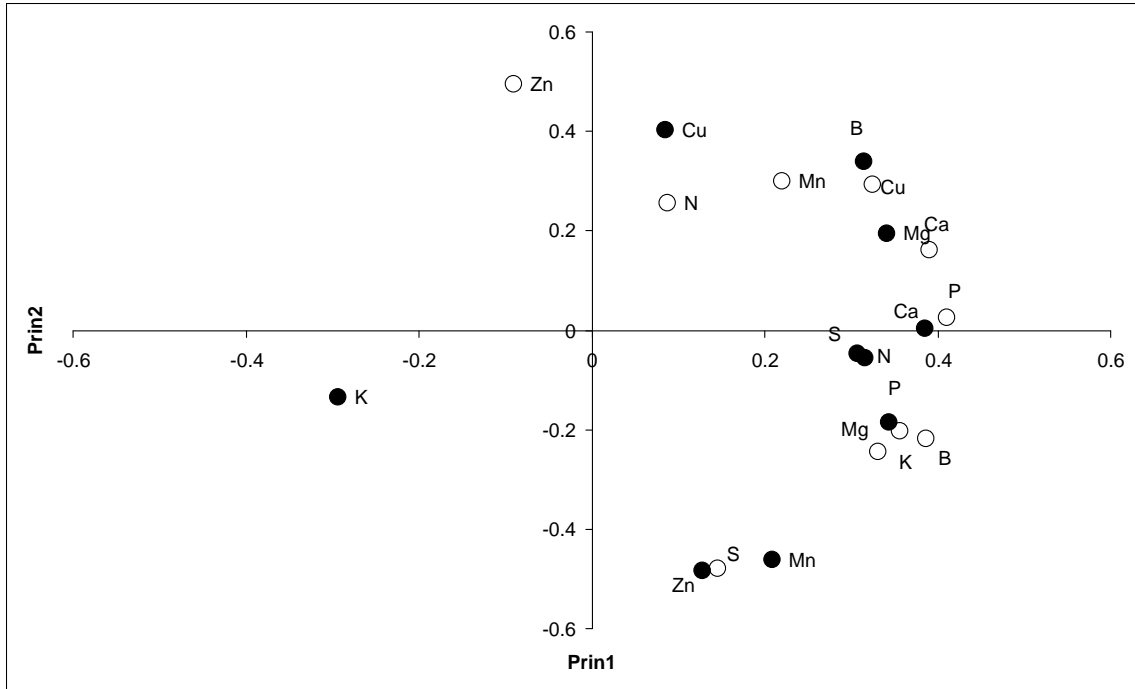
Figure 1-4. First vs. second principal component loadings from nutrients analysis of the woody tissue of newly expanding shoots from the 12th growing season by treatment at family-mean level for ACP provenance at SETRES-2. Nutrients from the control treatment are non-filled circles and nutrient from nutrient additions treatment are filled.
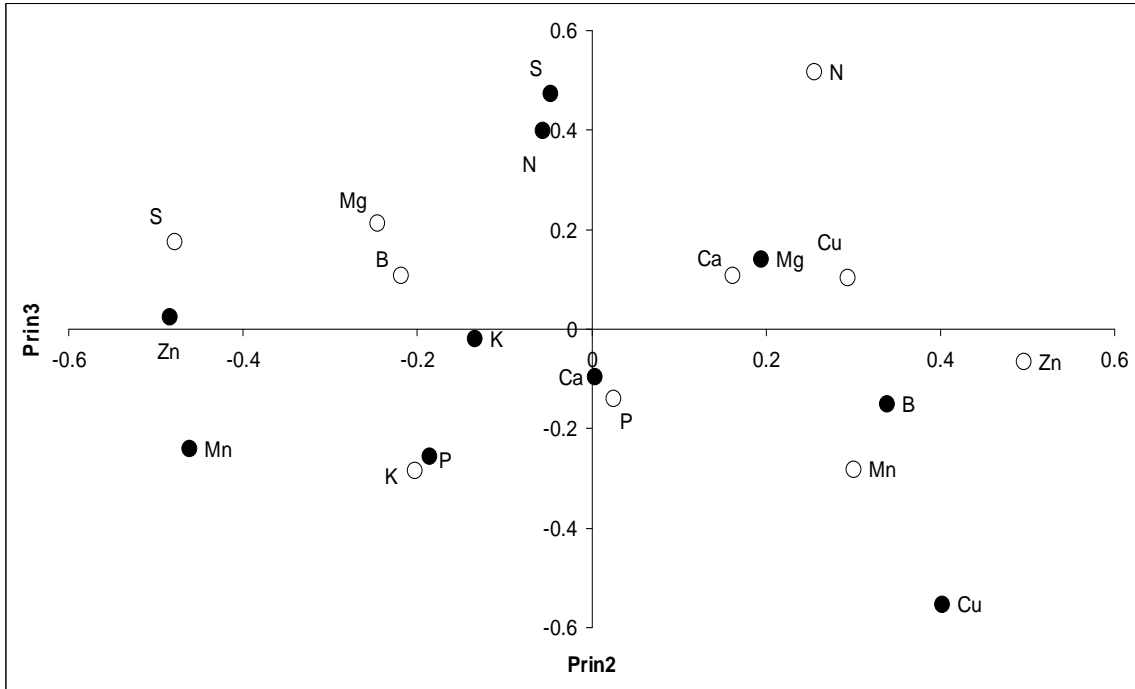
Figure 1-5. Second vs. third principal component loadings from nutrients analysis of the woody tissue of newly expanding shoots from the 12th growing season by treatment at family-mean level for ACP provenance at SETRES-2. Nutrients from the control treatment are non-filled circles and nutrient from nutrient additions treatment are filled.
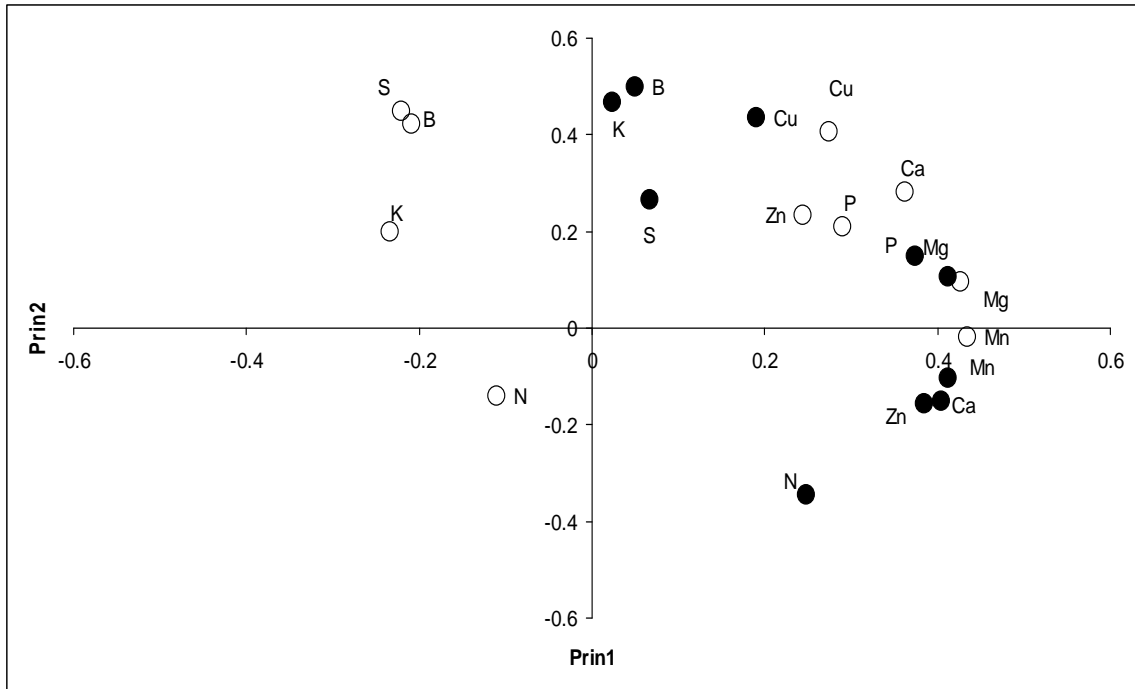
Figure 1-6. First vs. second principal component loadings from nutrients analysis of the woody tissue of newly expanding shoots from the 12th growing season by treatment at family-mean level for LPT provenance at SETRES-2. Nutrients from the control treatment are non-filled circles and nutrient from nutrient additions treatment are filled.
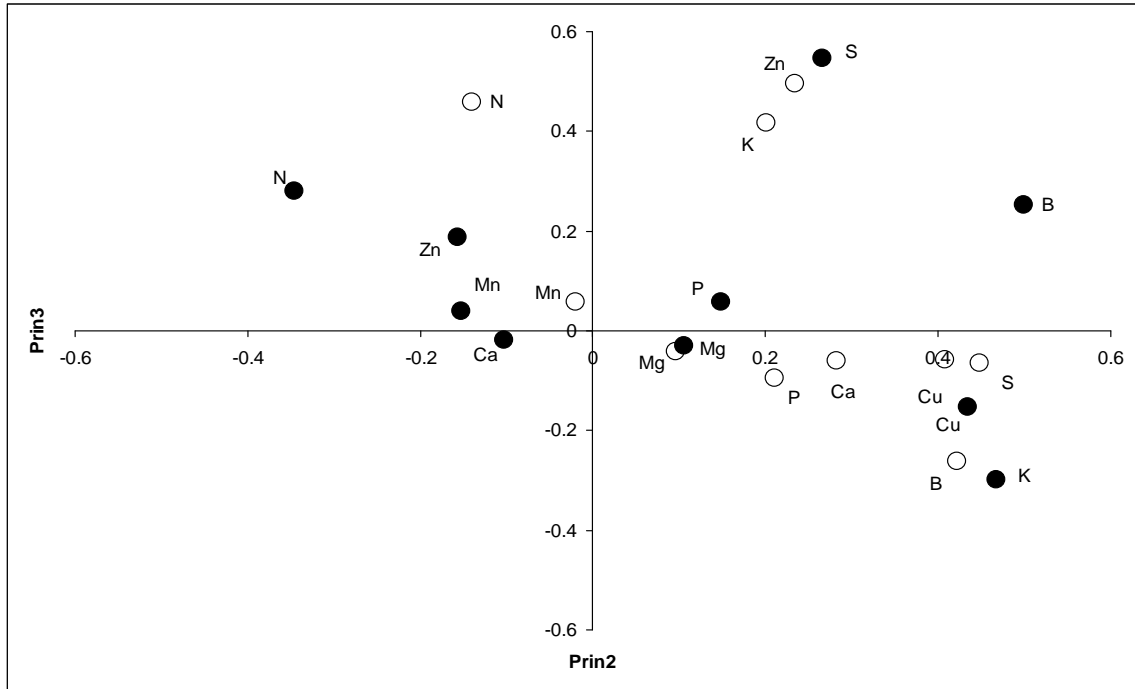
Figure 1-7. Second vs. third principal component loadings from nutrients analysis of the woody tissue of newly expanding shoots from the 12th growing season by treatment at family-mean level for LPT provenance. Nutrients from the control treatment are non-filled circles and nutrient from nutrient additions treatment are filled.
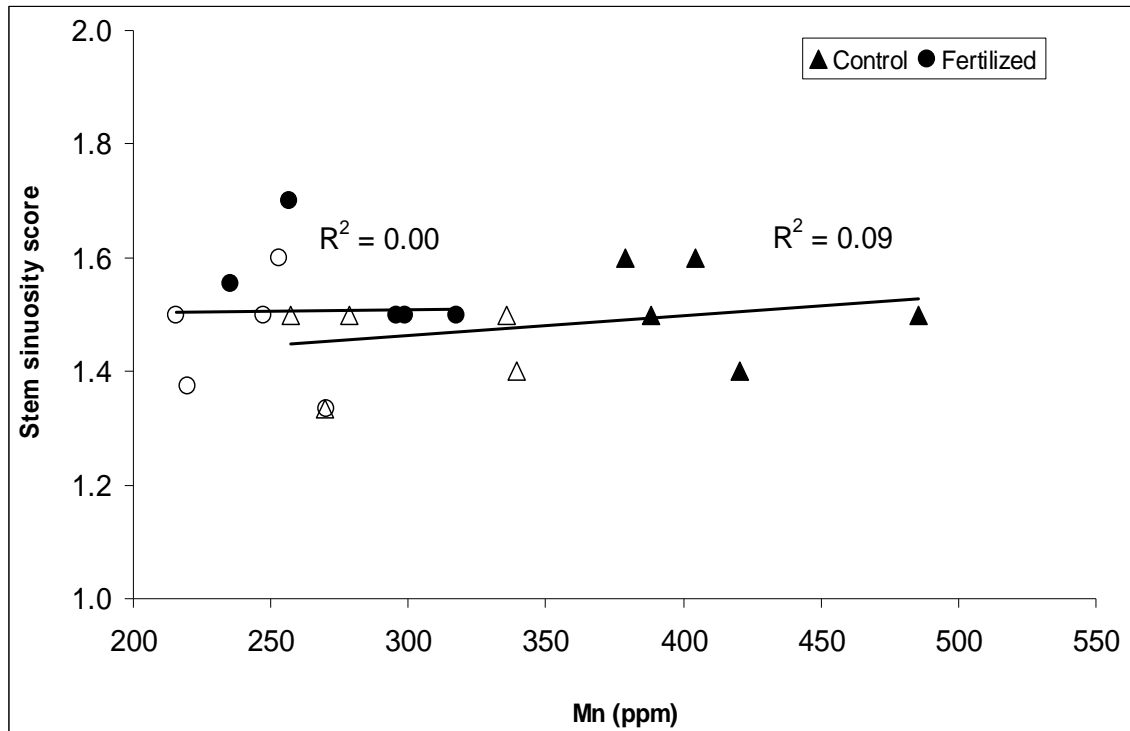
Figure 1-8. Family-mean plot correlations between stem sinuosity and manganese (Mn) concentration by treatment across provenances. Circles correspond to plots from nutrient additions treatment and triangles to plots from control treatment. The ACP provenance plots are non-filled circles and triangles and the LPT provenance plots are filled.

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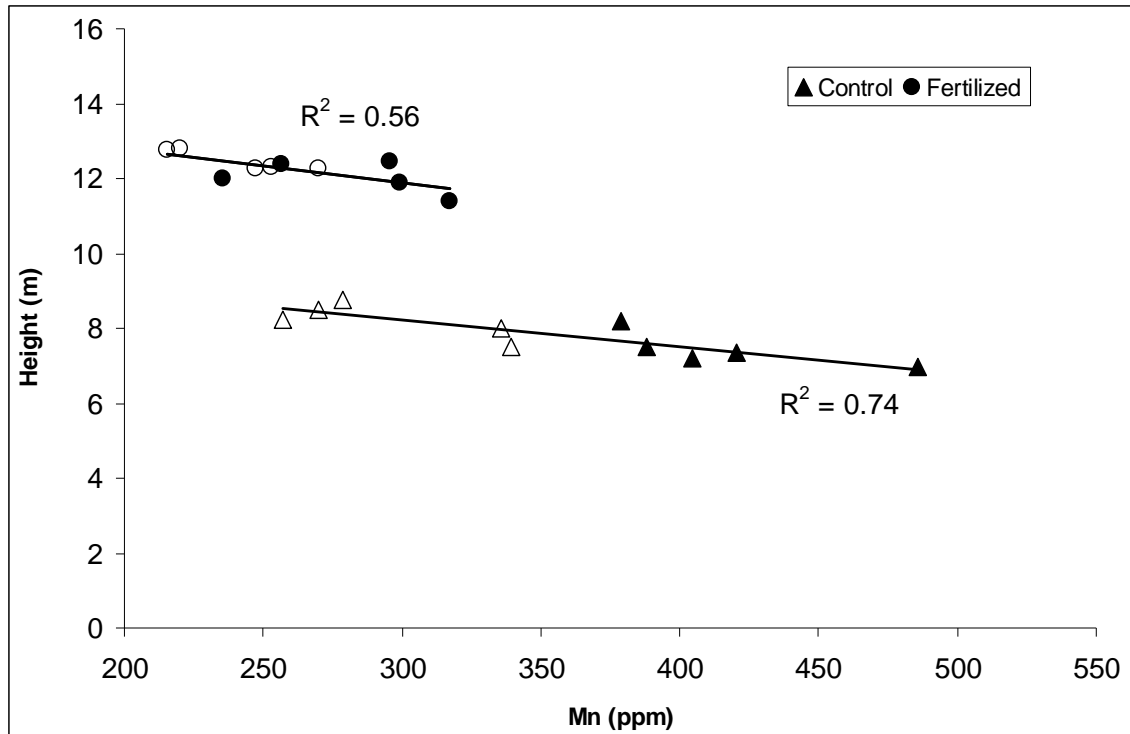
Figure 1-9. Family-mean plot correlations between height and manganese (Mn) concentration by treatment across provenances. Circles correspond to plots from nutrient additions treatment and triangles to plots from control treatment. The ACP provenance plots are non-filled circles and triangles and the LPT provenance plots are filled.

2. CHAPTER

Stem Sinuosity in Loblolly Pine with Nitrogen and Calcium Additions

Abstract

Stem sinuosity is a stem deformation that occurs in loblolly pine *(Pinus taeda* L.*)*, as well as many other species, and has been associated with nutritional and physiological problems. Nitrogen (N) and calcium (Ca) are two important elements affecting the formation, growth, membrane stability and maintenance of tree cell integrity. A trial was established at the ArborGen research area near Summerville, South Carolina with one open-pollinated (OP) family, 3 MCP families (full-sib families from Mass Control Pollination), and two clonal varieties of loblolly pine to determine whether high N and low Ca availability caused sinuous growth. In early spring (2007), eight family blocks were fertilized with N (200 lbs ha^{-1}) as (NH4)$_2$SO$_4$ and Ca (150 lbs ha^{-1}) as CaSO$_4$. Nutrient concentrations from flushing shoot tissue were examined and then correlated with stem sinuosity. During the same growing season, sinuosity and height were assessed three times. Nitrogen additions caused significant increases in both stem sinuosity and N concentrations. Calcium additions reduced stem sinuosity and mitigated the negative effect of N addition. Calcium addition did not significantly change Ca concentrations in the woody tissue from the flushing shoots. Manganese (Mn), Ca, N and phosphorus (P) concentrations were correlated with stem sinuosity.

2.1. Introduction

Sinuosity is defined as any stem crookedness that occurs in the segment between two whorls (Campbell, 1965) resulting in slight to severe curvature affecting the stem quality. In pine species, the presence of sinuosity has been reported and studied principally in radiata pine (*Pinus radiata* D. Don) (Bail and Pederick 1989, Carlyle et al. 1989, Downes and Turvey 1990, Downes et al. 1991, Hopmans et al. 1995, Hopmans 1990). However, this problem has been also reported in loblolly pine (Harrington et al. 1999, Murphy and Harrington 2004), slash Pine (*Pinus elliottii* Engelm.) (Harrington et al. 1999, Murphy and Harrington 2004) and in Douglas–fir (*Pseudotsuga menziesii* Mirb.) (Littke and Zabowski 2007b, Middleton et al. 1989, Spicer et al. 2000, Vargas-Hernandez et al. 2003).

Stem deformation has been widely studied in *Pinus radiata* because intensively managed plantations often develop moderate to severe stem deformations. Carlyle et al. (1989) studied 10-year-old *Pinus radiata* plantations growing on former pasture sites in Australia, and found that over 90% of the trees in some areas were affected by stem deformations and over 40% of these trees were severely deformed and were unacceptable for commercial use. The first symptoms of stem deformation in radiata pine have been observed as early as one and a half years after planting, although the more severe deformations occurred in the leading shoot at three to six years of age (Bail and Pederick 1989). Bail and Pederick (1989) also reported that this deformity has been called 'severe stem deformity', 'speed wobble', 'stem distortion', 'poor form', and even 'Toorour Syndrome' after the locality in which it was first described. Trees affected by this type of stem deformation are characterized by kinking and

twisting of stems, looping (which is caused when leaders lose the ability to maintain vertical growth), a loss of apical dominance, and the formation of numerous thick branches (Bail and Pederick 1989, Hopmans et al. 1995). This deformation appears to be under genetic control and is stimulated by high nitrification in the soil (Turvey et al. 1993). In addition, strong genetic variation in radiata pine with the Toorour syndrome has been reported (Pederick et al. (1984), Hopmans et al. 1995).

Spicer et al. (2000) found, in Douglas-fir, that trees that were highly sinuous one year were more likely to be sinuous in following years, suggesting a genetic predisposition to sinuosity. Shelbourne et al. (1969) also reported that bole straightness and the spiral crooks in loblolly pine were strongly heritable.

Several studies have been conducted to determine the possible causes of stem sinuosity. In radiata pine, the syndrome has been associated with previous land use, high-N levels, and copper (Cu) deficiency. It has also been reported in New Zealand, where deformed trees appeared to be adequately supplied with Cu (Hopmans et al. 1995, Hopmans 1990). Birk (1991) found that stem deformities were correlated with foliar Mn, Al, and Ca. Recent investigations suggest that sinuosity could be caused by biomechanical factors such as elongation direction, amount and position of axillary loads (branches), stem dimensions, wood elasticity, radial growth dynamics and active re-orientation due to reaction wood. In addition, differences in nutritional and physiological levels within the tree and surrounding soil, such as a deficiency in Cu, could cause poor lignification of woody tissue and cause stem deformation (Gartner and Johnson 2006, Almeras et al. 2004, Spicer et al. 2000, Downes et al. 1994).

During the last 25 years, there have been an increasing number of reports regarding the physiological disorders of plants associated with inadequate Ca nutrition (Kirkby and Pilbeam 1984). Calcium is a relatively large divalent cation, and most plants can adapt to a range in its supply (Marschner, 1995, Tinker and Läuchli, 1984). In contrast with other macronutrients, a high proportion of the total Ca in the plant tissue is often located in the cell walls (apoplasm). In the apoplasm, some Ca is firmly bound in structures; while other Ca is exchangeable through the cell walls and the exterior surface of the plasma membrane. The mobility of Ca in the plant is low. It is moved largely in the xylem, and to a very limited extent, the phloem (Marschner, 1995). Physiological disorders resulting from localized Ca deficiencies within plants have been attributed to poor Ca distribution, rather than restricted uptake. Factors that influence the distribution of Ca, such as soil moisture (Ca uptake by the whole plant is mostly passive and follows the influx of water), root pressure, and phytohormone activity, can also affect the occurrence of these disorders (Kirkby and Pilbeam 1984).

Calcium plays an important role in wood formation. Calcium is absorbed by the plant as Ca^{2+} from the soil solution and is supplied to the root surface by mass flow and root interception. Calcium, bound as pectate in the middle lamella, is essential in the structure and permeability of cell membranes, strengthening the cell walls and plant tissue (Havlin et al. 1999, Marschner, 1995). Calcium deficiency may cause the disintegration of cell walls and the collapse of the affected tissues, such as the petioles and upper parts of the stems (Marschner 1995).

Calcium uptake is depressed by NH_4^+, K^+, Mg^{2+}, Mn^{2+}, and Al^{3+}. It also has been reported (Kirkby and Pilbeam 1984) to be dependent on of counter anion (any ion that accompanies an ionic species in order to maintain electric neutrality), being highest with NO_3^-, followed by Cl^-, and then SO_4^{2-}. Conditions impairing the growth of new roots will reduce root access to Ca^{2+} and induce deficiency. Plants with smaller root systems are more likely to have problems related to inadequate Ca^{2+} uptake than those with more highly developed root systems (Havlin et al. 1999). Nitrogen fertilization is commonly used to increase growth in forest plantations. However, this increment in growth by N additions places higher demands on the soil for Ca and other nutrients. Inadequate Ca nutrition has been associated with physiological disorder of plants affecting the structure of cell membranes.

Some studies have shown a negative effect of N on stem sinuosity (Turvey et al. 1993, Carlyle et al. 1989, Downes and Turvey 1990) and the importance of Ca in the stiffness of the cell wall. Our study was conducted to determine the effects of N and Ca additions on stem sinuosity in different loblolly pine genotypes. Our hypotheses were that genotypes growing on soils with high N availability have higher stem sinuosity, and genotypes growing on soils with low Ca availability exhibit higher stem sinuosity.

2.2. Materials and Methods

2.2.1 Site description

The study was undertaken on two ArborGen research sites in South Carolina. Site 1 was located in Berkeley County on a poorly drained clayey Byars soil (fine, kaolinitic, thermic

umbric paleaquult). Precipitation averaged 1120 mm/year, and was evenly distributed throughout the year. Long-term mean air temperatures were 26°C in the summer and 14°C in the winter. Site 2 was located in Orangeburg County on a moderately well-drained loamy Goldsboro soil (fine-loamy, siliceous, subactive, thermic aquic paleudult) Precipitation at this site averaged 1257 mm. Long-term mean air temperatures were 24°C and 11°C in the summer and winter, respectively.

Site preparation at site 1 consisted of a broadcast application of glyphosate (41%) (11.5 liters / hectare) in July 2004, double bedding in September, and planting in October. The following years (2005 – 2008) in spring (March) and summer (July) 0.22 liter/hectare of Oust[®] (sulfometuron) and 0.07 liter/hectare of Escort[®] were applied in bands to control grasses, vines, and herbaceous vegetation. The plantation spacing was 7.3 m between rows and 1.83 m within the rows.

The site preparation at site 2 was done with similar weed control to that done in site 1. However, unlike site 1, site 2 had only one family-block (Fam-3) planted on double-bedded beds. The other two family blocks were not planted on beds. The weed control at site 2 was the same manner as for site 1. The plantation spacing was 5.5 m between rows or beds and 1.83 m within the rows.

2.2.2 *Experimental design and treatments*

The study was established when the plantations were 2 years old. In March 2007, one open-pollinated family (OP-1), three mass control pollinated (e.g. full-sib) families (MCP-1, MCP-2, MCP-3), and two clone varieties (CV-1, CV-2) were used as genetic material. At site

1, OP-1, MCP-2, MCP-3, CV-1, and CV-2 were used. At site 2, only MCP-1, MCP-3 and OP-1 were present. MCP-1 and MCP-2 represent reciprocal crosses of the same parents.

In each family block, twenty consecutive plantation rows were selected, and every other row was established as a block (resulting in 10 blocks (replicates) nested within each family block). Within each rep (study block), four treatment plots (3 trees per plot) were randomly established. Calcium (0 and 168 kg/ha) and N (0 and 224 kg/ha) were added as a 2 x 2 factorial design with calcium sulfate ($CaSO_4$) and ammonium sulfate [$(NH_4)_2SO_4$] as Ca and N sources. The nutrients were manually applied in two bands on both sides of the trees in the direction of the plantation row on March 30, 2007. Two or three non-treated trees were used as buffer between treatment plots.

2.2.3 *Measurements, sampling and nutrient analysis*

The scale used to assess stem sinuosity ranged from 1 to 5, with 1 being the straightest trees and 5 the most sinuous trees (Figure 2-1). The presence or absence of branch sinuosity was also assessed; 0 being the trees that did not exhibit branch sinuosity and 1 as the trees that exhibited branch sinuosity. After all the trees were scored for stem and branch sinuosity, the middle tree from each 3-tree plot was selected for tissue sampling for nutrient analysis. From each sample tree, tissue samples were collected from the newly expanding shoots (1st flush) closest to the leader or apical meristem of the 2007 growing season. Collections were carried out during the first and third weeks of April 2007, as well as the first week of May 2007. The collection times were selected during the early growing season, when N

concentrations have been found to be high and Ca concentrations low (Price 2000). Samples were labeled to indicate their family block, rep within family, treatment, and collection time.

Only one sinuosity assessment was done for the first two tissue collections due to short time intervals between the collections. However, stem sinuosity was assessed four more times from May 2007 to June 2008. In each assessment, stem sinuosity was scored by flush on the terminal shoot of 2007 growing season, and later these scores were averaged to obtain a mean stem sinuosity score for each tree.

The shoot samples were oven-dried at $70^{o}C$ for 72 hours. After oven-drying, needles were removed from the woody shoot and the woody tissues were ground in a Wiley mill to pass a 0.25-mm mesh sieve. Each sample, which contained ~ 8 mg of tissue, was pulverized, encapsulated in an aluminum tin, and combusted to determine nitrogen (N) concentrations using the CHN elemental analyzer (CE Instruments-NC 2100, CE Elatech Inc., Lakewood, NJ) at $1000^{o}C$. Phosphorus (P), potassium (K), Ca, magnesium (Mg), Mn, sulfur (S), B, and Zn concentrations were determined by wet–digesting a 0.16 or 0.4 g sample (based on the tissue availability) with a mixture of nitric acid and hydrogen peroxide (Zarcinas et al. 1987). Next, spectrometry analysis was done using an inductively coupled plasma atomic emission spectrometer (IPS-AES, Varian ICP, Liberty Series 2, Varian analytical instruments, Walnut Creek, CA) (Zerpa 2005).

2.2.4 Statistical analysis

The effects of family block, nutrient additions, and their interaction on stem sinuosity were examined using the MIXED procedure in SAS software (V 9.1.3, 2003), for main and

interaction effects. Blocks within families, family, Ca addition, N addition, assessment day, and their interactions were considered fixed effects. Random effects correspond to, i) plot to plot variation receiving the same treatment combination (block*treatment(family)), and ii) residual random variation associated with individual assessments within each assessment day. Residual random variation among assessment days was modeled as an unstructured 5x5 covariance matrix, with values on the diagonal representing the residual variance for each assessment (day) and off-diagonal values representing the covariance between two distinct-day assessments. Residual correlation between distinct-day assessments, after controlling for other effects in the model, was calculated using the residual covariance matrix. The covariance parameter for block x treatment(family) was estimated to be zero and it was pooled with the residual variance in the final model. Satterthwaite's rule for calculation of denominator mean square (MS) and degrees of freedom in Type 3 F-test of fixed effects was used.

An analysis of variance was carried out for each dependent variable (stem sinuosity, branch sinuosity, height, and nutrient concentrations in the shoot woody tissue) to analyze the effects of N and Ca additions on these dependent variables. Because only two families (OP-1 and MCP-3) were present in both sites, and one of these families (OP-1) was planted using different site preparation in each site (site1 on beds, site 2 without beds), it was not possible to carry out analyses across sites to assess the genotype by environment interaction. Therefore, we ignore the site effects, and the analyses were conducted across the 8 family blocks across the two sites. Our primary objective was to examine the effect of N and Ca additions on stem sinuosity using the genetic materials available, not to test the genetic

differences. In fact, we are using only six different genotypes, so the genetic base is narrow regarding the genetic variation in stem sinuosity.

The phenotypic correlations between stem sinuosity and branch sinuosity, stem sinuosity and height, and branch sinuosity and height were estimated at the family-block mean level using the correlation procedure from SAS software (V 9.1.3, 2003).

The significance level used in all the analyses was 0.1 and the model used was the following:

$$
\begin{aligned}
y_{ijklm} = {} & \mu + F_i + B_{j(i)} + Ca_k + N_l + (Ca*N)_{kl} + (F*Ca)_{ik} + (F*N)_{il} + (F*Ca*N)_{ikl} + d_m + (F*d)_{im} \\
& + (Ca*d)_{km} + (N*d)_{lm} + (Ca*N*d)_{klm} + (F*Ca*d)_{ikm} + (F*N*d)_{ilm} + (F*Ca*N*d)_{iklm} + \varepsilon_{ijklm}
\end{aligned}
$$

Where:

y_{ijkl} is a trait's value in the mth assessment within the plot in the jth rep for the ith family receiving the nutrient treatment given by the combination of lth nitrogen level and k^{th} calcium level.

μ is the overall mean;

F_i, is the ith family effect ($\sum F_i = 0$), $i = 1, \ldots, 8$

$B_{j(i)}$ is the jth block effect within i^{th} family ($\sum B_{j(i)} = 0$), $j = 1, \ldots, 10$

Ca_k is the kth effect of calcium nutrient ($\sum Ca_k = 0$), $k = 1, 2$

N_l is the lth effect of nitrogen nutrient ($\sum N_l = 0$), $l = 1, 2$

d_m is the mth assessment effect ($\sum d_m = 0$), $m = 1, \ldots, 5$

$(Ca*N)_{kl}$ is the interaction effect of kth calcium and l^{th} nitrogen nutrients ($\sum Ca*N_{kl} = 0$);

$(F*Ca)_{ik}$ is the interaction effect of kth calcium nutrient level and i^{th} family ($\sum (F*Ca)_{ik} = 0$)

$(F*N)_{il}$ is the interaction effect of lth nitrogen nutrient level and i^{th} family ($\sum (F*N)_{il} = 0$)

$(F*Ca*N)_{ikl}$ is the interaction effect of kth calcium nutrient level and l^{th} nitrogen nutrient level

and i^{th} family ($\sum(F*Ca*N)_{ikl}=0$)

$(F*d)_{im}$ is the interaction effect of mth assessment and i^{th} family ($\sum(F*d)_{im}=0$)

$(Ca*d)_{km}$ is the fixed interaction effect of the mth assessment with the kth calcium nutrient

level ($\sum(Ca*d)_{km}=0$)

$(N*d)_{lm}$ is the interaction effect of the mth assessment with the lth nitrogen nutrient level

($\sum(N*d)_{lm}=0$)

$(Ca*N*d)_{klm}$ is the interaction effect of the mth assessment with the lth nitrogen level and k^{th}

calcium level ($\sum(Ca*N*d)_{klm}=0$)

$(F*Ca*d)_{ikm}$ is the interaction effect of the mth assessment with the kth calcium nutrient level

and i^{th} family ($\sum(F*Ca*d)_{ikm}=0$)

$(F*N*d)_{ilm}$ is the fixed interaction effect of the mth assessment with l^{th} nitrogen nutrient level

and the ith family ($\sum(F*N*d)_{ilm}=0$)

$(F*Ca*N*d)_{iklm}$ is the interaction effect of the mth assessment with k^{th} calcium level and l^{th}

nitrogen level and the ith family ($\sum(F*Ca*N*d)_{iklm}=0$)

e_{ijklm} is the random effect of the mth assessment with k^{th} calcium level and l^{th} nitrogen level in

the jth block and the ith family; $e_{ijklm} \sim N(0,\sigma_m^2), \quad Cov(e_{ijklm},e_{ijklm'})=\sigma_{m,m'}$

The relationship among the levels of multiple nutrients measured within the sample tissues and their effects on stem sinuosity and branch sinuosity were examined using canonical correlation analysis (CANCORR procedure) in SAS software (V 9.1.3, 2003). Canonical correlation assesses the relationship between independent variables and multiple

dependent measures. It allows for an examination of the relationships among the linear combination of a set of explanatory variables (N, P, K, Ca, Mg, Mn, Zn, B, Cu, and Zn) and the response or dependent variables (stem sinuosity and branch sinuosity). In the canonical correlation analysis, our first attempt was to derive a linear combination (canonical variate) of the variables for each data set (nutrients and dependent variables) so that their correlations would be maximized and at least as large as the multiple correlations between any variable in one data set and those from the other data set. In addition, this procedure determined sets of canonical variates (vectors) that contained the coefficient (weight) of each nutrient and each dependent variable to maximize the correlation among the data sets. The first pair of canonical variates always has the highest canonical correlation. Next, the second set of canonical variables, uncorrelated with the first pair, which produced the second highest correlation coefficient (second canonical correlation), was found. Since the dependent data set contained two variables (stem sinuosity and branch sinuosity), consequently two pairs of canonical variates were produced. In this way, we obtained the most precise relationship between the nutrient dynamics of the tissue located at the tree's deformation site, along with stem and branch sinuosity.

2.3. Results and Discussion

2.3.1 *Sinuosity responses to nutrient additions*

Nitrogen, and Ca additions and their interactions had significant effects on stem sinuosity (Table 2-1, Figure 2-2 and 2-3). Given these effects, only the application of N alone resulted in significant changes in stem sinuosity as compared to the control (Figure 2-3). Nitrogen

increased the sinuosity score from 1.73 in the control to 1.83 in the N-only treatment and increased the proportion of trees affected by severe sinuosity by 12%; 21% of trees with scores of 4 or 5 in the control, vs. 33% of trees with scores of 4 or 5 in the N-only treatment. In addition, it is also important to point out that across treatments that 90% of the trees in study exhibited sinuosity to some extent.

These findings are similar in the effect of nutrient additions on stem sinuosity found in chapter 1. Our findings are also similar to those results found in other studies where high N levels were associated with incremental stem deformation in radiata pine (Birk 1991, Carlyle et al. 1989, Hopmans et al. 1995, Turvey et al. 1993). In fact, Hopmans et al. (1995) reported that in a study where high doses of N (200 and 600 kg ha^{-1}) were added, stem sinuosity increased from 12% to 56% affecting the potential loss in merchantable volume due to the severe deformations.

Calcium reduced the negative effect of N addition on stem sinuosity when it was applied with N. The Ca plus N treatment reduced the proportion of trees affected with severe sinuosity by 12% compared to trees in the N-only treatment. Our results were similar to those found in a study of Douglas-fir, where Ca additions did not reduce stem sinuosity when applied alone (Littke and Zabowski 2007b). The small but consistent reduction in sinuosity with Ca additions (Figure 2-4) suggest that Ca additions may maintain this same trend during the future years as the Ca is absorbed by the roots and is transported up the tree where new tissues are growing, and nutrient demands are high.

Havlin et al. (1999) pointed out that plants with smaller root systems are more likely to have problems related to inadequate Ca^{2+} uptake than those with more highly developed root

systems, and that Ca is transported slowly within trees. Therefore, the positive effect of Ca on stem sinuosity when it was added with N could be due to N additions increasing the leaf area index (LAI) (Vose and Allen 1988) and hence the amount of solar radiation intercepted by the canopy and the subsequent increase in the amount of carbon fixed (Dewar et al. 1994). This increment in the amount of carbon fixed may increase the amount of carbon allocated to the root system and increase the Ca uptake by the plants. In fact, Nadelhoffer et al. (1985) found that when N availability increased, the belowground carbon allocation was also increased. Zhang and Zak (1998) found that nitrogen fertilization increased root growth.

Family block differences were significant (Table 2-1). The family blocks that showed less stem sinuosity were MCP-1, OP-1 (in both sites), and MCP-2 (1.5, 1.6, 1.6, and 1.7) respectively. The family blocks with the highest stem deformation were MCP-3 (2) (site 2), followed by the variety CV-1, MCP-3 (1) (site 1) and variety CV-2 (2.0, 1.9, 1.9, and 1.8) respectively (Figure 2-5). Family-block differences were significant, but these differences were confounded with location and site preparation methods, since some of the families were present on only one site and different silvicultural treatments were applied to some families. Assessment times (date) and the interactions of N and Ca by date were significant. The family block by N and the family block by Ca interactions were significantly different for branch sinuosity. Family block by date interaction was significant for stem sinuosity, branch sinuosity, and height (Table 2-1).

Even though only six family blocks were included in this study, and different site preparation among family block was done, these results suggest that that there may be genetic potential to improve stem deformation. However, studies that include more families

and an appropriate experimental design should be used to assess the genetic potential to improve stem form such as the study used in Chapter 1 and the studies used by McKeand and Jett (1993), where they showed that there are family differences in stem sinuosity.

Family block means for stem sinuosity and height were not significantly correlated (r = 0.26, p-value < 0.1), nor were they correlated for branch sinuosity and height (r = 0.18, p-value <0.1). These results corresponded with the results found within provenances in a study where four provenances and different families within provenances of loblolly pine were assessed for growth and stem sinuosity (McKeand and Jett, 1993). However, the authors found a significant correlation when all the families were combined (McKeand and Jett 1993). Results from a study with Douglas-fir showed a positive correlation between stem sinuosity and height growth during the first growing season, yet there was very weak correlation the following year (Littke and Zabowski 2007b).

There was a strong positive family-mean correlation between stem sinuosity and branch sinuosity (r=0.87) (Figure 2-6). This strong positive correlation can be exploited in a breeding program because, depending on the selection age, the magnitude of stem sinuosity in mildly affected trees could become disguised by the subsequent diameter growth. Therefore, use of the branch sinuosity assessment could be very helpful during selection time to improve assessment of stem sinuosity or form. This corresponds with the observation of McKeand and Jett (1993) that if a tree displays sinuous branches but not a sinuous stem, then there is still a strong likelihood that its progeny will have sinuous stems.

2.3.2 *Nutrient concentration responses to nutrient additions*

Nitrogen addition affected only the N concentrations (1.63% no N added vs. 1.81% when N was added) (Table 2-1). Similar results were found in a study with similar objectives (sinuosity assessment) and where the treatments were control (no nutrient addition) and fertilized using five different families from two different provenances (Chapter 1). Unlike N, Ca addition did not affect the concentration of the different nutrients analyzed (Table 2-2). Date (days of sample collection) was significant in all of the nutrient analyses. The interaction of N addition by date was significant only for N and Mn elements. N levels increased through time while Mn decreased, showing a possible negative effect of one nutrient with respect to the other nutrient. This negative effect corresponds with the finding in the study described before (Chapter 1). The family block by N addition interaction was significant only for N and K elements.

These findings indicate that N additions changed the N levels of the woody tissues significantly, with N exhibiting a negative effect on stem sinuosity. Even though a high Ca amount (168 Kg/ha) was added as $CaSO_4$, there was no evidence of any change in the Ca concentrations or other nutrients within the tree. A possible explanation is that the effects of Ca addition require more time to be exhibited within the tree. The assessments of this study should be continued for at least 2 to 4 more years to determine if Ca addition exhibits any effect on stem sinuosity once the Ca has been absorbed by the roots and redistributed within the tree. The results from the nutrient analyses of the woody tissue agree with the results obtained from the phenotypic assessment where N addition had effects on the N levels in the

woody tissues and on stem sinuosity, while Ca addition did not show any effect on nutrient levels in the woody tissue nor stem sinuosity.

Results from the canonical correlation (CANCORR) analysis used to examine the relations between the ten nutrient variables and two dependent variables showed that the two canonical variate pairs extracted were significant ($p < 0.1$). The first canonical variate accounted for 79% of the total variation and a canonical correlation of 0.79, while the second canonical variate accounted for 21% of the total variation and its canonical correlation was 0.55. The correlation between stem sinuosity and the two canonical variates (vectors) were -0.76 and 0.13, and branch sinuosity was -0.56 and 0.38. The coefficients (weights) of the vector, V1, suggest that changes in P, Mn, Zn, and Ca levels could have a greater effect on stem and branch sinuosity (Table 2-3).

These findings indicate that several nutrients may be involved in the stem deformation, and high concentrations of P could be associated with stem sinuosity. Zinc also showed the potential to be associated with stem sinuosity; however, the Zn level from the flushing tissue indicated that it was adequate when it was compared with the value reported as adequate for radiata pine by Tausz et al. 2004. Therefore, it could be unlikely that Zn could have caused stem deformation in the families tested.

Low concentrations of Mn and Ca also showed the potential for increasing stem deformation. The correlations between low Ca concentrations and increased stem sinuosity are different from those found in the two studies with Douglas-fir (Littke and Zabowski 2007a, Littke and Zabowski 2007b), where Ca addition increased soil and foliar Ca, but did not cause a decrease in sinuosity. The relationship between low Mn and increased stem

sinuosity is in agreement with those reported by Raupach et al. (1978) and the found in Chapter 1 where low Mn was associated with stem sinuosity. However, in contrast to those results, Birk (1991) found that Mn, P, N, and Ca concentration were higher in deformed trees than non-deformed trees. The low Mn level found in this study could be associated with the N source used [$(NH_4)_2SO_4$]. It is a well-known phenomenon that NH^{+4} has an antagonistic effect on the uptake of most cationic elements that are essential to plants such as Mg, Cu, Zn, and Ca (Husted et al. 2004). The high N level added may also have been a possible cause of low Mn. In addition, Mn level showed significant changes (reduction) throughout the time with the addition of N (Table 2-1). Nitrogen is one of the most important nutrients to increase growth in the southern areas of US (Fox et al. 2007, Allen et al. 2005). This increase in growth promotes a higher demand for other nutrients such as Mn, which are characterized as having limited mobility in the soil, and are transported solely by slow diffusion (Rengel and Marschner, 2005). This may have resulted in a Mn deficiency.

2.4. Conclusions

Our results indicated a significant effect of nutrient additions on stem sinuosity. Nitrogen additions caused a substantial increase in the stem sinuosity score and on the N level in the woody tissues. Trees treated with Ca only did not exhibit a significant reduction in stem sinuosity; however, Ca additions reduced stem sinuosity when N was also added. Based on the correlation analysis, the elements that exhibited the highest associations with stem and branch sinuosity formations were P, Mn, and Ca. In addition to significant effect that N showed on stem sinuosity, it also affected the ratios of N with the other nutrients (data not

shown), which may cause further nutrient imbalances within the tree. It could be due to high dose of N added as reflected in the woody tissues. A study with higher number of families, sites, and different doses of N, P, Mn, and Ca additions throughout several growing seasons may show whether the ratio of these nutrients is directly related to stem sinuosity formation. So far, Ca addition has not changed the concentration levels of any of the nutrients assessed, suggesting that this study should be continued for at least 2 – 4 more years to evaluate better whether Ca only has any effect on stem sinuosity. Based on our findings, stem deformation is clearly affected by environmental differences and probably affected by genetics. This highlights the importance of matching appropriate site and cultural treatments with suitable genotypes. The choice of these three components has a large impact on the productivity and quality of the plantation.

2.5. Acknowledgements

2.6. Literature Cited

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Table 2-1. Analysis of variance results (P-values) for stem and branch sinuosity scores, height, and nutrients level in the woody tissues. Type 3 tests of fixed effects were used. Bold and shaded numbers indicate significant difference at P-value <0.1.

Source	Stem sinuosity	Branch sinuosity	Height	Dependent variables — Woody shoot nutrient concentrations									
				N	P	K	Ca	Mg	Mn	Zn	B	Cu	S
Family-block	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**
Rep(Family-block)	**<.01**	**<.01**	**<.01**	**<.01**	**0.02**	**<.01**	**<.01**	**<.01**	**<.01**	**0.03**	0.19	0.17	**<.01**
N	**<.01**	0.18	0.46	**<.01**	0.16	0.70	0.89	0.53	0.17	0.72	0.78	0.99	0.25
Ca	**0.01**	0.91	0.80	0.15	0.50	0.95	0.68	0.58	0.10	0.39	0.21	0.46	0.47
N*Ca	**0.05**	0.48	0.11	0.55	0.98	0.45	0.46	0.31	0.77	0.29	0.40	0.38	0.22
Date	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**
N*Date	**<.01**	0.80	0.55	**<.01**	0.42	0.86	0.13	0.78	**0.04**	0.67	0.21	0.09	0.13
Ca*Date	**0.07**	0.75	0.93	0.64	0.40	0.98	0.33	0.39	0.20	0.86	0.27	0.26	0.67
N*Ca*Date	0.66	0.82	0.92	0.21	0.92	0.55	0.72	0.50	0.48	0.66	0.50	0.46	0.98
Family-block*N	0.33	0.14	0.52	**0.08**	0.74	**0.07**	0.19	0.37	0.60	0.91	0.65	0.48	0.38
Family-block*Ca	0.44	**0.06**	0.55	0.14	0.44	0.98	0.33	0.27	0.84	0.75	0.72	0.22	0.75
Family-block*N*Ca	0.77	**0.04**	1.00	0.92	0.43	0.45	0.98	0.27	0.16	0.50	0.65	0.81	0.83
Family-block*Date	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**
Family-block*N*Date	0.96	0.98	0.44	0.32	0.95	0.52	0.19	0.83	0.32	0.93	0.40	0.94	0.84
Family-block*Ca*Date	0.64	0.63	0.98	0.80	0.12	0.53	0.49	0.21	0.64	0.40	0.89	0.57	0.71
Family-block*N*Ca*Date	0.76	0.95	0.96	0.55	0.39	0.43	0.65	0.83	0.97	0.54	0.52	0.44	0.63

Table 2-2. LS means of nutrient concentrations from the woody tissue of the newly expanding shoots (flush) closest to the leader or apical meristem for main effects.

Effects	Woody shoot nutrient concentrations									
	N	P	K	Ca	Mg	Mn	Zn	B	Cu	S
	%	%	%	%	%	ppm	ppm	ppm	ppm	%
N not added	1.63	0.22	1.15	0.10	0.08	137.86	41.53	0.53	5.59	0.11
N added	1.81	0.21	1.16	0.10	0.08	131.91	41.28	0.53	5.59	0.12
Ca not added	1.74	0.22	1.15	0.10	0.08	138.46	41.70	0.52	5.64	0.12
Ca added	1.70	0.22	1.15	0.10	0.08	131.31	41.11	0.53	5.55	0.12
N&Ca not added	1.64	0.22	1.16	0.10	0.08	136.11	42.19	0.74	5.69	0.11
N&Ca added	1.79	0.21	1.16	0.10	0.08	134.92	41.35	0.74	5.60	0.12

Table 2-3. Standardized canonical coefficients for nutrient variables. The highlighted numbers correspond to those coefficients of the variables that mainly drive the values of the vectors V1 and V2 at $P < 0.1$. V1 was driven mainly by four variables while V2 was driven by six variables.

Nutrient	V1	V2
N	0.06	0.37
P	-1.06	1.01
K	0.48	0.28
Ca	0.69	-1.09
Mg	-0.07	-0.23
Mn	-0.98	0.14
Zn	0.85	-1.91
B	0.30	0.72
Cu	0.43	0.97
S	0.12	0.65
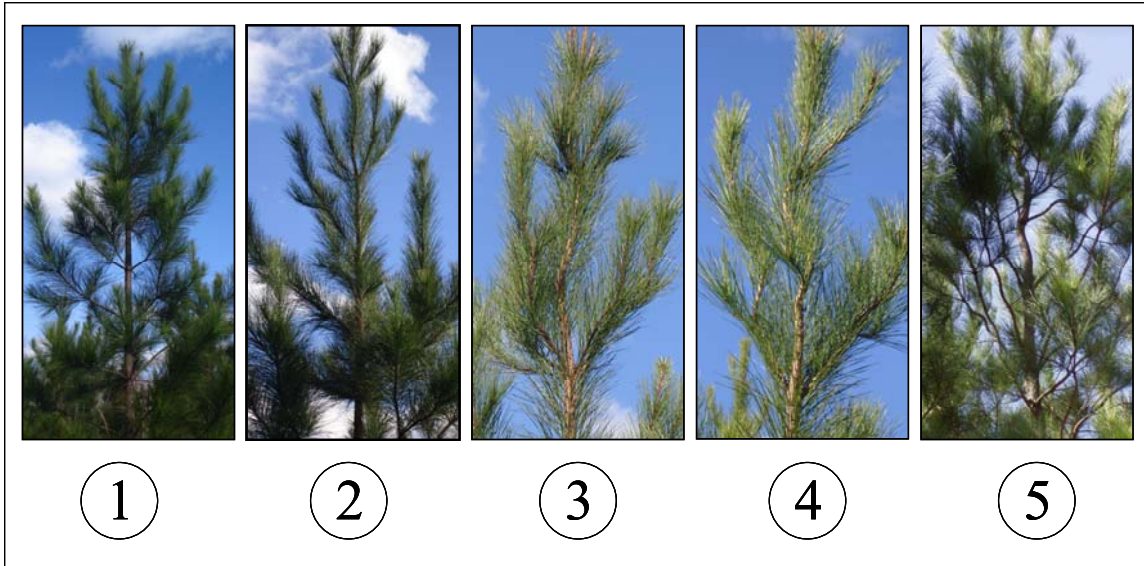
Figure 2-1. Stem sinuosity scores used to assess the trees, with 1 being the straightest and 5 the most sinuous trees.
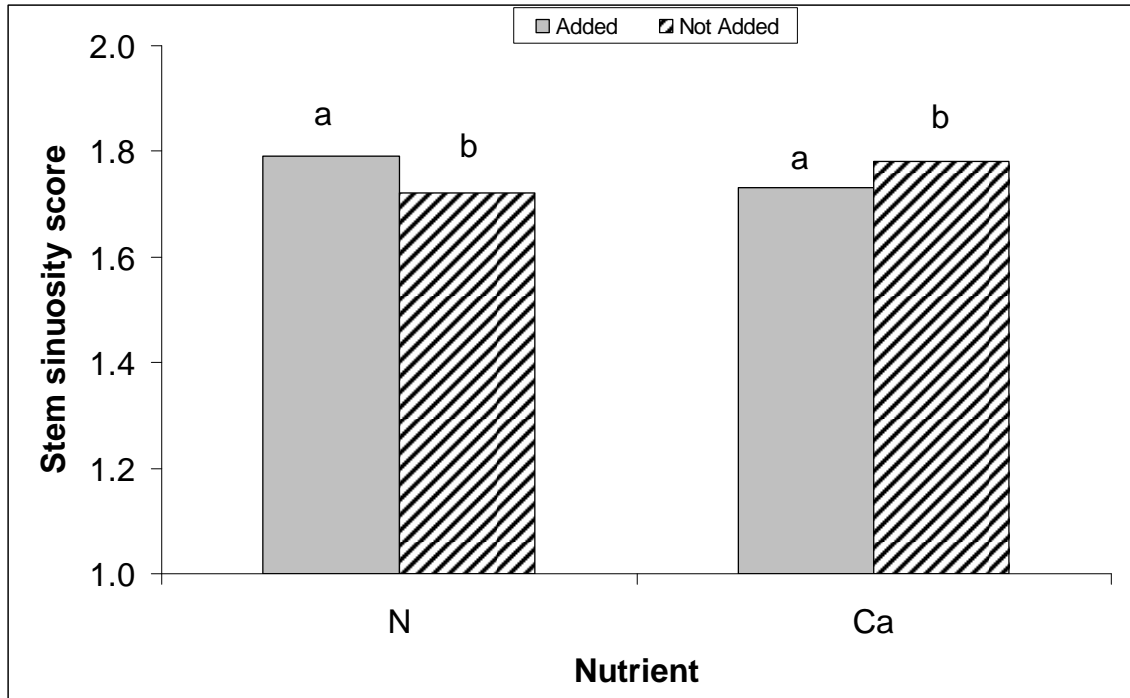
Figure 2-2. Mean stem sinuosity score versus N and Ca main effects. Bars with the same letter are not significantly (P<0.1) different.
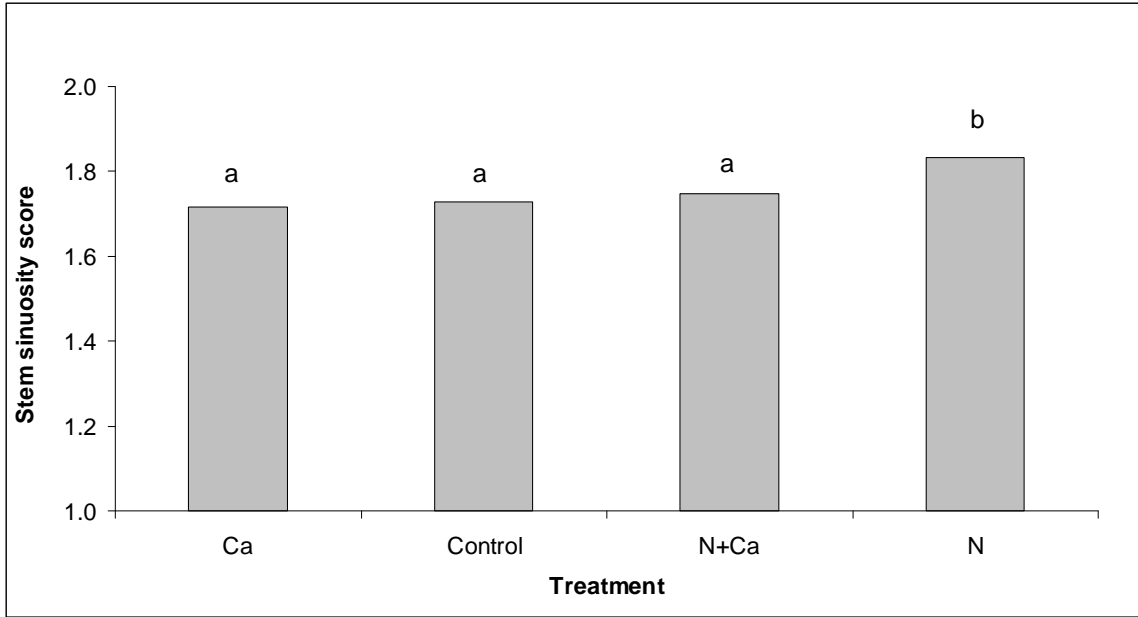
Figure 2-3. Mean stem sinuosity score by nutrient treatment. Treatment means with the same letter were not significantly (P<0.1) different.
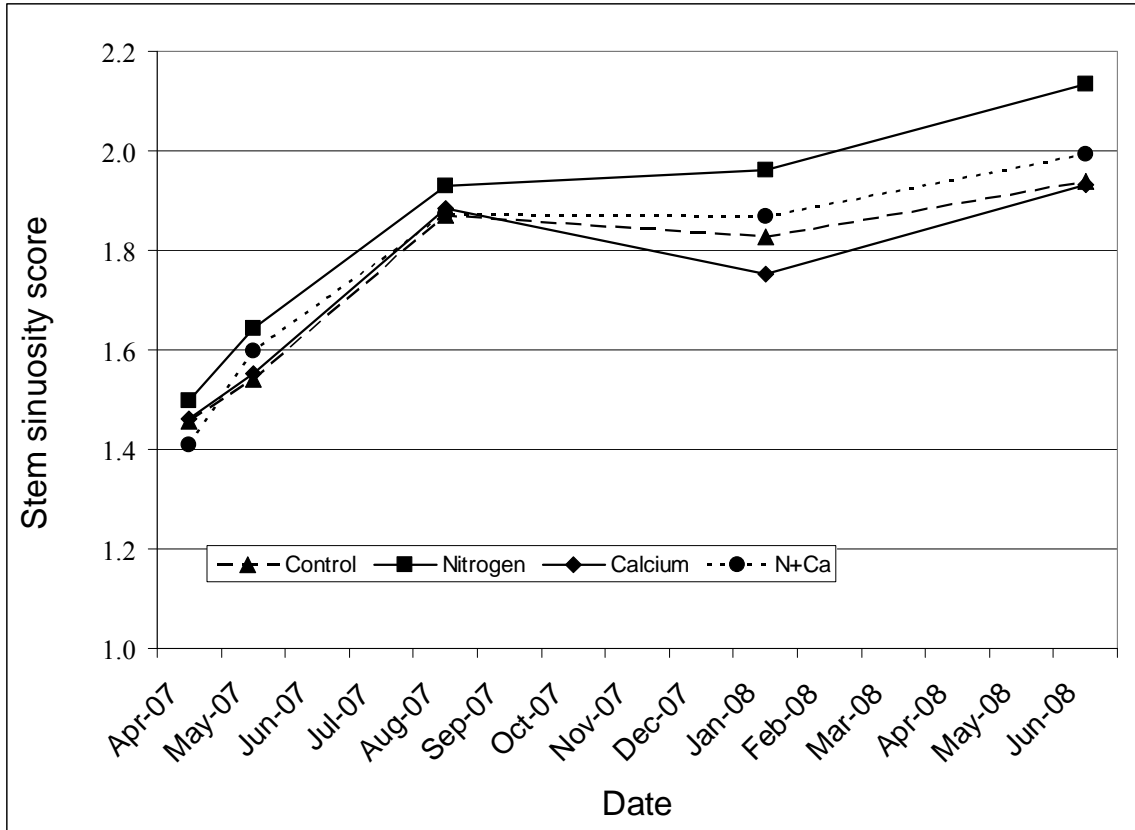
Figure 2-4. Mean stem sinuosity score by assessment date for different treatments.
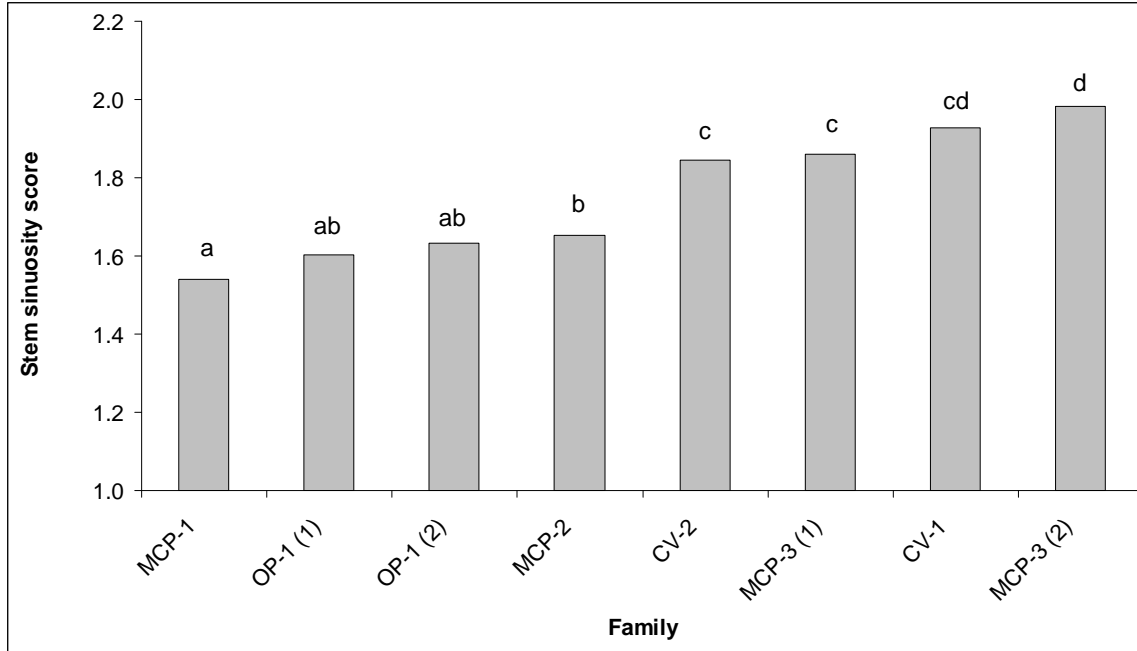
Figure 2-5. Mean stem sinuosity score for each family block. The family block effect was highly significant. The number within the bracket corresponds to site where the family block was planted. Family block means with the same letter are not significantly (P<0.1) different.
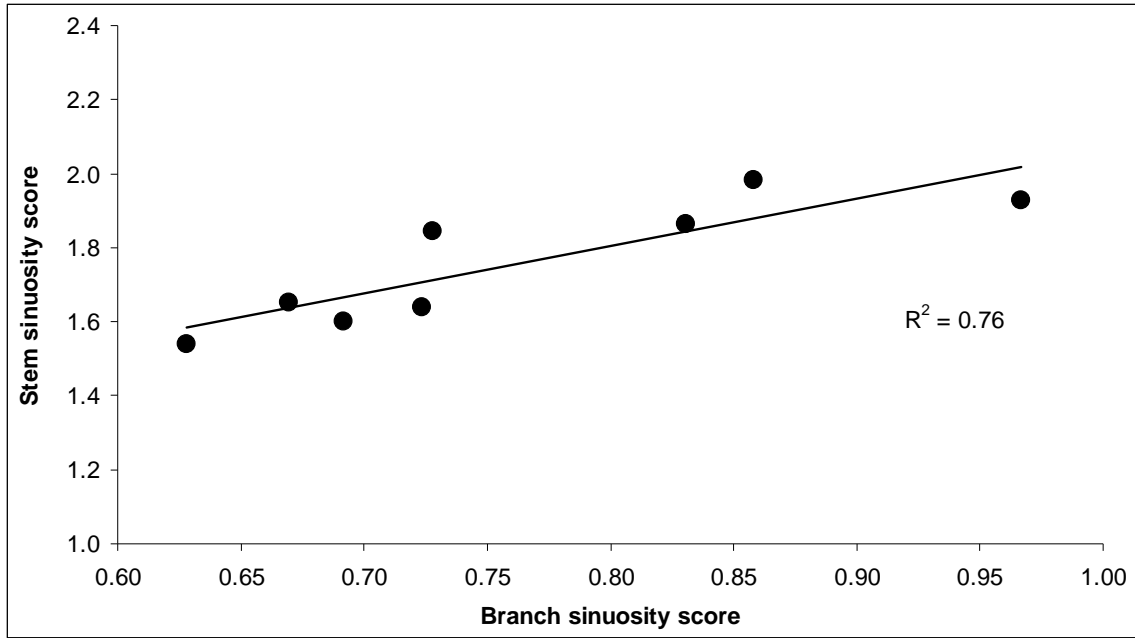
Figure 2-6. Phenotypic correlation between stem sinuosity and branch sinuosity. Each point is for a family block. (P<0.1).

3. CHAPTER

Responsiveness of Diverse Families of Loblolly Pine to Intensive Fertilization: Stem Form

Traits at Twelve Years

Abstract

Genetically improved loblolly pine *(Pinus taeda* L.) trees under intensive silviculture have demonstrated dramatic increases in wood production, but the effect of intensive culture on tree form and wood quality traits is less well understood. The responses of five loblolly pine families from each of two provenances [Atlantic Coastal Plain (ACP) and Lost Pines Texas (LPT)] to fertilization were assessed at age twelve years in a test located in the Sandhills region of North Carolina. Quantitative traits were assessed for all of the inner 64 trees in each plot. Tree height and diameter at breast height were recorded as well as presence of fusiform rust galls, and the stem form traits of straightness, sweep, forking, and ramicorn branching. This site has severe nutrient limitations, and all the development and stem form traits assessed, except sweep, changed with the fertilization throughout the study period. Trees were 66% taller in the fertilized plots than in the control plots, and volume per hectare was 2.6 times greater in the fertilized trees than in the non-fertilized plots. Stem straightness, forking, and ramicorn branching were all negatively impacted by the fertilizer treatment, especially in the LPT provenance. The ACP provenance showed 34% more susceptibility to fusiform rust (caused *by Cronartium quercuum* f. sp. *fusiforme* (Berk) Miyabe ex Shirai) than the LPT provenance. So far, the results are consistent with other trials where western seed sources were more disease and drought resistant but were slower growing than eastern sources. In addition, the results of this study show that a combination of appropriate silviculture and genetics is needed to optimize performance for both growth and quality traits.

3.1. Introduction

The southern United States produces more industrial timber than any other region of the world from a forest base of almost one-half of the world's industrial forest plantations (Prestemon and Abt 2002). Loblolly pine is the most important pine species used in the Southern United States, and in the early 2000's, more than 1 billion genetically improved loblolly pine seedlings were planted annually by forest industry and non-industrial private forest landowners (McKeand et al. 2003a). Genetic gains from tree improvement programs have been large, with average estimated gains in volume over non-improved loblolly pine between 15 to 20% for Coastal Plain and Piedmont regions of North Carolina, South Carolina, and Georgia (McKeand et al. 2006a). When improvements in stem form and disease resistance (such as fusiform rust) are considered, the gains in value are much higher.

Nevertheless, realization of genetic potential is only obtainable in combination with appropriate silvicultural inputs. Increased input intensity has been associated with higher propensity of certain genotypes to increase stem and branch deformities. This is true for *Pinus radiata* (Bail and Pederick 1989, Carlyle et al. 1989, Downes et al. 1991, Downes and Turvey 1990, Hopmans 1990), slash pine (*Pinus elliottii*) (Gatch et al. 1999, Murphy and Harrington 2004), and Douglas-fir (*Pseudotsuga menziesii*) (Middleton et al. 1989, Spicer et al. 2000, Vargas-Hernandez et al. 2003).

The degree of environmental and genetic control of stem deformation is not well understood in loblolly pine, even though many studies have been established to select the best genetic material to increase gains in volume production and in stem form, wood quality,

and disease resistance (Zobel and Talbert 1984, McKeand et al. 2003b, McKeand et al. 2006b).

Loblolly pine has a wide geographic range, from the Atlantic Coast to East Texas and from southern Maryland and Delaware south into northern Florida. This wide range of distribution includes wide variation in soils and climate, resulting in large geographic and within-provenance variation for growth and adaptive traits. This genetic variation provides opportunities to obtain significant genetic gains from tree breeding programs. In general, families from western seed sources are more disease and drought resistant but slower growing than eastern sources (McKeand et al. 1989, Wells 1983, Wells and Lambeth 1983, Schmidtling 1994, 2003).

Contrasting the response to nutrient stress of two very different provenances of loblolly pine such as from the ''Lost Pines'' region of Texas and the Atlantic Coastal Plain may give us insight into the adaptive significance of different ecophysiological traits. The objective of this study was to assess spatial and temporal variation in response of loblolly pine genotypes to environmental stress. In this report, we describe the effect of nutrient additions and genotypes on stem form traits (straightness, sweep, forking, and ramicorn branching) and fusiform rust incidence after the trees completed twelve growing seasons in the field.

3.2. Materials and Methods

3.2.1 Site description

The study site is located in the Sandhills region of Scotland County, North Carolina adjacent to the U.S. Forest Service / N.C. State University SETRES (Southeastern Tree

Research and Experiment Site) study (34^0 48$^{'}$ N, $79^0$12$^{'}$ W). The soil is an infertile, well-drained, sandy, siliceous, thermic Psammentic Hapludult soil (Wakulla series) with a water-holding capacity of 12–14 cm in a 2 m profile. Mean annual precipitation at this site is 1220 mm, evenly distributed throughout the year. Long-term mean air temperatures are 26°C and 9°C in the summer and winter, respectively (see Albaugh et al. 2004 for details).

3.2.2 *Experimental design and treatments*

Seedlings of open-pollinated families from two provenances (the Coastal Plain of North Carolina and South Carolina, and the "Lost-Pines" area of Texas) were planted in November/December 1993. Five families from each provenance with average or slightly above average breeding values for volume production were established in a split-split-plot design with two nutrient treatments (fertilized and non-fertilized (control), Table 3-1) as main plots, provenances as sub-plots, and families within provenances as sub-sub-plots. Fertilizer was applied most years (Table 3-1) to maintain a balanced supply of all nutrients in the fertilized plots based on foliage nutrient analysis (McKeand et al. 2000). Each family plot consisted of 100 trees planted at 1.5 m by 2 m spacing. Only the interior 64 trees were measured at age twelve. Initially, the study was replicated across 10 blocks; however, data from block 10 were eliminated due to removal of sample trees for other experiments. A total of 11,520 measurement trees were used in the current study.

3.2.3 *Measurements*

Quantitative traits were assessed for all of the inner 64 trees in each plot. Tree height and diameter at breast height were recorded as well as presence of fusiform rust galls, and the

stem form traits of straightness, sweep, forking, and ramicorn branching. The following scales were used to assess the stem form traits. Straightness was assessed using the score from 1 to 6, with 1 being the straightest trees and 6 the most crooked trees. Sweep was measured as maximum deviation (measured in cm) in the 4 m log starting 0.3 m above the ground. Rust score was 0 for trees that did not show rust infection; and 1 for trees with rust gall(s) present on branches while score 2 was assigned to trees with rust gall(s) present on stem (or on stem and branches). Zero score was assigned for trees without a fork, 1 for trees with presence of one fork and 2 for trees that had more than one fork. Ramicorn branching, which is defined as an abnormally steep and undesirable large branch, which tends to persist on the stem (Smith 1986), was assessed by assigning 0 for absence and 1 for presence.

In this paper, we only evaluated height data. Tree volume analyses are presented by Smith et al. (2009, in preparation). For all traits, individual tree data were averaged to obtain plot mean values for each trait for further analysis. Means and within family-plot standard deviations and coefficients of variation were calculated for height for each 64-tree family plot. Within family-plot standard deviations and coefficients of variation were also subjected to analyses of variance to determine if sub-sub-plot uniformity varied. Correlation analyses between straightness, sweep, forking, ramicorn branching and stem sinuosity (using the data from Chapter 1 with only five reps) were carried out to assess the relationship among stem form traits.

3.2.4 Data analysis

The statistical significance of the treatments, provenances, family within provenance and their interaction effects were determined using analysis of variance (SAS, 2003). All significance levels were <0.05. The analyses of variances were done with plot level data using SAS GLM and Mixed procedures. The fertilizer treatment and provenances were considered fixed effects, and families were considered random effects nested within provenance. The model used was:

$$y_{ijkl} = \mu + r_i + \tau_j + r\tau_{ij} + \rho_k + r\rho(\tau)_{ijk} + t\rho_{jk} + f(\rho)_{l(k)} + \tau f(\rho)_{jl(k)} + \varepsilon_{ijkl}$$

Where:

y_{ijkl} is a trait's value in the lth family within the kth provenance in the jth treatment in the ith block.

μ is the overall mean;

r_i, is the random effect due to i^{th} block [$r_i \sim N(0, \sigma^2_r)$],

$r\tau_{ij}$ is the random interaction effect of the ith block with the jth treatment [$(r\tau_{ij}) \sim N(0, \sigma^2_{r\tau})$]

$r\rho(\tau)_{ijk}$ is the random interaction effect of the ith block with the jth treatment with the kth provenance [$r\rho(\tau)_{ijk} \sim N(0, \sigma^2_{rp(t)})$];

$f(p)_{l(k)}$ is the random effect due to l^{th} family within k^{th} provenance [$f(\rho)_{lk} \sim N(0, \sigma^2_{f(\rho)})$];

$rf(\rho)_{il(k)}$ is the random interaction effect of the lth family within k^{th} provenance with the ith block [$(rf_{lk}) \sim N(0, \sigma^2_{rf(\rho)})$];

$\tau f(\rho)_{jl(k)}$ is the random interaction effect of the lth family within k^{th} provenance with the jth treatment [$\tau f(\rho)_{jl(k)} \sim N(0, \sigma^2_{\tau f(\rho)})$],

e_{ijkl} is the random error effect of the lth family within k^{th} provenance with j^{th} treatment in the ith block $[\varepsilon_{ijkl} \sim N(0, \sigma^2_\varepsilon)]$;

t_j is the fixed effect for treatment $(\sum \tau_i = 0)$,

ρ_k is the fixed effect for provenances $(\sum \rho_k = 0)$.

An analysis of variance by provenance was carried out to determine how the families within each provenance respond to the treatments. The model used was:

$$y_{ijk} = \mu + r_i + \tau_j + r\tau_{ij} + f_k + rf_{ik} + \tau f_{jk} + \varepsilon_{ijk}$$

Where:

y_{ijk} is a trait's value in the kth family in the jth treatment in the ith bock.

μ is the overall mean;

r_i, is the random effect due to i^{th} block $[r_i \sim N(0, \sigma^2_r)]$,

τ_j is the fixed effect for treatment $(\sum \tau_i = 0)$.

$r\tau_{ij}$ is the random interaction effect of the ith block with the jth treatment $[(r\tau_{ij}) \sim N(0, \sigma^2_{r\tau})]$

f_k is the random effect due to k^{th} family $[f_k \sim N(0, \sigma^2_f)]$;

rf_{ik} is the random interaction effect of the kth family with the ith block $[(rf_{ik}) \sim N(0, \sigma^2_{rf})]$;

τf_{jk} is the random interaction effect of the jth treatment with k^{th} family $[\tau f_{jk} \sim N(0, \sigma^2_{\tau f})]$,

e_{ijk} is the random error effect of the kth family with j^{th} treatment in the ith block $[\varepsilon_{ijk} \sim N(0, \sigma^2_\varepsilon)]$.

Similarly, an analysis of variance by treatment and provenance (all terms with τ_j were dropped in the analysis of variance by provenance model above) was carried out to determine

significance of family differences within treatments and provenance using Tukey multiple range test (adjustment for multiple comparisons).

3.3. Results and Discussion

3.3.1 Response to nutrient additions

Height was significantly increased with nutrient additions (Table 3-2, Figures 3-1). Height was 66% higher in the fertilized trees compared to the non-fertilized trees (12.2m vs. 7.3m). At the treatment level, ACP had significantly greater height than LPT for both control and fertilized plots (7.6m vs. 7m and 12.6m vs. 11.80m) respectively Table 3-3.

These differences in height results are consistent with those of Allen et al. (2005), who found that on loamy or sandy soils, low nutrient levels limit growth. At the SETRES site adjacent to our study, nutrient limitations were much stronger than water limitations. In fact, Albaugh et al. (2004) reported that nine years after treatment initiation, standing stem biomass was increased 100% by fertilization and 25% by irrigation, while current annual increment of stem biomass production was increased 119% and 23% by fertilization and irrigation, respectively.

Fertilization not only increased height but also the uniformity of this within the 64-tree family plots. The average within-plot coefficient of variation for twelve-year height was 24% for the control plots and 11% for the fertilized plots, with standard deviations of 1.7 and 1.3, respectively. This increase in height uniformity associated with fertilization was significant and was probably due to a reduction in the microsite variation for nutrients. It is important to

mention that the height uniformity effect on the fertilized plots was found at age four (McKeand et al. 2000), and this uniformity effect was still present at age 12.

In contrast to the positive effect of nutrient addition on height, nutrient addition negatively affected stem quality. Trees with nutrient additions were more crooked (poorer straightness scores), and had a greater amount of forking and ramicorn branching than non-fertilized trees. Trees from fertilized plots had an average straightness score of 3.8, and in the control plots the score was significantly higher at 4.1. Stem sweep did not show significant difference between treatments in the combined analysis of variance (Table 3-2). The differences between fertilized and control treatments were 8.3% vs. 1.6% for forking, 8.5% vs. 2.5% for ramicorn branching, and 31.5% vs. 25.1% for rust infection. In addition, fast growth generally results in more succulent tissue, which is more susceptible to attack by insect and diseases which in turn may affect stem quality and form.

Results from the correlation analyses between stem sinuosity and other stem form traits (straightness, sweep, forking, and ramicorn branching) showed that stem sinuosity was not correlated with any of these traits. This suggests that sinuosity (as measured) is a unique trait. In relation to the family-mean correlations among the other stem form traits, the only significant correlations were between straightness and sweep which were r = 0.68 and r = 0.49 for the control and fertilized treatments respectively. One of the traits can be predicted from the other trait which may save time and money in assessments.

3.3.2 Provenance and family variation

Provenance had significant effects on height and stem form. Trees from the ACP provenance were taller than trees from the LPT provenance (10.1 vs. 9.4 m). The Atlantic Coastal Plain provenance showed better stem form than Lost Pines Texas (straightness 3.4 vs. 3.9, sweep 3.5 vs. 4.4, forking 2.1% vs. 6.1%, and ramicorn 3.7% vs. 7.4% for ACP and LTP, respectively). When the analysis of variance was carried out by provenance, significant variation among families within provenance were observed for straightness, sweep, and rust infection traits in the ACP provenance; while in the LPT provenance, all the traits were significant except ramicorn branching and rust infection (Table 3-4). Families within each provenance all had higher (poorer) straightness scores in the fertilized plots than in the control plots (Figure 3-2). However, in contrast with all other quality traits, fertilization had positive effect on sweep (reduction of the deviation) at the family level for ACP provenance (Figure 3-3, Table 3-4).   It is possible that the sweep deviations were masked by the increased diameter growth of trees in the fertilized plots.

Trees from the control treatment were straighter and with less forking and ramicorn branching than trees from the fertilized treatment (Figures 3-2, 3-3, 3-4, and 3-5). The interaction of family(provenance) by treatment was significant only for forking in both provenances (Table 3-4). Nutrient addition responses were more pronounced in the LPT provenance than ACP provenance.

When the data were analyzed by provenance and treatment, the ACP provenance showed significant family differences in straightness, sweep and rust infection in the control treatment and significant family differences in straightness, fork, and rust infection in the

fertilized treatment (Table 3-5, Figures 3-2, 3-3, 3-4, and 3-6). In the LPT provenance, family difference were found in sweep and forking in the control treatment; while in the fertilized treatment, families differences were found in sweep, fork and ramicorn branching traits (Table 3-5, Figures 3-3, 3-4, 3-5). Straightness, in the ACP provenance, varied from 3.0 to 3.5 and from 3.3 to 3.9 in the control and fertilized treatments, respectively. In the LPT provenance, it varied from 3.6 to 3.9 and from 4.0 to 4.3 in the control and fertilized treatments, respectively. Sweep varied from 3.2 to 4.3 and from 3.0 to 3.8 in the control and fertilized treatments for the ACP provenance. In the LPT provenance, sweep varied from 3.9 to 4.8 and from 4.1 to 5.0 in the control and fertilized treatment. Forking varied from 0% to 1% in the control plots and from 2% to 10% for fertilized plots in the ACP provenance. In the LPT provenance, forking varied from 1% to 5% in the control and from 7% to 18% in the fertilized plots. Ramicorn branching varied from 1% to 2% in the control plots and from 4% to 7% for fertilized plots in ACP provenances; while, in the LPT provenance, ramicorn branching varied from 2% to 5% in the control plots and from 8% to 15% in the fertilized plots.

Provenance differences for fusiform rust infection were large. The overall rust infection in the ACP provenance was 45%, while the LPT provenance had 11% infection. Rust infection did not differ between treatments for either provenance (Figure 3-6), indicating that the level of fusiform rust was primarily due to genetics rather than nutrient additions. Treatment by family interactions were not significant for fusiform rust, with the ACP family ranks being identical for the two treatments (Figure 3-6). Percent rust infection in ACP families varied from 20% to 65% for control plots and from 41% to 69% for plots where

nutrients were added. In contrast, rust infection in families from LPT provenance only varied from 6% to 15% in the unfertilized trees and from 9% to 15% in the fertilized plots (Figure 3-6).

Our results are consistent with previous studies that found that rust resistance is strongly related to longitude of the seed source. Western sources are more resistant than the eastern ones (Lambeth et al. 2005, McKeand et al. 1999, Burn and Honkala 1990). However, our lack of differences in rust infection with fertilization contrast with results from other studies (Brown and Coder 2001, Carson and Young 1987) where rust infection increased with N fertilization. Our focus on maintaining balance nutrition rather than just adding N may account for our lack a fertilizer effect on rust infection.

3.4. Conclusions

Based on the results in this and other tests (Albaugh et al. 2004, Amishev and Fox 2006) as well as commercial plantations where intensive silviculture management has been used (Fox et al. 2007, Allen et al. 2005), integration of both the management of site (nutrients and water) and genetic resources (McKeand et al. 2006a, Lambeth et al. 2005) is now recognized as essential to improving tree growth and stem quality in a effective and environmentally sustainable manner.

The results of this experiment indicate that specific combinations of silviculture techniques and genetics will permit optimization of gains to both growth and also in stem form and disease resistance. Families from the Atlantic Coastal Plain provenance exhibited better stem form traits than the Lost Pine Texas source; however, the Lost Pine Texas

provenance had a greater resistance to fusiform rust. Nutrient additions had negative effects on most stem quality traits. This could be due to fast growth induced by the fertilization, which may cause an imbalance in the amount of nutrients required by the trees for development. Because of this, we emphasize the need to apply balanced and site-specific nutrient additions to optimize the gain in production and maintain good stem form traits and disease resistance.

Finally, even though we used only five families in each provenance, some families exhibited good growth and good form. The ACP-1 family had the highest height growth, the best straightness, lowest percent in forking and ramicorn branching, and low percent of rust infection among the ACP families. The family that exhibited the best performance (average) in height and stem form in the LPT provenance was LPT-4. Between these two families, ACP-1 exhibited better performance than LPT-4. Our results show the potential to improve not only growth but also stem form traits through the selection of the best provenances and best families within provenance in the breeding program and using the appropriate silviculture tools.

3.5. Acknowledgements

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Table 3-1. Type and amount of fertilizer applied from 1994 to 2006 at SETRES-2.

| Year | Fertilizer | Nutrient additions (Kg ha^{-1}) | | | | | |
		N	P	K	Ca	Mg	S
1994	10/10/10	52	22	43	0	0	<0.9
1995	12-6-6 + Micros[1]	41	9	17			
1996	Urea, TSP, KMagS	56	6	28	3	17	40
1997	Urea, TSP, Mg	90	9			18	11
1998	Urea, Mg	90				17	11
1999	Urea, DAP, B[2]	95	9				12
2000	Ammonium Sulfate	91	10		6		103
2001	Urea, DAP	90	9				
2002	Urea, DAP	90	9				
2003	Urea	123					
2004	Urea, TSP, KMagS	86	11	38		24	46
2005	Urea (estimate)	112					
2006	None						
Total		**1015**	**94**	**126**	**9**	**75**	**224**

[1]Micros 0.5 B, 2.0 Cu, 5.0 Fe, 5.0 Mn, 2.0 Zn, [2] 1.2 B

Table 3-2. P-values for nutrient addition and genetic effects from combined ANOVA for stem traits at 12-years at SETRES-2.

Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Treatment	**<.01**	**<.01**	0.38	**<.01**	**<.01**	0.07
Provenance	**0.02**	**<.01**	**<.01**	**0.03**	**<.01**	**<.01**
Family(Provenance)	**0.02**	**<.01**	**<.01**	0.24	0.27	**<.01**
Treatment * Provenance	0.63	0.27	**0.02**	0.07	**0.04**	0.06
Treatment*Family(Provenance)	0.96	0.30	0.32	**<.01**	**0.04**	0.26

Table 3-3. P-values for nutrient addition and genetic effects from ANOVA by treatment for stem traits at 12-years at SETRES-2.

a.- Control treatmenrt

Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Provenance	**0.05**	**0.01**	**0.01**	0.06	**0.04**	**<.01**
Family(Provenance)	**<.01**	**<.01**	**<.01**	**<.01**	0.18	**<.01**

b.- Fertilized treatment

Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Provenance	**0.02**	**<.01**	**<.01**	**0.04**	**0.01**	**<.01**
Family(Provenance)	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**	**<.01**

Table 3-4. P-values for nutrient addition and genetic effects from ANOVA by provenance for stem traits at 12-years at SETRES-2.

a.- Atlantic Coastal Plain provenance

Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Treatment	**<.01**	**0.05**	**0.03**	**0.03**	**<.01**	0.06
Family(Provenance)	0.63	**<.01**	**0.03**	0.31	0.31	**<.01**
Treatment*Family(Provenance)	0.95	0.16	0.57	**0.01**	0.42	0.23

b.- Lost Pine Texas provenace

Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Treatment	**<.01**	**<.01**	0.61	**<.01**	**<.01**	0.49
Family(Provenance)	**0.04**	**0.02**	**0.02**	**<.01**	0.32	0.09
Treatment*Family(Provenance)	0.74	0.58	0.22	**<.01**	0.09	0.59

Table 3-5. P-values for nutrient addition and genetic effects from ANOVA by treatment and provenance for stem traits at 12-years at SETRES-2.

a.- Family mean effect from the Atlantic Costal Plain provenace in the control treatment

| Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Family(Provenance)	0.19	**<.01**	**<.01**	0.33	0.93	**<.01**

b.- Family mean effect from the Lost Pine Texas provenace in the control treatment

Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Family(Provenance)	0.75	0.07	**<.01**	**<.01**	0.33	0.07

c.- Family mean effect from the Atlantic Costal Plain provenace in the fertilized treatment

Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Family(Provenance)	**<.01**	**<.01**	0.08	**<.01**	0.39	**<.01**

d.- Family mean effect from the Lost Pine Texas provenace in the fertilized treatment

| Effect	Height	Straightness	Sweep	Fork	Ramicorn	Rust infection
Family(Provenance)	0.31	0.09	**0.04**	**<.01**	**0.03**	0.16
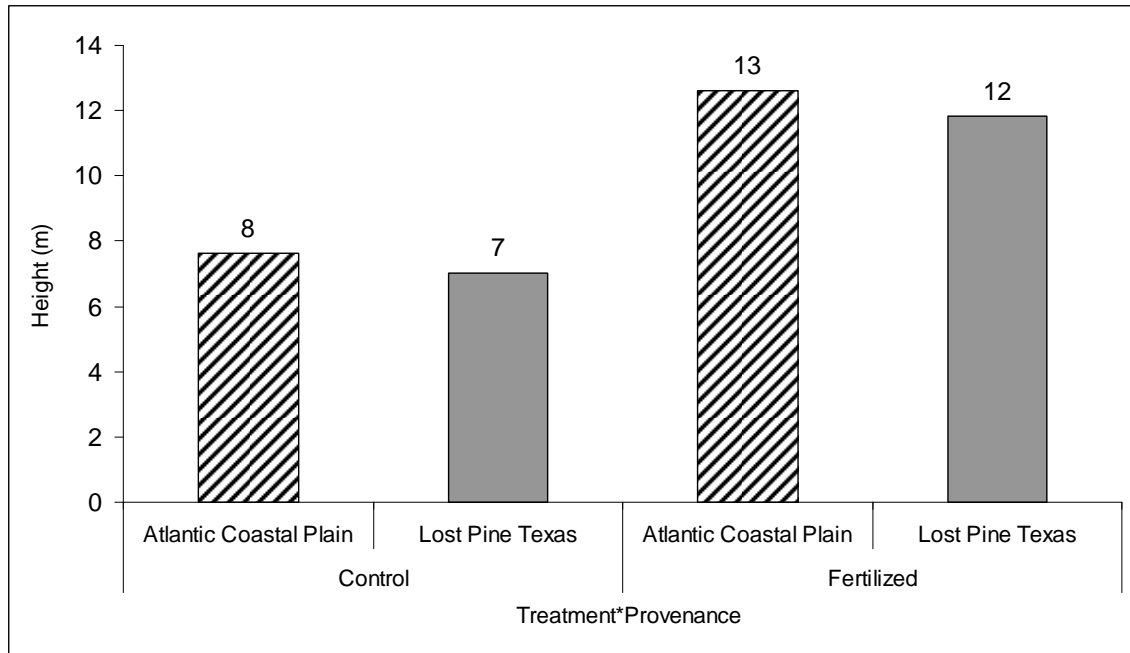
Figure 3-1. Mean tree height for Atlantic Coastal Plain (ACP) and Lost Pines Texas (LPT) provenances in the fertilized and control treatments at age twelve years. Treatment and provenance effects were highly significant (Tables 1-2, 1-3).
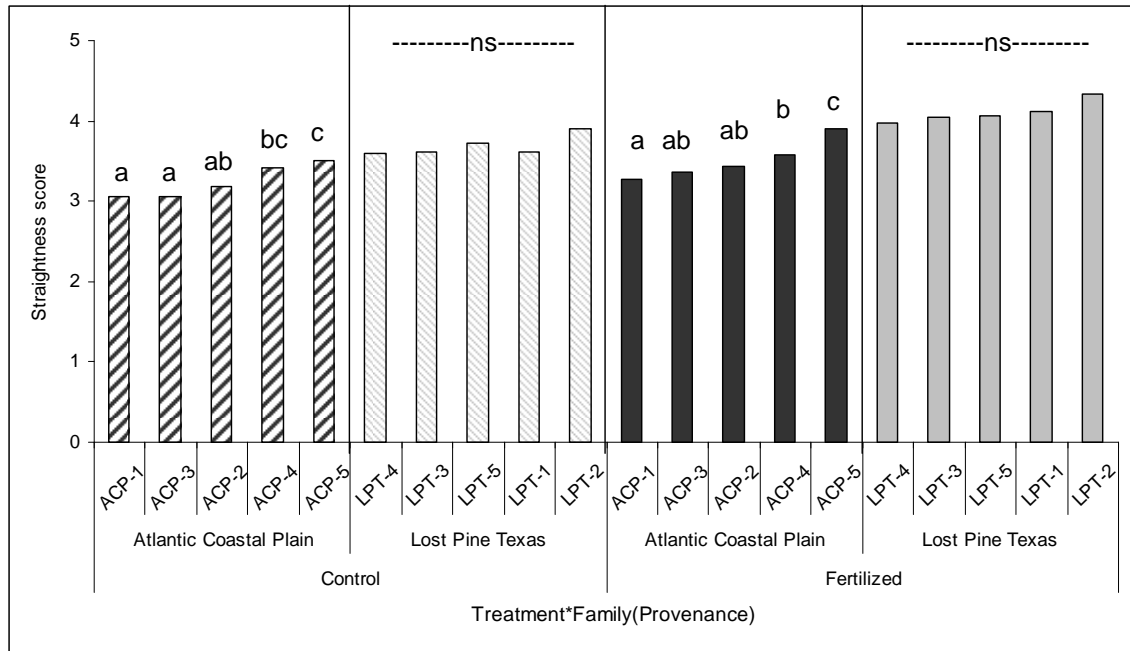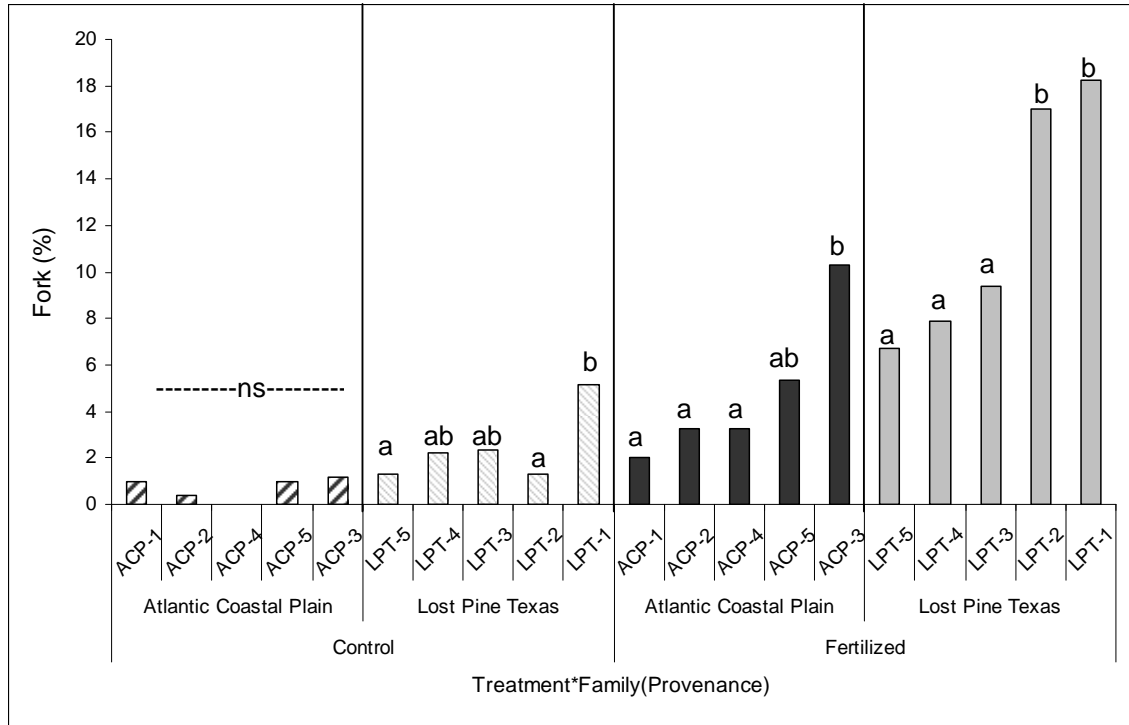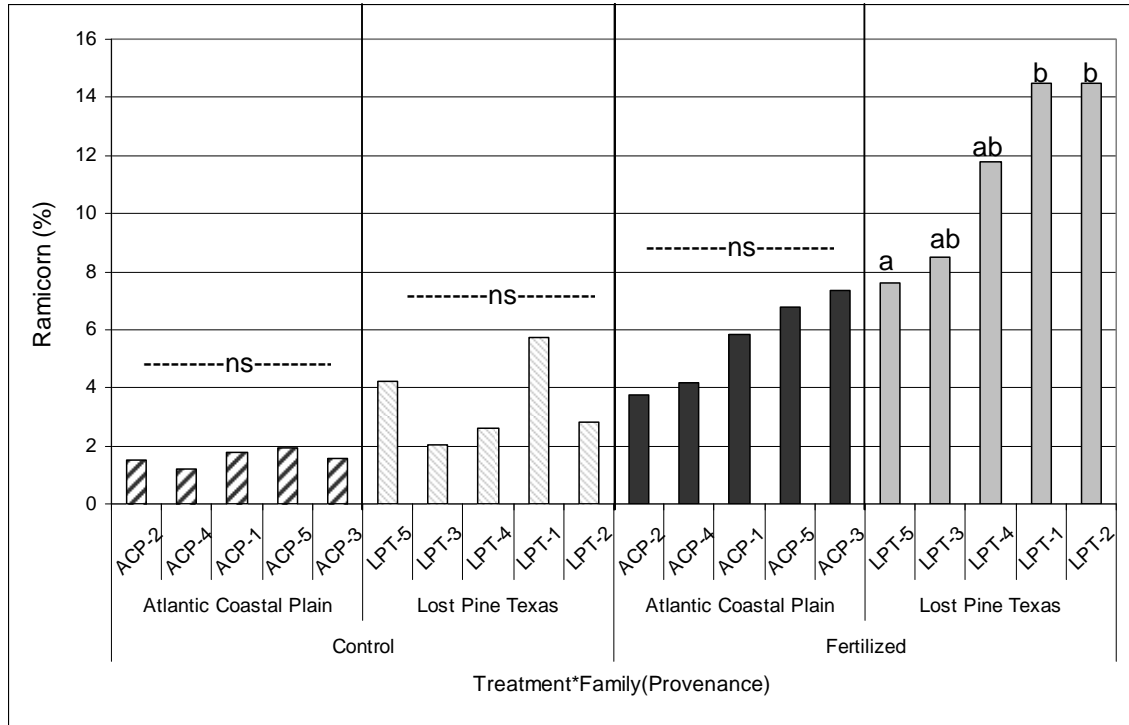
Figure 3-2. Mean of straightness scores for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots at age twelve years. Treatment, Provenance, and Family within Provenance effects were highly significant, and interaction effects were not (Table 1-2). Families within provenance are ordered by their straightness score in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination are not significantly (P<0.05) different (Table 1-5).
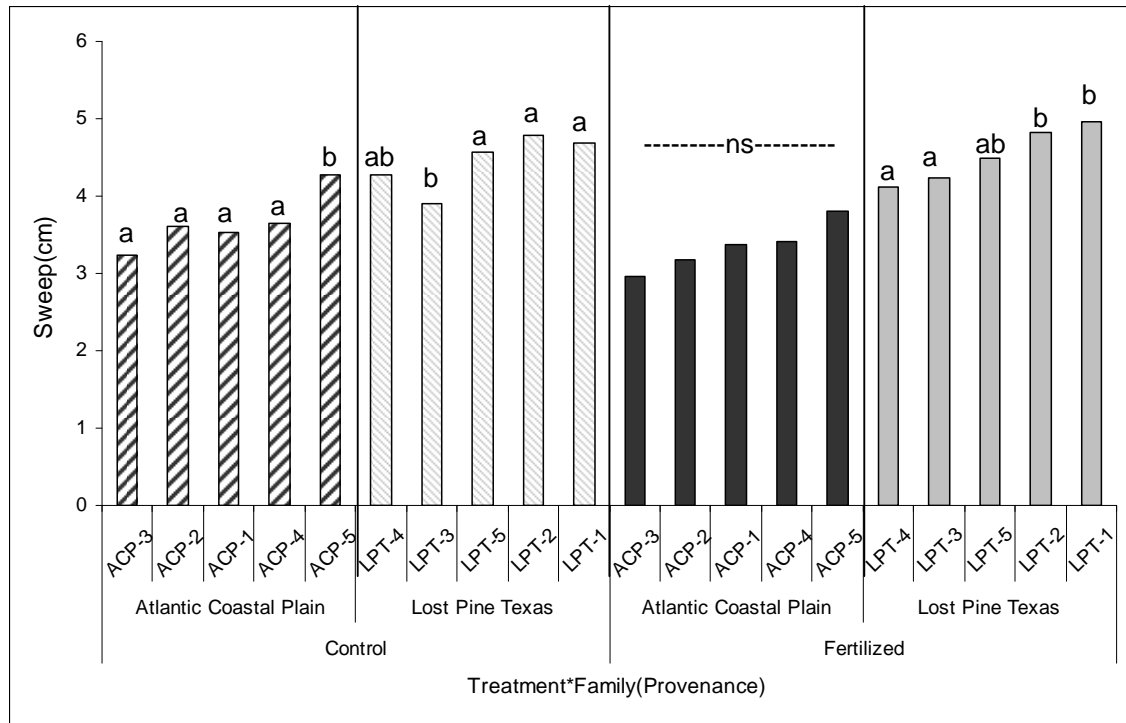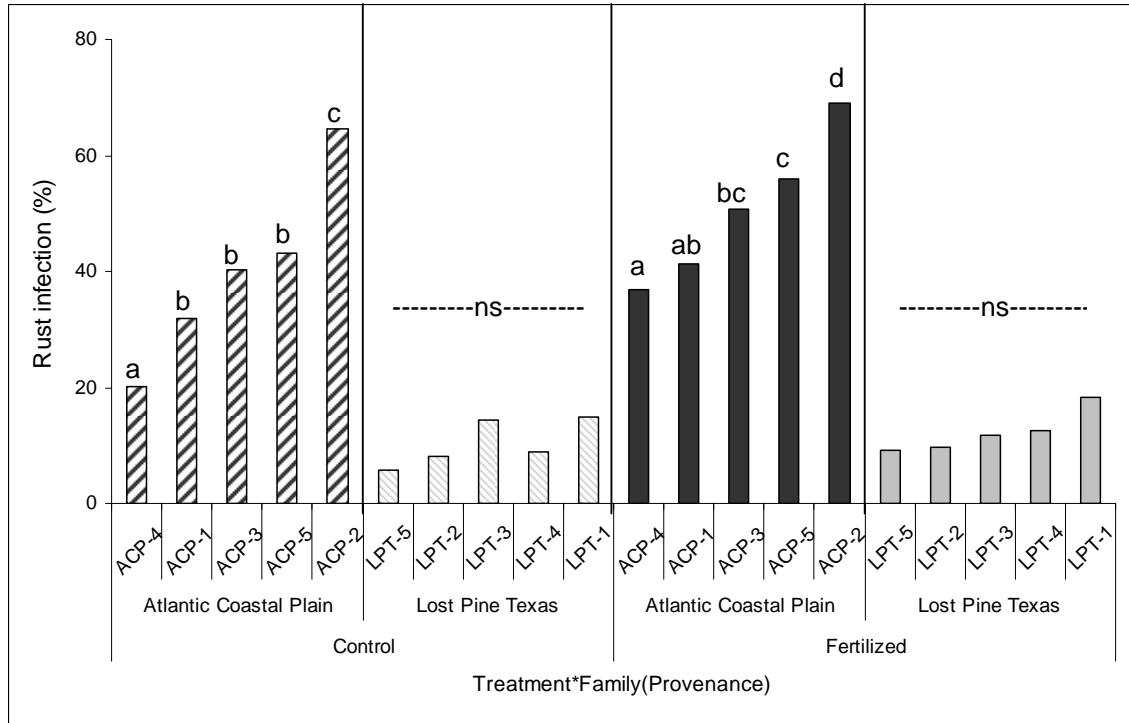
Figure 3-3. Mean sweep for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots at age twelve years. Provenance, Family within Provenance, and interaction Treatment by Provenance effects were highly significant, and interaction Treatment by Family within Provenance effects was not (Table 1-2). Families within provenance are ordered by their mean sweep in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination are not significantly ($P<0.05$) different (Table 1-5).

Figure 3-4. Mean forking for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots at age twelve years. Treatment, Provenance, and interaction Treatment by Family within Provenance effects were highly significant. Family within Provenance and interaction Treatment by Provenance effects were not (Table 1-2). Families within provenance are ordered by their mean forking in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination are not significantly (P<0.05) different (Table 1-5).

Figure 3-5. Mean ramicorn branching for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots at age twelve years. Treatment, Provenance, interaction Treatment by Provenance, and Treatment by Family within Provenance effects were highly significant. Family within Provenance effects was not (Table 1-2). Families within provenance are ordered by their mean ramicorn in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination are not significantly (P<0.05) different (Table 1-5).

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Figure 3-6. Mean rust infection for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots at age twelve years. Provenance and Family effects were highly significant. Treatment and all interaction effects were not (Table 1-2). Families within provenance are ordered by their mean rust infection in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination are not significant (Table 1-5).