SPITZER, JESSE ETHAN. Broad-Based Testing Compared to Local Testing of Families of Loblolly Pine for Resistance to Fusiform Rust and Growth Traits. (Under the direction of Dr. Steven E. McKeand and Dr. Fikret Isik).

Breeding loblolly pine (*Pinus taeda L.*) trees with resistance to fusiform rust *Cronartium quercuum f. sp. fusiforme* is a central focus of Southeast tree improvement programs. Annual losses from this disease are estimated in the range of $24 to $135 million, making it the most economically important disease of loblolly pine. Identification and deployment of pine families with enhanced resistance to fusiform rust across a broad range of sites are critical for the full benefit of breeding programs to be realized. Interactions of families’ susceptibility/resistance and specific pathogen avirulence/virulence among sites has been reported both in field trials and in controlled inoculations at the USDA Forest Service Resistance Screening Center in Asheville, NC, but the consistency and utility of these interactions have been limited.

We compared rust breeding values from a series of trials planted across a broad geographic range of sites (Plantation Selection Seed Source Study - PSSSS) to breeding values from trials established in more narrow geographic regions. The PSSSS used 80+ families that were also tested locally in Plantation Selection Diallel trials. By comparing the two breeding values for each family to see how much genotype by environment (e.g. family by virulence) interaction is occurring, we can elucidate any evidence for virulence variation in the locally tested breeding values. No significant GxE was found for fusiform resistance, and the regression between the diallel and PSSSS breeding values had an $R^2$ of 0.75, indicating
locally derived rust breeding values are reliable predictors of the families’ performance across a broad range of sites.

We also investigated if GxE interaction were occurring for straightness, height, DBH, and volume in loblolly pine. No significant GxE was found for straightness. We found evidence that some GxE may be occurring for the growth traits (height, DBH, and volume) but not likely enough to warrant site-dependent testing and breeding.
Broad-Based Testing Compared to Local Testing of Families of Loblolly Pine for Resistance to Fusiform Rust and Growth Traits

by
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DEDICATION

This thesis is dedicated to my parents for all of their support.
BIOGRAPHY

Jesse Spitzer was raised in Ossining, New York. He graduated from Ossining High School in 2008. He then attended The State University of New York College of Environmental Science and Forestry for undergraduate study. He graduated with his Bachelor of Science in Forest Health in 2012. He was then admitted to North Carolina State University graduate program in 2012 to pursue a Master’s degree in Forestry focusing on tree breeding with the Tree Improvement Cooperative.
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Chapter 1:

Review of Literature on Fusiform Rust Disease in Loblolly Pine
Fusiform Rust and Loblolly Pine Background

Loblolly pine (*Pinus taeda* L.) is the major timber species in the Southeastern United States, with almost 1 billion seedlings produced annually (McKeand et al. 2003). The native range of loblolly pine extends through 14 states from southern New Jersey south to central Florida and west to eastern Texas, and it is the second most common species of tree in the United States after red maple (*Acer rubrum*) (Kershner et al. 2008). Genetic improvement through breeding and selection of loblolly pine since the 1950’s has improved the productivity, form, and disease resistance in the species. Since the late 1970’s, nearly 100% of loblolly pine plantations in the southeast U.S. have been established with genetically improved loblolly pine (Aspinwall et al. 2012). Breeding loblolly pine with resistance to fusiform rust (caused by the fungus *Cronartium quercuum f. sp. fusiforme*, and hereby referred to *C.q.f.*) is a central focus of Southeast tree improvement programs.

Fusiform rust is commonly recognized as the most economically important disease of loblolly pine in the southern United States (Powers, 1991). Annual losses from this disease are estimated to be 562 million board feet of sawtimber and 194 million cubic feet of growth stock which monetarily translates to $150 million (Cubbage et al. 2000). Conversely, the economic benefits of increased resistance to fusiform rust are substantial. Planting rust resistant seedlings can save $40-60 million per year by preventing product losses and degrades (Cubbage et al. 2000).
Fusiform rust disease is a disease endemic to oaks and pines in the Southeastern United States. Fusiform rust occurs throughout most of loblolly pine’s native range from Maryland to Florida and west to Texas and southern Arkansas. The highest rust hazard areas occur in Georgia, Alabama, South Carolina, and Mississippi, especially in the central and southern regions of Georgia and Alabama (Dinus et al. 1977). Loblolly and slash pine (P. elliottii) are the major economically important hosts that are affected. From late March to mid-April, the fungus produces galls on pine trees (the aecial or nonrepeating host of the fungal disease) which produce orange aeciospores. In pines, these spindle-shaped swellings or galls which develop on branches or main stems of the trees are the disease. On older trees, infections are somewhat depressed on one side. Galls result in cankers which can result in mortality on young trees as well as deform older trees, reducing growth and weakening the stems until breakage in windstorms becomes likely. Cankered stems have greatly reduced sawtimber value. Heavily infected stands may need to be thinned earlier and more often with greater logging expenses. Residual sawtimber volume may be low, resulting in lower prices at harvest. Infections within the first 5 years can cause tree death. Infected areas may grow for many years until they eventually girdle and kill the branch or stem, or they may become inactive (Czabator, 1971). Spermagonia (pycnia) and spermatia (pycniospores) are produced on galls two years after infection in the fall or early spring and appear as small golden droplets on the gall surface. The year after successful fertilization, aecia appear as orange-yellow blisters on the gall breaking through the bark in the spring (Snow, 1968).
Aeciospores are wind disseminated to newly formed oak leaves. Various oak species serve as alternative hosts for *C. q.f.*, and they are chiefly species in the red oak subgenus. The damage on the telial or repeating host which is commonly red oak (*Quercus rubra L.*), is confined to leaf spotting, making fusiform rust disease not commercially important for oak species. On oaks, orange spores appear on lower surface of leaves. Two to three weeks after infection, uredinia are formed on the underside of oak leaves. In late spring to early summer, 2-4 weeks after uredinia are formed, telial columns are formed from the same mycelium in the oak leaf often pushing up through old uredinia. Next, telial columns appear on the underside and petioles of oak leaves. The telial column produces teliospores which germinate to form a basidium and basidiospores. These basidiospores are wind disseminated and infect susceptible pines through cotyledons, needles, or succulent bark tissues (Czabator, 1971). The pathosystem is discontinuous; pine-to-pine infection does not occur (Squillace, 1976). The timing of this process will vary from year to year and in different geographic areas, beginning earlier if the temperature is higher (Phelps, 1973).

*Fusiform Rust Resistance in Loblolly pine*

Substantial genetic variation in expression of host resistance to fusiform rust is present in loblolly pine, and use of resistant planting stock is the only feasible means of controlling fusiform rust disease in the forest environment (Zobel and Talbert, 1984). Appropriate use of resistant planting stock can increase grower profits by $36 to $192 million per year (Kinloch and Walkinshaw, 1991). Phenotypic selection and breeding of resistant trees have
allowed for an increase in the supply of resistant seedlings, but understanding the genetic mechanism of the host-pathogen interaction is important to carry out more successful breeding for fusiform resistance, since there are many varieties of the *C.q.f.* fungus that can occur in various different areas of fusiform rusts range (Isik et al. 2012).

The polygenic model of inheritance was originally assumed to be the genetic mechanism of fusiform resistance in loblolly pine. It was thought that though the resistance to different isolates of fusiform rust among loblolly pines can vary, it is not controlled by a major gene mechanism (Zobel and Talbert, 1984). In this polygenic model, an individual tree must possess many minor genes at many resistance loci which additively build its resistance. Each minor gene, additively, has a small effect. In general, disease resistance in forest trees is controlled by many genes because forest trees must be durable and effective in long term resistance (Robinson, 1987). Conversely, major gene, or qualitative gene for gene resistance mechanisms are known for many crop species (Thompson and Burdon, 1992). In a gene-for-gene system, a discrete resistance gene in the host blocks infection by strains of the pathogen, except for strains carrying a specific virulence gene. Individual, major, discrete resistance genes are frequently overcome within a few years in agronomic crops, where the pathogen's asexual repeating stage is present on the economically important host (Vanderplank, 1982). Because of these generalities, previous breeding efforts to improve the level of fusiform rust resistance in loblolly pine assumed a polygenic basis for resistance and followed quantitative breeding models (Zobel and Talbert, 1984). This line of thinking was challenged, when on the basis of infection percentages and reciprocal specificities with single gall isolates of the
fungus, Kinloch and Walkinshaw (1991) proposed that host resistance to fusiform rust in slash pine followed a gene-for-gene model. This theory was strengthened when detection of a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping was reported (Wilcox et al. 1996). This study was able to show that effective resistance to fusiform rust can be obtained from a single qualitative resistance gene, despite the presence of virulence in the pathogen population. The use of single genotype lines, or single aeciospore isolates, of the rust pathogen greatly reduces genetic variability in the basidiospore population used for pine inoculations and had allowed identification of a single genetic locus, Fr1, that behaved as a single gene having a major effect on host resistance to fusiform rust disease (Wilcox et al. 1996). A dominant allele at the Fr1 locus was shown to confer resistance against one single genotype line of the rust pathogen (avirulent against Fr1 resistance) and to lack effectiveness against another single genotype line that was virulent against Fr1 resistance (Wilcox et al. 1996). Later work with the same Fr1 allele by Wilcox et al. (1996) reported that the resistance conferred by this allele remained stable under increasing density of spore inoculum, provided that inoculation used a single genotype line of the pathogen against which the resistance allele is effective (Kuhlman et al. 1997). Host trees carrying an R allele at an Fr gene (fusiform rust disease resistance gene) are resistant when the challenging pathogen carries only an avirulence (Avr) allele at the corresponding pathogen gene. However, they are susceptible (barring other undetected resistance genes), if the challenging pathogen carries a virulence (avr) allele at that gene (Kubisiak et al. 2005).
Additional work with loblolly pine families from breeding programs extended these findings to identify eight loci with major effects on resistance of loblolly pine to fusiform rust disease (Amerson et al. 2005). These results strongly suggest that at least some of the resistance to fusiform rust disease detected in progeny tests can be explained by an oligogenic or major gene model of a few dominant alleles with major effects, rather than the many alleles with small additive effects assumed by the polygenic model. It is not yet clear whether the oligogenic model can explain most or all of the variation in rust resistance detected in field trials, or if polygenic mechanisms contribute some portion of the disease resistance detected in tests. A study of pine progeny test data compared the fit of a mixed-inheritance model that included both polygenic and oligogenic effects, with the fit of a model that included only polygenic effects; the conclusion was that the mixed inheritance model with both oligogenic and polygenic effects better described host disease resistance (Li et al. 2006). Gene-for-gene interactions do not preclude the modifying influence of other non-specifically acting genes in the final outcome of the host-pathogen encounter. But major genes have major effects, and identification of these genes is more useful for describing and predicting the dynamics of a pathosystem than are estimating quantitative genetic parameters for the host alone. With knowledge of specifically interacting genes and their frequencies, strategic deployment of major host genes can be made and the epidemiological consequences predicted. Identification and deployment of pine families with enhanced resistance to fusiform rust across a broad range of sites are critical for the full benefit of breeding programs to be realized.
**Fusiform Rust Testing**

Testing for fusiform rust resistance can be conducted in both field tests and controlled greenhouse inoculations. In the field, resistance to fusiform rust disease is measured by what southern pine tree breeders call an “R50” score. This means, if an unimproved loblolly pine seedlot would be expected to have 50% rust (% galled) on a site, then its R50 equals 50. A resistant family would have an R50 value lower than 50. A very resistant family could have an R50 value of 20% meaning that it should have 20% rust (% galled) when the unimproved seedlot would have 50% rust. A susceptible family would have a R50 value above 50%.

The USDA Forest Service’s Resistance Screening Center (RSC) in Ashville, NC evaluates seedlings for resistance to fusiform rust in a controlled environment. This sort of testing enables information to be obtained on the relative resistance of seedlings in much less time than is possible in field progeny tests. Trees producing disease resistant progeny are identified from the results of these tests within 6 to 9 months. The RSC cultivates the fungus by harvesting basidiospores from the alternative host and then inoculates the pine seedlings with a controlled basidiospore suspension system. They have the ability to test families of loblolly pine with bulk inoculum of a large variety of *C. q.f.* bulked galls (mixed basidiospore inoculum derived from aeciospores collected from several different geographic regions) to be able to determine a family’s resistance against many forms of the fungus that it could come in contact within any area. The RSC can also test families with single gall isolates of *C. q.f.* to determine the family’s resistance to that specific strain. Most of these methods provide a
moist atmosphere around seedlings exposed to basidiospores released from excised telial columns, or oak leaves, or oak branchlets. The percentage of seedlings showing visible galls after several months in a greenhouse or nursery bed is taken as an inverse measure of rust resistance (Cowling and Young, 2013).

Despite some of these advantages of controlled testing at the RSC, such as getting results in a shorter amount of time, there has been some discrepancy between the results from field testing and the results from the RSC. Loblolly pine families that are found to be resistant in field tests are not always found to be resistant in controlled inoculations at the RSC (Isik et al. 2008). This raises questions as to whether the RSC is too conservative in their designation of resistance, or if there are family by inoculum (GxE) interactions occurring in the field. This is a type of genotype by environment interaction and describes a situation where there is a change in the performance ranking of given genotypes when grown in different environments (Zobel and Talbert, 1984). It has been clearly demonstrated that there is virulence variation in the fusiform rust population (Isik et al. 2012, Kubisiak et al. 2005). Some aeciospore isolates of C.q.f. from single fusiform rust galls are significantly more virulent on seedlings of certain resistant pine families than are other isolates (Powers et al, 1979, Griggs and Walkinshaw, 1982, Kuhlman, 1992). It still remains to be demonstrated that the virulence variation in the rust population causes important family by inoculum interactions in field trials.
It is important to understand if genotype by environment interactions are the cause of the discrepancy between RSC and field results. If the methods of the RSC are causing field resistant families to be designated as susceptible, then a significant issue arises because good families are being disregarded for field planting. If genotype by environment interactions are in fact the cause, then this, too, is a significant issue, because most of the selections in breeding programs are based on field testing in restricted geographic areas.

The strategy of assigning the best loblolly pine families to the best sites rather than site specific assignments is common since large genotype by environment differences are not common for loblolly pine (Duzan and Williams 1988). Other past studies have noted lack of evidence of GxE interactions for fusiform rust resistance in loblolly pine (Wells et al. 1982, Roth et al. 2007), but there have been notable caveats. Wells et al. 1982 found small non-significant GxE interaction for fusiform rust resistance in loblolly pine, however, the plantings were poorly replicated. Roth et al 2007 found no significant GxE interaction for fusiform rust resistance in loblolly, although there was low overall incidence of fusiform rust between locations.

The discrepancies, however, found between field results and controlled inoculations (Isik et al. 2008) raise a concern that GxE interactions could have tangible manifestations in the field. Furthermore, the most rust resistant loblolly families (based on field test results) were found to be the least predictable for performance across sites (McKeand et al. 2003). For some families, the regression of family means for rust infection versus site means for rust
infection had low coefficients of determination ($r^2$). It has been hypothesized that the low correlation of some loblolly family means with site rust infection means, may be due to interactions of families’ susceptibility/resistance and specific pathogen avirulence/virulence among sites (McKeand et al. 1999). This causes additional concern that meaningful GxE interactions may exist, although these findings come with caveats as well. Although significant genotype by environment interactions were found for fusiform rust resistance, the overall variance was small in comparison to the family variance, and much of the genotype by environment interaction was due to heterogeneous regression of families across different sites and not family rank change (McKeand et al. 1999). While various studies have shown some evidence of GxE interactions occurring for fusiform rust resistance in loblolly pine, it has yet to be demonstrated that these interactions suggest local testing to assess rust resistance is not sufficient. It is important that this issue is investigated so that we can confidently determine if local testing is suitable for determining fusiform rust resistance in loblolly pine.
REFERENCES


McKeand, S.E., Amerson, H.V., Li, B., and Mullin, T.J. 2003. Families of loblolly pine that are the most stable for resistance to fusiform rust are the least predictable. Can. J. Res. 33: 1335–1339.


Chapter 2:

Broad-Based Testing Compared to Local Testing of Families of Loblolly Pine for Resistance to Fusiform Rust
Abstract

Breeding loblolly pine (*Pinus taeda* L.) trees with resistance to fusiform rust disease (caused by *Cronartium quercuum f. sp. fusiforme*) is a central focus of Southeast tree improvement programs. Annual losses from this disease are estimated in the range of $24 to $135 million, making it the most economically important disease of loblolly pine. Identification and deployment of pine families with enhanced resistance to fusiform rust across a broad range of sites are critical for the full benefit of breeding programs to be realized. Interactions of families’ susceptibility/resistance and specific pathogen avirulence/virulence among sites has been reported both in field trials and in controlled inoculations at the USDA Forest Service Resistance Screening Center in Asheville, NC, but the consistency and utility of these interactions have been limited.

We compared rust breeding values from a series of trials planted across a broad geographic range of sites (Plantation Selection Seed Source Study - PSSSS) to breeding values from trials established in more narrow geographic regions. The PSSSS used 80+ families that were also tested locally in Plantation Selection Diallel trials. By comparing the two breeding values for each family to see how much genotype by environment interaction is occurring, we can test for evidence that virulence variation among fungal pathotypes contributes to variation in the rust resistance breeding values estimated from local tests. No meaningful GxE was found for fusiform resistance and the regression between the diallel and PSSSS
breeding values had an $R^2$ of 0.75, indicating locally derived rust breeding values are reliable predictors of the families’ performance across a broad range of sites.
Introduction

Loblolly pine (*Pinus taeda* L.) is the major timber species in the Southeastern United States, with almost 1 billion seedlings produced annually (McKeand et al. 2003). The native range of loblolly pine extends through 14 States from southern New Jersey south to central Florida and west to eastern Texas and is the second most common species of tree in the United States after red maple (*Acer rubrum*) (Kershner et al. 2008). Genetic improvement through breeding and selection of loblolly pine since the 1950’s has improved the productivity, form, and disease resistance in the species. Since the late 1970’s, essentially 100% of loblolly pine plantations in the southeast U.S. have been established with genetically improved loblolly pine (Aspinwall et al. 2012). Breeding loblolly pine with resistance to fusiform rust (caused by the fungus *Cronartium quercuum f. sp. fusiforme*, and hereafter referred to *C.q.f.*) is a central focus of Southeast tree improvement programs.

Fusiform rust is commonly recognized as the most economically important disease of loblolly pine in the southern United States (Powers, 1991). Annual losses from this disease are estimated to be 562 million board feet of sawtimber and 194 million cubic feet of growth stock which monetarily translates to $150 million (Cubbage et al. 2000). Conversely, the economic benefits of increased resistance to fusiform rust are substantial. Estimates from almost 20 years ago indicate that planting rust resistant seedlings could save $40 to $60 million per year by preventing product losses and degrades (Cubbage et al. 2000).
Fusiform rust disease is endemic to oaks and pines in the Southeastern United States. Galls result in cankers on pines which can result in mortality on young trees as well as deform older trees, reducing growth and weakening the stems until breakage in windstorms becomes likely. Various oak species serve as alternative hosts for \textit{C. q.f.}, but loblolly and slash pine are the major economically important hosts that are affected. Pines produce orange aeciospores from fusiform galls that are wind-disseminated to infect oak leaves which eventually produce basidiospores that are wind-disseminated to infect susceptible pines through cotyledons, needles, or succulent bark tissues (Czabator, 1971). The pathosystem is discontinuous; pine-to-pine infection does not occur. Fusiform rust occurs throughout most of loblolly pine’s native range from Maryland to Florida and west to Texas and southern Arkansas. The highest rust hazard areas occur in Georgia, Alabama, South Carolina, and Mississippi, especially in the central and southern regions of Georgia and Alabama.

Substantial genetic variation in expression of host resistance to fusiform rust is present in loblolly pine, and use of resistant planting stock is the only feasible means of controlling fusiform rust disease in the forest environment (Zobel and Talbert, 1984). There are many varieties of the \textit{C. q.f.} fungus which can occur in different areas of the range of fusiform rust disease (Kuhlman, 1992; Kubisiak, 2005). The polygenic model of inheritance was originally assumed to be the genetic mechanism of fusiform resistance in loblolly pine. It was thought that resistance was not controlled by a major gene mechanism (Robinson, 1987). In this polygenic model, an individual tree must possess many minor genes that additively build its resistance. On the basis of infection percentages and reciprocal specificities with
single gall isolates of the fungus, Kinloch and Walkinshaw (1991) proposed that host resistance to fusiform rust in slash pine followed a gene-for-gene model. Additionally, Wilcox et al. (1996) were able to detect a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping. This study was able to show that effective long-term resistance to fusiform rust can be obtained from a single qualitative resistance gene, despite the presence of virulence in the pathogen population.

It is not yet clear whether the oligogenic model can explain most or all of the variation in rust resistance detected in progeny tests carried out by loblolly pine breeding programs, or if polygenic mechanisms contribute some portion of the disease resistance detected in field tests. A study of pine progeny test data that compared the fit of a mixed-inheritance model that included both polygenic and oligogenic effects, with the fit of a model that included only polygenic effects, concluded that the mixed inheritance model with both oligogenic and polygenic effects better described host disease resistance (Li et al. 2006). Identification and deployment of pine families with enhanced resistance to fusiform rust across a broad range of sites are critical for the full benefit of breeding programs to be realized.

Testing for fusiform rust resistance can be done in both field tests and controlled greenhouse inoculations. In the field, resistance to fusiform rust disease is measured by what southern pine tree breeders call an “R50” score; if an unimproved loblolly pine seedlot would be expected to have 50% rust (% galled) on a site, then its R50 equals 50. A resistant family would have an R50 value lower than 50. A very resistant family might have an R50 value of
20% meaning that it should have 20% rust (% galled) when the unimproved seedlot would have 50% rust. A susceptible family would have a R50 value above 50%. Breeding values for resistance can only be estimated from field tests with rust incidence between 20% and 80%, and not all field tests are suitable for estimation of rust resistance breeding values. This uncertainty about whether field tests will yield useful data for estimating rust resistance is another advantage to RSC screening.

The USDA Forest Service’s Resistance Screening Center (RSC) in Asheville, NC evaluates seedlings for resistance to fusiform rust in a controlled environment. This sort of testing enables information to be obtained on the relative resistance of seedlings in much less time than is possible in field progeny tests. Trees producing disease resistant progeny are identified from the results of these tests within 6 to 9 months. The RSC cultivates the fungus by harvesting basidiospores from the alternative host and then inoculates the pine seedlings with a controlled basidiospore suspension system. They have the ability to test families of loblolly pine with bulk inoculum of a large variety of \textit{C. q.f.} bulked galls to be able to demonstrate that family’s resistance against many forms of the fungus that it could contact in any area. The RSC also can test families with single gall isolates of \textit{C. q.f} to determine the family’s resistance to that specific strain.

Despite some of these advantages of controlled testing at the RSC, there has been some discrepancy between the results from field testing and the results from the RSC. Loblolly pine families that were resistant in field tests are not always found to be resistant in
controlled inoculations at the RSC (Isik et al. 2008). This raises questions as to whether the RSC is too conservative in their designation of resistance, or if there are family by inoculum (GxE) interactions occurring in the field. This is a type of genotype by environment interaction and describes a situation where there is a change in the performance ranking of given genotypes when grown in different environments (Zobel and Talbert, 1984). It is important to understand if GxE is the cause of this discrepancy between RSC and field results. If the methods of the RSC are causing field resistant families to be designated as susceptible, then a significant issue arises because good families are being disregarded for field planting. If GxE is in fact the cause, then this too is a critical issue, because most of the selections in breeding programs are based on testing in narrow geographic regions.

The discrepancies found between field results and controlled inoculations (Isik et al. 2008) raise a concern that GxE interactions could have tangible manifestations in the field. Furthermore, the most rust resistant loblolly families (based on field-test data) were found to be the least predictable for performance across sites (McKeand et al. 2003). For some families, the regression of family means for rust infection versus site means for rust infection had low coefficients of determination ($r^2$). It was hypothesized that the low correlation of some loblolly family means with site rust infection means may be due to interactions of families’ susceptibility/resistance and specific pathogen avirulence/virulence among sites (McKeand et al. 1999, Isik et al. 2012). This causes additional concern that meaningful GxE interactions may exist, although these findings come with caveats as well. Although significant genotype by environment interactions were found for fusiform rust resistance, the
overall variance was small in comparison to the family variance, and much of the genotype by environment interaction was due to heterogeneous regression of families across different sites and not family rank change (McKeand et al. 1999). While various studies have shown some evidence of GxE interactions occurring for fusiform rust resistance in loblolly pine, it has yet to be demonstrated that these interactions cause significant manifestations suggesting local testing is not sufficient. It is important that this issue is investigated, so that breeders can confidently determine if local testing is suitable for determining fusiform rust resistance in loblolly pine.

The purpose of this study is to investigate if there are genotype by environment interactions for fusiform rust resistance in loblolly pine. It has previously been found that rust incidence is reasonably predictable across sites due to expected individual and family mean heritabilities and non-substantial genotype by environment interactions (e.g. Chamblee 2011). These results were based on the Plantation Selection Seed Sources Study (PSSSS). The PSSSS aimed to gain information on the performance, adaptability, and patterns of variation of loblolly families from various geographic sources, planted in different regions of the southeastern US. The study looked to determine if there were genotype by environment interactions involved with moving a families across regions, and to determine if gain could be made by replacing local sources with better ones.

Second-generation selections and plantation selections were bred in 6-parent diallels and tested by members of the North Carolina State University Cooperative Tree Improvement
Program in the early stages of their breeding program. The families used in this diallel testing effort were only tested in narrow geographic regions. The diallel testing effort consisted of many test series in locations throughout much of the loblolly pine native range, but each family was only tested in a single test series in a narrow geographic region. These diallel test series are an example of local testing.

Using data from a set of families that were tested in both the PSSSS and diallel trials provides a unique opportunity to compare rust breeding values that are based on local tests (diallel trials) to those same families that were widely tested in the PSSSS trials. This will allow the breeding values for each family to be compared to see if a genotype by environment interaction is occurring and to determine if there is evidence of important virulence variation in the locally estimated breeding values. Even though previous studies have found that within the PSSSS there is little significant genotype by environment interaction occurring for the majority of quality traits and fusiform rust resistance (Chamblee 2011), studies showing a discrepancy between rust resistance in field trials and controlled inoculations at the RSC suggest there still may be important interactions occurring (e.g. Isik et al. 2008).

The majority of selections in the NCSU Cooperative Tree Improvement Program’s breeding populations are based on local testing, so elucidating any problems with family by rust inoculum interactions is of vital importance. Additionally, comparing rust breeding values from families in the PSSSS to the same families in the plantation selection diallel will
provide a stronger determination of genotype by environment interactions than the results based solely on the PSSSS. This is because the plantation selection diallel has many more replicates of the families at each of the locally tested sites which will statistically strengthen the significance of this analysis. This study aims to determine if testing families of loblolly pine for fusiform rust resistance in narrow geographic areas is reliable for predicting performance across a wide geographic range.

Materials and Methods

Experimental designs

*Plantation Selection Dials*les*

The plantation selection effort began in 1975 to supplement the Cooperative’s relatively small and genetically less diverse second-generation population (see Weir and Zobel, 1975 for details). Selections were mated in 6-parent half dials with no selves (e.g. 15 crosses per diallel), and the crosses from 2 and occasionally 3 dials were tested in a test series that consisted of 4 sites (tests) that are all very close to each other, usually within no more than a few adjacent counties. At each of the four test sites within a series, the design was a randomized complete block with 6 replications of 6-tree plots for a total of 36 seedlings per cross per test or 14 seedlings per cross per test series.
This analysis used a set of 31 of the plantation selection diallel test series that contain the 56 half-sib families of interest that were also tested in the PSSSS trials. The selections in the plantation selection diallels were tested as full-sib families. Some of the diallel test series only contain 1 family of interest, while others contain several. The 31 diallel test series used were located in North Carolina, South Carolina, Georgia, Alabama, Mississippi, and Florida. For any given diallel test series, most tests are located in just one state although some have tests in adjacent states, and each test is located relatively close to the other tests in the same series. Each test series included checklots for the region where the test series is located. Each test contained 4 local checklots that were randomly distributed throughout each rep. The same 4 checklots were used in each of the 4 tests per each test series. Across the 31 test series, 11 checklots from different seed sources were used (Table 2.1). See NCSU Tree Improvement Cooperative 28\textsuperscript{th} Annual Report (1984) for details of checklots (http://treeimprovement.org/sites/default/files/annual_reports/1984_Annual_Report_28.pdf).

Traits that were measured at age 6 included rust incidence (the presence or absence of fusiform rust galls on main stems or branches), height, diameter at breast height (DBH), sweep, straightness, and survival. Rust was measured as a binary trait with a 1 indicating a tree with rust infection and a 0 indicating a tree without rust infection. Straightness was measured on a scale from 1 to 6 with lower scores being straighter. Straightness was converted to a binary trait by设计ating a selection with a straightness class below the average as a 1 and a selection with a straightness class above average as a 0; 1 being straight
and 0 being not straight. Height and DBH were used to calculate individual tree volume (dm$^3$) using the equation of Sherrill et al. (2008).

*Plantation Selection Seed Source Study (PSSSS)*

The Plantation Selection Seed Source Study was established to gain information on the performance, adaptability, and patterns of variation of loblolly families from various geographic sources, planted in different regions of the southeastern US. The study looked to determine if there were genotype by environment when moving families across regions, and to determine if gain could be made by replacing local sources with better ones. The PSSSS used 140 families of loblolly pine, and a subset of those families came from Coastal seed sources and were mated in 6-tree diallels and established in field trials in local region.

The selections used in the PSSSS were randomly chosen from a pool of 3300 plantation selections in 7 breeding regions throughout the southeastern United States. In each region, 20 selections were randomly chosen as females for a total of 140 families. These selections were mated with a pollen mix of 40 pollen parents for each region giving a total of 280 pollen parents. A randomized complete block design with single tree plots was used for all sites. Each test had 24 blocks, with a single tree from each family and four seedlings from the local unimproved checklot randomly distributed throughout each block. All families were tested throughout the loblolly pine native range east of the Mississippi. There are 7 regions
in the PSSSS (1-VA and northern NC, 2-Coastal NC, 3-Coastal SC, 4-Coastal GA & FL, 5-Lower Gulf, 6-Upper Gulf, 7-Piedmont GA, SC, NC). The seedling were planted at 21 sites.

For this study, only families from the Coastal Plain breeding region were used. Of the 140 families originally contained in the PSSSS, 83 families that were from the following Coastal seed sources were used: 2-Coastal NC, 3-Coastal SC, 4-Coastal GA & FL, 5-Lower Gulf. There were 3 families out of the 83 that were in only a few tests and were excluded from the analysis. Out of these 80 families, 56 families were chosen that were well tested among all sites and had rust breeding values.

A subset of the original PSSSS tests sites was used for this analysis. The PSSSS originally consisted of 21 sites, but 14 were chosen to be included in this analysis. Some sites were not included because they were not in or near the Coastal Plain, and other sites were not included because of a lack of 8-year rust data recorded for them. The 14 sites were chosen based on their location in the Atlantic or Gulf Coastal Plain and lower Piedmont of South Carolina.

Traits that were measured at age 8 years included rust incidence (the presence or absence of fusiform rust galls on main stems or branches), height, diameter at breast height (DBH), sweep, straightness, forking, and survival. Rust was measured as a binary trait with a 1 indicating a tree with rust infection and a 0 indicating a tree without rust infection. Straightness was measured on a scale from 1 to 6 with lower scores being straighter. Straightness was converted to a binary trait by designating a selection with a straightness
class below the average for the PSSSS as a 1 and a selection with a straightness class above average as a 0; 1 being straight and 0 being not straight. Forking was determined as the presence or absence of forks or major ramicorn branching for a tree. Height and DBH were used to calculate individual tree volume (dm$^3$) using the equation of Sherrill et al. (2008)

**Statistical Analysis**

*Plantation Selection Seed Source Study (PSSSS)*

Summary statistics were calculated to determine the 8-year family means for rust as well as 8-year site means for rust, height, DBH, volume, and straightness in the PSSSS. Mean survival for the PSSSS was also calculated. In this chapter, only the analyses for rust are presented.

A linear mixed model was used to analyze rust incidence with assumptions that errors are independently and identically distributed with mean of zero. Rust at age 8 was modeled with a binomial distribution and a logit function. Seed source, test site, the interaction of seed source and test site, and block nested in test site were treated as a fixed effects in the model. Family nested in seed source and the interaction of test site with family nested in seed source were treated as random effects.
\[ Y_{ijklm} = \log(\pi / (1 - \pi)) = \mu + T_i + S_j + TS_{ij} + B(T)_{k(i)} + F(S)_{l(j)} + TF(S)_{li(j)} + E_{ijklm} \]

Where,

\( Y_{ijklm} \) = response variable of the m-th tree of the l-th family at the k-th block in j-th seed source of the i-th test site

\( \mu \) = overall mean

\( T_i \) = i-th test site

\( S_j \) = j-th seed source

\( TS_{ij} \) = interaction of the i-th test site and the j-th seed source

\( B(T)_{k(i)} \) = k-th block nested in the i-th test site

\( F(S)_{l(j)} \) = l-th family nested in the j-th seed source

\( TF(S)_{li(j)} \) = interaction of the i-th test site with the l-th family nested in the j-th seed source

with expectations NID \( \sim (0, \sigma^2) \)

\( E_{ijklm} \) = random error term with expectations NID \( \sim (0, \sigma^2_e) \)

Using the variances components, the family mean heritabilities for rust incidence across all 14 test sites were estimated as follows:

\[ h_f^2 = \frac{\sigma^2_f(s)}{\sigma^2_f(s) + \frac{\sigma^2_{tf(s)}}{t} + \frac{\sigma^2_e}{tn}} \]

Where \( h_f^2 \) is the pooled family mean heritability across all sites, \( \sigma^2_f(s) \) is the family nested in seed source variance, \( \sigma^2_{tf(s)} \) is the family nested in seed source by site or GxE interaction.
variance, $\sigma^2_e$ is the error variance, $t$ is the number of test sites used in this study, and $n$ is the average number of individuals for a family. This equation has been used commonly to estimate family mean heritabilities (Isik et al. 2008). A value of 3.29 was used as the error variance because rust incidence is a binomial trait.

Using the family by environment interaction and the additive genetic variance, type B correlation was calculated to examine the GxE interaction for rust incidence across all the sites:

$$r_{Bg} = \frac{\sigma^2_f(s)}{\sigma^2_f(s) + \sigma^2_{lf(s)}}$$

[3]

Where $r_{Bg}$ is the type B correlation coefficient across all sites, $\sigma^2_f(s)$ is the family variance, and $\sigma^2_{lf(s)}$ is the family by location, or GxE interaction variance. This is a ratio of family variance components over the sum of the family and genotype-by-environment interaction variance (Yamada 1962). This ratio has been used commonly to quantify the level of genotype-by-environment interaction in progeny trials (Burdon 1977). The type B correlation coefficient gives a general prediction of importance for interaction terms where a value less than 0.67 suggests that family by test site (GxE) interactions could be considerable and warrants closer analysis to understand the underlying causes (Burdon 1977, Shelbourne 1972, Isik et al. 2008, Komakech 2009).
Summary statistics were calculated to determine the mean rust incidence at age 6 for each of the 31 test series, the tests within each test series, as well as each parent and checklot. Additionally, 6-year test series means for height, DBH, volume, and straightness were calculated for each test series. Mean survival for the 31 test series as a whole was also calculated.

The dataset used to analyze the families tested in the plantation selection diallels was a subset of all of the diallels in the study. The 56 families of interest from the PSSSS were only tested in 31 diallel test series, so the dataset used was a subset containing only data from those 31 test series. Although we were only interested in the 56 families shared between the PSSSS and the plantation selection diallels, the dataset for the diallels contained all of the 375 parents that were tested in the 31 diallel test series of interest. The pedigree file contains all of these 375 parents as well as the 11 checklots that were used within those 31 diallel test series. It is necessary to include to checklots in the pedigree file because they are what connect all of the parents and allows them to be compared to others in different diallel test series.

A linear mixed model was used to analyze rust with assumptions that errors are independently and identically distributed with mean of zero. Rust at age 6 was modeled with a binomial distribution and a logit function. Test series and test site nested in test series were
considered fixed effects. Parent 1, parent 2, the interaction of parent 1 and test site nested in test series, the interaction of parent 2 and test site nested in test series, cross, and the interaction of cross and test site nested in test series, were considered random effects.

\[ 4 \] \( Y_{ijklm} = \log\left[ \pi / (1-\pi) \right] = \mu + S_i + T(S)_{j(i)} + GCA_k + GCA_l + SCA_{kl} + T^*GCA_{jk} + T^*GCA_{jl} + T^*SCA_{jkl} + E_{ijklm} \)

Where,

\( Y_{ijklm} \) = the response variable of the m-th observation for the kl-th cross of the j-th test site in the i-th series;
\( \mu \) = overall mean;
\( S_i \) = the i-th fixed series effect;
\( T(S)_{j(i)} \) = the fixed effect of the j-th test site within the i-th series;
\( GCA_k, GCA_l \) = the random general combining ability (GCA) effect of the k-th female or the l-th male ~Normally, Independently Distributed (NID) (0, \( \sigma^2_{G} \));
\( SCA_{kl} \) = the random specific combining ability (SCA) effect of the k-th and the l-th parents ~NID (0, \( \sigma^2_{S} \));
\( T^*GCA_{jk}, T^*GCA_{jl} \) = the random GCA by test site interaction ~NID (0, \( \sigma^2_{TG} \));
\( T^*SCA_{jkl} \) = the random SCA by test site interaction effect ~NID (0, \( \sigma^2_{TS} \));
\( E_{ijklm} \) = the random error term ~NID (0, \( \sigma^2_{E} \));
Using the variance components, the family mean heritabilities for rust incidence across all 31 sites (diallel test series) were estimated as follows:

\[
\hat{h}_f^2 = \frac{\sigma_G^2}{p \sigma_G^2 + \sigma_S^2 + \frac{p \sigma_{TG}^2}{t(p-1)} + \frac{\sigma_{TS}^2}{t(p-1)} + \frac{\sigma_E^2}{tn(p-1)}}
\]

Where \(\hat{h}_f^2\) is the pooled family mean heritability across all diallel series, \(\sigma_G^2\) is the GCA (family) variance, \(\sigma_S^2\) is the SCA variance, \(\sigma_{TG}^2\) is the GCA by site variance (GxE interaction), \(\sigma_{TS}^2\) is the SCA by site variance (GxE interaction), \(\sigma_E^2\) is the error variance, \(t\) is the number of test sites used in this study, \(p\) is the average number of parents per diallel, \(t\) is the number of test sites, and \(n\) is the average number of individuals for a family per site. A value of 3.29 was used as the error variance because rust incidence is a binomial trait.

Using the family by environment interaction and the additive genetic variance, type B correlation was calculated to examine the GxE interaction for rust incidence across all the sites:

\[
\gamma_{BG} = \frac{\sigma_{f(s)}^2}{\sigma_{f(s)}^2 + \sigma_{If(s)}^2}
\]

[6]
Where \( r_{bg} \) is the type B correlation coefficient across all sites, \( \sigma^2_{(s)} \) is the family variance, and \( \sigma^2_{I(s)} \) is the family by location, or GxE interaction variance.

**Estimation of Breeding Values**

In the PSSSS tests, eight-year rust breeding values for the 56 families were calculated using ASReml (Gilmour et al. 2006). For the diallels, six-year rust breeding values for the 56 families were calculated using ASReml (Gilmour et al. 2006). ASReml output a solution file containing GCA estimates for each family. For the families of interest, breeding values were calculated on the logit scale by multiplying the GCA estimates by two, adding the provenance effect, and centering on the mean incidence.

\[
BV_{\text{logit}} = \hat{\text{prov}} + 2\text{GCA} + \mu
\]

No provenance effect was estimated for diallel tests, so \( \text{prov} \) was dropped from the above formula. The breeding values on the logit scale were then converted to probability scale.

\[
BV_{\text{prob}} = \frac{1}{1 + \exp(BV_{\text{logit}})}
\]

A regression analysis between the rust breeding values of locally tested families in the planation selection diallels and the broadly tested families in the PSSSS was used to examine
how well the locally-estimated breeding values predicted the breeding values from the broadly-estimated breeding values. The following linear regression model was used:

\[ y = \beta_0 + \beta_1 x + \epsilon \]

where \( y \) is the PSSSS breeding values, \( \beta_0 \) is the intercept, \( \beta_1 \) is the coefficient for the diallel breeding values, and \( \epsilon \) is the error. Model significance was tested with the null hypothesis \( H_0: \beta_1 = 0 \) and \( H_1: \beta_1 \neq 0 \) with the significance level set at \( \alpha=0.05 \). The model was run using the REG procedure of SAS (SAS Institute Inc. 2008).

**Results**

*Summary Statistics*

The site means for survival for the 14 PSSSS tests were relatively high, averaging 89.6% across all test series. The PSSSS test sites also had a balanced spread of rust incidence with a mean rust incidence of 0.50 across the sites (Table 2.2).

The diallel test series means for survival were high, averaging 91.5% across all test series. The families were well tested and had a balanced spread of rust incidence at these test series with a mean rust incidence of 0.41, and minimum of 0.27 and a maximum of 0.73 (Table 2.3).
Genetic Variation for Rust

There was substantial genetic variation for rust incidence across the 14 test sites in the PSSSS, with family means ranging from 0.08 to 0.83. There was also substantial genetic variation for rust incidence across the 31 diallel test series. Test series means ranged from 0.27 to 0.73 in the diallel tests (Table 2.3). Using variance components from the linear mixed models run by ASReml, the family mean heritabilities for rust incidence across all sites were estimated for both the PSSSS and the plantation selection diallels. The average number of individuals in a half-sib family (n) was 330 for the PSSSS and 629 for the plantation selection diallels. The family mean heritability was 0.96 in the PSSSS and 0.87 in the plantation selection diallels (Table 2.4) (Table 2.5).

Type B Genetic Correlations

Using the family by environment interaction and the additive genetic variance, type B genetic correlations were calculated to examine the magnitude of the GxE interaction for rust incidence across all the sites for both the PSSSS and the plantation selection diallels. The type B genetic correlation was 0.85 for the PSSSS and 0.91 for the plantation selection diallels (Table 2.4) (Table 2.5). This indicates that there are not GxE interactions important to breeding for rust incidence in either study since the type B correlations are much higher than the 0.67 value that would suggest meaningful GxE interactions (Shelbourne 1972, Isik et al 2008, Komakech 2009).
Correlation of Rust Breeding Values

The overall regression model showed a significant correlation between the rust breeding values in the PSSSS and the diallels (P< 0.0001). The regression between the diallel and PSSSS breeding values had an $r^2$ of 0.75 (Figure 2.2). The intercept estimate of -0.06 was not significantly different from 0 (P=0.18). The slope estimate of 1.15 was significantly different from 0 (P< 0.0001).

Discussion

The objective of this study was to explore if local testing of loblolly pine families is suitable for estimating fusiform rust resistance across a broad geographic range of sites. The plantation selection seed source study and the plantation selection diallel study provided us with the unique opportunity to compare fusiform resistance of families of loblolly pine tested both broadly and locally. Both the PSSSS and the plantation selection diallel trials are useful studies to examine fusiform rust resistance. Both studies had high survival of the trees as well as a balanced spread of rust incidence across sites, indicating that measurements were taken in areas with a variety of fusiform rust intensity. The high degree of variation across sites is important because we want to insure the families are tested under a variety of inoculum pressure. This balance was expected because both studies had tests in many locations throughout much of loblolly pine’s natural range east of the Mississippi River. There were 56 families that were tested in both the PSSSS and the plantation selection diallels, which
should be an adequate sample size to be confident in our results. These 56 families that were analyzed were tested in 31 diallel test series which is another indicator that our sample size and study design is reliable for testing if local testing is suitable for determining fusiform resistance in a variety of sites. Additionally, the average number of individuals tested in a family was 330 for the PSSSS and 629 for the plantation selection diallels, so the family sizes were large enough to give us reliable rust breeding values.

The family mean heritability for rust resistance was 0.96 in the PSSSS and 0.87 in plantation selection diallel tests indicating that almost all of the variation among families for rust resistance in both studies is explained by additive genetic variation. Therefore, rust resistance in both the PSSSS and diallels is a highly heritable trait that can be greatly manipulated through breeding and selection. These high family mean heritabilities for rust resistance are expected because past studies have shown that fusiform rust susceptibility/ resistance is under a high degree of genetic control (Kinloch and Walkinshaw, 1991; Kinloch and Stonecypher, 1969).

The type B genetic correlation was 0.85 for the PSSSS and 0.93 for the plantation selection diallels. This suggests that no genotype by environment interactions are occurring for fusiform rust resistance that are important for tree breeding in either study since biologically important GxE interactions are indicated by a type B genetic correlation of less than 0.67 (Shelbourne 1972, Isik et al 2008, Komakech 2009). The high type B genetic correlation of 0.93 is the diallels is not very surprising due to the fact that all of the families were tested in a
narrow geographic region. The type B genetic correlation of 0.85 for the PSSSS is also fairly high, and this figure is much more meaningful and revealing concerning the absence of important GxE interactions for fusiform resistance in loblolly pine. In the PSSSS, we analyzed 56 families that were each tested at the same 14 sites throughout a broad range of sites dispersed around the coastal Southeast (Figure 2.1). If meaningful GxE interactions were occurring, they would surely be elucidated by the analysis of this comprehensive study. The fact that no meaningful GxE interactions were identified in the analysis of the PSSSS gives us confidence that resistant families can be deployed with confidence to a range of sites.

The regression analysis performed comparing the rust breeding values calculated for PSSSS and the diallels also supports that no significant GxE interaction is occurring. The coefficient of determination ($r^2$) of 0.75 suggests that approximately 75% of the variation of rust breeding values in the PSSSS is explained by the variation of rust breeding values in the plantation selection diallels. These breeding values are highly correlated with each other enough to suggest a lack of meaningful GxE occurring between the families tested. It appears that regardless of where the families were tested, their fusiform rust resistance remains relatively consistent. This regression analysis is also particularly robust and reliable due to the high degree of replication of families tested in the diallels. Because of this high degree of replication, we are very confident that the breeding values for the families in the diallels are highly accurate for their performance in the location in which they are tested. The fact that the breeding values for those same families, tested broadly, in the PSSSS are fairly consistent
with those from the diallels, suggest to us, that in general, we would not expect the relative rankings of families to change.

This finding that locally derived rust breeding values hold true across a broad range of sites is very important for tree breeding. Tree breeders have relied on the accuracy of local testing, and if there are meaningful family by inocula interactions occurring for fusiform rust resistance in the field, then tree breeders could be telling foresters to plant families that they think are resistant but may not be resistant in all environments.

In other studies, it has clearly been shown that significant family by inocula interactions exist for various loblolly families with major gene resistances (Isik et al. 2012). Families bearing the R allele at Fr loci did not perform the same with various inocula in controlled inoculations, suggesting that the underlying reason for family by inocula interaction is the variable response of R genotypes (Isik et al. 2012). Other past studies showing evidence of GxE interactions have raised concerns that the current methodology for testing fusiform rust resistance may not be sufficient (Isik et al. 2008, McKeand et al. 2003, McKeand et al. 1999). If this was the case, it would cause a major problem for tree breeders and their ability to test fusiform rust resistance. Our findings, however, can give tree breeders confidence that the current methodology is accurate, and locally derived rust breeding values are reliable predictors of the families performance across a broad range of sites.
If we are seeing robust evidence that important GxE interactions are not occurring for fusiform rust resistance in the field, then why are field resistant families appearing susceptible when tested in controlled inoculations at the Resistance Screening Center (e.g. Isik et al. 2008)? The RSC study used inocula from 10 different geographic collection areas in the southern United States, and each of the inocula was a 30 gall mix, derived by blending 10 gall collections from three different sites within a collection area; a spore density of 20,000 basidiospores/ml was applied (Knighten et al. 1988; Isik et al. 2008). When a family is designated as resistant at the RSC it is almost always resistant in the field, however, some families designated as susceptible at the RSC are in fact resistant in the field (Isik et al. 2008).

One hypothesis for why this may be occurring is that in the field the families may only encounter a limited number of pathotypes of the fungus, while at the RSC, the bulked inocula exposes the family to a large variety of many pathotypes. The results from our research showing no meaningful GxE interactions in the field suggest, however, that this is not the case. An alternative hypothesis is that the density of inoculum used at the RSC may be so high that it is overcoming polygenic resistance mechanisms that would normally be effective in the field.

Past studies have suggested that the mixed inheritance model with both oligogenic and polygenic effects best describe the disease resistance mechanism in loblolly pin (Li et al. 2006). Gene-for-gene interactions do not preclude the modifying influence of other nonspecifically acting genes in the final outcome of the host-pathogen encounter. If the effect of this non-specific polygenic resistance can be overcome by high inoculum pressure,
the high inoculation density used at the RSC may be causing families that are functionally field resistant to be falsely categorized as susceptible.

The bottom line for tree breeders is the need to deploy families that will be rust resistant in the field. The RSC is a very useful tool for getting quick results on families’ resistance/susceptibility, but it is a concerning issue if field resistant families are being falsely designated as susceptible. Further research efforts should investigate if its current methods are too discerning, and if they need to be revised to better serve its purpose of providing accurate information on family deployment.
REFERENCES


McKeand, S.E., Amerson, H.V., Li, B., and Mullin, T.J. 2003. Families of loblolly pine that are the most stable for resistance to fusiform rust are the least predictable. Can. J. Res. 33: 1335–1339.


Table 2.1: Checklots used in diallel test series

<table>
<thead>
<tr>
<th>Checklot ID</th>
<th>Region</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC1</td>
<td>Virginia</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC2</td>
<td>Coastal NC</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC3</td>
<td>Coastal SC</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC4</td>
<td>Coastal GA &amp; FL</td>
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</tr>
<tr>
<td>CC5</td>
<td>Lower Gulf</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC6</td>
<td>Upper Gulf</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC7</td>
<td>Piedmont GA, SC, NC</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>SOM2</td>
<td>Coastal NC</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
<tr>
<td>SOM3</td>
<td>Coastal SC</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
<tr>
<td>SOM4</td>
<td>Coastal GA &amp; FL</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
<tr>
<td>SOM5</td>
<td>Lower Gulf</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
</tbody>
</table>
Table 2.2: Test site means for Rust Incidence, Height, diameter at breast height (DBH), Stem Volume, and Straightness at age 8 from PSSSS trials.

<table>
<thead>
<tr>
<th>Site</th>
<th>Rust Incidence</th>
<th>Height (m)</th>
<th>DBH (cm)</th>
<th>Stem Volume (dm$^3$)</th>
<th>Straightness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northampton, NC</td>
<td>0.83</td>
<td>7.9</td>
<td>15.2</td>
<td>56.9</td>
<td>0.47</td>
</tr>
<tr>
<td>Laurens, SC</td>
<td>0.44</td>
<td>8.9</td>
<td>14.2</td>
<td>52.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Newberry, SC</td>
<td>0.68</td>
<td>9.2</td>
<td>16.5</td>
<td>75.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Robeson, NC</td>
<td>0.45</td>
<td>10.4</td>
<td>16.3</td>
<td>80.1</td>
<td>0.48</td>
</tr>
<tr>
<td>Autauga, AL</td>
<td>0.68</td>
<td>8.8</td>
<td>15.5</td>
<td>61.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Williamsburg, SC</td>
<td>0.42</td>
<td>7.7</td>
<td>12.2</td>
<td>36.2</td>
<td>0.63</td>
</tr>
<tr>
<td>Randolph, GA</td>
<td>0.61</td>
<td>7.0</td>
<td>11.4</td>
<td>28.0</td>
<td>0.65</td>
</tr>
<tr>
<td>Escambia 1, AL</td>
<td>0.08</td>
<td>10.5</td>
<td>13.7</td>
<td>60.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Escambia 2, AL</td>
<td>0.27</td>
<td>10.6</td>
<td>16.5</td>
<td>84.4</td>
<td>0.60</td>
</tr>
<tr>
<td>Screven, GA</td>
<td>0.54</td>
<td>8.9</td>
<td>14.5</td>
<td>55.8</td>
<td>0.58</td>
</tr>
<tr>
<td>Berkeley, SC</td>
<td>0.69</td>
<td>11.9</td>
<td>17.3</td>
<td>103.4</td>
<td>0.40</td>
</tr>
<tr>
<td>Tattnall, GA</td>
<td>0.48</td>
<td>12.3</td>
<td>16.3</td>
<td>96.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Bradford, FL</td>
<td>0.62</td>
<td>8.4</td>
<td>11.2</td>
<td>33.7</td>
<td>0.51</td>
</tr>
<tr>
<td>Ware, GA</td>
<td>0.21</td>
<td>8.0</td>
<td>10.4</td>
<td>26.6</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td><strong>0.50</strong></td>
<td><strong>9.32</strong></td>
<td><strong>14.37</strong></td>
<td><strong>60.78</strong></td>
<td><strong>0.54</strong></td>
</tr>
</tbody>
</table>
Table 2.3: Mean rust incidence, height, DBH, stem volume, and straightness score at age 6 for 31 diallel test series

<table>
<thead>
<tr>
<th>Series</th>
<th>Rust Incidence</th>
<th>Height (m)</th>
<th>DBH (cm)</th>
<th>Volume (dm³)</th>
<th>Straightness</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0073</td>
<td>0.27</td>
<td>5.55</td>
<td>8.43</td>
<td>14.68</td>
<td>0.58</td>
</tr>
<tr>
<td>S0074</td>
<td>0.28</td>
<td>6.24</td>
<td>9.47</td>
<td>18.40</td>
<td>0.67</td>
</tr>
<tr>
<td>S0081</td>
<td>0.30</td>
<td>5.72</td>
<td>10.18</td>
<td>19.73</td>
<td>0.32</td>
</tr>
<tr>
<td>S0121</td>
<td>0.44</td>
<td>7.57</td>
<td>12.77</td>
<td>37.22</td>
<td>0.46</td>
</tr>
<tr>
<td>S0135</td>
<td>0.34</td>
<td>6.46</td>
<td>11.43</td>
<td>27.56</td>
<td>0.50</td>
</tr>
<tr>
<td>S0139</td>
<td>0.37</td>
<td>5.87</td>
<td>NA</td>
<td>NA</td>
<td>0.55</td>
</tr>
<tr>
<td>S0149</td>
<td>0.31</td>
<td>6.64</td>
<td>NA</td>
<td>NA</td>
<td>0.49</td>
</tr>
<tr>
<td>S0150</td>
<td>0.27</td>
<td>4.33</td>
<td>5.89</td>
<td>7.65</td>
<td>NA</td>
</tr>
<tr>
<td>S0151</td>
<td>0.21</td>
<td>4.25</td>
<td>6.17</td>
<td>7.88</td>
<td>NA</td>
</tr>
<tr>
<td>S0157</td>
<td>0.46</td>
<td>6.74</td>
<td>10.59</td>
<td>22.99</td>
<td>0.41</td>
</tr>
<tr>
<td>S0158</td>
<td>0.63</td>
<td>7.63</td>
<td>13.52</td>
<td>43.13</td>
<td>0.30</td>
</tr>
<tr>
<td>S0168</td>
<td>0.34</td>
<td>8.78</td>
<td>14.75</td>
<td>57.24</td>
<td>0.53</td>
</tr>
<tr>
<td>S0191</td>
<td>0.34</td>
<td>6.84</td>
<td>11.68</td>
<td>28.56</td>
<td>0.38</td>
</tr>
<tr>
<td>S0197</td>
<td>0.41</td>
<td>8.48</td>
<td>14.28</td>
<td>51.73</td>
<td>0.57</td>
</tr>
<tr>
<td>S0200</td>
<td>0.29</td>
<td>6.34</td>
<td>10.52</td>
<td>24.23</td>
<td>0.54</td>
</tr>
<tr>
<td>S0201</td>
<td>0.36</td>
<td>6.85</td>
<td>11.09</td>
<td>27.89</td>
<td>0.55</td>
</tr>
<tr>
<td>S0244</td>
<td>0.31</td>
<td>8.75</td>
<td>14.50</td>
<td>54.95</td>
<td>0.54</td>
</tr>
<tr>
<td>S0266</td>
<td>0.33</td>
<td>6.91</td>
<td>11.01</td>
<td>28.80</td>
<td>0.38</td>
</tr>
<tr>
<td>S0267</td>
<td>0.44</td>
<td>7.74</td>
<td>12.16</td>
<td>36.50</td>
<td>0.41</td>
</tr>
<tr>
<td>S0279</td>
<td>0.49</td>
<td>5.32</td>
<td>8.12</td>
<td>13.49</td>
<td>0.50</td>
</tr>
<tr>
<td>S0348</td>
<td>0.54</td>
<td>5.95</td>
<td>9.93</td>
<td>20.42</td>
<td>0.52</td>
</tr>
<tr>
<td>S0349</td>
<td>0.56</td>
<td>5.88</td>
<td>9.69</td>
<td>19.54</td>
<td>0.48</td>
</tr>
<tr>
<td>S0353</td>
<td>0.73</td>
<td>5.53</td>
<td>10.11</td>
<td>18.29</td>
<td>0.43</td>
</tr>
<tr>
<td>S0355</td>
<td>0.54</td>
<td>8.93</td>
<td>14.10</td>
<td>71.49</td>
<td>0.43</td>
</tr>
<tr>
<td>S0356</td>
<td>0.31</td>
<td>6.46</td>
<td>11.82</td>
<td>29.35</td>
<td>0.59</td>
</tr>
<tr>
<td>S0370</td>
<td>0.32</td>
<td>8.52</td>
<td>13.92</td>
<td>50.98</td>
<td>0.46</td>
</tr>
<tr>
<td>S0408</td>
<td>0.48</td>
<td>6.85</td>
<td>11.53</td>
<td>29.23</td>
<td>0.66</td>
</tr>
<tr>
<td>S0414</td>
<td>0.30</td>
<td>7.00</td>
<td>11.28</td>
<td>27.94</td>
<td>0.66</td>
</tr>
<tr>
<td>S0430</td>
<td>0.54</td>
<td>6.90</td>
<td>11.59</td>
<td>29.28</td>
<td>0.64</td>
</tr>
<tr>
<td>S0431</td>
<td>0.72</td>
<td>6.31</td>
<td>10.94</td>
<td>24.75</td>
<td>0.56</td>
</tr>
<tr>
<td>S0438</td>
<td>0.53</td>
<td>6.10</td>
<td>9.70</td>
<td>18.96</td>
<td>0.48</td>
</tr>
<tr>
<td>Means</td>
<td>0.41</td>
<td>6.69</td>
<td>11.07</td>
<td>29.75</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Table 2.4: Variance components (standard errors follow ±) for family, family by environment interaction (GxE), type B genetic correlation ($r_B$), individual tree ($h^2$) and family mean ($h^2_f$) heritabilities for rust incidence, height, DBH, volume, and straightness across all 14 PSSSS sites.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Family ± SE</th>
<th>GxE ± SE</th>
<th>$r_B$</th>
<th>$h^2$ ± SE</th>
<th>$h^2_f$ ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rust Incidence</td>
<td>0.365 ± 0.063</td>
<td>0.058 ± 0.014</td>
<td>0.85</td>
<td>NA</td>
<td>0.96 ± 0.007</td>
</tr>
<tr>
<td>Height</td>
<td>53.33 ± 9.52</td>
<td>21.49 ± 3.24</td>
<td>0.71</td>
<td>0.21 ± 0.04</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>DBH</td>
<td>0.037 ± 0.007</td>
<td>0.034 ± 0.004</td>
<td>0.52</td>
<td>0.17 ± 0.03</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>Volume</td>
<td>6.97 ± 1.31</td>
<td>7.12 ± 0.66</td>
<td>0.49</td>
<td>0.19 ± 0.03</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>Straightness</td>
<td>0.117 ± 0.022</td>
<td>0.035 ± 0.012</td>
<td>0.77</td>
<td>NA</td>
<td>0.90 ± 0.02</td>
</tr>
</tbody>
</table>
Table 2.5: Variance components (standard errors follow ±) for family, family by environment interaction (GxE), type B genetic correlation ($r_B$), individual tree ($h^2$) and family mean ($h^2_f$) heritabilities for rust incidence, height, DBH, volume, and straightness across all 31 diallel test series.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Family± SE</th>
<th>GxE ± SE</th>
<th>$r_B$</th>
<th>$h^2$ ± SE</th>
<th>$h^2_f$ ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rust Incidence</td>
<td>0.22 ± .015</td>
<td>0.022 ± .003</td>
<td>0.91</td>
<td>NA</td>
<td>0.87 ± 0.005</td>
</tr>
<tr>
<td>Height</td>
<td>0.60 ± 0.04</td>
<td>0.096 ± 0.007</td>
<td>0.86</td>
<td>0.21 ± 0.04</td>
<td>0.87 ± 0.005</td>
</tr>
<tr>
<td>DBH</td>
<td>0.03 ± 0.002</td>
<td>0.008 ± 0.0006</td>
<td>0.79</td>
<td>0.18 ± 0.01</td>
<td>0.84 ± 0.007</td>
</tr>
<tr>
<td>Volume</td>
<td>2494.0 ± 191.1</td>
<td>1145.4 ± 72.4</td>
<td>0.69</td>
<td>0.18 ± 0.01</td>
<td>0.81 ± 0.009</td>
</tr>
<tr>
<td>Straightness</td>
<td>0.218 ± 0.015</td>
<td>0.029 ± 0.003</td>
<td>0.88</td>
<td>NA</td>
<td>0.87 ± 0.005</td>
</tr>
</tbody>
</table>
Figure 2.1: Test sites (12 Coastal and 2 Piedmont) used from the Plantation Selection Seed Source Study (PSSSS)
Figure 2.2: Regressing rust breeding values from PSSSS (BVp) on rust breeding values from diallels (BVd). The model was BVp = -0.06 + 1.15*BVd. The intercept was not significantly different from 0 (P=0.18). The slope was significantly different from 0 (P< 0.0001). The model F test was significant at Pr<0.0001 and explained 75% of the variance (r² = 0.75).
Chapter 3:

Broad-Based Testing Compared to Local Testing of Families of Loblolly Pine for Height, DBH, Volume, and Straightness.
Abstract

Breeding loblolly pine *Pinus taeda L.* for traits that impact productivity and stem form is a fundamental goal of breeding programs. Height, diameter at breast height (DBH), volume, and straightness are very important traits for breeding due to both their heritability and economic importance. While the majority of studies have found a lack of evidence that GxE interactions are important for growth traits and stem straightness, there have been studies that suggest otherwise. The objective of this study is to determine if testing families of loblolly pine for growth and form traits (height, DBH, volume, and straightness) in narrow geographic areas yields estimates of breeding values for those traits in accordance with values estimated by testing across a wide geographic range in the southern USA. We compared height, DBH, volume and straightness breeding values from a series of trials planted across a broad geographic range of sites (Plantations Selection Seed Source Study - PSSSS) to breeding values from trials established in more narrow geographic regions. The PSSSS used 83 families that were also tested locally in Plantation Selection Diallel trials. By comparing the breeding values estimated from both types of field trials for a subset of these families, we can elucidate if any important genotype by environment interaction is occurring. No meaningful GxE was found for straightness. We found evidence that some GxE may be occurring for the growth traits (height, DBH, and volume) but not likely enough to warrant site dependent testing and breeding.
Introduction

Loblolly pine (Pinus taeda L.) is the major timber species in the Southeastern United States, with approximately 1 billion seedlings produced annually (McKeand et al. 2003). The native range of loblolly pine extends through 14 states from southern New Jersey south to central Florida and west to eastern Texas. According to U.S. Forest Service surveys, loblolly pine is the second most common species of tree in the United States after red maple (Acer rubrum). Genetic improvement through breeding and selection of loblolly pine since the 1950s has improved the productivity, form, and disease resistance in the species. Since 1977, nearly 100% of loblolly pine plantations in the southeast U.S. have been established with genetically improved loblolly pine (Aspinwall et al. 2012).

Natural variation exists within tree species growing in natural stands. Nearly 90% of this variation is accounted for by provenance variation and tree-to-tree differences (Zobel and Talbert, 1984). Because genetic tests have shown that for many characteristics much of the tree-to-tree variation is genetically controlled, good gains can be achieved from a breeding program that concentrates on tree-to-tree differences. Taking advantage of this genetically controlled natural variation is the basis of tree breeding. In order to make economic gains, it is essential to breed for traits that are heritable with at least moderately strong genetic control as well as traits that are economically important for growing marketable trees. Resistance to fusiform rust, as discussed in Chapter 2, is an example of a heritable trait with natural variation that is economically important for breeding. In addition to disease resistance,
breeding for traits that impact productivity and stem form of loblolly pine is also a fundamental goal of breeding programs. Height, diameter at breast height (DBH), volume, and straightness are very important traits due to both their heritability and economic importance.

Of the three primary categories of traits (productivity, form, and disease resistance) that are desired for selection, productivity related traits are arguably the most important. Growth rate is nearly always the key characteristic in a selection program (Bouvarel, 1966). Height, DBH, and volume are all traits that have weak to moderate genetic control and are of great economic importance (Zobel and Talbert, 1984). Height and volume are the traits most highly correlated with the sawtimber potential score of individual loblolly pine trees (Cumbie et al. 2010). In addition to being economically important, growth and form traits are important to select for due to their relatively high heritability. Half-sib family mean heritability estimates for growth and form traits such as height, DBH, volume and straightness typically fall with the range of 0.89 to 0.95 (Li et al. 1996, Isik et al. 2005, Baltunis et al. 2007, McKeand et al. 2008, Cumbie et al. 2010).

Some traits such as forking are unfavorably correlated with growth traits (Xiong et al. 2010). Additionally, branch diameter is unfavorably correlated with stem diameter (Cumbie et al 2010). While breeding for both good form and growth are important, there are often direct tradeoffs associated with getting favorable results in one category or the other. Earlier author stated that growth, disease resistance, form, and wood properties are all typically
weakly if at all correlated with each other (Zobel and Talbert 1984; McKeand and Bridgwater 1998). By deploying seedlings from families based on only one type of trait, such as growth traits, the potential sawtimber quality of the plantation may be reduced if other quality traits are not emphasized appropriately. It is important to find a balance when breeding for favorable traits at the family level in order to obtain optimal sawtimber quality.

While it is important to balance breeding for a variety of traits which support optimal sawtimber quality, it is also essential that the tests measuring the performance of families for various traits are reliable across a broad range of sites. If tree breeders want to test the performance of families on a local scale and apply those results to a broad range of sites, it is essential that no substantial genotype by environment (GxE) interactions are occurring. Genotype by environment interactions describe a situation where there is a change in the relative performance of given genotypes when grown in different environments (Zobel and Talbert, 1984). Generally GxE has been of little practical significance for most traits in loblolly pine for relatively broad climatic zones. For growth traits, GxE interactions have been found to be of limited importance, even for material originating from widely different sources and site conditions (Yeiser et al. 1981, Li and McKeand 1989, and McKeand et al. 1990). In a comprehensive study of over 7500 full sib families of loblolly pine, relatively little GxE for height and volume was found (McKeand et al., 2006). Furthermore, no G×E was found for total standing volume at age 12 in loblolly pine (Martin and Shiver, 2002), and none was found at age 4 for five open-pollinated loblolly pine families (McCready and Jokela, 1996). Likewise, an analysis of whole tree biomass of 5-year-old loblolly pine from two seed
sources did not demonstrate G×E (Retzlaff et al., 2001). Though less research has been done examining GxE interactions for form traits than growth traits, studies have suggested that GxE interactions are not significant for straightness (Gwaze et al. 2000, Chamblee 2011).

While the majority of studies have found a lack of evidence that GxE interactions are important for growth traits, there have been studies that suggest otherwise. In 15-year-old trials of open pollinated families from central and Gulf Coastal Florida, significant rank changes for stem volume among seven sites were found (Sierra-Lucero et al. 2002). GxE is relatively common, however, when loblolly pine families are moved long distances such as from North Carolina into Arkansas and Oklahoma. Even when a seed source or a provenance generally performs well, thorough testing of families in exotic climatic zones is recommended (Lambeth et al., 2005). The significant GxE interactions found in the study by Sierra-Lucero et al., (2002) for growth traits may be because the loblolly pine families from Florida sources were moved northward one USDA Plant Hardiness Zone. Few other studies have documented GxE interactions for growth traits (Duzan and Williams 1988, Yeiser et al., 2001), but available evidence suggests that when GxE did occur in these situations, it was caused by a limited number of genotypes in the population that were highly sensitive to environmental variation (Zas et al., 2004).

Strong and significant GxE interactions in basal area and volume have been found in loblolly pine in a study where all test locations were located within USDA Plant Hardiness Zone 8b (Roth et al., 2007). Adaptation problems across sites, therefore, would not be expected to
cause GxE interactions in this experiment (Schmidtling, 2001 and Lambeth et al., 2005). This study, however, only tested limited genotypes at few locations. Therefore, it appears that GxE may become significant only under extremes in seed source movement and that relatively few genotypes from the population contribute to this response. Since tree breeders have relied on the performance of genotypes when they are tested in relatively narrow geographic regions, it is important that we achieve a more complete picture concerning the presence or absence of important GxE interactions for various traits in loblolly pine.

The purpose of this study is to investigate if there are genotype by environment interactions for growth (height, DBH, and volume) and form (straightness) traits in loblolly pine. It has previously been found that straightness is reasonably predictable across sites due to non-substantial GxE interactions while growth traits are not due meaningful GxE interactions (e.g. Chamblee 2011). These results were based on the Plantation Selection Seed Sources Study (PSSSS) that was designed to gain information on the performance, adaptability, and patterns of variation of loblolly pine families from various geographic sources, planted in different regions of the southeast US. The study looked to determine if there were genotype by environment interactions involved with moving families across regions and to determine if gain could be made by replacing local sources with better ones.

Diallel breeding and testing was used by the North Carolina State University Cooperative Tree Improvement Program in the second cycle of the breeding program with most of the plantation selections. The families used in this diallel testing effort were only tested locally,
but the replications of the families at each site is at a much larger scale than the PSSSS. The plantation selection diallel testing effort consisted of many test series in different locations throughout much of the loblolly pine native range, but each family was only tested in a single test series in a narrow geographic region. These diallel test series are an example of local testing.

Using data from both the PSSSS and diallel trials provides a unique opportunity to compare breeding values for various traits that are based on local tests (diallel trials) to those same families that were widely tested in the PSSSS trials. This will allow the two breeding values for each family to be compared to see if a genotype by environment interaction is occurring. Even though previous research has found evidence that within the PSSSS there was important GxE interactions for growth traits (Chamblee 2011), other studies with results contrary to these findings suggest that further research is needed to elucidate the presence or absence of meaningful interactions.

The majority of selections in the NCSU Cooperative Tree Improvement Program’s breeding populations are based on local testing, so obtaining a clearer picture concerning GxE interactions is important to determine if site-dependent testing is necessary. Additionally, comparing breeding values from families in the PSSSS to the same ones in the plantation selection diallel will provide a stronger determination of genotype by environment interactions than the results based solely on the PSSSS. This is because the plantation
selection diallel tests have many more replicates of the families at each of the locally tested sites which will strengthen the statistical significance of this analysis.

The first objective of this study is to determine if testing families of loblolly pine for growth and form traits (height, DBH, volume, and straightness) in narrow geographic areas yields breeding value estimates in accordance with estimates based on testing across a wide geographic range in the southern USA. The second objective is to compare the genetic parameters (genotype by environment interaction and heritability) from the same set of families tested in a narrow and in a wide geographic area.

**Materials and Methods**

**Experimental Designs**

*Plantation Selection Dialels*

The plantation selection effort began in 1975 to supplement the Cooperative’s relatively small and genetically less diverse second-generation population (see Weir and Zobel, 1975 for details). Selections were mated in 6-parent half diallels with no selves (e.g. 15 crosses per diallel), and the crosses from 2 and occasionally 3 diallels were tested in a test series that consisted of 4 sites (tests) that are all very close to each other, usually within no more than a few adjacent counties. At each of the four test sites within a series, the design was a
randomized complete block with 6 replications of 6-tree plots for a total of 36 seedlings per cross per test or 140 seedlings per cross per test series.

This analysis used a set of 31 of the plantation selection diallel test series. The data included a subset of families that were also tested in the PSSSS trials. The selections in the plantation selection diallels were tested as full-sib families. Some of the diallel test series only contain 1 family of interest, while others contain up to 3. The 31 diallel test series used were located in North Carolina, South Carolina, Georgia, Alabama, Mississippi, and Florida. For any given diallel test series, most tests are located in just one state. Although some series have tests in adjacent states, each test site is located relatively close to the other test sites in the series. Each test contained 4 local checklots relative to the region and were randomly distributed throughout each replication. The same 4 checklots were used in each of the 4 tests per each test series. Across the 31 test series, 11 checklots from different seed sources were used (Table 3.1). See NCSU Tree Improvement Cooperative 28th Annual Report (1984) for details of checklots (http://treeimprovement.org/sites/default/files/annual_reports/1984_Annual_Report_28.pdf).

Traits that were measured at age 6 years include rust incidence (the presence or absence of fusiform rust galls on main stems or branches), height, diameter at breast height (DBH), sweep, straightness, and survival. Rust was measured as a binary trait with a 1 indicating a tree with rust infection and a 0 indicating a tree without rust infection. Straightness was usually measured on a scale from 1 to 6 with lower scores being straighter. Since
straightness was not measured on the full 1 to 6 scale on all tests, it was converted to a binary trait by designating a selection with a straightness class below the average as a 1 and a selection with a straightness class above average as a 0; 1 being straight and 0 being not straight. Height and DBH were used to calculate individual tree volume (dm$^3$) using the equation of Sherrill et al. (2008).

*Plantation Selection Seed Source Study (PSSSS)*

The Plantation Selection Seed Source Study was established to gain information on the performance, adaptability, and patterns of variation of loblolly families from various geographic sources, planted in different regions of the southeastern US. The study looked to determine if there were genotype by environment interactions involved with moving families across regions and to determine if gain could be made by replacing local families with better ones. The PSSSS tested 140 families of loblolly pine; 83 of those families came from Coastal seed sources and were also included in the diallel test series described above.

The selections used in the PSSSS were randomly chosen from a pool of 3300 plantation selections in 7 breeding regions throughout the southeastern United States. In each region, 20 selections were randomly chosen as females for a total of 140 families. These selections were mated with a pollen mix of 40 pollen parents for each region giving a total of 280 pollen parents. A randomized complete block design with single tree plots was used for all sites. Each test had 24 blocks, with a single tree from each family and four seedlings from
the local unimproved checklot randomly distributed throughout each block. All families were tested throughout the native range of loblolly pine east of the Mississippi. There are 7 regions in the PSSSS (1-VA and northern NC, 2-Coastal NC, 3-Coastal SC, 4-Coastal GA & FL, 5-Lower Gulf, 6-Upper Gulf, 7-Piedmont GA, SC, NC). The seedling were planted at 21 sites.

For this study, only families from the Coastal Plain breeding region were used. Of the 140 families originally contained in the PSSSS, 83 families that were from the following Coastal seed sources were used: 2-Coastal NC, 3-Coastal SC, 4-Coastal GA & FL, 5-Lower Gulf. There were 3 families out of the 83 that were in only a few tests and were excluded from the analysis. Out of these 80 families, 56 families were chosen that were well tested among all sites and had rust breeding values (see chapter 2 for details).

A subset of the original PSSSS tests sites was used for this analysis. The PSSSS originally consisted of 21 sites, but 14 were chosen to be included in this analysis. Some sites were not included because they were not in or near the Coastal Plain, and other sites were not included because of a lack of 8-year rust data recorded for them. The 14 sites were chosen based on their location in the Atlantic or Gulf Coastal Plain and lower Piedmont of South Carolina.

Measurements of various traits were taken at age 8 years. Traits that were measured include rust incidence (the presence or absence of fusiform rust galls on main stems or branches), height, diameter at breast height (DBH), sweep, straightness, forking, and survival. Rust was
measured as a binary trait with a 1 indicating a tree with rust infection and a 0 indicating a tree without rust infection. Straightness was measured on a scale from 1 to 6 with lower scores being straighter. Since straightness was not measured on the full 1 to 6 scale on all tests, it was converted to a binary trait by designating a selection with a straightness class below the average for the PSSSS as a 1 and a selection with a straightness class above average as a 0; 1 being straight and 0 being not straight. Forking was determined as the presence or absence of forks or major ramicorn branching for a tree. Height and DBH were used to calculate individual tree volume (dm$^3$) using the equation of Sherrill et al. (2008).

**Statistical Analysis**

*Plantation Selection Seed Source Study – (PSSSS)*

Summary statistics were calculated to determine the 8-year family means and 8-year site means for rust, height, DBH, volume, and straightness in the PSSSS. Mean survival for the PSSSS was also calculated. A linear mixed model was used to analyze height, DBH, volume, and straightness - with assumptions that errors are independently and identically distributed with mean of zero. The incidence of fusiform rust was not included in this analysis because it is the subject of chapter 2. Height, DBH and volume at age 8 were modeled with a Gaussian distribution. Straightness at age 8 was modeled with a binomial distribution and a logit function. Seed source, test site, the interaction of seed source and test site, and block nested in test site were treated as a fixed effects in the model. Family nested in
seed source and the interaction of test site with family nested in seed source were treated as random effects.

\[ Y_{ijklm} = \log[\pi / (1 - \pi)] = \mu + T_i + S_j + TS_{ij} + B(T)_{k(i)} + F(S)_{l(j)} + TF(S)_{il(j)} + E_{ijklm} \]

Where,

\( Y_{ijklm} = \) response variable of the \( m \)-th tree of the \( l \)-th family at the \( k \)-th block in \( j \)-th seed source of the \( i \)-th test site

\( \mu = \) overall mean

\( T_i = i \)-th test site

\( S_j = j \)-th seed source

\( TS_{ij} = \) interaction of the \( i \)-th test site and the \( j \)-th seed source

\( B(T)_{k(i)} = k \)-th block nested in the \( i \)-th test site

\( F(S)_{l(j)} = l \)-th family nested in the \( j \)-th seed source

\( TF(S)_{il(j)} = \) interaction of the \( i \)-th test site with the \( l \)-th family nested in the \( j \)-th seed source

with expectations NID ~ (0,\( \sigma^2_{ilj} \))

\( E_{ijklm} = \) random error term with expectations NID ~ (0,\( \sigma^2_e \))

Using the variance components, the family mean heritabilities for height, DBH, volume and straightness across all 14 test sites were estimated as follows:

\[ h_f^2 = \frac{\sigma^2_f(s)}{\sigma^2_f(s) + \frac{\sigma^2_{tf(s)}}{t} + \frac{\sigma^2_e}{tn}} \]
Where $h_t^2$ is the pooled family mean heritability across all sites, $\sigma^2_{f(s)}$ is the family nested in seed source variance, $\sigma^2_{t(f(s))}$ is the family nested in seed source by site or GxE interaction variance, $\sigma^2_e$ is the error variance, $t$ is the number of test sites used in this study, and $n$ is the average number of individuals for a family. This equation has been used commonly to estimate family mean heritabilities (Isik et al. 2008). A value of 3.29 was used as the error variance for straightness because it is a binomial trait.

Using the family by environment interaction and the additive genetic variance, the type B correlation was calculated to examine the GxE interaction for height, DBH, volume, and straightness across all the sites:

$$r_{Bg} = \frac{\sigma^2_f(s)}{\sigma^2_f(s) + \sigma^2_{t(f(s))}}$$

[3]

Where $r_{Bg}$ is the type B correlation coefficient across all sites, $\sigma^2_{f(s)}$ is the family variance, and $\sigma^2_{t(f(s))}$ is the family by location, or GxE interaction variance. This is a ratio of family variance components over the sum of the family and genotype-by-environment interaction variance (Yamada 1962). This ratio has been used commonly to quantify the level of genotype-by-environment interaction in progeny trials (Burdon 1977). The type B correlation coefficient gives a general prediction of importance for interaction terms where a value less than 0.67 suggests that family by test site (GxE) interactions could be considerable and warrants closer analysis to understand the underlying causes (Shelbourne 1972, Burdon 1977, Isik et al. 2008, Komakech 2009).
**Plantation Selection Diallels**

Summary statistics were calculated to determine the mean rust incidence, height, DBH, volume, and straightness at age 6 for each of the 31 test series, the tests within each test series, as well as each parent and checklot. Mean survival for the 31 test series as a whole was also calculated.

The dataset used to analyze the families tested in the plantation selection diallels was a subset of all of the diallels in the study. The 56 families of interest from the PSSSS were only tested in 31 diallel test series, so the dataset used was a subset containing only data from those 31 test series. Although we were only interested in the 56 families shared between the PSSSS and the plantation selection diallels, the dataset for the diallels contained all of the 375 parents that were tested in the 31 diallel test series of interest. The pedigree file contains all of these 375 parents as well as the 11 checklots that were used within those 31 diallel test series. It is necessary to include to checklots in the pedigree file because they are what connects all of the parents and allows them to be compared to others in different diallel test series.

A linear mixed model was used to analyze height, DBH, volume, and straightness - with assumptions that errors are independently and identically distributed with mean of zero. Height, DBH and volume at age 6 were modeled with a Gaussian distribution. Straightness at age 6 was modeled with a binomial distribution and a logit function. Test series and test site
nested in test series were considered fixed effects. Parent 1, parent 2, the interaction of parent 1 and test site nested in test series, the interaction of parent 2 and test site nested in test series, cross, and the interaction of cross and test site nested in test series, were considered random effects.

\[ Y_{ijklm} = \log\left(\frac{\pi}{1-\pi}\right) = \mu + S_i + T(S)_{j(i)} + GCA_k + GCA_l + SCA_{kl} + T*GCA_{jk} + T*GCA_{jl} + T*SCA_{jkl} + E_{ijklm} \]

Where,

- \(Y_{ijklm}\) = the response variable of the m-th observation for the kl-th cross of the j-th test site in the i-th series;
- \(\mu\) = overall mean;
- \(S_i\) = the i-th fixed series effect;
- \(T(S)_{j(i)}\) = the fixed effect of the j-th test site within the i-th series;
- \(GCA_k, GCA_l\) = the random general combining ability (GCA) effect of the k-th female or the l-th male \(\sim\) Normally, Independently Distributed (NID) \((0, \sigma^2_G)\);
- \(SCA_{kl}\) = the random specific combining ability (SCA) effect of the k-th and the l-th parents \(\sim\) NID \((0, \sigma^2_S)\);
- \(T*GCA_{jk}, T*GCA_{jl}\) = the random GCA by test site interaction \(\sim\) NID \((0, \sigma^2_{TG})\);
- \(T*SCA_{jkl}\) = the random SCA by test site interaction effect \(\sim\) NID \((0, \sigma^2_{TS})\);
- \(E_{ijklm}\) = the random error term \(\sim\) NID \((0, \sigma^2_E)\);
Using the variance components, the family mean heritabilities for rust incidence across all 31 sites (diallel test series) were estimated as follows:

\[
\hat{h}_f^2 = \frac{\sigma_G^2}{p - 1} + \frac{p\sigma_{TG}^2}{t(p - 1)} + \frac{\sigma_{TS}^2}{t(n - 1)} + \frac{\sigma_E^2}{(p - 1)n}
\]

Where \( \hat{h}_f^2 \) is the pooled family mean heritability across all diallel series, \( \sigma_G^2 \) is the GCA (family) variance, \( \sigma_S^2 \) is the SCA variance, \( \sigma_{TG}^2 \) is the GCA by site variance (GxE interaction), \( \sigma_{TS}^2 \) is the SCA by site variance (GxE interaction), \( \sigma_E^2 \) is the error variance, \( t \) is the number of test sites used in this study, \( p \) is the average number of parents per diallel, \( t \) is the number of test sites, and \( n \) is the average number of individuals for a family per site. A value of 3.29 was used as the error variance for straightness since it is a binomial trait.

Using the family by environment interaction and the additive genetic variance, type B correlation was calculated to examine the GxE interaction for height, DBH, volume and straightness across all the sites:

\[
r_{Bg} = \frac{\sigma_{f(s)}^2}{\sigma_{f(s)}^2 + \sigma_{lf(s)}^2}
\]

Where \( r_{Bg} \) is the type B correlation coefficient across all sites, \( \sigma_{f(s)}^2 \) is the family variance, and \( \sigma_{lf(s)}^2 \) is the family by location, or GxE interaction variance.
Estimation of Breeding Values

In the PSSSS tests, eight-year height, DBH, volume, and straightness breeding values for the 56 families were calculated using ASReml (Gilmour et al 2006). In the diallel tests, six-year height, DBH, volume, and straightness breeding values for the 56 families were calculated using ASReml (Gilmour et al 2006). ASReml outputs a solution file containing GCA estimates for each family. The families of interest were isolated, and their breeding values for height, DBH, and volume were calculated on the probability scale by multiplying the GCA estimates by two and adding the provenance effect.

\[ BV_{prob} = \hat{prov} + 2GCA \]  

For straightness the breeding values were calculated on the logit scale by multiplying the GCA estimates by two, adding the provenance effect, and centering on the mean.

\[ BV_{logit} = \hat{prov} + 2GCA + \mu \]  

For diallel tests, no estimate was made of provenance effect, so prov was dropped from the above formula. The breeding values on the logit scale were then converted to probability scale.
Regression analyses between the height, DBH, volume and straightness breeding values of locally tested families in the planation selection diallels and the broadly tested families in the PSSSS was used to examine their relationships using the following linear regression model:

\[ y = \beta_0 + \beta_1 x + \epsilon \]

Where \( y \) is the PSSSS breeding values, \( \beta_0 \) is the intercept, \( \beta_1 \) is the coefficient for the diallel breeding values, and \( \epsilon \) is the error. Model significance was tested with the null hypothesis \( H_0: \beta_1 = 0 \) and \( H_1: \beta_1 \neq 0 \) with the significance level set at \( \alpha=0.05 \). The model was run using the REG procedure of SAS (SAS Institute Inc. 2008).

Results

Summary Statistics

In the 14 PSSSS test sites, the site means for survival were relatively high, averaging 89.6% across all tests. The 14 PSSSS test sites also had a balanced spread of rust incidence, height, DBH, volume and straightness with a mean rust incidence of 0.50, mean height of 9.3 m,
mean DBH of 14.4 cm, mean volume of 60.8 dm$^3$, and mean straightness of 0.54 across the sites (Table 3.2).

The diallel test series means for survival were high, averaging 91.5% across all test series. The families were well tested and had a balanced spread of rust incidence, height, DBH, volume and straightness at these test series with a mean rust incidence of 0.41, mean height of 6.6 m, mean DBH of 11.1 cm, mean volume of 29.7 dm$^3$, and mean straightness of 0.50 (Table 3.3). DBH and volume were not measured in series S0139 and S0149. Straightness was not measured in series S0150 and S0151.

**Genetic Variation and Heritabilities**

There was notable genetic variation for all traits across the 14 test sites in the PSSSS; family means ranged from 7.0 to 12.3 m for height, from 10.4 to 17.3 cm for DBH, 26.6 to 103.4 dm$^3$ for volume, and 0.40 to 0.63 for straightness (Table 3.2). There was also notable genetic variation for all traits analyzed across the 31 diallel test series. Test series means ranged from 4.3 to 8.9 m for height, from 5.9 to 14.8 cm for DBH, 7.7 to 71.5 dm$^3$ for volume, and 0.30 to 0.66 for straightness in the diallel tests (Table 3.3). Using variance components from the linear mixed models run is ASReml, the family mean heritabilities for each of these traits across all sites was estimated for both the PSSSS and the plantation selection diallels. The average number of individuals in a half-sib family (n) was 330 for the PSSSS and 629 for the plantation selection diallels. The family mean heritabilities in the PSSSS were 0.92 for
height, 0.88 for DBH, 0.88 for volume, and 0.90 for straightness (Table 3.4). The family mean heritabilities in the plantation selection diallels were 0.87 for height, 0.84 for DBH, 0.81 for volume, and 0.87 for straightness (Table 3.5).

**Type B Genetic Correlations**

Using the family by environment interaction and the additive genetic variance, type B genetic correlations were calculated to examine the magnitude of GxE interaction for height, DBH, volume, and straightness across all the sites for both the PSSSS and the plantation selection diallels. In the PSSSS, the type B genetic correlations were 0.71 for height, 0.52 for DBH, 0.49 for volume, and 0.77 for straightness (Table 3.4). In the plantation selection diallels, the type B genetic correlations were 0.86 for height, 0.77 for DBH, 0.71 for volume, and 0.83 for straightness (Table 3.5). Meaningful GxE interactions are indicated by a type B genetic correlation of less than 0.67 (Shelbourne 1972, Isik et al 2008, Komakech 2009).

**Breeding Values**

The overall regression models showed significant correlations among all pairs of breeding values in the PSSSS and the diallel tests, with r² values of 0.55 for height, 0.51 for DBH, 0.50 volume, and 0.69 for straightness. (Figures 3.1 to 3.4). For height, the intercept estimate of 12.27 and slope estimate of 0.99 were significantly different from 0 (P<0.0001). Likewise for DBH and straightness, the intercepts and slopes were significantly different from 0.
(P<0.0001). For volume, the intercept estimate of -0.01 was not significantly different from 0 (P=0.98), but the slope estimate of 1.97 was significantly different from 0 (P<0.0001).

Discussion

The objective of this study was to examine if meaningful GxE interactions exist for growth (height, DBH, and volume) and stem straightness traits in loblolly pine. This information can give breeders insight concerning the suitability of local testing of loblolly pine families for prediction of future performance across a broad geographic range of sites. The plantation selection seed source study and the plantation selection diallel study provided us with the unique opportunity to compare breeding values of families of loblolly pine tested both broadly and locally. The initial analysis of the data sets indicated that both the PSSSS and the diallel study are useful studies to examine the performance of loblolly pine families for various traits. Both studies had high survival as well as a wide range of site means for the traits measured. This balance was expected because both studies had tests in many locations throughout a wide geographic area across the loblolly pine natural range east of the Mississippi River. There were 56 families that were tested in both the PSSSS and the plantation selection diallels, which should be an adequate sample size to be confident in our results. These 56 families were tested in 31 diallel test series which is another indicator that our sample size and study design is reliable for examining if site specific testing is warranted for the form and growth traits of interest. Additionally, the average number of individuals
tested in a half-sib family was 330 for the PSSSS and 629 for the plantation selection diallels, so the family size was large enough to give reliable breeding values.

The family mean heritabilities in the PSSSS were 0.92 for height, 0.88 for DBH, 0.88 for volume, and 0.90 for straightness; the family mean heritabilities in the plantation selection diallel tests were 0.87 for height, 0.84 for DBH, 0.81 for volume, and 0.87 for straightness. This suggests that most of the variation among families in the PSSSS and diallel tests is explained by additive genetic effects, and all traits can be readily manipulated through breeding and selection. These family mean heritabilities fall within the same range as those obtained in past studies (Li et al. 1996, Isik et al. 2005, Baltunis et al. 2007, McKeand et al. 2008, Cumbie et al 2010). The high family mean heritabilities for these traits in both studies indicate that the breeding values calculated in this study will be highly reliable.

In the plantation selection diallels, the type B genetic correlations were 0.86 for height, 0.79 for DBH, 0.69 for volume, and 0.88 for straightness, suggesting that no genotype by environment interactions that are important for tree breeding are occurring for any of these traits in the diallels since meaningful GxE interactions are indicated by a type B genetic correlation of less than .67 (Shelbourne 1972, Isik et al 2008, Komakech 2009). These high type B genetic correlations are not very surprising since all of the families in the diallel test series were tested in a restricted geographic region.
The PSSSS can give us much more meaningful and revealing information concerning the absence of important GxE interactions for these traits in loblolly pine. In the PSSSS, 56 families were each tested at the same 14 sites throughout a broad range of sites dispersed around the Coastal and lower Piedmont of the Southeast (Figure 3.5). If meaningful GxE interactions exist for these traits, they should be elucidated by the analysis of this comprehensive study. In the PSSSS, the type B genetic correlations were 0.71 for height, 0.52 for DBH, 0.49 for volume, and 0.77 for straightness. This suggests that there are no meaningful GxE interactions for height or straightness, but there are meaningful GxE interactions for DBH and volume. Though there has been limited research on GxE for straightness and other form traits in loblolly, past studies support our findings that there are not important GxE interactions for straightness (Gwaze et al. 2000, Chamblee 2011). This type B genetic correlation for straightness indicates GxE interaction is not critical and straightness does not vary enough between sites to warrant widespread site dependent testing and breeding. Most of the literature suggests that there are not meaningful GxE interactions for growth traits (e.g. McKeand et al. 2006), though there are several reports of significant GxE interactions (Duzan and Williams 1988, Yeiser et al., 2001, Sierra-Lucero et al 2002, Roth et al., 2007, Chamblee 2011). It is surprising that DBH and volume were found to have important GxE interactions while height was not, since growth traits are typically highly correlated with each other (Cumbie et al, 2010). Also, though no meaningful GxE was found for height, its type B genetic correlation was 0.71, which only slightly surpasses the 0.67 threshold of non-importance (Shelbourne 1972, Isik et al 2008, Komakech 2009). This may mean that some GxE for height may be occurring due to a possible few interactive families.
The substantial GxE interaction for DBH and volume indicates the possible need for site dependent breeding and testing.

The regression analysis performed comparing the breeding values calculated for PSSSS and the diallels resulted in coefficient of determination ($r^2$) values of 0.55 for height, 0.51 for DBH, 0.50 for volume, and 0.69 for straightness. The high correlation for the straightness breeding values along with the high type B genetic correlation for straightness makes a strong case that there are not significant GxE interactions for straightness. This supports past findings that have also not found meaningful GxE interactions for straightness in loblolly pine (Gwaze et al. 2000, Chamblee 2011). The correlations for the growth traits suggest that around half of the variation of these trait’s breeding values in the PSSSS are explained by their variation in the plantation selection diallels. While these $r^2$ values are not particularly high, they are not very low either. These regressions show evidence that, though the diallel breeding values are somewhat predictive of the PSSSS breeding values, there may be some GxE interaction occurring for each of these traits. The result of the regressions paired with the results from the type B genetic correlations do not suggest a complete lack of GxE nor undeniable presence of meaningful GxE either for all growth traits analyzed. It is likely that there may be some GxE occurring for all of these traits as several past studies have indicated.

We do not have enough evidence to conclude that site dependent breeding or testing is necessary for these traits, but we cannot rule out the possibility. This study has been able to identify that some interaction is occurring, but we are not sure how meaningful these
interactions are. Further analysis is warranted to obtain a clearer picture on the degree of GxE interaction occurring for growth and form traits in loblolly pine.
REFERENCES


McKeand, S. E., Li, B., Hatcher, A.V., Weir, R.J. 1990. Stability parameter estimates for stem volume for loblolly-pine families growing in different regions in the Southeastern United States


Table 3.1: Checklots used in diallel test series

<table>
<thead>
<tr>
<th>Checklot ID</th>
<th>Region</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC1</td>
<td>Virginia</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC2</td>
<td>Coastal NC</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC3</td>
<td>Coastal SC</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC4</td>
<td>Coastal GA &amp; FL</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC5</td>
<td>Lower Gulf</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC6</td>
<td>Upper Gulf</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>CC7</td>
<td>Piedmont GA, SC, NC</td>
<td>Bulked non-improved</td>
</tr>
<tr>
<td>SOM2</td>
<td>Coastal NC</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
<tr>
<td>SOM3</td>
<td>Coastal SC</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
<tr>
<td>SOM4</td>
<td>Coastal GA &amp; FL</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
<tr>
<td>SOM5</td>
<td>Lower Gulf</td>
<td>Bulked first generation seed orchard mix</td>
</tr>
</tbody>
</table>
**Table 3.2:** Mean rust Incidence, height, DBH, stem volume, and straightness at age 8 for 14 PSSSS tests sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Rust Incidence</th>
<th>Height (m)</th>
<th>DBH (cm)</th>
<th>Stem Volume (dm³)</th>
<th>Straightness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northampton, NC</td>
<td>0.83</td>
<td>7.9</td>
<td>15.2</td>
<td>56.9</td>
<td>0.47</td>
</tr>
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<td>Laurens, SC</td>
<td>0.44</td>
<td>8.9</td>
<td>14.2</td>
<td>52.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Newberry, SC</td>
<td>0.68</td>
<td>9.2</td>
<td>16.5</td>
<td>75.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Robeson, NC</td>
<td>0.45</td>
<td>10.4</td>
<td>16.3</td>
<td>80.1</td>
<td>0.48</td>
</tr>
<tr>
<td>Autauga, AL</td>
<td>0.68</td>
<td>8.8</td>
<td>15.5</td>
<td>61.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Williamsburg, SC</td>
<td>0.42</td>
<td>7.7</td>
<td>12.2</td>
<td>36.2</td>
<td>0.63</td>
</tr>
<tr>
<td>Randolph, GA</td>
<td>0.61</td>
<td>7.0</td>
<td>11.4</td>
<td>28.0</td>
<td>0.65</td>
</tr>
<tr>
<td>Escambia 1, AL</td>
<td>0.08</td>
<td>10.5</td>
<td>13.7</td>
<td>60.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Escambia 2, AL</td>
<td>0.27</td>
<td>10.6</td>
<td>16.5</td>
<td>84.4</td>
<td>0.60</td>
</tr>
<tr>
<td>Screven, GA</td>
<td>0.54</td>
<td>8.9</td>
<td>14.5</td>
<td>55.8</td>
<td>0.58</td>
</tr>
<tr>
<td>Berkeley, SC</td>
<td>0.69</td>
<td>11.9</td>
<td>17.3</td>
<td>103.4</td>
<td>0.40</td>
</tr>
<tr>
<td>Tattnall, GA</td>
<td>0.48</td>
<td>12.3</td>
<td>16.3</td>
<td>96.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Bradford, FL</td>
<td>0.62</td>
<td>8.4</td>
<td>11.2</td>
<td>33.7</td>
<td>0.51</td>
</tr>
<tr>
<td>Ware, GA</td>
<td>0.21</td>
<td>8.0</td>
<td>10.4</td>
<td>26.6</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td><strong>0.50</strong></td>
<td><strong>9.32</strong></td>
<td><strong>14.37</strong></td>
<td><strong>60.78</strong></td>
<td><strong>0.54</strong></td>
</tr>
</tbody>
</table>
Table 3.3: Mean rust incidence, height, DBH, stem volume, and straightness score at age 6 for 31 diallel test series

<table>
<thead>
<tr>
<th>Series</th>
<th>Rust Incidence</th>
<th>Height (m)</th>
<th>DBH (cm)</th>
<th>Volume (dm³)</th>
<th>Straightness</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0073</td>
<td>0.27</td>
<td>5.55</td>
<td>8.43</td>
<td>14.68</td>
<td>0.58</td>
</tr>
<tr>
<td>S0074</td>
<td>0.28</td>
<td>6.24</td>
<td>9.47</td>
<td>18.40</td>
<td>0.67</td>
</tr>
<tr>
<td>S0081</td>
<td>0.30</td>
<td>5.72</td>
<td>10.18</td>
<td>19.73</td>
<td>0.32</td>
</tr>
<tr>
<td>S0121</td>
<td>0.44</td>
<td>7.57</td>
<td>12.77</td>
<td>37.22</td>
<td>0.46</td>
</tr>
<tr>
<td>S0135</td>
<td>0.34</td>
<td>6.46</td>
<td>11.43</td>
<td>27.56</td>
<td>0.50</td>
</tr>
<tr>
<td>S0139</td>
<td>0.37</td>
<td>5.87</td>
<td>NA</td>
<td>NA</td>
<td>0.55</td>
</tr>
<tr>
<td>S0149</td>
<td>0.31</td>
<td>6.64</td>
<td>NA</td>
<td>NA</td>
<td>0.49</td>
</tr>
<tr>
<td>S0150</td>
<td>0.27</td>
<td>4.33</td>
<td>5.89</td>
<td>7.65</td>
<td>NA</td>
</tr>
<tr>
<td>S0151</td>
<td>0.21</td>
<td>4.25</td>
<td>6.17</td>
<td>7.88</td>
<td>NA</td>
</tr>
<tr>
<td>S0157</td>
<td>0.46</td>
<td>6.74</td>
<td>10.59</td>
<td>22.99</td>
<td>0.41</td>
</tr>
<tr>
<td>S0158</td>
<td>0.63</td>
<td>7.63</td>
<td>13.52</td>
<td>43.13</td>
<td>0.30</td>
</tr>
<tr>
<td>S0168</td>
<td>0.34</td>
<td>8.78</td>
<td>14.75</td>
<td>57.24</td>
<td>0.53</td>
</tr>
<tr>
<td>S0191</td>
<td>0.34</td>
<td>6.84</td>
<td>11.68</td>
<td>28.56</td>
<td>0.38</td>
</tr>
<tr>
<td>S0197</td>
<td>0.41</td>
<td>8.48</td>
<td>14.28</td>
<td>51.73</td>
<td>0.57</td>
</tr>
<tr>
<td>S0200</td>
<td>0.29</td>
<td>6.34</td>
<td>10.52</td>
<td>24.23</td>
<td>0.54</td>
</tr>
<tr>
<td>S0201</td>
<td>0.36</td>
<td>6.85</td>
<td>11.09</td>
<td>27.89</td>
<td>0.55</td>
</tr>
<tr>
<td>S0244</td>
<td>0.31</td>
<td>8.75</td>
<td>14.50</td>
<td>54.95</td>
<td>0.54</td>
</tr>
<tr>
<td>S0266</td>
<td>0.33</td>
<td>6.91</td>
<td>11.01</td>
<td>28.80</td>
<td>0.38</td>
</tr>
<tr>
<td>S0267</td>
<td>0.44</td>
<td>7.74</td>
<td>12.16</td>
<td>36.50</td>
<td>0.41</td>
</tr>
<tr>
<td>S0279</td>
<td>0.49</td>
<td>5.32</td>
<td>8.12</td>
<td>13.49</td>
<td>0.50</td>
</tr>
<tr>
<td>S0348</td>
<td>0.54</td>
<td>5.95</td>
<td>9.93</td>
<td>20.42</td>
<td>0.52</td>
</tr>
<tr>
<td>S0349</td>
<td>0.56</td>
<td>5.88</td>
<td>9.69</td>
<td>19.54</td>
<td>0.48</td>
</tr>
<tr>
<td>S0353</td>
<td>0.73</td>
<td>5.53</td>
<td>10.11</td>
<td>18.29</td>
<td>0.43</td>
</tr>
<tr>
<td>S0355</td>
<td>0.54</td>
<td>8.93</td>
<td>14.10</td>
<td>71.49</td>
<td>0.43</td>
</tr>
<tr>
<td>S0356</td>
<td>0.31</td>
<td>6.46</td>
<td>11.82</td>
<td>29.35</td>
<td>0.59</td>
</tr>
<tr>
<td>S0370</td>
<td>0.32</td>
<td>8.52</td>
<td>13.92</td>
<td>50.98</td>
<td>0.46</td>
</tr>
<tr>
<td>S0408</td>
<td>0.48</td>
<td>6.85</td>
<td>11.53</td>
<td>29.23</td>
<td>0.66</td>
</tr>
<tr>
<td>S0414</td>
<td>0.30</td>
<td>7.00</td>
<td>11.28</td>
<td>27.94</td>
<td>0.66</td>
</tr>
<tr>
<td>S0430</td>
<td>0.54</td>
<td>6.90</td>
<td>11.59</td>
<td>29.28</td>
<td>0.64</td>
</tr>
<tr>
<td>S0431</td>
<td>0.72</td>
<td>6.31</td>
<td>10.94</td>
<td>24.75</td>
<td>0.56</td>
</tr>
<tr>
<td>S0438</td>
<td>0.53</td>
<td>6.10</td>
<td>9.70</td>
<td>18.96</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td><strong>0.41</strong></td>
<td><strong>6.69</strong></td>
<td><strong>11.07</strong></td>
<td><strong>29.75</strong></td>
<td><strong>0.50</strong></td>
</tr>
</tbody>
</table>
**Table 3.4:** Variance components (standard errors follow ±) for family, family by environment interaction (GxE), type B genetic correlation ($r_B$), individual tree ($h^2$) and family mean ($h^2_f$) heritabilities for rust incidence, height, DBH, stem volume, and straightness across all 14 PSSSS sites.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Family± SE</th>
<th>GxE ± SE</th>
<th>$r_B$</th>
<th>$h^2$ ± SE</th>
<th>$h^2_f$ ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rust Incidence</td>
<td>0.365 ± .063</td>
<td>0.058 ± .014</td>
<td>0.85</td>
<td>NA</td>
<td>0.96 ± 0.007</td>
</tr>
<tr>
<td>Height</td>
<td>53.33 ± 9.52</td>
<td>21.49 ± 3.24</td>
<td>0.71</td>
<td>0.21 ± 0.04</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>DBH</td>
<td>0.037 ± 0.007</td>
<td>0.034 ± 0.004</td>
<td>0.52</td>
<td>0.17 ± 0.03</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>Volume</td>
<td>6.97 ± 1.31</td>
<td>7.12 ± 0.66</td>
<td>0.49</td>
<td>0.19 ± 0.03</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>Straightness</td>
<td>0.117 ± 0.022</td>
<td>0.035 ± 0.012</td>
<td>0.77</td>
<td>NA</td>
<td>0.90 ± 0.02</td>
</tr>
</tbody>
</table>
Table 3.5: Variance components (standard errors follow ±) for family, family by environment interaction (GxE), type B genetic correlation ($r_B$), individual tree ($h^2$) and family mean ($h^2_f$) heritabilities for rust incidence, height, DBH, stem volume, and straightness across all 31 diallel test series.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Family ± SE</th>
<th>GxE ± SE</th>
<th>$r_B$</th>
<th>$h^2$ ± SE</th>
<th>$h^2_f$ ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rust Incidence</td>
<td>0.22 ± 0.015</td>
<td>0.022 ± 0.003</td>
<td>0.91</td>
<td>NA</td>
<td>0.87 ± 0.005</td>
</tr>
<tr>
<td>Height</td>
<td>0.60 ± 0.04</td>
<td>0.096 ± 0.007</td>
<td>0.86</td>
<td>0.21 ± 0.04</td>
<td>0.87 ± 0.005</td>
</tr>
<tr>
<td>DBH</td>
<td>0.03 ± 0.002</td>
<td>0.008 ± 0.0006</td>
<td>0.79</td>
<td>0.18 ± 0.01</td>
<td>0.84 ± 0.007</td>
</tr>
<tr>
<td>Volume</td>
<td>2494.0 ± 191.1</td>
<td>1145.4 ± 72.4</td>
<td>0.69</td>
<td>0.18 ± 0.01</td>
<td>0.81 ± 0.009</td>
</tr>
<tr>
<td>Straightness</td>
<td>0.218 ± 0.015</td>
<td>0.029 ± 0.003</td>
<td>0.88</td>
<td>NA</td>
<td>0.87 ± 0.005</td>
</tr>
</tbody>
</table>
Figure 3.1: Regressing the breeding values (BVP) from the PSSSS on breeding values from the diallel (BVd) for height. The model was $BVP = 12.27 + 0.98 \times BVd$ with an $r^2$ of 0.55. Both intercept and slope estimates were significantly different from 0 ($P<0.0001$).
Figure 3.2: Regressing the breeding values (BVp) from the PSSSS on breeding values from the diallel (BVd) for DBH. The model was BVp = 2.38 + 1.16*BVd with an $r^2$ of 0.51. Both intercept and slope estimates were significantly different from 0 (P<0.0001).
Figure 3.3: Regressing the breeding values (BVP) from the PSSSS on breeding values from the diallel (BVd) for stem volume. The model was BVp = -0.01 + 1.97*BVd with an r² of 0.50. The intercept estimate was not significantly different from 0 (P=0.98), but the slope estimate was significantly different from 0 (P< 0.0001).
Figure 3.4: Regressing the breeding values (BVp) from the PSSSS on breeding values from the diallel (BVd) for straightness. The model was BVp = -0.35 + 1.54*BVd with an $r^2$ of 0.69. Both intercept and slope estimates were significantly different from 0 ($P<0.0001$).
Figure 3.5: Test sites (12 Coastal and 2 Piedmont) used from the Plantation Selection Seed Source Study (PSSSS)