

12-9-2022

Disease resistance and productivity in genetically improved loblolly pine: Results from a resistance screening trial and a midrotation comparison of genetic improvement levels

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Disease resistance and productivity in genetically improved loblolly pine: Results from a resistance screening trial and a midrotation comparison of genetic improvement levels

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Forestry
in the Department of Forestry

Mississippi State, Mississippi

December 2022

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2022

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Title of Study: Disease resistance and productivity in genetically improved loblolly pine:
Results from a resistance screening trial and a midrotation comparison of genetic
improvement levels

Pages in Study: 71

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Decades of tree improvement has resulted in genetic gains in loblolly pine productivity, form, and resistance to fusiform rust. The goal of this study was to advance the understanding and applied use of genetic improvement by analyzing inter- and intra-provenances hybrids' rust resistance and evaluating midrotation performance of varying levels of genetically improved stock types. The first study compares 16 seedlots at the USDA Resistance Screening Center and evaluates rust resistance of controlled-pollinated inter- and intra-provenances crosses, and open-pollinated seedlots from three provenances: Western Gulf, Atlantic Coastal, and Interior Piedmont. Post inoculation, one Coastal OP seedlot was resistant and ten of the seedlots were susceptible to the disease. The second study compares three levels of improved stock types: second-generation open-pollinated, controlled pollinated, and varietal material. After the fifteenth growing season, all three improved stock types were not significantly different from one another in defects, height, diameter, volume, and exhibited site index.

DEDICATION

I want to dedicate this research to my dear deceased friend Robert Glen (1984-2010).
You were a massive inspiration in my pursuit to fulfill a graduate degree in forestry.

ACKNOWLEDGEMENTS

I want to thank Dr. Adam Polinko and Dr. Randy Rousseau for their support and the opportunity to further my education at Mississippi State. I would also like to thank the multitude of graduate students who assisted in collecting data that contributed to this research.

I would like to thank ArborGen and International Forest Company (IFCO) Seedlings for their industry support and contributions to this genetic evaluation research.

I want to thank the USFS Resistance Screening Center in Asheville, North Carolina, for their contributions to this research project.

I want to thank PRT Growing Services for their employee educational support while completing this graduate degree.

I would also like to thank my wife, Celise Goodfellow, and my family members for their continued support as I worked to further my education and research goals.

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CHAPTER I

INTRODUCTION

The natural range of loblolly pine (*Pinus taeda* L.) extends across the southeastern United States (US) and includes portions of 14 states. Loblolly pine can be found east and west of the Mississippi River throughout the Atlantic Coastal Plains, Piedmont Plateau, and Western Gulf regions. During the Pleistocene era, loblolly pine originated in two separate refugia resulting in two separate populations with unique characteristics (Schmidting 2007). A distinct western loblolly population was geographically confined between northeastern Mexico and southwestern Texas. A second loblolly pine population, in the east, spread across parts of southern Florida and inhabited the Caribbean Islands. Throughout the period of genetic isolation, these populations individually adapted to local climates and soils resulting in population movement, advancing from these regions to what we know today to be the natural range of loblolly pine. As a result, loblolly pine seed sources collected east of the Mississippi River are genetic derivatives from the Florida and Caribbean refugia populations, and seed sources west of the Mississippi River were derived from the Texas and northern Mexico refugia population (Schmidting et al. 1999). The 100,000-year separation of these two distinct populations resulted in the many genetic and geographic differences in provenances that we see today (Schmidting 2007).

Pinaceae species are well adapted to respond to changing climatic conditions since pine pollination requires favorable temperatures and humidity levels (Hocker 1956). At the time of pine pollen release, the current wind patterns maximize the effective breeding range of the

species and allow for a significant potential for gene movement (Schmidtling 2007). Throughout the twentieth and twenty-first centuries, studies of racial and geographic variation within loblolly pine have been conducted to determine acceptable seed source options for artificial forest regeneration in the southeastern US. These provenance studies have shown distinctions across loblolly pine seed sources for traits like survival, productivity, disease resistance, and drought or cold hardiness (Schmidtling 2001).

The southern yellow pine forests have been the ideal geographic location for practicing forest conservation and timber production as a renewable resource in the southeastern US. Loblolly pine is the fastest growing of the southern yellow pines and is the primary commercial timber species in the southeast. On average, more than one billion loblolly pine seedlings are produced annually for artificial regeneration (McKeand et al. 2003). Forest managers who are focused on timber production favor loblolly pine due to the natural range, adaptability to climates, form characteristics, resistance to disease, and superior growth (Lantz and Hofmann 1969).

For more than half a century, forest genetics research across the US has hinged itself upon implementing genetics as a means of increasing stand productivity through genotype and phenotype selection (Wheeler et al. 2015). Over the last two decades, the rise of commercial nursery vendors offering varying levels of genetically improved seedlings has increased significantly (Barnett 2013; Bell 2015). Many of these improved pine seedlings have noticeable improvements in growth, form, and disease resistance making them marketable to landowners interested in improving forest health and stand productivity (Barry 2011; Wheeler et al. 2015).

Southern fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex. Sharai f. sp. *fusiforme*) is a virulent pathogen that severely impacts tree health and stand productivity of loblolly pine in

the southeast, as a result, there is significant economic loss from forest investments within areas of high rust presence (Sniezko et al. 2014). Fusiform rust infects all southern yellow pine species found throughout the southeastern pine range but is most severe in slash (*Pinus elliottii* Engelm.) and loblolly pine (Enebak et al. 2004). Regions with high humidity and warm temperatures provide more favorable conditions for developing fusiform rust and result in higher rust presence (Czabator 1971). Like loblolly pine, fusiform rust also has a high genetic diversity dependent on its provenance and exhibits different levels of virulence. To address this disease, loblolly pine tree improvement programs have employed breeding, testing, and selection strategies to identify and develop fusiform rust resistance within genetic selections (Sniezko et al. 2014). In areas of high rust presence, disease resistant planting stock remains an important factor for landowners (McKeand et al. 2003).

The southeastern US is supported by three tree improvement cooperatives that have advanced the development of loblolly pine over the last 50 years (Wheeler et al. 2015). As a result, forest managers have a wide variety of loblolly pine planting stock options to choose from which include different levels of genetic improvement touting substantial genetic gain increases when compared to unimproved nursery-run seedlings (Li et al. 2000).

Objectives

The goal of this study is to advance the understanding and applied use of genetically improved loblolly pine planting stock by analyzing inter- and intra-provenance hybrids' rust resistance and evaluating midrotation performance of varying levels of improved stock types. Two studies were conducted.

The first study compares 16 unique loblolly pine seedlots at the USDA Resistance Screening Center. The study evaluates fusiform rust resistance of inter- and intra-provenances

hybrid crosses, and open-pollinated seedlots from three provenances: Western Gulf, Atlantic Coastal, and Interior Piedmont.

The second study was established in 2007 in northern Mississippi and compares age 15 performance of three different levels of genetically improved loblolly pine stock types: a second-generation open-pollinated family, controlled mass pollinated (CMP) family, and varietal material. The study also explores the differences in midrotation growth (i.e., 15 years) performance of multiple unique varieties.

CHAPTER II

LITERATURE REVIEW

Seed Source Movement Studies

The history of southern pine genetics research began in the early twentieth century with seed source studies (Wakeley and Barnett 2016). Loblolly pine's sizeable natural range, genetic variation, and patterns of geographic variation allow for wide-ranging options of genetic stock for artificial regeneration (Jayawickrama et al. 1998). Foresters recognized the opportunity to improve pine plantations and the economic value of their timberlands by replanting with pine seed collected from varying geographic regions. To determine the best performance of the geographic regions, seed source study trials were conducted (Wells and Wakeley 1966). In the early nineteen-twenties, the first trials were established to identify seed sources with superior growth adaptable for widespread deployment (Wakeley 1954).

Under the United States Forest Service (USFS) direction, the first trial to understand seed source deployment of loblolly pine was established in Bogalusa (Louisiana) in 1926. The study collaborated with Great Southern Lumber Co. and USFS Southern Forest Experiment Station (Wakeley 1944) and incorporated plantation installments using four native loblolly pine seed sources collected from Texas, Louisiana, Arkansas, and Georgia. Early-age results for survival, volume, and rust resistance suggested that local seed sources were best adapted for their regions (Wakeley and Bercaw 1965). These results also supported the 1939 USFS seed policy advising forest managers to plant only native seed sources (Long 1980). Later midrotation age

measurements concluded that a local seed source from Livingston Parish, Louisiana was superior in volume growth and fusiform rust resistance. Based on these findings, loblolly seed from Livingston Parish, Louisiana was deployed across the Atlantic and Lower Gulf Coastal Plains. Later research would suggest that the Livingston Parish seed source was likely a naturally occurring provenance hybrid resulting from the combination of eastern and western loblolly populations (Wells et al. 1991).

In the nineteen-fifties, a comprehensive seed deployment guide for loblolly pine seed sources was developed through extensive collaboration across the forest industry (Wakeley and Barnett 2016). As a result, a sub-committee was formed under the Southern Forest Tree Improvement Committee (SFTIC) to further understand loblolly pine's geographic variation and origin. The SFTIC subcommittee established the well-known Southwide [Southern] Pine Seed Source Study (SPSSS/SSPSSS). The SPSSS's purpose was to guide seed source selection for forest regeneration activities. From the study, significant geographic variation in growth and disease resistance was discovered within seed sources of loblolly pine. Western loblolly seed sources were both more resistant to fusiform rust and had better survival in drier climates when compared to eastern seed sources (Wakeley 1953). Western loblolly seed sources were generally slower growing, resistant to fusiform rust, and drought tolerant, however, Atlantic Coastal Plains seed sources are superior in volume production when deployed on both eastern and western sites (Schmidtling 1987). As a result of this study, a phenomenon known as the "coastal-continental" effect suggested that moving loblolly pine seed sources between the eastern and western regions had a high potential for success if annual precipitation regimes were similar (Wells 1983). Artificial regeneration of forests represented an option for landowners to reestablish forests without waiting on natural regeneration to occur and managed seed orchards would be needed to

support southern pine nurseries with seed supply to meet forest planting demand. The first grafted southern pine seed orchard was established in the nineteen fifties by the Texas Forest Service. Seed orchards serve to provide seeds for pine seedling nurseries and establish parent selections used for the next generation of improved genetic families (Fox et al. 2007).

The nineteen-sixties brought about new ideas for the potential of moving seed sources across provenances. Ron Schmidting, a USFS research scientist and geneticist, suggested that the effects of large-scale movement of loblolly pine seed sources would positively impact the genetic integrity and diversity of loblolly pine. In 2001, Schmidting published a guide advising forest managers and consulting foresters in selecting appropriate seed sources for planting southern pines. Schmidting pioneered the early understanding and general concepts of geographic variation in loblolly pine. Moving western seed sources east of the Mississippi River mimicked natural gene flow, however, moving eastern seed sources west of the river may be problematic due to harsher climates (Schmidting and Myszewski 2003).

Through the nineteen-seventies, the significant benefits of the advancements of deploying specific seed sources was recognized for its economic impact. Timber companies began internal research that utilized internal seed source studies and field tests to guide the deployment of loblolly pine on company lands. This was an important transition in the history of forest genetics research because previously, studies were typically government-funded research. Motivated by the SPSSS results, Container Corporation, established a localized study combining SPSSS trials and in-house trials on company land (Draper 1975). Superior rust resistance and impressive growth were observed from Livingston Parish, Louisiana, and east Texas seed sources when planted in Florida and Georgia (Pait and Draper 1983).

In the late nineteen-seventies, Weyerhaeuser Company had interest in superior seed orchard selections to guide artificial regeneration plantings on company timberlands. Weyerhaeuser established progeny tests with eastern seed sources across Arkansas and eastern Oklahoma (Lambeth et al. 2005). The Atlantic Coastal Plains sources grew taller than local Arkansas and Oklahoma seed sources but lacked hardiness for the local disease pressure and were not well adapted to the harsh climates in Arkansas and Oklahoma (Wells and Lambeth 1983). It was advised that silvicultural practices in the Western Gulf would need to be modified when using Atlantic Coastal Plains seed sources. Eastern and western seed sources showed significant differences in a growth trajectory for height-age relationships and as a result, rotation lengths would need to be adjusted (Talbert and Strub 1987). Following the widespread deployment of Atlantic Coastal Plains plantings west of the Mississippi, approximately 60% of Arkansas and Oklahoma landscape was identified as suitable for planting Northern Atlantic Coastal seed sources (Lambeth et al. 1984).

Forest Genetics and Tree Improvement

Pine improvement and genetic interest led to the development of tree improvement programs within industry and university-based cooperatives. The first tree improvement cooperative was established in 1951 at the Texas A&M University in College Station, Texas (Schmidtling et al. 2004). Today, three primary tree improvement cooperatives focus on southern pine species:

1. The North Carolina State University Tree Improvement Program (NCSUTIP) is based in Raleigh, North Carolina, at North Carolina State University. NCSUTIP's focus is on the Atlantic Coastal Plain, Piedmont, and Northern provenances of loblolly pine.

2. Western Gulf Forest Tree Improvement Program (WGFTIP) is based in College Station, Texas, at Texas A&M University. WGFTIP's focus is on the Western Gulf Upper and Lower Gulf region provenances of loblolly and slash pine.
3. Cooperative Forest Genetics Research Program (CFGRP) is based in Gainesville, Florida, at the University of Florida. CFGRP's focus is on Florida sourced loblolly pine and eastern Atlantic Coastal Plains slash pine.

Tree improvement programs are comprised of personnel from private industry universities, state, and federal levels. Cooperatives focus on the step-wise progression of long-term breeding strategies to manage genetic diversity and genetic gain within their perspective breeding populations (White et al. 2018).

The nineteen-eighties advancements in biotechnology and tree improvement programs resulted in further knowledge for the proper deployment of loblolly pine seed sources. The SPSSS trials were adequate but the design had its limitations and needed further refinement. The SPSSS tests were broadly spaced across the southeast with varying uniformity (Falkenhagen et al. 1984; Cao 2014). To illustrate geographical trends in growth and survival for eastern and western seed sources, the NCSUTIP established 52 trials across the natural range of loblolly pine. In these trials, local open-pollinated seed sources were used in addition to four unimproved seed sources from Livingston Parish (Louisiana), Gulf Hammock (Florida), Marion County (Florida), and Eastern Shore (Maryland and Virginia). The results showed Livingston Parish seed source was consistent across regions in productivity and resistance to fusiform rust (McKeand et al. 1989).

Florida sourced loblolly was not included in the SPSSS and other seed source trials and the CFGRP and NCSUTIP sought to expand on Florida seed sources' performance compared to

Atlantic Coastal Plains and Western Gulf. Data suggested that seed sources from central Florida and Marion County, Florida, respectively outperformed Atlantic Coastal Plains and Western Gulf seed sources in volume production but lacked disease resistance to fusiform rust (Sierra-Lucero et al. 2002).

The establishment of the first southern pine seed orchards comprised of first-generation selections from wild loblolly pine plantations. Wild seeds from natural stands were collected from desirable trees that exhibited favorable phenotypic characteristics. The seeds were then tested in replicated progeny tests and planted across a wide geographic region. The resulting information indicated genetic gain potential and represented the parent trees' ability to pass down their genetic traits to their progeny. Scion from those wild selections was collected and grafted into seed orchards for mass seed production. First-generation orchards produced wind-pollinated also known as "open-pollinated" seed. Seed from these first-generation orchards could achieve 7% to 13% more volume at the end of the rotation compared to unimproved seed (Schultz 1999).

In the nineteen-eighties, the advancement of forest genetics and tree improvement programs led to the development of the second-generation loblolly seed orchards. Observed genetic gains from selections within second-generation orchards within the Atlantic Coastal Plains boasted double that of the first-generation orchards (McKeand et al. 2006). By the early twenty-first century, more than 50% of the loblolly pine seedlings deployed were produced from second-generation seed orchards. Today, tree improvement programs continue to advance their breeding programs to present the best genetic options for landowners to deploy (McKeand 2019).

Tree Breeding

Successful tree breeding techniques first occurred in the mid-nineteen-fifties and resulted in a new way to control seed source selections for the improve loblolly pine (Wheeler et al. 2015). Pine tree breeding involves controlled measures to isolate female strobili within a pollination bag and injecting known pollen from a desirable parent to create a full-sibling family in which both parents are known. Previously, seed source selections were open-pollinated by the wind and the paternal parents are unknown. Due to the lower cost, open-pollinated seedlings are still a standard option for landowners and represent a moderate level of genetic gain along with increased genetic variation (Rousseau et al. 2015).

Controlled-pollinated breeding is currently the standard in tree improvement programs for progeny production. The resulting controlled-pollinated families are progeny tested across numerous locations, with the best families and genotypes possessing the greatest trait values selected for the next generation of breeding (Wheeler et al. 2015).

Today, landowners have numerous commercially available genetically improved planting stock options to choose from, including controlled-pollinated families (Barry 2011). These genetically improved seedlings are marketed as Mass Controlled Pollinated (MCP®) or Control Mass Pollinated (CMP) and are comprised of two known parents of superior genetic traits (McKeand 2019).

Varietals

Loblolly pine varietal seedlings currently represent the highest level of genetic improvement available to landowners (Rousseau et al. 2012). Varietal pine seedlings are produced through clonal asexual propagation methods; hedging or somatic embryogenesis (SE). Hedging is a propagation technique in which juvenile seedlings are cut back to produce

numerous growing shoots. The shoots are harvested and propagated using rooting hormones and developed into seedlings with containerized plugs. Somatic embryogenesis is another propagation technique in which embryos are removed from a seed and the embryonic tissue is cultured to propagate in a controlled environment. The resulting propagates of both techniques produced identical genetic copies of the donor thus resulting in a clone or a varietal of the foundation stock type (Greenwood et al. 1991; Rousseau et al. 2012).

The history of clonal propagation dates back six thousand years ago with olive (*Olea* spp.) tree species in the middle east (Burdon and Libby 2006). Clonal forestry practices in the US were first explored in the early twenty-first century. The development of loblolly pine varietal clones was derived from the desire to produce lines of superior genetic genotypes in large quantities (Frampton et al. 2000). The use of varietal material could result in productivity gains greater than 60% for landowners (McKeand et al. 2006). Although, there are suggestions that a lack of harvest-age data to support varietal performance expectations in forest plantations (Dougherty and Wright 2009).

ArborGen is the primary commercial producer of loblolly pine varietal seedlings available to landowners (Watson 2020). Nursery production costs for varietal seedlings are higher than traditional seed germination techniques. The cost of varietal pine seedlings is about ten times that of open-pollinated second-generation seedlings and nearly four times that of CMP (Rousseau 2017).

Fusiform Rust

Southern fusiform rust (*Cronartium quercuum* [Berk.] Miyabe ex Shirai f sp. *fusiforme*) is a virulent pathogen that has caused significant decreases in southern yellow pine forests. Fusiform rust galls are the signs seen on southern pine trees as a result of infection from the

fusiform rust pathogen (Warren and Covert 2004). Resistance to fusiform rust is an important factor in selecting loblolly pine planting stock, particularly in areas with moderate to high rust hazards (Walker and McKeand 2017).

Like most *Cronartium* fungi species, southern fusiform rust requires an alternate host species, often a red oak (*Quercus* spp.), to complete its lifecycle. The complex cycle of this pine-oak fungi involves four stages of spores and begins with yellow aeciospores derived from pine galls that infect the alternate host. The infection results in the development of urediospores which reinfect the new growth of the alternate host and create columns of teliospores giving rise to the pine infecting basidiospores (Vogler 2008). The infection of mature trees is not always fatal and results in the development of spindle-shaped rust galls causing deep fissures in the wood and restricting xylem movement throughout the vascular system, however, the infection of saplings or juvenile trees is usually fatal immediately or within a few years (Sinclair and Lyon 2005). Larger severely infected pine trees that have a distorted stem where the pathogen creates a rust gall are prone to mortality, windthrow, and wood defects resulting in lower value at harvest (Cowling and Young 2013).

Tree improvement programs consider fusiform rust resistance a highly desirable and heritable trait. Using traditional tree breeding techniques, resistance genes can be passed to their progeny (Isik et al. 2008). To combat the impact of fusiform rust, seed orchards designed from rust-resistant families have been established and these orchards can be found throughout the southeast and contain selections from the most resistant clones (Powers 1984).

Resistance screening center

In the nineteen-seventies, the USDA Southeastern Forest Experimental Station established the Resistance Screening Center (RSC) in Asheville, North Carolina. The RSC was

tasked with developing an accelerated rust screening process for southern yellow pine species. The purpose of the RSC is to perform controlled disease resistance screening tests for organizations engaged in pine seed production, tree improvement, disease resistance, and forest research activities (Cowling and Young 2013).

The RSC uses fusiform rust aeciospores collected from galls of loblolly and slash pine in specific geographic regions and use them to inoculate northern red oak (*Quercus rubra*) saplings (Young et al. 2018). The leaves of the infected oak sapling develop the basidiospores used to inoculate first year pine seedlings. Once pine seedlings are inoculated, the occurrence of infection is analyzed and genetic family differences are examined (Cowling and Young 2013).

A key element to producing rust-resistant pine trees is the reliable and rapid procedure for evaluating the relative resistance of host families. Field trials represent the best way to observe rust resistance, however, field trials are time-consuming, and the results are dependent on disease exposure and virulence (Young et al. 2018). Families that show resistance in resistance screening trials are also likely to show resistance when outplanted in plantations due to a strong correlation between the RSC and field trial resistance data (Powers and Matthews 1980). The RSC has played a vital role in pioneering the development and understanding of loblolly pine fusiform rust resistance and exploring the genetic interaction with host and pathogen (Bronson 2012).

Fusiform rust resistance genes

Historically, progeny tests and controlled screenings have been used to locate fusiform rust resistance genes (Wilcox et al. 1996; Nelson et al. 2010). Recent resistance research suggests that there is a high provenance variation within both the pine host and the pathogen variant (Wilcox et al. 1996). Genetic resistance to fusiform rust occurs on a gene-to-gene interaction level (Nelson et al. 2010). It's been suggested that long-term resistance can be

obtained from a single qualitative resistance gene, despite the presence of virulence in the pathogen population (Wilcox et al. 1996). However, this is largely dependent on the relationship between host resistance genes and virulence of the pathogen variant since these are allele-specific interactions between host and pathogen (Ence et al. 2022). For example, host mortality occurs when the pine host has a homozygous recessive allele (*fr/fr*) for a major fusiform rust resistance gene or if a pathogen with a virulence allele (*avr*) that is unaffected by the specific dominant allele (*Fr*) resistance gene. Host resistance is expressed when the pine host carries a dominant allele (*Fr*) for resistance as heterozygous dominant allele (*Fr/fr*) or homozygous dominant allele (*Fr/Fr*), and the pathogen is avirulent (*Avr*) to that specific resistance gene (Quesada et al. 2014).

The recent biotechnology advances and the emergence of gene mapping technology have provided powerful tools for characterizing the genetic interactions with host and pathogen where previous conventional genetic analysis was limited (Wilcox et al. 1996). Recently, researchers have discovered the existence of numerous *Fr* genes within the loblolly pine genome. Given the complex relationship between these gene-for-gene interactions between specific *Fr* genes and *Avr* genes, researchers are working to map and identify sequences that make up the multiple identifiable *Fr* genes (Quesada et al. 2014; Ence et al. 2022). With the emergence of biotechnology and advanced understanding of the gene-for-gene interaction variation between the host's resistance genes and virulence in the pathogen, research for identifying fusiform rust resistance genes is ongoing (Ence et al. 2022).

Livingston Parish

The evaluation of fusiform rust resistance in Livingston Parish, Louisiana seed sources have been well documented. Historically, early seed source movement trials and resistance

screening studies showed strong resistance to fusiform rust within Livingston Parish seed sources (Wells and Wakeley 1966). Rust resistance of Livingston Parish has been evaluated in field trials in the Georgia Piedmont, Georgia Coastal, and Alabama, and field performance has supported the findings that Livingston Parish seed sources are inherently rust resistant (Powers 1984). As a result, the widespread planting of Livingston Parish seed sources for universal rust resistance has occurred (Wells 1985). More recently, several studies have found conflicting results for Livingston Parish seed sources resistance in field trials and inoculation screenings although the lack of resistance is often attributed to the seed source collection zones being different than those in which earlier seed source studies based their analysis on (Powers and Matthews 1980; Pait and Draper 1983; Snow et al. 1990).

Schmidting and Nelson (1996) established field trials in Mississippi, Georgia, and Florida to explore the possibility of combining desirable traits from wide crosses within Livingston Parish (LA), Marion County (FL), and Coastal Plain (NC) populations. The focus was specifically on the Livingston Parish loblolly population due to its known fusiform rust resistance and superior growth attributes. The goal was to produce a variety of widely adaptable planting stock for the southeastern US by exploring the growth performance and disease resistance of various provenance hybrids (inter- and intra-provenances hybrids). In all three plantings, the Livingston Parish source showed the least amount of infection from fusiform rust. Provenance hybrids that included Livingston Parish as a maternal parent expressed similar rust occurrence as the paternal parent in the study, but showed better resistance than the estimated mid-parent value suggesting heterosis (Schmidting and Nelson 1996).

Interprovenance Hybrids

Interprovenance hybrids are the result of breeding two distinct provenance parents together to create a hybridized cross. Loblolly interprovenance hybrids present a feasible method for increasing genetic diversity and incorporating desirable traits such as resistance to disease and insects with fast-growing or hardy provenances (Schmidting and Nelson 1996). Eastward gene flow across the southern end of the Mississippi Valley has resulted in natural occurring interprovince hybrids between eastern and western loblolly populations (Wells et al. 1991; Schmidting and Myszewski 2003; Lu et al. 2019). The first investigation of the potential benefits of loblolly interprovenance hybrids occurred in the nineteen-seventies. Suggestively, interprovenance hybrids could increase geographic and genetic variation through forwarding selections within tree improvement programs. The greatest possibility for interprovenance hybrids is with wide crosses that include western sourced families, like Livingston Parish, that show resistance to fusiform rust (Woessner 1972).

NCSUTIP staff investigated the growth potential of loblolly pine interprovenance hybrids between Piedmont and Coastal provenances (McKeand et al. 2004). This research concluded that the interprovenance hybrids grew similar to the Coastal seed source and also were well adapted to cooler climates like the Piedmont source. The results also concluded that interprovenance hybrids with a Piedmont parent survived better and had less cold damage. Interprovenance hybrids with a Coastal parent were affected greater by cold damage and had poor survival (Alizoti et al. 2006; Zapata-Valenzuela et al. 2015). Today, it has been suggested that an interprovenance mating strategy within tree improvement breeding programs may allow for a broader geographic planting range for loblolly pine.

Nursery Planting Stock

Southern pine seedlings are produced at tree nurseries in the form of bareroot or container plugs (Allen et al. 2017). Pine cones are collected from grafted seed orchards in the fall, the seed is extracted from the cone, and stored in the freezer until its intended use at the nursery (Bonner and Karrfalt 2008). There are some crucial distinctions that differentiate between bareroot and container planting stock options and it is important to note that nursery cultural practices, climate, and field site conditions directly affect seedling morphological and physiological attributes correlated to their field performance (Grossnickle 2017).

Bareroot seedlings

Bareroot and container seedlings are the primary reforestation planting stock type for loblolly pine. Historically, bareroot seedlings have been the standard planting stock and millions of seedlings are still produced annually through federal, state, and private nurseries (Barnett 2013). Bareroot seedlings are grown in sandy beds in open field nurseries which allow for water drainage and the maneuverability of nursery equipment during wet periods. Bareroot seedlings are sown in spring at densities between 210 to 280 seedlings per m² and are lifted for planting in early winter. Planting densities at the nursery have a direct effect on the morphology of seedlings, when grown at wider spacing, seedlings are larger in both diameter and biomass (Brissette and Roberts 1984; South et al. 1990). Bareroot seedlings typically grow a larger shoot system than containerized seedlings and nurseries target a shoot length of around 30 cm and a root-to-shoot ratio of 3:1 (Brissette 1986; South et al. 2016). To meet target seedling height specifications, bareroot and container seedlings are mechanically top pruned which increases uniformity and reduces the number of cull seedlings (South and Blake 1994).

Bareroot pine seedlings perform well when planted on ideal sites with adequate moisture between February through April. Bareroot seedlings are a cost-effective option that when established on high-quality sites under normal growing conditions can perform well. However, concerns for survival when growing conditions are less than ideal resulted in the development and subsequent production of a planting stock option that uses trays to produce seedlings with containerized plugs (Grossnickle and El-Kassaby 2016).

Containerized seedlings

The use of container seedlings has significantly increased in southern forestry in the last thirty-five years (Watson 2020). Large-scale planting of container seedlings began in the southern US as a solution to poor survival rates of bareroot longleaf seedlings (Boyer 1988).

Containerized seedlings are grown in molded Styrofoam® or plastic linear trays within raised open-compound sites or greenhouses. The trays are set on raised T-posts to allow airflow and air-pruning of roots to occur on the underside. Seed is sown into a media mixture that acts as a growing medium for root development comprised of bark, peat, perlite, and vermiculite. (Landis et al. 2010; Sung and Dumroese 2013). Loblolly pine container seedlings have a shorter targeted shoot height than bareroot but the same root-to-shoot ratio (Brissette 1986; South et al. 2016). In addition, no undercutting or mechanical lifting occurs with containerized production resulting in fine root structures and a more developed root system (Mathers et al. 2007).

The southeastern US forest industry has experienced large-scale changes in climate, timber markets, silviculture, and genetic technology over the last twenty-five years, there are many reasons that container seedlings add value to forest management (Grossnickle 2005). The length of the window in which containerized seedlings can be planted is much wider than bareroot seedlings (Grossnickle 2012). Most importantly, planting containerized seedlings under less

desirable conditions such as unusually dry or wet periods has a higher likelihood of success than bareroot seedlings (Barnett and McGilvray 1993).

CHAPTER III
RESISTANCE SCREENING CENTER

Introduction

Fusiform rust is caused by the fungus *Cronartium quercuum* (Berk.) Miyabe ex. f. sp. *fusiforme* and is a major concern in forest health due to the loss in stand productivity in the southeastern US. The timber loss inflicted by fusiform rust due to mortality is estimated to be around \$134M annually (Cubbage et al. 2000). Resistance to fusiform rust is an important factor in selecting loblolly pine planting stock, particularly in areas with moderate to high rust hazards (Randolph et al. 2015). Rust resistance is traditionally evaluated through field trials or controlled inoculation screening at the USDA Resistance Screening Center in Asheville, North Carolina (Young et al. 2018). Lab results from artificially inoculated screenings offer an efficient and cost-effective approach to rapidly identify rust-resistant loblolly families (Nelson et al. 2010).

A novel approach to modern-day loblolly pine tree improvement programs is to breed select elite performing families across provenances to create wide-cross interprovenance hybrids. The hybridization of loblolly pine across provenances may prove to be beneficial when breeding for site adaptability, growth, and disease resistance traits. The exploration into interprovenance mating strategies can provide opportunities to further evaluate disease resistance and the geographic adaptability of loblolly pine since the relative rust resistance of interprovenance hybrids is unclear.

This study examines rust resistance through artificial inoculation of full-sibling inter- and intra-provenances hybrids, and half-sibling open-pollinated seedlots from parental selections within the Western Gulf, Atlantic Coastal Plains, and Interior Piedmont provenances. The use of this information can further the understanding and applied use of interprovenance hybrids across the natural range of loblolly pine.

Materials and Methods

The USDA Resistance Screening Center (RSC) in Ashville, North Carolina, was selected to screen various seedlots for fusiform rust susceptibility and resistance. The main purpose of the RSC is to perform controlled fusiform rust inoculation screening tests for organizations engaged in pine seed production, tree improvement, disease resistance, or further forest research and development activities (Cowling and Young 2013).

In the Spring of 2019, seed from 16 loblolly families was sent to the RSC to be artificially inoculated with a bulk inoculum consisting of 20k spores/ml from 13 spore collection zones across the natural range of loblolly pine (Figure 3.1). Following standard operating procedures, the RSC included two additional known checklot families that represent controls for known susceptible and resistant loblolly families to fusiform rust.

Experimental design

Seedlots included in the trial were provided by IFCO and are representative of elite loblolly pine parents and included controlled crosses of inter- and intra-provenances and open-pollinated (OP) families within pedigrees representative of the Western Gulf, Atlantic Coastal Plains, and Interior Piedmont provenance origins (Table 3.1).

Seedlots included in the study were a Western Gulf intraprovenance cross (W4xW10), an intraprovenance Piedmont cross (P14xP15), an intraprovenance Coastal cross (C12xC5), three interprovenance Coastal by Western Gulf hybrids (W4xC5, C5xW10, and C12xW4), an interprovenance Piedmont by Western Gulf hybrid (P4*xW4), and two interprovenance Coastal by Piedmont hybrids (C12xP13 and P14xC5). In addition, two OP Coastal (C5xOP and C12xOP), two OP Western Gulf (W10xOP and W4xOP), and three OP Piedmont (P4*xOP, P14xOP, and P15xOP) seedlots were included. It was noted that the pedigree of Piedmont family P4* includes parents from Livingston Parish, Louisiana (Table 3.1).

The study design was a randomized complete block (RCB) with matched-pairs. There were sixteen seedlot treatments in the study, plus one known rust-resistant seedlot and one known rust-susceptible seedlot. Each seedlot treatment was separated into two identical replicates and evenly distributed across growing trays. In most cases, there were six observations and 120 seedlings per seedlot (2 replicates x 3 trays x 20 seedlings per tray).

Screening center protocol involved the collection of rust aeciospores and the growing of northern red oak saplings. Once saplings produced juvenile leaves, they were inoculated with a wide geographic mixture of fusiform rust spores in a bulk inoculum (Figure 3.1). Infected saplings developed telial columns on the undersides of the leaves and the resulting teliospores were germinated to discharge basidiospores (sporidia). Basidiospores were collected into a suspension and diluted to a density of 20k spores/ml using an electronic particle counter (Young et al. 2018).

Loblolly seedlots were stratified for approximately six weeks and grown in SC10 Ray Leach® “Super Cell” container plugs. Seven weeks later, the seedlings were inoculated with the 20k spores/ml basidiospore suspension and placed in a humidity chamber ideal for fungal

infection, and then moved to the RSC greenhouses. Post inoculation, seedlings were fertilized with Miracle-Gro® (15-30-15), a high phosphate fertilizer to increase the susceptibility of infection. The absence or presence of fusiform rust galls was assessed nine months post inoculation through visual inspection by RSC personnel (Young et al. 2018).

Table 3.1 Parental provenance origin locations and cross abbreviations for loblolly pine seedlots; checklot for rust resistant (RR) and rust susceptible (RS), controlled crosses of inter- and intra-provenances, and open-pollinated (OP) families within the Western Gulf, Atlantic Coastal Plains, and Interior Piedmont provenance evaluated at the US Forest Service Resistance Screening Center (RSC) in Asheville, North Carolina.

Provenance Origin	Cross Abbr.
Western Gulf (4) x Coastal (5)	W4 x C5
Western Gulf (4) x Western Gulf (10)	W4 x W10
Coastal (12) x Coastal (5)	C12 x C5
Coastal (5) x Western Gulf (10)	C5 x W10
Coastal (12) x Piedmont (13)	C12 x P13
Piedmont (14) x Piedmont (15)	P14 x P15
Piedmont* (4) x Western Gulf (4)	P4* x W4
Coastal (5) x Open Pollination	C5 x OP
Coastal (12) x Open Pollination	C12 x OP
Piedmont* (4) x Open Pollination	P4* x OP
Piedmont (14) x Open Pollination	P14 x OP
Piedmont (15) x Open Pollination	P15 x OP
Western Gulf (10) x Open Pollination	W10 x OP
Western Gulf (4) x Open Pollination	W4 x OP
Piedmont (14) x Coastal (5)	P14 x C5
Coastal (12) x Western Gulf (4)	C12 x W4
Check – Rust Resistant (RR) x Open Pollination	RR x OP
Check – Rust Susceptible (RS) x Open Pollination	RS x OP

P4* - Piedmont Family with Livingston Parish family in pedigree

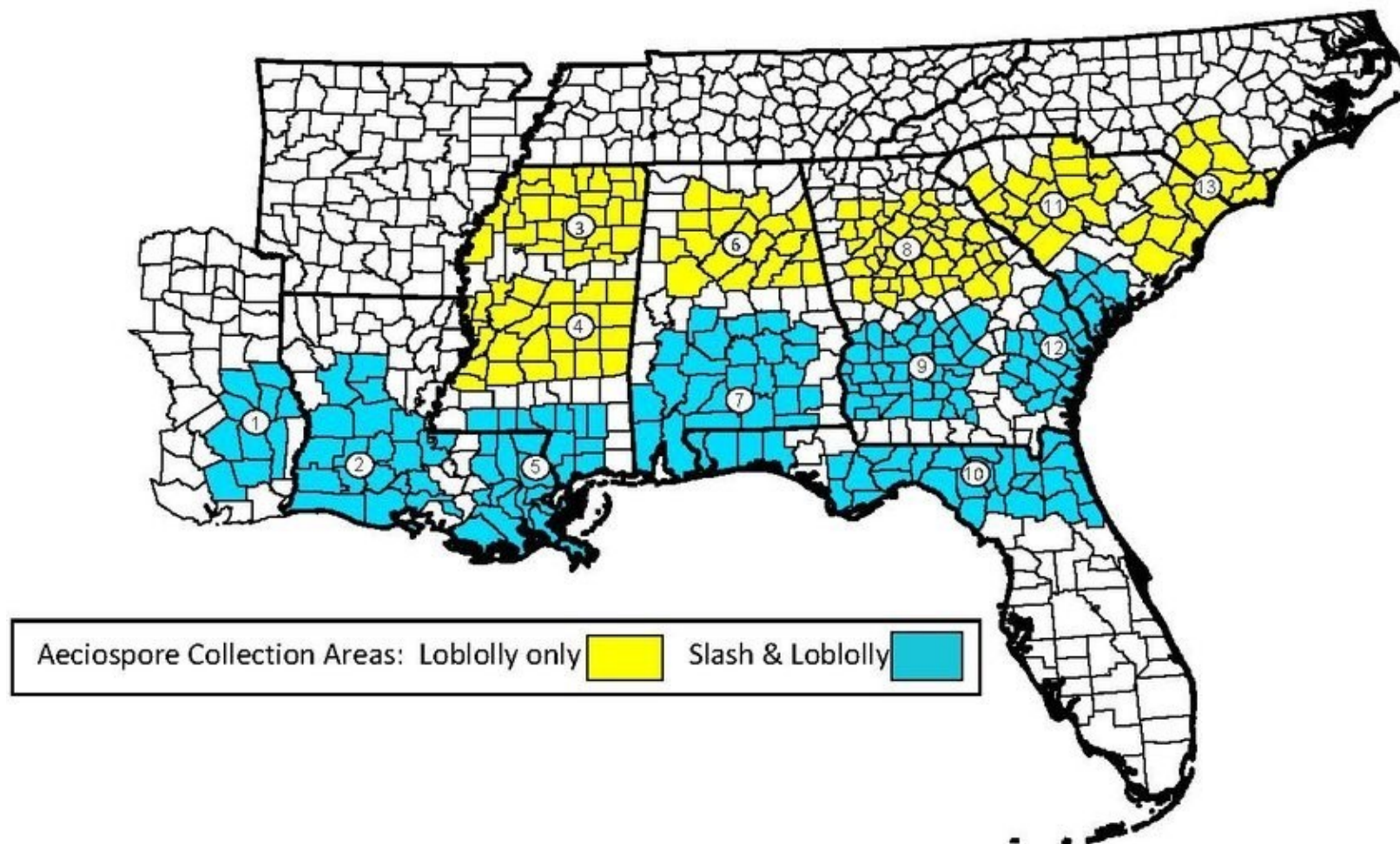


Figure 3.1 Distribution map of the locations of the thirteen fusiform rust aeciospore collection zones that made up the bulk inoculum used in the disease resistance study conducted at the US Forest Service Resistance Screening Center (RSC) in Asheville, North Carolina (Young et al. 2018).

Evaluation and Statistical Methods

Data manipulation and analysis were completed in the R version 4.2 statistical computing environment (R Core Team 2020). Disease incidence was observed as a binary variable. The effect of disease incidence represented as rust gall presence relative to sixteen genetically improved seedlots were tested using a mixed effects logistic regression model using the lme4 (Bates et al. 2015) and emmeans (Lenth 2022) packages. Genetically improved seedlots were evaluated as fixed effects and replicates were evaluated as random effects.

Results

In total, eighteen loblolly pine seedlots were tested at the screening center. Two of the eighteen loblolly seedlots were known controls representing Rust Susceptible (RS) and Rust Resistant (RR) families. One Coastal open-pollinated seedlot (C12xOP) was similar to the Rust Resistance (RR) checklot. Ten of the seedlots were similar to the Rust Susceptible (RS) checklot, and the remaining five seedlots were not similar to either of the checklots (Figure 3.2).

One intraprovenance Western Gulf x Western Gulf (W4xW10) seedlot expressed significantly more rust than the other seedlots ($p < 0.05$; Table 3.2). Six seedlots (RRxOP, C12xOP, C12xC5, P14xC5, C12xW4, and C12xP13) expressed significantly less rust than the other seedlots ($p < 0.05$; Table 3.2).

Seedlot infection ranged from 12.5% to 79.2%. The seedlot with the highest rate of infection was intraprovenance Western Gulf x Western Gulf (W4xW10) seed lot, followed by open-pollinated Western Gulf (W10xOP) seedlot, and Rust Susceptible (RS) seedlot. The Rust Resistant (RRxOP) checklot was the least infected seedlot in the study followed by open-pollinated Coastal (C12xOP) seedlot (Table 3.2).

Table 3.2 Statistical results and coefficients for fusiform rust among loblolly pine seedlots; checklot for rust resistant (RR) and rust susceptible (RS), controlled crosses of inter- and intra-provenances, and open-pollinated (OP) families within the Western Gulf, Atlantic Coastal Plains, and Interior Piedmont provenance evaluated at the US Forest Service Resistance Screening Center (RSC) in Asheville, North Carolina.

	Dependent variable
Intercept	0.384 (0.365)
RR x OP	-2.336 (0.455)*
C12 x OP	-1.315 (0.415)*
C12 x C5	-1.080 (0.410)*
P14 x C5	-0.979 (0.410)*
C12 x W4	-0.932 (0.408)*
C12 x P13	-0.861 (0.408)*
P14 x P15	-0.791 (0.407)
P14 x OP	-0.250 (0.405)
C5 x W10	-0.080 (0.406)
C5 x OP	-0.012 (0.407)
P4 x OP	0.048 (0.441)
W4 x OP	0.350 (0.411)
P4 x W4	0.350 (0.411)
P15 x OP	0.388 (0.412)
RS x OP	0.507 (0.414)
W10 x OP	0.547 (0.415)
W4 x W10	0.956 (0.426)*

Table 3.2 (continued)

Dependent variable	
sd(REPID)	0.016
Observations	106
Akaike Inf. Crit.	456.7
Bayesian Inf. Crit.	507.3

Note: * $p < 0.05$

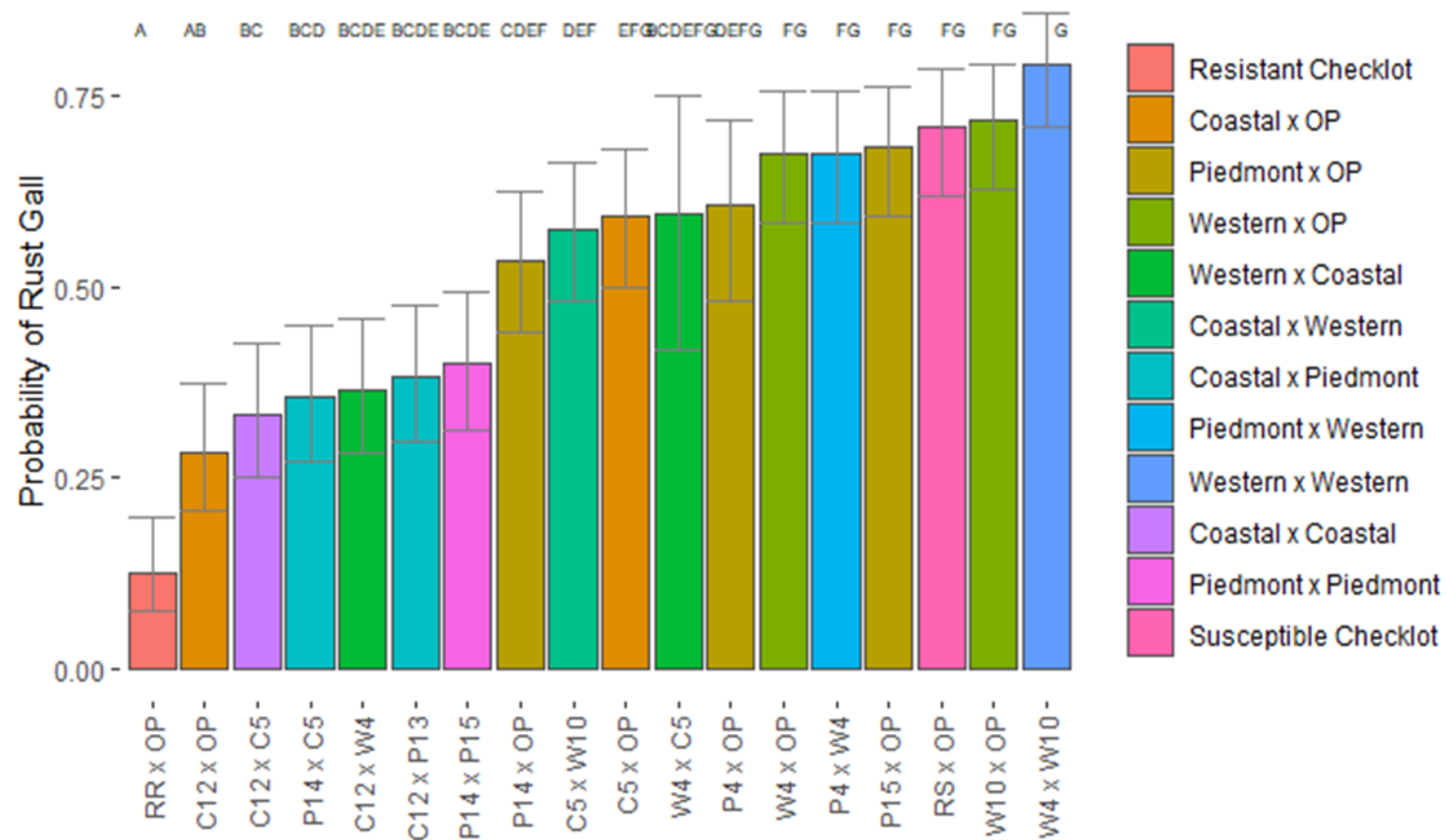


Figure 3.2 Probability of fusiform rust gall occurrence among loblolly pine seedlots; checklot for rust resistant (RR) and rust susceptible (RS), controlled crosses of inter- and intra-provenances, and open-pollinated (OP) families within the Western Gulf, Atlantic Coastal Plains, and Interior Piedmont provenance evaluated at the US Forest Service Resistance Screening Center (RSC) in Asheville, North Carolina.

Discussion

Our results conclude that there was only one rust-resistant seedlot family out of the sixteen tested. Ten of the seedlots were found to be susceptible and the remaining five seedlots were found to be neither resistant nor susceptible to the disease. Our results show no clear geographic or provenance trends in rust resistance given the unexpectedly low number of rust-resistant families in the study.

The Coastal seedlot C12xOP showed significantly low ($p < 0.05$) incidence of rust compared to the other seedlots tested and was the only seedlot similar to the rust-resistant checklot. C12xOP would likely also be resistant to fusiform rust when outplanted in plantations since research suggests a strong correlation between the RSC and field trial resistance data (Powers and Matthews 1980). Field testing would provide the most reliable prediction of disease resistance for family deployment in regions with a high rust pressure (Spitzer et al. 2017). In conjunction with this research project, five field trials were established with similar seedlots and field observations of rust within blocked tests were collected at age three. However, there was an extremely low incidence of rust presence within the trials which limited the statistical inference of the study and no scientific inference was formed.

Rust resistance has been shown to be a highly heritable trait and has a high family-mean heritability (Isik et al. 2008). The rust-resistant Coastal seedlot pedigree included family C12 as the maternal parent. In addition to C12xOP, three other seedlots containing C12 in their pedigree (C12xC5, C12xW4 and C12xP13) were found to express significantly lower ($p < 0.05$) incidence of rust than the other seedlots but were not similar to the susceptible or resistant checks (Table 3.2). Inherited resistance genes may be a contributing factor resulting in the lower percentage of rust infection for the seedlots that have C12 in their pedigree.

Given the diversity of families and provenances, the resulting low number of rust-resistant seedlots in the analysis was largely unexpected. Early resistance screening data suggests that as a source of inoculum is varied, the ranking of seedlots change (Walkinshaw and Anderson 1987). In order to minimize the variation in seedlot ranking and detect virulence variation among inocula standard protocol for RSC incorporate a bulk inoculum from collection zones ranging from east Texas to South Carolina (Isik et al. 2012; Young et al. 2018). The bulk inoculum from the 13 collection zones well-represented the virility of inocula across the loblolly range but may not have been reflective of seedlot genetic resistance for localized variants of the pathogen. Recent rust resistance research suggests that there is a high provenance variation within both the host species and the pathogen (Wilcox et al. 1996) indicating tested seedlots may still have resistance at the local level that was not observed in this study. Fusiform rust resistance occurs on a gene-to-gene interaction level (Nelson et al. 2010) and is largely dependent on the relationship between host resistance genes and virulence of the pathogen variant (Ence et al. 2022).

The pedigree in Piedmont family P4* includes parents from Livingston Parish, Louisiana. In the study, two seedlots included P4* as a parent; P4* \times W4 and P4* \times OP. Early USFS seed source movement studies also support the Livingston Parish sourced seed has an inherent resistance to the disease (Wells and Wakeley 1966). Research for rust resistance within Livingston Parish seed sources has been well document across multiple field trials and screening studies, including the SPSSS (Wells 1985; Powers 1986). Previous studies have concluded a strong resistance to fusiform rust disease within Livingston Parish seed sources and found similar results for their provenance hybrids (Schmidtling and Nelson 1996) However, the exhibited occurrence of rust in the interprovenance hybrid P4* \times W4 was 68% (81 out of 120

seedlings) and the open-pollinated half-sibling P4*xOP seedlot showed 61% infection (40 out of 66 seedlings). P4*xOP was one of two seedlots in the study that had less than 120 seedlings germinate from the provided seed. The RSC requires a minimum of 120 seedlings per seedlot to effectively evaluate rust presence. W4xC5 and P4*xOP were deemed to be susceptible to rust but the results are unreliable given the low number of germinated seedlings for both seedlots.

The lack of resistance in seedlots with Western Gulf parents was unexpected. These results were similar to Powers Jr and Matthews (1980) findings when evaluating loblolly pine seed sources from six geographic areas resistance in inoculation screenings. The highest presence of rust occurred within the Louisiana seed source and the lack of resistance was attributed to the seed source collected. The RSC suggests the screening center results are best utilized for determining seedlots that are rust-resistant and not for determining which are most susceptible (Carson and Young 1987). It is possible that both the Western Gulf seedlots and P4* seedlots screened in this study may have also not originated from the same region of Livingston Parish that earlier research based their resistance assumptions on.

Rust gall incidence was evaluated subjectively through visual inspection by RSC personnel and the results were subject to human error. The development of high-throughput phenotyping of loblolly pine seedlings for fusiform rust presence will likely become an integral part of the standard methods used for resistance screening (Pandey et al. 2021).

Our results further support the significance that the RSC contributes in evaluating various loblolly pine provenance and their subsequent hybrids. A larger analysis would likely need to be conducted to evaluate the interaction between various interprovenance hybrids and their rust resistance. The use of interprovenance hybrids to develop rust-resistant planting stock remains

unclear but may have a strong potential to increase the genetic diversity of loblolly pine and subsequent expression of resistance genes.

Research suggests that the relative resistance of loblolly families is directly affected by the geographic and genetic variation of virulence of the rust pathogen. In addition, there is evidence to suggest that planting a diverse mixture of resistant families would be more effective at limiting fusiform rust infections than the widespread planting of a single resistant family (Kuhlman et al. 1995). Fusiform rust will continue to be an important trait of interest in southern pine tree improvement programs and the ongoing research into genetic resistance can pave a path forward for the future management of destructive forest pathogens.

CHAPTER IV

GENETIC COMPARISON FIELD TRIAL

Introduction

Loblolly pine trees provide a wide range of materials for forest products and is the most widely planted pine species in the southeastern US (Schultz 1999). In southern plantation forestry, forest managers typically deploy genetically improved loblolly pine as a tool to increase stand productivity (Zhao et al. 2016). The correct genetically improved pine planting stock provides forest landowners the opportunity to improve the quality and quantity of wood. Landowners who invest in the correct genetically improved seedlings along with proper silvicultural techniques will see favorable returns from wood products due to increases in growth rates, disease resistance, and wood properties (Dougherty and Wright 2009).

The increases in stand productivity resulting from improved loblolly pine is due to decades of tree improvement work. Forest research along with cooperative efforts in applied forest genetics has resulted in the production of multiple generations of improved loblolly pine populations (Wheeler et al. 2015). Seed collected from these orchards can result in substantial increases in forest productivity when outplanted in the field. At the end of rotation, first-generation open-pollinated seed orchards can produce 7% to 12% more volume per acre over unimproved wild seed (Talbert 1982) and second-generation orchards can produce 13% to 21% more volume per acre than the first-generation (Li et al. 1999) . Controlled-pollinations of selected first-generation parents to construct the best second-generation parents can increase

volume production by more than 50% over unimproved wild seed (Jansson and Li 2004). The question is will varietal selections material result in possible genetic gains of 60% or greater than unimproved wild seed as suggested by McKeand et al. (2006).

Historically, pine seedling production of loblolly pine has been limited to vertically integrated timber companies and state/federal nurseries (Barnett 2013). Over the last couple decades, the rise of commercial nursery vendors offering varying levels of genetically improved seedlings to the public has increased significantly (Barnett 2013; Bell 2015). In order to market higher genetic gain value to landowners, pine seedling vendors offer the commercial sale of various genetically improved options such as mass control-pollinated aka Controlled mass-pollinations (MCP/CMP) and varietals material (Barry 2011). The process to commercially mass-produce MCP/CMP planting stock as well as varietal material is expensive, and as a result, the planting stock is often more expensive than the standard open-pollinated stock types (Rousseau et al. 2012).

The goal of this study is to advance the understanding and applied use of genetically improved planting stock by evaluating mid-rotation performance of three levels of genetic improvement. The information presented in this study is part of an ongoing study and expands on earlier measurements providing value for genetically improved stock type performance differences between early-age (age 6) and midrotation-age (age 15) measurements.

Materials and Methods

A genetic comparison study was established in 2007 in northern Mississippi at Mississippi State University's North Mississippi Branch Experiment Station in Marshall County near Holly Springs, Mississippi (34°48'56" N, 89°25'40" W).

The site topography was uniform and according to the SSURGO NRCS Soil Series is comprised of 75% Loring silty loam and 25% Cahaba-Providence complex. SSURGO database suggests that the (Coile and Schumacher 1953) site index (SI₅₀) curve is 24.4 m for Loring silty loam and 29.0 m for Cahaba-Providence complex for loblolly pine plantation (Coile and Schumacher 1953; Soil Survey Staff 2022).

The site was previously used as grazing pastureland and as a result, had a heavily compacted subsoil layer. To mitigate compaction, the site was sub-soiled at a depth of 14 inches in order to break up the compacted plow pan. Prior to planting, a site-prep herbicide application of 64 oz (per acre) of glyphosate was applied in three-foot bands centered on the subsoil line. The site was hand planted using dibble bars in early April 2007. The site was planted at a tree spacing of 3.7 m x 2.7 m. Each seedling received a 20 mg tablet of Silvashield® (imidacloprid) as a proactive measure to reduce the impact of Nantucket pine tip-moth (*Rhyacionia frustrana*) predation (Rousseau et al. 2015).

Post site establishment, a banded herbicide application of Select® (Clethodim) was applied at 32 oz per acre to control Bermuda grass (*Cynodont dactylon* L.) competition. In addition, a broadcast release herbicide application of Oustar® (hexazinone and sulfometuron methyl) was applied at a rate of 6 oz per acre in May 2008 (Herrin 2012).

In the first growing season, substantial Nantucket pine tip moth damage was observed across the study. In response to the significant insect damage, an application of PTM® insecticide (fipronil) was injected at the base of each tree at a rate of 1.4 ml per position in late April 2009 (Herrin 2012).

Experimental design

The study comprised of three treatments of genetically improved loblolly pine genetic planting stock types; second-generation open-pollinated (GEN2 OP) family MWV356, controlled mass pollinated (CMP) family M0023, and 56 unique varieties. Both the GEN2 OP and CMP seedlings were produced and provided by MeadWestvaco Corporation as a bareroot seedling stock type. The varietal material was produced and provided by ArborGen, LLC in the form of containerized plug seedlings (Herrin 2012).

The trial was laid out as a randomized complete block (RCB) design with three genetically improved stock types as the treatments. Each treatment was replicated six times in 100-tree (10 x 10) block plots (Rousseau et al. 2015). Within each block, the internal 64 trees (8 x 8) were designated as measurement trees surrounded by two rows of unmeasured border trees. Each varietal plot was designed to contain a single ramet of each of the 56 varieties and additional check families.

Trial measurements were collected in December 2021 and consisted of diameter at breast height (DBH) using a diameter tape and total tree height using a Haglöf® Vertex III hypsometer. Common defect like broken tops, crooked stems, fusiform rust, forks, and ramicorns were noted in the field.

Evaluation and Statistical Methods

Data manipulation and analysis were completed in the R version 4.2 statistical computing environment (R Core Team 2020). Measurements were analyzed on a plot means basis and total tree volume (inside bark) was calculated using the Amateis and Burkhart (1987) combined-variable equation. Data entry errors were cleaned from the dataset and observations that were labeled as fillers or checks were not considered in the analysis.

Exhibited site index was evaluated to estimate optimal stock type height growth as a measure of site productivity (Hann and Scrivani 1987). Top height was evaluated using the adjusted largest tree sample plot method used for estimating top height ($n = 10$; García and Batho 2005). Exhibited site index (SI_{25}) was calculated for the GEN2 OP, CMP, and varietal material stock types the using the Lenhart and Clutter (1968) curve for loblolly in the Georgia Piedmont region.

The growth characteristics of the varietal material were evaluated in two separate groups; Aggregated Varietals (AV) and Elite Varietals (EV). Aggregated Varietals (AV) comprised of all varieties in the varietal plots. A second analysis was conducted to explore the performance of the best varieties in the study. The five varieties ranked largest in volume after the fifteenth growing season and had five or more surviving ramets at the end of the second growing season were evaluated separately as the elite varietals (EV) subgroup. The five elite varieties included in the EV analysis consisted of varieties: 575, 573, 484, 586, and 567. Tree growth characteristics represented as height, diameter, volume, survival and defects relative to genetically improved stock type levels represented as GEN2 OP, CMP, and AV were tested using a linear mixed effect regression model. Genetically improved stock types were evaluated as fixed effects and replicates were evaluated as random effects.

The effect of improved stock type levels on exhibited site index (SI_{25}), tree height, diameter, volume, survival and defects were tested using a linear mixed effect regression model. Genetically improved stock types were evaluated as fixed effects and replicates were evaluated as random effects. Mixed effects models were constructed and evaluated using the lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017) packages. An alpha level of 0.05 was used for

all statistical tests. The GEN2 OP and CMP stock types were compared to each varietal group (AV and EV) independently.

Results

Overall average survival after the fifteenth growing season was 82.3%. The AV stock type survival was significantly lower when compared with GEN2 OP and CMP stock types ($p < 0.05$; Table 4.1). Average survival was the lowest (72.3%) in AV stock type and highest (90.1%) in CMP stock type (Table 4.2; Figure 4.1). Defect was not significant between GEN2 OP, CMP and AV stock types ($p < 0.05$; Table 4.1). Average defect presence was the lowest (14.1%) in AV and highest (24.5%) in CMP stock type (Table 4.2; Figure 4.2).

At the end of the fifteenth growing season, there was no significant difference in height, DBH, volume, and site index (SI_{25}) between GEN2 OP, CMP and AV stock types ($p < 0.05$; Table 4.1). Of all stock types, the AV stock type consistently represented the lower range for average height, diameter and volume. The average height ranged from 18.9 m (AV) to 19.9 m (CMP). Average DBH ranged from 27.0 cm (AV) to 27.5 cm (CMP). Average volume ranged from 0.43 m³ (AV) to 0.45 m³ (CMP; Table 4.3; Figures 4.3, 4.4, and 4.5). Exhibited site index (SI_{25}) ranged from 33.82 m (GEN2 OP) to 33.94 m (AV; Tables 4.1 and 4.3).

The EV stock type significantly outperformed the CMP and GEN2 OP stock types in every measurement ($p < 0.05$; Table 4.4; Figures 4.3, 4.4, and 4.5). On average, the EV stock type was 0.62 m taller than the CMP stock type and 0.82 m taller than the GEN2 OP stock type. EV stock type diameter (DBH) was 3.77 cm larger than the CMP stock type and 4.14 m taller than the GEN2 OP stock type. The EV stock type averaged 25.0% and 28.3% more volume per tree in the CMP and GEN2 OP, respectively (Table 4.4).

Varieties in the AV group showed a wide distribution in growth performance and the average tree height by family ranged from 20.7 m (family 141) to 16.2 m (family 587). Average height for the GEN2 OP stock type was 19.1 m and the CMP stock type were 19.3 m (Figure 4.6).

Table 4.1 Statistical coefficients and standard error for frequency of Survival, frequency of defect, height (m), diameter at breast height (cm), volume (m³), and exhibited site index (SI₂₅; m) among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), and aggregated varieties (AV) at the end of fifteenth growing season.

	Survival	Defect	Height (m)	DBH (cm)	Volume (m ³)	SI ₂₅ (m)
Intercept	0.846 (0.030)*	0.188 (0.029)*	19.096 (0.199)*	27.167 (0.345)*	0.433 (0.014)*	33.816 (0.294)*
CMP	0.055 (0.036)	0.057 (0.028)	0.205 (0.228)	0.3813 (0.304)	0.020 (0.012)	0.093 (0.334)
Aggregated Varietals (AV)	-0.123 (0.036)*	-0.046 (0.028)	-0.165 (0.228)	-0.1966 (0.304)	-0.004 (0.012)	0.0123 (0.334)
sd(Block)	0.001	0.003	0.435	0.462	0.0007	0.186
sd(Residual)	0.004	0.002	0.278	0.221	0.0004	0.334

Note: * p<0.05

Table 4.2 Means and standard errors for survival and defect presence among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), and aggregated varieties (AV) at the end of fifteenth growing season.

	GEN2 OP (se)	CMP (se)	AV (se)
Survival	84.6 (0.088)	90.1 (0.068)	72.3 (0.138)
Defects	18.8 (0.121)	24.5 (0.094)	14.1 (0.103)

Table 4.3 Average height (m), diameter at Breast Height (cm), volume (m³), and exhibited site index (SI₂₅) among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), and aggregated varieties (AV) at the end of fifteenth growing season.

	Height (m)	DBH (cm)	Volume (m ³)	SI ₂₅ (m)
GEN2 OP	19.10 (0.304)	27.17 (0.322)	0.43 (0.070)	33.82 (0.337)
CMP	19.30 (0.263)	27.54 (0.339)	0.45 (0.077)	33.91 (0.337)
Aggregated Varietals (AV)	18.93 (0.283)	26.97 (0.433)	0.43 (0.076)	33.94 (0.364)

Note: Mean exhibited site index (SI₂₅) used Lenhart and Clutter (1968) curve.

Table 4.4 Statistical coefficients and standard error for height (m), diameter at breast height (cm), and volume (m³) among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), and elite varieties (EV) at the end of fifteenth growing season.

	Height (m)	Diameter (cm)	Volume (m ³)
Intercept	19.096 (0.221)*	27.167 (0.643)*	0.433 (0.003)*
CMP	0.205 (0.240)	0.181 (0.738)	0.009 (0.031)
Elite Varietals (EV)	0.820 (0.240)*	4.139 (0.738)*	0.165 (0.031)*
sd(Block)	0.120	0.845	0.002
sd(Residual)	0.173	1.633	0.003

Note: * p<0.05

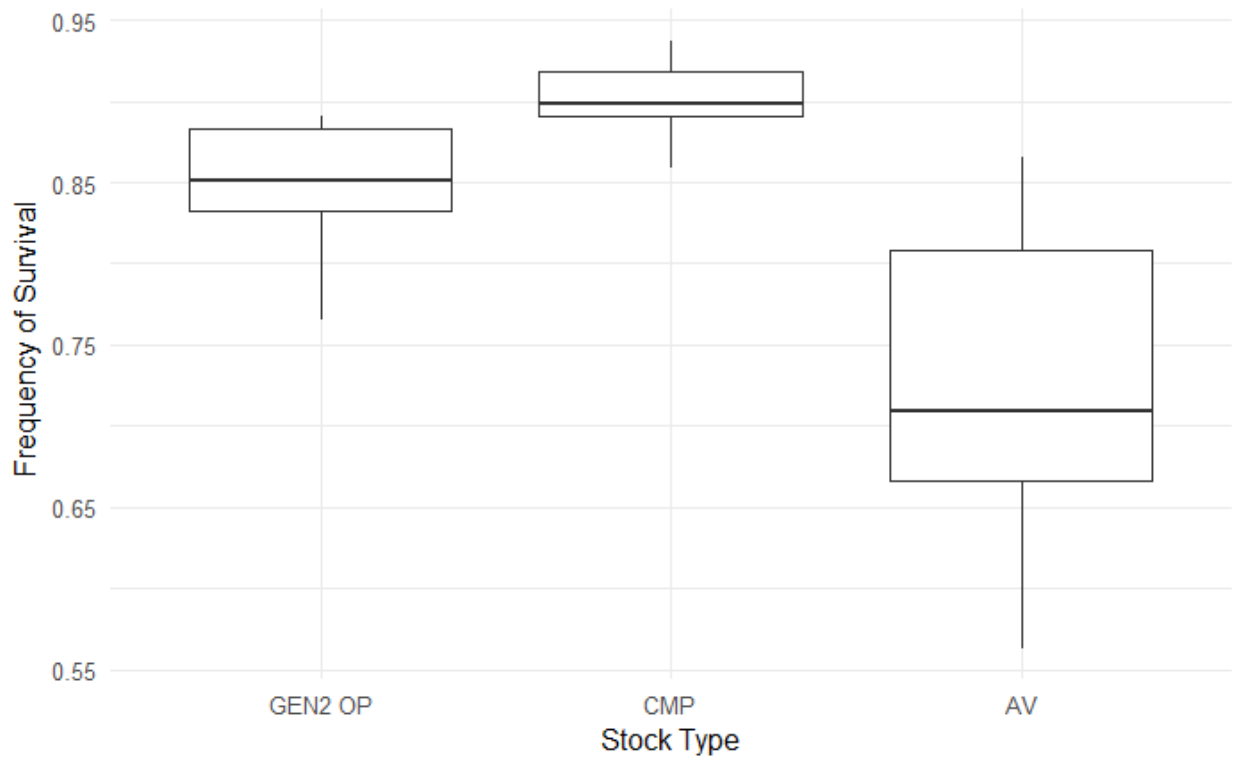


Figure 4.1 Boxplot showing frequency of survival distribution among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), and aggregated varieties (AV) at the end of fifteenth growing season.

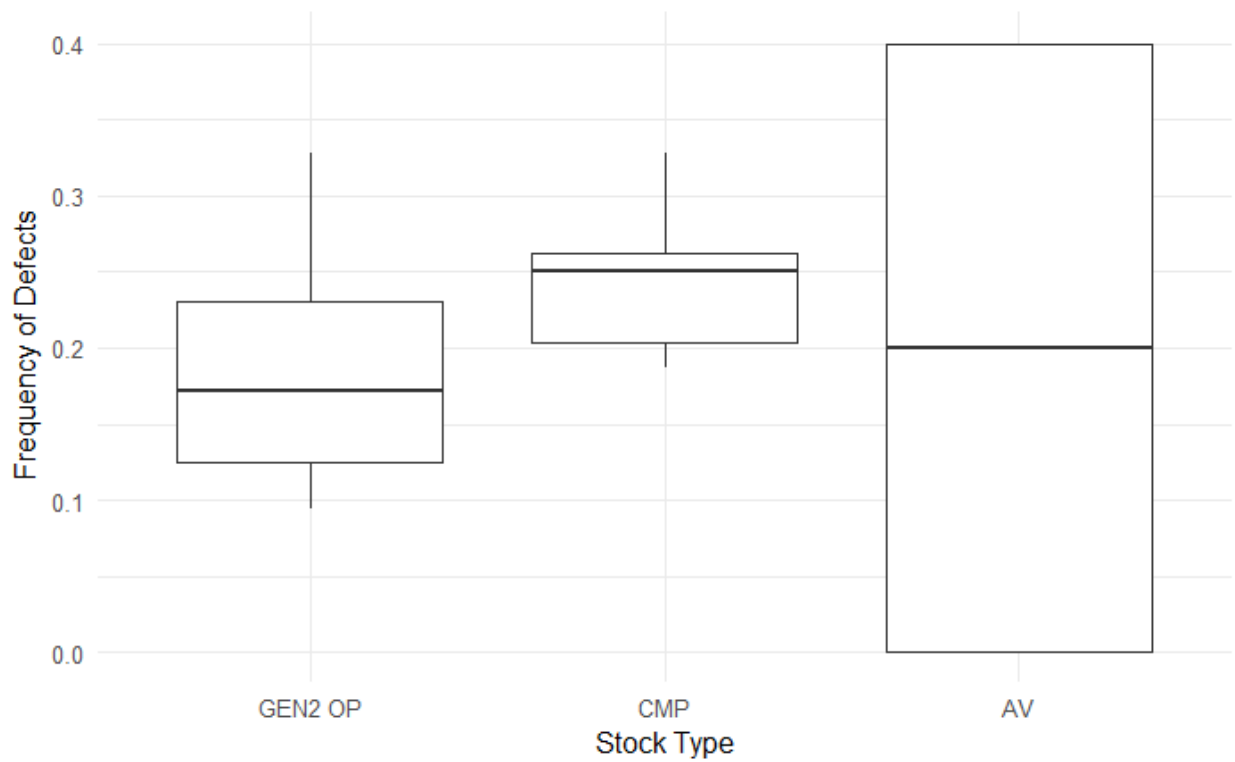


Figure 4.2 Boxplot showing frequency of defect distribution among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), and aggregated varieties (AV) at the end of fifteenth growing season.

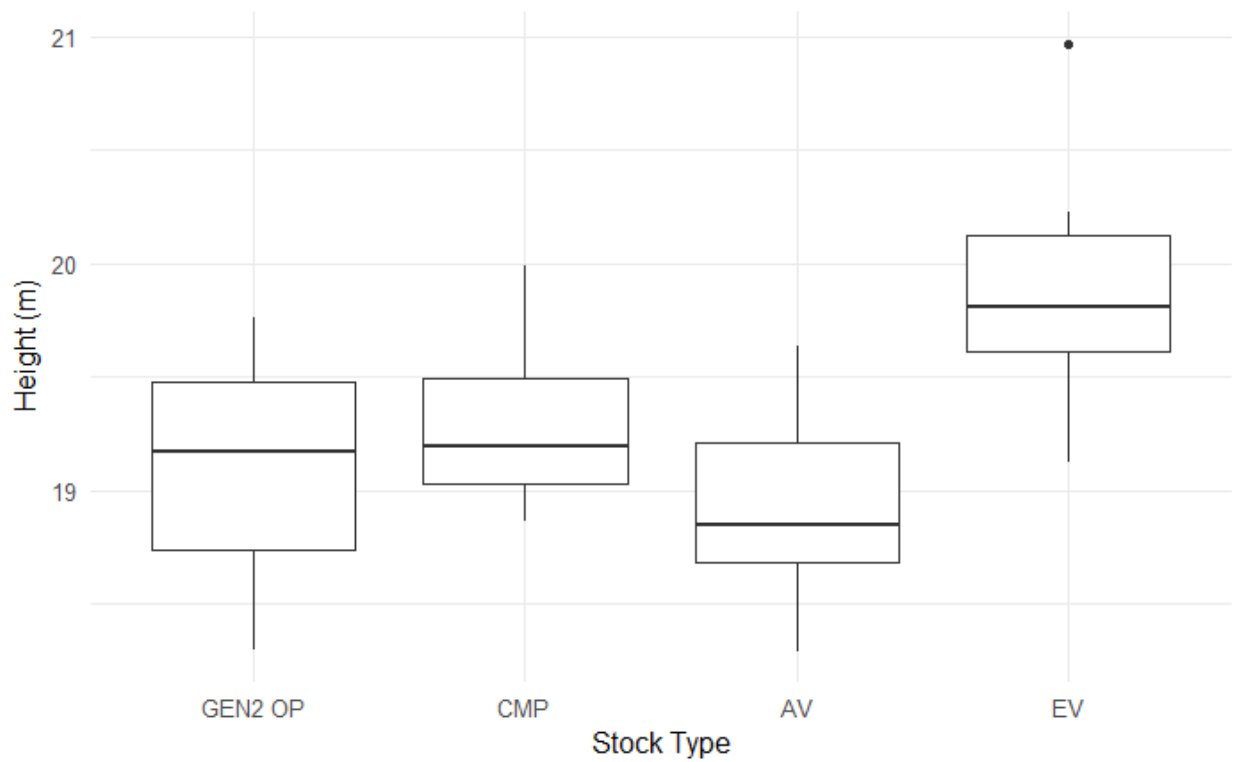


Figure 4.3 Boxplot showing height (m) distribution among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), aggregated varieties (AV), and elite varieties (EV) at the end of fifteenth growing season.

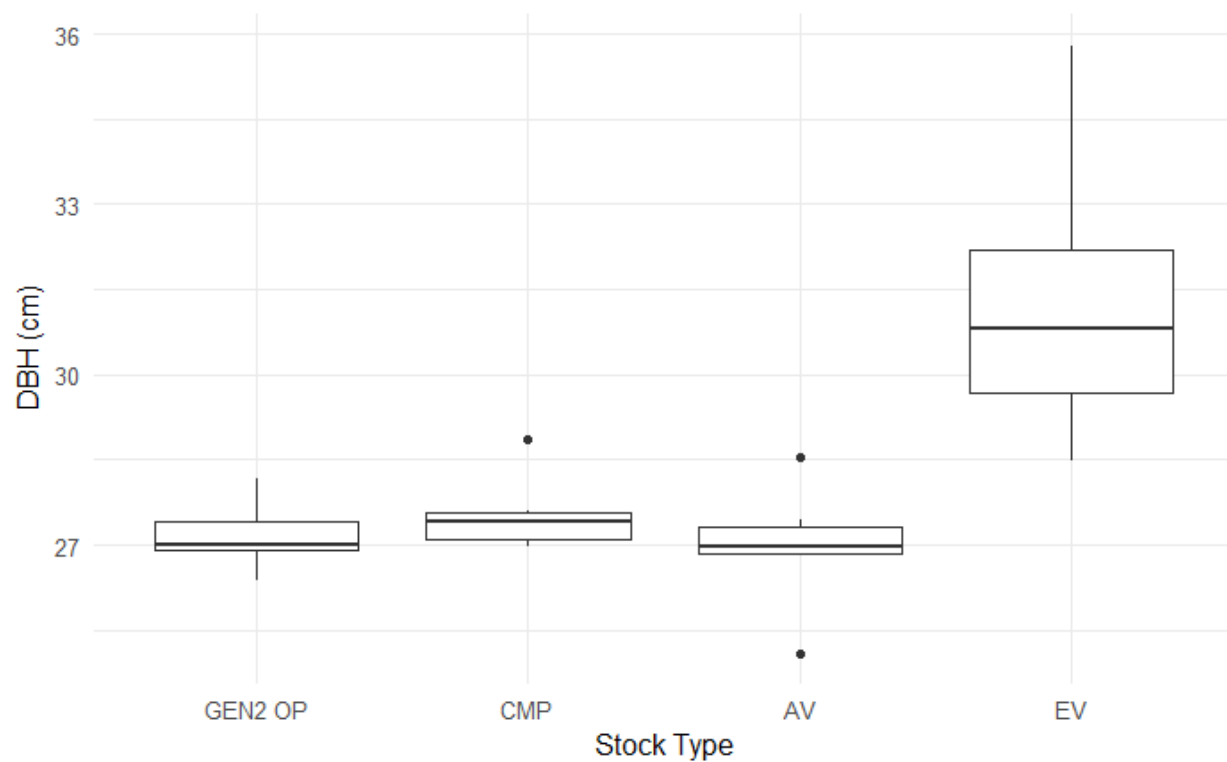


Figure 4.4 Boxplot showing diameter (DBH; cm) distribution among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), aggregated varieties (AV), and elite varieties (EV) at the end of fifteenth growing season.

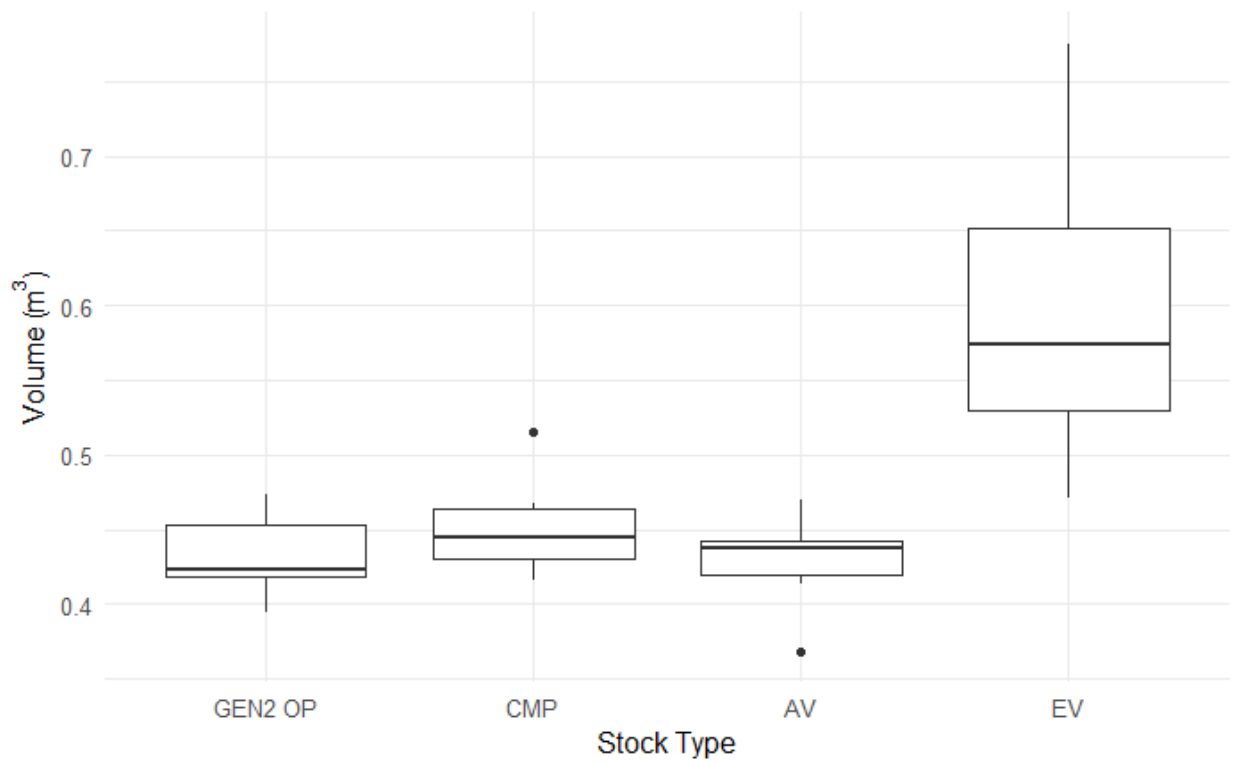


Figure 4.5 Boxplot showing individual tree volume (m^3) distribution among loblolly pine stock types; controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP), aggregated varieties (AV), and elite varieties (EV) at the end of fifteenth growing season.

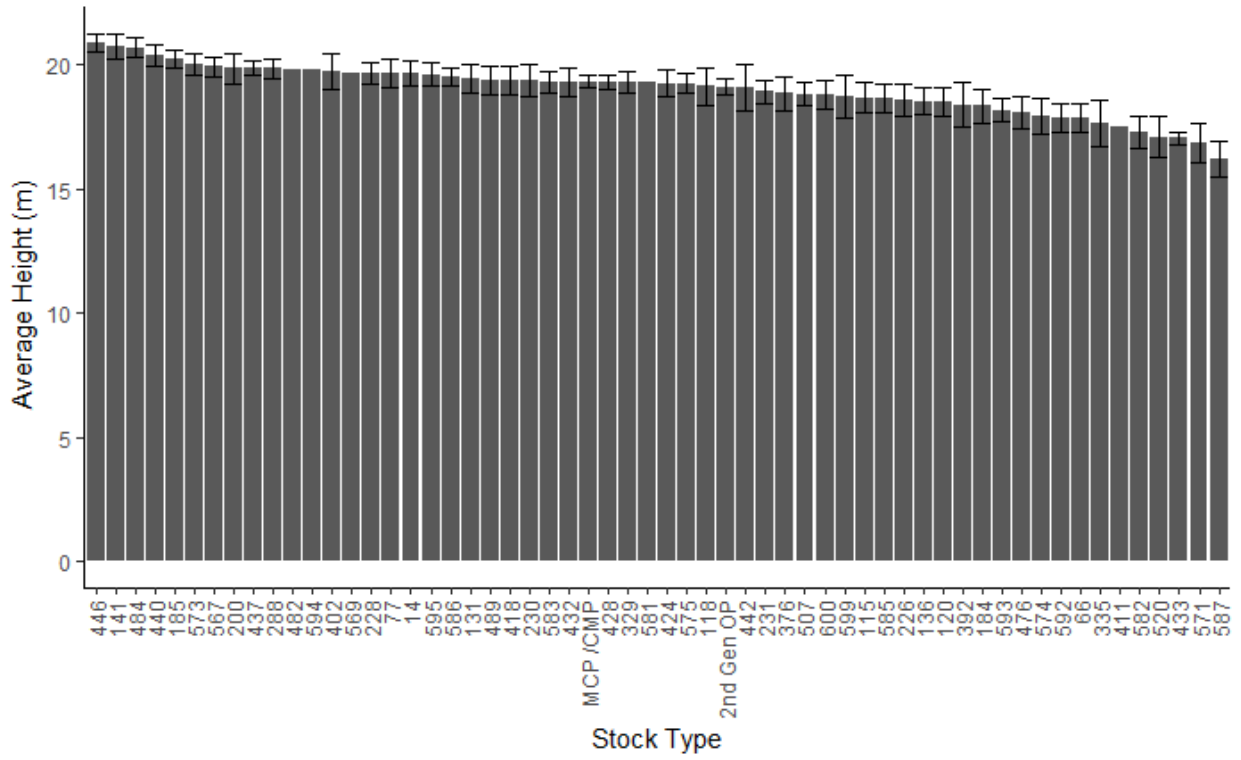


Figure 4.6 Bar graph showing mean height (m) for unique 56 varieties, controlled mass pollinated (CMP), second-generation open-pollinated (GEN2 OP) stock types at the end of fifteenth growing season.

Discussion

Our results conclude that at the end of the fifteenth growing season, all three genetically improved seedling stock types evaluated in this study were not significantly different in defect, height, diameter (DBH), volume, and site index (SI₂₅). The AV stock type had significantly lower survival than GEN2 OP and MCP/CMP stock types. The top-five performing varieties (EV) analysis were taller, larger in diameter, and produced more volume relative to the mean performance of the other genetically improved stock types.

Our mid-rotation results are significant since there was not a clear statistical difference between the three genetically improved planting stock types. This study is one of the few field trials in literature to explore a direct comparison between loblolly pine planting stock derived from MCP, GEN2 OP, and Varietals at midrotation. The lack of significant differences among stock types was unexpected. Early-age measurement of this study, suggested greater productivity in the MCP stock type compared to the GEN2 OP stock type. Our results did not observe the same patterns of growth and distinct differences seen after the sixth growing season (Rousseau et al. 2015). Mid-rotation measurements are more indicative of rotation age performance, providing a more robust basis for evaluating and projecting growth metrics than early-age (Joo et al. 2020).

This analysis was focused on a single site, which limits the ability to effectively evaluate and predict site performance. Since this trial was established on a single site the genotype by environment (GxE) relationship cannot be explored. A single trial is unable to compare genotype performances to represent the varying degrees of gene expressions and their corresponding differences that can occur across different environments (Braga et al. 2020). Several similar but younger trials have been established across Mississippi and will make for a more valid comparison in the future.

Our objective was to equally compare genetically improved stock types with corresponding growth performance and determine the best improved stock option suitable for the site. Due to the great amount of diversity across varieties that make up the varietal stock type, it is difficult to make an accurate comparison to the CMP and GEN2 OP stock types. This study was established in the early 2000's and that period was the beginning of operation CMP and varietal production in the southeast. The varieties selected for this research project were provided by ArborGen, LLC and the corresponding family pedigree and provenance of origin is unknown. In addition, we do not know the genetic pedigree of the CMP family or the genetic mixture of families that made up the pollen cloud of the GEN2 OP seedlot provided by MeadWestvaco Corporation. As a result, it is difficult to draw a formidable conclusion for performance differences since stock types may not have been well-adapted to the site.

The survival within the varietal blocks was unusually low, especially in block-1 (56.3%). Herrin (2012) investigated early-age measurements in the study and suggests the lower survival observed in the varietal stock type was the result of a combination of poor seedling quality and heavy grass competition (Rousseau et al. 2015). The nursery planting stock types used for GEN2 OP and CMP were both bareroot seedlings and the varietal planting stock types were grown in container plugs. It was noted during establishment, that the container varieties produced by ArborGen, LLC were smaller, less developed than the other two bareroot seedlings types provided by MeadWestvaco Corporation. Previous research suggests that the less developed roots and smaller seedling heights within container stock types may account for higher mortality rates and slower growth rates (South et al. 1990). In addition, competition from Bermuda grass establishment impacted pine seedling establishment and survivability resulting in the loss of

numerous valuable observations that would otherwise provide additional information to strengthen the analysis.

The design of the study limited the inference due to varietal blocks containing 56 unique varieties. The observations for each variety was limited to a single ramet per block, resulting in a maximum of six observations of each variety across the entire study. Alternatively, CMP and GEN2 OP blocks contained 64 observations from the same seedlot that were related and similar in growth potential. It is unfortunate that the study design did not included a single variety to represent the varietal performance potential in comparison to CMP and GEN2 OP.

The EV analysis sought to explore the performance variation of the best varieties in the study. The five varieties ranked largest in volume after the fifteenth growing season that had five or more surviving ramets at the end of the second growing season were evaluated separately as the elite varietals (EV) subgroup. Each variety in the EV analysis had six surviving ramets, with the exception of Family 575 which had five, resulting in 35 total observations. As a result, the EV subgroup was pseudoreplicated in the analysis.

We evaluated site index as a measure of site productivity of each genetically improved stock type in order to evenly compare top height from each stock type and exclude variation from stand density. The USDA soil series site index (SI_{50}) estimated a weighted average of 25.5 m for loblolly pine when planted in Loring silty loam and Cahaba-Providence complex (Coile and Schumacher 1953). Our results estimated average exhibited site index (SI_{25}) was 33.9 m (Lenhart and Clutter 1968) for the three stock types. Although there were no significant differences between the stock types, there was an increase in site index over site productivity estimates likely a result of previous genetic improvement work between the three stock types. Previous genetic improvement of the stock types may have resulted in the observed increase in

site index estimates since the differences in the height-age equations are likely nominal. However, recent research suggests that the effects of genetic improvement on site index curves are polymorphic due to stand dynamics (Burkhardt and Tomé 2012) and adjusting the site index anamorphically is likely to over predict these growth potentials (Sabatia 2011). Site index curves estimate crop tree height by using a subset of the tallest trees of each stock type (García and Batho 2005), the nominal differences between GEN2 OP, CMP, and varietal material suggest that the EV subgroup stock type was likely truncated and the superior growth performance were influenced by stand dynamics and competition due to lack of survival within the varietal blocks.

Tree improvement practices have resulted in substantial genetic gains in controlled crosses and varietal selections. As a result, private seedling vendors have increased the scale of production of improved genetics like CMP to market higher genetic gain value to landowners (Bridgwater et al. 1998; Bell 2015). Choosing the best stock type for landowners requires careful consideration of seedling genetic stock type performance and costs. The actual genetic performance of improved seedlings will vary site to site and are dependent on proper deployment (Rousseau 2017).

Our results conclude that at the end of the fifteenth growing season, all three genetically improved seedling stock types evaluated in this single site study were not significantly different to one another in height, diameter, volume, defects, survival or exhibited site index. The results from the EV analysis suggest that the top-five best performing varieties were higher in height, diameter (DBH), and volume relative to the mean performance of the other genetically improved stock types. Landowners should make reforestation decisions based on sound research and evaluate their management intensity, goals, financial limitations, and personal circumstances when selecting genetically improved planting stock.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Introduction

The southern yellow pine forests have been the ideal geographic location for practicing plantation forestry. Reality is that a lot of this land was abandoned farmland because of unsuitable techniques for continual farming without the addition of fertilization. Once these sites diminished in fertility and abandoned natural native pine reseeded the area. It was at this time that Wakley and others began to put forth the idea of plantation forestry with silvicultural techniques as well as tree improvement. Armed with a long history of conservation using forest management practices, land managers favor the soils and climates in the southeastern US since they are conducive for growing softwood pines quickly yielding favorable returns for forest investments.

Today forest land has become even more fragmented, with land use changes like agriculture and urban expansion coupled with climate changes and forest health concerns resulting in a need for landowners to be more productive. Additionally, forest management practices have evolved and adapted, integrating silviculture and genetic improvement as a strategic approach for improving forest health and productivity.

Of the southern yellow pines, loblolly pine is the major commercial timber species and is the most widely planted pine in the southeastern US (McKeand et al. 2003). Forest genetics research and tree improvement programs are focused on improving loblolly pine as a means of

increasing forest stand productivity through genetic selection (Wheeler et al. 2015). Forest managers favor planting genetically improved loblolly pine due to the natural wide range, adaptability to climates, form characteristics, resistance to disease, wood qualities, and superior growth (Dougherty and Wright 2009).

Over the last couple of decades, there has been a significant increase in commercial nurseries offering varying levels of genetically improved seedlings for reforestation (Barnett 2013; Bell 2015). In order to market the value of increased genetic gain to landowners, seedling vendors offer the sale of numerous genetically improved options such as mass pollinated controlled crosses and varieties (Rousseau 2017). These seedlings boast significant genetic improvement for growth, form and disease resistance but are more expensive than the standard second-generation open-pollinated options. This research serves as a tool in furthering the understanding and applied use of genetically improved loblolly planting stock in southeastern forestry.

Resistance Screening Center

Our results found that none of the interprovenance hybrids tested were deemed rust-resistant and there was only one Coastal OP seedlot that was, as a result, no distant geographic or provenance resistance trend was observed. Today, the use of intraprovenance hybrids in forestry is not well documented in white literature. A potential exists for interprovenance hybrid's use in forestry and the growth and disease resistance traits need to be explored further. However, assuming two elite parents from different provenances will be perform superior in rust resistance can be problematic. This information is valuable to understand loblolly pine provenances' and their subsequent disease resistance to fusiform rust, and to further explore the applied use of loblolly pine interprovenance hybrids in plantation forestry in the south.

The RSC is a valuable tool for evaluating fusiform rust resistance within seed sources. As tree improvement managers continue to explore resistance among loblolly pine provenance and their hybrids, the RSC remains an effective tool and provides valuable rust resistance information in a timely manner. Our results indicate that under extreme rust pressure interprovenance hybrids tested were not a viable solution for broad scale rust resistant planting stock and further exploration into interprovenance hybrids' gene-to-gene interaction with the disease is needed.

A larger multi-year study that investigates the relationship between interprovenance hybrids and rust resistance is needed. In order to determine site-specific resistance, a more diverse collection of inocula from each of the 13-collection location would provide a sounder evaluation of resistance. Seedlots tested across multiple degrees of inoculum densities specific to each collection site and the traditional bulked inoculum would help to represent the varying degrees of rust pressure within the natural range of loblolly pine. In addition, it would be advantageous to include known resistance and susceptible check from the Western Gulf, Coastal and Piedmont provenances to explore geographic variation for disease resistance.

In addition to the RSC study, a multi-year and multi-site replicated field trial study located across the 13 collection locations in varying degrees of rust pressure (high, moderate, and low) with the same seedlots as the screening study could correlate expressed symptoms of fusiform rust with the RSC and explore growth performances to further the understanding and applied use of interprovenance hybrids in southern forestry practices.

Genetic Comparison and Varietal Field Trial

Our results conclude that at the end of the fifteenth growing season, the genetically improved seedling stock types evaluated in this single site study were not significantly different to one another in defects, height, diameter, volume and site index.

However, this is only a single site with very limited genetic stock types. Where possible genetic stock types should be selected based on performance across a wider array of genetic variability and geographic areas. Thus, it becomes imperative for forestland owners to base their selections on known research results. These results suggest that while genetic improvement plays a vital role in deployment of seedlings, midrotation operational plantings will be important to evaluate real-world expectation of performance on a single site. Adjusting the site index in growth and yield models to assume genetic productivity potential is problematic due to polymorphic effect of genetics and stand dynamics on the curve. Field trial results from this study suggest that further exploration is needed for rotation-age yield expectations from operational plantings of genetically improved seed sources. Our results further suggest there is an important implication between genetically improved stock types and their corresponding influence on stand dynamics. A study exploring the polymorphic effect on the relationship effect between levels of genetic improvement and stand dynamics is needed to provide strong correlations between genetic gain and expected volume production at rotation.

The investigation into the performance comparisons between different stock types, families and provenances are best when replicated equally across a wide range of sites. To appropriately evaluate the performance differences between the three genetically improved levels of loblolly pine evaluated in this study, a future research study would need to be designed with a single variety per replicate to appropriately compare performance differences, in addition,

multiple study sites and the inclusion of unimproved checks lots would further strengthen the analysis and provide a more concrete perspective of the growth differences of genetically improved levels of loblolly pine at midrotation.

Conclusion

Southern pine tree improvement programs will continue to incorporate breeding strategies that focus on the development of fusiform rust-resistant planting stocks. Many of these selections are inherently resistant to fusiform rust, however, tree form, volume production, and adaptability may not be optimal. Currently, those areas that have no or little need for pulpwood, forest landowners should focus more on improving the quality of their pine plantations. This begins with the landowners being fully aware of the tree quality from numerous sites. The exploration of rust resistance through the use of interprovenance hybrids may provide a solution to genetically improved planting stock that expresses strong family heritability for rust resistance, site adaptability, and desirable growth and quality attributes.

Therefore, in addition to volume and rust resistance traits, tree form and wood quality characteristics are extremely important to determining the potential for sawtimber value from genetically improved loblolly planting stock. The collection of phenotypic measurements such as; tree form and wood quality, in addition to standard disease presence and productivity would provide a better differentiation of potential value derived from genetic improvement. The collection of midrotation measurements from blocked plots will be important for evaluating genetically improved stock type potential until the polymorphic relationship between genetics and stand dynamics is fully understood.

Forest management that incorporates genetics as a means of improving financial returns should be selective about the genetic merit and integrity of material deployed. Choosing the best

stock type for landowners requires careful consideration of establishment costs and the subsequent return on investment. Actual genetic performance will vary site to site and depend on proper deployment.

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