

## ABSTRACT

FORD, GRAHAM ANDREW. Effect of Inbreeding on Growth and Wood Properties in Loblolly Pine. (Under the direction of Dr. Steven McKeand and Dr. Fikret Isik.)

Inbreeding can increase rapidly in intensively selected breeding populations and is detrimental in outcrossing species such as loblolly pine (*Pinus taeda* L.). Managing inbreeding and the deleterious effects on metric traits is a primary objective in forest tree breeding programs. In these studies, the effects of inbreeding were quantified for 10 Coastal and 10 Piedmont loblolly pine lines. Each of 10 selected parents was bred to other related and non-related selections to provide a gradient of inbreeding coefficients (F); F = 0, 0.125, 0.25, 0.5. Progeny from each cross were planted in field trials, and measurements were collected at age 9. There was generally a decrease in metric traits with increase in inbreeding coefficient, but probability of fusiform rust incidence and straightness were not affected by inbreeding.

Following early measurements, all test sites were either lost or abandoned, except one Coastal installation. Height, diameter, survival, and wood properties were measured at age 23. Growth traits showed significant inbreeding depression, but wood properties were not strongly affected by increased inbreeding. Wood specific gravity did not change with inbreeding, and wood bending strength decreased slightly, but significantly. Genetic entries (referred to as lines) did not respond equally to increased inbreeding.

Growth and survival traits are more closely tied to fitness than resistance to fusiform rust, straightness, and wood properties. Significant inbreeding depression is seen in growth and survival likely because of the selective advantage on fitness.

Response to inbreeding depended on genetic background, indicating that a single approach to managing inbreeding in breeding populations is not appropriate.

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Effect of Inbreeding on Growth and Wood Properties in Loblolly Pine

by  
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A thesis submitted to the Graduate Faculty of  
North Carolina State University  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

Forestry and Environmental Resources

Raleigh, North Carolina

2012

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## **DEDICATION**

To Callie, Mama, Daddy, Adam, Maria, Grandma, Granddaddy, Mom, Dada, Andy, Matt, Greg, and of course, Penny and Tullie.

## **BIOGRAPHY**

Graham Andrew Ford is a native of Clayton, North Carolina. His mother, Cynthia, is a kind, honest Christian woman who taught Graham to pray, be patient, generous, and forgiving. His father, Jimmy, is a strong Christian man with a warrior's heart, who taught Graham how to hunt, fish, be honest, brave, and sincere in all that he does. The rest of Graham's closest of kin are a home-grown variety of quirky Southerners and the sweetest Italian lady you will ever meet.

## ACKNOWLEDGMENTS

I would like to thank Steve, Fikret, and Jeff for all of the encouragement and direction you have given me. I was not born knowing this stuff. I feel very fortunate to have such a great team in my corner that has held me to a high standard. Thank you for your patience and belief in me.

I greatly appreciate the cooperating members of the North Carolina State University Cooperative Tree Improvement Program for their work in breeding efforts and installation of the experiments. Thank you Dr. J.B. Jett for your instrumental role in coordinating the breeding efforts and establishing the field trials. Thank you to all who helped in the field collecting data: Tori Brooks, Josh Steiger, Jadie Andrews, David Barker, Alfredo Farjat, and Bo Dixon. Thank you Dr. Tony LaPasha for working with me in the process of drying wood cores.

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## Chapter 1 : Review of Literature on Inbreeding and Inbreeding Depression in Conifers

### Introduction to Inbreeding and Inbreeding Depression

Inbreeding is the mating of individuals that are related by ancestry. Individuals with a common ancestor can both have a replicate of the same gene found in the ancestor, and mating of these related individuals can pass the replicates to offspring. Therefore, inbred individuals can have two genes that are identical by descent (IBD) at a locus found in the ancestor in a previous generation (Falconer and Mackay 1996).

The coefficient of inbreeding,  $F$ , is the probability that the two alleles at any locus in an individual are identical by descent. This measure refers to an individual and describes the degree of relation between the parents. For the coefficient of inbreeding to be meaningful, a point of reference is needed, at which all genes in the population are regarded as independent. This point is referred to as the base population, and  $F$  is considered equal to zero (Falconer and Mackay 1996). Coefficient of inbreeding in progeny of a self is equal to 0.50. Between full-sibs,  $F = 0.25$ . For half-sibs,  $F = 0.125$  (Wright 1922).

Inbreeding results in a redistribution of the genetic variance of a metric trait. With inbreeding, the proportion of homozygotes increases, because heterozygotes are reduced each generation by a factor of  $(1 - F)$ . With successive generations of inbreeding, variances of means between lines increase, while within-line variance decreases. These changes in variances are dependent upon initial frequencies of the recessive alleles and the degree of dominance associated with the alleles (Falconer and Mackay 1996).

Matings between related individuals are more likely to occur in smaller populations than in larger populations (Falconer and Mackay 1996). In a population of hermaphroditic, self-fertile organisms, there are  $N$  individuals. In mating generation 1, the probability that a pair of gametes coming together at random carry IBD genes at a locus is  $1/2N$ . Falconer and

Mackay (1996) show that in any generation following generation 1, the total probability of identical homozygotes can be expressed as

$$[1] \quad F_t = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_{t-1}$$

Inbreeding depression is an observable consequence of inbreeding. It is the reduction in mean phenotypic values of traits related to fitness: reproductive capacity, vigor, and physiological efficiency (Falconer and Mackay 1996, Wright 1922, Charlesworth and Willis 2009). However, traits not closely connected with fitness often show no change due to increased inbreeding (Falconer and Mackay 1996).

There are many methods used to calculate changes in mean with inbreeding. A method of calculating inbreeding depression, shown by Williams and Savolainen (1996) and Byers and Waller (1999), is as follows:

$$[2] \quad \delta = 1 - (w_s / w_c)$$

where,

$\delta$  = inbreeding depression

$w_s$  = fitness of selfed individuals

$w_c$  = fitness of outcrossed individuals

Another method to describe degree of inbreeding depression is to fit a regression model of mean phenotypic values against F. When loci combine additively to influence a trait, change in mean upon inbreeding will be proportional to the inbreeding coefficient. If the mean values were plotted against F, the relationship would be linear. Epistatic interactions between loci would cause the relationship to be non-linear, due to double or multiple heterozygotes (Falconer and Mackay 1996).

## Cases of Inbreeding and Resulting Inbreeding Depression in Conifer Species

### *Seed and Cone Traits*

One of the most serious consequences of self-fertilization seen in conifers is reduction in the proportion of filled seed (Kärkkäinen 1996, Durel et al. 1996, Franklin 1968, Sniezko 1984, Bramlett and Pepper 1974, Orr-Ewing 1965, Andersson 1974, Snyder 1968, Bingham and Squillace 1955, Sorensen et al. 1976, Franklin 1969). Proportion of filled seed continued to decline after a second generation of selfing (Sniezko 1984, Andersson 1974). This failure to produce sound seed is attributed to embryonic death. Embryonic death is a result of damaging genes that are lethal when made homozygous by inbreeding, not due to self-incompatibility (Williams and Savolainen 1996, Sorensen and Miles 1982, Hagman and Mikola 1963, Orr-Ewing 1957, Savolainen et al. 1992).

Andersson (1974) found that matings between half-sibs and full-sibs produced proportions of filled seed near that of matings of unrelated individuals. Griffin and Lindgren (1985) show a similar result. Percent filled seed relative to outcrosses is 102% for half-sibs, 97% for full-sibs, 43% for S1's (one generation of selfing), and 42% for S2's (two generations of selfing). See Franklin (1970) for a wide review of inbreeding effects on cone, seed, and seedling traits in the Pinaceae family.

Estimates of the number of embryonic lethals per zygotic embryo are high in many conifer species, ranging from 0.1 (Fowler 1965) in red pine (*Pinus resinosa*), 8.5 in loblolly pine (*Pinus taeda*) (Franklin 1972), 9 in scots pine (*Pinus sylvestris*) (Savolainen et al. 1992), to 10.3 (Park and Fowler 1982) in tamarack (*Larix laricina*). These high numbers of lethals can be attributed to relatively high mutation rates in conifers, arising from mutations in meristematic tissues in long-lived individuals (Williams and Savolainen 1996). Number of lethals also varies among individual trees within a species (Bramlett and Pepper 1974).

Inbreeding reduces the size of cones and pollen strobili in conifers (Sniezko 1984, Orr-Ewing 1965), resulting in the inevitable reduction in total number of seeds produced and seed weight in loblolly pine (Sniezko 1984, Franklin 1968). Franklin (1968) showed that self-fertilization of loblolly pine resulted in reduced germination of filled seed and a longer time to reach germination. Seed weight and germination were not adversely affected by inbreeding in a study involving Noble fir (*Abies procera*) (Sorensen et al. 1976). Germination was also decreased upon selfing for both Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). Douglas-fir seed weight was decreased following selfing, but ponderosa pine seed weight showed no change (Sorensen and Miles 1982).

### *Reproductive Traits*

Ability to produce reproductive organs can be impacted by inbreeding. There is a delay in age of onset of flowering and reduction in number of cones produced upon inbreeding in radiata pine (*Pinus radiata*). This effect was more pronounced at earlier ages (4 and 5 years) than at a later age (18 years). Delay in reproductive age was seen in 8.3% and 8.5% of trees in S1 and S2 generations, respectively. In S1's and S2's, number of trees producing no cones, was higher than outcrosses by 6.7% and 13.1%, respectively. There was a significant interaction of clone and inbreeding level; some clones showed a greater inbreeding depression than others (Matheson et al. 2002). Maritime pine (*Pinus pinaster*) also showed a significant inbreeding depression. Number of cones produced was reduced by 53% and 89% following one and two generations of selfing, respectively (Durel et al. 1996). Western white pine (*Pinus monticola*) has been shown to exhibit no reduction in cone yield upon inbreeding (Bingham and Squillace 1955).

### *Seedling and Early Life-Stage Development and Survival*

Inbreeding depression is detectable in many conifer species at an early age. Six-month-old progeny of selfed loblolly pine were 87% as tall as progeny of outcrosses (Franklin 1968).

Snieszko (1984) reports similar inbreeding depression in loblolly pine for height and root collar diameter at 17 weeks in progeny following one and two generations of selfing. Overall means for seedling height and root collar diameter decreased by 1% and 1.2%, respectively, with each 0.1 increase in F (Snieszko 1984, Snieszko and Zobel 1988). A linear regression model significantly explained the decrease of seedling height and root collar diameter, respectively, upon inbreeding. This linear association is concurrent with genetic models involving additivity and dominance over loci. As explained previously, epistatic interactions would result in a model with significant quadratic terms. These results suggest that as loblolly pine is increasingly inbred, height and diameter will continue to decrease (Snieszko and Zobel 1988).

Seedling mortality in loblolly pine is greater in inbred progeny than progeny of unrelated matings (Franklin 1968; Snieszko 1984). Self-fertilization of Noble fir also resulted in reduced seedling growth but not survival rate (Sorensen et al. 1976).

Comparison between one-year-old selfed and outcrossed seedlings of Douglas-fir and ponderosa pine showed reduction in seedling height of 18% and 21%, respectively. One-year-old seedlings of selfs also had significantly lower survival than outcrosses for both species (Sorensen and Miles 1974). Two-year-old Sierra lodgepole pine (*Pinus contorta* var. *murrayana*) demonstrated inbreeding depression of 24.4% for height and 21.9% for diameter (Sorensen 2001). Slash pine selfed seedlings showed a similar inbreeding depression of 21% at one-year-old (Snyder 1972). Radiata pine seedling height was reduced by 18% upon selfing, but some selfed individuals showed as much as a 60% reduction in height (Wilcox 1983).

Self-fertilization results in mutant phenotypes at the seedling stage. These mutant forms retard growth, normal development, and can prove to be lethal. In a study of 119 experimentally self-fertilized families of loblolly pine, twenty-two different mutant forms

were observed. Some of these mutants included: fused cotyledons (usually lethal), pale-green cotyledons (non-lethal), dieback of primary needles (lethal), pale droopy cotyledonary stage (lethal), dwarf (non-lethal), albino (white) cotyledons and pink hypocotyl (lethal), pale yellow cotyledons and pink hypocotyl (lethal). The patterns of segregation for the mutant types suggested that there were relatively high numbers of mutant alleles carried in heterozygote form in the population (Franklin 1969).

#### *Later Life-Stage Growth and Survival*

Inbreeding depression in growth traits has been consistently shown in the majority of conifer species. Douglas-fir, ponderosa pine, and Noble fir showed a 24% to 30% inbreeding depression in height at outplanting, and at 10 years, inbreeding depression ranged from 29% to 36%. These species still showed inbreeding depression in growth rate at 5 years of 35%, and 28% as of age 9 (Sorensen and Miles 1982). Selfing Norway spruce (*Picea abies*) in 3 diallel crosses resulted in inbreeding depression of height at ages 5, 7, and 10 of 25.5%, 30.5%, and 33%, respectively (Skrøppa 1996).

Norway spruce diameter showed an inbreeding depression of 37.5% at year 10. Furthermore, these selfed families showed a delay in onset of growth and an earlier cessation of growth compared to outcrossed families; beginning growth approximately 2 days later and ending approximately 6 days earlier than outcrosses. This equated to an approximate loss of 8.5 days out of the growing season, or one-third of the total growing period (Skrøppa 1996).

Research into the effects of inbreeding on growth of radiata pine has been conducted by many authors. Wilcox (1983) showed that diameter of selfed radiata pine trees was significantly reduced by 12%, compared to the outcrossed trees. At age 7, this resulted in a 30% reduction in volume. Four-year-old progeny of selfs were 91% as tall as the outcrosses. Of the 25 clones utilized in the test, 15 showed significant inbreeding depression in growth at 7 years. The remaining clones showed either no change in mean or an increase in mean

(although non-significant). This variation in response to selfing resulted in a significant clone by inbreeding coefficient interaction (Wilcox 1983).

Radiata pine showed inbreeding depression at age 12 for DBH of 5%, 6%, 15%, 19% relative to outcrosses for half-sibs, full-sibs, S1's, and S2's, respectively. The differences in diameter growth between the five inbreeding levels were statistically significant. Regression of DBH on inbreeding coefficient was linearly significant. Per each 0.1 increase in F, DBH decreased by 0.55 cm, or 2.7%. Response of mean DBH was different among the eight founder clones (Wu et al. 1998). Matheson et al. (1995) showed that in slash pine, inbreeding depression remained constant from ages 2 to 13 for height and diameter, but it increased for tree and plot volume (Matheson et al. 1995).

At early and later ages, degree of inbreeding depression in diameter is similar for radiata pine. In progenies of 41 radiata pine plus trees, inbreeding depression in diameter was seen to be 12.5% at age 9, and was similar at 26 years, 17% (Kumar 2004).

Slash pine exhibited an inbreeding depression in height of between 4% and 8% per each 0.1 increase in F. Diameter showed a slightly greater inbreeding depression per each 0.1 increase in F, between 5% and 8%. Tree and plot volume showed even greater inbreeding depression, ranging between 6% and 19%. Families responded differently to inbreeding for all of these growth traits. The inbreeding depression was linearly related to inbreeding coefficient, with no significant quadratic effects for any traits (Matheson et al. 1995). Earlier research by Snyder (1972) shows a greater degree of inbreeding depression at five-years-old. Progeny of selfed slash pines were 34% shorter than outcrossed progeny.

Height, following the second generation of selfing, is depressed in jack pine (*Pinus banksiana*). Progenies of S2 individuals were 20 centimeters shorter than outcrossed progeny at four years. This translated to an average inbreeding depression of 18.1%, but ranged to 39% (Rudolph 1981).

Western white pine shows inbreeding depression for height at 9 – 12 years of age, but degree of inbreeding depression was different among populations. Among parents from lower-elevation sites (914 meters), selfs were 26.33% shorter than outcrosses. Among parents derived from higher-elevation sites (1463 meters), inbreeding depression for height in the progeny was greater, 35.67% (Barnes 1964).

Eleven-year-old maritime pine exhibits inbreeding depression for growth traits. Relative to outcrosses, progeny of half-sibs, full-sibs, S1's and S2's displayed 3%, 5%, 15%, and 27% reduction in height, respectively, and 11%, 20%, 45%, and 63% reduction in volume, respectively. Inbreeding depression in these growth traits was linearly related with F (Durel et al. 1996).

Inbreeding effects on survival of older trees is variable. Survival of radiata pine trees was not significantly affected by inbreeding. Progeny of half-sib and full-sib matings were not significantly different from outcrosses. Survival declined slightly following one (7%) and two cycles (11%) of self-pollination in radiata pine (Wu et al. 1998). Relatively self-compatible slash pines showed no reduction in survival at 5 years. But, more self-incompatible clones showed a 20% reduction in survival of selfs at 5 years (Snyder 1972). Survival of 11 and 12-year-old slash pine was generally not affected by inbreeding of half-sibs and full-sibs (Matheson et al. 1995). Across three sites, survival of self-pollinated western white pine was on average 16.33% lower than outcrossed progeny (Barnes 1964). Modest reductions in survival were expressed following one and two generations of selfing maritime pine. Relative to outcrosses, survival of S1's and S2's was reduced by 7% and 20%, respectively (Durel et al. 1996).

### *Stem Straightness*

Effects of inbreeding on stem quality and straightness are extremely variable among conifer species. Selfed progeny of 7-year-old radiata pine showed inbreeding depression for

straightness (12% reduction in straightness) and a poorer branching habit (20% worse than outcrosses) (Wilcox 1983). This pattern of inbreeding depression in straightness was confirmed by Kumar (2004), who showed 12.4% and 13.2% reduction in straightness at ages 9 and 26, respectively. Selves had 21.7% and 17% fewer branch clusters at ages 9 and 26, respectively.

However, straightness in maritime pine improved with increasing levels of inbreeding. Relative to outcrosses, half-sibs, full-sibs, S1's, and S2's were 13%, 18%, 20%, and 23% straighter, but these differences were not significant (Durel et al. 1996). Slash pine at 11-12 years old showed no change in straightness or forking with breeding of half-sibs and full-sibs (Matheson et al. 1995).

#### *Disease Resistance*

Upon inbreeding, there are inconsistent trends in disease incidence among and within conifer species. Wilcox (1983) showed that radiata pine selves were less resistant to two types of needle diseases. Kumar (2004) showed no clear pattern of incidence of *Dothistroma* in radiata pine between selves and outcrosses, and attributed this result to environmental variation among sites.

Snyder (1972) reported that progeny of selfed slash pine had lower occurrences of infection of fusiform rust, 18% in selves compared to 34% for wind-pollinated individuals. Matheson et al. (1995) showed that half-sib and full-sib mated slash pines generally showed no difference from outcrosses in incidence of fusiform rust, but families were not identical in their responses to increased inbreeding.

### *Wood Properties*

Change in wood density upon increased inbreeding is variable in radiata pine. A population of eight radiata pine founder clones were inbred to levels of  $F= 0, 0.125, 0.25, 0.5,$  and  $0.75$ . Across the five inbreeding levels, there was a minor, but non-significant, decrease in wood density among progeny of all clones (Wu et al. 2002). Kumar (2004) showed that relative to progeny of outcrosses, radiata pine selfs had 3.3% and 3.1% more dense juvenile and mature wood (Kumar 2004). Seven-year-old progeny of selfed radiata pines had slightly, but not significantly denser, wood than progeny of outcrosses (Wilcox 1983).

At age 10 in 3 diallel crosses of Norway spruce, wood density of selfs was 5% greater than in outcrosses (Skrøppa 1996).

The increase in wood density upon inbreeding has been interpreted as a product of the slower diameter growth of inbred trees (Wilcox 1983, Wu et al. 2002, Skrøppa 1996). Wu et al. (2002) offer some additional interpretation of the effects of inbreeding on wood density. It is possible that wood density is not as fundamental to fitness as growth traits, and/or that wood density has fewer damaging or lethal recessive alleles to arise upon increased homozygosity. Furthermore, there could be less variation among the genes that control wood density.

Wood density is not only affected by genetics, but it is also heavily dependent upon environmental factors. Wood density is a complex characteristic determined largely by cell size and earlywood to latewood ratio. Detrimental alleles that reduce the growth of these cells may have no impact on the resulting density of the wood (Wu et al. 2002).

### Changes in Variances with Inbreeding

For all growth, branching, needle retention, and wood density traits in radiata pine, among-family variance of selfs was greater than among-family variance of outcrosses. Furthermore, among-family variance of selfs was significantly different from zero for every trait, while among-family variance of outcrosses consistently was not. Among-family variance increased the most in growth traits and needle retention (Wilcox 1983).

Standard deviation of mean DBH in radiata pine increased, relative to outcrosses, by 10%, 10%, 30%, and 25% for progeny of half-sibs, full-sibs, S1's, and S2's, respectively. Coefficient of variation (CV) also increased with greater inbreeding (17%, 16%, 53%, and 55%, respectively). Generally, standard deviation and CV increased linearly with inbreeding. Among the 8 founder clones used in the experiment, there was variation in behavior of within-family standard deviation and CV with increased inbreeding coefficient. In two clones, however, within-family standard deviation and CV increased to  $F = 0.5$ , then decreased slightly at  $F = 0.75$ . A single clone showed little change in metric trait means or within-clone variances upon inbreeding (Wu et al. 1998).

In diameter growth of radiata pine among-family and within-family variance increased with inbreeding. Among-family variance increased to  $F = 0.25$ , then decreased at  $F = 0.75$ . Among and within-family variance together was within-clone variance, which increased slowly with inbreeding as well (Wu et al. 1998).

Changes in variances have also been found to hold true in other economically-important traits. Variation of wood density among trees increased with inbreeding in 17-year-old radiata pine. Relative to outcrosses, standard deviation increased by 3.7%, 3.4%, 15.74%, and 29.01% for half-sibs, full-sibs, S1's, and S2's, respectively (Wu et al. 2002).

Observation of selfed and outcrossed loblolly pine seedlings also support the theoretical changes in variances upon inbreeding. In general, within-plot variance of seedling height and diameter is higher for selfed families and is smaller for outcross progeny. Family variance components increased with inbreeding for seedling diameter; showing that increased inbreeding resulted in greater differentiation among families. This trend was not evident for seedling height (Sniezko and Zobel 1988). The increase in within-plot variance in inbred progeny could be attributed to a greater environmental variance, as explained in Falconer and Mackay (1996).

Among-family standard deviation for height in four-year-old jack pine was greater in S2's than in outcrosses. However, within-family standard deviation was similar for S2's and outcrosses (Rudolph 1981).

### Genetic Basis of Inbreeding Depression

Selection against deleterious alleles often maintains them at low frequencies in populations. Inbreeding depression arises by increases in the proportion of the less-fit homozygotes in the population (Charlesworth and Willis 2009). Any change in the mean value of a trait, associated with increased inbreeding, must be a result of these changes of genotype frequencies and differences in genotypic values between homozygotes and heterozygotes (Falconer and Mackay 1996).

The chief genetic expression that results in a change in mean upon inbreeding is directional dominance; where dominance of the alleles is in one direction. If alleles that cause an increased value of a trait are dominant to the alleles that reduce the value, inbreeding will result in a decreased population mean, in the direction of the recessive alleles (Falconer and Mackay 1996).

The cause of inbreeding depression is predominantly interpreted through two genetic models: the overdominance model and the partial dominance model (Charlesworth and Willis 2009, Charlesworth and Charlesworth 1987, Falconer and Mackay 1996, Williams and Savolainen 1996). In the overdominance model, heterozygotes have a higher fitness than both homozygotes (Charlesworth and Charlesworth 1987). This condition originates by optimum fitness occurring with complementary alleles at a locus. Overdominance is not a prevalent factor in inbreeding depression (Charlesworth and Charlesworth 1987, Johnston and Schoen 1995), and there is little evidence for overdominance in conifer species (Williams and Savolainen 1996).

In the partial dominance model, the superior homozygote is either fully or partially dominant to the other homozygote that is completely or partially recessive. The inferior homozygote is either fully or partially masked in the heterozygote form (Williams and Savolainen 1996). Williams and Savolainen (1996) assert that explanation of inbreeding depression by the partial dominance model is based on the existence of deleterious recessive alleles (e.g. Jones 1917), that are then increased in proportion upon inbreeding. Genetic models including recessive lethal and near lethal alleles, along with mutation in quantitative characters, can explain most observations of inbreeding depression and heterosis (Lande and Schemske 1985, Sorensen 2001).

Remington and O'Malley (2000) conducted a mapping study of embryonic survival and seedling growth and survival from selfed progeny of loblolly pine. Nineteen QTLs were detected having deleterious to lethal effect on embryos and followed the model of recessive action. These loci equated to >13 lethal equivalents; higher than the loblolly pine's average of 8.5 reported by Franklin (1972). No evidence was found for epistasis playing an important role in inbreeding depression and "weak evidence at best" for the role of overdominant gene action (Remington and O'Malley 2000).

### *Evolution and Variability of Inbreeding Depression*

The genetic architecture of inbreeding depression determines the evolutionary path of the species. Outcrossing is maintained in a species due to inbreeding depression in the progeny of self-fertilized individuals, as a result of deleterious recessive alleles in homozygotes (Lande and Schemske 1985, Charlesworth and Charlesworth 1998, Charlesworth and Charlesworth 1979). Magnitude of inbreeding depression is dependent upon the mutation-selection balance. As deleterious mutations arise, they are subsequently selected against and removed from the population (Charlesworth and Charlesworth 1987). This action is called purging. If inbreeding depression is caused by rare, recessive alleles, purging of these alleles is possible (Wang et al. 1999, Charlesworth and Willis 2009).

Selection against and purging of deleterious alleles will act differently among life stages. Highly deleterious and lethal alleles are expressed at early life stages (Husband and Schemske 1996). Purging will act quickly on severely deleterious alleles of large effect (Wang et al. 1999). Later life stage deleterious mutations are often less damaging (Husband and Schemske 1996). Therefore, selection will not be as efficient in purging these alleles of moderate effect and reducing the amount of inbreeding depression (Charlesworth and Willis 2009, Charlesworth, Morgan, and Charlesworth 1990, Husband and Schemske 1996).

This theoretical difference in expressed inbreeding depression among traits has been shown to exist in loblolly pine. In a self-fertilization trial, the most significant inbreeding depression was exhibited between fertilization and germination, with percent filled seed showing the most obvious effect (Franklin 1969).

For deleterious alleles to be successfully purged, selfing must exceed a certain threshold level. At this point, selection can effectively act on the inbred progeny (Lande et al. 1994). Wang et al. (1999) confirm that there is a theoretically optimum inbreeding rate to reduce inbreeding depression and number of lethals.

Purging of deleterious alleles can account for decreased inbreeding depression found in species capable of self-fertilization. This suggests that inbreeding depression should be lower in populations with a history of some self fertilization and greater in more outcrossing populations (Charlesworth and Charlesworth 1987, Lande and Schemske 1985, Wang et al. 1999, Husband and Schemske 1996). Husband and Schemske (1996) found that predominantly selfing species had lower inbreeding depression than outcrossing species at four separate life stages.

Some natural inbreeding will occur in conifer species capable of self fertilization (Williams and Savolainen 1996). Yadzani et al. (1985) found a self-fertilization rate of 11.8% in a scots pine seed-tree stand. Sarvas (1962) and Koski (1971) explain that proportions of seeds produced originating from self-fertilization may range from 10% to 25% in scots pine (Williams and Savolainen 1996). Inbreeding coefficient among parent trees of a loblolly pine stand was found to be 0.026 (Franklin 1968).

Genetic load is high in nearly all pine species. This leads to inbred progeny being readily selected against in the wild. Therefore, the mating system of most pines is essentially completely outcrossing. For this system to change, deleterious recessive alleles would need to be purged (Vogl et al. 2002). Franklin (1968) further confirms that low natural inbreeding in stands of loblolly pine accompanied by selection against inbreds, results in a completely outcrossing population generating the successive generation.

Effectiveness of purging is a very complicated action, given all of the parameters at play. Purging of the genetic load appears to be a highly variable and inconsistent action when considering the many experiments that have been conducted in attempts to observe it (Byers and Waller 1999).

Historical population structure is another important selective force in determining a population's mating system. Species and/or populations with a reduction in size, such as

bottlenecks or pollinator failure, are expected to exhibit lower inbreeding depression and favor more self-fertilization. Historically large populations facilitate outcrossing and will result in greater inbreeding depression of inbred progeny (Lande and Schemske 1985).

Probability of inbreeding increases as population size decreases (Falconer and Mackay 1996). Therefore, small populations are more vulnerable to the negative effects of inbreeding. If deleterious alleles are removed, they are simply replaced again due to the inevitable inbreeding (Byers and Waller 1999, Lynch et al. 1995). The increasing proportion of deleterious alleles causes their effects to become essentially neutral. Therefore, selection is dramatically less efficient at selecting against the deleterious mutations. Mildly deleterious alleles have a better chance in this situation to increase in frequency and possibly become fixed. Once these alleles become fixed, they reduce the fitness of both selfed and outcrossed individuals; making the distinction of inbreeding depression much more difficult to ascertain, based on the relative performance of the individuals (Byers and Waller 1999). This “mutational meltdown” greatly increases the risk of extinction for these small populations. For these reasons, inbreeding in small populations is alarming to conservation biologists (Lynch et al. 1995).

#### Differences in Inbreeding Depression among Conifer Species

Collectively, there is great variation in the scale of inbreeding depression in conifer species. Magnitude of inbreeding depression depends on the trait being assessed and the age of development of the tree. Outcrossing species exhibit greatest inbreeding depression at early (seed development) and late life stages (growth/reproduction), and slightly lower inbreeding depression at germination and survival to reproduction (Husband and Schemske 1996). Degree of inbreeding depression varies between species, among families within a species,

and among individuals within a family. This pattern of variation could be due to variation in number of lethal and damaging alleles (Skrøppa 1996, Bramlett and Pepper 1974).

Variation in environmental factors affects the expression of inbreeding depression (Kärkkäinen 1996, Byers and Waller 1999, Sniezko and Zobel 1988, Bower and Aitken 2007, Husband and Schemske 1996). Generally, inbreeding depression is greater in more stressful environments. Furthermore, inbreeding depression in sub-optimal conditions varies among populations of a species and among lineages within a population. This is evidence that different alleles and/or loci are responsible for vulnerability to stresses in different lineages or populations (Armbruster and Reed 2005).

Wilcox (1983) speculated that somewhat modest inbreeding depression in radiata pine was owed in part to excellent growing conditions. This has been confirmed by Durel et al. (1996) who developed a “biological index” to assess growing season conditions during an experiment with maritime pine. Inbreeding depression was expressed more strongly during years of higher environmental stress. During unfavorable years, statistical differences between means at different inbreeding levels were more easily identified. In better years, statistical differences were not as easily detected (Durel et al. 1996).

Some species of conifers consistently show lower levels of inbreeding depression in many fitness traits, relative to other species. Extensive work in inbreeding has been conducted with radiata pine, and inbreeding depression for growth and fitness traits is lower than in other species (Wu et al. 1998, Wu et al. 2004). Durel et al. (1996) also assert a relatively low degree of inbreeding depression is seen in maritime pine.

The low degree of inbreeding depression in radiata pine is interpreted as a product of deleterious recessive alleles being purged from radiata pine populations, as a result of its evolutionary mating history (Wu et al. 1998). Greatly restricted population sizes and bottlenecks have enabled much of the genetic load in radiata pine to be purged. Accordingly,

the mating system has shifted from predominantly outcrossing to a mixed mating system; outcrossing and low to moderate levels of self-fertilization. Smaller, island populations of radiata pine have a greater proportion of individuals arising from selfing, 0.15, compared to larger, mainland populations, 0.05 (Vogl et al. 2002).

Red pine is apparently homozygous for a large number of alleles, and progeny of self-pollinations exhibit little to no inbreeding depression (Fowler 1965). Mosseler et al. (1992) and Fowler and Morris (1977) confirm that red pine has very low levels of genetic diversity and heterozygosity.

Reasons for low genetic diversity and resilience to inbreeding in red pine are based on reduction in the number of founders of successive generations; on a long or short time scale. Red pine naturally regenerates following large fires that greatly reduce the density of the stands. Seedlings regenerating after these events arise from a limited number of individuals. Repetition of this process greatly increases the likelihood of inbreeding in successive generations. Deleterious mutant genes would thus arise and be removed through selection (Fowler 1965).

The range of red pine is hypothesized to have been pushed south of its current range during periods of glaciations. It is very possible that recolonization of the modern red pine range could have come from a single, refuge population. This evolutionary bottleneck could be the reason for absence of genetic diversity seen in red pine (Fowler and Morris 1977).

When grown in a common garden setting, whitebark pine (*Pinus albicaulis*) showed very little to no inbreeding depression (Bower and Aitken 2007). Whitebark pine has a lower outcrossing rate (0.73) (Krakowski et al. 2003) than other wind-pollinated conifers that are typically  $>.90$  (Schemske and Lande 1985).

This negligible inbreeding depression and lower outcrossing rate in whitebark pine is hypothesized to be a product of whitebark pine's mating history (Bower and Aitken 2007). Clark's Nutcracker (*Nucifraga columbiana*) feeds on ripe cones and stores the wingless seeds in small caches in the soil. The pattern of seed dispersal results in the clustering of related individuals, greatly enhancing the chances for natural inbreeding to occur (Tomback and Linhart 1990, Krakowski et al. 2003). Even milder forms of inbreeding, such as half-sib mating, can be effective at purging deleterious alleles (Fu et al. 1998). It is possible that historical purging of some deleterious alleles has taken place in these high-elevation populations of whitebark pine, reducing the genetic load and facilitating reduced rates of outcrossing (Bower and Aitken 2007).

Scots pine in Finland exhibits geographic variation in inbreeding depression. Northern populations have lower abortion rates of experimentally self-pollinated individuals than in central and southern populations. The northern populations had on average two fewer embryonic lethal equivalents and a slightly lower outcrossing rate than the populations to the south (Kärkkäinen 1996).

Lower outcrossing rate in northern populations suggests that the northern populations have experienced a longer history of inbreeding and subsequent purging of deleterious alleles. Progeny of selfed scots pine from the northern part of the natural range would be exposed to more severe environmental conditions than selfed progeny in southern regions. Harsher environments in the north would be more efficient at selecting against inbred individuals, resulting in faster purging of deleterious alleles (Kärkkäinen 1996).

Inbreeding depression in Sierra lodgepole pine also has been shown to vary between populations. Effect of inbreeding was less severe in progeny of individuals sourced from environmentally-stressful conditions; relative to progeny of individuals from populations native to ecologically-productive conditions. The differentiation in inbreeding depression

between the populations is because the marginal population has purged alleles of lethal and large effect, but not the alleles of smaller effect (Sorensen 2001).

### Implications of Inbreeding and Inbreeding Depression for Breeding Programs

Conifer tree improvement programs have historically been managed with large population sizes and the goal of avoiding inbreeding (Williams and Savolainen 1996). Inbreeding is not a concern in the first generation of conifer tree improvement programs, because trees selected from the wild to form the breeding population are typically selected from diverse stands and are assumed unrelated. In successive generations, due to intense selection and reduced population size, the probability of mating two related selections greatly increases. Therefore, consciously addressing the issue of inbreeding must become a part of the population management strategy (Wu et al. 1998). In advanced-generation breeding programs, population subdivision strategies have been implemented to manage inbreeding (e.g. McKeand and Bridgwater 1998).

Williams and Savolainen (1996), Lindgren (1975), Snieszko (1984), and Wu et al. (2002) explain that advances in corn breeding through inbreeding and hybrid development have enticed interest in the use of inbreeding in forest tree breeding programs. Interests in the theoretical gains for tree breeding by using inbreeding and outcrossing increased in the 1950's and 1960's. By the 1970's and 1980's, interest was growing and theoretical plans were being set forth (Lindgren 1975, Franklin 1969). Research into the effects of inbreeding and evidence for its use as a tree breeding strategy continue.

The success seen in corn breeding programs may not directly transfer to conifer breeding efforts. The early generations of corn inbred line development were accompanied by a suite of complications and setbacks. Grain yield in corn is a reproductive fitness trait and is

economically valuable. Typically in forest trees, reproductive traits are not directly economically valuable. It is traits such as growth and wood quality that are economically valuable. If conifer breeding programs were to include inbreeding and selfing as breeding strategies, a temporary shift to selection for fitness traits would be necessary. This could possibly come at a detriment to traits of greater economic value (Williams and Savolainen 1996).

There are serious economic and biological issues that limit the use of inbreeding as a breeding tool in conifer species. Reduction in percent filled seed of inbred trees is identified to be the most serious (e.g. Franklin 1968, Snieszko and Zobel 1988). This will significantly increase the cost of obtaining sufficient numbers of filled seed (Franklin 1968). Longer intervals of breeding and selection will prove to be a limiting factor (Snieszko and Zobel 1988).

Wu et al. (2004) give some imperative criteria if inbreeding is to be an efficient breeding tool in a tree improvement program. They are as follows: 1) production of superior inbred lines is possible through purging of deleterious alleles, 2) heterosis is obtained in crosses between lines 3) inbreeding must not seriously delay reproduction, and 4) early selection between lines is effective.

Radiata pine has been shown to exhibit relatively low levels of inbreeding depression for metric traits. Degree of inbreeding depression has been shown to be pedigree-specific, with some clones exhibiting superior growth and no change in mean with increased inbreeding (Kumar 2004, Wu et al. 1998, Wilcox 1983). This is evidence that these clones may be fixed for a large number of beneficial alleles and devoid of deleterious recessive alleles. These factors make the case for the selections to be prized for advanced generation breeding, especially breeding plans involving inbreeding (Wu et al. 1998, Williams and Savolainen 1996).

### *Inbreeding as a Tool in Breeding Programs*

The theoretical increase in genetic variance among families upon inbreeding has been demonstrated in conifer species (Wilcox 1983, Sneizko and Zobel 1988, Rudolph 1981; Wu et al. 1998). This effect is expected to be advantageous for selection efficiencies in developing inbred lines of trees (Rudolph 1981).

Predominantly, tree breeders are concerned with avoiding inbreeding because of the loss of fecundity in individuals in breeding populations (McKeand and Bridgwater 1998). The impact of inbreeding on fecundity for several species was discussed previously. There is variability among species in the effect of inbreeding on reproductive capabilities. In a study of radiata pine, inbreeding depression was seen in onset of flowering and cone production. Again, different clones responded differently to increasing levels of inbreeding, with one clone constituting a significant portion of the inbreeding depression. Differences among clones were greater than differences across inbreeding levels (Wu et al. 2004). This is encouraging evidence that inbreeding can be affectively used in breeding populations, if appropriate clones are identified and utilized.

### *Exploiting Purging*

Intentional inbreeding to purge deleterious alleles could aid in generating superior lines (Sniezko 1984). Severe forms of inbreeding, like selfing, would be quickly effective at purging, but more conservative forms may also be effective. Half-sib mating could be as effective in purging, while greatly reducing the risks of losing superior selections or populations of individuals. This approach is attractive to programs breeding for conservation purposes (Fu et al. 1998). Purging through continued full-sib mating would act quickly and

effectively in removing recessive lethals (Hedrick 1994). These methods could essentially be extended to programs breeding for genetic gain.

The effectiveness of purging through intentional inbreeding is directly commensurate on the underlying genetic architecture that is responsible for inbreeding depression. If the majority of the genetic load in a species is due to lethals, it is conceivable that successful purging could be attained through directed inbreeding. Alleles of deleterious effect would be much more difficult to purge and have a higher probability of becoming fixed. Therefore, the prospects of using directed inbreeding to rid this type of genetic load are not as bright (Hedrick 1994).

### *Selection*

Utilizing selfing to make within-family selections for traits under stronger additive control could be effective. Wood density, straightness, branching quality and needle retention traits showed largely additive genetic variance (additive by additive is important for needle retention). With these traits, the correlation in phenotypes of selfs and outcrosses was high. Growth traits, in which non-additive genetic variance is most important, showed poor correlation between selfs and outcrosses (Wilcox 1983).

Kumar (2004) observed stronger correlations in radiata pine for estimates of narrow-sense heritability between selfs and outcrosses for wood density, form traits, and *Dothistroma* infection than correlations of the same sort for diameter growth.

Inbred families of radiata pine produced higher age-age correlation values for breast-height cross-sectional area than outcrossed families did. Correlation values increased with higher levels of inbreeding. This is evidence that early selection in radiata pine would be more efficient in inbred families than in outcrossed families (Matheson et al. 2002).

Other species show different correlations in families' selfed and outcrossed progeny.

Phenotypic correlations between means of selfed and outcrossed families were weak and not significant in loblolly pine seedlings. This is evidence that the means of selfed families would not be good indicators of general combining ability for loblolly pine (Sniezko and Zobel 1988).

In radiata pine, age-age correlations have been found to be higher for selfed families than for outcrossed families. Estimated DBH breeding values at ages 9 and 26 had higher correlation in selfed families, 0.79, than in outcrossed families, 0.34 (Kumar 2004).

### Conclusion

There are myriad theoretical gains to be made with inbreeding as a tree improvement tactic, but there are realistic obstacles to overcome (Lindgren 1975). Further research into the basic effects on growth and reproduction traits in conifers is critical in planning future generations of tree improvement. Reliable estimates of inbreeding depression are essential in designing breeding strategies. Also, these estimates will give a more firm understanding of the genetic architecture of inbreeding depression and the mating history of the species. Examination of less severe levels of inbreeding is as important as comparing selfs with outcrosses, since it is the less severe instances that will most likely be encountered first in breeding populations (Sniezko 1984, Wu et al. 1998, Williams and Savolainen 1996).

Expression of inbreeding depression is environmentally sensitive (e.g. Armbruster and Reed 2005). Identification of families that exhibit low levels of inbreeding depression would require replication across multiple environments. If many families are to be tested, the scale of experimentation rapidly increases. Implementing and managing this effort will require a lot of work.

Undoubtedly, inbreeding is a serious impediment for most conifer species. Mild and severe inbreeding results in decreased health and vigor at all life stages. For these reasons, breeding programs have avoided inbreeding outright and maintained large breeding populations to conserve genetic diversity. Evolutionary mating history has sculpted some conifer species through time to have reduced amounts of deleterious alleles and modest amounts of inbreeding depression; even no inbreeding depression in the case of red pine. Other species have maintained geographically large populations through time; maintaining a significant genetic load and favoring outcrossing.

In species and families within species that are not impacted severely by inbreeding depression, it is possible to explore inbreeding, purging, and crossing as a breeding strategy. Economical, biological, and logistical obstacles are replete. However, theoretical evidence and foundational experiments have laid the groundwork for the next steps to be taken and further advances to be made by modern day forest tree breeders.

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## Chapter 2 : Effect of Inbreeding on Growth and Quality Traits in Coastal and Piedmont Loblolly Pine

### Abstract

Successful breeding strategies must directly address the risk of accruing inbreeding in breeding populations. Therefore, quantifying the magnitude of inbreeding depression that can occur in economically-important traits is necessary. It is critical to determine the degree of inbreeding depression that can be expected at lower levels of inbreeding as well as in selfs.

Ten Coastal and ten Piedmont selections were sourced from the North Carolina State University Cooperative Tree Improvement Program. Each selection was bred to other related and unrelated selections to achieve four levels of inbreeding coefficient (F); outcrossed (F = 0), half-sib (F=0.125), full-sib (F=0.25), and self (F=0.5). A split-plot design was installed at two Coastal locations and four Piedmont locations, each with six replications. Four inbreeding levels were assigned to main plots and ten lines were planted in six-tree row plots within each main plot. Growth and quality traits were measured in progeny of the selections from each of the four inbreeding levels.

At the F = 0.125, 0.25, and 0.5 inbreeding levels, mean height was shorter than outcrosses by 5.5%, 12.7, and 20.6% in the Coastal provenance and 3.7%, 5.9%, and 17.8% in the Piedmont provenance. Volume at the F = 0.125, 0.25, and 0.5 inbreeding levels was reduced 12.4%, 26%, and 33.2% in the Coastal provenance and 9%, 10.9%, and 32.7% in the Piedmont provenance. Inbreeding did not significantly affect fusiform rust incidence (caused by the fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme*) or stem straightness in either provenance. The effect of inbreeding varies for different genetic backgrounds. Some lines showed greater tolerance to inbreeding but most had decreased growth. One Piedmont line showed a constant increase in metric growth traits with increased inbreeding.

Inbreeding poses a serious risk to growth traits in loblolly pine, but the response to increased inbreeding depends on genetic background. The presence of this variability implies that a breeding population management scheme to controlling inbreeding is not as appropriate as a more diversified plan that could account for differing genetic backgrounds.

## Introduction

The southeastern United States is an important timber-producing region, responsible for a majority of the country's wood products. Timber market models suggest that United States timber production, from 1995 – 2040 will increase by one third, with virtually all of the increased production coming from the South ([srs.fs.usda.gov/sustain/](http://srs.fs.usda.gov/sustain/)). Of the approximately 1.2 billion pine seedlings planted annually, 80% are genetically improved loblolly pine (*Pinus taeda* L.) from forest-tree breeding programs (McKeand et al. 2003). Use of this genetically improved stock allows for greater productivity and improved timber quality from plantations.

Nearly all forest products companies and state forest services in the South have active forest tree breeding programs. Most loblolly pine breeding programs have progressed into advanced generations of selection and breeding, and genetic gains continue to increase (McKeand et al. 2003, McKeand and Bridgwater 1998). With these gains come an increase in the number of related individuals in the breeding population and the potential increase in the number of related matings. Matings between related individuals are more likely to occur in smaller populations than in larger populations (Falconer and Mackay 1996). Hence, there is an increased probability of inbreeding in future generations of breeding.

Conifer tree improvement programs have historically been managed with large population sizes with the goal of avoiding inbreeding (Williams and Savolainen 1996). Inbreeding is not a concern in the first generation of conifer tree improvement programs, because trees selected from the wild to form the breeding population are typically selected from diverse stands and are assumed unrelated (Wu et al. 1998).

In addition to their larger mainline populations, many conifer tree improvement programs have commissioned “elite” breeding populations (Williams and Hamrick 1996). Focusing breeding among the best selections from the breeding population is an attempt to yield

increased gain in shorter spans of time and emphasize selection on more specific product goals (McKeand and Bridgwater 1998). The greatly restricted size of elite populations allows the possibility for inbreeding to occur more rapidly.

Inbreeding is the mating of individuals that are related by ancestry. Inbred individuals can have two genes that are identical by descent (IBD) at a locus found in the ancestor in a previous generation (Falconer and Mackay 1996). The coefficient of inbreeding,  $F$ , is the probability that the two alleles at any loci in an individual are identical by descent (Falconer and Mackay 1996).

Inbreeding depression is an observable effect of inbreeding. It is the reduction in mean phenotypic values of traits related to fitness, such as reproductive capacity, vigor, and physiological efficiency (Falconer and Mackay 1996, Wright 1922, Charlesworth and Willis 2009). Traits not closely connected with fitness often show no change due to increased inbreeding (Falconer and Mackay 1996).

Addressing the issue of inbreeding must become part of the breeding population management strategy (Wu et al. 1998). Further research into the basic effects on growth and reproduction traits in conifers is critical in planning successful future generations of tree improvement. Reliable estimates of inbreeding depression will give a more firm understanding of the genetic architecture of inbreeding depression and the mating history of the species. Examination of less severe levels of inbreeding is as important as comparing selfs with outcrosses, since it is the less severe instances that will likely be encountered first in breeding populations (Sniezko 1984, Wu et al. 1998, Williams and Savolainen 1996).

Progeny of related matings in conifers generally exhibit inbreeding depression for growth traits. Selfing Norway spruce (*Picea abies* (L.) Karst) in 3 diallel crosses resulted in inbreeding depression of height at ages 5, 7, and 10 of 25.5%, 30.5%, and 33%, respectively (Skrøppa 1996). Slash pine (*Pinus elliottii* Engelm.) exhibited an inbreeding depression in

tree and plot volume ranging between 6% and 19% per each 0.1 increase in F (Matheson et al. 1995). Eleven-year-old maritime pine (*Pinus pinaster* Aiton) exhibits inbreeding depression, relative to outcrosses, in progeny of half-sibs, full-sibs, S1's (one generation of selfing) and S2's (two generations of selfing) of 11%, 20%, 45%, and 63% in volume, respectively (Durel et al. 1996).

Trends in the effect of inbreeding on stem straightness and disease incidence in conifers is much more variable. Progeny of selfed radiata pine were less resistant than outcrosses to two types of needle diseases (Wilcox 1983). Slash pine selfs had lower occurrences (18%) of infection of fusiform rust than wind-pollinate individuals (34%) (Snyder 1972). Half-sib and full-sib-mated slash pines showed no difference from outcrosses in incidence of fusiform rust (caused by the fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme*), but families were not identical in their responses to increased inbreeding (Matheson et al. 1995). Selfed progeny of 7-year-old radiata pine showed inbreeding depression of 12% for straightness (Wilcox 1983). Relative to maritime pine outcrosses, progeny of half-sibs, full-sibs, S1's, and S2's, respectively, were 13%, 18%, 20%, and 23% straighter (Durel et al. 1996). Slash pine at 11-12 years old showed no change in straightness or forking with breeding of half-sibs and full-sibs (Matheson et al. 1995).

This study was designed to address two objectives.

- 1) Assess the response of loblolly pine to various degrees of related mating on height and volume, fusiform rust incidence, and stem straightness.
- 2) Examine genetic background and inbreeding levels interactions.

The nature of variability in response to inbreeding depression among genetic entries or lines is important to understand. If all lines respond similarly to the various levels of inbreeding, general prescriptions can be implemented across the breeding population. However, if

significant variation in line responses exist, general prescriptions of managing inbreeding will not be as appropriate.

## Methods and Materials

### *Genetic Material*

Effects of inbreeding were quantified for 10 Piedmont and 10 Coastal loblolly pine selections sourced from the North Carolina State University Cooperative Tree Improvement Program. Matings for these 10 selections generated different genetic groups or lines as follows: each selection was mated with two unrelated selections, two half-sibs, two full-sibs, and was self-fertilized (Figure 2.1).

Mating unrelated selections is referred to as outcrossing, and  $F = 0$ . Half-sibs are selections with one common parent, and when mated, produce progeny with  $F = 0.125$ . Full-sibs are selections with both parents in common, and when mated, produce progeny with  $F = 0.25$ . Selfing or self-fertilization generates progeny with  $F = 0.5$ .

Upon completion of breeding, seeds were collected and stratified. Seeds were sowed into 164 ml containers in a greenhouse in late 1987 and early 1988. The containerized seedlings were hand planted at the test sites through April and May 1988.

### *Experiment Design*

In each provenance, five test sites were established. Lines were planted at test sites according to their provenance of origin. One Piedmont and three Coastal Plain test sites were abandoned due to poor survival of experiment trees primarily as a result of drought conditions and competition from weeds.

The surviving Coastal Plain test sites were established by Weyerhaeuser Co. in Beaufort County, North Carolina and Georgia Pacific, now Plum Creek Timber Co., in Robeson County, North Carolina. Surviving Piedmont test sites were established by Bowater Land Company, now Abitibi Bowater Inc., in Whitfield County, Georgia, Catawba Timber Co., now Abitibi Bowater Inc., in Chesterfield County, South Carolina, the South Carolina Forestry Commission in Sumter County, South Carolina, and Federal Paper Board Co., Inc., now International Paper Co., in Chatham County, North Carolina.

Prior to planting, sites were mechanically and chemically prepared to control competing vegetation. Fertilizer was applied to ensure proper growth of experiment trees. Insects were controlled with normal rates of appropriate pesticides.

At each test site, a split-plot design was installed with six replications. In each replication, there were 4 main plots, each representing the four inbreeding levels: outcross, half-sib, full-sib, self. Within each of the main plots, the ten lines were planted in six tree row plots. The six-tree row plots were comprised of three trees from each of two crosses of Outcrosses, Half-Sibs, and Full-Sibs, respectively. In this experiment design, the four inbreeding levels are the main plot factor and lines are the sub-plot factor. Replications are nested within test sites.

### *Traits Measured*

Test sites were measured at age 9, except for the Weyerhaeuser test site, which was measured at age 10. Total tree height was recorded using a clinometer. Diameter at breast height (DBH) was recorded at 1.4 meters from the ground using a diameter tape.

Survival of each experiment tree was assessed as a binary trait; with 1 indicating survival and 0 indicating a dead tree. Incidence of fusiform rust was recorded; 1 indicating infection with rust and 0 indicating no rust.

Stem straightness was recorded on the binary scale. Trees above average for straightness were given a 1, and trees below average for straightness were given a 0. “Average” straightness was relative only to trees at a given test site.

### *Statistical Analysis*

Inbreeding coefficient “F” was assigned to the four mating types. The F values are as follows: unrelated = 0, half-sib = 0.125, full-sib = 0.25, self = 0.5. Filler trees were deleted from the data set. Outside-bark total tree volume (in cubic decimeters) was calculated using Sherrill et al. (2011).

One line in the Coastal Plain and two lines in the Piedmont were removed from the data set prior to analysis, due to poor survival.

Survival was converted to a scale of 0 for dead trees and 100 for live trees; to obtain percent survival of experiment trees in provenances and test sites.

Analysis of Coastal Plain and Piedmont lines was conducted separately. The MEANS procedure of SAS software was used to produce summary statistics for each trait (SAS Institute Inc. 2008). Summary statistics for the traits observed were produced for each provenance and each test site. Trait summary statistics were computed for each of the four inbreeding levels, across all test sites.

A linear mixed model (equation 1) was used to analyze height and volume - with assumptions that errors are independently and identically distributed with mean of zero. The MIXED procedure in the SAS system was used (SAS Institute Inc. 2008). Test site and inbreeding level were treated as fixed effects. Replication and line were considered random effects.

$$[1] \quad Y_{ijklm} = \mu + T_i + R(T)_j + I_k + (T * I)_{ik} + (I * R(T))_{kj} + \\ L_l + (L * T)_{li} + (L * R(T))_{lj} + (L * I)_{lk} + \varepsilon_{ijklm}$$

where,

$Y_{ijklm}$  = response variable of the m-th tree of the l-th line at the k-th inbreeding level in j-th replication at the i-th test site

$\mu$  = overall mean

$T_i$  = i-th test site

$R(T)_j$  = j-th replication effect with expectations NID  $\sim (0, \sigma^2_r)$  (j=6)

$I_k$  = k-th inbreeding level effect (k=4)

$(T * I)_{ik}$  = interaction of i-th test site with k-th inbreeding level

$(I * R(T))_{kj}$  = interaction of k-th inbreeding level with j-th replication with expectations NID  $\sim (0, \sigma^2_{ir})$

$L_l$  = l-th line (Coastal l=9 Piedmont l=8) with expectations NID  $\sim (0, \sigma^2_l)$

$(L * T)_{li}$  = interaction of l-th line with i-th test site with expectations NID  $\sim (0, \sigma^2_{li})$

$(L * R(T))_{lj}$  = interaction of l-th line with j-th replication with expectations NID  $\sim (0, \sigma^2_{lr})$

$(L * I)_{lk}$  = interaction of l-th line with k-th inbreeding level with expectations NID  $\sim (0, \sigma^2_{li})$

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

Fusiform rust incidence and straightness were analyzed as binary traits. The probability of these traits were modeled by fitting a generalized linear mixed model using the GLIMMIX procedure (SAS Institute Inc. 2008). The generalized linear mixed model and expectations, as shown by Isik (2011) are as follows:

$$[2] \quad E[\mathbf{y}|\mathbf{u}] = g^{-1}(\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u})$$

where,

$\mathbf{y} = (n \times 1)$  response vector

$\mathbf{X} = (n \times p)$  design matrix of rank  $k$  for the  $(p \times 1)$  fixed effects  $\boldsymbol{\beta}$

$\mathbf{Z} = (n \times q)$  design matrix for the  $(q \times 1)$  random effects  $\mathbf{u}$

Random effects,  $\mathbf{u}$ , are assumed to be normally distributed with mean equal to 0 and variance matrix  $\mathbf{G}$ . The link function,  $g$ , used to model the binomially distributed response is as follows:

$$[3] \quad g = \text{Logit} = \ln(\mu_i(1 - \mu_i))$$

Therefore, the inverse link function was used to obtain the probability of survival as follows:

$$[4] \quad g^{-1} = e^n = 1/(1 + e^n)$$

For traits with a significant F-statistic for inbreeding level, the GLM procedure and Tukey's means separation procedure were used to show the significant differences among the four levels of inbreeding (SAS 2008).

Significance of the fixed effect, Inbreeding, was assessed using F-statistics and p-values output from the MIXED and GLIMMIX procedures. Significance of the random terms Line and Inbreeding\*Line was assessed by the ratio of the estimate of the term divided by the standard error. Official tests of significance were not run, due to the small sample size of genetic lines.

Percent inbreeding depression was calculated for each trait. It is defined as percent reduction in mean of the trait, relative to the mean of progeny from unrelated crosses.

## Results

Height and volume of experiment trees was comparable between the provenances (Table 2.1). Coastal trees averaged 9.8 meters tall ( $n = 2219$ ), and Piedmont trees averaged 9.6 meters tall ( $n = 3808$ ). Volume of Coastal trees averaged  $83.5 \text{ dm}^3$ , and Piedmont trees' average volume was  $92.4 \text{ dm}^3$ . Overall survival of Coastal and Piedmont trees was 92.86% and 94.38%, respectively.

Among test sites of the same provenance, mean height and volume were similar (Table 2.2). Probability of fusiform rust and straightness was more variable among test sites ranging from 9% to 50%. Survival was greater than 90% at all test sites that were kept for analysis (Table 2.2). This incidence level did not allow for appropriate analysis of this binary trait.

### *The Effect of Inbreeding on Growth*

Inbreeding had a significant effect on height for both Coastal ( $p = 0.0034$ ) and Piedmont ( $p = 0.003$ ) provenances (Table 2.3). Progeny of Coastal half-sibs, full-sibs, and selfs were 5.5%, 12.7%, and 20.6%, respectively, shorter than progeny of Coastal outcrosses. All inbreeding

levels were significantly different from one another. Progeny of Piedmont half-sibs, full-sibs, and selfs were 3.7%, 5.9%, and 17.8%, respectively, shorter than progeny of Piedmont outcrosses.

Individual-tree volume was also significantly affected by inbreeding in Coastal ( $p = 0.0455$ ) and Piedmont ( $p = 0.0098$ ) provenances (Table 2.3). Relative to progeny of Coastal outcrosses, volume in progeny of half-sibs, full-sibs, and selfs, respectively, was reduced by 12.4%, 26%, and 33.2%. Mean volume in progeny of outcrosses and progeny of half-sibs differed significantly from all other levels of inbreeding. Mean volume did not differ significantly among progeny of full-sibs and progeny of selfs (Table 2.5). Progeny of Piedmont half-sibs, full-sibs, and selfs, respectively were 9%, 10.9%, and 32.7% smaller than progeny of Piedmont outcrosses. Volume in progeny of Piedmont selfs was significantly different from the other levels of inbreeding (Table 2.5).

In Coastal and Piedmont provenances, the estimate/standard error ratio suggests that variation among lines for height or volume was not statistically significant (Table 2.4, Figures 2.2, 2.3, 2.4, and 2.5). The approximate t values for line variance component and its ratio were less than 1.

#### *Interaction of Inbreeding Level and Line for Growth*

The Line by Inbreeding interaction for height was significant for the Coastal and Piedmont provenances (Table 2.4). Lines in the Coastal and Piedmont provenances responded differently to increased inbreeding (Figures 2.2 and 2.3). Responses of height to increased inbreeding in each line are plotted independently in Appendix 1.

In the Coastal provenance, three lines (TIP2280651, TIP2357867, and TIP2472543) exhibited a constant decrease in mean height as inbreeding increased. Mean height in three other lines (TIP230370, TIP2312374, and TIP2714551) decreased to the  $F = 0.25$  level, but

recovered somewhat at the  $F = 0.5$  level. Upon inbreeding in other lines, mean height of inbred progeny was less than mean height in progeny of outcrosses, with some rank changes among inbreeding levels.

Mean height in four Piedmont lines (TIP2132366, TIP2333057, TIP2579098, and TIP279417) decreased constantly with increased inbreeding. Line TIP2140401 had the greatest height at  $F = 0$  inbreeding level, and reduced heights in the inbred progeny. Line TIP2836017 exhibited a constant increase in mean height as inbreeding increased. Upon inbreeding in other lines, mean height generally decreased with inbreeding, with some rank changes among inbreeding levels.

Interaction of Line and Inbreeding was significant for volume for the Coastal and Piedmont provenances (Table 2.4). Lines of Coastal and Piedmont provenances responded differently to increased inbreeding. Figures 2.4 and 2.5 show interactions of line and inbreeding levels for the Coastal and Piedmont provenances. Volume responses to increased inbreeding in each line are plotted independently in Figures Appendix 1.

In three Coastal lines (TIP2280651, TIP2472543, and TIP292525) mean volume decreased constantly as inbreeding increased. In three other lines (TIP230370, TIP2357867, and TIP2714551), mean volume decreased to the  $F = 0.25$  level with some recovery at the  $F = 0.5$  level. With increased inbreeding, mean volume in other lines showed more variable patterns.

Mean volume in four Piedmont lines (TIP2132366, TIP2333057, TIP2579098, and TIP279417) decreased constantly with increased inbreeding. Mean volume in line TIP2836017 increased constantly with inbreeding. Other Piedmont lines showed variable patterns in mean volume with increased inbreeding.

### *Probability of Fusiform Rust and Straightness*

Inbreeding did not have a significant effect on probability of fusiform rust incidence (Table 2.3) in either the Coastal ( $p = 0.0542$ ) or the Piedmont ( $p = 0.3157$ ) provenances. In the Coastal provenance, probability of fusiform rust incidence ranged from 0.23 to 0.35 for different inbreeding levels (Table 2.6). In the Piedmont provenance, probability of fusiform rust ranged from 0.18 to 0.23 (Table 2.6).

Straightness was not significantly affected by inbreeding in either the Coastal ( $p = 0.6193$ ) or the Piedmont ( $p = 0.9894$ ) provenance (Table 2.3). In the Coastal provenance, probability of straightness was above average and ranged from 0.60 (outcross) to 0.66 (self) (Table 2.6). In the Piedmont provenance, probability of straightness ranged from 0.45 (outcross) to 0.47 (self) (Table 2.6).

Line main effect was not significant on probability of fusiform rust in the Coastal or Piedmont provenances (Table 2.4 and Figures 2.6 and 2.7). Similarly, line main effect was not significant for straightness in the Coastal or Piedmont provenances (Table 2.4 and Figures 2.8 and 2.9).

### *Interaction of Inbreeding Level and Line for Fusiform Rust and Stem Straightness*

The interaction of Inbreeding Level and Line was significant for fusiform rust incidence in the Coastal provenance, but marginally significant in the Piedmont provenance (Table 2.4). Increased inbreeding affected lines differently. Figures 2.6 and 2.7 are interaction plots of the Coastal and Piedmont lines' mean probability of fusiform rust, across the four inbreeding levels. The responses of each line to increased inbreeding are displayed independently in Appendix 1.

In five Coastal lines (TIP230370, TIP2419552, TIP2472543, TIP2714551, and TIP292525) probability of fusiform rust was decreased in inbred progeny, relative to progeny of outcrosses. Other lines exhibited variable patterns in probability of fusiform rust upon increased inbreeding.

Two Piedmont lines (TIP2132366 and TIP2140401) exhibited a steady decrease in probability of fusiform rust with increased inbreeding. Probability of rust in three lines (TIP2169531, TIP2469791, and TIP2579098) was equal at  $F = 0$  and  $F = 0.125$  and decreased in  $F = 0.25$  and  $F = 0.5$  levels. Other Piedmont lines displayed variable patterns in probability of fusiform rust with increased inbreeding.

Interaction of Inbreeding Level and Line was significant in the probability of straightness being above population mean for the Coastal provenance, but marginally significant in the Piedmont provenance (Table 2.4). Increased inbreeding affected lines differently. Figures 2.8 and 2.9 are interaction plots of the Coastal and Piedmont lines' mean probability of straightness, across the four inbreeding levels. The responses of each line to increased inbreeding are displayed independently in Appendix 1.

In three Coastal lines (TIP2227582, TIP2714551, and TIP292525) probability of straightness was consistently lower in inbred progeny, relative to progeny of outcrosses. Two other lines (TIP2357867 and TIP2472543), probability of straightness was nearly equal at all four levels of inbreeding. In all other Coastal lines change in probability of straightness across the four inbreeding levels was extremely variable.

In the Piedmont, although the interaction of Inbreeding Level\*Line was marginally significant for stem straightness, lines showed some differences in response to increased inbreeding. Mean probability of straightness increased in lines TIP2140401 and TIP279417, decreased in TIP2579098, and TIP2469791 showed no change. Probability of straightness in

all other Piedmont lines showed more variable trends in straightness with increased inbreeding.

### Discussion

The significant inbreeding depression in height and volume, in both Coastal and Piedmont provenances, is a product of the distinct selective advantage these traits have on fitness. Large trees have a better chance at reproducing successfully than small, slow-growing trees. This selective advantage will increase the proportion of favorable, dominant alleles and decrease the proportion of detrimental, recessive alleles. Inbreeding depression arises through increased proportions of these deleterious alleles (Charlesworth and Charlesworth 1987, Wright 1922, Charlesworth and Willis 2009). With increased homozygosity upon inbreeding, the change in the mean of height and volume will be reduced, in the direction of the recessive allele (Falconer and Mackay 1996).

Inbreeding did not affect the probability of fusiform rust or straightness probably because these traits may not impact fitness nearly as much as growth traits. Presence of a branch rust gall or a crooked stem is not a serious impediment to loblolly pine reproduction success. Only extremely poor phenotypes would be selected against in the wild. A high degree of variation exists in loblolly pine for these traits (Zobel and Talbert 1984, Schultz 1997). Therefore, increased homozygosity upon inbreeding will not cause a directional change in mean. Rare, recessive alleles increasing in proportion upon inbreeding may increase, decrease, or not change mean phenotypic value of these traits.

The magnitude of inbreeding depression for height and volume shown in this experiment are commensurate with the breeding history of loblolly pine. Loblolly pine inhabits a native range extending from southern New Jersey south to central Florida and west to eastern Texas

(Baker and Langdon 1990). Historically large populations facilitate outcrossing and will result in greater inbreeding depression of inbred progeny (Lande and Schemske 1985).

The population and mating history of large ranges is contrasted with species and/or populations with a reduction in size. Populations with a history of bottlenecks or pollinator failure are expected to exhibit lower inbreeding depression and favor more self-fertilization (Lande and Schemske 1985). The low degree of inbreeding depression in radiata pine (*Pinus radiata* D. Don) is interpreted as a product of deleterious recessive alleles being purged from radiata pine populations; induced by its evolutionary mating history in small, remote populations (Wu et al. 1998, Vogl et al. 2002).

Genetic lines did not respond equally to increased inbreeding. The significant Inbreeding Level by Line interaction is consistent with previous research. Wu et al. (1998) showed a significant interaction between inbreeding level and radiata pine genotypes for growth. Wilcox (1983) reported a significant interaction between inbreeding level and founder clones in radiata pine for growth. Matheson et al. (1995) explained that slash pine families responded differently to inbreeding for growth traits.

Degree of inbreeding depression varies between species, among families within a species, and among individuals within a family. This pattern of variation could be due to variation in number of lethal and damaging alleles (Skrøppa 1996, Bramlett and Pepper 1974). Variation in environmental factors also affects the expression of inbreeding depression (Kärkkäinen 1996, Byers and Waller 1999, Sniezko and Zobel 1988, Bower and Aitken 2007, Husband and Schemske 1996).

Lines responded differently to increased inbreeding for growth. The Piedmont line TIP2836017 was the only line, in either provenance, that showed an increase in height and volume mean as inbreeding increased. With increased inbreeding all other lines showed decrease in growth traits in a conspicuous pattern or had a moderate degree of variability. As

suggested by Wu et al. (1998), lines that show no inbreeding depression in growth traits may be fixed for a number of favorable alleles. These types of selections could be valuable in future generations of breeding.

Degree of inbreeding depression has been shown to be pedigree-specific in radiata pine, with some clones exhibiting superior growth and no change in mean with increased inbreeding (Kumar 2004; Wu et al. 1998; Wilcox 1983). This may suggest that these clones may be fixed for a large number of beneficial alleles and devoid of deleterious recessive alleles. These factors might make the selections prized for advanced generation breeding, especially breeding plans involving inbreeding (Wu et al. 1998; Williams and Savolainen 1996).

Loblolly pine forests in the Piedmont region of the southeastern United States arose after abandonment of agricultural operations. Reforestation of old fields came from small numbers of parent trees along fence rows, near houses and roads, or in areas too wet to farm. This small founder population may have resulted in relatedness among the majority of individuals in the new forests (Schultz 1997, Zobel and Talbert 1984). Mating among related individuals in subsequent generations and dispersal of inbred progeny could have promoted selection against and purging of deleterious alleles in these populations. Purging of deleterious alleles can account for decreased inbreeding depression in species capable of self-fertilization. This suggests that inbreeding depression should be lower in populations with a history of some self fertilization and greater in more outcrossing populations (Charlesworth and Charlesworth 1987, Lande and Schemske 1985, Wang et al. 1999, Husband and Schemske 1996).

## Conclusion

Inbreeding poses a serious detriment to growth traits in loblolly pine. Inbreeding depression in height ranged from 20.6% to 17.8% in the progeny of Coastal selfs and Piedmont selfs, respectively. Reduction in loblolly pine volume of was more severe than height. Coastal and Piedmont selfs showed inbreeding depression of 33.2% and 32.7%, respectively.

Lines varied significantly in their susceptibility to change in mean for growth traits with increased inbreeding. Several lines, in both provenances, showed a consistent decrease in growth with increased inbreeding. Other lines had variable patterns, except one line in the Piedmont that showed increased height and volume growth with inbreeding.

Inbreeding did not affect fusiform rust incidence and straightness. These traits might not be related to fitness of loblolly pine, and there is great genetic diversity for these traits.

This research sets an important benchmark for future breeding plans of loblolly pine in the southeastern United States. The comparison of multiple levels of inbreeding, across two major provenances, has asserted the degree of inbreeding depression that can be expected in genetically superior selections of loblolly pine. The results of this experiment also assert that there is significant variation in the effect of inbreeding on different selections. The presence of this variability indicates that population-wide inbreeding management strategies are not appropriate. Rather, individualized plans must be implemented to either manage and avoid inbreeding, or explore inbreeding as a breeding tool.

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**Table 2.1** Summary statistics of traits measured and percent survival for each provenance.

<b>Provenance</b>	<b>Statistic</b>	<b>Height (m)</b>	<b>Volume (dm<sup>3</sup>)</b>	<b>Fusiform Rust</b>	<b>Straightness</b>	<b>Survival (%)</b>
Coastal Plain	<b>Mean</b>	9.8	83.5	0.29	0.64	92.9
	<b>Std. Error</b>	0.042	0.947	0.005	0.004	0.516
	<b>N</b>	2219	2219	2219	2154	2494
Piedmont	<b>Mean</b>	9.6	92.4	0.21	0.46	94.4
	<b>Std. Error</b>	0.027	0.72	0.003	0.002	0.357
	<b>N</b>	3808	3808	3808	3808	4149

**Table 2.2** Test-site means and standard errors in parentheses of the traits measured.

<b>Provenance</b>	<b>Test</b>	<b>Height (m)</b>	<b>Volume (dm<sup>3</sup>)</b>	<b>Fusiform Rust</b>	<b>Straightness</b>	<b>Survival (%)</b>
Coastal Plain	<b>G-P</b>	9.2 (0.1)	74.6 (1.3)	0.50 (.006)	0.53 (.005)	91.9
	<b>Weyco.</b>	10.4 (0.1)	92.1 (1.4)	0.09 (.002)	0.74 (.004)	93.9
Piedmont	<b>Catawba</b>	9.7 (0.1)	91.3 (1.5)	0.26 (.004)	0.50 (.004)	93.8
	<b>Federal</b>	9.9 (0.1)	107.7 (1.5)	0.09 (.001)	0.36 (.003)	93.8
	<b>Hiwasse</b>	8.7 (0.1)	76.4 (1.2)	0.09 (.001)	0.48 (.004)	94.1
	<b>SCFC</b>	10.2 (0.1)	95.1 (1.3)	0.40 (.005)	0.50 (.002)	95.9

**Table 2.3** F-tests for inbreeding levels (Inbr.) for Coastal and Piedmont provenances. F-test values with probability of 0.05 or less are considered significant.

<b>Provenance</b>	<b>Effect</b>	<b>Height</b>		<b>Volume</b>		<b>Fusiform Rust</b>		<b>Straightness</b>	
		F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Coastal Plain	<b>Test</b>	75.91	<.001	31.08	<0.001	173.85	<.001	23.36	0.001
	<b>Inbr.</b>	7.13	0.003	3.40	0.046	3.19	0.054	0.61	0.619
	<b>T*I</b>	0.95	0.414	0.65	0.600	2.01	0.101	2.75	0.042
Piedmont	<b>Test</b>	58.49	<.001	29.04	<.001	43.83	<.001	6.89	0.002
	<b>Inbr.</b>	12.17	<.001	5.45	0.001	1.28	0.316	0.04	0.989
	<b>T*I</b>	5.29	<.001	5.78	<.001	1.10	0.356	1.35	0.205

**Table 2.4** Variance estimates (Est.), their standard errors (SE), and ratios of estimate and standard error (Est./SE) for random terms.

<b>Provenance</b>	<b>Effect</b>	<b>Height</b>			<b>Volume</b>		
		Est.	SE	t (Est./SE)	Est.	SE	t (Est./SE)
Coastal Plain	<b>Line</b>	0.11	0.19	0.58	54.01	96.61	0.56
	<b>Inbr.*Line</b>	0.83	0.25	3.28	424.79	131.27	3.24
Piedmont	<b>Line</b>	0.09	0.13	0.69	68.26	105.60	0.65
	<b>Inbr.*Line</b>	0.50	0.16	3.15	426.79	135.15	3.16

<b>Provenance</b>	<b>Effect</b>	<b>Fusiform Rust</b>			<b>Straightness</b>		
		Est.	SE	t (Est./SE)	Est.	SE	t (Est./SE)
Coastal Plain	<b>Line</b>	0.26	0.23	1.09	0.28	0.23	1.25
	<b>Inbr.*Line</b>	0.43	0.16	2.64	0.21	0.09	2.35
Piedmont	<b>Line</b>	0.17	0.13	1.31	0.09	0.07	1.29
	<b>Inbr.*Line</b>	0.11	0.06	1.89	0.05	0.03	1.81

**Table 2.5** Means, 95% confidence intervals, number of observations (N), and percent inbreeding depression, are given at each of the four inbreeding levels for height and volume traits. Inbreeding levels with same letter are not significantly different.

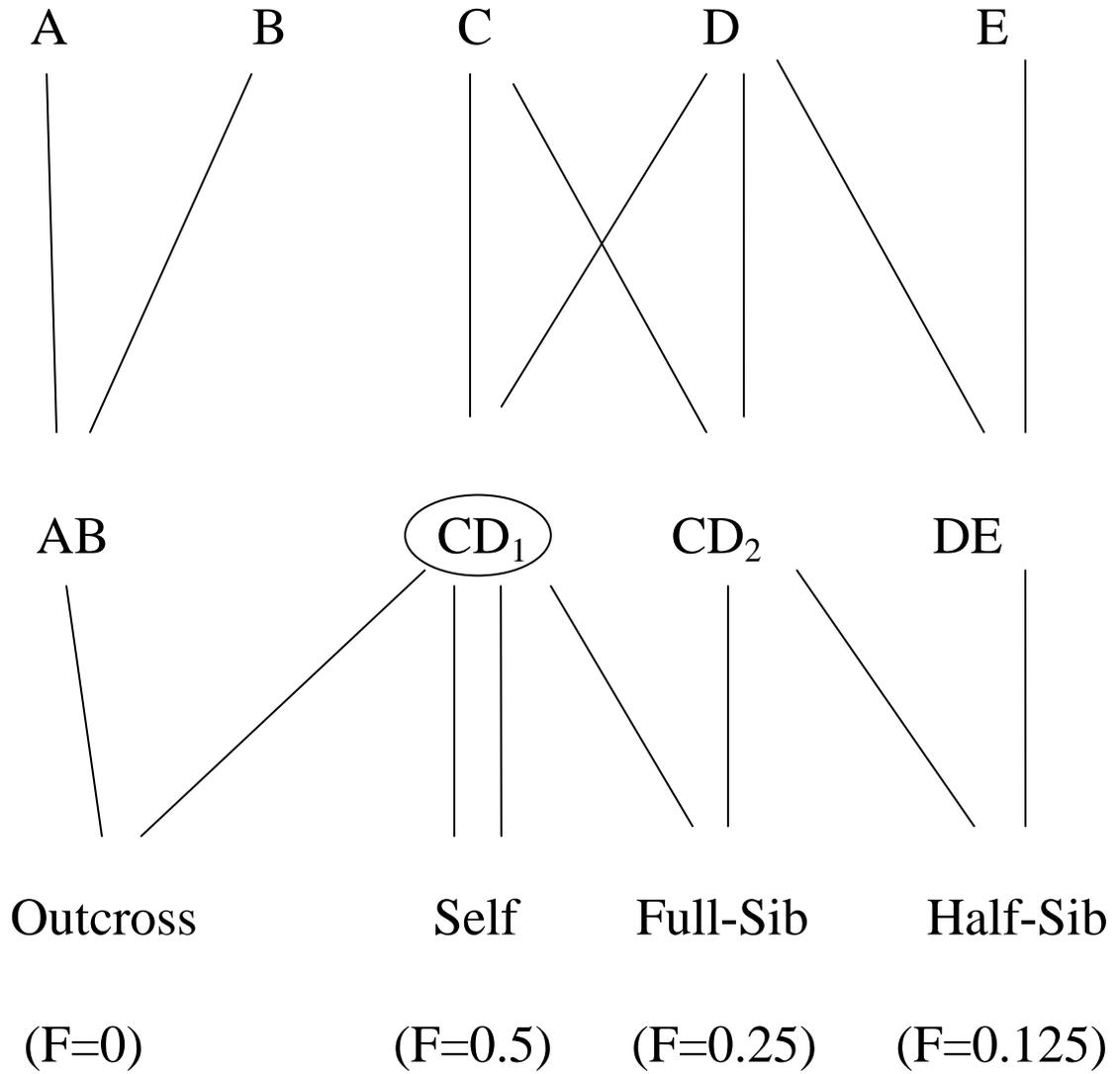
<b>Provenance</b>	<b>Mating Type</b>	<b>Height (m)</b>	<b>95% CI</b>		<b>N</b>	<b>% ID*</b>
Coastal Plain	Outcross (F = 0)	10.8 <b>a</b>	10.7	10.9	585	--
	Half-Sib (F = 0.125)	10.2 <b>b</b>	10.1	10.3	590	5.5
	Full-Sib (F = 0.25)	9.4 <b>c</b>	9.3	9.6	576	12.7
	Self (F = 0.5)	8.6 <b>d</b>	8.3	8.8	468	20.6
Piedmont	Outcross (F = 0)	10.2 <b>a</b>	10.2	10.3	1079	--
	Half-Sib (F = 0.125)	9.8 <b>ab</b>	9.8	10.0	999	3.7
	Full-Sib (F = 0.25)	9.6 <b>b</b>	9.5	9.7	979	5.9
	Self (F = 0.5)	8.4 <b>c</b>	8.3	8.6	751	17.8

<b>Provenance</b>	<b>Mating Type</b>	<b>Volume (dm<sup>3</sup>)</b>	<b>95% CI</b>		<b>N</b>	<b>% ID</b>
Coastal Plain	Outcross (F = 0)	100.6 <b>a</b>	97.3	104.0	585	--
	Half-Sib (F = 0.125)	88.1 <b>b</b>	85.0	91.3	590	12.4
	Full-Sib (F = 0.25)	74.5 <b>c</b>	71.5	77.6	576	26.0
	Self (F = 0.5)	67.2 <b>c</b>	62.3	72.0	468	33.2
Piedmont	Outcross (F = 0)	104.6 <b>a</b>	102.4	106.7	1079	--
	Half-Sib (F = 0.125)	95.2 <b>a</b>	92.8	97.5	999	9.0
	Full-Sib (F = 0.25)	93.2 <b>a</b>	90.3	96.1	979	10.9
	Self (F = 0.5)	70.4 <b>b</b>	66.6	74.1	751	32.7

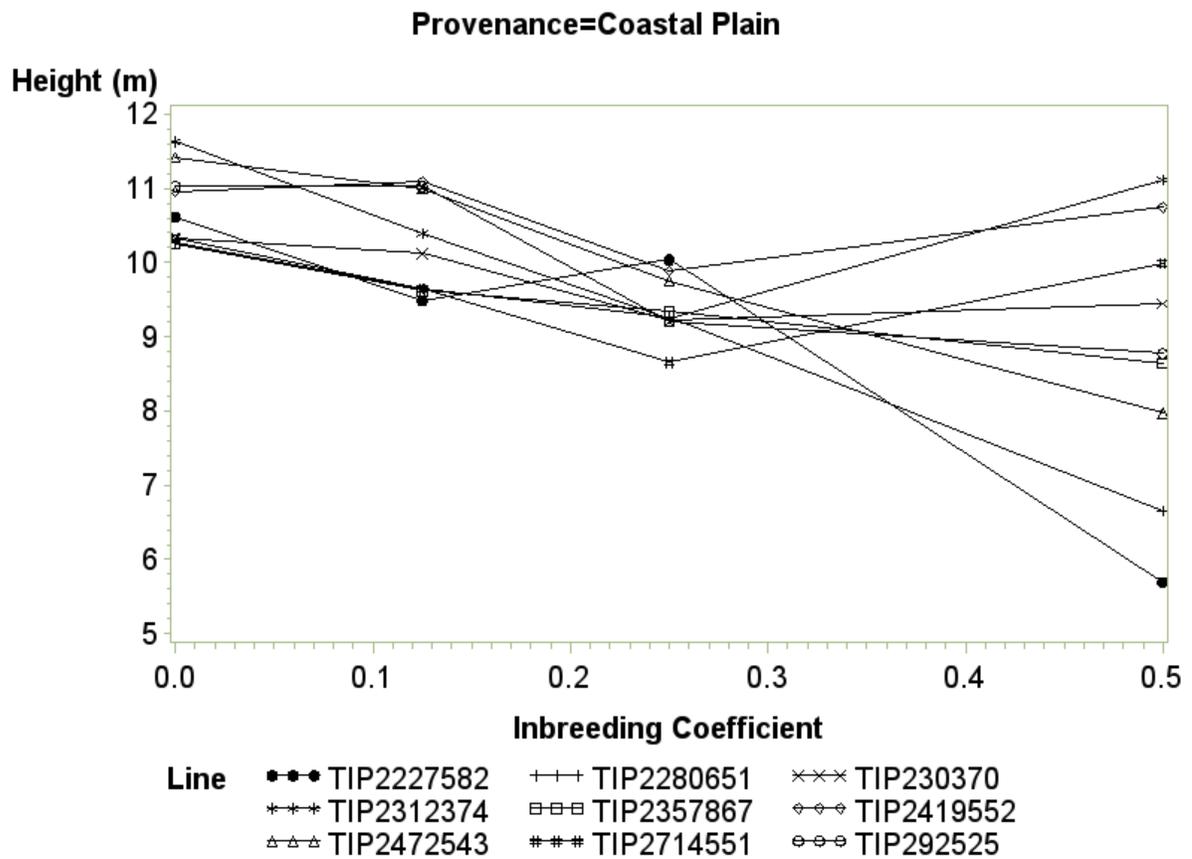
**Table 2.6** Means, 95% confidence intervals, number of observations (N), and percent inbreeding depression, are given at each of the four inbreeding levels for fusiform rust and straightness. Inbreeding levels with same letter are not significantly different.

<b>Provenance</b>	<b>Mating Type</b>	<b>Fusiform Rust</b>	<b>95% CI</b>		<b>N</b>	<b>% ID</b>
Coastal Plain	Outcross (F = 0)	0.35 <b>a</b>	0.33	0.37	585	--
	Half-Sib (F = 0.125)	0.30 <b>a</b>	0.28	0.32	590	13.3
	Full-Sib (F = 0.25)	0.23 <b>a</b>	0.21	0.25	576	34.5
	Self (F = 0.5)	0.28 <b>a</b>	0.26	0.31	468	19.8
Piedmont	Outcross (F = 0)	0.23 <b>a</b>	0.22	0.24	1079	--
	Half-Sib (F = 0.125)	0.23 <b>a</b>	0.22	0.25	999	-2
	Full-Sib (F = 0.25)	0.19 <b>a</b>	0.18	0.20	979	17.6
	Self (F = 0.5)	0.18 <b>a</b>	0.17	0.19	751	21.8

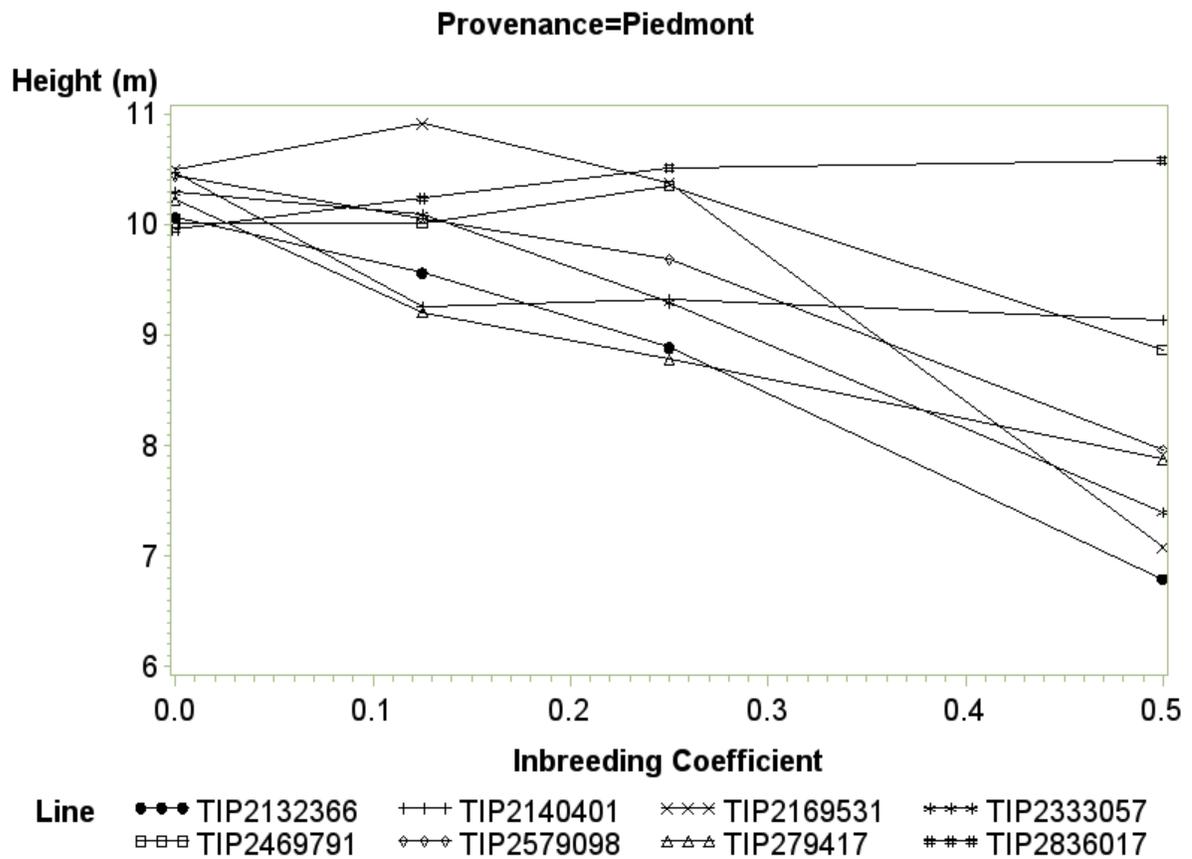
<b>Provenance</b>	<b>Mating Type</b>	<b>Straightness</b>	<b>95% CI</b>		<b>N</b>	<b>% ID</b>
Coastal Plain	Outcross (F = 0)	0.66 <b>a</b>	0.64	0.68	576	--
	Half-Sib (F = 0.125)	0.63 <b>a</b>	0.61	0.64	571	5
	Full-Sib (F = 0.25)	0.65 <b>a</b>	0.63	0.67	559	1.34
	Self (F = 0.5)	0.60 <b>a</b>	0.58	0.61	448	9.7
Piedmont	Outcross (F = 0)	0.46 <b>a</b>	0.45	0.46	1079	--
	Half-Sib (F = 0.125)	0.46 <b>a</b>	0.46	0.47	999	-0.75
	Full-Sib (F = 0.25)	0.45 <b>a</b>	0.45	0.46	979	1.18
	Self (F = 0.5)	0.47 <b>a</b>	0.46	0.48	751	-1.32



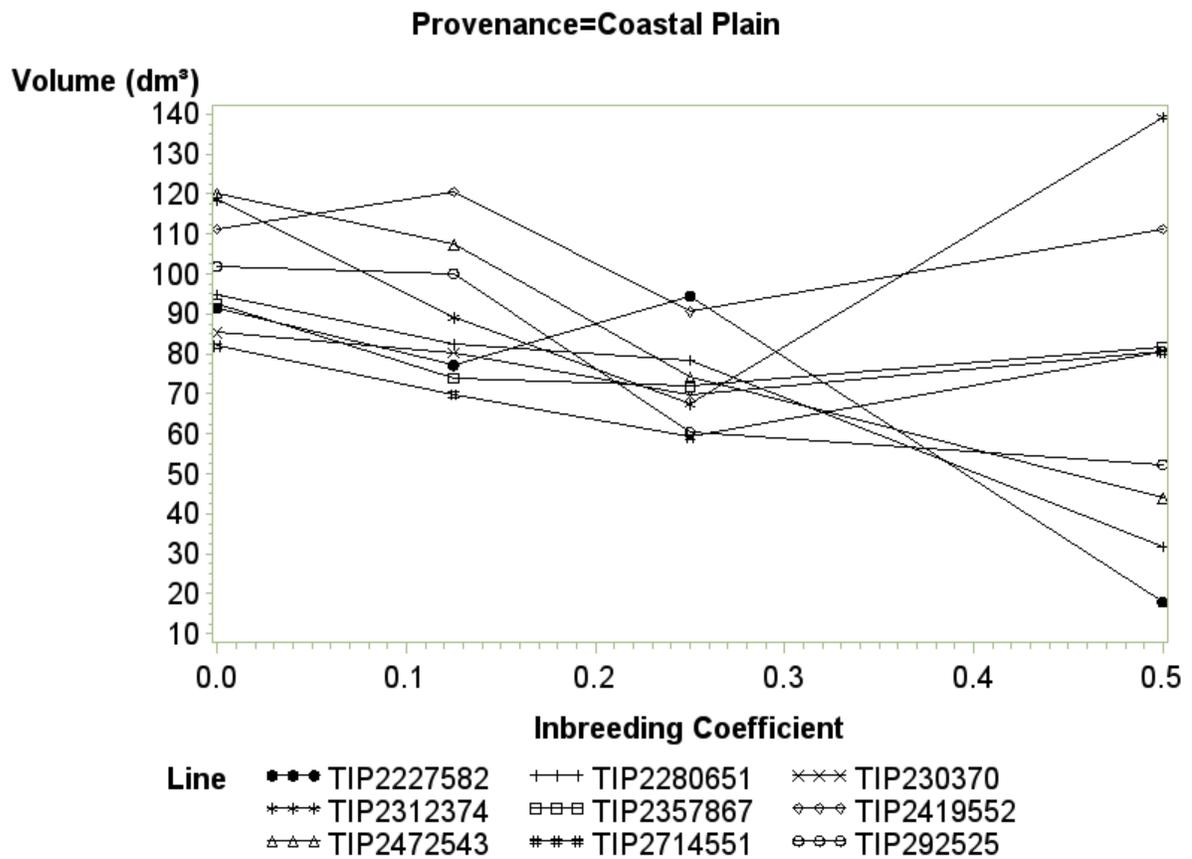
**Figure 2.1** Mating design illustrating the production of the four levels of inbreeding; where A, B, C, D, and E are founder selections. These crosses are for line CD1.



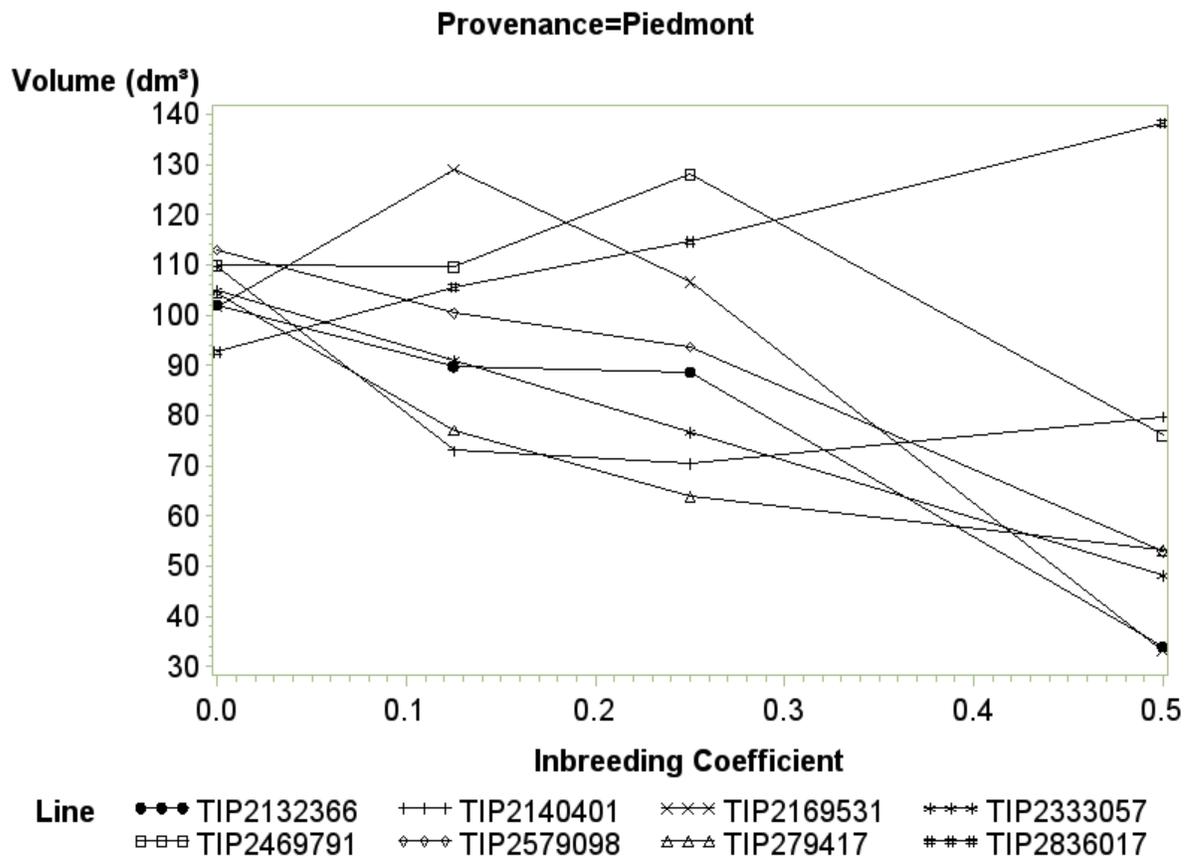
**Figure 2.2** Mean height for the nine Coastal lines for different inbreeding levels. The line by inbreeding interaction was clear from rank changes among lines for different inbreeding levels.



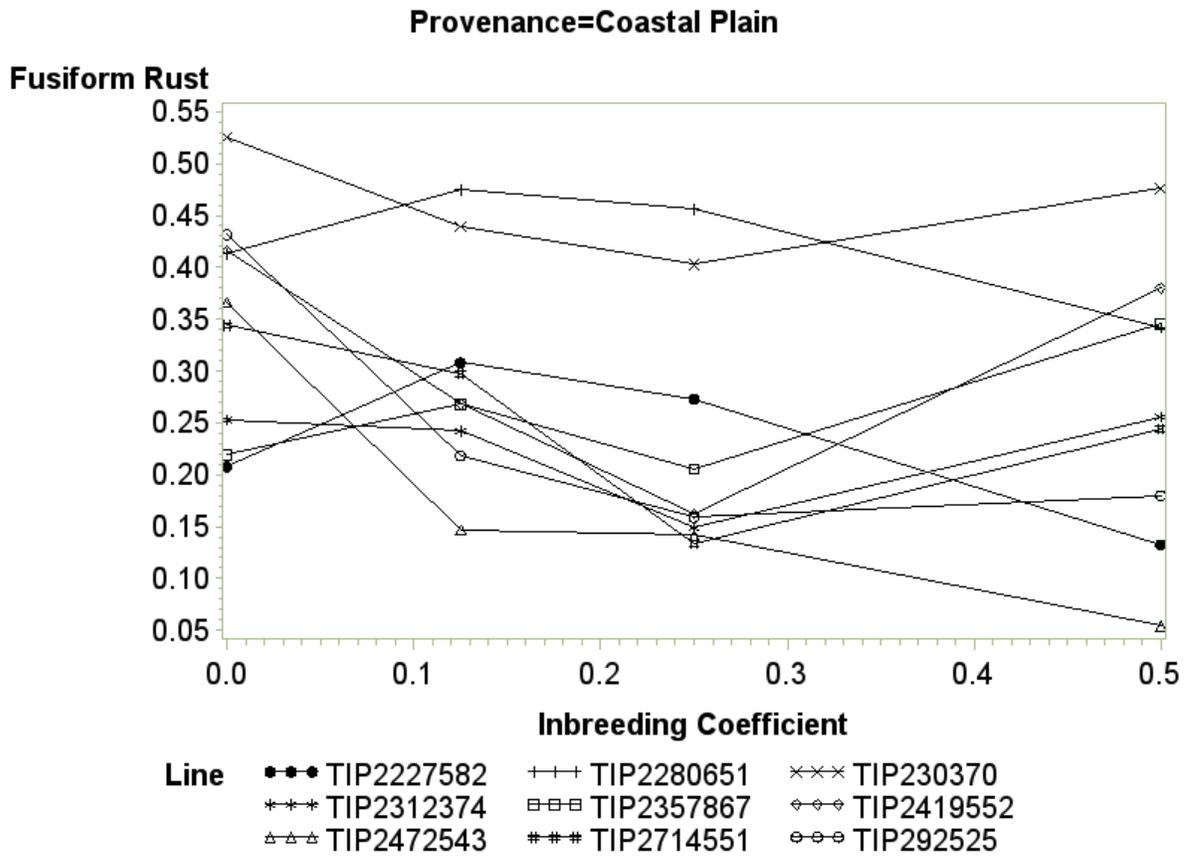
**Figure 2.3** Mean height for the eight Piedmont lines for different inbreeding levels. The line by inbreeding interaction was clear from rank changes among lines for different inbreeding levels.



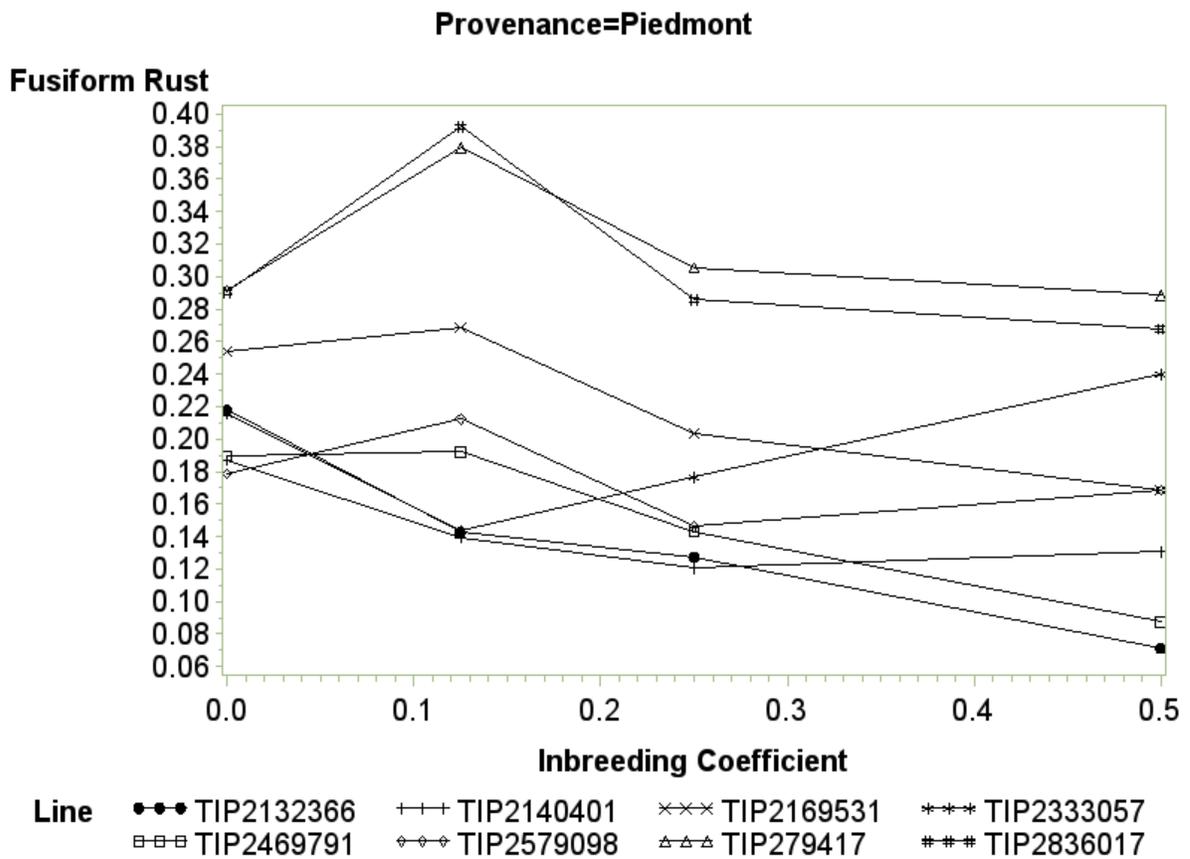
**Figure 2.4** Mean volume for the nine Coastal lines for different inbreeding levels. The interaction of line by inbreeding levels is significant.



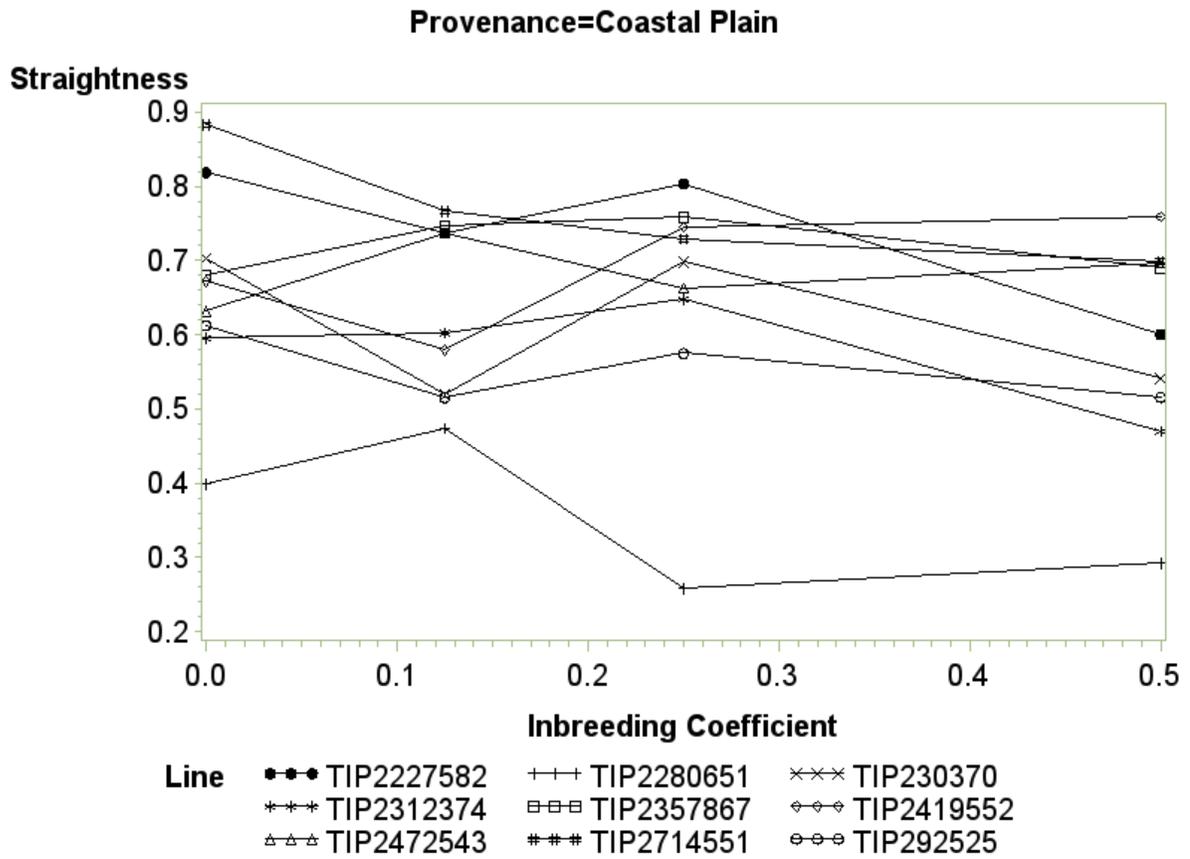
**Figure 2.5** Mean volume for the eight Piedmont lines for different inbreeding levels. The interaction of line by inbreeding levels is significant.



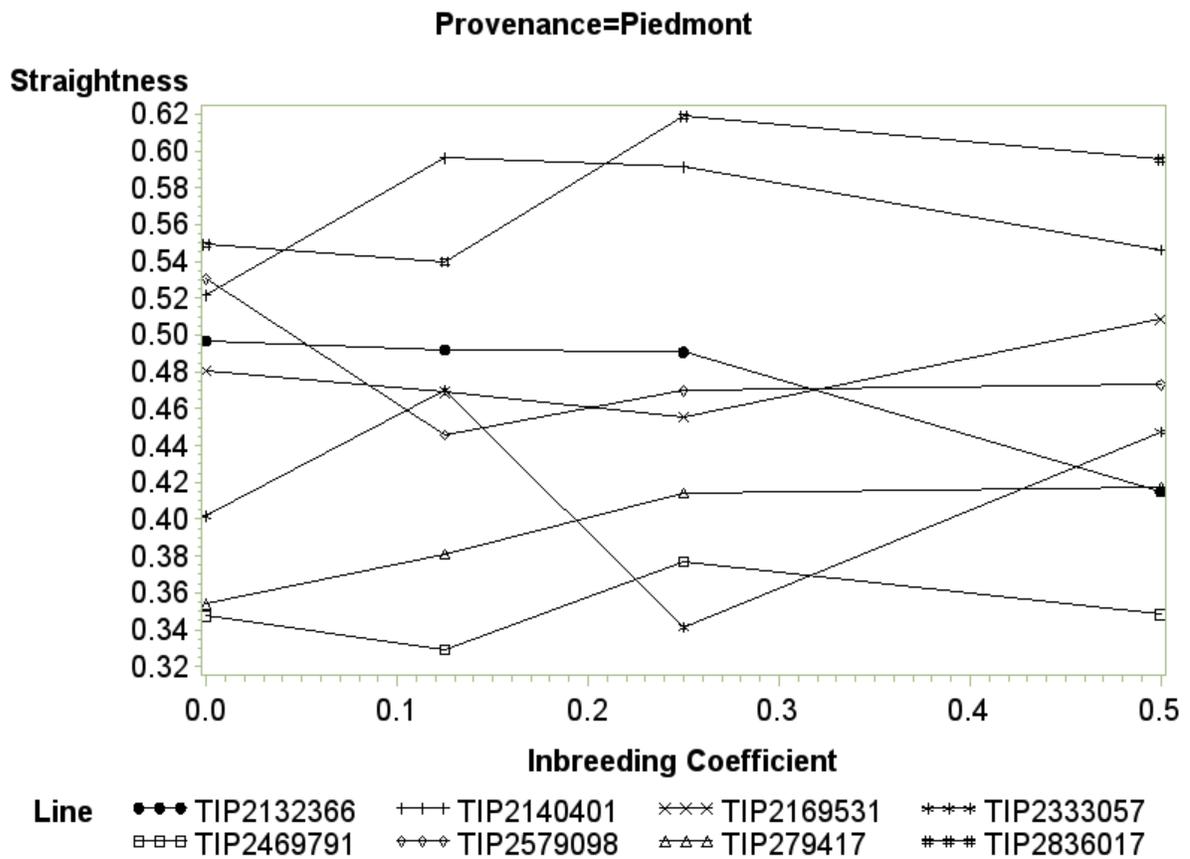
**Figure 2.6** Mean probability of fusiform rust for the nine Coastal lines for different inbreeding levels. Response of lines to inbreeding is dependent on the line as suggested by crossover interactions.



**Figure 2.7** Mean probability of fusiform rust of the eight Piedmont lines for different inbreeding levels. Response of lines to inbreeding is dependent on the line as suggested by crossover interactions.



**Figure 2.8** Mean probability of straightness for the nine Coastal lines at different inbreeding levels.



**Figure 2.9** Mean probability of straightness for the eight Piedmont lines at different inbreeding levels.

### **Chapter 3 : Effect of Inbreeding on Loblolly Pine Growth and Wood Properties**

#### Abstract

Quantifying the magnitude of inbreeding depression in various traits is necessary to understand the risks related matings pose for genetically superior selections. Progeny from 10 Coastal selections from the North Carolina State University Cooperative Tree Improvement program were assessed to study the effect of inbreeding. Each selection was bred to other related and unrelated selections to achieve four levels of inbreeding coefficient (F); outcrossed (F = 0), half-sib (F=0.125), full-sib (F=0.25), and self (F=0.5). A split-plot design was used at one location with six replications. Four inbreeding levels were assigned to main plots and ten lines were planted in six-tree row plots within each main plot. Growth, survival, and wood attributes were measured in progeny of the selections from each of the four inbreeding levels.

Relative to progeny of outcrosses, height showed significant inbreeding depression. Progeny of full-sibs and selfs were 5.44%, and 4.14% shorter than outcrossed progeny. Probability of survival also significantly declined to 0.52 and 0.39 in progeny of full-sibs and progeny of selfs. Diameter at breast height at F = 0.125 and F = 0.25, respectively, was 1.92% and 7.3% smaller than outcrossed progeny, but progeny of selfs had the greatest DBH of all mating types; 2.48% larger than outcrossed progeny. This increase was due to low survival in the selfed plots in the split-plot design.

Wood specific gravity was not significantly affected by inbreeding. Bending strength (measured by acoustic stress-wave velocity) decreased with inbreeding. Stress-wave velocity was 2.75%, 2.50%, and 2.71% less in progeny of half-sibs, full-sibs, and selfs relative to outcrosses.

For all traits measured, there was significant variation in response to inbreeding among the ten lines. Some lines show greater susceptibility to inbreeding than others. The presence of this variability implies that a single approach to managing inbreeding in a breeding population is not optimal. A multifaceted strategy that accounts for or utilizes this variability is more appropriate.

## Introduction

The southeastern United States is an important timber-producing region, with approximately 1.2 billion pine seedlings planted annually. Of these seedlings, approximately 80% are genetically improved loblolly pine from forest-tree breeding programs (Li et al. 1999). Use of this genetically improved stock allows for greater productivity and improved timber quality from plantations (McKeand et al. 2003).

Breeding programs of loblolly pine (*Pinus taeda* L.) have progressed into advanced generations, and genetic gains continue to increase (e.g. McKeand and Bridgwater 1998). With these gains come an increase in the number of related individuals in the breeding population and the potential increase in the number of related matings. Falconer and Mackay (1996) confirm that matings between related individuals are more likely to occur in smaller populations than in larger populations. Hence, there is an increased probability of inbreeding in current and future generations of breeding.

In addition to their larger mainline populations, many conifer tree improvement programs have commissioned “elite” breeding populations (Williams and Hamrick 1996). Focusing breeding among the best selections from the breeding population is an attempt to yield increased gain in shorter spans of time and emphasize selection on more specific product goals (McKeand and Bridgwater 1998). The greatly restricted size of elite populations increases the possibility for inbreeding to occur more rapidly.

Inbreeding is the mating of individuals that are related by ancestry. Inbred individuals can have two genes that are identical by descent (IBD) at a locus found in the ancestor in a previous generation. The coefficient of inbreeding,  $F$ , is the probability that the two alleles at any locus in an individual are identical by descent (Falconer and Mackay 1996).

Inbreeding depression is an observable effect of inbreeding and is the reduction in mean phenotypic values of traits related to fitness, such as reproductive capacity, vigor, and physiological efficiency (Falconer and Mackay 1996, Wright 1922, Charlesworth and Willis 2009).

Conifer tree improvement programs have historically been managed with large population sizes with the goal of avoiding inbreeding (Williams and Savolainen 1996). Inbreeding is not generally a concern in the first generation of conifer tree improvement programs, because trees selected from the wild to form the breeding population are typically selected from diverse stands and are assumed unrelated (Wu et al. 1998).

Addressing the issue of inbreeding must become part of the breeding population management strategy (Wu et al. 1998). Further research into the basic effects on growth and reproduction traits in conifers is critical in planning successful future generations of tree improvement. Reliable estimates of inbreeding depression will give a more firm understanding of the genetic architecture of inbreeding depression and the mating history of the species. Examination of less severe levels of inbreeding is as important as comparing selfs with outcrosses, since it is the less severe instances that will likely be encountered first in breeding populations (Sniezko 1984, Wu et al. 1998, Williams and Savolainen 1996).

Progeny of related matings have been shown to exhibit inbreeding depression at many life stages. Loblolly pine expresses inbreeding depression for seedling height and root collar diameter, with a significant interaction between family and inbreeding level (Sniezko 1984; Sniezko and Zobel 1988). Wu et al. (1998) show that radiata pine (*Pinus radiata* D. Don) exhibited inbreeding depression at age 12 for DBH, and response of mean DBH with increased inbreeding was different among the eight founder clones.

Traits not closely connected with fitness, such as wood properties, often show no change due to increased inbreeding (Falconer and Mackay 1996). For wood properties, less is known

about the impacts of inbreeding on wood specific gravity and bending strength. In the few studies reported in the literature, change in wood density upon inbreeding varies. A population of eight radiata pine founder clones were inbred to levels of  $F= 0, 0.125, 0.25, 0.5,$  and  $0.75$ . Inbreeding caused a minor, but not statistically significant, decrease in wood density among progeny of all clones (Wu et al. 2002). Selfed progeny, relative to outcrosses of radiata pine had 3.3% and 3.1% more dense juvenile and mature wood, respectively (Kumar 2004). Seven-year-old progeny of selfed radiata pines had slightly, but not significantly, denser wood than progeny of outcrosses (Wilcox 1983). Wood density for selfs was 5% greater than outcrosses at age 10 in 3 Norway spruce (*Picea abies* (L.) Karst.) diallel crosses (Skrøppa 1996).

Except for specific gravity, we are not aware of any assessment of the effect of inbreeding on other wood quality traits in other conifer species. Nor are we aware of any assessments of inbreeding effects for any wood quality traits in loblolly pine.

This study was designed to address two objectives.

- 1) Assess the response of loblolly pine to various degrees of related mating on growth and the wood property traits of specific gravity and bending strength.
- 2) Describe genetic differences in inbreeding depression for these traits.

The nature of variability in response to inbreeding depression among genetic entries or lines is important to understand. If all lines respond similarly to the various levels of inbreeding, general prescriptions can be implemented across the breeding population. However, if significant variation in line responses exist, general prescriptions of managing inbreeding will not be as appropriate.

## Methods and Materials

### *Genetic material*

Effects of inbreeding were quantified for 10 Coastal loblolly pine selections sourced from the North Carolina State University – Cooperative Tree Improvement Program. The selections were mated each with two unrelated selections, two half-sibs, two full-sibs, and were self-fertilized. This mating scheme provided a gradient of inbreeding coefficients (F) of 0, 0.125, 0.25, and 0.5 (Figure 3.1). Progeny from the four crosses are the genetic entries used in this experiment, and are called lines in the following sections.

Mating unrelated selections is referred to as outcrossing, and  $F = 0$ . Half-sibs selections have one common parent, and when mated, produce progeny with  $F = 0.125$ . Full-sibs selections have both parents in common, and when mated, produce progeny with  $F = 0.25$ . Selfing or self-fertilization generates progeny with  $F = 0.5$ .

Upon completion of breeding, seeds were collected and stratified for 60 days. The seeds were then sown into containers in a greenhouse in January 1988. The containerized seedlings were hand planted at the test site in May, 1988 at 2.4 by 2.7 meters (8 by 9 feet) spacing.

### *Experiment design*

The test site is located on Weyerhaeuser Company land in the Coastal Plain of North Carolina in Beaufort County. The soil series is a Leaf Silt Loam soil, and is characterized by areas of flat terrain, poor natural drainage, and high amounts of organic matter. Forestry activities are successful only after the installation of a drainage system. Prior to planting, the site was mechanically and chemically prepared to remove competing vegetation, bedded, and fertilized.

A split-plot design was installed with six replications. In each replication, there were 4 main plots, each representing the four inbreeding levels: Outcross, Half-Sib, Full-Sib, Self. Within each of the main plots, the ten lines were planted in six tree row plots. The six-tree row plots were comprised of three trees from each of two crosses of Outcrosses, Half-Sibs, and Full-Sibs, respectively. In this experiment design, the four inbreeding levels are the main plot factor and lines are the sub-plot factor.

### *Traits measured*

In winter of 2012 when trees were at age 23 years, height and diameter at breast height (DBH) measurements were tallied. Total tree height was measured with a laser vertex hypsometer. Diameter at breast height was measured by wrapping a diameter tape around the main stem, 1.4 meters from the ground.

Survival of each experimental tree was assessed as a binary trait; with 1 indicating survival and 0 indicating a dead tree.

Wood strength data were collected using a TreeSonic (<http://www.fakopp.com/site/treesonic>) that measures the time of travel for a stress wave sent between two transducers that are inserted into a tree. Three hits per tree were recorded and an average value was calculated. This average time of flight was then converted to calculate the velocity (meters per second) of the stress wave. The velocity of acoustic stress waves directly correlates with bending strength or modulus of elasticity in loblolly pine (e.g. Eckard et al. 2010).

Specific gravity was measured on 12mm cores that were extracted from trees using a gas-powered drill with a hollow drill bit. Coring was done at approximately breast height (1.4 meters above ground). Branch scars and resin pockets were avoided to yield clean cores. Cores were labeled, placed in plastic bags, and put into a cooler of ice until they were put into refrigeration.

In the lab, cores were split at the pith, and bark, cambium, and pith tissue were removed. Specific gravity was calculated on both core pieces using a standard volumetric method (e.g. USDA 1956) then averaged to give one density measurement per tree.

To assess stem quality, sweep of the first merchantable log was assessed as the amount of perpendicular deviation of a 2.4 meter (8 feet) straight edge held up against the tree. Forking and ramicorn branching in the lower-stem (5.5 meter and below) and in the upper-stem (above 5.5 meters) were recorded. Incidence of fusiform rust galls (caused by the fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme*) the main stem were also recorded. Forking and ramicorn branch characteristics were recorded on a binary scale: 0 = event did not occur, 1 = event occurred.

### *Statistical Analysis*

Inbreeding coefficient “F” was assigned to the four mating types. The F values are as follows: Unrelated = 0, Half-Sib = 0.125, Full-Sib = 0.25, Self = 0.5. Filler trees were deleted from the data set.

The MEANS procedure in the SAS system was used to produce summary statistics of DBH, height, percent survival, specific gravity, and stress wave velocity (SAS Institute Inc. 2008). Summary statistics of these variables were generated for the test site.

A linear mixed model (equation 1) was used to analyze height, DBH, specific gravity, and stress wave velocity- with assumptions that errors are independently and identically distributed with mean of zero. The MIXED procedure in the SAS system was used (SAS Institute Inc. 2008). Inbreeding level and line were treated as fixed effects. Replication was considered a random effect.

$$[1] \quad Y_{ijkl} = \mu + R_i + I_j + (R * I)_{ij} + L_k + (L * I)_{jk} + (R * L)_{ik} + (R * I * L)_{ijk} + \varepsilon_{ijkl}$$

where,

$Y_{ijkl}$  = response variable of the l-th tree of the k-th line at the j-th inbreeding level in i-th replication

$\mu$  = overall mean

$R_i$  = i-th replication effect with expectations NID  $\sim (0, \sigma_r^2)$  (i=6)

$I_j$  = j-th inbreeding level effect (j=4)

$L_k$  = k-th line (k=10)

$(L * I)_{jk}$  = interaction of j-th inbreeding level with k-th line

$(R * I)_{ij}$  = interaction of i-th replication with j-th inbreeding level with expectations NID  $\sim (0, \sigma_{ri}^2)$

$(R * L)_{ik}$  = interaction of i-th replication with k-th line with expectations NID  $\sim (0, \sigma_{ri}^2)$

$(R * I * L)_{ijk}$  = interaction of i-th replication with k-th line with l-th line with expectations NID  $\sim (0, \sigma_{ril}^2)$

$\varepsilon_{ijkl}$  = random error term with expectations NID  $\sim (0, \sigma_e^2)$

Survival was analyzed as a binary trait. The probability of survival was modeled by fitting a generalized linear mixed model using the GLIMMIX procedure (SAS Institute Inc. 2008). The generalized linear mixed model and expectations, as shown by Isik (2011) are as follows:

$$[2] \quad E[\mathbf{y}|\mathbf{u}] = g^{-1}(\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u})$$

where,

$\mathbf{y} = (n \times 1)$  response vector

$\mathbf{X} = (n \times p)$  design matrix of rank  $k$  for the  $(p \times 1)$  fixed effects  $\boldsymbol{\beta}$

$\mathbf{Z} = (n \times q)$  design matrix for the  $(q \times 1)$  random effects  $\mathbf{u}$

Random effects,  $\mathbf{u}$ , are assumed to be normally distributed with mean equal to 0 and variance matrix  $\mathbf{G}$ . The link function,  $g$ , used to model the binomially distributed response is as follows:

$$[3] \quad g = \text{Logit} = \ln(\mu_i(1 - \mu_i))$$

Therefore, the inverse link function was used to predict survival on the inverse scale:

$$[4] \quad g^{-1} = e^n = 1/(1 + e^n)$$

For traits with a significant F-statistic for inbreeding level, the GLM procedure and Tukey's means separation procedure were used to show the significant differences among the four levels of inbreeding (SAS 2008). The Waller-Duncan adjustment was used in the means separations procedures for stress wave velocity because of complications and inconclusive results using Tukey's adjustment.

Percent inbreeding depression was calculated for each trait. It is defined as percent reduction in mean of the trait, relative to the mean of progeny from unrelated crosses.

## Results

At age 23, the trees in the test had an average height of 22 meters and DBH of 24 centimeters. Overall survival in the test was 55%. Mean specific gravity was 0.455. Mean stress wave velocity was 4135.8 meters/second. Test-site summary statistics of each trait analyzed are in Table 3.1.

Occurrences of forking and ramicorn branching characteristics and stem rust galls were very low. The incidence levels for these branching characteristics were too low for appropriate or successful analysis to be performed (see Appendix 2).

### *Growth and Survival*

Height was significantly different ( $p = 0.0079$ ) among the four levels of inbreeding (Tables 3.2). Relative to outcrosses, half-sibs, full-sibs, and selfs were 1.09%, 5.44%, and 4.14% shorter, respectively (Table 3.3). Average height of progeny of outcrosses is significantly greater than average height of progeny of full-sibs and selfs, respectively. Average height of outcrosses and half-sib progeny were not significantly different. Average height in progeny of full-sibs and selfs were not significantly different.

There were significant differences in inbreeding levels ( $p = 0.0162$ ) for DBH (Table 3.2). Average DBH of half-sib and full-sib-mated progeny were 1.92% and 7.3% smaller than outcrossed progeny, respectively. Progeny of selfs had the greatest DBH of all mating types; 2.48% larger than outcrossed progeny (Table 3.3). The large DBH of the selfs appears to be due to the low stocking levels in the selfed plots. Mean DBH was significantly different between progeny of full-sibs and all other inbreeding levels, and between progeny of half-sibs and selfs.

Probability of survival differed significantly ( $p = 0.0004$ ) among the four inbreeding levels (Table 3.2). Progeny of self-fertilizations had the lowest probability of survival, 0.40. Probability of survival was decreased in progeny of full-sibs to 0.52. Probability of survival between progeny of outcrosses and half-sibs was no different. The probabilities of survival for progeny of full-sibs and selfs, respectively, differed significantly from all other levels of inbreeding (Table 3.3).

Line was statistically significant for traits height, DBH, and probability of survival (Table 3.2). Figures 3.2, 3.4, and 3.6 show the variation in line means at each of the four inbreeding levels for these growth and survival traits.

#### *Interaction of Inbreeding Level and Line for Growth and Survival*

The interaction of line by inbreeding level was highly significant ( $p = 0.0002$ ) for height (Table 3.2). Response of height growth to increased inbreeding varied significantly among selections that were used as lines. Figure 3.2 is an interaction plot, showing variable trends in the mean height of the ten lines as inbreeding increases.

Variation in line response can also be seen in Figure 3.3, with each family displayed independently. For lines TIP2472543, TIP268415 and TIP292525, average heights decreased severely with increased inbreeding. Lines TIP2714551 and TIP2227582 showed a more gradual decrease in height with increased inbreeding. Average height of other lines was characterized by variability and rank changes among the inbreeding levels.

Interaction of line and inbreeding level was highly significant ( $p < 0.0001$ ) for DBH (Table 3.2). Figure 3.4 is an interaction plot showing the variable trends in mean DBH of the ten lines as inbreeding increases.

The non-identical responses to increased inbreeding among the ten families are illustrated independently in Figure 3.5. Four lines (TIP2227582, TIP2472543, TIP268415, and TIP292525) showed a steady decrease in mean DBH with increased inbreeding. Two lines, TIP230370 and TIP2419552, exhibited no change in mean DBH with increased inbreeding. Mean DBH in other lines was variable and inconsistent with increased inbreeding.

There is significant ( $p < 0.0001$ ) line by inbreeding level interaction for the probability of survival (Table 3.2). As inbreeding increased, the ten lines differed in their probabilities of survival. This interaction is plotted collectively in Figure 3.6.

Response to increased inbreeding on probability of survival is shown for each line independently in Figure 3.7. Probability of survival declined directly with increased inbreeding in only one line, TIP2280651. No consistent patterns of survival upon increased inbreeding are detectable in other lines.

#### *Wood Property Traits*

Inbreeding had no significant effect ( $p = 0.6884$ ) on specific gravity (Table 3.2). The means for the different inbreeding levels are shown in Table 3.4.

Mean stress wave velocity was significantly greater ( $p = 0.0026$ ) in outcrossed progeny, relative to outcrosses (Table 3.2). Relative to progeny of outcrosses, mean stress wave velocity flight decreased by 2.75%, 2.50%, and 2.71% in progeny of half-sibs, full-sibs, and selfs, respectively (Table 3.4).

Line was statistically significant for specific gravity and stress wave velocity (Table 3.2). Figures 3.8 and 3.10 show the variation in line means at each of the four inbreeding levels for these wood property traits.

### *Interaction of Inbreeding Level and Line in Wood Property Traits*

For specific gravity, there is a significant ( $p < .0001$ ) interaction between line and inbreeding level. Not all lines responded the same to increased inbreeding. Figure 3.8 is an interaction plot showing trends of line specific gravity means with increased inbreeding.

Figure 3.9 shows the trend of each line's specific gravity response to increased inbreeding independently. Lines TIP2280651, TIP2419552, and TIP2714551 showed greater mean specific gravity with increased inbreeding. Lines TIP230370, TIP2357867, and TIP292525 specific gravity remained constant as inbreeding increased. Specific gravity steadily decreased in lines TIP2472543 and TIP268415 as inbreeding increased. With inbreeding in other lines, specific gravity followed no consistent pattern.

For mean stress wave velocity, there is a significant interaction ( $p = 0.0008$ ) between line and inbreeding level. Lines responded differentially to increased inbreeding. Figure 3.10 shows the variable trends in lines' mean stress wave velocity change with increased inbreeding.

Effect of increased inbreeding for each line is shown independently in Figure 3.11. Lines TIP2714551, TIP292525, and TIP2227582 showed decreases in mean stress wave velocity in the inbred progeny. Stress wave velocity in all other lines remained relatively constant, with some slight rank changes among inbreeding levels.

### Discussion

The design of the experiment has facilitated survival of some of the inbred progeny. The split-plot design relegated competition among trees and among lines to those of the same mating type. This was important for obtaining non-biased growth measurements of the four levels of inbreeding. Competition would be much different in a natural stand; where inbred

trees would be competing with outcrossed progeny at all life stages. Most inbred individuals are outcompeted in early life-stages. Competitive disadvantage against inbred trees is exacerbated in wild environments (Vogl et al. 2002).

Neighboring trees are competition for vital resources. Death of a neighboring tree redistributes resources to remaining trees. With approximately 60% of the trees in the self-fertilized main plots being dead, remaining trees have access to more resources; relative to moderately-inbred trees in plots with more surviving trees. Patterns of mortality within the selfed plots were irregular, thus increasing environmental heterogeneity within the selfed plots.

This impact on survival of experiment trees skewed the results of growth traits at this age, most clearly seen in DBH. Decrease in stand density in the selfed plots appears to be the reason that mean DBH is greatest among progeny of self-pollinations.

In stands of loblolly pine at moderate planting densities, height growth is not strongly dependent upon density (MacFarlane et al. 2000). Height is also a trait closely linked to fitness. These factors help explain the downward trend in mean height with increased inbreeding and the significant inbreeding effect present in the mixed model.

The relatively modest amount of inbreeding depression on height in the progeny of full-sibs and selfs is a likely a result of the age at which the test was measured. Since establishment of the test 23 years ago, self-thinning through mortality of less-fit trees has had ample time to occur. Only the fittest individuals in the inbreeding-level main plots remain. Non-fit individuals have been outcompeted and are dead. This likely gives a reduced measure of the inbreeding depression on height seen at this mature age.

Across all ten loblolly pine lines, specific gravity was not affected by increasing amounts of inbreeding. The presence of inbreeding depression for growth and survival but not wood

density is likely due to the implications that these traits have on fitness. Falconer and Mackay (1996) explain that inbreeding depression is seen predominantly in traits that impact either “reproductive capacity or physiological efficiency.”

Differences in selective advantage between growth traits and wood density have likely resulted in dissimilar proportions of damaging alleles between these two classes of traits. Growth traits and survival have a positive influence on reproductive capability and physiological efficiency of a tree. Large, vigorous trees reproduce more successfully than small trees. Therefore, alleles resulting in small trees would be selected against and driven to relatively low frequencies. Charlesworth and Charlesworth (1987) and Charlesworth and Willis (2009) confirm that as mutations deleterious to fitness arise, they are subsequently selected against and maintained at low frequencies in populations. Inbreeding depression arises by an increased proportion of these less-fit homozygotes in the population (Charlesworth and Willis 2009). As the rare alleles controlling growth increase in frequency upon inbreeding, population mean height is reduced.

The density of wood is not an important fitness trait (Wu et al. 2002). Wood properties will vary greatly from tree to tree (Zobel and Talbert 1984). Therefore, rare alleles of wood density would not likely cause strictly negative effects. As inbreeding occurs, rare homozygous alleles will not change the population mean in one direction. Rare alleles may increase, decrease, or not change the mean value of a wood property, because there has been no selective advantage towards any single value.

Inbred progeny had lower stress wave velocity than progeny of unrelated matings. Eckard et al. (2010) explain that this stress wave velocity correlated directly with bending strength of wood. Therefore, lower mean stress wave velocity signifies lower wood strength in the inbred progeny. Although the reduction in stress wave velocity was modest, less than 3%, the differences in mean velocities were statistically significant and should not be overlooked.

Barnett and Bonham (2004) explain that microfibril angle (MFA) has a profound effect on wood stiffness, but the factors that result in changes or differences in MFA are not clearly understood. Strength of wood is a result of an extremely complex suite of factors involving genetics and environment (Bowyer et al. 2003).

Herman et al. (1999) explain that MFA in Norway spruce increased following thinning. This thinning-induced increase in MFA would result in a decrease in wood strength. If MFA increases with thinning in loblolly pine, the lower survival in the full-sib and self plots in this trial could help explain the decrease in stress wave velocity in these inbred progeny. However, probability of survival was equal in progeny of outcrosses and half-sibs, respectively, but half-sib progeny had significantly lower stress wave velocity than outcrosses. These results suggest that the decrease in stress wave velocity is likely attributed to other factors in addition to within-stand thinning.

This research has resulted in identifying disparity in the presence of inbreeding depression between the wood property traits specific gravity and wood strength (measured by stress wave velocity). This should serve as motivation for further research into the effect of inbreeding on wood properties in loblolly pine.

The second objective of this experiment was to assess whether there is variation among lines of loblolly pine in response to increased inbreeding. This interaction between line and inbreeding levels was present for all traits.

Degree of inbreeding depression varies between species, among families within a species, and among individuals within a family. This pattern of variation could be due to variation in number of lethal and damaging alleles (Skrøppa 1996; Bramlett and Pepper 1974).

Magnitude of inbreeding depression is dependent upon the mutation-selection balance (Charlesworth and Charlesworth 1987). If inbreeding depression is caused by rare, recessive alleles, purging of these alleles is possible (Wang et al. 1999; Charlesworth and Willis 2009).

Purging of deleterious alleles can account for decreased inbreeding depression found in species capable of self-fertilization. This suggests that inbreeding depression should be lower in populations with a history of some self fertilization and greater in more outcrossing populations (Charlesworth and Charlesworth 1987; Lande and Schemske 1985; Wang et al. 1999; Husband and Schemske 1996).

Historically large populations facilitate outcrossing and will result in greater inbreeding depression of inbred progeny. But, species and/or populations with a reduction in size, such as bottlenecks or pollinator failure, are expected to exhibit lower inbreeding depression and favor more self-fertilization (Lande and Schemske 1985).

The theories explained above allow some inferences and speculation into the mating history of the founder selections used in this experiment. There were varying responses to increased inbreeding among the ten lines in this experiment. Amount of inbreeding depression is a reflection of the genetic load for a particular trait. Purging of deleterious alleles, induced by environmental factors, reduces inbreeding depression. Therefore, the degree of inbreeding depression in a particular line is also a representation of the mating history of its ancestors.

It is likely through time that environmental conditions have enabled deleterious alleles to be purged from ancestors of some selections of loblolly pine. The large geographic range of loblolly pine has facilitated exclusively outcrossing with no purging in ancestors of some other selections. This would be the nature of the variability in inbreeding depression seen in the ten lines in this experiment.

## Conclusion

With advanced generations of tree improvement on the horizon, it is necessary for forest tree breeders to address how inbreeding shapes breeding strategies. Reliable estimates of inbreeding depression are essential for these breeding strategies to be successful.

Examination of less severe levels of inbreeding is as equally important as comparing selfs with outcrosses, because it is the less severe instances that will most likely be encountered first in breeding populations (Sniezko 1984; Wu et al. 1998; Williams and Savolainen 1996).

This study has asserted that loblolly pine exhibits inbreeding depression for growth and survival at a mature stand age. Wood strength, as measured through acoustic stress wave velocity is also decreased with inbreeding. Effect of inbreeding depression on DBH is confounded because DBH is a stand density-dependent trait. High mortality in progeny of self-fertilizations has increased the growing space of remaining trees, and as a result, diameters were large for the selfed trees

Wood density in loblolly pine was not adversely affected by increased inbreeding. This is a new, valuable result for loblolly pine tree breeders. However, lines show different patterns in response of wood properties to increased inbreeding.

The significant interactions between lines and inbreeding level, for all traits, imply that no general prescriptions can be assigned for an entire breeding population. The effect of inbreeding depression depends on the genetic background.

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**Table 3.1** Summary statistics for each trait observed.

Statistics	Height (m)	DBH (cm)	Survival (%)	Specific Gravity	Stress Wave Velocity (m/s)
Mean	22	24	55	0.455	4135.8
Std. Error	0.10	0.18	1.37	0.001	17.29
CV%	12.57	20.72	90.14	6.62	11.24
N	723	723	1310	716	723

**Table 3.2** F-tests for inbreeding levels (Inbr.), line, and their interaction. F-test values with probability of 0.05 or less are considered significant.

<b>Effect</b>	<b>Height</b>		<b>DBH</b>		<b>Survival</b>		<b>Specific Gravity</b>		<b>Stress Wave Velocity</b>	
	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
<b>Inbr.</b>	5.76	0.0079	4.73	0.0162	11.09	0.0004	0.50	0.6884	7.57	0.0026
<b>Line</b>	5.25	<.0001	3.48	0.0024	6.46	<.0001	10.75	<.0001	7.34	<.0001
<b>I*L</b>	2.64	0.0002	4.22	<.0001	3.67	<.0001	3.21	<.0001	2.39	0.0008

Numerator DF for Inbreeding, L and IL were 3, 9, and 26, respectively.

**Table 3.3** Means, 95% confidence intervals, number of observations (N), and percent inbreeding depression are given at each of the four mating types (inbreeding levels) for traits. Inbreeding levels with the same letter are not significantly different. (%ID is inbreeding depression and calculated as percent reduction in mean relative to progeny of outcross.)

Mating Type	Height (m)	95% CI		N	% ID*
		Lower	Upper		
Unrelated (F = 0)	22.7 <b>a</b>	22.39	23.09	219	--
Half-Sib (F = 0.125)	22.5 <b>ab</b>	22.07	22.84	215	1.09
Full-Sib (F = 0.25)	21.5 <b>c</b>	21.06	21.87	184	5.44
Self (F = 0.5)	21.8 <b>bc</b>	21.25	22.27	105	4.14

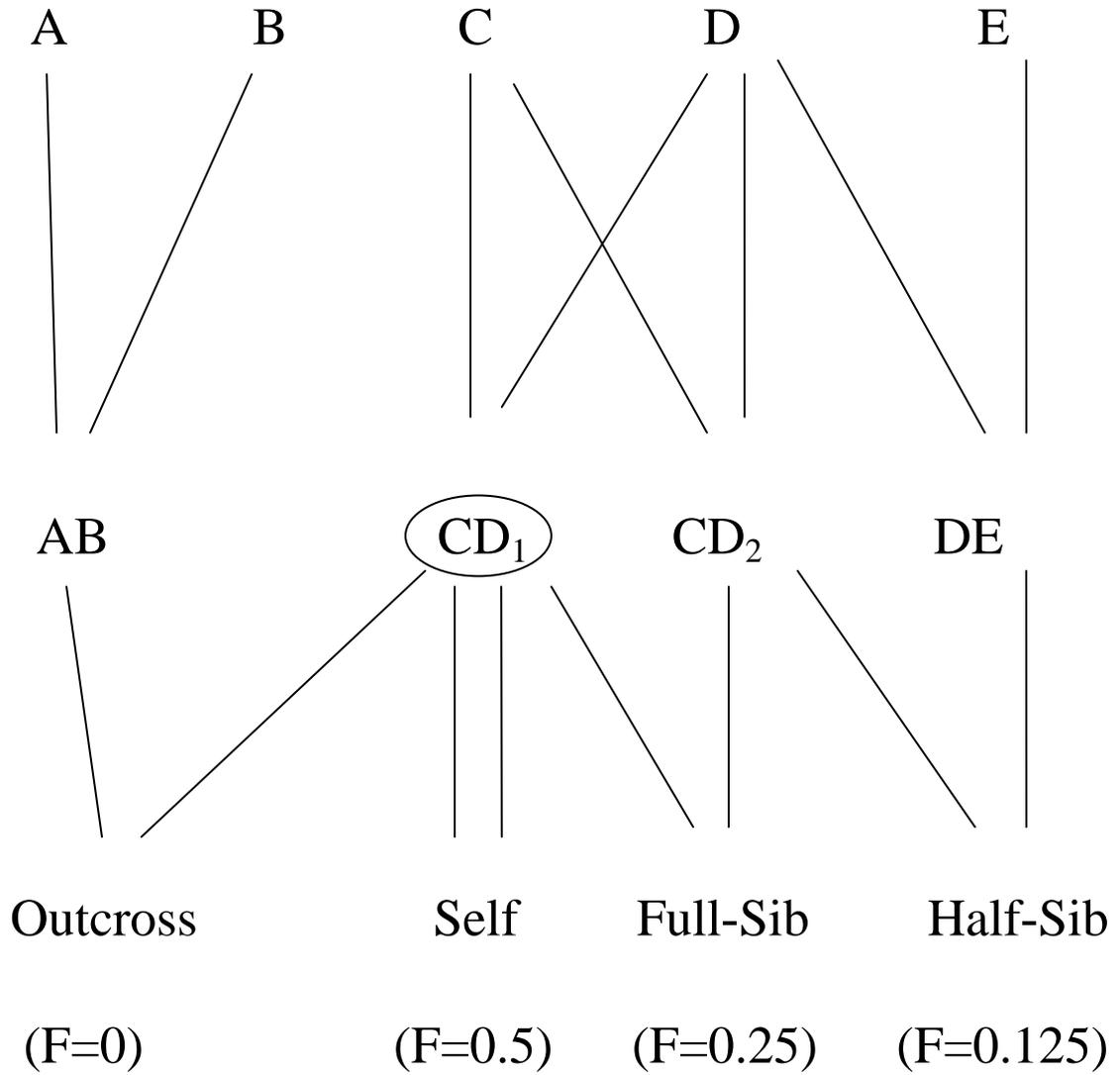
Mating Type	DBH (cm)	95% CI		N	% ID
		Lower	Upper		
Unrelated (F = 0)	24.5 <b>ab</b>	23.87	25.07	219	--
Half-Sib (F = 0.125)	24.0 <b>b</b>	23.35	24.65	215	1.92
Full-Sib (F = 0.25)	22.7 <b>c</b>	22.01	23.36	184	7.30
Self (F = 0.5)	25.1 <b>a</b>	23.89	26.27	105	-2.48

Mating Type	Survival freq.	95% CI		N	% ID
		Lower	Upper		
Unrelated (F = 0)	0.63 <b>a</b>	0.62	0.64	348	--
Half-Sib (F = 0.125)	0.63 <b>a</b>	0.61	0.65	342	0.00
Full-Sib (F = 0.25)	0.52 <b>b</b>	0.51	0.53	354	17.46
Self (F = 0.5)	0.40 <b>c</b>	0.36	0.43	266	36.51

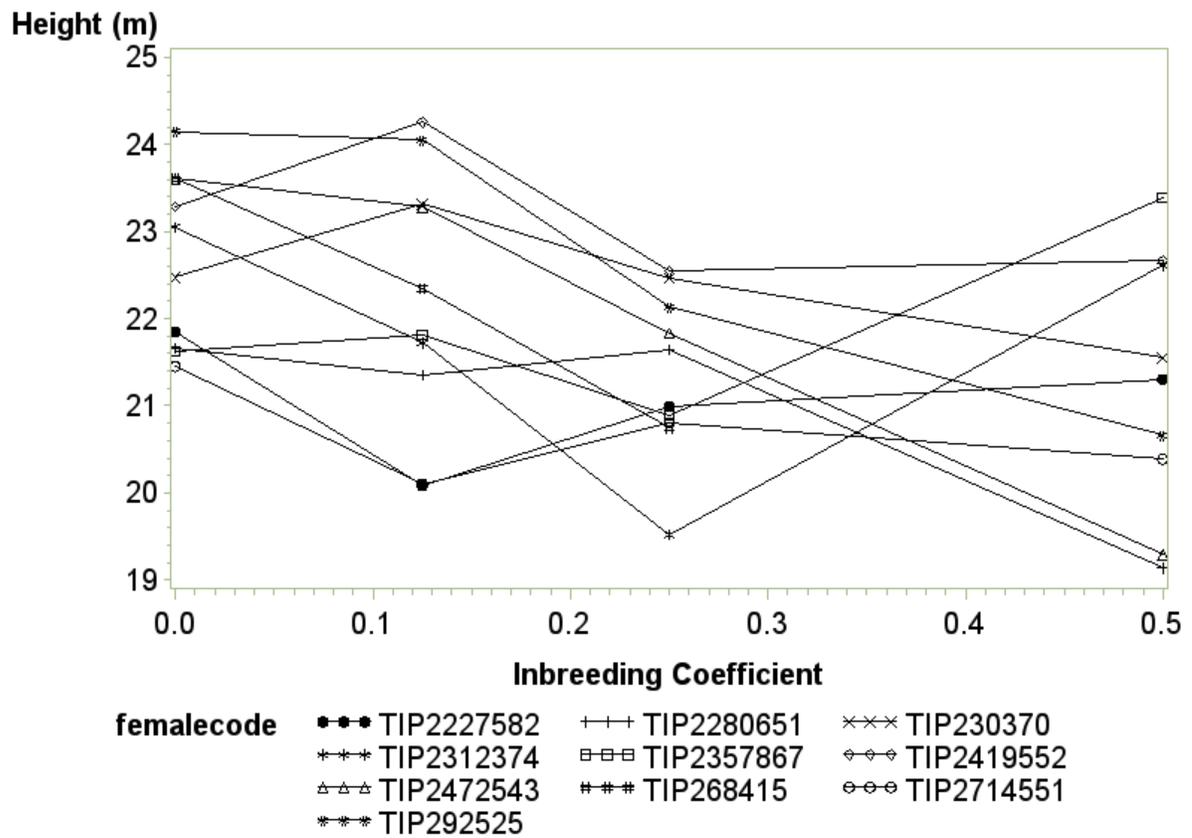
**Table 3.4** Means, 95% confidence limits, number of observations (N), and percent inbreeding depression are given at each of the four inbreeding levels. Inbreeding levels with the same letter are not significantly different.

Mating Type	Specific Gravity	95% CI		N	% ID
		Lower	Upper		
Unrelated (F = 0)	0.454 <b>a</b>	0.45	0.458	218	--
Half-Sib (F = 0.125)	0.458 <b>a</b>	0.454	0.462	213	-0.88
Full-Sib (F = 0.25)	0.457 <b>a</b>	0.452	0.461	183	-0.66
Self (F = 0.5)	0.452 <b>a</b>	0.446	0.457	102	0.44

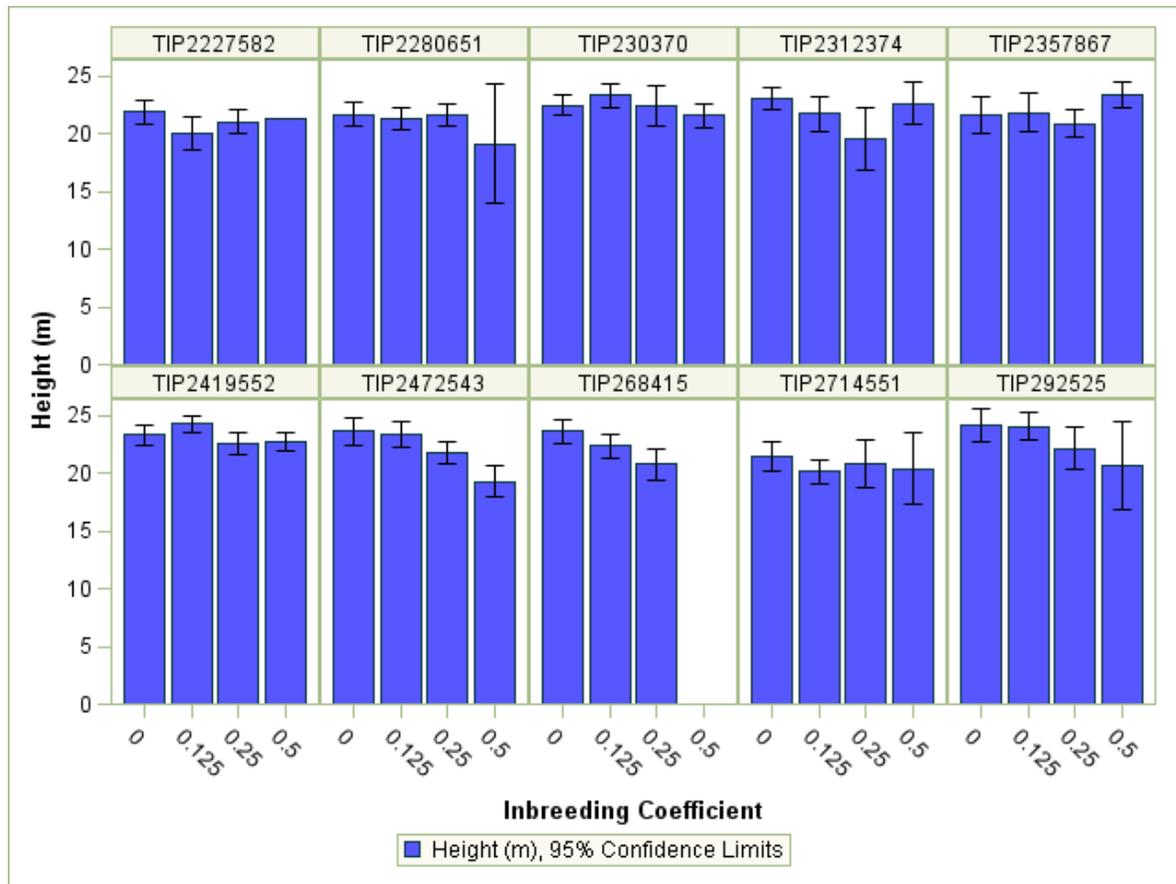
Mating Type	Stress Wave Velocity (m/s)	95% CI		N	% ID
		Lower	Upper		
Unrelated (F = 0)	4213.7 <b>a</b>	4151.8	4275.6	219	--
Half-Sib (F = 0.125)	4097.8 <b>b</b>	4035.9	4159.8	215	2.75
Full-Sib (F = 0.25)	4108.3 <b>b</b>	4041.3	4175.3	184	2.50
Self (F = 0.5)	4099.3 <b>b</b>	4009.0	4189.7	105	2.71



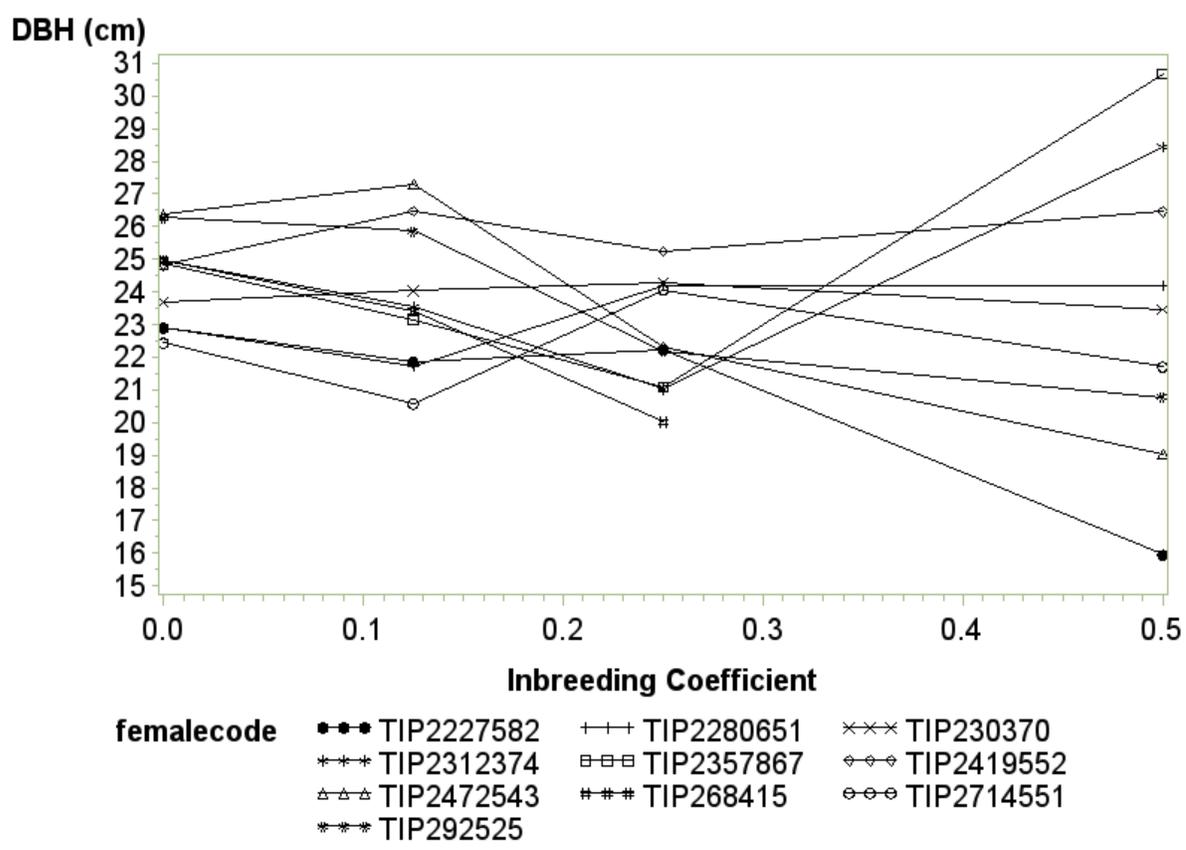
**Figure 3.1** Mating design illustrating the production of the four levels of inbreeding; where A, B, C, D, and E are founder clones and lines denote mating together of individuals. These crosses are for line CD1.



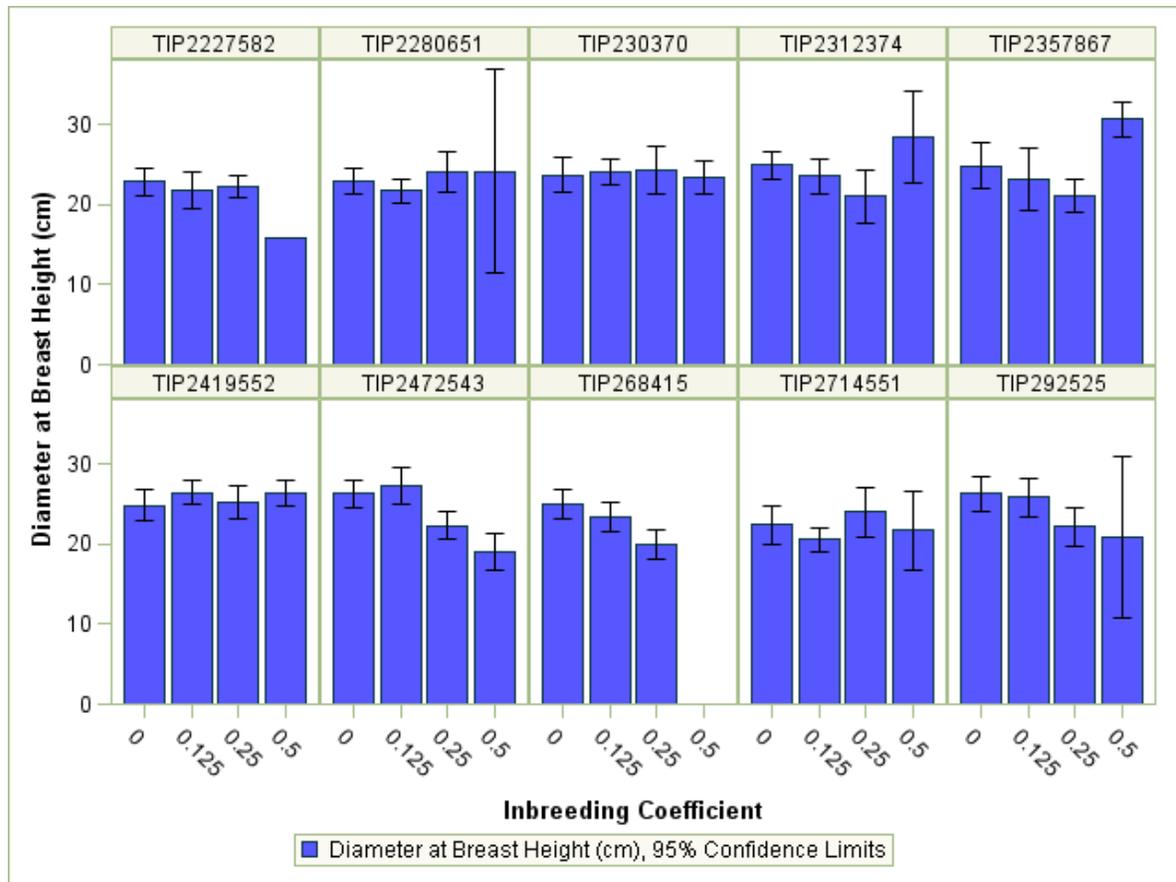
**Figure 3.2** Mean heights for different lines across different inbreeding levels. The line by inbreeding interaction was clear from rank changes among lines for different inbreeding levels.



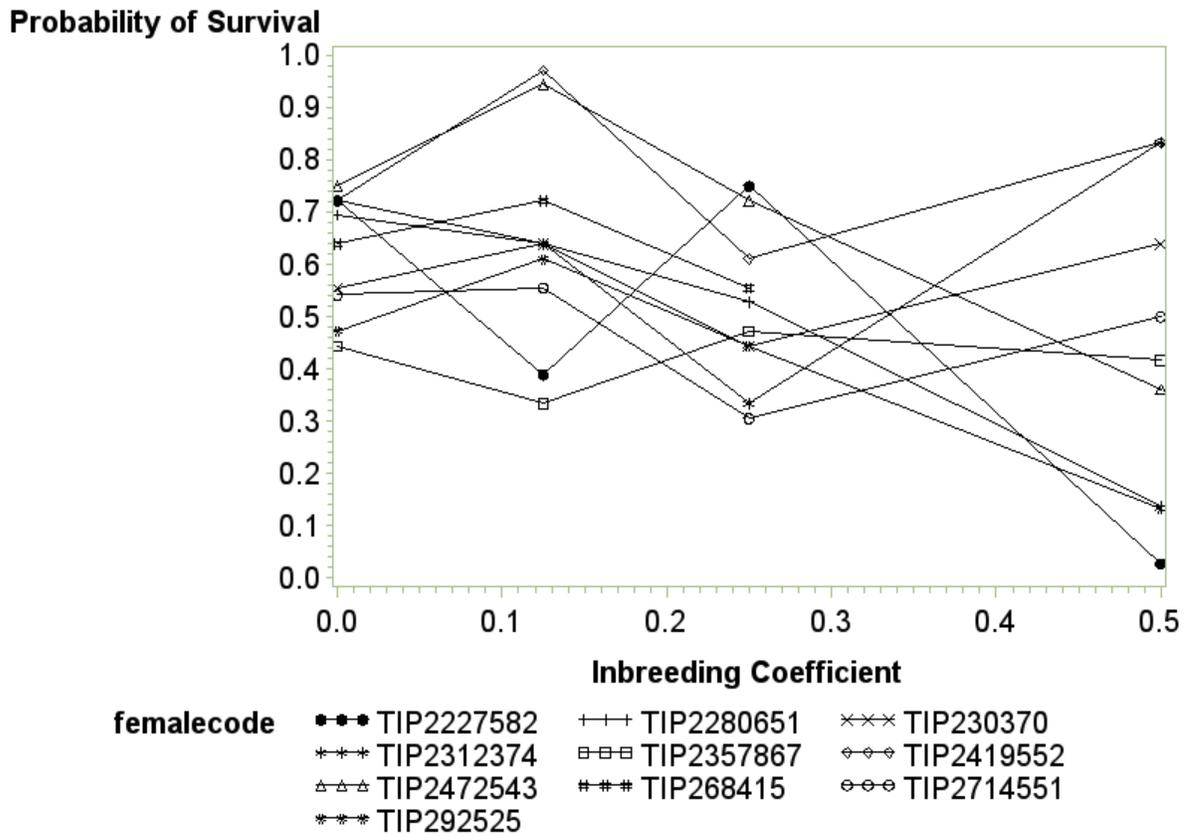
**Figure 3.3** Mean height at each of the four inbreeding levels is shown for all ten lines independently, illustrating the response of lines to increased inbreeding levels.



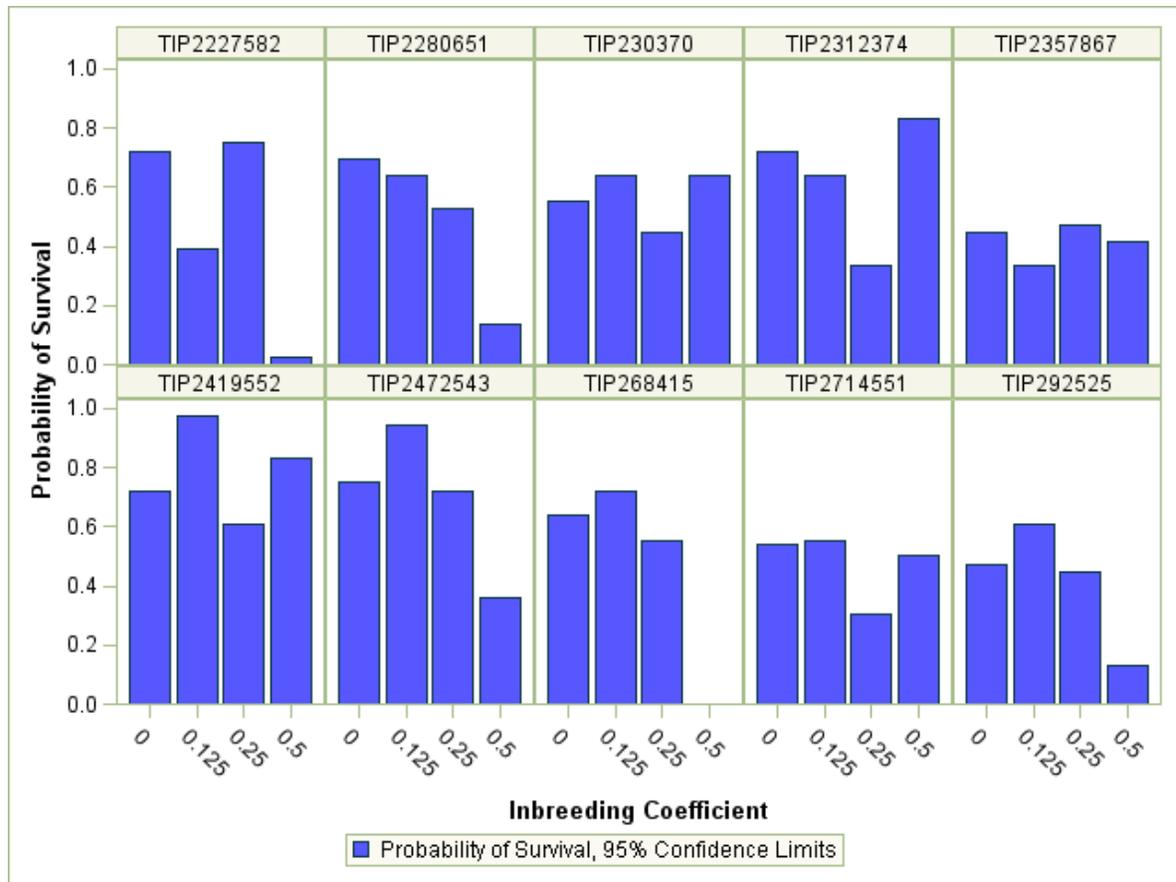
**Figure 3.4** Mean DBH for different lines across different inbreeding levels. The interaction of line by inbreeding levels is significant.



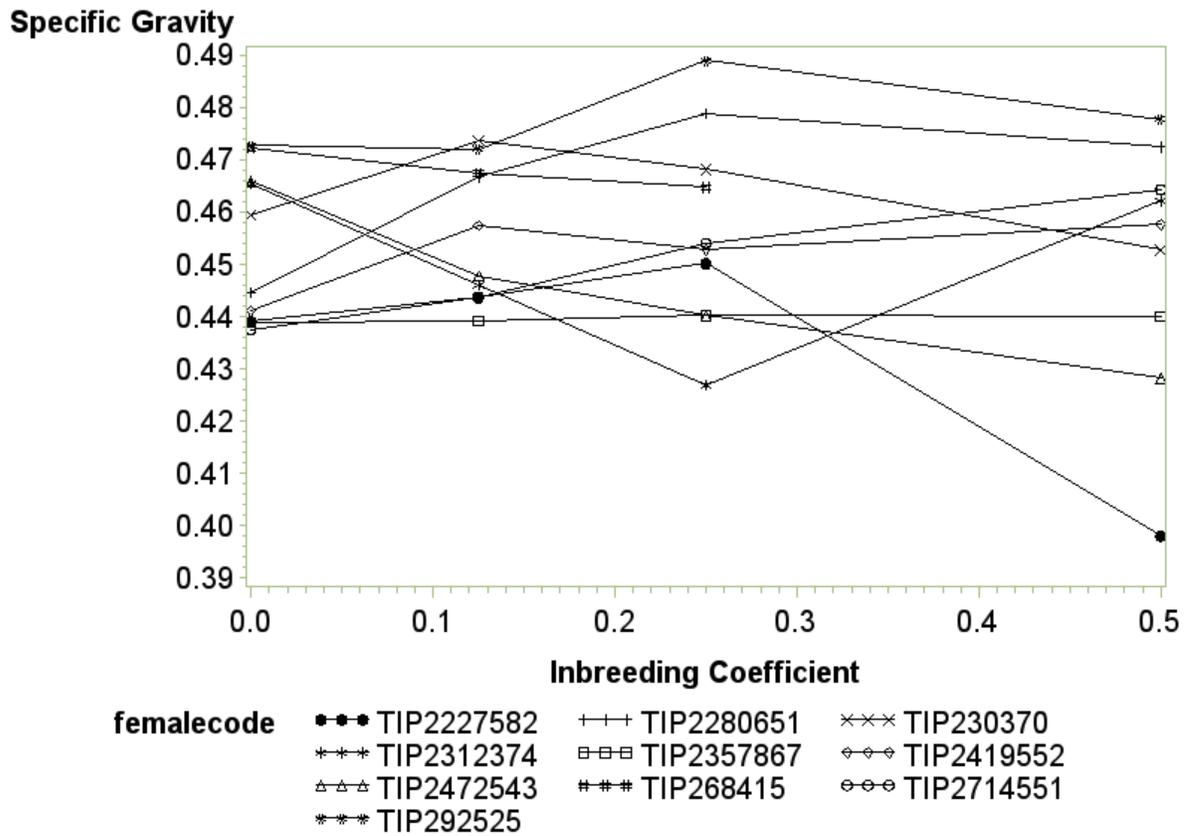
**Figure 3.5** Mean DBH at each of the four inbreeding levels is shown for all ten lines independently, illustrating the response of lines to increased inbreeding levels.



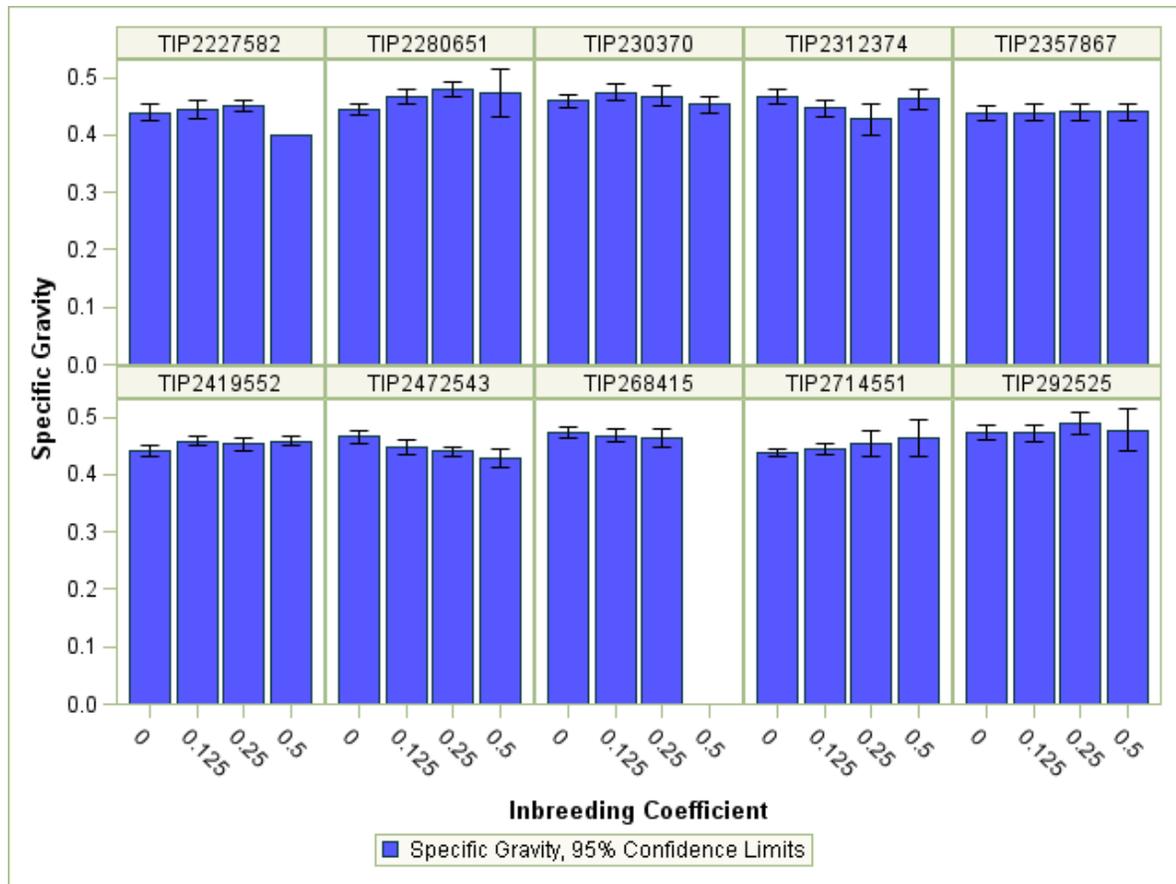
**Figure 3.6** Mean probability of survival for different lines across four inbreeding levels. Response of lines to inbreeding is dependent on the line as suggested by crossover interactions.



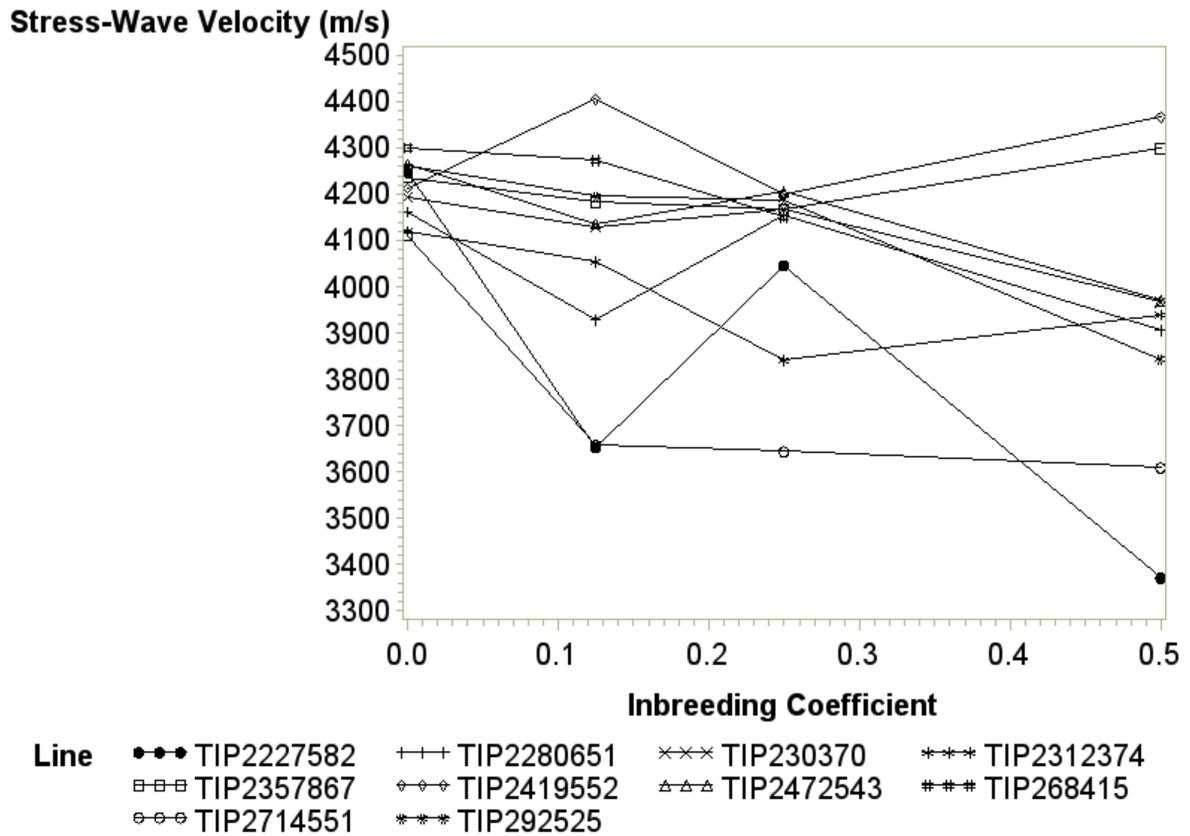
**Figure 3.7** Percent survival at each of the four inbreeding levels is shown for all ten lines independently, illustrating the variable patterns in response to increased inbreeding among the ten lines.



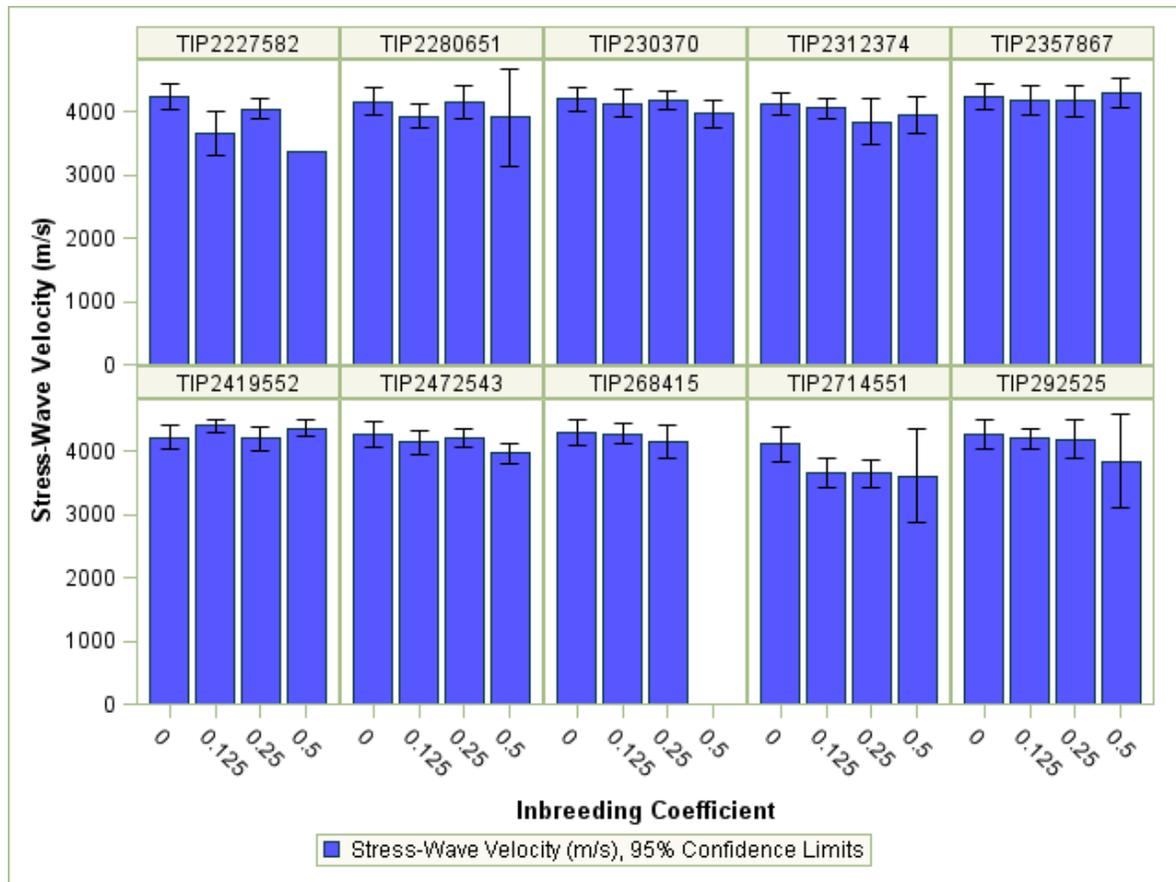
**Figure 3.8** Plot of line mean specific gravity for different lines across the four inbreeding levels.



**Figure 3.9** Mean specific gravity at each of the four inbreeding levels is shown for all ten lines independently, illustrating response of lines to increased inbreeding.



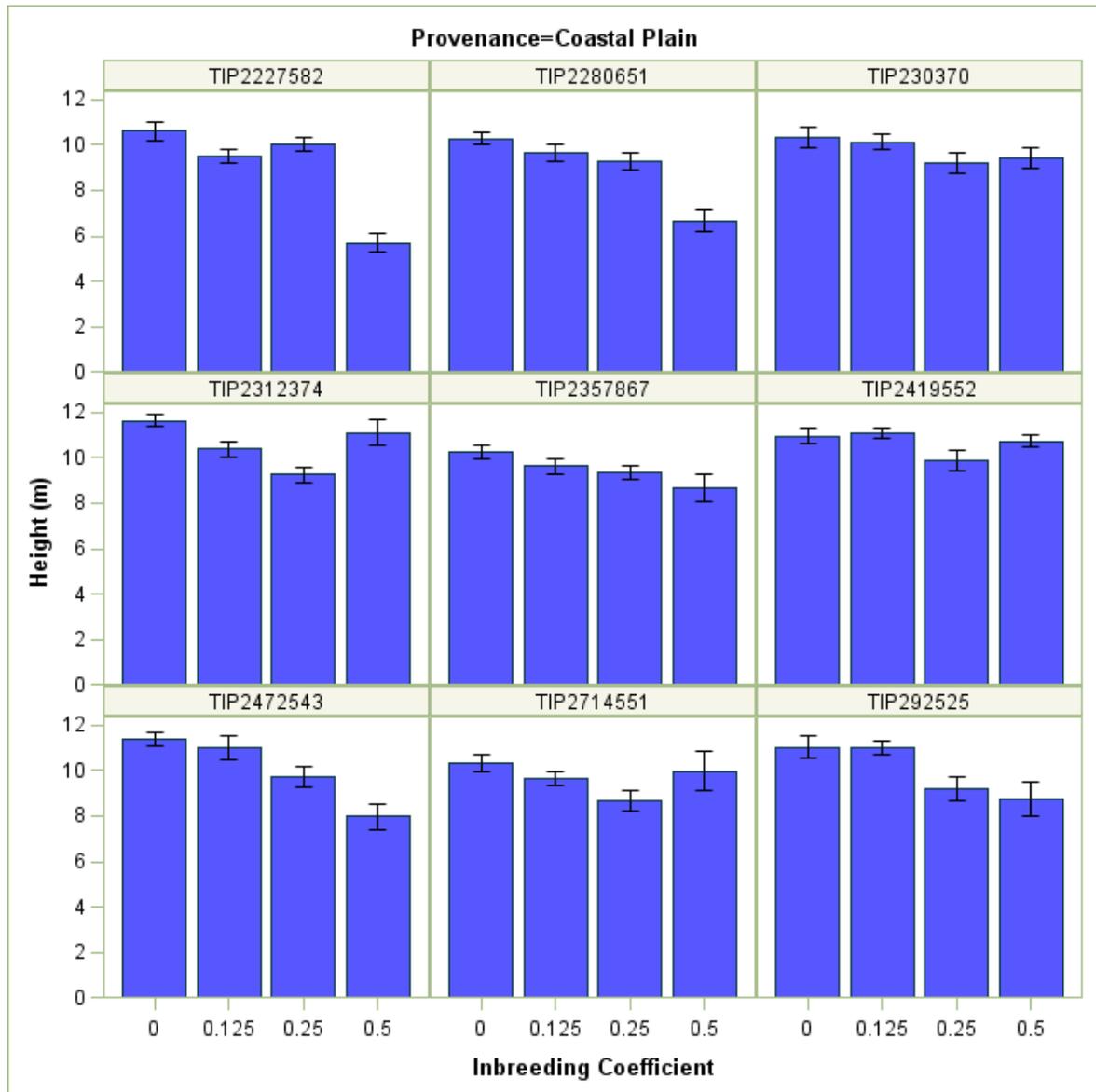
**Figure 3.10** Plot of line mean stress wave velocity for different lines across the four inbreeding levels.



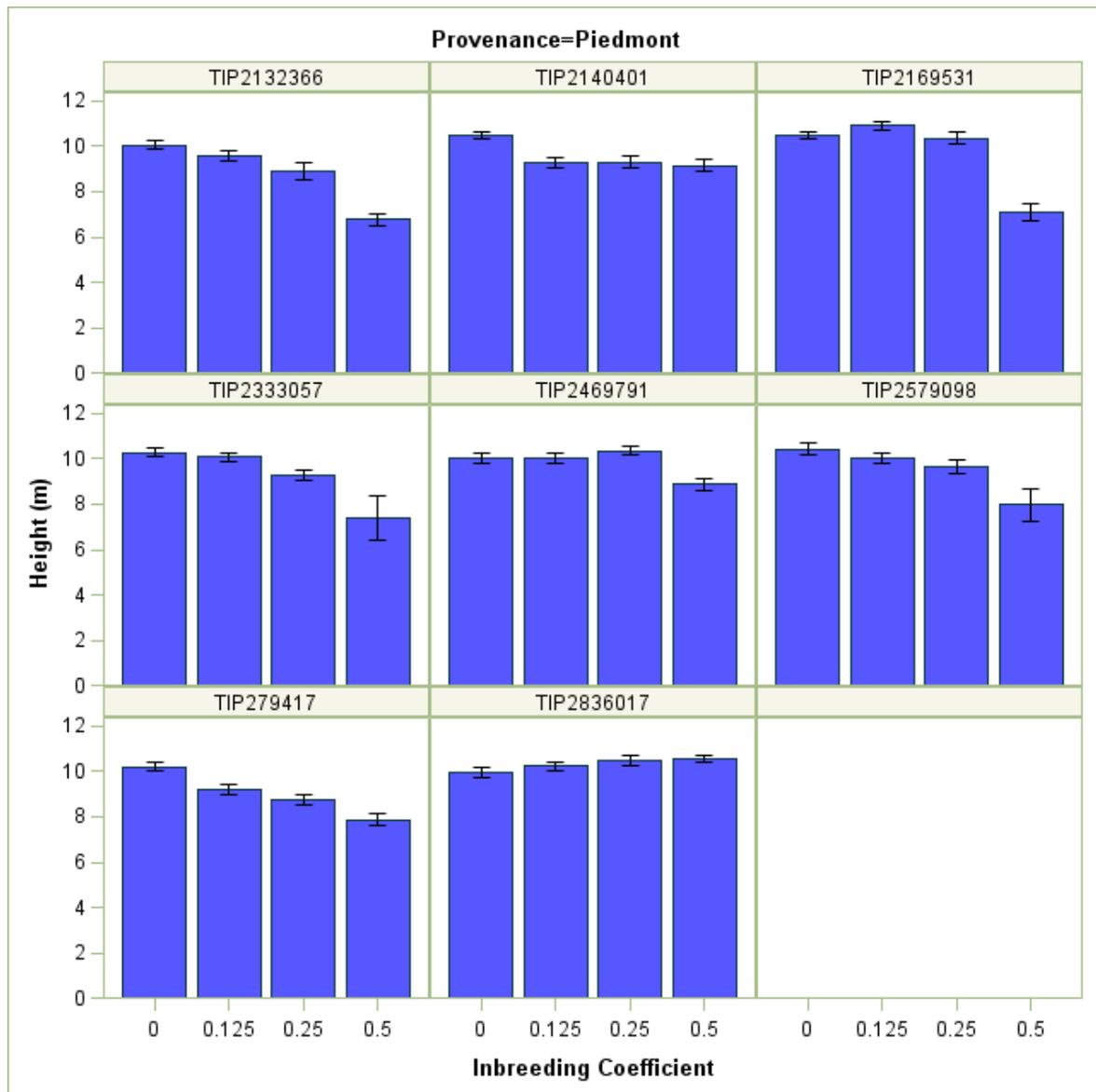
**Figure 3.11** Mean stress wave velocity at each of the four inbreeding levels is shown for all ten lines independently, illustrating the response of lines to increased inbreeding.

## APPENDICES

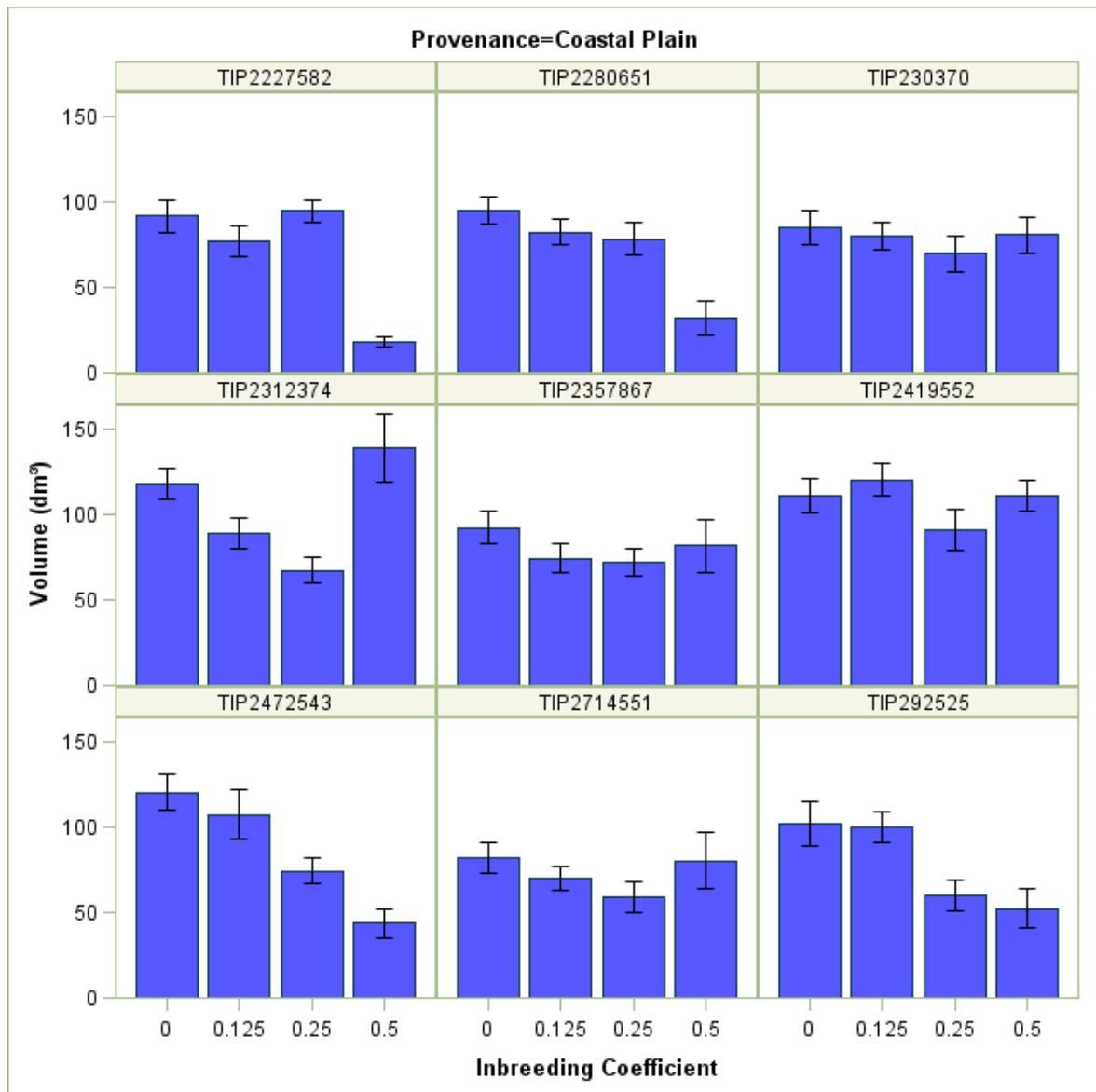
Appendix 1. Age 9 trait means at each of the four inbreeding levels.



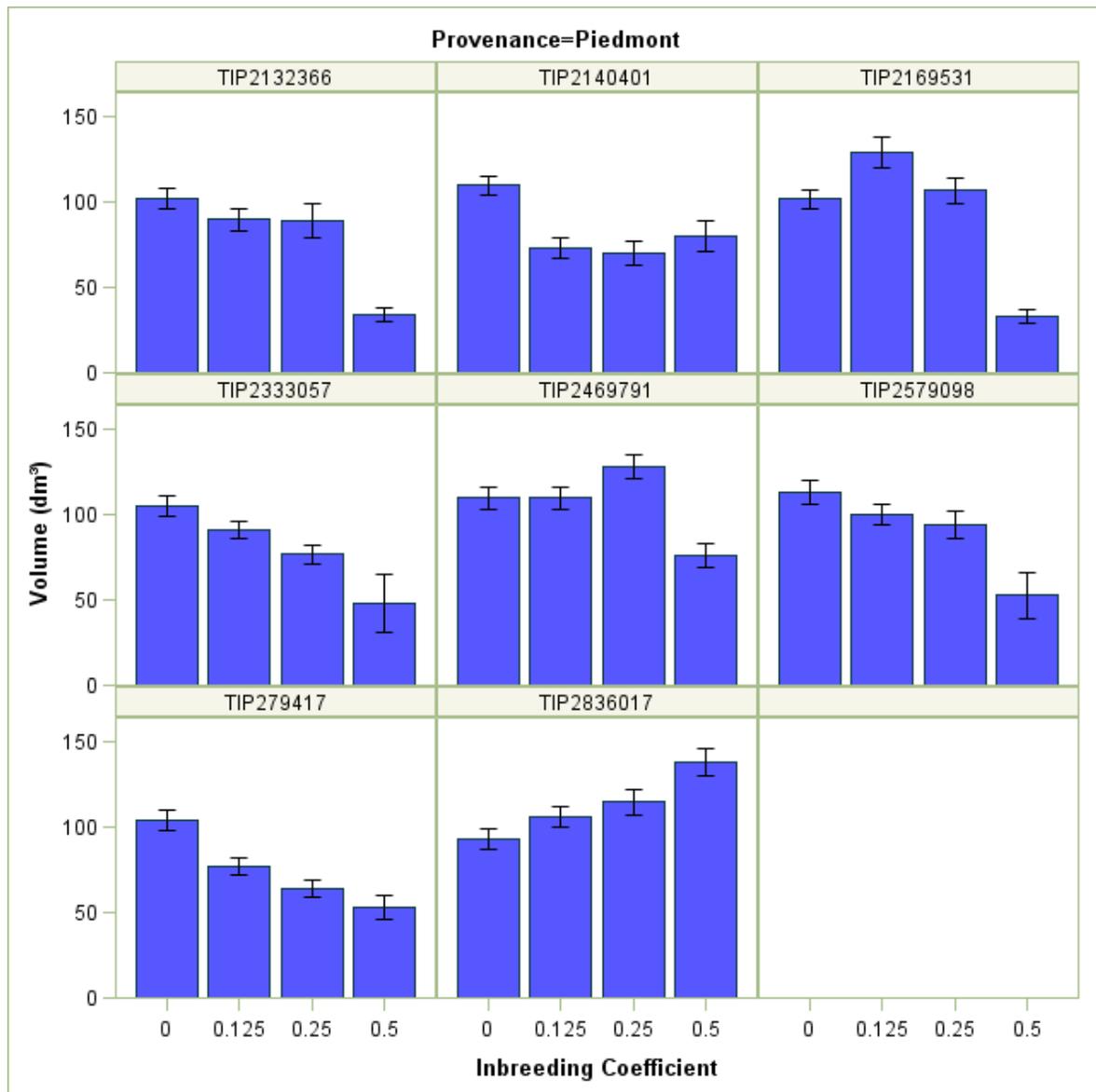
**Figure A1.** Mean height at each of the four inbreeding levels is shown for all Coastal lines independently, illustrating the different responses of lines to increased inbreeding levels.



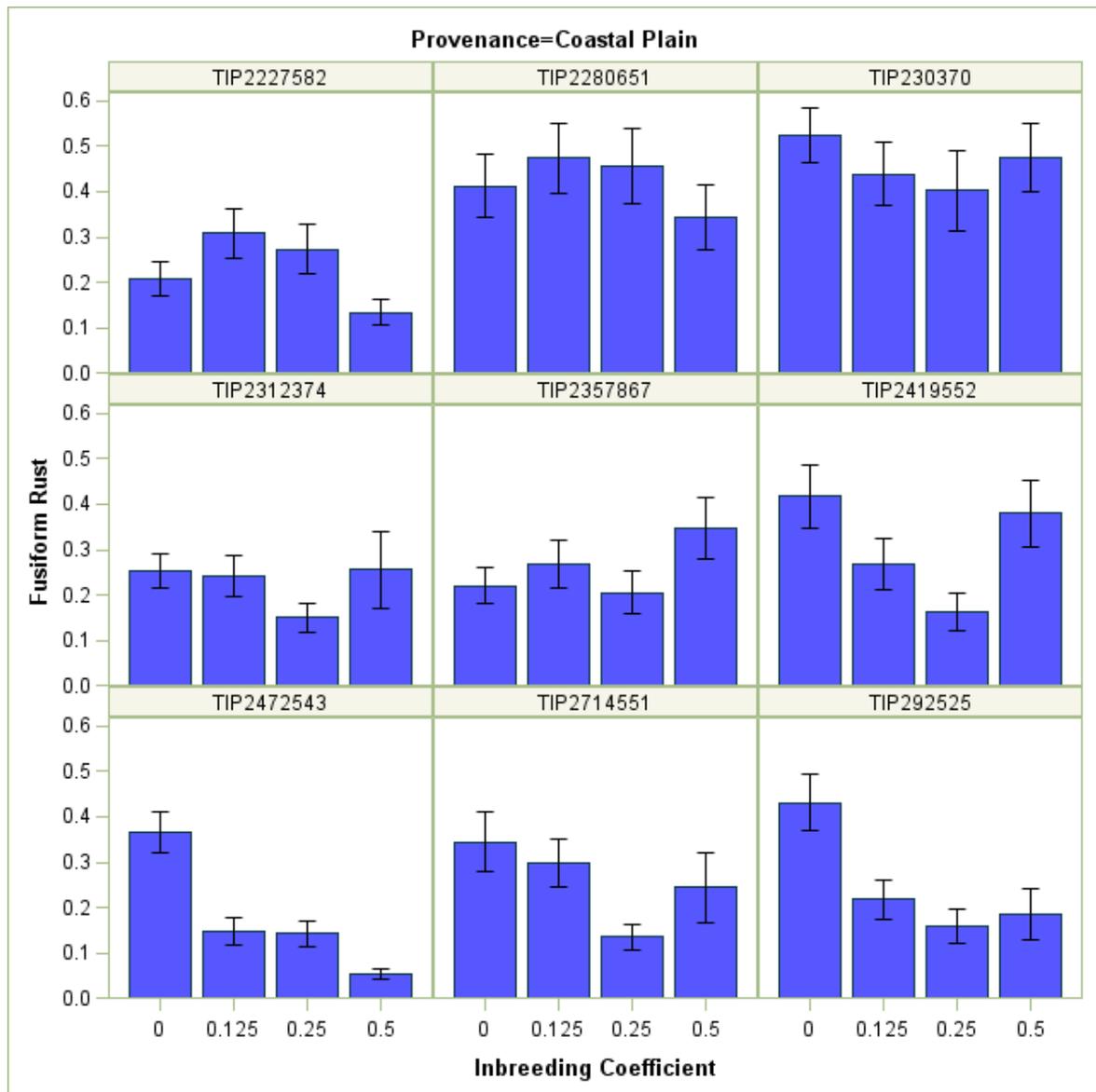
**Figure A2.** Mean height at each of the four inbreeding levels is shown for all Piedmont lines independently, illustrating the different responses of lines to increased inbreeding levels.



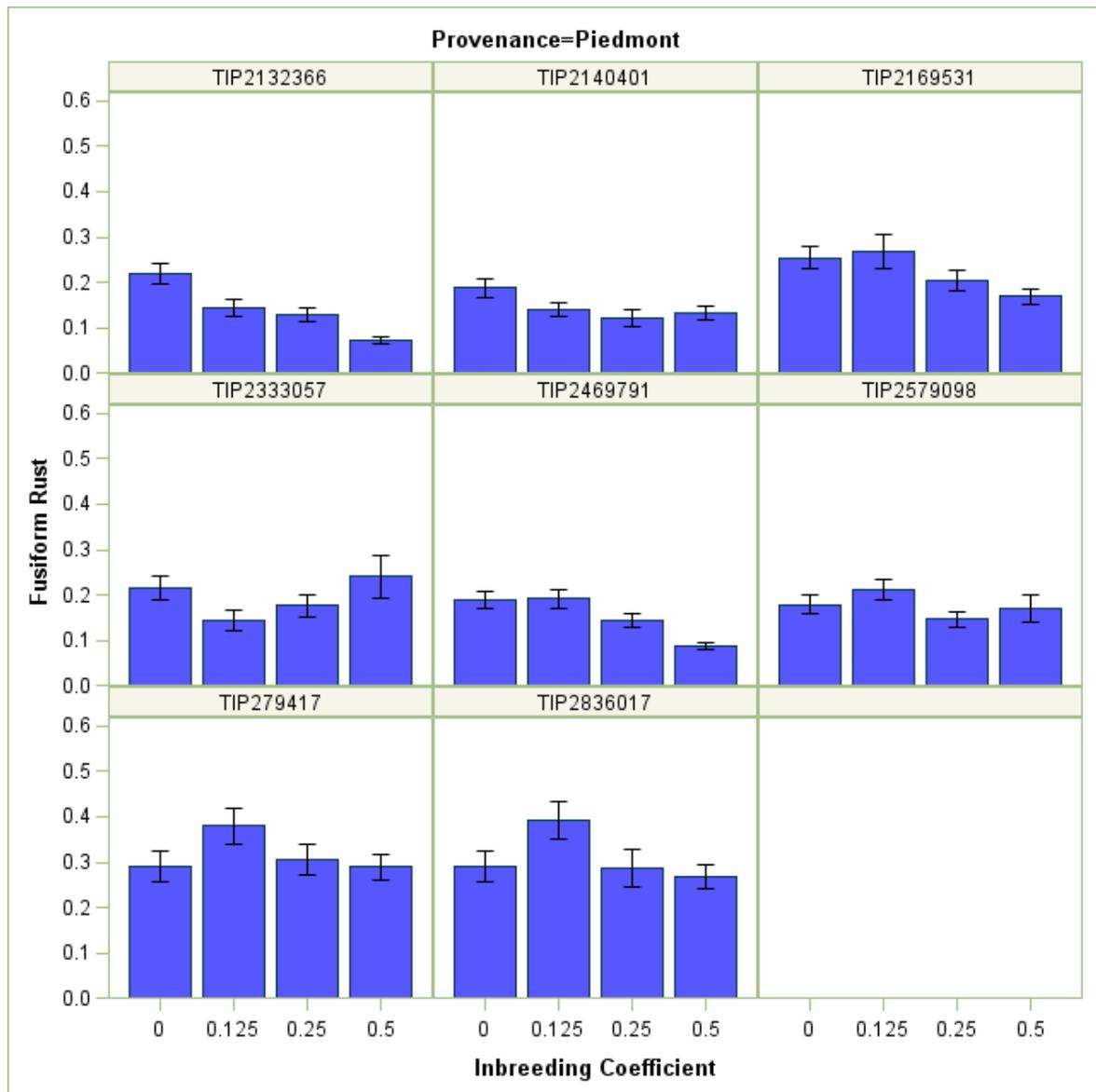
**Figure A3.** Mean volume at each of the four inbreeding levels is shown for all ten Coastal lines independently, illustrating the response of lines to increased inbreeding levels.



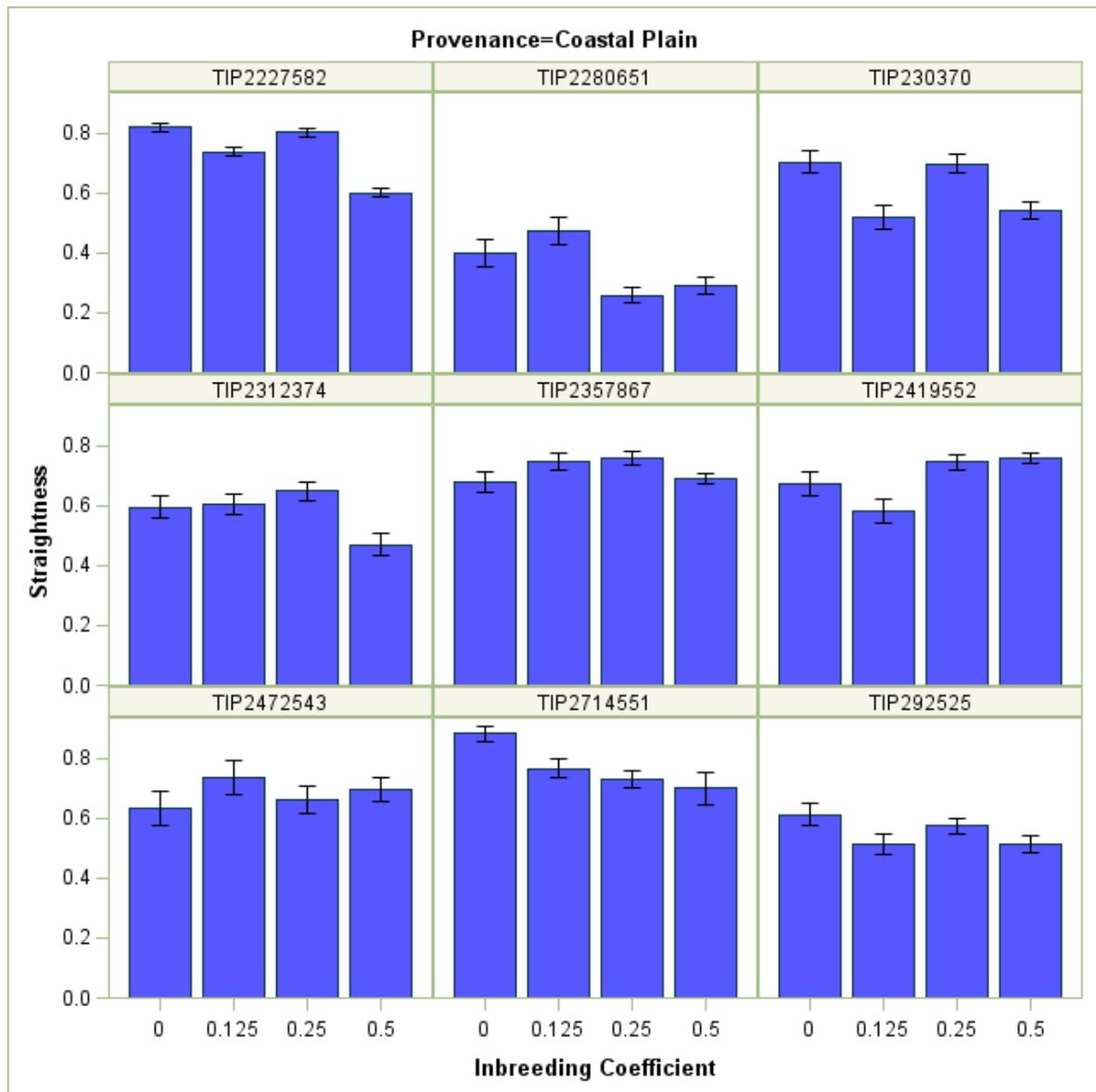
**Figure A4.** Mean volume at each of the four inbreeding levels is shown for all ten Piedmont lines independently, illustrating the response of lines to increased inbreeding levels.



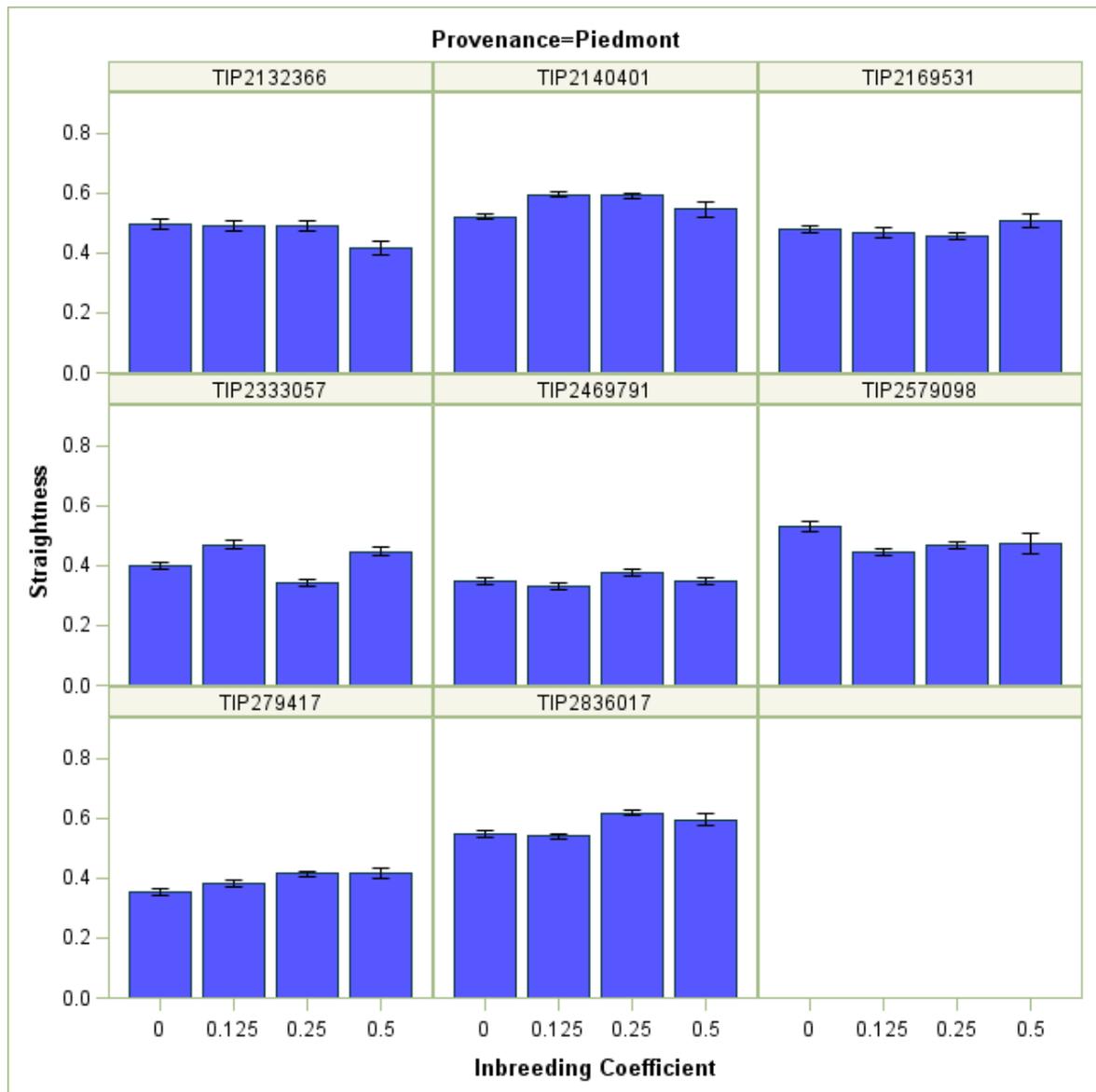
**Figure A5.** Mean fusiform rust at each of the four inbreeding levels is shown for all Coastal lines independently, illustrating the patterns in response to increased inbreeding among the lines.



**Figure A6.** Mean fusiform rust at each of the four inbreeding levels is shown for all Piedmont lines independently, illustrating the patterns in response to increased inbreeding among the lines.



**Figure A7.** Mean straightness at each of the four inbreeding levels is shown for all Coastal lines independently, illustrating the variable patterns in response to increased inbreeding among the lines.



**Figure A8.** Mean straightness at each of the four inbreeding levels is shown for all Piedmont lines independently, illustrating the variable patterns in response to increased inbreeding among the lines.

Appendix 2. Age 23 number of occurrences of forking and ramicorn branch characteristics and fusiform rust at each of the four inbreeding levels.

**Table A1.** Incidences of forking, ramicorn, and fusiform rust.

Mating Type	Low Forking	High Forking	Low Ramicorn	High Ramicorn	Fusiform Rust
Unrelated (F = 0)	5	5	2	0	14
Half-Sib (F = 0.125)	10	6	6	1	8
Full_Sib (F = 0.25)	6	3	6	1	2
Self (F = 0.5)	2	5	2	1	6