Age Affects Genetic Gain Estimates in *Pinus taeda* L. Progeny Tests

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Abstract

In forest tree breeding, genetic gain is often estimated from progeny tests at juvenile ages comparing the performance of specific families against a non-improved checklot. This study evaluated the effects of progeny test age on genetic gain estimates in *Pinus taeda* L. Growth, disease, and stem quality traits were assessed at ages 4, 6, 8, and 12 years across 103 trials planted in the southeastern United States. Results showed that heritability estimates generally increased with age, particularly for growth traits and stem straightness. Breeding values for height and volume increased over time, with stable rankings, while fusiform rust disease incidence (caused by the fungus *Cronartium quercuum* f. sp. *fusiforme*) remained consistent. Stem straightness exhibited less stability. Mean age-to-age correlations of breeding values were high for height (0.81-0.95), volume (0.76-0.96), and rust (0.81-0.95) but were slightly lower for straightness (0.67-0.89). Genetic gain for volume and straightness showed a significant upward trend over ages for a large number of parents. For height, about half of the parents showed a positive change in gain, whereas for fusiform rust disease, more than half showed a decrease in gain over time. The study underscores the importance of considering measurement age in progeny tests, as it impacts genetic gain estimates and selection decisions. Findings suggest that while early measurements accelerate gain per year in breeding cycles, older measurements for growth and stem quality traits provide more reliable estimates of gain for deployment. The linear relationship between gain estimates from older ages can be used to rescale gains at younger ages.

Keywords: *Loblolly pine, tree improvement, breeding value, age effect, gain adjustment, family deployment*

Introduction

Forest tree improvement programs utilize progeny tests to assess the genetic merit of trees for breeding and deployment. Progeny tests are primarily established using experimental block designs with single-tree plots (one tree per genetic entry per block) or row-plots (e.g. six trees per entry per block) to accommodate the testing of hundreds to thousands of genetic entries (White et al., 2007). Progeny tests are also assessed at young ages to maximize genetic gain per unit time in the breeding program. The data from these tests are used to estimate breeding values to measure the genetic performance of seed orchard parents and/or the families collected from orchard parents. Genetic gain, derived from progeny tests, offers an alternative means of presenting the genetic merit of families by comparing the performance (genetic value or breeding value) of a specific family against that of a commonly used genetic entry, which is often a wild non-improved checklot as is the case for loblolly pine (*Pinus taeda*).

In the NC State University Cooperative Tree Improvement Program (NCSUCTIP), genetic gain for *Pinus taeda* is published in a Performance Rating System (*PRS*™). Members of the NCSUCTIP rely on these gain values for seed orchard establishment and the deployment of families in plantations. In contrast to breeding values, gains are more readily understood by foresters and landowners who may not have a background in genetics. These gain values serve as a valuable tool to convey complex information from genetic analyses in a more standardized and understandable manner.

In the *PRS*™, gains are expressed as a percentage improvement over the checklot at the age of six years (McKeand, 2019; Shalizi et al., 2023). For growth traits such as height and volume, the checklot is defined to have 0 % gain and serves as a baseline. Positive values indicate faster growth rates than the checklot, while negative values signify slower growth rates compared to the checklot. Concerning fusiform rust disease,

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caused by the fungus *Cronartium quercuum* f. sp. *fusiforme*, the infection rate for the checklot on a site was expected to be 50 %, which signifies that half of the checklot trees are infected with the disease. A value of 50 % would represent the infection level in a rather high-hazard environment (Walker and McKeand, 2018). *PRS*™ rust values exceeding 50 % imply more susceptibility than the checklot, while values below 50 % indicate more resistance than the checklot. Stem straightness gain represents the improvement of a family over the checklot (set at 0 %) in percentage points, reflecting the probability that a tree is straighter than the wild non-improved tree in the population. The formulas for these gain calculations are presented in the methods section of this manuscript.

In the second and most of the third breeding cycles of *P. taeda*, the age of progeny test measurement for selection and gain estimation was 6 years (McKeand, 1988, McKeand and Bridgewater, 1998). Further research showed that the age of selection could be brought down to 4 years due to high age-to-age correlation of breeding and genetic values (Balocchi et al., 1993; Foster, 1986; Xiang et al., 2003a). Age-to-age correlation of breeding and genetic values have been extensively studied in many forest trees species such as *P. taeda*, *P. elliottii*, *P. contorta*, *P. sylvestris*, *Abies alba*, *A. sachalinensis*, *Picea abies*, *Pseudotsuga menziesii*, *Larix kaempferi*, *Cryptomeria japonica*, and *Chamaecyparis obtusa* (Foster, 1986; Isik et al., 1995; Balocchi et al., 1993; Magnussen and Yanchuk, 1994; Sato, 1994; Jansson et al., 1998, 2003; Xiang et al., 2003ab; Xiaoyang et al., 2003; Vergara et al., 2007; Lai et al., 2014; Diao et al., 2016; Mihai and Mirancea, 2016; Hiraoka et al., 2019; Takahashi et al., 2023). In general, these studies reported strong correlation between breeding values from younger ages with the breeding values from older ages; mean of correlations ranged between 0.50 and 1.00 (averaged 0.77). These studies suggested that trees can be selected at younger ages to accelerate the breeding cycle and increase gain per year. The studies also indicated that measurements on very young trees did not produce similar rankings of breeding values with older ages. In *P. taeda*, age four measurements showed high genetic correlation with data from ages five to eight (Xiang et al., 2003a). The genetic correlation between age four and age eight measurements ranged between 0.80 and 0.90. As a result, the NCSUCTIP decided to measure tests of *P. taeda* trees at age four years to accelerate the breeding program.

While time trends in genetic parameters and correlations among breeding values have been extensively studied, a notable gap exists in our understanding of how genetic gain estimates may change with measurement age. A preliminary comparison of gain values from analysis based on age six- and four-year data revealed unexpected changes in gain over non-improved checklots for a substantial number of genetic entries in the NCSUCTIP breeding program (unpublished data). The changes were attributed to the reduced GCA effect in age four data. This suggests that reducing the measurement age for progeny tests could impact gain values. It was suspected that competition among genotypes might exaggerate gains over the slower-growing checklots at older ages where crown closure has occurred. It was also suspected that gains for straightness might increase for older ages where larger stems would better reflect their genotype.

During the 2nd-Cycle of *P. taeda* breeding in the NCSUCTIP, a subset of progeny tests were repeatedly measured as part of an experiment called the Early Diallel Measurement Study (EDMS) (see Xiang et al. 2003ab for details). The present study utilized the EDMS data to address the change in genetic gain estimated from *P. taeda* progeny tests over time, specifically focusing on growth traits, fusiform rust disease incidence, and stem quality traits.

Materials and methods

Plant population and measurements

The EDMS trials were planted by members of the NCSUCTIP between 1984 and 1994. Detailed information regarding the study design can be found in Xiang et al. (2003ab). In summary, a total of 103 trials from 27 test series (four tests per series) were established across the Atlantic Coastal and Gulf Coastal Plains as well as the Piedmont region in the southeastern US (Figure 1).

The original intention of the study was to evaluate genetic parameters and age-age correlations of traits over time. As a result, these traits were measured at various ages, with some tests extending up to age 20. The majority of the tests had data at ages 4, 6, 8, and 12 years, which were utilized in this study. The number of test series and test sites varied based on trait and age (Table 1). Height had the greatest number of test sites (88), followed by volume (72), fusiform rust incidence (44), and stem straightness (44).

Almost all of the EDMS trials employed in this study were established using full-sib crosses from the 2nd-Cycle Atlantic Coastal, Piedmont, and/or Northern breeding populations of *P. taeda*. One test series was planted with open-pollinated (OP) families from the same cycle. The full-sib crosses were generated through a disconnected six-parent half-diallel mating design (Talbert, 1979). In each half-diallel, a total of 15 full-sib crosses (excluding selfs and reciprocals) were produced (Xiang et al., 2003a). Roughly 30 full-sib crosses, representing two half-diallels, were planted within each test series. Additionally, seedlings from two to four checklots (CC1 - CC8) were also planted in each test series. The checklots consisted of bulked seed from multiple non-improved wild seed sources representing the eight 2nd-Cycle testing areas (CC1: VA Coastal Plain and Piedmont, CC2: NC Coastal Plain, CC3: SC Coastal Plain, CC4: GA-FL Coastal Plain, CC5: Lower Gulf Coast, CC6: Upper Gulf Coast, CC7: GA and SC Piedmont, and CC8: NC Piedmont) (Figure 1). Details about the checklot system are in the Cooperative's 1984 Annual Report (p. 19-22) (Anonymous, 1984). Strong connections existed within each series, where all parents and families were shared in common, but pedigree connections among series were weak, and only checklots (ranging from 0 to 4 checklots, average of 1) could be used to connect the series. Therefore, each test series was analyzed individually.

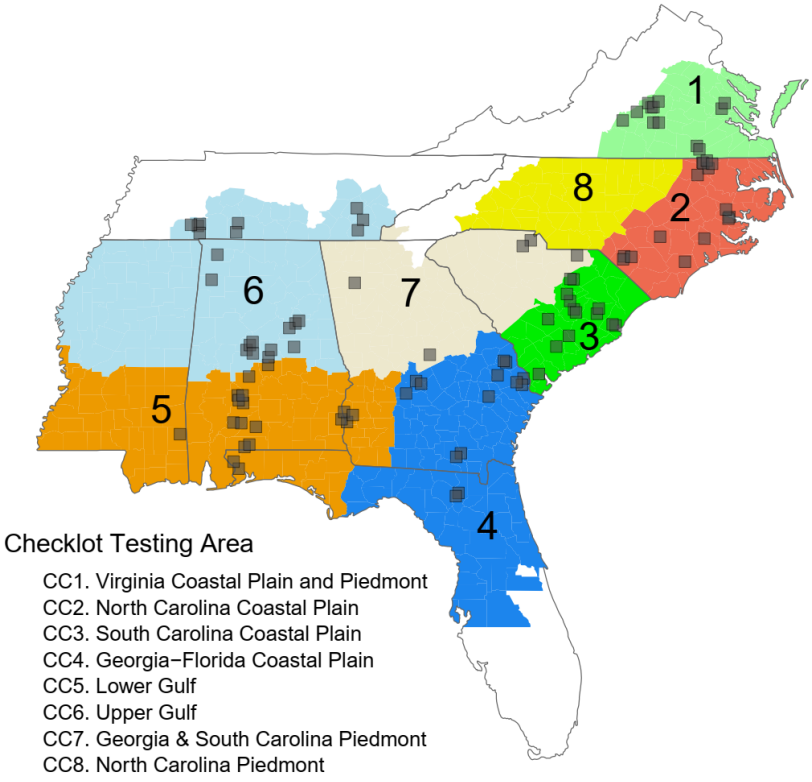

Figure 1
Geographic location of the Early Diallel Measurement Study (EDMS) test sites across the southeastern United States. Each square represents a test site. Background colors represent geographic location of the eight *P. taeda* checklot areas from the 2nd-Cycle testing, east of Mississippi river. The checklots consisted of bulked seed from multiple non-improved wild seed sources within each testing area.

Initially, the EDMS study included 336 parents, 628 full-sib crosses, and the eight checklots. However, the number of parents and full-sib crosses varied among traits in this study due to differences in data dimensions (Table 1). The number of parents ranged from 288 (height) to 132 (straightness), while the number of crosses ranged from 627 (height) to 299 (rust).

Each test site was established using a randomized complete block design with six blocks, each containing all the respective families and checklots in 6-tree row plots. Trees were assessed for stem height (m), diameter at breast height (DBH, cm), the presence of rust galls caused by *Cronartium quercuum f. sp. fusiforme* on the main stem or branches (coded as 1 for diseased and 0 for not diseased), and the incidence of stem straightness (coded as 0 for not straighter than the average tree, and 1 for straighter than the average tree). Individual-tree volume was calculated using the Goebel and Warner (1962) volume equation, expressed in metric (dm^3) form.

Table 1
Number of series, test sites, parents, crosses, and blocks per site in the EDMS trials. Data dimensions were different by trait and age.

Variable	Height	Volume	Rust	Straightness
Number of series	22	18	11	11
Number of sites	88	72	44	44
Number of parents	288	240	157	132
Number of crosses	627	507	299	326
Number of blocks per site	6	6	6	6
Number of trees at age 4	98,881	70,462	52,706	51,480
Number of trees at age 6	97,374	82,630	48,584	48,101
Number of trees at age 8	96,003	82,341	44,631	45,507
Number of trees at age 12	59,515	49,020	33,942	29,899

Statistical analysis

Estimation of breeding values and heritability

Breeding values were estimated using generalized linear mixed models. Each series was analyzed separately by age, which was necessitated by the lack of test connectivity among some test series and the presence of unbalanced data between years. The following linear mixed model was used to analyze height and volume:

$$y = \mu + \mathbf{X}t + \mathbf{Z}_1 b + \mathbf{Z}_2 p + \mathbf{Z}_3 c + \mathbf{Z}_4 pt + \mathbf{Z}_5 ct + \mathbf{Z}_6 bc + e \qquad (1)$$

where *y* is the vector of response variable, *μ* is the overall mean, **X** and **Z** are incidence matrices for the fixed and random effects, respectively, *t* is the vector of fixed test site effect, *b* is the vector of random block effects nested within test site with $b \sim NID(0, \mathbf{I}\sigma_b^2)$, *p* is the vector of random general combining ability (GCA) effect from female and male parents (or checklot) with $p \sim NID(0, \mathbf{A}\sigma_p^2)$, *c* is the vector of random specific combining ability (SCA) from full-sib crosses with $c \sim NID(0, \mathbf{I}\sigma_c^2)$, *pt* is the vector of random GCA by test site interaction effect with $pt \sim NID(0, \mathbf{A}\sigma_{pt}^2)$, *ct* is the vector of random SCA by test site interaction effect with $ct \sim NID(0, \mathbf{I}\sigma_{ct}^2)$, *bc* is the vector of random plot error with $bc \sim NID(0, \mathbf{I}\sigma_{bc}^2)$, and *e* is the vector of random error with $e \sim NID(0, \mathbf{R})$. The random plot effect corresponds to the effect of 6-tree row plot of families nested within rep and test site. The random *b*, *p*, *c*, *pt*, *ct*, and *bc* effects were associated with independent and identically distributed (IID) variance ($\mathbf{I}\sigma^2$), where $\mathbf{I}$ is an identity matrix of its proper dimensions. The GCA variance was associated with the numerator relationship matrix (**A**) calculated from the pedigree. The random residual effect was fit with block diagonal (DIAG) variance structure ($\bigoplus_{i=1}^{t} \sigma_i^2 \mathbf{I}_{n_i}$). This structure provided independent variance for each test site.

The following generalized linear mixed model was used to analyze fusiform rust disease and stem straightness incidence:

$$\eta = log\left(\frac{\pi}{1-\pi}\right) = \mu + \mathbf{X}t + \mathbf{Z}_1 b + \mathbf{Z}_2 p + \mathbf{Z}_3 c + \mathbf{Z}_4 pt + \mathbf{Z}_5 ct + \mathbf{Z}_6 bc + e \quad (2)$$

where η is the logit link function for the vector of response variable (incidence or no incidence), π is the probability of trait incidence, $log\left(\frac{\pi}{1-\pi}\right)$ is the log of odds of the trait incidence, and *μ* is the conditional mean. Other terms in the model were defined previously. Binary traits have no residual variance (*e*); thus, a fixed dispersion parameter $\frac{\pi^2}{3} \approx 3.29$ was used as an alternative error variance in the phenotypic variance (Gilmour et al., 1985).

The random SCA (*c*) and SCA by test site (*ct*) interaction effects were not included in the analysis of the test series that involved only open-pollinated families. The models were fit in ASReml v4.2 statistical software, which uses the Restricted Maximum Likelihood (REML) method to estimate the parameters (Gilmour et al., 2015).

The individual-tree narrow-sense heritability was calculated by utilizing the variance components from equations one and two, along with the derivations outlined in Isik et al. (2017).

$$h_i^2 = \frac{4\sigma_p^2}{2\sigma_p^2 + \sigma_c^2 + 2\sigma_{pt}^2 + \sigma_{ct}^2 + \sigma_{bc}^2 + \bar{\sigma}_e^2} \qquad (3)$$

where σ_p^2 is the GCA variance, σ_c^2 is the SCA variance, σ_{pt}^2 is the GCA by test site interaction variance, σ_{ct}^2 is the SCA by test site interaction variance, σ_{bc}^2 is the plot error variance, and $\bar{\sigma}_e^2$ is the average residual variance. Standard error of heritability was approximated using the delta method (Lynch and Walsh, 1998, Gilmour et al., 2015). The σ_c^2 and σ_{ct}^2 were not included in heritability calculation for the test series that involved only open-pollinated families.

Gain calculation

The breeding values derived from equations one and two were used to calculate genetic gain for each parent. The following equations were used to calculate gain for growth traits (height and volume), fusiform rust disease, and stem straightness, respectively.

$$G_{growth} = \left[\left(\frac{BV_{Parent}}{BV_{Checklot}}\right) - 1\right] \times 100 \qquad (4)$$

$$G_{rust} = [BV_{Parent} - (BV_{Checklot} - 0.50)] \times 100 \qquad (5)$$

$$G_{straightness} = (BV_{Parent} - BV_{Checklot}) \times 100 \qquad (6)$$

These are the same equations used in the *PRS*™, although here they are presented for parents rather than families. Almost all the parents were tested in one series only. However, the checklots were tested in multiple series in more than one 2nd-Cycle testing area, often in the adjacent checklot zones. Thus, each parent was compared against two to four wild non-improved checklots within a series. For simplicity of interpretation, the gain values of parents were averaged within that broad-based locally adapted blended checklots region (e.g., average of CC5, CC6, CC7 for the Upper Gulf Coastal Plain and the Piedmont). The blended gain values were consolidated into a composite dataset for graphical analyses and for calculations of correlations among measurement ages.

Random regression coefficients model

To evaluate the impact of progeny test measurement age on genetic gain, we employed a random regression coefficients model. These models are efficient for assessing changes in gain over time because they allow linear (or non-linear) relationships between gain and measurement age to vary among different parents. As a result, unique intercept and slope estimates are provided for each parent. The following random regression coefficients model was applied to estimate gains in height, volume, fusiform rust disease resistance, and stem straightness.

$$y = \mathbf{X}_1\alpha + \mathbf{X}_2\beta + \mathbf{Z}_1 a_i + \mathbf{Z}_2 b_i + e \qquad (7)$$

where *y* is the gain value (for a particular trait, age, and parent), **X** and **Z** are incidence matrices for the fixed and random effects respectively, α is the overall fixed intercept, β is the overall fixed slope for the logarithmic age effect, a_i is the random intercept deviation for i^{th} parent with $a_i \sim NID(0, \mathbf{I}\sigma_a^2)$, b_i is the random slope deviation for the logarithmic age effect for i^{th} parent with $b_i \sim NID(0, \mathbf{I}\sigma_b^2)$, and e is the random error with $e \sim NID(0, \mathbf{I}\sigma_e^2)$. The random intercepts (a_i) and slopes (b_i) were assumed to be correlated with each other as:

$$V = \begin{pmatrix} a_i \\ b_i \end{pmatrix} = \begin{bmatrix} \sigma_a^2 & \sigma_{ab} \\ \sigma_{ab} & \sigma_b^2 \end{bmatrix} \qquad (8)$$

A separate model was fit for each trait. The logarithmic age values were transformed back to normal values for the results.

Results

Heritability trends across the years

Narrow-sense heritability estimates for height, volume, fusiform rust disease, and stem straightness generally increased from age four to age twelve in most test series (Figure 2). The average heritability increased up to age eight and then plateaued at age twelve for height and volume. Average heritability of fusiform rust did not change after age six. Even at the oldest measurement ages, growth traits and rust had some series with low heritability estimates, and the distribution of estimates had a large range at the oldest age measured (12 years). For straightness, average heritability continued to increase through the oldest measurement age. Further, straightness had consistently higher heritability estimates at the oldest measurement ages compared to younger ages. Higher heritability estimates were correlated with greater general combining ability (GCA) variance (results not shown), which increased over time within most test series (Figure S1). The GCA variance of height and volume increased substantially after age four in many test series and was largely due to the increased means on older trees (Figure S2).

Time trends in growth traits, disease incidence, and stem straightness

Height and volume breeding values increased with stand age and displayed very stable rankings (Figure 3). The average height and volume breeding values of the checklots were lower than the vast majority of parents in this population. The range in height breeding values between the slowest and fastest growing families increased slightly with age, while the range in volume breeding values increased considerably.

To delve deeper, the difference in breeding values for parents and checklots was calculated at each age (Figure 4). In general, the difference in height and volume between parents and checklots increased over time. Concerning height, the magnitude of difference between parents and checklots was large at age four with a difference of 0.79 m between two extreme parents. That difference increased to 2.57 m at age twelve. The magnitude of the difference between parents and checklots increased for most parents as time progressed.

For volume, the magnitude of the difference between parents and checklots at age four was small (3.6 dm^3 between two extreme parents) but increased exponentially at age six and beyond. By age twelve, the magnitude of the difference in volume between parents and checklots was significantly higher (82.2 dm^3 between two extreme parents) than at age four. The majority of parents exhibited greater volume in comparison to the checklots through time.

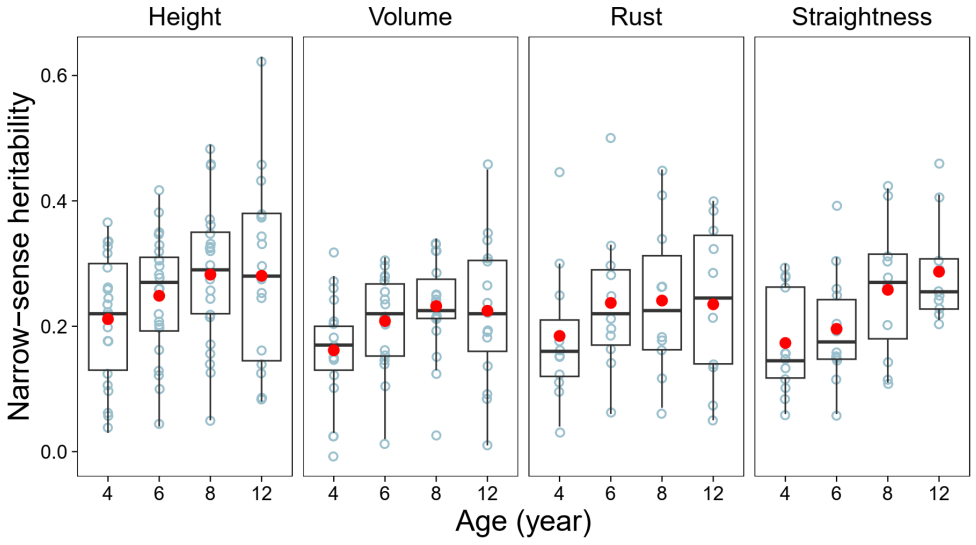

Figure 2

Distribution of individual-tree narrow-sense heritability estimates at each age for height, volume, fusiform rust disease, and stem straightness. Circles correspond to individual series, and the red point represents overall mean. Narrow-sense heritability generally increased from age four to eight and then plateaued for height, volume, and rust but continued to increase for straightness.

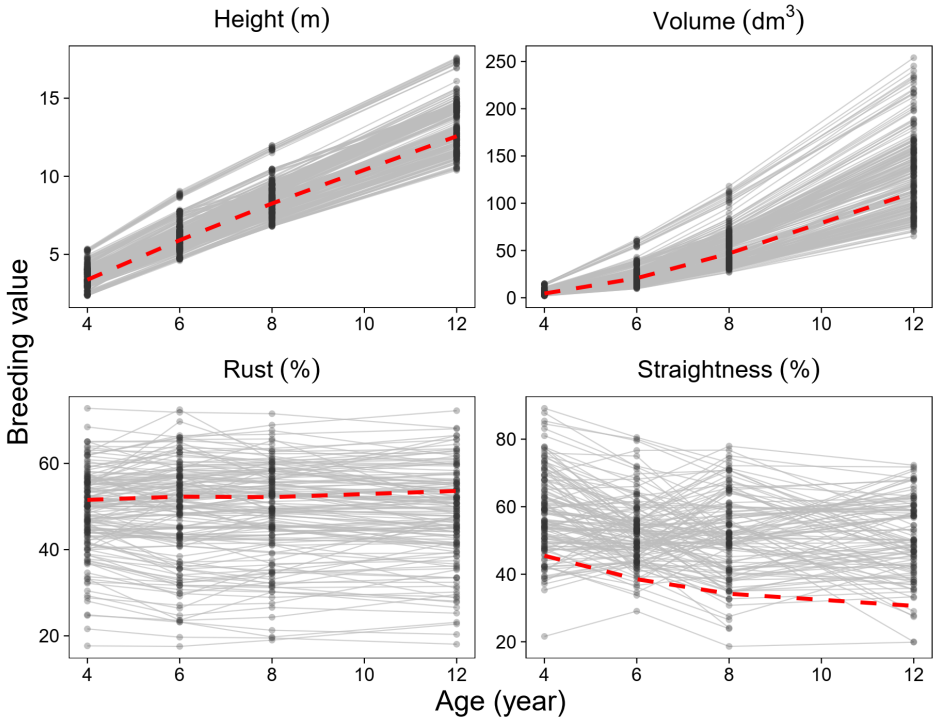

Figure 3
Line plots of height, volume, fusiform rust disease incidence, and stem straightness breeding values for parents over time. The dashed red line represents the CCC checklot which is the average of CC1 – CC8 checklots. Each line corresponds to a parent tested in the EDMS trials. As expected, the non-improved checklots have lower height, volume, and straightness, and higher disease incidence compared to most parents.

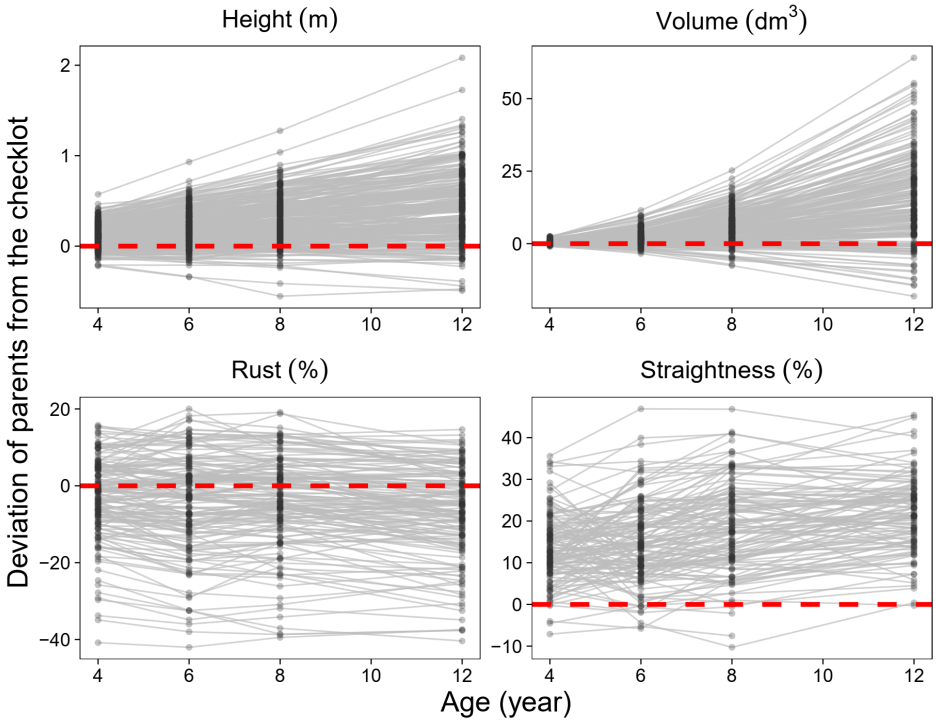

Figure 4
Line plots of the difference in parent and checklot height, volume, fusiform rust disease incidence, and stem straightness breeding value over time. The dashed red line represents the CCC checklot which is the average of CC1 – CC8 checklots. Each line corresponds to a parent tested in the EDMS trials. The difference between a family and checklot growth increases for most families through time, especially for volume. Disease incidence and stem straightness for the non-improved checklot is poor compared to most parents.

For fusiform rust disease, the difference between parents and checklot breeding values was stable over time (Figure 4). This was because fusiform rust breeding values remained very stable for most parents over time (Figure 3). Resistant parents consistently maintained lower breeding values for disease incidence, whereas susceptible parents consistently had higher breeding values through time. Recall that the checklot has a breeding value scaled to 50 % at every age.

Stem straightness breeding values were less stable over time, and there were considerable changes in rankings at different ages (Figures 3 and 4). The stem straightness breeding values decreased for some parents, while others increased over time. However, the average of the checklots' straightness breeding values consistently ranked very poorly at all ages. The checklot average stem straightness decreased consistently from age four to eight and appeared to approach a low plateau at age twelve.

Age-to-age correlation of breeding values

Breeding values estimated at each age within the test series were correlated among age pairs to assess the stability in the performance of parents over time. The mean and range (min-max) of pairwise age-to-age correlations are summarized in Figure 5. Overall, the age-to-age correlations of breeding values were high for height (averaging 0.88), volume (averaging 0.87), and fusiform rust disease (0.89), indicating a stable performance of parents across ages four to twelve. The correlations, though slightly lower, remained high when comparing age-four breeding values with age twelve. Age-to-age correlations of breeding values were slightly lower for stem straightness compared to the other traits. This was particularly notable when comparing the age four straightness breeding values to ages six to twelve (average ranging from 0.67 to 0.73). Of particular interest are the correlations between age four and age six, which averaged 0.88, 0.82, 0.85, and 0.73 among the series for height, volume, rust, and straightness, respectively.

Dynamics of genetic gain over time

The estimated gain for each parent through time is displayed for each trait in Figure S3. Height gain estimates showed slight fluctuations, increasing or decreasing, for most parents between the ages of four and twelve. On the other hand, volume gains exhibited an overall upward trend for the majority of parents through the last age of measurement (twelve years). The trends over time for changes in fusiform rust disease and stem straightness gains were less definitive, as some parents showed increasing gains, some decreasing, some remaining stable, and others experiencing fluctuations over time.

To quantify the impact of age on gain estimates, random regression models provided an overall fixed intercept and slope, along with unique random intercepts and slopes for the parents. The overall fixed slope estimate was not significant for height, but it was highly significant for volume (P-value <

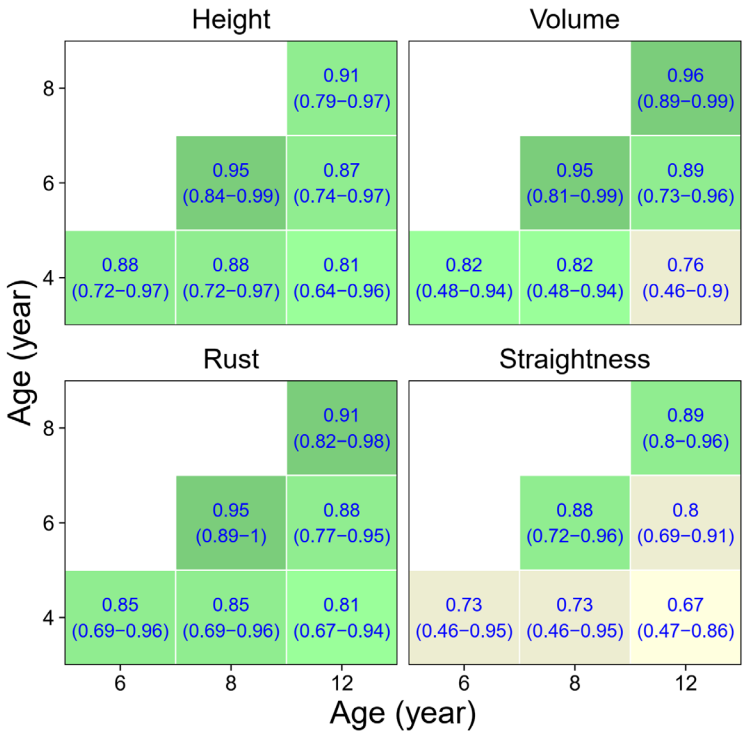

Figure 5

Average age-age correlation of breeding values across test series for height, volume, fusiform rust disease incidence, and stem straightness (minimum and maximum are in parentheses). Age-age correlations of breeding values were higher between adjacent age classes and were higher for growth traits and fusiform rust disease compared to straightness.

0.001). The variance component for the random intercepts was highly significant for both height and volume, explaining approximately 90 % of the variation. The slope variance was also significant, explaining 9 % of the total variation in height and 1 % in volume. The covariance between the slope and the intercept indicated correlations of -0.88 and -0.23 for height and volume, respectively. For rust and straightness, the overall slope estimates were also significant (P-value < 0.001), indicating that gain estimates depended on the age of measurement. The correlation between the slope and the intercept was -0.11 and -0.65 for the two traits, respectively.

Density plots depicting the distribution of parents' slope estimates (Figure 6**)** indicate the change in genetic gain per year (in percentage points) for each parent over time and are positive for increasing gain estimates and negative for those decreasing with age. Parents exhibited a wide range of slope values, suggesting variability in how age affects gain estimates among parents. The random slope estimates ranged from -6.3 to 5.4 percentage points per year for height and from -1.6 to 4.2 percentage points per year for volume. For the trait height, about half of the parents had negative slope estimates, while the other half had positive slopes. In contrast, for volume, the majority of parents (190 out of 240) had positive slope estimates, indicating an overall increase in genetic gain estimates over time for this trait in most cases. For fusiform rust, the slope estimates were negative for most parents, but the magnitude of the estimates was not large (ranged between -1.5 to 0.6). For straightness, the majority of the parents had positive slope estimates (≥ 1) indicating increase gain estimates when tests are measured at older ages.

The regression of age-six gain values on age four revealed a robust linear relationship for height, volume, and fusiform rust disease (Figure 7). Although gains at age four serve as a strong predictor of gains at age six overall, the relationship is not perfect, indicating some changes in gain estimates between these two ages. Specifically, for volume, the gains, on average, were approximately 3 % higher compared to the gain values at age four. The correlation between age four and age six gain values was high (0.91 to 0.95) for height, volume, and fusiform rust disease. However, straightness gain values at ages four and six showed a weaker correlation (0.57), with the regression model explaining only 33 % of the variation.

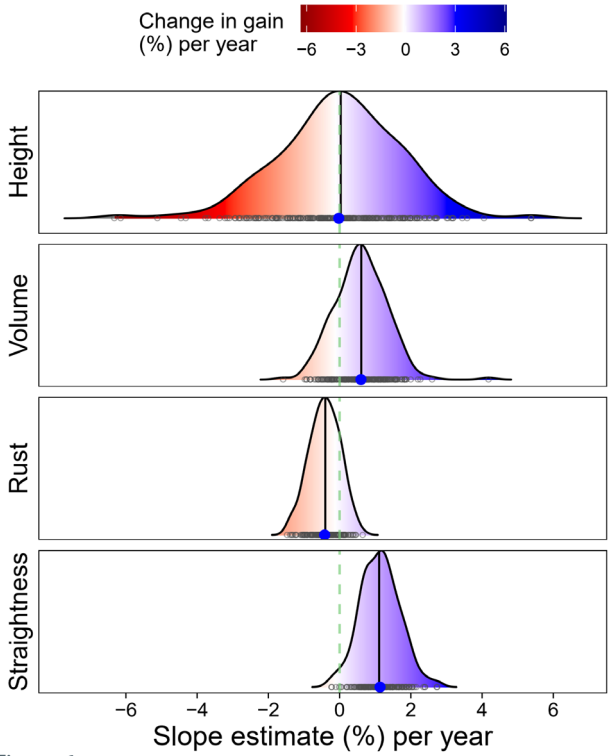

Figure 6

Distribution of parents' slope estimates from random regression of genetic gain (over checklots) against age for height, volume, fusiform rust, and stem straightness. The random slopes were adjusted for the overall fixed slope effect. The units are change in percentage points per year. The vertical solid black lines in the middle of density plots correspond to the median, the blue points are the overall mean, the dashed green lines are the zero-reference, and the gray circles beneath density plots are parent-wise slope estimates. Annual change in gain estimates varied among parents for all traits, especially height. The volume and straightness gain estimates tended to increase with age, while height did not have a trend among parents.

Discussion

This study evaluated repeated measurements of progeny tests from ages four to 12 years. Genetic variance and heritability estimates for growth traits, fusiform rust disease incidence, and stem straightness increased with older measurements (age six and beyond). This was especially true for stem straightness, where heritability was increasing at the oldest age evaluated, while the other traits had plateaued in their estimates of heritability. Increasing heritability for growth traits (height, DBH, volume) has been widely reported in the literature for forest tree species including *P. taeda* (Foster, 1986; Isik et al., 1995; Balocchi et al., 1993; Magnussen and Yanchuk, 1994; Jansson et al., 2003; Xiang et al., 2003ab; Xiaoyang et al., 2003; Lai et al., 2014; Diao et al., 2016; Mihai and Mirancea, 2016; Hiraoka et al., 2019). To the best of our knowledge, heritability trends for fusiform rust disease and straightness have had less attention in the literature.

There are a few supposable reasons behind the increased heritability estimates for all traits from older-age measurements. Firstly, in the absence of a major disturbance (hurricane, fire, thinning, etc.), it is reasonable to expect that the phenotypes of older trees should be more reflective of their genotype. As an extreme example, very young trees, say one or two years after planting, will have phenotypes that are highly dependent on small differences in their microenvironment, such as differences in available soil due to variability in planting depth, site preparation, bed/mound size, presence/absence of rocks, soil nutrients, among other factors. Secondly, for growth traits, measurements made in trials with mixed family plots (i.e. the 6-tree family row plots in this study) where crown closure has

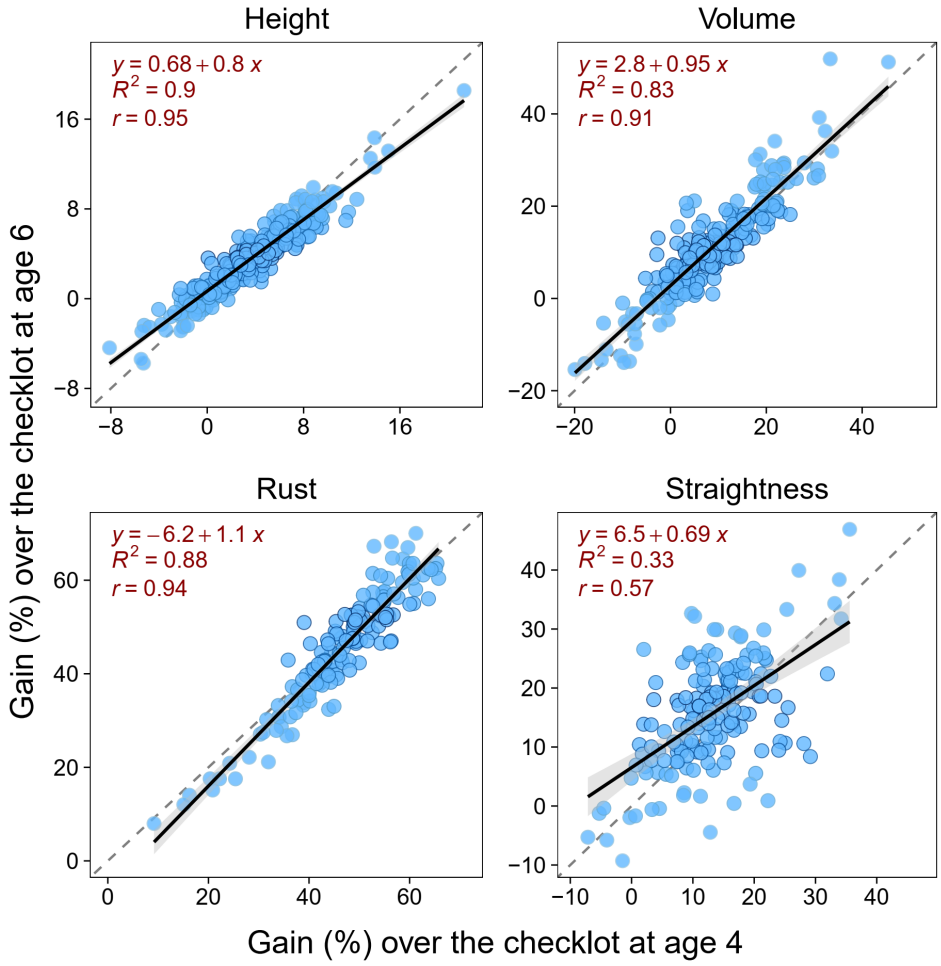

Figure 7
The relationship between age four and age six gain values for height, volume, fusiform rust disease incidence, and stem straightness. The black solid line is the linear regression slope, and the dashed gray line is the 1:1 reference line. Regression equation, model R2, and correlation coefficient are shown on the top left of the figures.

occurred, will have exaggerated genetic variance estimates due to competition among genotypes, where the slower growing entries will tend to be suppressed and overtopped. For fusiform rust disease, trees become infected over time, and it may take some time for symptoms to manifest. Measuring straightness on small trees is challenging, since it is a subjective score, and consistently assessing small deviations in stem sweep and crook on trees with living lower branches is difficult. The continued increase in heritability estimates for straightness through age 12 may be due to more accurate assessment of larger trees with fewer live branches. Another reason may be that trees have had more exposure to stresses that cause deviations from straightness, such as high winds from hurricanes. In more recent cycles, the testing protocol was updated to assess straightness using a 1-6 categorical scale to improve precision.

The difference between parents and the checklot became more pronounced for height and volume as the stands aged. Volume gain was the most sensitive trait affected by age, with the difference in checklot and parent volume increasing substantially at age six and beyond for most parents. The increasing volume gain can be explained in part by competition effects in the row-plot trials, which will influence diameter and hence volume. While the competition was not extreme at these young ages, it did occur as the crowns were closing. The non-improved checklots were among the slowest-growing genetic entries, so they likely became more suppressed over time, and the gain values increased. The ranking of the parents did not change substantially (Figures 5 and S3), but the scale changes as the trees get older over time. This is similar to what is shown in McKeand et al. (1997) for trees of different sizes where family rankings were very similar across tests, but the differences among families were larger on the faster growing sites. The effect of age on height was less sensitive, certainly because tree height is largely independent of competition.

For growth traits, the increasing gain estimates and increasing heritability estimates are co-dependent. When heritability is low, the breeding values will have a reduced spread due to the shrinkage property of the best linear unbiased prediction (Henderson, 1984), so gain estimates will be reduced. Conversely, measurements with higher heritability estimates will result in more confidence in the breeding value prediction, less

shrinkage, and thus a larger gain estimate. However, heritability estimates for growth traits will also be higher in stands with intense competition due to exaggerated differences between the faster and slower growing genetic entries. These two aspects are confounded, although methods to untangle the effects of competition in tree progeny tests have been developed (Cappa and Cantet, 2008; Ferreira et al., 2023). Competitive progeny test models partition the genetic variation among trees' phenotypes into direct and indirect genetic effects, where the direct effect affects the tree's own phenotype, and the indirect effect affects the phenotypes of neighboring trees. Robust estimates of the direct and indirect genetic effects require many blocks per genetic entry, so that there is opportunity for being neighbors with many other genetic entries.

Gain estimates for fusiform rust disease incidence were stable through time, in part due to the definition of the gain calculation (estimated infection for a genotype when the checklot is set at 50 %). Breeding values had strong correlations among ages for fusiform rust disease, suggesting that selection at age four years will be effective. Age-age correlation of breeding values for fusiform rust disease are not common in the literature.

Stem straightness gain estimates fluctuated considerably as the stands aged with no clear trend. While not weak, the correlations between straightness breeding values at age four and older ages were the lowest of the traits evaluated. The lower correspondence among ages is likely due in part to the low precision of how the trait was assessed (0 = less straight than the average tree in the test, 1 = more straight than the average tree in the test). This rather coarse scale resulted in trees changing categories at different ages. In the third and fourth cycles of progeny testing in the NCSUCTIP, straightness was assigned on a six-point scale to improve precision. There is a dearth of literature evaluating the age-age correlations for stem straightness.

Age four and six are two important measurement ages of interest in the NCSUCTIP *P. taeda* breeding program. Trees have been measured at age four years in the fourth-cycle progeny trials to hasten their breeding (Isik and McKeand, 2019), but the vast majority of tests in the second and third cycles were measured at age six years. Thus, most deployment decisions have been based on age six performance. The results of this study indicate that a simple linear regression is appropriate to convert the volume and height gain estimates between age four to six. Such an adjustment factor does not alter the ranking of parents, but it puts the gain values from age four on the same scale as age six gain values. Further research, such as that described by other studies (Carson et al., 1999; Vergara et al., 2004; Kimberley et al., 2015; Shalizi et al., 2023) seek to translate gains estimated from progeny tests to pure family block or clonal plantations.

Conclusions

While correlations among ages were strong, the estimated genetic gains for stem volume consistently increased through time due to a combination of increasing heritability and competition among genetic entries in the progeny tests. Height and fusiform rust disease incidence gain estimates were less affected by age, and age four gain estimates were highly correlated with older age measurements. Stem straightness gains were less consistent among age four and older ages, and heritability estimates suggest older measurement ages may be warranted. Gains estimated from progeny tests are a proxy for the realized gain in plantations, where only the best genetic entries are planted in pure blocks or highly-selected composites. Nevertheless, understanding how gain estimates change depending on age of measurement is important for choosing and managing genetic options.

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