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ORIGINAL PAPER

Phenotypic analysis of first-year traits in a pseudo-backcross {(slash x loblolly) x slash} and the open-pollinated families of the pure-species progenitors

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Abstract A single test, including one pseudo-backcross (*Pinus elliottii* x *Pinus taeda*) x *P. elliottii* and open-pollinated families of the pure species progenitors, was established in North Central Florida in December 2007 to study the transfer of the fast-growing characteristics from a *P. taeda* L. (loblolly pine) parent into the *P. elliottii* Engelm. (slash pine) background. Several traits were measured in the first growing season: height growth, phenology, tip moth incidence, stem traits, crown architectural and needle traits. Heterosis was evaluated for each trait using analyses of variance by fitting a linear mixed model. All traits were significantly (p value < 0.05) different among families while the significance for heterosis varied by trait. Positive heterosis was found for average rate of shoot elongation (ASRE), total growth (TG), total height and number of needles per fascicle while the opposite was true for base diameter, top diameter, fascicle length, fascicle diameter, crown projected area and phenological traits (cessation, duration and day to reach 50% of the height). Average performance (i.e., no heterosis) was found for initiation of growth, number of branches, number of nodes, tip moth incidence, sheath length and specific leaf area. The analyses indicated that introgression of loblolly pine alleles into slash pine was effective and novel trait combinations were achieved. The pseudo-backcross had larger variation in early height growth than the slash pine families and was taller than all open-pollinated families at the end of the first season. Tip moth incidence was much lower than the loblolly pine family.

Keywords Pseudo-backcross · Genetic transfer · Heterosis · *Pinus taeda* · *Pinus elliottii* · Southern pines

Introduction

In Queensland, Australia, the *Pinus elliottii* var. *elliottii* (PEE) x *Pinus caribaea* var. *hondurensis* (PCH) hybrid outperforms either parent on lowland sites with poor drainage (Nikles and Robinson 1989) and it has comparable wind firmness, stem form and wood quality to the parental species (Harding and Copley 2000). As a consequence, this hybrid has almost entirely replaced PEE and entirely replaced PCH in southeast Queensland (Nikles 2000). This hybrid also averages 2.5 times as much volume and with better form than pure PEE in several South African test sites at 13.5 years (Van der Sijde and Roelofsen 1986).

Motivated by the performance of PEE x PCH in Queensland and South Africa, in 1994, the Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida established 11 pine hybrid trials in the Lower Coastal Plain of the southeastern USA with seven taxa, including the F1 hybrid between PEE by *Pinus taeda* (PTA).

In general, a hybrid has phenotypic characteristics intermediate to its parents (Wright 1976; Zobel and Talbert

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1984). However, a hybrid can also strongly display a desired characteristic of one parent and not intermediacy, or sometimes the hybrid can be superior to both parents (Zobel and Talbert 1984). The terms "hybrid vigor" or "heterosis" apply if the hybrids perform better than the average of the parents. Negative hybrid vigor occurs when the hybrid performs worse than the parental average (Wright 1976; White et al. 2007). Nevertheless, tree hybrids have historically had more value as a source of new combinations of genes rather than extra vigor (Zobel and Talbert 1984) and heterosis in forest tree species hybrids is the exception rather than the rule (Fowler 1978).

A cross between a hybrid and either of its parents is called a "backcross" or "pseudo-backcross" if different parents from the same species are used. Backcross breeding is a well-known procedure for the introgression of a target trait from a donor line into the genomic background of a recipient line. The objective is to increase the recipient genome content of the progenies by repeated backcrosses to the recipient line (Bouchez et al. 2002) while maintaining desirable trait(s) from the donor line.

Kinghorn (2000) recommends using backcrossing when only two good parental breeds are available and/or when direct heterosis is not important. Yet many times, one of the two species being hybridized has commercial interest, and the second has only one or a few traits that are more desirable than those found in the first species. In that case, the hybridization followed by one or more backcrosses to the first species is the logical way to incorporate the trait(s) of interest from species two into species one.

Many experiments have used backcrossing to achieve the goal of recovery of the commercial species or the species of interest. The backcross of (*Pinus echinata* x *P. taeda*) x *P. taeda* showed that desirable traits can be combined. Most of the offsprings were rust-resistant (desirable trait from *P. echinata*) and fast-growing (desirable trait from *P. taeda*) (La Farge and Kraus 1980; Kraus 1986). The interspecific cross of *Eucalyptus grandis* and *Eucalyptus globulus* and the backcrosses to the parental species were made to combine favorable adaptability characteristics of *E. grandis* parents with superior wood qualities of *E. globulus* parents (Myburg et al. 2000).

Loblolly and slash pine have adaptative and morphological attributes which make one more suitable than the other in certain circumstances (Barnes and Mullin 1978). Loblolly pine is the fastest growing species of the southern pine group widely known for high-volume production, but generally has less desirable form (Dorman 1976). Results from many studies indicated that loblolly pine grows as well as or better than slash pine on most sites except for very poorly drained flatwood sites on which slash pine outperforms loblolly pine (Borders and Harrison 1989). Loblolly and slash pine have numerous differences in phenology and morphology. Loblolly pine has more branches than slash pine (Xiao et al. 2003) and its branches tend to be longer and fairly large in diameter (Dorman 1976) with shorter needles (Richardson 1998; Chmura et al. 2007). In addition, loblolly pine is more susceptible to tip moth (*Rhyacionia* spp.) than slash pine (Lopez-Upton et al. 2000), which can cause more than 20% decrease in volume yield in loblolly pine after 20 years (Stephen et al. 1982; Cade and Hedden 1987). On the other hand, loblolly pine is more resistant to fusiform rust (*Cronartium quercuum*) than slash pine (Lopez-Upton et al. 1999), has higher specific leaf area (SLA; McGarvey et al. 2004; Chmura et al. 2007) and larger whole tree leaf area than slash pine (Dallas-Tea and Jokela 1991; Xiao et al. 2003; Martin and Jokela 2004; Emhart et al. 2007). In areas prone to hurricanes, slash pine has been shown to be more resistant to wind damage than loblolly pine, although not as resistant as longleaf pine (*Pinus palustris*; Johnsen et al. 2009).

It is well known, from the CFGRP work, that the mean performance of the slash x loblolly hybrids (F1) at age 3 is inferior to that of improved slash pine (negative heterosis) for pest resistance and early growth (Lopez-Upton 1999; Gezan et al. 2004). However, by year 8, the F1 hybrid's growth was equal to the average of the two parental species, and several excellent individuals were found (Huber et al. 2000). Nikles (2000) had similar results reporting a very heterogeneous slash x loblolly hybrid in Queensland with no heterosis but with some outstanding individuals. Barnes and Mullin (1978) also have indicated greater within-family variation in the hybrid between slash and loblolly pine than for the pure species for third-year height.

This study examines the transfer of traits from a loblolly pine parent into a slash pine background utilizing an outstanding F1 individual selected from the CFGRP pine hybrid trials. Growth, pest incidence, crown architecture and needle traits were measured in year 1 at a single North Florida site. This first-stage evaluation allows some examination of the potential utility of introgression of loblolly alleles into slash pine to achieve larger gains in future slash pine breeding. This study is part of the US Department of Agriculture Coordinated Agricultural Project (USDA CAP) grant 'Conifer Translation Genetic Network'. Thus, such traits will be evaluated repeatedly until 6 years of age and SNP markers will be evaluated for effects of the loblolly alleles.

Materials and methods

Study area characteristics and description

The study was planted on December 18, 2007, in Alachua County, Florida (29°44.258′N 82°36.891′W) in a single block. The average annual precipitation for the area is 1,375 mm. The average annual temperature is 20.4°C (NCDC 1971–2000). The soil is classified as Lake fine sand with a slope from 0% to 5%. This Lake soil has low available water capacity, rapid permeability, low natural fertility, low organic matter content of the surface layer, low surface runoff, and a water table at a depth of more than 183 cm. Potential productivity of this soil is moderately high for slash, longleaf (*P. palustris*), or loblolly pines. Seedling mortality is usually moderate because of the droughty conditions of the soil. Weed competition is also moderate (Thomas et al. 1985).

Because the land was previously in grass, site preparation included two tillages (mowing plus disking on July 14, 2007, and disking plus leveling on October 31, 2007). The post-plant maintenance included: replacing dead seedlings on February 15, 2008, banded glyphosate 1.1% weed control in May and June, hand-weeding in July (for persistent weeds) and fertilization with NPK (10:10:10) at 270 kg/ha in June 30.

Genetic material and experimental design

The slash pine x loblolly pine (PEE x PTA) F1 hybrid progeny planted in the CFGRP pine hybrid trials in 1994 was produced by controlled pollination of first-cycle slash pine mother trees with a loblolly pine pollen mix (Lopez-Upton 1999). Within the PEE x PTA F1 hybrids, 30 selections were made for growth and disease resistance at year 8, and needle samples were sent to two genetic marker labs. The results showed that 5 of the 30 F1s were from a desirable loblolly male parent. Those genotypes were selected for inclusion in an introgression program (Gezan et al. 2005). The F1 hybrid (SL1) is one of these five elite selections where the slash pine female parent (Slash1) was an original slash pine selection. The pseudo-backcross (BC1) of SL1 with a third-cycle slash pollen parent (Slash3) was performed in 2005 (Fig. 1) with seeds available in fall 2006. These seeds along with open-pollinated seeds from the slash and loblolly pine ancestors of the cross were grown by Plum Creek Timber Company and were used to establish the trial (Huber et al. 2007).

The trial was planted in a single block (0.89 ha) using a Latinized row–column design with single-tree plots spaced at 1.82×3.05 m. The Latinized design was used to improve the allocation of the treatments across the field (Williams et al. 1999). The trial included the BC1, one open-pollinated family from the loblolly pine progenitor (Lob_OP) and two open-pollinated families from the slash pine progenitors (Slash1_OP and Slash3_OP) (Table 1).

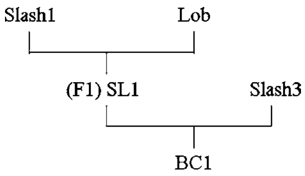

Fig. 1 Pedigree of the pseudo-backcross family (*BC1*). *Slash1* and *Lob* were first-cycle selections and *Slash3* was a third cycle. *SL1* corresponds to a slash x loblolly F1 hybrid selection

Table 1 Details of the families and the number of seedlings used in the backcross study

Family	Number of seedlings
Slash1_OP	282
Slash3_OP	228
Lob_OP	352
BC1	738
Total	1,600

Traits evaluated

Evaluation of height (cm) of the backcross and pure species seedlings began on February 15, 2008, and with repeated evaluations until the end of the first growth period, i.e., December 30, 2008 (Table 2). For each height measurement, a graduated pole was used to measure the distance from the ground to the tip of the highest bud. The phenological traits initiation day, 50% growth day (AG50) and cessation day for height growth were estimated using a linear interpolation to determine when the plants reached 5%, 50% and 95% of their annual growth, respectively

Table 2 Date and day of year for the height measurement of the backcross study

Number	Date	Days of year 2008
1	February 15	46
2	March 10	69
3	March 16	75
4	March 24	83
5	March 31	90
6	April 07	97
7	May 08	128
8[a]	June 10	161
9	July 14	195
10	August 13	225
11	September 11	254
12	October 17	290
13	November 14	318
14	December 30	364

[a] Due to sampling problems the eighth measurement was not used in analysis; January 1st is day of year 1

(Mirov et al. 1952; Hanover 1963; Jayawickrama et al. 1998; Emhart et al. 2006). The other traits considered were: duration of the growing season (*D*, days) estimated as the difference between the initiation and the cessation day; TG (cm) as the difference in height between initiation and cessation date and ASRE (cm/day) as the ratio of TG to *D*. Evaluation of pest incidence was part of the measurement protocol; however, tip moth was the only pest present. Tip moth incidence was recorded for each plant for all 14 measurements as present or absent and accumulated as once present always present.

Stem and crown architectural traits were recorded only at the last measurement; these were: basal diameter (mm) measured twice at the base of each seedling, one perpendicular to the other utilizing a digital caliper; top diameter (mm) measured just below the bud on the top woody part of the tree, also utilizing a digital caliper; total number of nodes on the primary stem; and number of primary branches. Other variables were derived from these measurements: taper, calculated as top diameter divided by base diameter; base diameter divided by total height (BDTH); number of nodes divided by total height (NTH); number of branches divided by total height (BRTH); and number of branches divided by number of nodes (BRN).

Crown projected area (cm^2) was estimated utilizing digital images and the threshold technique (King et al. 2008). Digital photographs of each tree were taken with a Nikon D40× camera at a fixed distance and elevation from the tree using a white background. A graduated ruler was used as a size reference in image processing. Images were captured from north and south (to avoid shadows) between October and November 2008 in five sessions. Images were processed with the software ImageJ (Rasband 1997–2005) by cropping to the desired area (live canopy of the tree), determining and setting the number of pixels of the known length reference into the software, converting the image to black and white and calculating the projected area of the crown.

A sample of fully expanded needles were collected from each tree to determine: total fascicle length (mm) measured with a graduated ruler to the nearest millimeter, sheath length (mm) measured with a digital caliper and number of needles per fascicle counted in each sampled fascicle. Individual needle surface area was obtained according to Murthy and Dougherty (1997) and was used to estimate SLA: needle radius was measured with a magnified graduated glass (10×) and length with a digital caliper. The sampled needles were then oven-dried for 48 h at 65°C and weighed to the nearest 0.0001 g (XA-100, Denver Instruments, Denver, CO). SLA (cm^{2}g^{-1}) was estimated using the ratio between surface area and dry weight of needles (Gonzalez 2008), and SLA was divided by π to obtain projected SLA.

Statistical analysis

Analyses of variances were performed using SAS/PROC MIXED® software (SAS Institute 2002–2003) except for needle traits that were analyzed with ASReml v.2 software (Gilmour et al. 2006). Distributional assumptions for the residuals for hypothesis testing were examined for all traits. Models with one to four residual variances (by family) were selected by trait using the Bayesian information criteria (BIC; Littell et al. 2002). The statistical linear mixed model for analysis was as follows:

$$Y_{ijk} = \mu + F_i + r_j + c_k + e_{ijk} \tag{1}$$

where Y_{ijk} is the value for the respective trait in the *i*th family (i=1–4) in the *j*th row (j=1–40) and *k*th column (k=1–40); μ is the overall mean; F_i corresponds to the fixed family effect; r_j is the random row effect ~IID(0, σ_{row}), c_k is the random column effect ~IID(0, σ_{col}) and e_{ijk} is the random residual effect of the *i*th family in the *j*th row and *k*th column ~IID(0, σ_e) and for some traits ~Diag(0, σ_{ei}) where i is the family. Additional factors in Eq. 1 were considered for the analysis of crown projected area (the photographic date factor) and for needle traits (repetition within the tree, i.e., fascicle factor).

Using repeatability analysis to estimate the gain in precision from multiple measurements (Falconer and Mackay 1996), five fully elongated fascicles were taken from a subsample of 100 trees. This analysis indicated that, for most of the needle traits, three fascicles were sufficient to reach the desired level of accuracy; however, the number of needles per fascicle trait required five fascicles per tree.

In addition to the hypothesis of differences among families tested for each trait (α=0.05), a heterosis hypothesis (α=0.05) was also tested, assuming minor gene effects for all traits except for a major effect for tip moth incidence (Lopez-Upton 1999). The hypotheses evaluated were:

$$\text{Minor gene effect } H_0\text{: } \mu_{\text{BC1}} = 0.5{*}[(\mu_{\text{Lob\_OP}} + \mu_{\text{Slash1\_OP}})/2] + 0.5{*}[\mu_{\text{Slash3\_OP}}] \tag{2}$$

$$\text{Major gene effect } H_0 : \mu_{\text{BC1}} = 0.25{*}[\mu_{\text{Lob\_OP}}] + 0.75{*}[(\mu_{\text{Slash1\_OP}} + \mu_{\text{Slash3\_OP}})/2] \tag{3}$$

For Slash1_OP, Slash3_OP and Lob_OP, their sample means correspond to the average of its progeny. These analyses make the assumption that the pollen source for the OP families was equal to the parental value for each family. For example, the first hypothesis assumes that, under no heterosis, the mean response of BC1 is the average between the F1 hybrid and Slash3_OP, where the F1 hybrid is represented by the average of Lob_OP and Slash1_OP.

Results

All phenology and growth traits were statistically significant (p value < 0.05) for differences among families and for the heterosis contrasts except for growth initiation. The mean levels for these traits along with tip moth incidence are shown in Fig. 2. The dashed line indicates the expected values for BC1 under no heterosis. Slash pine families initiated their growth earlier than the loblolly family, while BC1 had a value intermediate between its parents (Fig. 2a). The family patterns were the same as initiation for AG50 except that the BC1 demonstrated heterosis reaching 50% height growth 6 days before the parental average (Fig. 2b). Cessation and duration of growth had similar family patterns (Fig. 2c and d). Slash3_OP completed growth on day of year 281 (the longest duration of growth, 223 days). On the other hand, BC1 had the shortest duration (212 days) because the family stopped growing early in the season (less than the parental average). Total height, total growth and ASRE had similar patterns for the family means in the study (Fig. 2e, f and g). The performance of the two slash families differed considerably; Slash3_OP had the highest rate of growth (0.12 cmday^{-1}), total growth, and was the second tallest family at the end of the season, while Slash1_OP had the lowest rate of growth (0.09 cmday^{-1}), total growth, and was the shortest family at the end of the first growing season. Lob_OP had a lower rate of growth than BC1 and Slash3_OP, plus a shorter period of growth and completed the season shorter than Slash3_OP and BC1. Both slash pine entries had low tip moth incidence (10%), where Lob_OP had more than 50% of its individuals affected. The backcross BC1 showed no heterosis at 20% infection for the weighted parental average of 21% (Fig. 2h).

The coefficient of variation (CV) for height of Lob_OP and BC1 were lower than the slash families before the ninth measurement; however, after that, their CV more than doubled (Fig. 3). Slash families had the lowest final CV when compared with BC1 and Lob_OP performing consistently as a species regardless of the level of improvement.

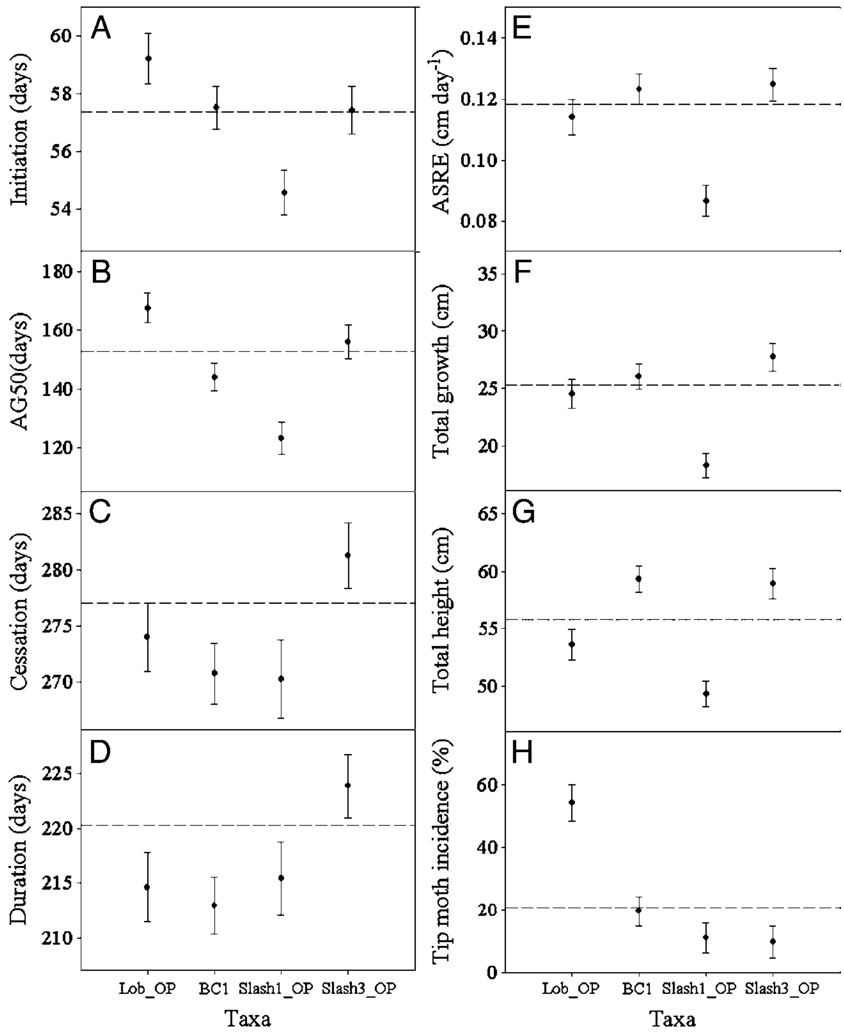

Fig. 2 Least square means and error bars for slash pine families (*Slash1_OP* and *Slash3_OP*), a loblolly pine family (*Lob_OP*) and the pseudo-backcross (*BC1*) for: **a** initiation; **b** *AG50* days for reaching 50% growth; **c** cessation; **d** duration; **e** *ASRE* average rate of shoot elongation; **f** total growth; **g** total height; and **h** accumulated tip moth incidence for 2008 growing season in the backcross study. The *dashed line* indicates the average expectation for BC1 under no heterosis. If the error bar (±2 standard errors) for BC1 does not touch the dashed line, then heterosis is significant

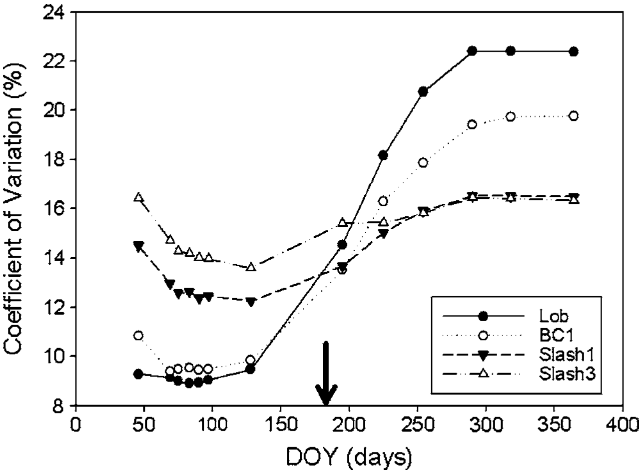

Fig. 3 Behavior of the coefficient of variation (*CV*) for height during the growing season for the four families in the backcross study. *Black arrow* indicates day 181, fertilization event

All crown architectural and foliar variables differed among families (p value < 0.05), while the significance of the heterosis hypothesis varied by trait (Fig. 4a through j). The number of nodes and the number of branches had similar patterns for family means (Fig. 4a and b). Slash3_OP had the highest number of nodes and number of branches, but only slightly higher than Lob_OP family, while Slash1_OP had the lowest values. Slash3_OP had the largest basal diameter. BC1 demonstrated negative heterosis for base and top diameter, performing 18 and 8 mm less than expected, respectively (Fig. 4c and d). The results for top diameter showed that the slash pine families had thicker diameters, and Lob_OP had the smallest mean value. Differences in top diameter among families drove the results for taper (Fig. 4g). Slash pine decreased less in diameter at the top; as an example, Slash1_OP has approximately half of its base diameter at the top. Lob_OP had the largest decrease in diameter from the base to the top, while there was no heterosis for taper for BC1. Lob_OP had more branches per node than the other families. Slash1_OP had the lowest number of branches per node (1.9), while BC1 performed at the parental average (Fig. 4e). The ratio of BDTH indicated that BC1 had the lowest diameter per unit height (negative heterosis) performing outside the parental range and more closely resembling loblolly pine (Fig. 4f). Number of nodes per unit of height and number of branches per unit of height had similar family mean patterns (Fig. 4h and i). Lob_OP had more nodes per unit height and more branches per unit height than other families. BC1 demonstrated negative heterosis for both ratios, and Slash1_OP had the lowest mean values. Slash3_OP had larger projected crown area than the other families in the study (Fig. 4j), more than 40% greater than Slash1_OP, while BC1 had negative heterosis at 82 cm^2 lower area than the expected.

Fascicle length and diameter had the same mean pattern for the families in the study (Fig. 5a and e) and were highly correlated (0.74). Slash families had larger and thicker needles than the loblolly family. There was negative heterosis for both length and diameter for BC1. Lob_OP had slightly more needles per fascicle (3.0) than the slash families (Fig. 5b), while BC1 showed heterosis for this trait. Slash families had lower projected SLA than the loblolly family, and heterosis was not present for this trait (Fig. 5c). In addition, BC1 did not show heterosis for sheath length performing as the parental average (Fig. 5d).

Discussion

The date of initiation and cessation determine the duration of growth, and those factors together with the daily rate of growth determine the total growth for the season (Emhart et al. 2006). BC1 started growth earlier than was estimated from the initial measurement. This was detected because at test establishment all families were near the same height (not recorded), but BC1 was larger at the first measurement (initial height). The phenological results for loblolly pine from this study were in partial accordance with Parisi (2006), where initiation and cessation of growth for seedlings at a Florida site in the second year were the 83rd and 266th day of year, respectively. Initiation differed by more than 20 days, whereas cessation only differed by 8 days. These differences could have been caused by site-to-site variation (Parisi 2006) or year-to-year variation (Jayawickrama et al. 1998; Emhart et al. 2006).

BC1 demonstrated positive and negative heterosis for several traits and no heterosis for others. While additive gene actions explain parental average traits, heterosis is supposed to be due to non-additive gene action (Shull 1908; Bruce 1910). Heterosis has been found to be primarily due to dominant gene action in maize, and there is some evidence for epistasis in rice (Franco et al. 2008). In this study, positive heterosis was present for all height growth traits, meaning that BC1 performed better than the parental average. In contrast, Lopez-Upton (1999) found that the F1 hybrid had negative or no heterosis for growth traits. Similar results have been seen in other species with Major et al. (2003) reporting negative heterosis in mature trees for the intercross between *Picea rubens* x *Picea mariana* F1 hybrid, whereas the backcross to *P. mariana* produced positive heterosis for seedlings and mature trees.

The goal of the backcross program was to introduce the fast growth properties of loblolly into slash pine. With a larger base of taxa and families within taxa with the same degree of improvement and several trials, Lopez-Upton (1999) found that loblolly pine grew faster and was consistently taller than slash pine at 3 years. In this study,

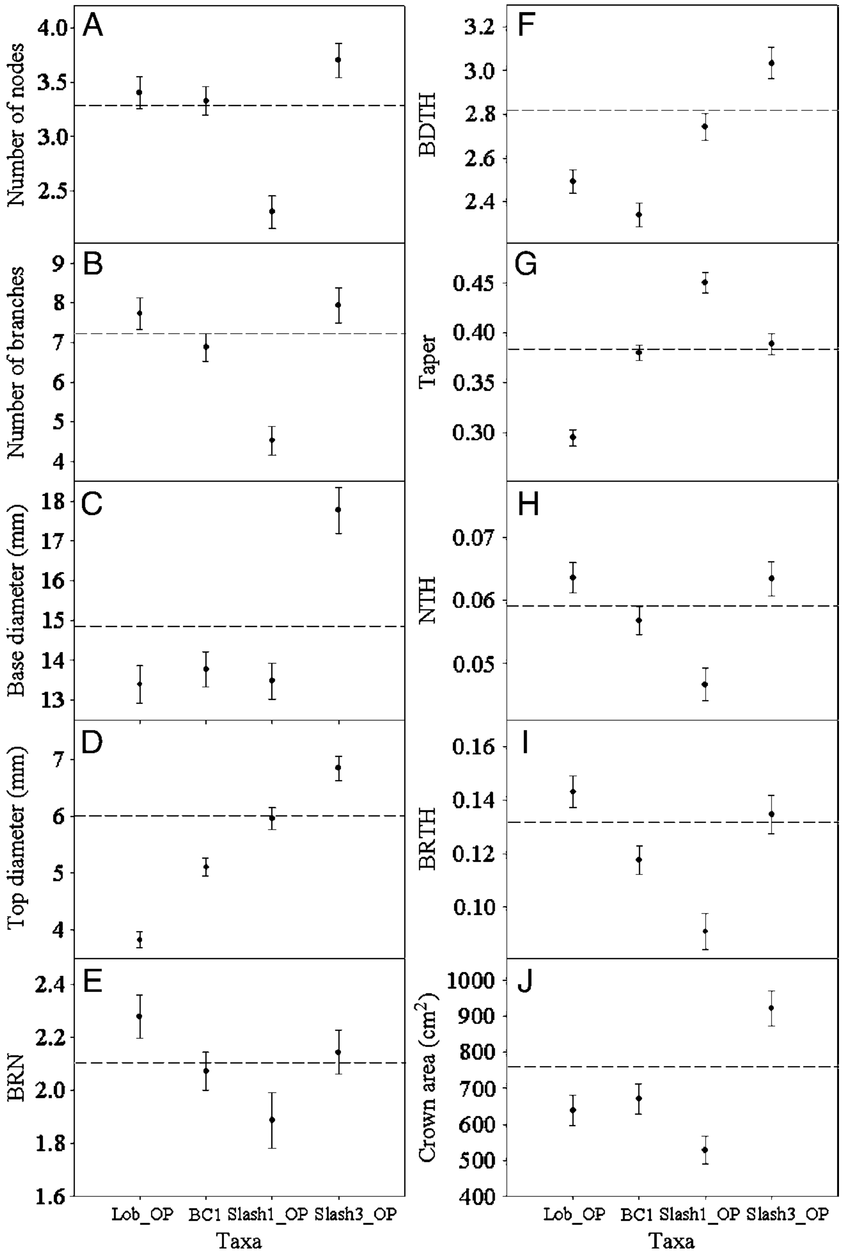

Fig. 4 Least square means and error bars for slash pine families (*Slash1_OP* and *Slash3_OP*), a loblolly pine family (*Lob_OP*) and the pseudo-backcross (*BC1*) for: **a** number of nodes; **b** number of branches; **c** base diameter; **d** top diameter; **e** *BRN* number of branches per node; **f** *BDTH* base diameter per unit height; **g** taper; **h** *NTH* number of nodes per unit height; **i** *BRTH* number of branches per unit height; **j** Crown-projected area for 2008 growing season in the backcross study. The *dashed line* indicates the average expectation for BC1 under no heterosis (see caption in Fig. 2)

the loblolly family was shorter than Slash3_OP but larger than Slash1_OP for height at age 1. This observation did not follow the expectation and could be due to early evaluation or the quality of the soil since it is expected that loblolly pine would outperform slash and the BC1 under moderate fertilization (Colbert et al. 1990).

The influence of loblolly pine alleles was evident in tip moth incidence for BC1; however, this was also a successful backcross because the performance of BC1 for tip moth incidence was much closer to slash than loblolly. The confirmation of the successful gene transmission by the recurrent slash parent comes from previous evaluations of the slash x loblolly F1 hybrid at age 1. Lopez-Upton et al. (2000) showed that loblolly pine families and the loblolly x slash F1 hybrid families had similar levels of incidence and much higher tip moth incidence than slash pine families. These results were consistent with the hypothesis that loblolly was conferring susceptibility to the hybrid. Even when the slash families came from different improvement cycles, there was no evidence of change in tip moth resistance. This result agrees with Lopez-Upton et al. (2000), where there was no statistical difference between improved (first cycle) and unimproved slash pine families for tip moth incidence, indicating that selection for growth and rust resistance had not changed the ability of the species to sustain low rates of tip moth incidence. In this study, it was shown that tip moth resistance could be transmitted from slash pine to the backcross. Considering

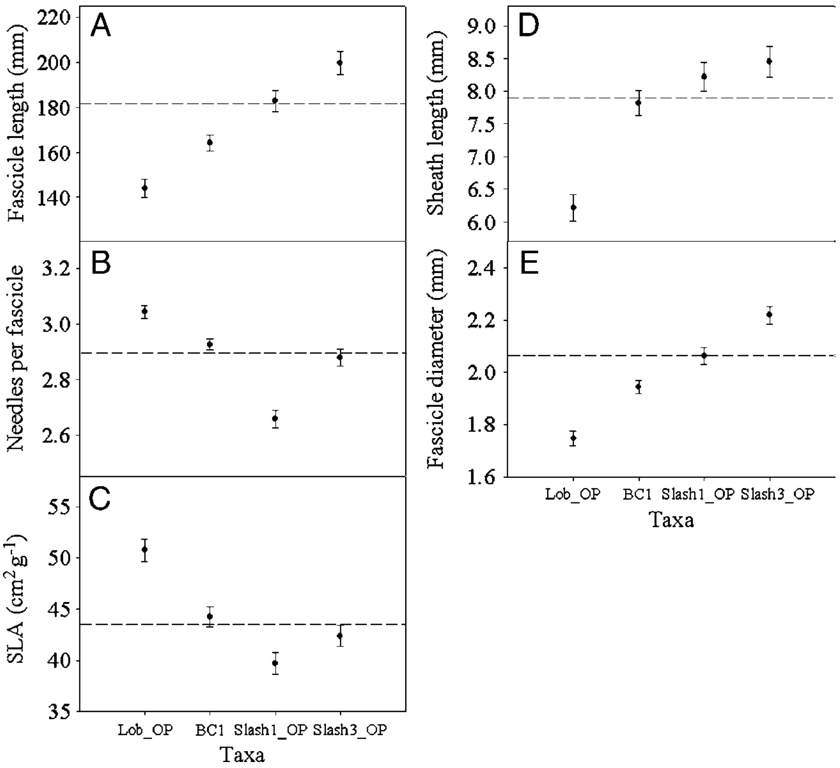

Fig. 5 Least square means and error bars for slash pine families (*Slash1_OP* and *Slash3_OP*), a loblolly pine family (*Lob_OP*) and the pseudo-backcross (*BC1*) for **a** fascicle length; **b** number of needles per fascicle; **c** *SLA* projected specific leaf area; **d** sheath length; **e** fascicle diameter for 2008 growing season in the backcross study. The *dashed line* indicates the average expectation for BC1 under no heterosis. If the error bar for BC1 does not touch the dashed line then BC1 demonstrated heterosis

the importance of this trait in volume yield in loblolly pine (Stephen et al. 1982; Cade and Hedden 1987) and the possible trait combination shown here, the use of slash hybrids appears to be a logical strategy to improve tip moth resistance in US southern pines.

Based on more than 200 progeny plants per family, it was expected that the CV were similar among families, although the BC1 family had more variation in early height growth than the slash families. BC1 was apparently influenced by the loblolly pine parent whose family had the largest CV for height growth, while the slash families had consistently low variation. Higher variation could potentially translate into higher genetic gains if the pattern continues. On the other hand, this BC1 could be introduced to a slash improvement program as an infusion to increase variation or provide greater environmental response.

In pure pine species, branch traits are under moderate to strong genetic control (Ehrenberg 1963; Strickland and Goddard 1965; Emhart et al. 2007). With no apparent heterosis, branch traits seemed to be controlled by additive effects. This could be an advantage in a potential improvement program, where, if the observed pattern continued, parents with desirable branch trait levels should result in a desirable hybrid.

Slash pine families had longer and thicker needles than the loblolly pine family similar to results reported by Richardson (1998) and Chmura et al. (2007). Chmura et al. (2007) found needle length of 228.3 and around 160 mm for slash and loblolly, respectively, in a second-year evaluation. These values were larger than for this study for slash (around 200 mm) and loblolly (144 mm). Once more, these differences demonstrate the genetic variation within species. More than 50% of the time slash pine had three needles per fascicle contrary to other studies that characterized slash pine as two needles per fascicle with a few threes (Dorman 1976). The higher SLA found in loblolly pine over slash pine families and BC1 was in accord with Chmura et al. (2007), Will et al. (2001) and McGarvey et al. (2004). McGarvey et al. (2004) found SLA of 155–187 and 128–131 $cm^2 g^{-1}$ upper and lower crown for loblolly and slash, respectively. SLA did not explain the growth differences between slash and loblolly families, as stated by Marron and Ceulemans (2006) for poplar hybrids, while between the slash pine families, higher SLA was associated with higher growth.

BC1 was more efficient in terms of height growth given the rate of growth and size at the end of the season with smaller crown area than Slash3_OP. Even when crown area was standardized by size (height and diameter, data not shown), BC1 was still more efficient.

The slash pine families were significantly different as was expected. Slash1_OP is from an original wild selection, and Slash3_OP is a third-cycle open-pollinated family. The CFGRP analyses indicated that Slash3_OP had approximately twice the breeding value for volume of Slash1_OP.

In conclusion, the analyses indicated that introgression of loblolly pine alleles into slash pine was effective and some novel trait combinations were achieved. The backcross had larger variation in early growth than slash pine and is taller than both slash families at the end of the season with lower tip moth incidence than the loblolly pine ancestor. A drawback was the presence of the negative heterosis for the ratio of diameter to height found in BC1; the backcross was taller with lower relative diameter than slash and loblolly pine families. This could affect an improvement program where the final goal is total volume. If similar trait patterns are maintained in future crosses, then the prediction of hybrid response traits that did not show heterosis will be possible, and hence selection of pure species parents to produce hybrids is recommended. On the other hand, for those traits that showed heterosis, predictions will not be possible, where cross-specific studies will be necessary to know the performance of the different traits. This study is based on the first measurement of a plan of annual measurement that will help to understand the developmental behavior of the BC1 and to estimate age–age correlations in this specific and unique cross, also QTL, analysis based on tracking the loblolly SNP alleles, which will be performed in the near future.

At present, the tree improvement programs of the southeastern USA have material with high levels of improvement for both species. The existing genetic material could be used to obtain hybrids improving the performance of those traits that appeared to be highly influenced by the poor performance of the first-cycle slash ancestor (Slash1_OP). A backcross, such as BC1, could be introduced in the slash improvement program as an infusion of new material to increase the variability and to introduce new alleles of commercial interest.

Finally, in this study, it was demonstrated that, for these southern pine species and if patterns observed here are maintained, hybridization and backcrossing have potential as a way to introduce novel traits from one species to the other. In this respect, hybrids can contribute by either increasing growth or maintaining actual growth gains while improving other traits of commercial importance.

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