Taxon and Family Differences in Survival, Cold Hardiness, Early Growth, and Rust Incidence of Loblolly Pine, Slash Pine and Some Pine Hybrids

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Summary

Seven taxa, including open-pollinated families of improved *P. taeda* (PTA), improved (PEE) and unimproved *P. elliottii* (PEU) and four hybrid's families, PEE x PTA, PEE x *P. caribaea* var. *bahamensis* (PEE x PCB), PEE x *P. caribaea* var. *hondurensis* (PEE x PCH), and the backcross PEE to PEE x PCH, were tested in eight field tests in the lower Coastal Plain of the southeastern (northern Florida and southern Georgia) USA. Each field test included 16 families per taxon and two silvicultural treatments, intensive and less intensive.

Results at three years of age indicated that PTA was the best taxon, and PEE was next. PEE x (PEE x PCH) and PEE x PCB were less affected by cold damage and taller than PEE x PCH, and had height growth similar to the PEE families. All slash x Caribbean hybrids were more susceptible to fusiform rust than PEE. The PEE x PTA hybrid was intermediate for rust incidence and height growth to the PEE and PTA parental species, but mean values were closer to PEE.

Taxon ranking for height growth was not affected by cultural treatments, but differences increased in the intensive treatment. Outstanding healthy trees were detected in all hybrid taxa. Larger hybrid heritabilities (h^2_h) and more within hybrid family variability for height compared to within families of parental species were detected and this may be exploited by selection within hybrid families to find outstanding recombinants. If these early results are confirmed in later years, the technique of reciprocal recurrent selection does not hold much promise since it is based on operational deployment of the F1 hybrids and none of the hybrids tested here outperformed the current pure taxa being deployed (PEE and PTA). The excellent individual trees within hybrid taxa might be useful for breeding purposes in hybrid programs that combine genes from the hybrids back with the parental PEE and PTA species in various combinations to form synthetic taxa.

Key words: Pinus elliottii, Pinus taeda, Pinus caribaea, hybrids, cultural intensity, heritability, early growth, cold resistance, rust resistance, progeny tests.

Introduction

Geneticists have long been aware that species hybridization has the potential to be a valuable approach in developing genetically-improved, domesticated tree varieties. Hybridization creates new gene combinations that can be useful and economically utilized (Namkoong and Kang, 1990). Many characters of hybrids are intermediate to both parents, but sometimes the crosses of two different genotypes produce hybrid vigor or heterosis, which is defined as an excess in vigor for the particular trait of the hybrid over the midpoint between its parents (Burton, 1980). However, heterosis in forest tree species hybrids is the exception rather than the rule (Fowler, 1978). Further, expression of hybrid vigor is dependent on environment, and sometimes due, apparently, to complementarity of desirable traits of the parents (Wright, 1976; Nikles and Robinson, 1988; Martin, 1989).

Species hybridization has proven to be a useful tool for combining specific attributes from one species, for example disease resistance or cold hardiness, with growth attributes of another species (Fowler, 1978). Some studies have shown that the cross of slash pine (*Pinus elliottii* Engelman var. *elliottii*) and loblolly pine (*P. taeda* L.) has at least some potential value (Barnes and Mullin, 1978). Each species has adaptative and morphological attributes that make it more suitable than the other in certain circumstances. Slash pine is more tolerant of poor soil drainage and damage by tip moth (*Rhyacionia* spp.) than loblolly pine, while loblolly pine responds better to intensive management and is more tolerant of fusiform rust caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Powers, 1975; Hood *et al.,* 1985; Colbert *et al.,* 1990).

Slash pine has been used successfully to produce hybrids with its subtropical relatives: *P. caribaea* Morelet var. *hondurensis* Barret *et.* Golfari and *P. caribaea* Morelet var. *bahamensis* Barret *et.* Golfari (van der Sijde and Roelofsen, 1986; Nikles, 1991; Rockwood and Nikles, 1996). Studies in Australia have shown that the slash pine x *P. caribaea* var. *hondurensis* hybrid is superior in volume production to slash pine and at least equal in stem straightness and windfirmness on both well-drained and poorly-drained soils (Nikles and Robinson, 1988). On the other hand, slash pine x *P. caribaea* var. *bahamensis* hybrid have not been tested extensively and *P. caribaea* var. *bahamensis* appears more cold hardy than *P. caribaea* var. *hondurensis* (Luckhoff, 1964; Nikles, 1966; Duncan *et al.,* 1996). The excellent performance of slash pine x *P. caribaea* var. *hondurensis* in other parts of the world motivated the Cooperative Forest Genetics Research Program (CFGRP) to begin a study to assess the potential of this and other hybrids in the lower Coastal Plain of the southeastern USA. In order to be commercially important in this area, any taxon has to be tested against improved materials of slash and loblolly pine. Seasonal freezing temperatures may be the most significant factor limiting potential utility of hybrids with *P. caribaea* var. *hondurensis* and *P. caribaea* var. *hondurensis* given that Caribbean pines occur naturally in frost-free zones and are sensitive to sub-freezing temperatures, hybridization with slash pine improved the resistance to cold temperatures in a greenhouse study (Duncan *et al.,* 1996). Therefore, these hybrids need to be tested in the field where plants could suffer freeze and pest damage.

The primary goals of this paper are to: 1) compare survival, cold damage, fusiform rust incidence and third-year height growth, of seven taxa including four slash pine hybrids [slash x

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loblolly, slash x *P. caribaea* var. *hondurensis,* slash x *P. caribaea* var. *bahamensis,* and the backcross slash x (slash x *P. caribaea* var. *hondurensis*)] against improved and unimproved slash pine and improved loblolly pine; 2) assess levels of hybrid vigor for cold damage, fusiform rust resistance and height growth; 3) determine if intensive management (including fertilization, weed control and tip moth control) changes taxon ranks or hybrid vigor; and 4) determine the amounts of among family and within-family variability in the hybrids relative to the pure native species.

Materials and Methods

Taxa

The cooperators of the CFGRP established a hybrid-species comparison test on 11 field sites in the lower Coastal Plains (LCP) of the southeastern USA. In the eight sites reported here, seven taxa were tested: improved loblolly pine (PTA), improved (PEE) and unimproved slash pine (PEU), the hybrid PEE x PTA and three slash x *P. caribaea* hybrids (*Table 1*).

Thirty superior slash pine clones from the CFGRP were used as female parents for all hybrid combinations. Each slash parent (PEE) was control pollinated with four different pollen mixes: 1) a 30-parent polymix of *P. caribaea* var. *hondurensis* (PCH) to produce the PEE x PCH; 2) a 25-parent polymix of the hybrid PEE x PCH to create the PEE x (PEE x PCH) backcross; 3) a 24-parent polymix of *P. caribaea* var. *bahamensis* (PCB) to produce the PEE x PCB; and 4) a 30-parent polymix of superior clones of *P. taeda* to make the PEE x PTA. All three sources of Caribbean pine pollen were obtained from the Queensland Forest Service in Australia. The loblolly pine pollen was obtained mainly from Atlantic Coastal Plain sources (Florida and Georgia).

Eighteen out of the 30 improved slash clones used to produce the hybrid families were the female parents of the PEE. Thus, the improved slash taxon (PEE) consisted of open-pollinated seed from 18 slash pine clones that are outstanding for volume growth or disease resistance. The PEE seedlot was estimated to have a mean 18.3% volume gain and 37.1% rust incidence (R50), as predicted by the CFGRP. The 18.3% gain is expressed for 15-year volume above unimproved material. A R50 value lower than 50% indicates resistance to fusiform rust (WHITE *et al.,* 1988) and specifically indicates that 37.1% of the seedlings would be rust infected when unimproved seedlings have 50% of rust incidence. The PEE seed came from 11 seed orchards in southeastern USA. There are all possible combinations of clones in these orchards, but no more than five parents were from the same orchard.

The unimproved slash pine families (PEU) consisted of open-pollinated seed from 17 clones intended to represent slash pine as it existed in 1955, before domestication. The PEU seed came from 9 seed orchards in SE USA. All orchards contain plus and unimproved clones and some orchards had been rogued one time, so we tried to compensate for the slight average superiority of the pollen cloud by choosing females that were slightly below average based on their breeding values. Thus, for comparison, the PEU seedlot was estimated to have 4.9% volume gain and an R50 of 51.3%. The improved loblolly taxon (PTA) consisted of open-pollinated seed from 17 of the 30 best available parents (the same 30 clones used in the pollen mix to produce PEE x PTA). Details are not available on breeding values of these parents.

The number of families in each taxon planted in the 11 field trials is indicated in *table 1.* However, sixteen families in each taxon were planted per location. Some of the 30 hybrid crosses did not yield sufficient seeds or seedlings for planting as a single family in all sites as initially designed. Thus, for the hybrids PEE x PCH and PEE x PCB only three sites have 16 „single“ families (*Table 1*).

Nursery Phase and Field Implementation

Pollination for all hybrids was conducted in February of 1991 and 1992, with subsequent seed collection in 1992 and 1993. Open-pollinated seeds of PEE, PEU and PTA were collected in 1992. Both years' seed harvests were combined, cleaned and cold-stored at 5°C. Germination was done in june 1994 in greenhouse conditions, and plants were moved outdoors after transplanting and seedling establishment.

All of the eight field test locations were planted in December 1994 in the lower Coastal Plain USA, which includes seven sites in north Florida and one site in the most southerly portion of Georgia, from 29°07' to 31°11' L.N. (See LÓPEZ-UPTON, 1999, for complete details). These sites covered a wide range of soil groups, from well to poorly drained and site indices from 17 to 20 (meters at a base age of 25 years).

At each location, the field design consisted of a randomized complete block, nested split-plot design, with two cultural treatment levels labeled as High (intensive) and Low (less intensive). High treatment included fertilizer, weed control and insecticide application (LÓPEZ-UPTON *et al.,* 2000). There is a buffer area of 21 m or more between High and Low management

Table 1. – Taxa and number of families tested across 11 field trials.

Taxon female	Male taxon (Pollen)					
	PEE	*PEU*	*PTA*	*PCH*	*PEE x PCH*	*PCB*
PEE	PEE 18		PEE x PTA 19	PEE x PCH[1] 18 + (6)	PEE x (PEExPCH) 20	PEE x PCB 17 + (5)
PEU		PEU 17				
PTA			PTA 17			

[1]) Due to the lack of seedlings, this taxon was planted in eight sites.The more northern sites were not planted.

Due to an insufficient number of seeds or seedlings, bulks lots of two or three families were formed. These are indicated in parenthesis.

treatments on each of the 8 sites to mitigate the effects of fertilizer and insecticide applications. Within each treatment there were 3 complete blocks and a total of 21 taxon plots (7 taxa x 3 blocks). Each taxon whole-plot consisted of 16 families with 5 trees per family row (80 trees per taxon plot). All together 3360 trees were planted at each site (2 treatments x 3 blocks x 7 taxa x 16 families x 5 trees/family). Additionally, two to four border rows of trees were planted to completely surround the three blocks of each treatment. Tree spacing varied by location from 1.5 m x 3.0 m to 1.8 m x 3.0 m.

Variables Measured

The following variables were measured at all eight sites: a) Survival was assessed at 1, 1.4, 2, 2.4, and 3 years of age (after planting) and scored as a binary trait: 0 (dead) and 1 (alive); b) Cold damage was assessed in the early spring of 1996 (1.4 years), and 1997 (2.4 years), and scored as: 0 = no damage, 1 = slight cold damage (less than 25% of the foliage with damage), 2 = severe cold damage (more than 25% of the foliage with damage), 3 = death from cold; c) Rust incidence was assessed at age three years and scored as 0 (absence of rust) and 1 (presence of rust, either branch or stem galls); and d) Height was assessed at age 3 years, and was measured as height of the highest bud.

Statistical Analysis

Data editing

Data from all eight sites were used for rust, cold damage and survival analyses. One site was eliminated from the height analyses due to extensive deer damage, which resulted in an extremely large mean square error (MSE). For all analyses raw, untransformed data were used because MSEs appeared similar across sites and treatments, and MSE did not increase as the mean increased for any trait.

Taxon comparisons and hybrid vigor

A series of analyses of variance (ANOVA) were performed by pooling data across sites and treatments to examine main effects and interactions of all taxa except PEU. This taxon was not included, because no female PEU clone was included in the hybrid crosses. Thus hybrid performance was compared against improved slash pine and improved loblolly pine. The pooled analyses for rust, cold damage and survival were based on individual data (0, 1 observations) using the following linear model:

$$Y_{tijkmn} = \mu + s_t + \alpha_i + s\alpha_{ti} + b_{j(ti)} + \tau_k + s\tau_{tk} + \alpha\tau_{ik} + s\alpha\tau_{tik} + b\tau_{tijk} + f_{m(k)} + sf_{tm(k)} + \alpha f_{im(k)} + s\alpha f_{tim(k)} + e_{tijkmn} \quad (1)$$

where: Y_{tijkmn} is the observation on a nth tree of the mth family of the kth taxon in the jth block in the ith treatment at the tth site,

μ is the population mean,

s_t is the random variable for site ~*NID* $(0, \sigma^2_s)$,

α_i is the fixed effect for treatment (High and Low),

$s\alpha_{ti}$ is the random interaction site by treatment ~*NID* $(0, \sigma^2_{s\alpha})$,

$b_{j(ti)}$ is the random variable for block within site by treatment ~*NID* $(0, \sigma^2_b)$,

τ_k is the fixed effect for taxon (PEE, PTA, PEE x PTA, PEE x (PEE x PCH), PEE x PCH and PEE x PCB),

$s\tau_{tk}$ is the random interaction site by taxon ~*NID* $(0, \sigma^2_{s\tau})$,

$\alpha\tau_{ik}$ is the fixed effect for the interaction treatment by taxon,

$s\alpha\tau_{tik}$ is the random three way interaction site by treatment by taxon ~*NID* $(0, \sigma^2_{s\alpha\tau})$,

$b\tau_{tijk}$ is the random interaction block(site by treatment) by taxon ~ *NID* $(0, \sigma^2_{b\tau})$,

$f_{m(k)}$ is the random variable for family within taxon ~*NID* $(0,\sigma^2_f)$,

$sf_{tm(k)}$ is the random interaction site by family within taxon ~*NID* $(0, \sigma^2_{fs})$,

$\alpha f_{im(k)}$ is the random interaction treatment by family within taxon ~*NID* $(0, \alpha^2_{f\alpha})$,

$s\alpha f_{tim(k)}$ is the random three way interaction site by treatment by family within taxon ~ *NID* $(0, \sigma^2_{s\alpha f})$,

e_{tijkmn} is the error term ~*NID* $(0, \sigma^2_e)$;

where t = 1,...8 sites; i = 1,2 cultural treatments; j = 1,2,3 blocks per treatment; k = 1,...6 taxa; m = 1,...20 families per taxon; and n = 1, ...5 trees per family.

Analyses for survival were performed separately for each date of assessment (5 dates). Also the number of trees that died from cold, rust or other causes was obtained for each measurement to explain mortality for some taxa. A pooled analysis for rust was done with data from the six sites that had more than 15% mean rust incidence in both treatments. The other two sites were not considered, because they had very low rust incidence levels (WHITE and HODGE, 1987). Nevertheless, rust data from all the eight sites were used to obtain the least square means of rust incidence for all taxa across sites. PEU was included in the latter analysis to get the rust incidence means for site-treatment-taxon combination. Thus, PEU was used as a baseline in regression analysis, ranking the infection of all other taxa relative to PEU.

Preliminary analyses for height with model (1), demonstrated that variances were different between pure and hybrid taxa. Therefore, to avoid unequal variances among taxa, the whole-plot means (i.e., the mean of 80 trees = 16 families x 5 trees if all trees living) were obtained to test height differences across sites and treatments. Heights of all trees and rust-free trees were used to compare taxa with the following model:

$$Y_{tijk} = \mu + s_t + \alpha_i + s\alpha_{ti} + b_{j(ti)} + \tau_k + s\tau_{tk} + \alpha\tau_{ik} + s\alpha\tau_{tik} + e_{tijk} \quad (2)$$

where Y_{tijk} is the mean of the 80 trees (if living) of the kth taxon in the jth block in the ith treatment at the tth site and t = 1,...7 sites; i = 1,2 management treatments; j = 1,2,3 blocks; k = 1,...6 taxa for a total of 252 observations. All other effects are as described in model (1).

Moreover, since the standard deviation values for height were increasing as site means increased, an ANOVA was performed to test taxon differences for the coefficient of variation (CV). So, the CV for third-year height was obtained for every five-tree row plot with PROC MEANS (SAS Institute, 1988) and used in an ANOVA (model 1) to test whether within family variance differed among taxa. Only heights from rust-free trees were used in the analysis. This analysis employed row plot CVs as the unit of observation, so in model (1) the subscript „n“ is excluded and the block by family effect is part of the error.

For all analyses, PROC GLM was used to test the significance of random effects (SAS Institute, 1988). Significance levels and estimated means (using LSMEANS option) for fixed effects (treatment, taxon, treatment by taxon) were obtained from PROC MIXED with the Satterthwaite option (LITTELL *et al.,* 1996). A default probability value of 0.05 was established to show significance unless otherwise specified.

To better understand taxon differences, several single degree-of-freedom contrasts were computed. Specific null hypotheses for contrasts were: 1) There are no differences between two taxa such as between PEE and any hybrid; 2) There are no differences between PEE x PTA and the average performance of PEE and PTA; and 3) There are no differences between PEE x (PEE x PCH) and the average performance of PEE and PEE x PCH. The last two contrasts were performed to determine whether or not hybrid vigor was expressed in the hybrid taxon for all traits measured. Those contrasts were calculated based on:

$$\text{Hybrid vigor} = H / ((P_1 + P_2)/2) \qquad (3)$$

Thus, the single degree-of-freedom compared each hybrid mean (H) to the mean of the two parental taxa (P_1 and P_2). A significant contrast indicated that H was not linearly intermediate to P_1 and P_2, and was taken as evidence of hybrid vigor.

Frequency polygons for height growth using only healthy trees were constructed to compare height distributions of different taxa across sites. All observations were adjusted by the height means for sites and blocks and then the height deviations were pooled across sites to form a single polygon for each taxon. Normality was tested with SHAPIRO-WILK tests in PROC UNIVARIATE (SAS Institute, 1990). Also, to test whether median values were different from means, the hypothesis „Median-mean = 0“ was tested by a T-test in PROC UNIVARIATE. To test differences in height medians among taxa, a BROWN-MOOD test (STEEL and TORRIE, 1980, p.543) was performed using PROC NPAR1WAY with the median option (SAS Institute, 1988).

Heritabilities

Heritabilities (h^2) for third-year height growth were estimated separately for both treatments and each taxon. Analysis was accomplished using the individual tree data pooled across sites. To remove the effects of scale, height was standardized by dividing each observation in a site-treatment-block combination by the corresponding square root of the phenotypic variance for that block (VISSCHER *et al.,* 1991; HODGE *et al.,* 1996). The goal in h^2 estimation was to determine variance among families in the hybrids relative to that in slash pine and loblolly pine.

Variance components were estimated by PROC MIXED with the restricted maximum likelihood method, REML (LITTELL *et al.,* 1996). Analyses of variance were performed for each treatment and taxon combination (2 treatments x 6 taxa = 12 analyses) to estimate the significance of family, site by family, treatment by family, and block by family interaction. The model was obtained by dropping the taxon effect from model (1) (i.e., dropping all terms with subscript *k*). The model for each treatment-taxon combination was the following:

$$Y_{tjmn} = \mu + s_t + b_{jt} + f_m + sf_{tm} + bf_{tjm} + e_{tjmn} \qquad (4)$$

where: Y_{tjmn} is the nth observation of the mth family in the jth block at the tth site; and t = 1,...7 sites; j = 1,2,3 blocks; m = 1,...20 families; and n = 5 trees. All other effects are as described in model (1) but site and block were considered as fixed effects.

The open-pollinated families in the PEE and PTA taxa were assumed to be half-sib families; hence the variance component for families (σ^2_f) can be interpreted as an estimate of one quarter of the additive genetic variance (σ^2_A) (FALCONER and MACKAY, 1996).

Individual tree heritabilities were estimated as:

$$h^2 \text{ or } h^2_h = 4^* \sigma^2_f \,/\, \sigma^2_f + \sigma^2_p + \sigma^2_w \qquad (5)$$

For the hybrids, a hybrid heritability (h^2_h), was calculated as the ratio of 4 times the hybrid family variance ($4^*\sigma^2_{fh}$) over the total phenotypic variance, also using Equation (5). These h^2_h values for hybrids may be larger than for pure species, because in the hybrids additive and non-additive variance are confounded in σ^2_{fh} and gene frequencies in parental species may be different (COCKERHAM, 1961; WEI *et al.,* 1991). In any case, the h^2_h ratio can be interpreted as the proportion of variation in the hybrid population that is attributable to genetic differences among hybrid families, and useful for selection.

Estimates of heritability were obtained in three ways with 1) heights of all trees, 2) heights of only healthy trees and 3) heights without dwarf trees. A dwarf tree was defined as any tree smaller than 3 standard deviations from the height mean in each cultural treatment. For PEE x PCH and PEE x PCB bulked lots were excluded from these analyses.

Results and Discussion

Survival and Cold Hardiness

Significant differences ($p<0.001$) for mean survival existed among the six taxa in all five measurement periods (from one year to three years after planting). Treatment and treatment by taxon interaction effects were not significant for any measurement (*Table 2*). Results were similar for mortality due to cold temperatures, with highly significant taxon differences ($p<0.001$).

The PEE x PCH hybrid was the most susceptible to low temperatures, and had the lowest survival percentage for each of the five survival measurements (*Figure 1*). At three years of age, PEE x PCH survival was 68% averaged across all sites (ranging from 49% to 83% at individual sites); its lower survival was partially caused by its low cold resistance, since 14.7% of the PEE x PCH trees were killed by freezing temperatures. Evaluation at 16 months (1.4 years) showed that an average of 14% of the trees in this taxon were killed by cold temperatures during the winter 1995 to 1996. Meteorological data indicated that this particular winter had more days with freezing temperatures than others in the period of measurement.

The PEE x (PEE x PCH) hybrid was more similar to PEE for survival, cold damage, and number of dead trees from cold than to the PEE x PCH hybrid (the other parental species of this hybrid taxon). Thus, backcrossing the PEE x PCH hybrid to PEE, a more cold resistant parent, was effective in increasing cold hardiness and survival in that taxon. DUNCAN *et al.* (1996), studying similar hybrids as young seedlings under controlled chamber conditions found that the PEE x PCH hybrid was more similar to the more freeze susceptible parent, PCH. This study showed that the PEE x (PEE x PCH) hybrid was more comparable to the most cold resistant parent, PEE.

PEE x PCB was more cold tolerant than PEE x PCH. DUNCAN *et al.* (1996) also found that PEE x PCB was more cold hardy than PEE x PCH. In fact, this was expected since the *bahamensis* variety inhabits colder areas than *hondurensis* (NIKLES, 1966). Mean values of cold damage and survival indicated that PEE x (PEE x PCH) was as cold resistant as PEE x PCB, but both hybrids had somewhat more cold damage than pure slash pine with the same survival.

PEE x PTA had significantly lower survival than both parental species (and also lower than PEE x [PEE x PCH] and PEE x PCB). Further, single-degree contrasts indicate a negative hybrid vigor for survival (*Table 2*). The generally lower survival of PEE x PTA seems associated with the large variability for vigor among trees in this taxon. Some hybrid trees were small trees and tended to be very susceptible to cold temperatures, rust or other causes.

Third-Year Rust Infection

The pooled analysis showed no significant differences between cultural treatments for average rust incidence. No rank changes were detected due to site-by-taxon or treatment-by-taxon interactions in the pooled analysis, but highly significant taxon differences existed (*Table 2*). The slash x Caribbean hybrids had a higher infection rate for fusiform rust than pure slash or loblolly pine (*Figure 2*). Overall, PEE had nearly twice the rust infection of PTA. Rust incidence coefficients from the regression analysis based on the rust incidence of unimproved slash pine (PEU), indicated that both the PEE x PCH and PEE x (PEE x PCH) hybrids were more rust resistant than PEU, but PEE x PCB was as susceptible as unimproved slash pine (*Figure 2*). The PEE x PCH hybrid had less rust incidence than PEE x PCB, although the single-degree contrast was only weakly significant (p<0.051). TAINTER and ANDERSON (1993) screening a sample of commercial seed found that *P. caribaea* var. *hondurensis* seedlings were very susceptible to fusiform rust. In fact, all varieties of *P. caribaea* were at least somewhat susceptible to fusiform rust compared with rust resistant slash pine (JEWEL, 1960; TAINTER and ANDERSON, 1993; DOUDRICK *et al.,* 1996).

Negative hybrid vigor was detected when comparing the PEE x (PEE x PCH) hybrid to the average rust incidence of its parental species, PEE and PEE x PCH hybrid. In fact, the var.

Table 2. – Analysis of third-year data pooled across sites and cultural levels for survival and rust damage incidence.

Source	SURVIVAL[a]			RUST INCIDENCE[b]		
	DF	F value	Pr > F	DF	F value	Pr > F
SITE	7	2.87	0.0256	5	12.71	0.0045
TREATMENT (High *vs.* Low)	1	0.45	N.S.	1	0.10	N.S.
SITE*TREATMENT	7	1.31	N.S.	5	5.02	0.0081
TAXON	5	21.80	0.0001	5	22.12	0.0001
[c]Hybrid Vigor in PEE x PTA hybrid	1	41.98	0.0001	1	1.14	N.S.
[d]Hybrid Vigor in PEE x (PEE x PCH) hybrid	1	5.46	0.0220	1	3.77	0.0548
PEE *vs.* PEE x PCH	1	62.70	0.0001	1	8.47	0.0044
PEE *vs.* PEE x PCB	1	3.42	0.0683	1	23.21	0.0001
PEE x PCH *vs.* PEE x PCB	1	38.55	0.0001	1	3.78	0.0508
SITE*TAXON	35	3.07	0.0001	25	1.30	N.S.
TREATMENT*TAXON	5	0.62	N.S.	5	0.22	N.S.
SITE*TREAT*TAXON	35	1.51	0.0460	25	1.08	N.S.
FAMILY(TAXON)	112	6.84	0.0001	112	8.38	0.0001
SITE*FAMILY(TAXON)	606	1.51	0.0001	428	1.05	N.S.
TREAT*FAMILY(TAXON)	112	0.95	N.S.	112	0.87	N.S.
SITE*TREAT*FAMILY	604	1.07	N.S.	425	1.21	0.0026
ERROR	21102			14066		

[a]) ANOVAs from measurements at 1, 1.4, 2, and 2.4 years showed similar results as at 3 years of age.

[b]) ANOVA for rust was for the six sites with more than 15% of rust incidence.

[c]) Hybrid vigor of the PEE x PTA hybrid was analyzed by: PEE x PTA / (0.5 PEE + 0.5 PTA).

[d]) Hybrid vigor of the PEE x (PEE x PCH) hybrid was analyzed by:
PEE x (PEE x PCH) / (0.5 PEE + 0.5 PEE x PCH).

Note: A significant contrast in c and d means that the hybrid mean is not equal to the mean parental taxa.

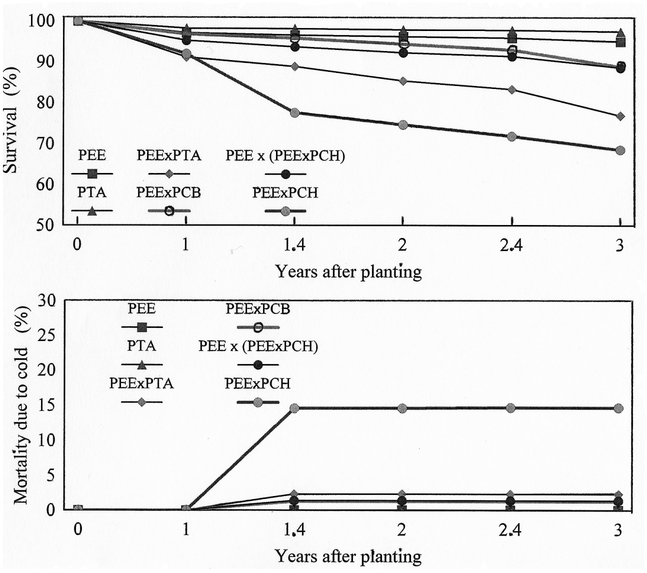

Figure 1. – Total cumulative survival and mortality due to cold in percentage for the pure slash (PEE), loblolly pine (PTA) and the hybrids slash x loblolly pine (PEE x PTA), slash x *caribaea* var. *bahamensis* (PEE x PCB), slash x *caribaea* var. *hondurensis* (PEE x PCH) and the backcross slash x (PEE x PCH). Evaluations at 1, 2, and 3 years of age were performed in January 1996, 1997 and 1998. Evaluations at 1.4 and 2.4 were done in May-1996 and May-1997, respectively. ANOVA's showed that significant differences ($p<0.05$) existed among the 6 taxa at evaluations 1.4 through 3 years of age in both traits. Treatments were not significant different at any age, so averages are combined across both treatments.

hondurensis backcross to PEE was as rust susceptible as PEE x PCH. The pollen mixed from PEE x PCH used to create the PEE x (PEE x PCH) was obtained from the Queensland Forest Service in Australia. This pollen, most likely, is the product of slash pines that have not been improved for fusiform rust resistance, since this pest is not present in Australia. So, it is likely that the (PEE x PCH) pollen was unimproved for rust resistance. In fact, two other tests in northeastern Florida indicated that the PEE x PCH hybrid formed in Australia was taller than PEE but very rust susceptible at three years of age (ROCKWOOD and NIKLES, 1996).

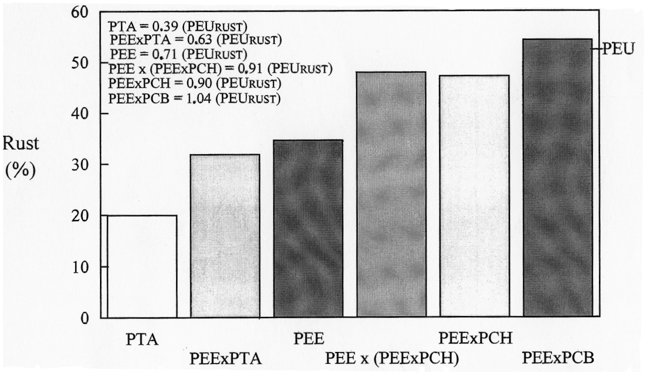

Figure 2. – Mean cumulative rust incidence at three years of age by taxa for improved loblolly pine (PTA), improved slash pine (PEE) and the hybrids: PEE x PTA, slash x *caribaea* var. *hondurensis* (PEE x PCH), slash x (PEE x PCH), and slash x *caribaea* var. *bahamensis* (PEE x PCB). Treatment effects and interactions with other independent variables were non significant, so averages are combined across both treatments and sites. Rust incidence coefficients based on unimproved slash pine (PEU) were obtained by regression of each taxon's rust against PEU. For the full model $y = PEU_{RUST} + PEU_{RUST}{*}TAXON$, $R^2 = 0.92$. Taxon differences are highly significant and specific contrasts are presented in *table 2*.

The single-degree of freedom contrast of hybrid vigor for the six sites with more than 15% rust incidence indicated that the PEE x PTA hybrid was intermediate for infection rate between the parental species. However, across all sites rust associated mortality at three years of age in this hybrid was four times greater than slash pine. Furthermore, when rust incidence was averaged across all 8 sites, the mean rust incidence of PEE x PTA was closer to slash pine, the susceptible parental species (*Figure 2*). Testing few crosses, SCHMITT (1968) found that the hybrid PEE x PTA was more susceptible to fusiform rust than both pure slash and loblolly pine. This was not the case here, but there was some indication that the hybrid PEE x PTA was more similar to the rust susceptible parent, PEE.

Third-Year Height

Taxa comparisons

The pooled analysis using data only from healthy trees showed that the intensive culture had effectively increased third-year height ($p = 0.0029$ in *Table 3*). While the interaction site by treatment was statistically significant, the interaction was caused by heterogeneous variances in the two treatments (commonly called scale effects). That is, the interaction was not caused by rank changes, because the rank order of the taxa are the same in both treatments. The fact that loblolly pine was most responsive to the high treatment, led to the significant interaction since its difference above the two slash pine taxa increased in the high treatment. In spite of the significant interactions of site by taxon and treatment by taxon ($p<0.01$), no important taxon rank changes were found across sites (data not shown) nor across treatments (*Figure 3*). Highly significant taxon differences were found in both treatments, and differences among taxa increased in the high treatment. Improved loblolly pine was the tallest taxon at three-years of age and was most responsive to the high cultural treatment.

Similar results were found when pooled analyses were done including all trees (healthy and diseased trees), but means were somewhat smaller when diseased trees were included. On average, native taxa were shorter by 1%, while the slash x Caribbean hybrids were shorter by 5% when fusiform rust infected trees were included. However, no taxon rank changes were noticed when comparing analyses employing only healthy trees to those including both healthy and diseased trees.

Positive hybrid vigor was detected for height in both treatments when comparing the PEE x (PEE x PCH) hybrid to the average third-year height of the parental species PEE and PEE x PCH. The backcross taxon was not significantly different from slash pine. Its faster growth relative to PEE x PCH may be partially due to the increased cold hardiness conferred by the backcross to PEE. In fact, PEE x PCH was the shortest taxon of all. PEE x PCB was taller than PEE x PCH in both treatments ($p<0.02$). This difference was also significant when including healthy and diseased trees in the ANOVA. The early advantage of PEE x PCB may be due to its superior cold hardiness.

The hybrid PEE x PTA was the second shortest taxon, and negative hybrid vigor was detected for height growth in both treatments. In the low treatment, the PEE x PTA was not different from PEE, but with intensive culture, PEE was taller than PEE x PTA ($p<0.02$). BARNES and MULLIN (1978) testing the PEE x PTA hybrid and both parental species in South Africa found that hybrid vigor was positive where environmental conditions were marginal for both slash and loblolly pine and

Table 3. – Analysis of variance for third-year height. Data were pooled across seven sites and two cultural levels. Values were analyzed based on whole-plot means of healthy trees (252 observations = 7 sites x 2 treatments x 3 blocks x 6 taxa). Contrasts below the line are specific to high and low cultural treatment.

Source	DF	F value	Pr > F
SITE	6	2.49	N.S.
TREATMENT (High *vs.* Low)	1	23.45	0.0029
SITE*TREATMENT	6	16.27	0.0001
TAXON	5	16.38	0.0001
[a] Hybrid Vigor in PEE x PTA hybrid	1	27.60	0.0001
[b] Hybrid Vigor in PEE x (PEE x PCH) hybrid	1	3.85	0.0590
PEE *vs.* PEE x PCH	1	11.99	0.0016
PEE *vs.* PEE x PCB	1	0.07	N.S.
PEE x PCH *vs.* PEE x PCB	1	11.77	0.0018
SITE*TAXON	30	2.81	0.0030
TREATMENT*TAXON	5	5.60	0.0009
HIGH Hybrid Vigor in PEE x PTA	1	36.88	0.0001
Hybrid Vigor in PEE x (PEE x PCH)	1	3.57	0.0648
PEE *vs.* PEE x PCH	1	17.84	0.0001
PEE *vs.* PEE x PCB	1	0.69	N.S.
PEE x PCH *vs.* PEE x PCB	1	11.52	0.0014
LOW Hybrid Vigor in PEE x PTA	1	8.70	0.0049
Hybrid Vigor in PEE x (PEE x PCH)	1	5.35	0.0252
PEE *vs.* PEE x PCH	1	2.97	0.0910
PEE *vs.* PEE x PCB	1	0.60	N.S.
PEE x PCH *vs.* PEE x PCB	1	6.24	0.0159
SITE*TREAT*TAXON	30	1.01	N.S.

[a])[b]) Definition of contrasts are in *table 2.* The error term with 140 degrees of freedom.

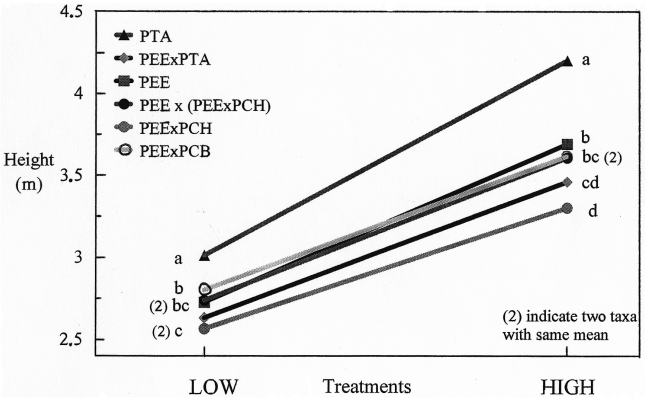

Figure 3. – Third-year height by taxon in the two sivicultural treatments across seven sites. The height values include only healthy trees. Mean height values using all trees were a little lower, but very similar to those presented. Treatment and taxon differences were highly significant and some specific contrasts are presented in *table 3.* The treatment by taxon interaction was significant (p<0.05). Taxon means with same letter are not significantly different within a treatment at p=0.05.

negative where environmental conditions were favorable for the two parental species. This may be the case for slash and loblolly pine growing in the lower Costal Plain of USA where conditions are favorable for both parental species.

The frequency for heights of the PEE x PTA hybrid revealed a skewed distribution to the left, meaning that many trees were very short (*Figure 4*). In the high treatment, PEE x PTA heights were somewhat outside of a normal distribution (W = 0.8962, where values lower than 0.90 may lead to rejection of the hypothesis of normality, SAS Institute, 1990). The PEE x PTA heights were close to a normal distribution in the low treatment (W = 0.9416). The greater frequency of very small trees reduced the mean height of the PEE x PTA hybrid. Median values for PEE x PTA were somewhat higher than for PEE (*Table 4*). The median is not as influenced by extreme values as is the mean (DANIEL, 1974), allowing a better comparison of these taxa. The PEE x PTA median was higher than its mean in both treatments (p<0.01), due to its skewed distribution. Thus, when median values were compared in the high treatment, the PEE x PTA hybrid was taller ($p<0.05$) than PEE. Similarly, the mode of the hybrid population was located between the two parental taxa in the high treatment, and closer to PTA in the low treatment (*Figure 4*).

If a dwarf tree is defined as any tree smaller than three standard deviations from the mean of all three taxa, then 5% of the PEE x PTA trees may be classified as dwarfs while 0.17% of the PEE and 0.14% of the PTA were dwarfs in the low treatment. Further, 8% of the PEE x PTA, 0.20% of the PEE and 0% of the PTA trees were dwarf trees in the high treatment. SCHMITT (1969) found that the combination of foreign genomes in slash x shortleaf pine (*P. echinata* MILL.) hybrids is apparently conducive to mixoploidy or polyploidy which may produce dwarf trees (SCHMITT and SNYDER, 1971). SAYLOR and SMITH (1966) found also higher chromosomal irregularities in many hybrid combinations of several pine species than in the pure species.

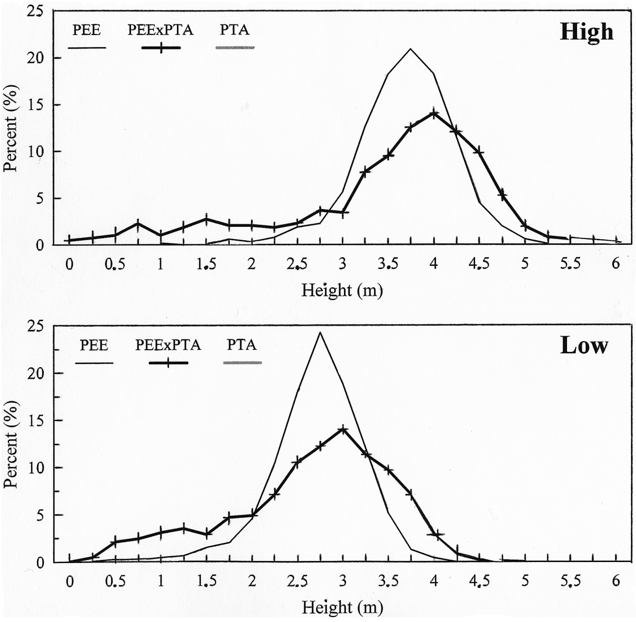

Figure 4. – Frequency polygon of third-year height for the improved loblolly (PTA), improved slash (PEE) and the F1 hybrid (PEE x PTA) by treatments. Frequencies are for all rust-free trees and height values were adjusted by the site and block means of these taxa. Percentage values were obtained by PROC FREQ (SAS Institute, 1988) with 24 intervals of 0.25 m.

Some very tall PEE x PTA hybrid trees were detected when adjusted by the site and block mean. For example, in the high treatment the tallest 2% of the PEE x PTA trees were taller than any slash pine tree and as tall as the best 4% of the loblolly pine trees. Further, 54% and 52% of the PEE x PTA hybrid trees were taller than the median PEE tree in the high and low treatments, respectively.

Frequency distribution for third-year height of the slash x Caribbean hybrids were quite similar to the PEE distribution. The frequency for PEE x PCH hybrid was skewed to the left with 1% and 2% of dwarf trees in the low and high treatment, respectively. When comparing the median, mode or superiority values (percentage of trees in each taxon that were bigger than the median of PEE), the hybrids PEE x (PEE x PCH) and PEE x PCB were as tall as PEE (*Table 4*). PEE x PCH had 31% of its trees taller than the PEE median. If this trend continues some individuals from these taxa have potential use in breeding activities.

Family comparisons

Heritabilities for height from pooled analyses were calculated for both pure species (PEE and PTA) and the hybrids. Hybrid heritability (h^2_h), calculated as the ratio of 4 times the hybrid family variance (σ^2_{fh}) over the total phenotypic variance, were larger than heritabilities for the pure species (*Table 5*). In general, when estimates were performed on healthy trees, h^2_h values were higher. Higher heritabilities were estimated in the high treatment, most probably resulting from a reduction in the error variance in the intensive culture (LÓPEZ-UPTON, 1999). These estimates of h^2 and h^2_h should be taken with caution due to the small number of families and young age.

Analysis of within-family variance of third-year height

To examine whether taxa differed in variability among trees within the same family, the within-family coefficient of variation (CV) was calculated for each taxon and treatment combination using the third-year height from the five trees of a given family in each row plot. There were 3598 of these plot-level, within-family CVs that were subjected to ANOVA (7 sites x 2 treatments x 6 taxa x 3 blocks x 16 families less missing plots). Less intensive culture (low) had more within-plot varia-

Table 4. – Measurements of central tendency for third-year height growth. Values in meters were obtained for healthy trees and values adjusted by the height means of site and block for PEE, PTA and their F1 hybrid. The mode was obtained from the most frequent interval in *figure 4.*

TAXON	LOW				HIGH			
	Mean	Mode	Median[a]	S[b] (%)	Mean	Mode	Median	S (%)
PTA	3.02	3.00	3.05* a	70	4.20	4.25	4.25** a	83
PEE x PTA	2.64	3.00	2.81**b	52	3.50	4.00	3.79** b	54
PEE	2.72	2.75	2.75* b	50	3.68	3.75	3.70 c	50
PEEx(PEExPCH)	2.71	2.75	2.75* b	49	3.62	3.75	3.71 bc	51
PEE x PCH	2.55	2.60	2.60* c	40	3.29	3.37	3.37**d	31
PEE x PCB	2.79	2.75	2.81 b	52	3.66	3.75	3.71 bc	51

[a]) Height medians were significantly different from the mean with $p<0.05$ * or $p<0.01$**.
Letters indicate medians among taxa, where taxon medians with same letters are not significant different at p = 0.05.

[b]) Superiority (S) was calculated as the percentage of trees in each taxon that were bigger than the median of PEE. Thus, PEE value is 50%.

Table 5. – Estimates of heritabilities for pure taxa (h^2) and estimates of hybrid heritabilities ($h^2_h=4*\sigma^2_{fh} / \sigma^2_{fh} + \sigma^2_p + \sigma^2_w$) for early height growth in all taxa by treatment. Values were obtained using standardized data in three different analyses: Including all trees, healthy trees and without dwarf trees. For PEE x PCH and PEE x PCB bulked families were not included.

TAXON	h^2 or h^2_h in High Treatment			h^2 or h^2_h in Low Treatment		
	All trees	Healthy trees	Without dwarfs	All trees	Healthy trees	Without dwarfs
PTA	0.14	0.14	0.13	0.07	0.08	0.07
PEE x PTA	0.18	0.28	0.13	0.14	0.16	0.08
PEE	0.10	0.15	0.10	0.04	0.16	0.03
PEE x (PEExPCH)	0.15	0.16	0.14	0.08	0.12	0.05
PEE x PCH	0.19	0.24	0.19	0.37	0.28	0.33
PEE x PCB	0.13	0.23	0.11	0.08	0.13	0.04

bility for height growth than intensive culture (high) when comparing the heights of healthy trees (*Figure 5*, $p<0.04$).

Taxa also differed markedly in the amount of within-plot variability and the trends were consistent across treatments. The PEE x PTA taxon was the most variable of all taxa. This hybrid had a mean coefficient of variation twice that of both PEE and PTA (*Figure 5*). Growing in South Africa, the slash x loblolly pine hybrid at three years of age had a larger phenotypical variance for height growth than the pure species (BARNES and MULLIN, 1978). In summary, PEE x PTA showed a negative hybrid vigor for height growth (*Figure 3*), but higher variability (*Figure 5*), probably due to high frequencies of both very short and very tall trees (*Figure 4*).

In general, the slash x Caribbean hybrids were more variable than pure slash pine in the low treatment. PEE x PCH was the second most variable taxon. This hybrid was significantly more variable than PEE in both treatments. Considerable phenotypic variation among and within families has been found for several traits in PEE x PCH including height growth in Australia (NIKLES and ROBINSON, 1988).

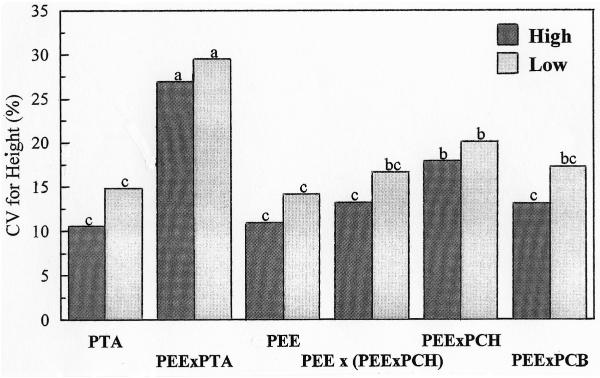

Figure 5. – Coefficient of variation (CV) for third-year height in the High and Low treatments for improved loblolly pine (PTA), improved slash pine (PEE) and the hybrids: PEE x PTA, slash x *caribaea* var. *hondurensis* (PEE x PCH), slash x (PEE x PCH), and slash x *caribaea* var. *bahamensis* (PEE x PCB). Data based on rust-free trees. Treatment differences were significant ($p<0.04$) but treatment by taxon interaction was not significant. Taxon differences were highly significant. Taxon means with same letter are not significantly different at $p=0.05$ (from single-degree of freedom contrasts) in each treatment.

General Discussion and Conclusions

In general, results indicated that all hybrid taxa are suffering some adaptation problems in the lower Coastal Plain of USA. Overall, height means of the hybrids indicated a lack of positive hybrid vigor. However, very outstanding trees and families may be found in all of these hybrid taxa, and evaluations at older ages are needed to draw more precise conclusions about their productivity.

On average, the PEE x (PEE x PCH) and the PEE x PCB were better adapted than PEE x PCH at three years of age. Both taxa were less affected by cold damage and taller than PEE x PCH, and had height growth similar to the pure-improved slash pine (PEE) families. Hybridization has shown to improve cold hardiness in other tree species, for example, *P. rigida* MILL. x *P. taeda* hybrids are more cold resistant than pure loblolly pine (HYUN, 1976; GENYS, 1988).

All varieties of *P. caribaea* seem relatively susceptible to fusiform rust (JEWEL, 1960; TAINTER and ANDERSON, 1993; DOUDRICK *et al.,* 1996). Var. *bahamensis* seems somewhat more susceptible than the var. *hondurensis*. *P. caribaea* has probably evolved in the absence of fusiform rust. No species of *Quercus* suitable as the alternate host of *Cronartium quercuum* f.sp. *fusiform* presently occurs in the Caribbean area (BURNS and HONKALA, 1990), but some *Quercus* species grow in the natural range of var. *hondurensis* (KELLMAN, 1976).

Overall, the PEE x PTA hybrid had rust infection similar to the most rust susceptible parent, PEE. The PEE x PTA hybrid taxon had a significant frequency of abnormal trees that reduced the taxon mean values, but median height is significantly superior to PEE in the high treatment. In any case, PEE x PTA was less well adapted than pure loblolly pine. The F1 hybrid had more rust incidence and almost the same incidence of tip moth attacks as pure loblolly pine (LÓPEZ-UPTON, 1999).

FOWLER (1978) hypothesized that when two species have evolved and adapted to similar local conditions, their hybrid progeny may have some degree of negative hybrid vigor. This may be the case for loblolly pine and slash pine in the LCP, where they are well adapted. Generally, when interspecific hybrids are planted in zones that are marginal for the pure species or as exotic in radically different environments from natural distributions of the parental species, the hybrids tend to show positive hybrid vigor (Martin, 1989). For example, in Texas, outside the range of slash pine and in drier environments, the hybrid PTA x PEE (the reciprocal cross) was taller than PTA although with lower survival (Long, 1973). In Queensland, Australia, on poorer sites the F1 hybrid PEE x PCH outperforms both pure species, but on better quality sites PCH is as productive as the F1 (Powell and Nikles, 1996). Barnes and Mullin (1978) concluded that the slash x loblolly hybrid may be good where the species are grown as exotics and where the F1 progeny are located in „hybrid habitats“.

Very tall hybrid families were detected when adjusted by the site and block mean of the native taxa. Using only healthy trees, one PEE x PCB family was better than the best family of PTA and two PEE x PCB families were better than any PEE family in the low treatment. The top 20% of families for height in the low treatment (23 families), were composed of 13 PTA families, 4 PEE x PCB families, 3 PEE x PTA families, 2 PEE and 1 PEE x PCH family. Meanwhile, in the high treatment the composition of the top 20% of the families was: 17 PTA families, 2 PEE x PTA, 2 PEE x PCB, 1 backcross PEE x (PEE x PCH), and 1 PEE family.

Clearly, there are some tall trees in all hybrid taxa that might be useful in a breeding program. For example, in the high treatment the tallest 2% of the PEE x PTA trees were taller than any slash pine tree and as tall as the best 4% of the loblolly pine trees. In any case, outstanding hybrid trees that are rust free may be found in each taxon. However, rust resistance needs to be improved before considering operational deployment of slash x Caribbean hybrids in areas where rust hazard is high.

The larger hybrid heritabilities (h^2_h) and more within-family variability compared to within families of parental species may be exploited by selection within hybrid families to find outstanding recombinants and increase genetic gain above that projected on the basis of average taxon performance.

Large gains may be obtainable via family and clonal selection of outstanding hybrid trees. Examples of this in pines are PEE x PCH in Queensland, Australia and *P. rigida* x *P. taeda* in Korea, where hybrids are mass propagated by seed (Nikles, 1992; Kim and Zsuffa, 1994). However, relatively few hybrids in pines have been developed to the stage of operational use, because the major constraint is the difficulty and extra expense of mass producing hybrids, either by seed or vegetatively. In this study, the PEE x (PEE x PCH) and PEE x PTA hybrids produced an average of 32 and 30 seeds per cone (Hodge *et al.,* 1993) which is acceptable for breeding purposes. Further, no seedling production problems were detected for the PEE x (PEE x PCH) and PEE x PTA hybrids.

Hybrid breeding programs are currently operational with several species in the genera *Eucalyptus, Populus* and *Salix* (Zobel, 1993; Bisoffi and Gullberg, 1996). These programs have been greatly facilitated by the ease of vegetative propagation in the species of these genera. As efficient means of large-scale vegetative propagation become more available with pines (Stelzer *et al.,* 1998), breeding for hybrids will almost certainly become more popular. This will allow outstanding individuals to be propagated operationally no matter what their genetic composition, and without using extensive controlled pollination.

The technique of reciprocal recurrent selection used to develop commercial F1 varieties does not hold much promise if these early results (showing poorer mean performance of all tested F1 hybrids) are borne out in the future. However, excellent individual trees in several of these taxa would be useful for breeding purposes in hybrid programs that combine genes from the hybrids back with the parental PEE and PTA species to form synthetic backcross taxa in various combinations.

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In vitro Propagation of *Bombax ceiba* L. (Silkcotton)

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Abstract

An *in vitro* protocol has been developed for multiplication from shoot tips of *Bombax ceiba* L., a multipurpose tree species belonging to the family Bombacaceae. High frequency bud break and multiple shoots were induced from shoot tips of *B. ceiba* on Murashige and Skoog (1962) medium supplemented with 2.0 mg/l 6-benzylaminopurine. One shoot was formed per explant in the initial cultures, however, on an average, 7.4 ± 0.5 shoots were formed from a single shoot tip over a period of 16 weeks. The shoots were successfully rooted on half strength MS medium containing 2.0 mg/l indole-3-butyric acid. *In vitro* raised plants were hardened for survival before transplantation to soil.

Key words: Bombax ceiba L., silkcotton, *in vitro,* micropropagation, shoot tips.

Abbreviations: BAP 6-benzylaminopurine, *Kn* kinetin, *IBA* indole-3-butyric acid, *NAA* α-naphthaleneacetic acid, *MS* Murashige and Skoog (1962) medium.

Introduction

Bombax ceiba L. (commonly known as silkcotton) is a deciduous, multipurpose tree, widely distributed in India. It is eminently suited for afforestation of new ground and grass lands. The tree is also useful in controlling soil erosion. It is a tree of immense horticultural importance as it produces clean floss and is planted in the gardens as avenue tree. The floss is suitable for mattresses, cushions, pillows, and quilts. Young twigs and leaves are looped for fodder and roots are eaten either raw or roasted as sweet potato. The flowers are astringent. The paste of flowers and leaves is employed as an application in cutaneous troubles. The paste of bark is applied to skin eruptions and credited with astringent, tonic and demulcent properties and also used for dysentery, haemoptysis in pulmonary tuberculosis, influenza and menorrhagia. The young fruits are reported to be employed as expectorant, stimulant, diuretic, and in chronic inflammation of the bladder. The

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