GENETIC AND RECIPROCAL EFFECTS ON FIRST-YEAR GROWTH AND LEAF AREA DEVELOPMENT OF SYCAMORE SEEDLINGS

Zhenmin Tang[1] and Samuel B. Land, Jr.[2]

Abstract:-Seedlings from control-pollinated crosses of American sycamore *(Platanus occidentalis L.)* were studied for genetic and reciprocal effects on seedling growth and leaf area development during the first growing season in a nursery. Reciprocal effects accounted for most of the variation among families in seedling heights during the first month, but these effects then disappeared . After a dry period during the fourth month (August), reciprocal effects were detected for stem volume and for number and leaf area of live leaves per seedling. Genetic effects were responsible for family variation detected in (1) seedling height and diameter during the other portions of the growing season, (2) seedling stem volume at the end of the season, and (3) leaf area development before the August dry period. Genetic differences in seedling size at the end of the first growing season were related to differences in leaf area expansion early in the growing season (r 0.97). The fastest-growing cross (pair of reciprocals) had the largest leaf area per seedling by three months (July) after the seeds were planted, and it maintained the greatest height, diameter, and stem volume throughout the growing season.

Keywords: Reciprocal crosses, Common environmental effects, Family variation, Seedling growth, Leaf expansion, *Platanus occidentalis L.*

INTRODUCTION

Early selection for genetic improvement of woody crops has recently received considerable attention from tree breeders, because it may be used to shorten generation intervals and reduce costs of progeny tests. Increased genetic gain per unit time may be achieved from selection of juvenile traits for improvement of mature traits, but this requires high juvenile-mature correlations and a reasonable heritability for each trait (McKinley and Lowe 1986). Strategies of early selection and breeding have been proposed for increasing productivity of sycamore *(Platanus occidentalis L.)* in short-rotation energy plantations (Land 1981).

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[1] School of Forestry, Wildlife, and Fisheries, Louisiana State University, LSU Agricultural Center, stationed at USDA Forest Service Southern Research Station, 2500 Shreveport Highway, Pineville, LA 71360.

[2] College of Forest Resources, Forest and Wildlife Research Center, Mississippi State University, Mississippi State, MS 39762. Approved for publication as Journal Article No. FA079-0597 of the Forest and Wildlife Research Center, Mississippi State University.

Seedling characteristics are juvenile traits and may be subject to maternal (non-nuclear genetic) and common environmental effects of the mother tree. Differences between progenies of reciprocal crosses (sex roles of parents reversed) provide a measure of these effects. Maternal and common environmental effects cause resemblances among offspring from the same mother tree that are not chromosomally inherited, and the effects can increase variation among different mother-tree families (Falconer 1989). Such effects can give overestimates of heritability and thereby provide erroneous conclusions about expected genetic gains from early selection. Several studies have reported maternal or environmental effects in forest tree species. Perry (1976) and Barnett (1991) have pointed out that maternal effects in coniferous species influenced seed size and germination rate, and early seedling performance. Large seeds of loblolly pine germinate more quickly and produce larger germinants than small ones (Dunlap and Barnett 1983). Robinson and van Buijtenen (1979) found a positive relationship between seed weight and 15-year tree height of loblolly pine in a progeny test. Khalil (1981) reported that seed weight of *Picea glauca* was positively correlated with annual terminal shoot growth of young trees. Land *et al.* (1989) conducted a germination test with control-pollinated seed of sycamore. They found significant differences in seed length and weight, rates of germination, and germination values between reciprocal crosses. However, no studies have been conducted to quantify the relative magnitudes of genetic and reciprocal influences on seedling performance of sycamore. Information on how genetic and reciprocal effects influence juvenile traits is needed for tree improvement programs of quasi-juvenile traits in short-rotation energy plantations. The objectives of the present research were to: (1) determine monthly change and genetic variation in height, root-collar diameter, stem volume, and leaf characteristics of sycamore seedlings during the first growing season under nursery conditions, and (2) evaluate the relative importance of reciprocal effects on seedling growth, leaf area, and root characteristics.

MATERIALS AND METHODS

Plant Material and Experimental Design. A single-pair mating scheme, with reciprocals, was used among 12 sycamore trees to produce 12 control-pollinated families (six pairs and two reciprocals per pair). The six pairs included L108 x G106 (L x G), T205 x T108 (T x T), H205 x S210 (H x S), B104 x B110 (B x B), B209 x N109 (B x N), and K110 x C204 (K x C). The 12 parent trees came from seed sources in Alabama, Arkansas, Louisiana, and Mississippi and were represented by grafts in a clonal orchard at Mississippi State University (MSU) in northeast Mississippi (33 °27' N. latitude and 88 °45' W. longitude). Four seeds per container from each of the 12 families were planted on April 14, 1989 in containers (15 cm x 15 cm x 40 cm deep) filled with a 1:1 mixture of coarse sand and a local fine sandy loam soil at the MSU outdoor nursery. Germinants were thinned to the tallest seedling per container on June 12 (three months after seeds were planted). In addition, the germinants were fertilized with 13-13-13 slow-release fertilizer and grown for eight months in the nursery with daily morning irrigations until mid-October.

The experimental design in the nursery was a randomized complete block with four replications. Each family plot within a replication consisted of a single row of eight seedlings (eight containers) and therefore an entire replication had a total of 96 seedlings. The see dling spacing was 15 cm by 15 cm.

Nursery Measurements. Germinant heights were recorded at 7, 14, 22, 28, and 56 days after seeds were planted. Measurements of heights and root-collar diameters continued on a monthly basis from three-months (July) to eight-months (December) seedling age. Number of branches, numbers of living and dead leaves on each branch and on the main stem, and leaf widths of each live leaf were taken for each seedling at two-week intervals from July to mid-November.

The eight-month-old seedlings were removed from the containers and measured for heights and root-collar diameters in mid-December. The roots were carefully washed and measured for (1) number and length of tap roots per seedling and (2) numbers of lateral roots in each of three size classes per seedling. The three size classes were based on lateral-root diameter adjacent to the connection with the tap root: (1) "fine" roots were less than 2 .0 mm diameter, (2) "medium" roots were between 2.0 and 5.0 mm diameter, and (3) "large" roots were greater than 5.0 mm diameter. Root volume per seedling was measured by water displacement in a graduated cylinder and recorded separately for tap roots and each of the three sizes of lateral roots.

Data Analyses. Leaf areas of seedlings were calculated from leaf widths with the following equation:

$$\text{Ln (leaf area)} = 1.8 \times \text{Ln (leaf width)} \qquad (1)$$

where Ln is the natural logarithm.

Equation (1) was developed using SAS regression analysis procedures (SAS Institute Inc. 1985) on a data set of 200 sample leaves that were measured for leaf widths and actual areas with a LI-COR LI-3000 portable leaf area meter (LI-COR, Inc., Lincoln, NE, USA) (Tang and Land 1996). The fit of these data to the equation was almost perfect ($r = 0.99$). Seedling stem volume (volume index) was estimated as one-third of seedling root-collar basal area multiplied by seedling height (the equation for volume of a cone).

Table 1. Format of analyses of variance used to study genetic and reciprocal effects on seedling growth and leaf area development of sycamore.

Source of Variation	d.f.	Expected Mean Squares[a]
Replications (R)	3	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+2\sigma^2_{RP}+12\sigma^2_R$
Pairs (P)	5	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+2\sigma^2_{RP}+4\sigma^2_{C(P)}+8\sigma^2_P$
Crosses within Pairs (C:P)	6	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+4\sigma^2_{C(P)}$
R x P	15	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}+2\sigma^2_{RP}$
R x C:P	18	$(1/h)\sigma^2_w+\sigma^2_{RC(P)}$
Within plots	336	σ^2_w

[a] (1) All effects assumed random, and σ^2 = variance component.
(2) The letter "h" is the harmonic mean number of living seedlings per block.

Analyses of variance with a format given in Table 1 were conducted using GLM procedures of SAS (SAS Institute Inc. 1985). Variation within plots was calculated on an individual seedling basis, whereas the analysis of other sources of variation was based on plot means due to the imbalanced data caused by differences in seedling survival among family plots within replications. F-tests were constructed and computed from the expected mean squares in Table 1 (Snedecor and Cochran 1980) to determine differences among six pairs of crosses (i.e., genetic effects) in seedling growth, leaf area expansion, and root characteristics. The approximate degrees of freedom were calculated for the F-tests of genetic differences using a Satterthwaite's procedure. Reciprocal effects were indicated by differences between two reciprocal crosses within each pair. Genetic and reciprocal effects were considered significant if F-test statistics revealed that the respective sources of variation had probability levels of 0.05 or less. When genetic and/or reciprocal effects were significant, Duncan's multiple range test (Steel and Torrie 1980) was conducted to test the ranked pair and cross means for significant differences at P 0.05.

RESULTS AND DISCUSSION

Monthly Trends in Seedling Growth and Leaf Area Development. Maximum rate of height growth occurred in July and was 26.1 cm for that month, while the greatest monthly diameter growth rate was 1.8 mm in August (Table 2). Stem volume had the largest monthly increment (6.5 cm^3) in October, but growth had almost ceased by mid November. Eight-month-old seedlings (end of the growing season) averaged 90 percent in survival, 64 cm in height, 8.9 mm in root-collar diameter, 16.8 cm^3 in stem volume, and 36 lateral roots.

Table 2. Average monthly rainfall, leaf area, and growth of sycamore seedlings during the 1989 growing season'.

Month	Average Rainfall (mm)	Current Living Leaves: Leaf area ($cm^2 \cdot seedling^{-1}$)	Current Living Leaves: No. leaves (seedling')	Monthly Growth Increment: Height (cm)	Monthly Growth Increment: Diameter (mm)	Monthly Growth Increment: Stem volume (cm 3)
May	180			0.2 (0.0)		
June	262			3.6 (0.2)		
July	160	858 (51)	10.4 (0.7)	26.1 (1.1)		
August	61	658 (48)	9.5 (0.7)	20.2 (0.3)	1.8 (0.0)	4.0 (0.2)
September	190	1060 (77)	14.5 (0.9)	10.8 (0.2)	1.2 (0.0)	4.4 (0.4)
October	10	516 (72)	7.8 (0.8)	2.8 (0.3)	1.6 (0.1)	**6.5 (0.5)**
November	159	213 (48)	2.6 (0.4)	0.0 (0.0)	0.1 (0.0)	0.2 (0.0)
December	124			0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a] The number in parentheses is the standard error of the mean.

Live leaves on main stems reached a maximum size of 118 $cm^2 \cdot leaf^{-1}$ in July and accounted for 74 to 88 percent of the total leaf area per seedling through the end of the growing season. Branch live leaves represented the remaining total during that period and became a full size of 29 cm²˙ leaf' in September. The greatest leaf area per seedling was 1060 cm² and occurred in September, because (1) both stem and branch leaves had been fully expanded at that time, and (2) the largest number of live leaves per seedling (14.5 leaves) was reached in that month (Table 2). However, the month of greatest leaf area may vary from year to year, depending on timing of drought periods. In the present study the leaf area declined by 27 percent from July to August during a dry period, when the seedlings lost seven percent of leaves on the stem, 24 percent of the branches that bore leaves, and 20 percent of leaves on the remaining branches. The losses of leaf area were followed by a 33-percent reduction in diameter growth increment during the subsequent period from mid August to early September. Number of live leaves and leaf area increased with increased rainfall in September (Table 2). Consequently, diameter growth increased in October, and stem volume growth reached a maximum rate at that time.

Genetic and Reciprocal Effects. *(1) Seedling growth* - Genetic variance components (among six pairs) were significant and larger than reciprocal components (between reciprocal crosses within each pair) for seedling height, diameter, and stem volume starting three months (July) after seeds were planted (Table 3). The only exception to this trend was for diameter in October and stem volume in September and October. Genetic effects, although not significant, also accounted for 66, 73, and 100 percent of the total family variation (among all 12 crosses) in numbers of fine, medium, and large lateral roots in December. Pair L x G had larger seedling height, diameter, and stem volume than other pairs at the end of the growing season (Figures la, lb, and lc).

Table 3. Relative sizes of estimated variance components for height, diameter, stem volume, and number of lateral roots of sycamore seedlings *as* measured on a given date during the 1989 growing season.

Measurement Date	Relative sizes of variance components as % of the family component'											
	Height		Diameter		Stem volume		Fine roots		Medium roots		Large roots	
	°	C:P	P	C:P	P	C:P	P	C:P	P	C:P	P	C:P
April 14	0.0	100**										
April 28	0.0	100**										
May 6	0.0	100**										
May 12	17.3	82.7**										
June 9	35.9	64.1										
July 14	97.0*	3.0	100**	0.0	100**	0.0						
August 11	100*	0.0	100**	0.0	100**	0.0						
September 15	100*	0.0	81.3*	18.7	51.3	48.7						
October 13	i00*	0.0	53.4	46.6	14.0	86.0*						
December 13	100*	0.0	82.4*	17.6	54.7**	45.3	65.8	34.2	73.3	26.7	100	0.0

• * = Significant at P 5 0.05 and ** = Significant at P 5 0.01.
° P = Variance component among pairs, and C:P = Variance component between two reciprocal crosses within pairs. Negative estimates were given a value of zero.

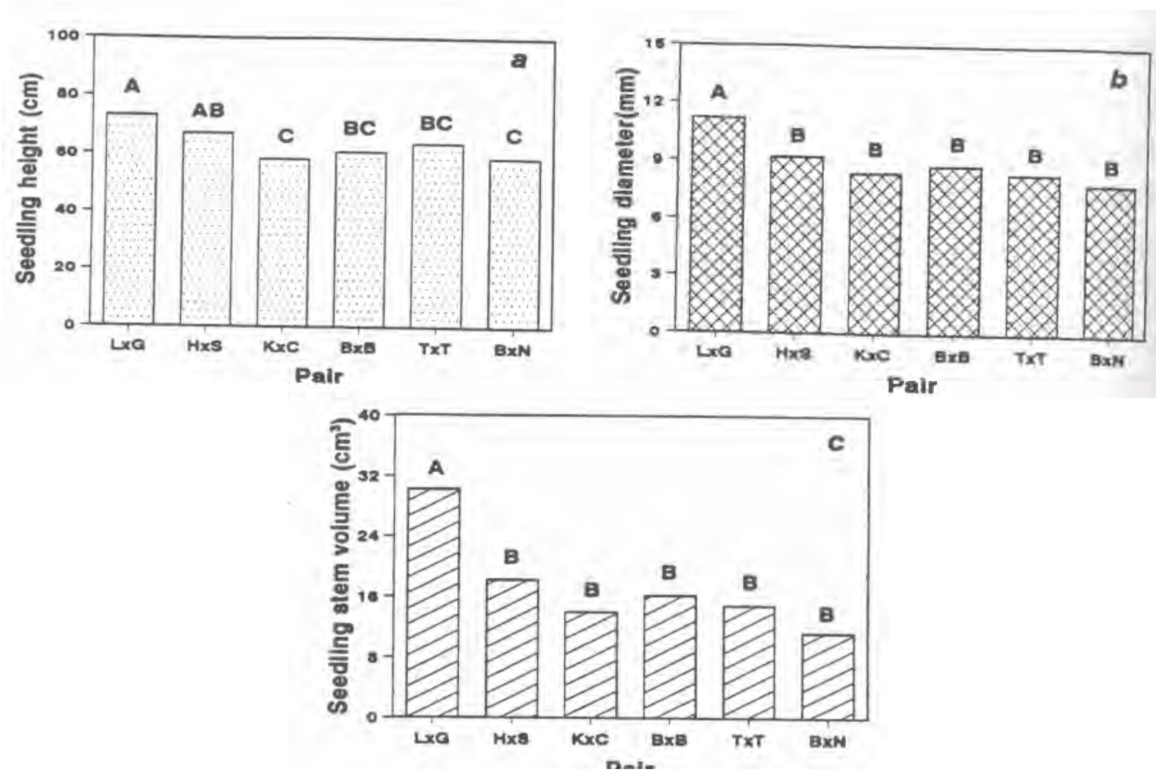

Figure 1. Mean comparisons of height (a), diameter (b), and stem volume (c) in December for six pairs of sycamore seedlings (abbreviations of the pairs are given in the Materials and Methods). Bars not labeled by the same letters differ at P 0.05 (experimentwise error at P S 0.23).

Reciprocal effects influenced seedling heights during the first month after planting and were responsible for 83 to 100 percent of the family variation during that period (Table 3). These effects decreased and eventually disappeared for seedling heights by mid July (three months after planting), but significantly affected stem volume in October following the August dry period. Reciprocal effects had little influence (zero to 34 percent of the family variation) on numbers of fine, medium, and large lateral roots at the end of the growing season (Table 3).

The reciprocal effects on one-month-old seedling height were due to significant reciprocal-cross differences in April and May for two pairs (L x G and B x B). Crosses L108 x G106 and B104 x B110 had larger seedling heights than did their reciprocal crosses during that period. Cross L108 x G106 also produced a significantly larger stem volume by October than the reciprocal G106 x L108 (33.4 cm^3 versus 16.9 cm^3). The two crosses showed greater seed weights and nursery germination values and germination peak values (Land *et* 1989). For instance, cross L108 x G106 had significantly lower full seed weight and empty seed weight than the reciprocal G106 x L108 (2.9 mg • seed' versus 4.2 mg • seed-1, P 0.01, and 1.9 versus 3.3 mg • $seed^{-1}$, P 0.01, respectively). Clone G106 exhibited poor vigor during the year of pollination and seed development, and it died during the year after seed collection as an apparent result of graft incompatibility. This poor vigor may have provided a poor common maternal environment for the seeds of cross G106 x L108. This study indicates that reciprocal-cross differences in seedling height and stem volume *may* result from reciprocal effects on seed sizes and germination rates. Similar results have been reported from several studies in pine species (Perry 1976, Dunlap and Barnett 1983, Barnett 1991). However, relationships between mother-tree vigor, seed quality, and reciprocal effects on first-year sycamore seedling growth and development cannot be proven from the present study. The observed coincidence of graft incompatibility and large reciprocal effects suggests that further studies of the relationships are needed.

(2) Leaf area development - F-tests and estimates of variance components indicated that genetic effects (variation among six pairs) accounted for all of the family variation in number of live leaves and total leaf area per seedling through August 7 (nearly four months after planting) (Table 4). Differences among pairs were also significant for leaf areas on main stems and branches during that period (Figures 2a and 2b). Genetic differences in total leaf area per seedling in July were linearly correlated with differences in seedling stem volume in December (r = 0.97) (Figure 3). The greater leaf area per seedling the pairs produced, the larger stem volume they had. Rapid leaf area expansion early in the growing season is apparently of direct importance to first-year seedling size, which is comparable to results found for *Populus* hybrids (Isebrands and Nelson 1982, Ridge *et al.* 1986, Michael *et al.* 1988).

Table 4. Relative sizes of estimated variance components for leaf area of sycamore seedlings during the 1989 growing season.

Measurement Date	Relative sizes of variance components as % of the family component[a]			
	Leaf area per seedling		Live leaves per seedling	
	P[b]	C:P	P	C:P
	%			
July 27	100**	0.0	100**	0.0
August 7	100**	0.0	100**	0.0
August 21	63.8	36.2	36.4	63.6
September 7	29.9	70.1*	0.0	100*
September 21	16.9	83.1**	0.0	100*
October 9	42.6	57.4*	23.0	77.0
October 24	0.0	100**	0.0	100**
November 9	0.0	100	0.0	100*

[a] * = Significant at P ^ 0.05 and ** = Significant at P ^ 0.01.
[b] P = Variance component among pairs, and C:P = Variance component between two reciprocal crosses within each pair. Negative estimates were given a value of zero.

Reciprocal effects (variation between reciprocal crosses within each pair) were found for number of live leaves and leaf area after August and increased toward the end of the growing season (Table 4). These effects were primarily related to the behavior of the two reciprocal crosses between G106 and L108 (Figure 4). Cross G106 x L108 demonstrated poor leaf

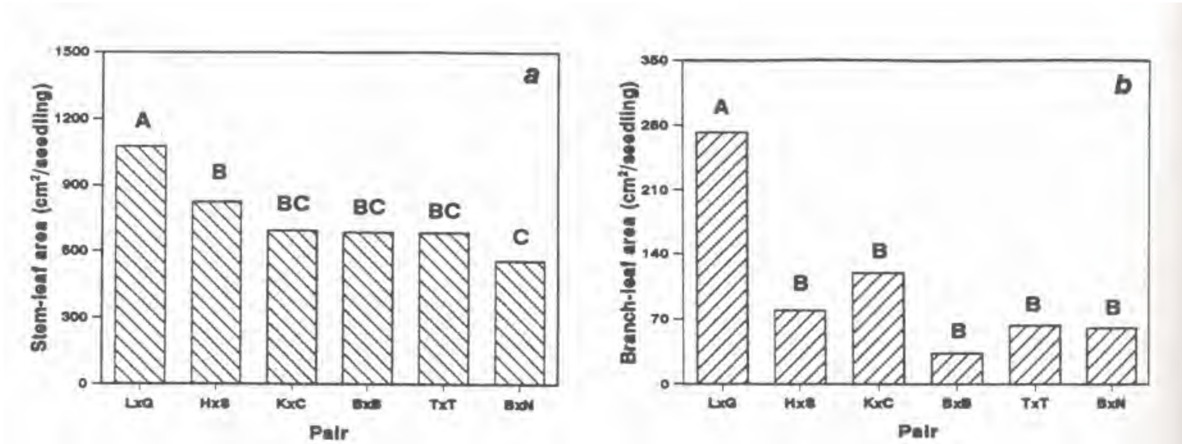

Figure 2. Mean comparisons of stem-leaf area (a) and branch-leaf area (b) in July for six pairs of sycamore seedlings (abbreviations of the pairs are given in the Materials and Methods). Bars not labeled by the same letters differ at $P < 0.05$ (experimentwise error at $P < 0.23$).

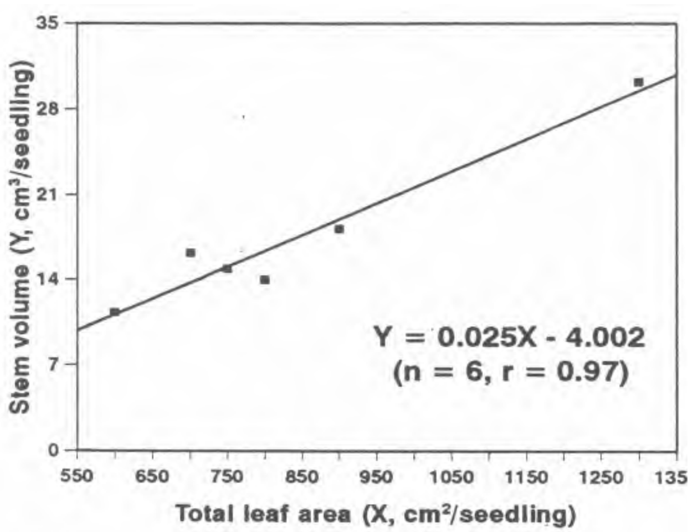

Figure 3. Relationship between leaf area per seedling in July and stem volume in December during the 1989 growing season (based on the means of six pairs of crosses).

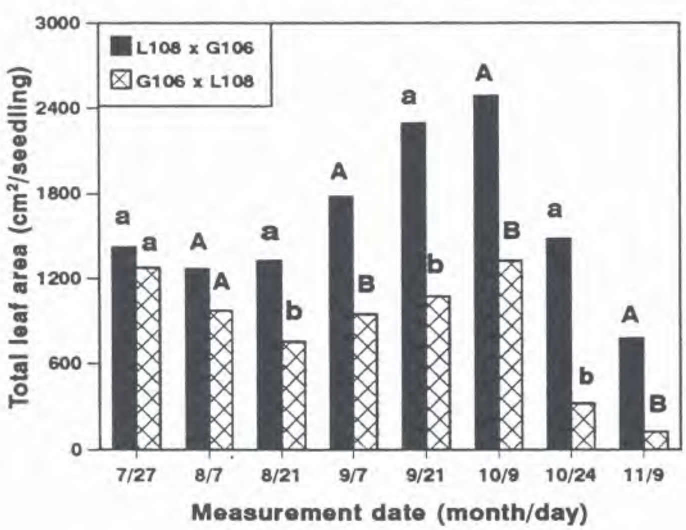

Figure 4. Mean comparison of total leaf area for two reciprocal crosses within pair L **x G** of sycamore seedlings from July 27 through November 9, 1989. Bars not labeled by the same letters at a given measurement date differ at $P < 0.05$.

retention during the August dry period, and it also had a slow recovery of leaf area during September to mid October after the dry period. However, cross L108 x G106 maintained nearly twice as much leaf area per seedling during the same time interval. The rea son for this reciprocal effect cannot be explained by the present study, but as noted earlier it coincides with the decline of mother clone G106 from apparent graft incompatibility.

SUMMARY

Genetic differences were detected for (1) seedling height and diameter during most of the growing season, (2) total number of live leaves and leaf area per seedling before mid -August, and (3) stem volume at the end of the growing season. Reciprocal effects were responsible for family variation in seedling height one month after planting and then gradually disappeared. Differences among families for stem volume in October and leaf area production after August were also associated with the reciprocal effects. The fastest-growing cross produced the largest total leaf area per seedling early in the growing season, and it sustained this advantage with the greatest height, diameter, and stem volume growth throughout the growing season. Future research efforts need to focus on relationships between mother tree vigor, seed production and quality, and reciprocal effects on first-year seedling growth and leaf area development.

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