EFFECTS OF ROOTSTOCK GENOTYPE AND SCION CHRONOLOGICAL AGE ON GROWTH AND FLOWERING OF SLASH PINE GRAFT'S.

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Abstract.—In 1988 and 1989, the University of Florida Cooperative Forest Genetics Research Program (CFGRP) established clone banks throughout the Southeastern U. S. for breeding and scion multiplication for seed orchards. This provided an opportunity to study rootstock-scion interactions, screen for potential seed orchard rootstock families and study the effects of scion maturation on growth and reproduction of grafted slash pine clones across many sites and ages. This study included nine clone banks, seventy-six open-pollinated slash pine rootstock families, approximately 460 scion clones of different chronological ages and over 3600 ramets. Comparisons among rootstocks were made for height and diameter growth, disease resistance, female strobili production, male strobili production and survival. The scion clones had chronological ages (age from time of seed germination) of 5 to over 40 years. Comparisons of height growth, diameter growth and female and male strobili production were made between older and younger scion clones.

Keywords: *Pinus elliottii,* seed orchard, rootstock-scion interaction, chronological age, juvenility.

INTRODUCTION

Industrial forestry in the Southern United States is primarily based on the use of genetically-improved seed for plantation establishment, which has significantly increased both the volume and quality of wood throughout the region. As a result, clones in breeding programs and seed orchards are of immense economic value. Most existing slash pine *(Pinus elliottii* Engelm. var *elliottii)* seed orchards were grafted using unselected rootstock, and some orchards have experienced incompatibility, poor growth and inadequate or delayed flowering. Many methods of controlling these problems have been tried such as top-pruning, fertilization and irrigation timing and partial girdling (Schmidtling 1980, 1985). Some methods have been effective (fertilization and irrigation), while others seem to be of limited use (top-pruning) (Varnel 1969, Greenwood and Bramlett 1989). The use of genetically-selected rootstock is another possible means of providing cost-effective control of scion characteristics for the life of grafted trees, which has proved effective with horticultural perennials such as apples and peaches (Schmidtling 1980, Simons 1987).

As woody plants age, their development begins with the juvenile phase, which can last from a few days to as long as 30-40 years and is characterized by vegetative growth with little or no reproductive growth. The mature phase follows and is characterized by consistent reproductive growth (Hackett 1985, Hackett et al. 1990, Greenwood and Hutchison 1993). This process is commonly called phase change or maturation and has many synonymous names such as ontogeny, cyclophysis, ontogenetic ageing, meristem ageing, ageing and juvenility (Brink 1962, Oleson 1973, Hackett 1985). A great deal of research on phase change has been done in horticultural crops especially citrus, prunus and apple species (Visser 1964, 1965, Zimmerman 1972, Hackett 1985, Oliveira and Browning 1993, Snowball et al. 1994). However, less work on maturation has been done with conifers and almost none with slash pine (Hood and Libby 1978, Greenwood and Nussbaum 1981, Greenwood 1981, 1984, Bolstad and Libby 1982, Greenwood et al. 1989, Burris et al. 1991).

There are three main objectives to this study: (1) To ascertain whether some genotypes, when used as rootstock in grafted seed orchards; will confer desirable characteristics to the scion; (2) To screen a large number of open-pollinated families for their potential as rootstocks; and (3) To examine the effects of scion chronological age on the growth and reproduction of slash pine in clone banks and seed orchards. Characteristics that would be desirable to screen for are: early flowering (precocity), heavy flower production (fecundity), graft compatibility, rust resistance, pitch canker resistance and dwarfing.

METHODS

The nine clone bank locations are planted on a 15 by 30 foot (4.6 by 9.2 meters) spacing with from 5 to 12 blocks (1 block is 0.62 acres or 0.25 hectares), 40 to 120 scion clones and 8 to 10 open-pollinated (OP) rootstock families per clone bank with between 30 and 72 ramets grafted onto each rootstock family. The scion clones have chronological ages from 5 to 30 years among the forward selections, and greater than 40 years among the backward selections. Backward (parental) selections are original selections made in the 1950's which have been maintained in orchards. Forward (offspring) selections are offspring of the original selections and were selected from progeny tests. The rootstocks are placed in 5 groups with 2 OP rootstock families in each, selected based on their parents' performance in various progeny tests and research studies. Each rootstock group was intended to test one of 5 performance traits which were: (A) growth, (B) flowering, (C) graft compatibility, (D) fusiform rust resistance and (E) pitch canker resistance. Each group consists of a high and low performer for each trait (i.e., a fast and slow grower in group A in each clone bank).

An ideal clone bank contains 10 randomized complete blocks, 10 rootstock families and 100 scion selections, with 5 blocks established in 1988 and 5 in 1989. Each block consists of 20 row plots, of three trees each. A given rootstock group was assigned to 4 row plots (12 ramet positions per block), and two scion clones were assigned to each rootstock group. Each scion clone was grafted onto one row plot of each rootstock within a group (i.e., 6 positions per block). Thus, each group of 4 row plots can be viewed as a 2 X 2 factorial (2 rootstocks by 2 scion clones). The two scion clones are therefore nested in each block by group combination, and cross classified with rootstock families within the group. There are five rootstock groups (A through E) and thus five different contrasts going on within each block. The rootstock groups are considered whole plot factors, and the two rootstock families within each group are considered subplot factors. Single degree of freedom contrasts within the groups measure the difference between the two rootstocks in each group. Having scion clones nested within rootstock groups within blocks allows for the many scion clones needed in these operational clone banks.

Measurements were taken each year from 1988 until 1995 of: planting code, status (whether living, dead, fusiform rust infected, pitch canker infected and graft incompatible), total height, height to graft union, scion and rootstock diameter (near the graft union), number of lateral branches, female strobili (flower) counts and male catkin cluster counts. Low disease incidence, high graft compatibility and good survival in the clone banks precluded the analysis of the disease, graft incompatibility and survival variables. The seven key variables that could be analyzed were: rootstock diameter, scion diameter, ramet height (total height minus height to graft union), ratio of scion diameter to rootstock diameter, ratio of ramet height to scion diameter, number of flowers per tree and number of male catkin clusters per tree.

Analysis of the Rootstock and Scion Effects

The rootstock, scion and the rootstock-scion interaction effects were examined to determine their overall effects on growth and reproduction. The linear model for the analysis was:

$$y_{ijkl} = b_i + g_j + bg_{ij} + r_{k(j)} + br_{ik(j)} + s_{l(ij)} + rs_{kl(ij)}$$

where
b_i = random effect of i^{th} block,
g_j = fixed effect of j^{th} rootstock group,
bg_{ij} = random effect of interaction of i^{th} block and j^{th} rootstock group,
$r_{k(j)}$ = fixed effect of k^{th} rootstock within j^{th} rootstock group,
$br_{ik(j)}$ = random effect of interaction of i^{th} block with k^{th} rootstock,
$s_{l(ij)}$ = random effect of the i^{th} scion within the i^{th} block and j^{th} rootstock group,
$rs_{kl(ij)}$ = random effect of interaction of k^{th} rootstock and l^{th} scion.

There was too little growth in year 1 to include it in the rootstock analysis. And there were
1 too few flowers and catkin clusters before years 5 and 6 to test the effects of flowers per ramet and male catkin clusters per ramet. All analyses was done using procedure GLM in the SAS® programming language (SAS Institute 1989). Tests were considered significant if the F-test was significant at the a=0.10 level. To strengthen the test of the within-group contrasts the rootstock scion interaction ($rs_{u(1)}$) was pooled with the error. Only 33% of the F-tests for the interaction were significant at the a =0.25 level, with no consistency by trait or measurement year, and their mean significance was greater than a =0.25, which is an acceptable level for pooling (Bozivich et. al. 1956, Bancroft 1968). This reduced linear model was used to test the single degree of freedom contrasts for each rootstock grouping in each clone bank. Due to low disease incidence, high graft compatibility and high survival only contrasts within the flowering and growth groups were used (ie., rootstock groups A and B). Further, only contrasts in measurement years 5 and 6 were analyzed since persistent rootstock effects are the ones of most interest.

In order to estimate the relative effects of the rootstock and scion on growth and reproduction of ramets in the clone banks, a ratio of the rootstock family variance component to the sum of the rootstock and scion variance components was calculated. The variance component estimates were obtained through the VARCOMP procedure in SAS ® (SAS Institute 1989). Since, due to common pollen parents, the intraclass correlation among rootstock OP families is probably closer to 0.30 than 0.25 the constant 3.3 ,instead of 4, was used to multiply the rootstock family variance to obtain an estimate of additive variance (Squillace 1974). The scion variance component is among scion clones and hence estimates total genetic variance. It was used in the denominator of the ration without amplification. Several methods of variance component estimation were tried and found to produce similar results, so the ANOVA Type I sums of squares method was used, since it is both simple and unbiased. The ratio gives the amount of rootstock variance compared to the total rootstock and scion variance. Thus, the closer the ratio is to 0, the less variance that is accounted for by the rootstock, and the closer to 1 the more variance accounted for by the rootstock.

<u>Scion Chronological Age Analysis</u>

To quantify the effects of scion chronological age (age from time of seed germination) on growth and flowering of clone bank ramets, four variables were analyzed at ramet ages 1 to 6 (age of a ramet since it was grafted). They were: scion diameter, ramet height (total height minus height to graft union), flowers per ramet and male catkin clusters per ramet. Since the only true replication of scion clones was due to the two different grafting years and the nine clone bank locations, the units of observation for this analysis were means by chronological age group (backward selections or forward selections) within year-grafted (1988 or 1989) within location (9 clone banks). For example, the mean of all forward selections grafted in 1988 in a given location is considered one observation. The linear model for the scion age analysis was:

$$y_{ijk} = l_i + y_j + ly_{ij} + g_k + lg_{ik} + yg_{jk} + lyg_{ijk}$$

where l_i = random effect of i^{th} location ($i=1,\cdots 9$),
y_j = random effect of j^{th} year ($j=1988$ or 1989),
ly_{ij} = random effect of interaction of i^{th} location and j^{th} year,
g_k = fixed effect of k^{th} age group (k= backward or forward),
lg_{ik} = random effect of interaction of i^{th} location and k^{th} age group,
yg_{jk} = random effect of interaction of j^{th} year and k^{th} age group.

The three way interaction was considered the error term to increase its power and the "sometimes pooling" technique was used to determine which two-way interactions could be pooled with it (Bozivich et. al. 1956, Bancroft 1968). The year-grafted by age group (ygjk) and the location by age group ($1g,_{k}$) interactions had few significant F-tests with no consistency by trait or measurement year and were thus acceptable for pooling (Bozivich et. al. 1956, Bancroft 1968). Pooling led to the use of a reduced model in which the residual error consisted of the pooled lyg_{uk} and yg_{jk} interactions for most F-tests and lyg_{ijk}, yg_{jk} and lg,k interactions for the few remaining F-tests. This model was used to test the difference between backward and forward selections.

RESULTS AND DISCUSSION

Overall rootstock effects were only occasionally significant at the a =0.10 level for the 4 growth variables, and effectively nonsignificant for the other 3 variables. However, overall scion effects were almost always significant. The interaction between rootstock and scion had more significant tests than the rootstock effects alone. The rootstock effects when present were small compared to the scion effects (Table 1). Only 18% of 92 rootstock group contrasts showed significance at the a=0.10 level. At the same time 19% of all possible contrasts (the 7 variables by 5 rootstock group contrasts by 9 clone banks) were significant at the a=0.10 level. That is, the high flowering or growth rootstock, chosen on the basis of its progeny test performance, was almost never significantly different than the low flowering or growth rootstock family in the same group. If the groups were well-chosen they should have a higher percentage of significant contrasts than for all contrasts. Thus, the choices of rootstocks to contrast in the flowering and growth groups were not effective, which is not surprising given the small overall rootstock effects relative to overall scion effects (Table 1). Scion effects seem to be so large that they seem to overwhelm the rootstock effects.

Table 1. Number and percentage of tests of the overall rootstock, scion and rootstock by scion interaction effects that were significant, at a =0.10, level for 7 key variables. The N is the total number of tests for the variable, while the % value is the percentage of tests that were significant at the a =0.10 level.

	Model effects					
	Rootstock		Scion		Interaction	
Variables	N	%	N	%	N	%
Flowers / ramet	17	6	18	83	18	22
Height / scion dia. ratio	41	24	41	78	40	23
Catkin clusters / ramet	18	6	18	83	18	39
Ramet height	43	9	43	95	43	21
Scion dia. / rootstock dia. ratio	41	34	41	54	41	22
Rootstock diameter	41	17	41	73	41	27
Scion diameter	40	23	41	78	41	24

The ratio of the rootstock variance to the sum of the rootstock and scion variance components indicates that the relative contribution of rootstock to the genetic variance is much less than the scion contributions. All variables, except the scion diameter to rootstock diameter ratio, had ratios of about 0.1 to 0.2. Also, the ratio for the ramet height and scion diameter started high, but fell after year 2 implying the rootstock effect diminished quickly as the ramets aged (Table 2). Height to scion diameter ratio diminished slowly over the 5 years. The ratio of scion diameter to rootstock diameter remained constant and fairly high over all 5 measurement years. Rootstock effects on the 2 flowering variables may be increasing, but with only 2 year's flowering data there is too little information to call it a trend (Table 2).

Table 2. Ratio of the rootstock variance to the sum of the rootstock and scion variance components for the 7 key variables averaged across the 9 clone banks. Each measurement year's value is the mean of the values of all 9 clone banks. Insufficient flowers were present in years 2 through 4 to estimate the flower per ramet and catkin clusters per ramet ratios.

	Measurement Year					
Variables	2	3	4	5	6	Mean
Flowers / ramet				0.05	0.15	0.11
Height / scion dia. ratio	0.29	0.36	0.12	0.19	0.02	0.19
Catkin clusters / ramet				0.14	0.23	0.19
Scion dia. / rootstock dia.	0.29	0.38	0.33	0.40	0.38	0.36
Ramet height	0.31	0.08	0.12	0.05	0.06	0.12
Rootstock diameter	0.29	0.29	0.22	0.13	0.22	0.23
Scion diameter	0.24	0.02	0.11	0.08	0.10	0.11

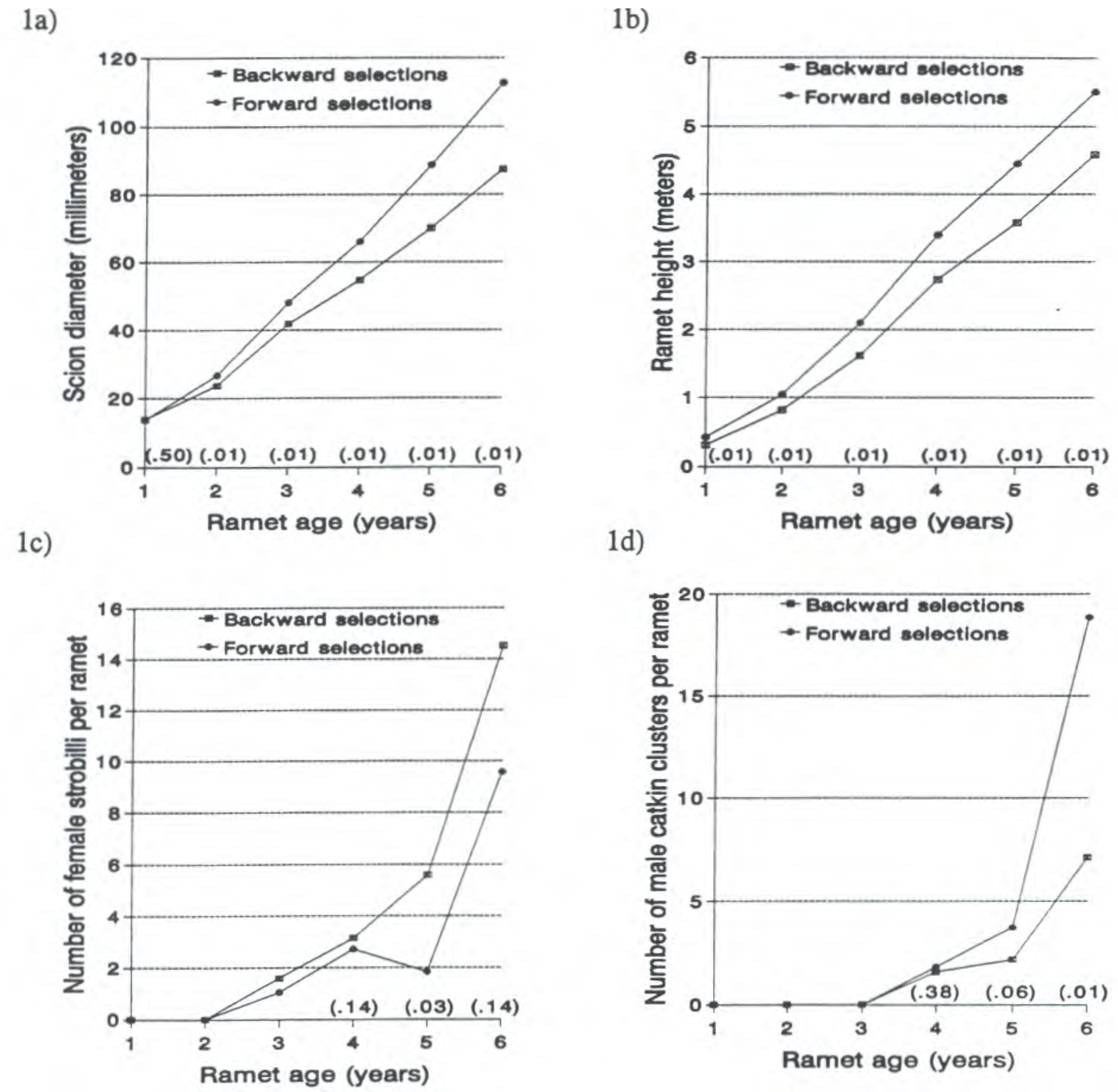
No ranking of the 76 different rootstock families was done for several reasons. First, there was too little disease incidence, graft incompatibility and too high survival to determine the rootstock effects on fusiform rust, pitch canker and graft compatibility. Second, the rootstock effects were too small relative to the scion effects to effectively rank the rootstocks for the remaining growth and flowering variables.

Scion Chronological Age Effects

For almost all dependent variables (across all 6 years) there was a statistically significant difference at the a =0.05 level between forward and backward selections. In all except the first measurement year the difference between scion diameters of backward and forward selections was increasing and significant at the a=0.01 level (Figure la). The lack of significance in the first year was probably because no appreciable growth had yet occurred. Differences between the ramet height of backward and forward selections were significant at the a=0.01 level in all 6 years (Figure lb). Also, in all but the first year the forward selections grew more in diameter than backward selections. Therefore, there were significant differences in growth rates between the backward and forward selections.

In years 1 through 3 there were not enough female strobili present to test the difference between backward and forward selections (Figure lc). In year 4, the difference in female strobili production between backward and forward selections was only significant at the a=0.14 level. But, by year 5 the backward selections produced significantly more female strobili at the a=0.05 level than the forward selections. However, in year 6 the difference became nonsignificant again, even though there was a greater number of flowers present on backwards selections despite them being smaller in diameter, shorter and having fewer branches. However, the absolute differences in number of female strobili per ramet was only about 3 flowers (Figure 1c).

Figure 1. Differences in growth and flowering between backward selections (chronologically older scion) and forward selections (chronologically younger scion) for 6 years after grafting: a) Total scion diameter growth; b) total ramet height growth; c) female strobili production; d) male strobili production; The values in the parenthesis are the a levels at which the contrasts between backward and forward selections are significant.



Forward selections produced significantly more catkin clusters per tree than the backward selections in years 5 and 6 (Figure 1d). This could be because most forward selections, though chronologically younger than the backward selections, were close to maturity. Of the forward selections 72% were 6 to 10-years-old. Also, in conifers most catkins are produced in the lower crown, which is chronologically younger than the upper crown where female strobili are formed. Thus, the forward selections may be at an excellent age to produce catkins, and the backward selections may be beyond the prime age to produce catkins.

CONCLUSIONS

This study leads to the following conclusions:

1. Rootstocks had a small but significant effect on the taper and diameter growth, and no noticeable effect on the height and flowering of clone bank ramets.

2. Scion had a large, highly significant effect on the height, diameter, taper and flowering of clone bank ramets. This effect was at least 5 to 10 times larger than the rootstock effect.

3. Interaction between the scion and rootstock was significant for all tested variables and larger than the rootstock effect.

4. Rootstock effects were too small relative to the scion effects to make it possible to effectively rank the rootstocks for the tested variables. The evidence here suggests that there is little reason to select for rootstocks in slash pine.

5. Scion chronological age effects were highly significant, even after six years of ramet growth. Chronologically older scion grew less in diameter and height than chronologically younger material. Chronologically younger scion grew more in both height and diameter and produced about 2.5 times as many male catkin clusters per tree as chronologically older material. Age effects on female flower production were small and generally not significant.

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