FLOWERING RESPONSE OF JUVENILE SELECTIONS IN LOBLOLLY PINE

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Abstract--Four types of loblolly pine scions were grafted and subjected to female strobilus induction. These scion types were: age one (1) from nursery-grown seedlings, age three (3S) from a genetic field test averaging one meter in height, age three (3L) from a genetic field test averaging 2.4 meters in height and age eight (8). At 14 and at 26 months after grafting, there was a positive scion age effect on female strobilus production but none on male strobilus production. There was more pollen produced on scion type 3L compared to all other scion types. Grafting and applying flower induction treatments in the same year could reduce the breeding interval from five to four years for selections which are at least three years old. However, this method is not effective in reducing the breeding interval for early selection methods which rely on one-year-old selections. All juvenile selections readily responded to flower induction at 26 months from grafting.

Additional Keywords: Early selection, *Pinus taeda* L., accelerated breeding technology

INTRODUCTION

The flowering response of juvenile selections influences practical use of early selection schemes for loblolly pine (*Pinus taeda* L.) in two ways. Use of juvenile selections can 1) delay flowering response, which offsets the time savings of early selection, or 2) reduce the flowering response which raises annual breeding costs, thus reducing gain per unit cost.

To date, there has been one report on the effect of selection age on flowering in loblolly pine (Greenwood 1984). Scions were grafted from selections made in first-generation genetic tests at ages 1, 4, 8 and 12 years. Flower induction treatments were applied one year after grafting. In spring 1981, 33 months from grafting, there was a marked reduction between ages 1 and 4 years in male and female flowering response for juvenile selections. Given refinements in flower induction technology over the past decade, it is not certain if poor flowering will delay operational use of early selection particularly if grafting and flower induction, normally separated by one year, are done in the same year (Figure 1A).

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The effect of juvenile selection on flowering is further clouded by the loose association between chronological age and seedling development (Poethig 1990). Early selection methodology is often based on accelerating seedling growth in a greenhouse (van Buijtenen 1986) or on accelerating seedlings in closely-spaced farm-field genetic tests (i.e. Li et al. manuscript in review; Williams 1987). Accelerated seedling growth may improve flowering response of juvenile selections.

The following study was conducted to address two questions:

1. Can female strobilus induction treatments applied in the same year as grafting be used to reduce the time for breeding juvenile selections?

2. Is height at selection more important than the tree's chronological age in determining flowering response?

METHODS AND MATERIALS

In February 1989, scions were collected for a total of 63 grafts. Four scion types (Table 1) were grafted onto two rootstock types, ages two and three years, using scion from two open-pollinated families from a first-generation North Carolina Coastal Plain seed orchard. Sample sizes of scion age types were nearly balanced: scion type 1 had 17 grafts, scion type 3S and 3L had 15 grafts each and scion type 8 had 16 grafts.

Table 1. Ortet location and site characteristics for each of four scion types.

Code	Scion Type	Selection Age From Seed	Mean Height	Ortet Location	Site Characteristics
1	1 year	10 months	0.20 m	Columbia Co. Arkansas	Weyerhaeuser Nursery
3S	3 year small	34 months	0.99 m	Washington Co. North Carolina	Genetics x silviculture interaction trial. No herbicide applied
3L	3 year large	34 months	2.40 m	Washington Co. North Carolina	Genetics x silviculture interaction trial. Herbicide applied
8	8 year	87 months	4.20 m	Saline Co. Arkansas	Droughty, slow growing site, some competition control

All scions came from the upper one-third of each crown. In May 1989, grafts were repotted from 12-liter pots to 60-liter galvanized metal pots using a mixture of sand, pine bark, peat moss and vermiculite (1:1:1:1 by volume). A slow release fertilizer (Osmocote 18-6-12) was incorporated. The trees had one month to stabilize before starting mid-June female strobilus induction treatments.

In the first year, 60% of the grafts received gibberellin applications and mild water stress using methods cited by Greenwood (1981). The untreated remainder (40%) served as the control. As described by Greenwood (1981), water stress levels were monitored using a Scholander pressure chamber to measure predawn stress. After reaching stress levels of 125 psi (8.5 atm) trees were watered thoroughly; this continued from mid-June until mid-September. This water stress level of 125 psi is lower than the 150 psi used by Greenwood (1981).

In the second year, all grafts received female strobili induction treatments. Water stress was allowed to reach 150 psi (10.5 atm) before re-watering. A second application of fertilizer was made in April 1990. 70 grams of Osmocote (18-6-12) was added as a top dressing to each tree. Eight applications of 0.01 ml of GA $_{4/7}$ per bud were also applied from mid-June to mid-September using a Cole-Palmer jet pipette model 3202. This was the same application regime used in year 1 flower induction treatment in both years. 1 gram of GA $_{4/7}$ was dissolved per 50 ml of 80% ethanol. All potential flower producing branches were treated as close as possible to the base of the bud.

Grafting scions and applying flower induction treatments within the same year is considered an abbreviated breeding schedule (Figure 1A); flower induction normally follows one year after grafting (Figure 1B). Under the abbreviated schedule, we began female strobili induction at five months after grafting.

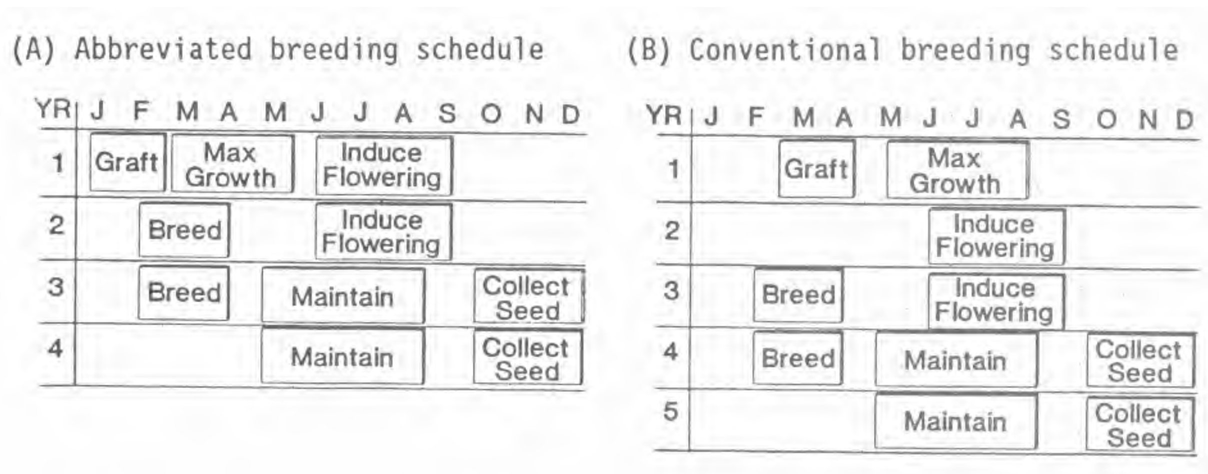


Figure 1. Timeline comparison between an abbreviated indoor breeding schedule (A) and conventional indoor breeding schedule (B) as proposed by Greenwood et al. (1986).

STATISTICAL METHODS

The study was blocked on a 2 x 2 rootstock-family combination. Blocks were considered fixed. A linear model with fixed treatment effects was used for analyses of variance:

$$Y_{ijk} = \mu + \beta_i + \tau_j + \beta\tau_{ij} + \epsilon_{ijk}$$

Where μ = overall mean
β_i = effect of block i
τ_j = effect of scion type j
$\beta\tau_{ij}$ = block i by scion type j interaction effect
ϵ_{ijk} = experimental error

A generalized linear model approach was used to adjust for imbalance across scion types for traits based on female and male strobilus clusters. Adjusted means were used to remove any effect of imbalance arising from unequal number of grafts per scion type or from unequal number of strobilus clusters per scion type.

The first-year flower induction treatment was tested as a covariate for all second-year flowering response (26 months after grafting) but demonstrated no statistically important effect on second-year traits. All statistical analyses were aided by PC-SAS version 6.03.

RESULTS

Selection Age Effect at 14 Months on Female Strobilus Production

Accelerated breeding techniques are still effective for loblolly pine; without female induction treatments no scion type would have produced female strobili at 14 months after grafting (Table 2).

Table 2. Response at 14 months after grafting to female strobilus induction treatment applied within six months after grafting across all scion types.

Trait Description	-- Treatment Response at 14 Mo. Female Strobilus Stimulation Treatment	No Treatment Control
female strobili per graft (count)	0.78	0.04
percentage of flowering grafts (%) (ratio)	34.15% (14/41)	4.50% (1/22)
female strobili per flowering graft (count)	2.30	1.00

Scion types 3S, 3L and 8 produced female strobili at 14 months after grafting (Table 2; Figure 2). The number of female strobili at 14 months was comparable to the 1981 study observed at 33 months after grafting (Figure 2). For example, scion type 8 has 1.2 female strobili per graft which was the same flowering response reported previously for age 8 (Figure 2).

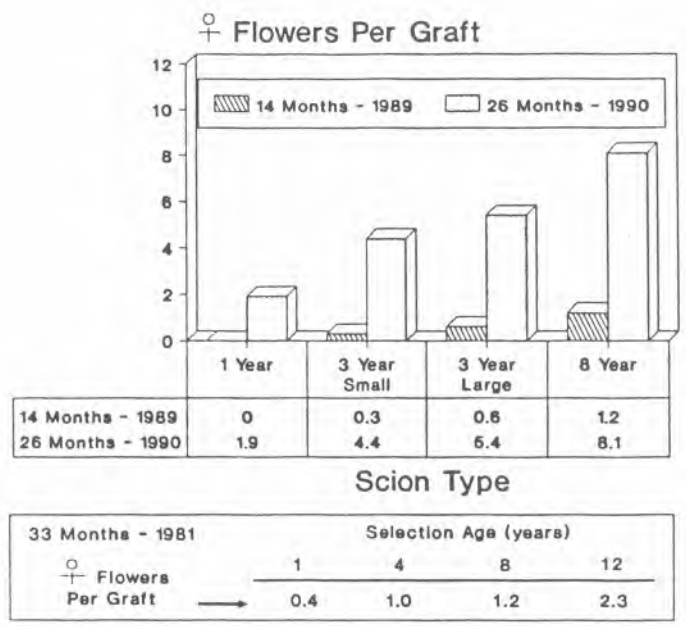


Figure 2. Female flowering response at 14 and at 26 months after grafting. Data are compared with a previous study reported by Greenwood (1984) in which female flowering response was assessed at 33 months after grafting.

However, the same-year graft/flower induction treatment did not induce any female strobili for scion type 1 (Figure 2). In this case, the number of female strobili is less than what was reported for the 1981 study (Figure 2). It is not clear whether this lack of flowering occurred because we used a water stress treatment (125 psi) which was lower than normal.

Selection Age Effect at 26 Months on Female Strobilus Production

Female strobili production increased for all scion types at 26 months after grafting (Figure 2). In absolute terms, scion type 1 in this study had as many female strobili as the 12-year-old scion and four times as many strobili as 1-year-old scion in the 1981 experiment (Figure 2). This was quite unexpected because scion type 1 is younger than the age one scion used in the 1981 study. Age one selections were actually collected from 1.5 to 2.0 seedlings potted from 1-0 nursery stock (Greenwood 1984).

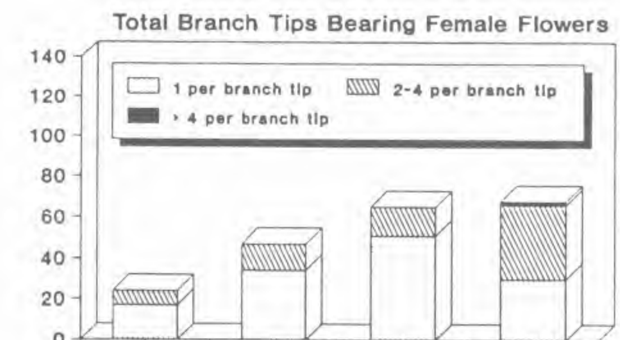
Scion types 3S and 3L had two to three times more female strobili per graft than the 12-year-old scion in the 1981 study and scion type 8 exhibited a four-fold increase compared to 12-year-old selections in the 1981 experiment. Family differences for female strobilus production were negligible (Table 3).

Tree Height Effect on Female Strobilus Production

Height at selection had less influence on female strobilus production than age. The differences between scion types 3S and 3L were statistically significant at the 60% level. This also proved to be the case at 26 months; the differences between scion types 3S and 3L were statistically significant at the 50% level.

Despite a low probability of a true difference due to tree height, scion type 3L did produce more female strobili per graft than scion type 3S at both 14 and 26 months after grafting (Figure 2). The increase in female strobili at 26 months appeared to be due to the number of branch tips bearing strobili although most of these flowering branches bore more singlets (Figure 3).

On a minor point, scion type 3S also had more non-flowering grafts at 14 and 26 months after grafting. At 14 months, 36.4% of the 3S grafts produced female strobili. Of these flowering grafts, there were 1.3 female strobili per graft. For scion type 3L, 44.4% of the grafts produced female strobili. Of these flowering grafts, there were 2.0 female strobili per graft. At 26 months, 73% of the scion type 3S grafts produced female strobili with 6.0 female strobili per flowering grafts. For scion type 3L, 100% of the grafts produced female strobili with a mean of 5.4 female strobili per graft.



	1 Year	3 Year Small	3 Year Large	8 Year
› 4 per branch tip	0	0	0	2
2-4 per branch tip	7	13	14	36
1 per branch tip	17	34	51	30

Scion Type

Figure 3. The total branch tips with female strobili are subdivided into three categories: branch tips with a single female strobilus, branch tips with two to four female strobili and branch tips with more than four female strobili. Branch tips bearing female strobili is defined as one terminal and its lateral buds, all of which would be enclosed in one bag for controlled-pollination.

Selection Age Effect on Male Strobilus Production

Pollen catkins did not appear on any grafts 14 months after grafting. This was not unexpected since no pollen induction treatments were applied. Until pollen induction is tested in the same year as grafting, it is uncertain whether pollen will be available in the first year of the abbreviated breeding schedule.

At 26 months, pollen production on all four scion types was adequate for controlled pollinations in a breeding orchard (Bramlett et al. 1985) and there was no detectable scion age effect (Table 4). This is in direct contrast with male strobilus clusters per graft reported in 1981 (Greenwood, 1984; Table 2). There were large differences between open-pollinated families for male strobilus production (Table 3).

Tree Height versus Tree Age for Male Strobilus Production

Selection height was more important than selection age for pollen production although the reverse was true for female strobilus production (Table 4; Figure 2). Scion type 3L produced 4.60 male catkin clusters per graft as opposed to 1.35 male catkin clusters per graft for scion type 3S (Table 4). Scion type 3L had fewer male strobili per cluster than any other type yet it produced the most male strobili on a whole-crown basis. The increase was due to height at selection age rather than selection age.

DISCUSSION

Selection Age Effect on Female Strobilus Production

These results suggested that breeding can be started one year sooner for scion types 3S, 3L or 8 by applying flower induction treatments soon after dormant-season grafting. Breeding could be completed in four years rather than in five if pollen is available (i.e. Greenwood et al. 1986). There are added advantage to the abbreviated schedule: the grafts will be smaller at the completion of breeding so that pollination work does not require a ladder. Also, Indoor breeding can be conducted in a smaller greenhouse at a considerable cost savings.

More grafts per selection will be needed if a selection is made in a test with a mean height of one meter. Also, it seemed likely that there will be more non-flowering grafts than if the selections came from a test averaging 2.4 meters in height. Results for scion types 3S, 3L or 8 are applicable to selections from conventional widely spaced tests and perhaps to closely spaced farm-field tests.

Annual breeding costs per graft will also be slightly higher for scion types 3S and 3L compared to age 8. There are more single female strobili per branch tip (singlets) so more isolation bags will be required to obtain the same amount of sound seed. A singlet is less desirable for controlled-pollination than two to four female strobili per branch tip because more isolation bags must be applied to obtain the same number of sound seed. Future refinements in accelerated breeding techniques would best be directed toward increasing numbers of female strobili per branch tip rather as opposed to a whole-crown basis.

For age one selections, same-year grafting and flower induction was not effective. There should be adequate female and male strobili to begin breeding on a conventional breeding schedule; an abbreviated breeding schedule is not an option using these flower induction treatments.

Breeding costs are likely to be highest for age one selections. 71% of the flowering branch tips bore a single female strobilus and many isolation bags would be needed per given quantity of sound seed. If age one selections are used, one option might be to reduce the required number of sound seed per cross and increase total number of crosses.

Effect of Height versus Age on Male Strobilus Production

Scion type 3L had more flowering branch tips than 3S and we observed that many of these tips bore both female and male strobili. This may explain why there were fewer male strobili per catkin cluster yet more catkin clusters in total (Table 4). We also observed in the greenhouse that the taller 3L grafts produced more growth cycles (and lateral branches) at a faster rate once grafted. By contrast, scion type 8 tended to produce fewer cycles therefore fewer higher-order lateral branch tips and scion type 3S had fewer branch tips capable of flowering (Figure 3).

Table 3. Adjusted open-pollinated family means (and standard errors) for female strobili per cluster and female strobili per graft and three male strobilus traits: pollen per graft, male strobili per cluster and male strobili clusters per graft.

Family ID	Female Strobili per Cluster	Female Strobili per Graft	Male Strobili per Cluster	Male Strobilus Cluster per Graft	Pollen per graft (ml)
A	1.33(±.086)	4.02(±.81)	6.45(±1.83)	0.98(±.76)	1.65(±2.53)
B	1.33(±.076)	5.94(±.85)	8.01(±1.08)	4.12(±.72)	10.03(±2.41)

Table 4. Male flowering response in March 1991, 26 months after grafting. Means and standard errors are shown for pollen quantity per graft number of male strobili per cluster and number of clusters per graft. Scion type differences were statistically significant at the 20% level in this study.

1991 Study 26 Mo. After Grafting				1981 Study 33 Mo. After Grafting	
Scion Type	Pollen per Graft (ml) 1/	Male Strobili per Cluster	Male Strobilus Cluster per Graft	Scion 2/ Age	Male Strobilus Cluster per Graft
1	3.8(±3.4)	6.07(±2.20)	1.90(+1.03)	1	.02 a
3S	3.3(±3.6)	8.67(±1.35)	1.35(±1.16)	4	.97 b
3L	9.3(±3.7)	4.76(±1.65)	4.60(±1.14)	8	.87 b
8	7.3(±3.5)	10.12(±1.58)	2.05(±1.07)	12	2.58 b

1/ 3.7 male strobili equal 1 ml fresh pollen

2/ Data from Greenwood (1984); scion age effects were statistically significant at .05 level; letters denote differences detected by Duncan's multiple comparison test.

In both the 1981 and 1991 studies, it would also appear that female strobilus induction techniques have a side effect on male strobilus production. We observed that heavy pollen production occurs when a graft's crown produces many higher-order laterals which are competent to flowering.

Comparison between 1991 and 1981 scion age studies

A comparison between the two studies suggests that the increased flowering in 1991 is due to improvements in accelerated breeding methodology. There are important similarities between the studies: 1) both studies were conducted in the same location in Hot Springs, Arkansas, 2) both sets of families were sampled from the same North Carolina Coastal Plain seed orchard and 3) both studies were tended by the senior author.

The families were not the same in each study but this seems unlikely to wholly account for the difference. The 1981 study sampled scion randomly from five half-sib families from operational full-sib genetic tests and the 1991 study sampled two additional open-pollinated families, chosen for putative "poor" and "good" flowering ability (J. Hunt, Weyerhaeuser Company, pers. comm.). Family differences may account for some variation between studies but one would expect the family effect to be larger (Table 3) if this were the sole basis for the fourfold increase in female strobilus production.

Refinements in accelerated breeding technology, which have occurred over a decade of steady practice, are thought to account for the difference. There are at least two important refinements. First, gibberellin is now applied as close to the base of the bud as possible, rather than at a constant distance from the bud. Gibberellin placement depends on the morphology of the bud rather than on a set distance from the bud tip. Secondly, the bud that forms in late summer must remain resting or quiescent until spring. This is critical to obtaining a treatment response. If watering or lighting is inadvertently changed, the resting bud may elongate, losing its treatment response for the following spring. It appears that accelerated breeding technology has continued to evolve to the point that breeding juvenile selections is no longer an obstacle.

SUMMARY AND CONCLUSIONS

Juvenile loblolly pine selections can be bred on the same schedule as older selections; there were four times as many female strobili at all ages compared to an earlier study conducted in 1981. These improvements in female strobilus induction technology have removed the adverse impact of scion age on accelerated loblolly pine breeding programs. Controlled-pollination would be more cost-effective if there were also refinements which increased the numbers of female strobilus on a branch cluster basis rather than on a whole-crown basis.

For all scion types except age one, breeding can be completed in four years rather than in five years under the abbreviated breeding schedule. We expect the shorter breeding schedule to reduce the need for 6-meter ceilings in indoor breeding orchards. Routine use of the abbreviated breeding schedule will depend on the availability of pollen at 14 months after grafting. Our next step will be to test same-year grafting and pollen induction treatments.

On a conventional five-year breeding schedule, selection age did not have an effect on pollen production. However, for age three selections, height at selection was more important than age at selection age for male strobilus production. If this proves to be consistent, then there may be an additional opportunity to reduce the breeding schedule by another year since pollen nor female strobili are limiting for three-year selections.

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