TOPWORKING YOUNG SCIONS INTO REPRODUCTIVELY-MATURE LOBLOLLY PINE

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Abstract.--Scions from young trees ranging in age from 1-5 years were grafted into the upper and lower crowns of reproductively-mature loblolly pine seed orchard trees. The scions were randomly collected from a 12-clone, first-generation mix of families used as a check lot in Weyerhaeuser Company's progeny tests. Scions for tree ages of 2-5 years were collected from four different progeny test sites. The 1-year-old scions were collected from nursery-bed seedlings. Ten scions of each age class were grafted into ramets of four second-generation clones in Weyerhaeuser Company's seed orchard at Lyons, GA. On each ramet, five grafts were in the lower crown and five were in the upper crown. A total of 200 grafts were completed in February 1994.

In March 1995, scions were measured for shoot growth, number of branches, number of female strobili, and number of pollen clusters. Survival of grafts in the upper crown was 97%. Female strobili were produced on scions from all age classes and ranged from 21% of age one grafts to 80% of the age four surviving grafts. A total of 247 female strobili were produced on 53 grafts in the upper crown. No female strobili were produced in the lower-crown grafts.

Pollen did not occur on any of the 1-year-old grafted scions. In the lower crown, scion ages from 2-5 years produced pollen clusters on 33 of the 75 surviving grafts. The percent of grafts with pollen ranged from 15% on age 2 scions to 75% on age 5 scions. Pollen was less frequent in the upper crown but did occur on age 3-5 grafts.

Topworking allows tree breeders to produce female strobili one year after selection on scions collected from trees that are 1-5 years old. Pollen can be produced in one year on topworked scions in lower crown from scions that are collected from trees 2-5 years old. The topworking procedure can be used to greatly reduce the generation interval in loblolly pine and to accelerate the breeding cycle for genetic improvement.

Keywords: *Pinus taeda,* female strobili, pollen, tree breeding, seed orchard, grafting

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INTRODUCTION

The breeding cycle in forest trees is the time required from the selection of a genotype in one generation until the selection of a new genotype from the succeeding generation. This breeding cycle depends on the generation interval of the species (time required to produce adequate seed for testing and selection) and the age that genetic selections can be made from the next generation. Obviously, tree breeders want to reduce the time required for the cycle. However, because forest trees typically have a long, non-flowering juvenile stage of development, the breeding cycle can be delayed for as long as 10 to15 years.

Topworking, or the grafting of young scions into mature trees, has been used in an attempt to reduce the generation interval in conifer species. Robinson and Wareing (1969) grafted scions from both juvenile seedlings and nine-year-old trees of European larch (Larix decidua) and Japanese larch (Larix leptolepis) on to aged and flowering shoots of older trees. There was very little cone initiation in juvenile scions of either species but some of the nine-year-old material did produce both female and male strobili.

Barnes and Bingham (1963) also attempted to induce flowering of seedling scions by grafting into the crowns of older trees. The scions were five years old when grafted into 28-year-old mature trees, but none of the scions produced strobili. Other methods tested also proved unsuccessful in inducing or stimulating early strobilus production in western white pine.

Greenwood and Gladstone (1978) demonstrated that both male and female strobili could be produced on scions from one-year-old loblolly pine (Pinus taeda L.) seedlings topworked throughout the crowns of large seed orchard trees. Two to three years after topwork grafting, 50 percent of the surviving grafts produced male strobili. Female strobili occurred on about 20 percent of the scions. Topworking has not been used for tree breeding because an accelerated breeding schedule of 5 years was developed by Greenwood (1993) using a greenhouse environment to stimulate early female and pollen production for breeding purposes.

Burris and Williams (1991) further reduced Greenwood's five-year breeding schedule to four years by applying flower stimulation treatments in the same year that scions were grafted to greenhouse rootstocks. Female strobili were produced one and two years after grafting but pollen was not available until 26 months after grafting. The time required for pollen production was reduced to 13 months by Bramlett et al. (1995) using a surrogate pollen induction (SPI) method. In SPI, scions from selected individual trees in five-year-old progeny tests were topworked into the lower crown of ramets in a second-generation seed orchard. The seed orchard trees had been established for 8 years and were producing abundant pollen in the lower crown. Topworked scions produced pollen strobili on 57% of the surviving grafts 13 months after grafting, and produced enough pollen for breeding four of the five selected individuals.

Based on the success of SPI, a similar method was tested to produce female strobili for breeding purposes. We observed limited female strobili production on scions grafted into the upper crowns of receptor clones in 1993 [2]. The objective of the 1994 study was to evaluate the strobilus production on young scions topworked into the upper and lower crowns of reproductively-mature seed orchard trees.

MATERIALS AND METHODS

Genetic Material

Scions were collected from trees that ranged in age from one to five years. Scions from each age class were part of a 12-clone, first-generation mix of trees used as a check lot in Weyerhaeuser Company's progeny tests. This mix had an equal number of seedlings from each of 12 clones, thus, each age class represented identical genetic material. Obviously, individual trees selected for scion collection could not be identified as an individual family, but the composite sample represented a minimum of 15 trees from the same genetic sources. Scions from tree age classes two-five were collected from progeny test sites. Scions from age class one were collected from seedlings growing in a nursery bed.

Four clones were selected in Weyerhaeuser Company's second-generation loblolly pine seed orchard at Lyons, GA. Receptor clones were 10 years from seed orchard establishment at the time of topworking in February 1994. The seed orchard is intensively managed with annual fertilization, mowing, herbicide application, and pest management. The receptor clones were known to be good female and pollen strobili producers.

Grafting Method

Scions were grafted in February 1994. The scion was prepared by removing all needles and making axial cuts starting just below the terminal bud on opposite sides. A wedge was created exposing cambium layers on both sides of the scion. A total of 10 rootstock branches were chosen, 5 in the upper crown and 5 in the lower crown, for each receptor tree. An axial cut was made just below the terminal bud of each rootstock branch reaching into the pith area. This cut continued downward for another three to four inches and the prepared scion was inserted into the slit. After matching at least one side of the cambium layers of the scions to the cambium layers of the exposed rootstock slit, the scion was secured in place with a rubber budding strip wrapped in an overlapping spiral pattern. Hot wax (175-200°F) was applied to the completed graft for protection from desiccation. Two to four weeks after the graft emerged through the wax, the rootstock branch was cut back and the rubber budding strip removed.

Experimental Design

A split-plot experimental design was used with receptor clones considered blocks and scion age the treatment variable. A whole plot was an individual ramet of the receptor clone with crown location as the split plots and individual branches as observations within the subplots. The receptor clone was considered a random variable and the treatment (age class) was a fixed variable.

'Data on file, U.S. Forest Service, Macon, GA.

Survival of scions in both the upper and lower crown locations was recorded 13 months after grafting. Shoot elongation and the number of branches that developed in 1994 were recorded in December of 1994. The number of new shoots in spring of 1995 was recorded in March 1995.

The number of female strobili was recorded on each surviving scion in March 1995. Pollen strobili were recorded as clusters on individual shoots and ranged from a single strobilus to 25 strobili per individual shoot. The observation date for male and female strobili was about two weeks after maximum receptivity and pollen release.

The data was analysed using the SAS (SAS Institute, Cary, NC) procedure for mixed models. Contrasts were computed for all possible comparisons (10) of the five scion age classes for both the upper and lower crown levels. For each response variable tested, mean separation was computed at the 5% level of probability. This value gives a rigorous t-test for significant differences between individual means as the comparisonwise error rate (CER) is equal to the experiment error rate (EER) divided by the number of comparisons. Thus, for means to be significantly different CER = EER/C or .05/20 = 0.0025. All contrast tests were completed with CER = 0.0025.

RESULTS AND DISCUSSION

Survival and Shoot Growth

The survival of topworked scions was excellent for all age classes. Ninety-seven percent of the grafts in the upper crown survived one year after grafting, and 91% of the grafts on the lower crown survived (Table 1).

Table I. Survival and growth of young scions topworked into the upper and lower crowns of reproductively-mature loblolly pine seed orchard trees.

RESPONSE VARIABLE	CROWN LEVEL	SCION AGE 1 YR	2 YR	3 YR	4 YR	5 YR
		(%)				
Survival	Lower	80a	95a	95a	100a	85a
	Upper	95a	100a	95a	100a	95a
		(in)				
Shoot lengh (December 1994)	Lower	6.0a	7.4a	6.4a	6.7a	5.4a
	Upper	15.8a	18.0a	18.3a	17.8a	14.6a

The amount of shoot growth on grafts in the upper crown was phenomenal during the 1994 growing season with an average length of 16.9 inches. Some grafts, in the most favorable locations in the upper crown, grew more than 30 inches (76cm) in length during the summer of 1994.

Position in the tree crown had a very pronounced effect on the growth of the grafted scion. Grafts placed directly on large primary branches in the upper crown produced the greatest shoot growth. In general, large branches originating from the buds at the base of the annual shoot, were the most vigorous branches in the tree crown and produced the greatest shoot growth.

Shoot growth in the lower crown was significantly less than in the upper crown with an average of 6.4 in. per branch. These lower crown grafts were on secondary and tertiary branches and associated with much less shoot growth, in general, in the lower crown.

The grafted scions exhibited juvenile growth characteristics even though they were in the upper crown of second-generation seed orchard trees. Although we did not quantify these juvenile characteristics, we observed that the needles were shorter, smaller in diameter, and frequently showed some winter chlorosis that would be characteristic of seedlings in comparison to mature foliage. Grafted scions from young trees also appeared to have more branches per shoot length than adjacent branches from the interstock. Thus, in appearance, the young scions looked like a seedling growing in the top of a large tree crown.

Number of Branches

As the amount of flower production may be a function of the number of shoots, we counted the number of shoots per branch in December of 1994 and again in March of 1995 (Table 2). The 1994 branches contained multiple buds, so the number of 1995 branches increased substantially from the 1994 count.

Table 2. Number of branches on young scions topworked into the upper and lower crowns of reproductively-mature loblolly pine seed orchard trees.

RESPONSE VARIABLE	CROWN LEVEL	SCION AGE 1 YR	2 YR	3 YR	4 YR	5 YR
		(no)				
Number of branches (December 1994)	Lower	1.9a	3.6a	2.2a	2.2a	2.3a
	Upper	8.2a	7.9a	5.9a	5.6a	4.7a
Number of branches (March 1995)	Lower	5.8a	6.3a	4.5a	4.6a	2.6a
	Upper	31.6a	30.8a	17.8ab	15.4b	12.8b

There was no statistical difference between crown levels or among scion ages for the number of branches in the upper or lower crown in December 1994. In 1995, the mean of 4.8 branches per shoot in the lower crown was significantly different from the mean of 21.7 shoots per branch in upper crown level. And, in the upper crown, the younger age scions also had more branches per shoot than the older age scions. Scion age one grafts averaged 31.6 shoots compared to scion-age five grafts with 12.8 shoots per branch.

Strobili Production

In the upper crown, scions from all age classes produced some female strobili one year after grafting. None of the 91 surviving grafts in the lower crown produced female strobili on any scions regardless of the age of the collected scion. Scion age influenced both the frequency and number of female strobili produced. Of 20 attempted grafts with age one scion material, 17 grafts survived and 5 of those produced a total of 11 female strobili (Table 3). In contrast, all 20 attempted grafts from age 4 scion material survived and 16 grafts (80%) had female strobili for a total of 90 strobili.

One-year-old scions in either the upper or lower crown did not produce pollen on any grafts. However, scions collected from 2-year-old trees produced pollen strobili on 3 of the 19 surviving grafts (15%) in the lower crown. Ages 3-5 scions produced pollen in both the upper and lower crown. For age 5 scions, 75% of the surviving grafts produced pollen strobili in the lower crown and 52% produced pollen in the upper crown (Table 3).

Table 3. Percentage of live grafts with strobili and number of female and male strobili on young scions topworked into reproductively mature loblolly pine seed orchard trees.

RESPONSE VARIABLE	CROWN LEVEL	SCION AGE 1 YR	2 YR	3 YR	4 YR	5 YR
		(%)				
Live grafts w/ female strobili	Lower	0a	0a	0a	0a	0a
	Upper	21b	70a	59a	80a	52ab
Live grafts w/ male strobili	Lower	0c	15bc	26b	55a	75a
	Upper	0b	0b	31ab	25ab	52a
		(no)				
Number female strobili (total no.)	Lower	0a	0a	0a	0a	0a
	Upper	11a	62ab	53ab	90b	31ab
Pollen clusters (total no.)	Lower	0a	6a	11a	20a	19a
	Upper	0a	0a	17a	6a	20a

The mechanism of strobili induction on young topworked scions into reproductively-mature trees is not clearly understood. It appears that the location of the grafted scion in the tree crown is the major factor. Furthermore, it appears that the flower-promoting stimulus, perhaps GA, is produced locally in the reproductively-mature branches and is translocated to the newly-grafted material. Bramlett et al. (1995) found that branch girdles had no effect on the survival or the number of pollen clusters produced per graft on branches that were already heavy pollen producers. This indicated a localized stimulation factor for pollen induction or perhaps transportation in the xylem, but not in the phloem, as the phloem would be blocked by the branch girdle.

Nineteen-ninety-five was considered a good flowering year and this obviously favored abundant female and male strobili production on the topworked scions. It is certainly plausible that a reduced effect would be observed in poorer flowering years and perhaps a greater response in bumper years. Our interstocks were not screened as high flower producers. Perhaps interstocks exist that are even more conducive to the promotion of male and female strobili. Further research is needed to evaluate many interstocks with the objective of identifying one or more specific interstocks that are especially effective in the promotion of strobili on topworked grafts.

We offer no rational explanation for the apparent, but not statistically significant, reduction in female strobili produced on age five scions compared to age four scions. This appears to be an artifact of the data and is possibly related to the random selection of the scions from the family mix. Or, by chance, the scions could have been grafted to less vigorous branches in the 5-year-old treatment. A larger study is needed with known genetic identification over a range of age classes to separate the components of variance attributed to scion age vs. scion genotype.

CONCLUSIONS

Topworking young selections into reproductivley-mature tree crowns greatly reduces the generation interval in loblolly pine. We think that the amount of strobili production observed on topworked scions was conservative because not all scions in the upper crown were grafted on the most vigorous branches, nor did we totally release the grafted scion by taking out all competing branches.

The scions that grew and flowered best were grafted on vigorous primary branches or on secondary branches with the tip of the primary branch removed. In this study only light pruning around the graft was done when the grafts were released by cutting away the tip of the branch distal to the graft location. Based on our observations of the 1994 topworked scions, we recommend that grafts for tree breeding be made, whenever possible, on the major branches in the crown. Typically, these branches are from the basal whorl of branches for a given year of stem growth. If grafts cannot be completed on the primary branch because of a large difference between scion and branch diameter, graft onto a secondary branch and prune out the primary branch tip. When the grafts are released, also cut away other branches that may compete with the grafted scion. The objective is to give the graft maximum opportunity to develop into a major branch within the tree crown.

The fact that pollen strobili were produced in the upper crown grafts suggests a somewhat different strategy than our original approach. We grafted scions in the upper crown for female strobili and scions in the lower crown for pollen production. The problem of using the lower crown is that even though these grafts consistently produced pollen, shoot development was rather restricted. For example, we observed an average shoot growth of 6.4 inches in the lower crown grafts compared to 16.9 inches in the upper crown. This greater growth is also associated with more branches and thus more flowering points the next year. Our recommendation is to graft in the mid crown on vigorous primary branches for pollen production. We suggest that 5-10 grafts be made for each cross where the selection will be used as a female parent, and about 5 grafts in the mid crown if the selection will be used as a pollen parent.

The utility for breeding very young genetic selections that have been topworked into reproductively-mature seed orchard trees remains speculative until validated with actual results. Previous to this study, female strobili had not been produced one year after grafting on scions collected from one-year-old seedlings. In this study, female strobili were produced on one-year old seedlings but pollen was not produced until scions were from two-year old seedlings. It would appear feasible to select as early as age two and complete the breeding one year later. This would then shorten the generation interval to a minimum of three years and the breeding cycle to five years for loblolly pine. This procedure assumes that early genetic evaluation procedures are valid for two-year-old progeny. Otherwise, the minimum breeding cycle using topworking is three years plus the age of selection from progeny tests.

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