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SHORT COMMUNICATION

INTERSTOCK EFFECTS ON STROBILUS INITIATION IN TOPGRAFTED LOBLOLLY PINE

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ABSTRACT

Grafting scions of new selections of loblolly pine (*Pinus taeda* L.) into the crowns of sexually mature seed orchard ramets (interstocks) has been a very effective tool for stimulating both male and female strobili one or two years after grafting. Two years of grafting studies evaluating six seed orchard clones as interstocks indicate that there is a strong influence of the interstock clone on the number of strobili produced by the topgrafts. The 1996 study utilized the same six clones in the seed orchard both as topgrafts and interstocks, and clones were grafted in all possible combinations. In 1997, ten 3rd-generation selections were used as the topgraft clones. Each scion clone was grafted into the crowns of three different ramets of the six interstock clones. Male and female strobili were counted each spring through 1999. In both studies, there were strong topgraft clonal effects and interstock clonal effects for total number of strobili. For example, the two best interstock clones stimulated four times as many females as the poorest interstock clone in the 1996 trial. In conventional grafts made on one-year-old seedling rootstocks, there were very few strobili produced. Although there were strong interstock effects, the flowering tendency of the clone was not related to its strobilus stimulation capacity as an interstock, indicating that choosing good flowering clones to use as interstocks will not necessarily result in the best flowering in the topgrafts.

Key words: breeding, flowering, grafting, *Pinus taeda*, rootstock, seed orchard

INTRODUCTION

Rapid generation turnover is a key to the economic viability of tree breeding programs (MCKEAND & WEIR 1981). While both the age of selection and methods to reduce the breeding cycle have been researched, the greatest benefit has been realized from intensive management of selected trees to produce flowers or strobili at early ages. When determining optimal selection age, there are biological and economic tradeoffs between early selection (fast generation turnover but lower correlation with rotation-age performance) and selection near rotation age (slower generation turnover but higher correlation with rotation-age performance). For the breeding cycle, there are no biological costs for rapid production of seed.

For the past 25 years, methods such as out of phase dormancy, application of gibberellins, water stress, and indoor breeding facilities have all been used to initiate flowering on young, grafted trees (LAMBETH & GREENWOOD 1987). Recently, a method long used by horticulturists has been used by tree breeders to accelerate breeding. Grafting scions of new selections of loblolly pine (*Pinus taeda* L.) into the crowns of sexually mature seed orchard ramets has proven to be a very effective tool for stimulating both male and female strobili (BRAMLETT *et al.* 1995, BRAMLETT 1997). Two years after grafting, an average of 9 females per scion were stimulated on the new grafts in the upper crown and 11 pollen clusters per graft in the lower crown. With a modest effort, breeding could be completed on these selections in only two or three years (BRAMLETT & BURRIS 1998). Many orchard managers in the South have successfully experimented with topgrafting, given the opportunity and strong incentive to shorten loblolly pine breeding cycles to less than five years.

In 1996, we initiated a study to evaluate various aspects of topgrafting on to different seed orchard clones. Since these trees were already grafted, they were considered interstocks. The primary objective was to determine if there is an influence of the interstock clone on the number of strobili produced by the top-

grafts. In horticultural orchard crops, the influence of rootstocks and interstocks on growth and reproduction is well documented (Garner 1979, Hartmann & Kester 1983). In general, the influence of rootstock on reproduction has not been great in most forest trees (Jayawickrama *et al.* 1991), but little information is available on the influence of interstocks.

MATERIALS AND METHODS

The study was conducted at the Smurfit-Stone Container Corporation (SSCC) Brewton Seed Orchard Complex in Brewton, AL (31.07 N latitude, 87.04 W longitude) where the soil type is a Ruston fine sandy loam. The experimental seed orchard was grafted in 1988 and was designed to evaluate the impact of various rootstocks on the growth and reproduction of the scions (see Jayawickrama *et al.* 1997 for details of the study). Six different second-generation clones were used as scion clones in the rootstock study, and they ranged from extremely high to almost sterile for cone production over the eight years of assessment. Standard orchard management practices were followed such as annual fertilization, pruning, and weed and insect control (e.g. Jett 1986).

For the current topgrafting study, grafting was carried out over a two year period using the six clones from the rootstock study as interstocks. The 1996 study utilized these six clones both as topgrafts and interstocks, and clones were grafted in all possible combinations. In 1997, ten third-generation selections were used as topgraft clones. Standard dormant-season grafting procedures for loblolly pine (McKeand & Jett 2000) were used with a few modifications. Cleft grafts were made in dominant branches in the upper quarter of the crowns. Rather than cover the graft union and scion bud with wax, scions were dipped in paraffin at 90°C to cover the bud prior to grafting. After grafting, the graft union was covered with Parafilm^R to prevent dessication.

Each scion clone was grafted into the crown of three different ramets of the six interstock clones in a completely randomized experimental design. The average size of the trees in 1996 was 10.5 m in height and 23.3 cm in diameter at breast height. To evaluate the potential of using large ungrafted seedlings for topgrafting, large ungrafted seedlings within the orchard were also used as rootstocks and were considered as "interstocks". These seedlings were the same size and age as the grafted ramets in the experimental orchard. A total of 126 topgrafts (6 scion clones × 7 interstocks × 3 grafts) was made in 1996, and 210 topgrafts (10 scion clones × 7 interstocks × 3 grafts) were made in 1997. In addition to the topgrafts, each scion clone was also grafted on to two or three one-year-old seedling rootstocks (approximately 1 m in height).

Female strobilus counts were made each spring from 1997 to 1999, and the number of male pollen strobilus clusters were counted only in 1999. Strobili were also counted on two ungrafted branches in the crown of each interstock. The latter counts and the strobilus and cone counts from the previous rootstock study data (Jayawickrama *et al.* 1997) were summed to give an index of the flowering tendency of each interstock clone. These indices of the flowering tendency were then correlated with the clonal means when the clones were used as an interstocks.

Data were subjected to analyses of variance (Table 1) using the GLM procedure in SAS (SAS Institute, Inc. 1989) to evaluate the impact of both scion and

Table 1. Analyses of variance to assess the scion and interstock effects on strobilus counts (the model for the1997 study, assuming complete balance, is shown). All effects were assumed to be random. REML estimates of variance components are given for total number of female strobili (2 years for 1997 topgrafts and 3 years for 1996 topgrafts) and number of male strobili clusters in 1999. The percentage of the total variance for each trait is given in parentheses.

Source	d.f.	Expected mean square	Variance component estimates (% of total)			
			1997		1996	
			Females	Males	Females	Males
Interstock[1]	6	$\sigma^2_{R/IS} + 3\sigma^2_{IS} + 30\sigma^2_I$	24.5*(4.2)	6.5*(7.0)	142.4*(14.9)	5.4(2.0)
Scion	9	$\sigma^2_{R/IS} + 3\sigma^2_{IS} + 21\sigma^2_S$	301.2*(52.2)	27.8*(29.8)	228.1*(23.9)	138.6*(52.5)
I × S	54	$\sigma^2_{R/IS} + 3\sigma^2_{IS}$	0(0)	0(0)	0(0)	0(0)
Ramet/I × S	140	$\sigma^2_{R/IS}$	251.6(43.6)	58.9(63.2)	583.6(61.2)	119.8(45.4)
Corr. Total	209					

*) Indicates a significant effect at $p \leq .05$ for the trait.
1) Interstocks consisted of 6 different clones and large seedlings.

Table 2. Clonal means for flowering tendency (combination of branch counts in current study and strobilus and cone counts from the experimental orchard) and for the number of female and male strobili for each clone when it was used as an interstock[1].

Interstock	Flowering tendency index	1996 Grafts total # female strobili (3yrs)	1996 Grafts # male strobili in 1999	1997 Grafts total # female strobili (2yrs)	1997 Grafts # male strobili in 1999
111154	38.7	38.6[a]	31.3[a]	16.2[b]	12.3[ab]
081166	72.0	7.9[b]	26.0[a]	13.1[b]	9.0[bc]
081218	93.7	24.1[ab]	29.8[a]	13.4[b]	8.3[bc]
071095	177.8	23.7[ab]	33.9[a]	29.5[a]	15.7[a]
111135	298.1	8.8[b]	24.9[a]	14.6[b]	10.0[b]
181210	466.9	38.7[a]	35.8[a]	21.1[ab]	10.6[b]
Large seedling	–	17.9[b]	20.8[a]	12.4[b]	5.1[c]

[1] Strobilus counts within a column followed by the same letter are not significantly different at $p \leq 0.05$.

interstock clone on female and male strobilus counts. The Waller-Duncan multiple range test within GLM was used to identify interstock clonal means that differed significantly ($p \leq 0.05$). To estimate the contribution of each component to the total variance in the study, the SAS VARCOMP procedure (SAS INSTITUTE, INC. 1989) using the REML estimation method was used.

RESULTS AND DISCUSSION

In both the 1996 and 1997 topgrafting studies, there were strong scion (or topgraft) clonal effects and interstock clonal effects for number of strobili (Table 1). Rather than present all the data, the total number of female strobili over the 2 or 3 years of each study and the number of male strobilus clusters in 1999 are presented. As has been found in other trials (e.g. BRAMLETT *et al.* 1995, BRAMLETT 1997), topgrafting was very effective at stimulating both female and male strobili two and three years after grafting (average of 17.2 female strobili over two years in 1997 grafts, 22.9 female strobili over three years in 1996 grafts). In the conventional grafts made on young seedlings, there were very few strobili produced (average of 4.7 females in 1997 grafts, 2.1 females in 1996 grafts), and these strobili were all produced in 1999 which was an exceptionally heavy flowering year.

As expected, the scion clone used for topgrafting had a very large effect (Table 1). In the topgrafts made in 1997, the scion clone means ranged from 5.0 to 55.2 females and 3.8 to 21.8 males, and in the topgrafts made in 1996, the scion clone means ranged from 5.0 to 45.9 females and 14.9 to 49.0 males. In earlier trials comparing rootstock and scion effects, JAYAWICKRAMA *et al.* 1997 found that scion effects completely overwhelmed the effects of rootstocks. In the current study, the scion effects on flowering were large, accounting for 24% to 52% of the variation in the study (Table 1), but the interstock effects were also substantial (as high as 15% of the total variation for female strobilus in the 1996 trial) and greater than previously found effects of rootstocks (typically < 1% of the variation).

The effect of grafting into crowns of different clones was generally significant and important (Table 2). The interstock clone means ranged from 13.1 to 29.5 females in the 1997 topgrafts, and from 7.9 to 38.7 in the 1996 topgrafts. Although there were differences between the two years of grafting, some general trends are apparent. When used as interstocks, clones 071095, 111154, and 181210 appear to be very effective at stimulating female strobili. Clones 081166 and 111135 tend to be poor clones to use as interstocks, but the effect in the 1997 trial was not as large. The effect on male strobili was not as great as for females; there were no interstock effects in the 1996 trial, and the significant differences in the 1997 trial were not very large (Table 2). There was no significant interaction between the interstock clonal effect and the topgraft clonal effect for any of the strobilus counts. Interstocks that are effective at stimulating strobili on one clone tended to be effective at stimulating strobili on all clones.

Although there were strong clonal interstock effects, the flowering tendency of the clone was not related to its flower stimulation capacity as an interstock. The correlations of clone means between the flowering tendency index and female strobilus counts were low and not significant $r = 0.19$ for the 1996 topgrafts, $r = 0.31$ for the 1997 topgrafts). When good flowering clones are used as interstocks, they will not necessarily

stimulate the best flowering in the topgrafts.

Another concern is the impact of the interstock clone on survival. While clone 071095 was very effective at stimulating female strobili (Table 2), the survival of topgrafts made on ramets of 071095 in the 1997 trial was significantly lower ($p \leq .05$) than the other interstocks and was only 67% compared to ≥80% for all the other clones. Clone 111154 was a particularly interesting clone in the trials. The clone itself is a very poor cone producer, but would be an excellent choice to use as an interstock. Survival of topgrafts on 111154 was 100%, and it was very effective at stimulating female strobili (Table 2).

While the topgrafts made on the large seedlings did not produce many strobili (Table 2), there were enough females and males to be useful for breeding. Some orchard managers do not have excess seed orchards or clone banks that can be used as topgrafting orchards, but some have thinned existing plantations to wide spacings and will use these as topgrafting orchards in the future.

CONCLUSIONS

Topgrafting will continue to be an extremely valuable tool in breeding programs with loblolly pine in the southern United States. It is an inexpensive method to stimulate both female and male strobili on new selections in only two or three years. While the choice of clones to use as interstocks appears to be important, the ability to identify clones *a priori* that will stimulate flowering does not look promising. Other breeders and orchard managers are evaluating various aspects of topgrafting, and the search for good interstock clones and methods to identify them will continue.

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