Survival and Promotion of Female and Male Strobili from Topgrafting in Third-Cycle Slash Pine (*Pinus elliottii* var. *elliottii*) Breeding Program.

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In January 2003 the Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida began establishing the slash pine third-cycle breeding population through topgrafting the selected clones. The topgrafting strategy, which has the potential to drastically reduce the breeding cycle, will also eliminate the need for a separate clone bank for breeding (White *et al*., 2003). A study of flowering response to topgrafting was conducted to obtain better understanding and refine the operational use of this technique in slash pine. The objectives of this study were to understand the effect of the genetic material (interstock clones and scion clones) and the interstock crown position on survival and flowering response of topgrafts. Quadrant direction, branch order of the interstock and scion age were assessed as survey data and their effects on survival and flowering response of the topgrafts were also estimated.

The topgrafts were established onto sexually-mature, insect-protected seed orchard trees in nine slash pine seed orchards (first and second generations). Scions were collected from 4 to 6 year-old full-sib block plots for forward selections, and from first generation seed orchards and second generation clone banks for backward selection. The recommended experimental design for each cooperator in a single orchard was one topgraft in each of three crown positions in four different seed orchard interstock clones for a total of 12 grafts per third-cycle selections. The three crown positions were defined as: top (the first two whorls), mid-top (about whorl 4), and mid-crown (usually about whorl 6). Survival, number of female strobili and number of male strobili one year after topgrafting were the response variables of this study. A variation of the modified cleft was the standard grafting method used with the exception of one cooperator that used a regular modified cleft. The statistical analysis was performed in two stages. In the first stage, an analysis of variance (ANOVA), using SAS PROC MIXED (SAS ® Institute 1996), was used to test the effects of the topgraft clone, interstock clone and crown position on the response variables. To test the significance of the variance components for the random predictors a Wald Test was used (Greene, 2000). In the second stage, the effects of quadrant, branch order and topgraft chronological age (adhoc variables) on the topgraft responses were assessed. Given a large number of missing values and inconsistency in the levels of these three new predictor variables among cooperators, the analysis was conduced using a more balanced subset of the full data set for each adhoc variable. The fitted linear model from the first stage analysis (experimental design factors) was the base model to which the three new independent variables (all of them treated as fixed effects) and their interactions were added and fitted in separate analyses using the backward elimination approach as in the first stage.

After one year from grafting a total of 1861 topgrafts (72.6%) from 200 topgraft clones out of 209 were alive. Significant differences in survival were found among cooperators (Table 1) with

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a minimum and maximum mean survival of 46.1% and 86.1%, respectively. Differences in survival among crown positions were also significant (p-value = 0.01) with higher survival rate obtained in the mid-top (75.3%), followed by the top (69.9%) and finally by the mid-crown position (67.2%).

Table 1. First stage analysis of variance, for survival, and female and male strobili production using a full model with all the experimental design factors that were significant at 25% for at least one response variable. P-values are shown for fixed effects and variance components, expressed as percentage of the total phenotypic variance, are shown for random effects.

Model effects [a]	**Response variables (p-value)**		
Fixed effects	**Survival**	**Female flowering**	**Male flowering**
Cooperator	0.0261	0.0122	0.0078
Crown	0.01	< 0.0001	0.18
Cooperator*crown	0.192	0.134	ns[b]
Random effects	**Variance (% of total variation)**		
Topgraft clone (Topgraft)	5.36%	23.7%	8.6%
Interstock	16.28%	12.5%	6.7%
Ramet	6.00%	ns	6.6%
Topgraft*interstock	ns	4.1%	3.9%
Interstock*crown	ns	2.0%	11.7%
Topgraft*crown	ns	ns	2.9%
Topgraft*ramet*crown	11.44%	ns	Ns
Residual	60.87%	57.7%	59.3%

[a] predictor variables in the model are: cooperator, crown (crown position), topgraft (topgraft clone nested within cooperator), interstock (nested within cooperator) and ramet (interstock replication, nested within interstock and cooperator). [b] effect not significant at the 0.25 level in the fitted model.

Topgrafting was a very effective tool for promoting both female and male strobili. While some topgrafts did not flower, the overall mean and maximum flowering yield per live topgraft was 2.52 and 43 female strobili, and 1.67 and 59 male strobili. After one year from grafting, 84% of the live topgraft clones produced strobili, and almost half of them bore both flower sexes. The expectation in the third-cycle slash pine is to breed 36 out of the 50 selections assigned to each breeding group. The first year results allow us to be optimistic about reaching this expectation in the next few years via topgrafting.

Consistent with topgrafting studies in *Pinus taeda* and *Pinus sylvestris* (Gooding *et al*., 1999; McKeand and Raley, 2000; Almqvist and Ekberg, 2001), the genetic material, scion and interstock clones, had large effects on the flowering response. More than 23 and 8% of the total female and male flowering variation, respectively, were due to differences among scion clones. Given this results it became clear that there is a different potential among slash pine clones to produce flowers, especially female strobili, when topgrafted. This fact might compromise the early incorporation of poor topgraft flowering clones into the breeding program throughout topgrafting. Thus, the suitability of topgrafting to shorten the breeding cycle, is also a function of the amount of selected clones that have good flowering response when topgrafted. Clonal differences among interstocks, also large, were less important than the scion clone effect, accounting for 12.5% of the female and 6.7% of the male total variation. In addition, with the large interstock effect on topgraft survival, it results important to identify and select the clones that as interstock promote good topgraft survival and flowering. A low correlation between the flowering capacity of a clone and its suitability as interstock on promoting topgraft flower initiation has been reported in *Pinus taeda* and *Pinus sylvestris* (Schmidtling, 1983; McKeand and Raley, 2000; Almqvist and Ekberg, 2001); and therefore, the practicability of selecting good interstock clones for their flowering performance has been discussed as not promising (McKeand and Raley, 2000; Almqvist and Ekberg, 2001). Given the low topgraft clone by interstock clone interaction on survival and flowering responses in our study, the problem of selecting a good interstock clone can be in part overcome by topgrafting selected genotypes into more interstock clones, this ameliorates the risk of having poor flower initiation and poor survival caused by interstock clone.

Topgraft survival and female strobili promotion showed significant differences among crown positions. The highest survival rate was reached by the mid-top followed by the top crown position. Grafting in the top of the crown was highly superior promoting female strobili followed by mid-top position. Flowering differences among crown position were not significant for male strobili; however, higher overall yields were observed in top and mid-top position compared to mid-crown. When combining survival and strobili production rates in a single index, the top of the crown resulted to be the most efficient promoting female strobili, while mid-top reached the highest efficiency producing male strobili; however, higher proportions of topgrafts allocated in the top and mid-top crown should increase male and female flower production in balanced proportions for breeding practices.

Quadrant showed no significant effect either in topgraft survival or in topgraft strobili promotion; consequently our results do not allow us to make any related recommendation about topgraft quadrant orientation. Branch order was not a relevant source of variation on topgraft response variables with the exception of female strobili production. When testing the effect of the three levels of branch order in a subset of three cooperators, first order branches were significantly superior promoting female strobili, followed by second order branches. Thus, higher proportions of scions grafted on first and second order branches should increase the efficiency for promoting female strobili; however, given that first and second order branches might not be abundant enough for large scale crossing, a lower proportion of scions should also be grafted into third order branches, especially if there is a higher need for male strobili in which the production was not related to the order of the branches. Chronologically older scions (backward selections) produced significantly more female and male strobili when topgrafted. The higher efficiency of older scions promoting both female and male strobili may represent an additional plus in incorporating backward selections in the breeding population; and hence, further studies using a more balanced and larger data is recommended to support these results.

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