Graft survival and promotion of female and male strobili by topgrafting in a third-cycle slash pine (*Pinus elliottii* var. *elliottii*) breeding program

Alex M. Medina Perez, Timothy L. White, Dudley A. Huber, and Timothy A. Martin

Abstract: A total of 2561 slash pine (*Pinus elliottii* Engelm. var. *elliottii*) grafts were topgrafted in the winter of 2003 and evaluated in January of 2004. The objectives of this study were to understand the effect of the genetic material and crown position on survival and flowering response of topgrafts. Also, the effects of geographic direction, branch order, and scion age on topgraft response were assessed. Topgrafting was an effective tool for promoting both female and male strobili. The genetic material (scion and interstock clones) and the crown position had large effects on the promotion of female flowering and topgraft survival. More than 23% of the total variation in female flowering and 16.3% of the total variation in topgraft survival were due to differences among scion clones and among interstock clones, respectively. The highest survival rate was reached by grafting in the mid-top followed by the top crown position. Grafting in the top of the crown was highly superior in promoting female strobili followed by the mid-top position. First-order branches showed a significantly superior production of female strobili. Chronologically older scions, from selections made in the first and second generations of the tree improvement program, produced more female and male strobili than third-cycle forward selections.

Résumé : Un total de 2561 greffes ont été faites à l'hiver 2003 sur les rameaux supérieurs d'arbres déjà plantés en verger puis ont été évaluées en janvier 2004. Les objectifs de cette étude visaient à comprendre les effets du matériel génétique et de la position des greffons dans la couronne sur la survie et la floraison des greffons sur les rameaux supérieurs d'arbres déjà plantés en verger. Les effets de l'orientation, de l'ordre des branches et de l'âge des greffons sur la réponse des greffes ont aussi été évalués. Le surgreffage a été un outil efficace pour promouvoir le développement des strobiles femelles et mâles. Le matériel génétique (le greffon et les clones de greffons intermédiaires) et la position dans la couronne ont eu des effets importants sur la promotion de la floraison femelle et la survie des greffons. Plus de 23 % de la variation totale dans la floraison femelle et 16,3 % de la variation totale de la survie des greffons étaient respectivement dues à des différences entre les clones de greffons et les clones de greffons intermédiaires. Le plus haut taux de survie a été atteint chez les greffes situées au milieu de la couronne suivi des greffes situées au sommet de la couronne. Le greffage au somment de la couronne a fortement favorisé le développement des strobiles femelles suivi du greffage au milieu de la couronne. Les branches de premier ordre ont produit significativement plus de strobiles femelles. Les greffons chronologiquement plus âgés, issus de sélections faites dans la première et la seconde génération du programme d'amélioration des arbres, ont produit plus de strobiles femelles et mâles que ceux qui provenaient d'un troisième cycle de sélections dans la descendance.

[Traduit par la Rédaction]

Introduction

The Cooperative Forest Genetics Research Program (CFGRP) began a slash pine (*Pinus elliottii* Engelm. var. *elliottii*) tree improvement program in 1953. As in most long-term breeding programs, generation intervals, costs, and genetic gains are the key elements to be optimized under a basic principle "the faster the breeding starts, the shorter the cycle turnover and the greater the gain per unit of time" (Almqvist and Ekberg 2001). Following this principle, the breeding cycle interval for slash pine was shortened from 32 to 15 years from the first to the second cycle (White et al. 2003). Now in the third breeding cycle, which began in January 2003, the strategy seeks higher efficiency by reaching similar or better genetic gains in an even shorter time, 10–11 years. To help accomplish the goal of shortening the generation length, "topgrafting" of the selected clones has been incorporated into the breeding strategy. Topgrafting, also called topworking, is the grafting of selected material onto mature rootstock (such as flowering seed orchard trees) and serves as a tool to accelerate breeding, allowing flowering to start as soon as 1 year after grafting (Bramlett and Burris 1995; Lott et al. 2003). Given that topgrafts are made in existing seed orchards of the cooperative members, they also serve as temporary third-cycle clone banks. The incorporation of topgrafting into the third-cycle strategy of slash pine has the potential to accelerate the cycle turnover by as many as 5 years (Gezan et al. 2003).

When scion pieces from selections are grafted onto seed orchards trees, the resulting composites consist of three different genotypes: the rootstock of the seed orchard tree, the

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A.M. Medina Perez.[1] Los Canelos 1244, Villarrica, Chile.
T.L. White, D.A. Huber, and T.A. Martin. School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences, University of Florida, P.O. Box 110410, Gainesville, FL 32611, USA.

[1] Corresponding author (e-mail: alexfitz1@gmail.com).

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Table 1. Seed orchard location, number of interstock clones, mean age of interstock clones, and number of topgrafts by cooperator for topgrafts made in winter 2005.

			Number of topgrafts		
Seed orchard location (county, state)	Number of interstock clones	Interstock mean age in years (coefficent of variation)	Backward selections	Forward selections	Total
Santa Rosa, Florida	10	63 (24%)	6	338	344
Taylor, Florida	10	21 (0%)	125	220	345
Dooly, Georgia	7	32 (35%)		146	146
Decatur, Georgia	5	54 (47%)		463	463
Charlton, Georgia	11	40 (30%)	77	384	461
Hamilton, Florida	6	41 (1%)		136	136
Tattnall, Georgia	8	72 (25%)		140	140
Nassau, Florida	16	42 (14%)		366	366
Toombs, Georgia	2	34 (0%)	160		160
	75		368	2193	2561

interstock that was initially grafted in the seed orchard onto the juvenile rootstock, and the topgraft. The underlying principle of topgrafting is that the fitness, physiological stability, and reproductive competence of the interstock are transferred to the topgraft, while the topgrafted scion carries the desirable genetic information for breeding (Hartman and Kestler 1983; Almqvist and Ekberg 2001).

Scion and interstock genotypes have been reported as important sources of variation in topgraft survival, development, and flowering response in several southern pine species (Schmidtling 1983; Greenwood 1994; Clarke and Malcolm 1998; McKeand and Raley 2000). In conifers, numerous studies have reported significant differences in natural flowering associated with varying crown positions (Greenwood 1994; Kozlowski and Pallardi 1996; Parker et al. 1998). In species of the genera *Araucaria*, *Picea*, *Larix*, *Pinus*, and *Abies* (monoecious species), the unisexual female strobili are typically borne higher in the crown of the previous year's twig growth, while male strobili clusters develop lower in the crown; however, as the trees age and the whole crown reaches maturity, male and female flower zones come closer together and usually overlap in the mid-crown (Tosh and Powell 1991; Young and Young 1992; Greenwood 1994; Clarke and Malcolm 1998).

Branch character or branch order (Tosh and Powell 1991; Clarke and Malcolm 1998) and scion chronological age (Greenwood 1981, Parker et al. 1998) have also been reported as factors affecting production and distribution of seed and pollen cones.

The CFGRP cooperators topgrafted the selected third-cycle clones onto sexually mature, insect-protected seed orchard trees. These topgrafted selections represented an opportunity to study many of the unanswered questions related to topgrafting in slash pine and constitute the experimental material for this study. Thus, the overall goal of this experiment was to understand the effects of the genetic material (interstock clones and topgraft clones) and crown position within the interstock on survival and flowering response in slash pine topgrafting. The specific objectives of this study were to determine the effects of the following factors on topgraft survival and production of male and female strobili: (*i*) genotype of scion and interstock, (*ii*) interstock crown position (top, mid-top, and mid-crown), (*iii*) geographic direction of topgrafts (north, south, east, west, and their combinations), (*iv*) branch order of the interstock branch where the topgraft was placed (primary, secondary, and so on), and (*v*) scion chronological age.

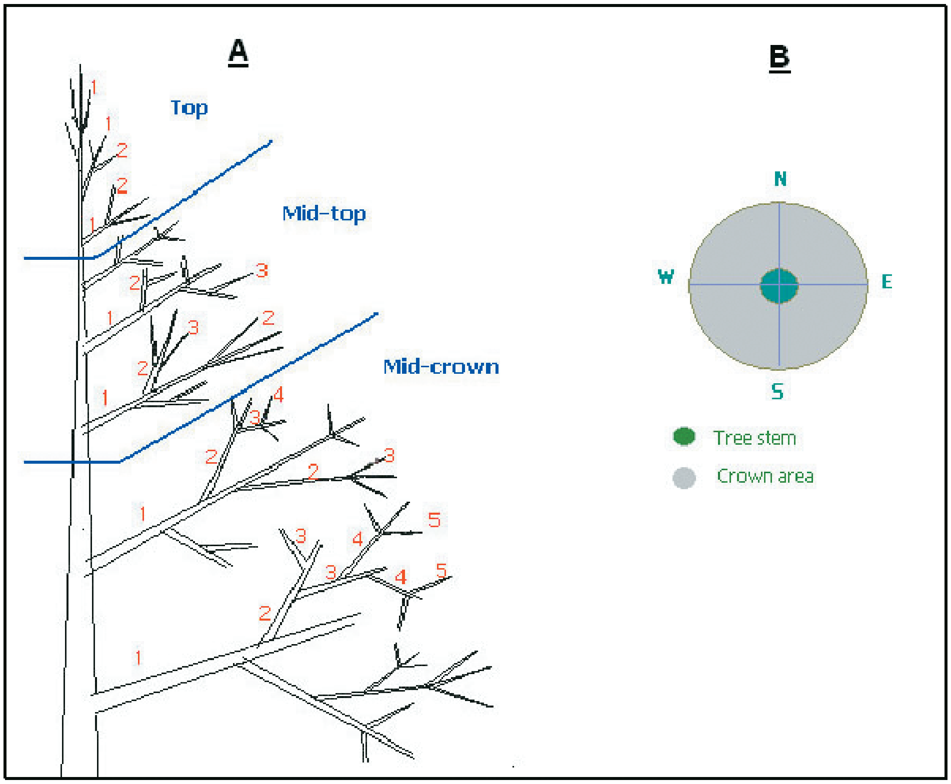
Materials and methods

Topgrafting in the CFGRP slash pine breeding program began in January 2003 immediately after the first third-cycle selections were made. A total of 2561 topgrafts from 209 selections were grafted in the winter of 2003 and were used as experimental material for this study. Of the 209 third-cycle selections, 34 were selections made during the first and second cycles of tree improvement (called backward selections) and 175 were forward selections from 4- to 6-year-old seedling tests. The topgrafts were established in eight slash pine seed orchards (first and second cycles) and one slash pine clone bank by the CFGRP members. The seed orchards are located in northern Florida and southern Georgia (Table 1). These sites were intensively managed with herbaceous weed control, fertilization, and insect protection. Healthy ramets with well-developed crowns that had demonstrated heavy cone production were selected as interstocks. Cone production was assessed visually based on cones retained or seed orchard records.

Scions were collected in January 2003 from 4- to 6-year-old seedling tests for forward selections and from first-cycle seed orchards and second-cycle clone banks for backward selections. Scions were chosen from the upper one third of the crown from primary branches or, in case of shortage, from secondary branches. The chronological age of the backward selection scions ranged from 22 to over 80 years old. After scion collection and before grafting, buds of the scions were immersed in paraffin at 80–90 °C to protect the bud from physical damage and dehydration. The modified cleft was the standard grafting method used. Once grafted, the graft union was wrapped with Parafilm to avoid desiccation.

The recommended experimental design for each cooperator for a single seed orchard was one topgraft per selection in each of three crown positions in four different interstock clones for a total of 12 grafts per selection. The three crown positions were defined as (Fig. 1) top (the top two whorls), mid-top (about whorl 4 from the top), and mid-crown (usu-

Fig. 1. Crown categories for slash pine topgrafting. (A) Interstock crown position (top, mid-top, and mid-crown) and interstock branch order 1, ..., 5. (B) Cardinal directions for topgraft geographic direction definition.



ally about whorl 6 from the top). About 3–4 months after grafting, the first survival assessment was completed and living grafts were released by removing grafting materials and pruning branches surrounding the graft until the branch was not shaded.

Survival and female and male strobili production were measured on topgrafted branches in January of 2004 and were the response variables analyzed in this study. To relate topgraft survival and flowering to branch character and light exposure, the branch order and crown geographic direction for each topgraft were also recorded by some cooperators and were treated as ad hoc variables (not controlled as part of the true experimental design). Branch order corresponds to the order of the interstock receptor branch. Thus, the first branch coming out from the main stem of the tree is branch order 1, a branch coming from an order 1 branch is order 2, and so on (Fig. 1).

The statistical analysis was performed in two stages. In the first stage, an analysis of variance, using SAS PROC MIXED, was used to test the effects of the topgraft clone, interstock clone, and crown position effects as designed variables on survival and female and male strobili production as response variables. For strobili production, data from one of the nine cooperators were removed from the analysis because there were very few living topgrafts and little strobili production. Topgraft clones and interstock clones were unique to each cooperator, and for a broader level of inference, they were considered as random effects in the model.

The initial full linear model for the first-stage analysis was as follows:

$$\begin{aligned} y_{ijklmo} = {} & \mu + O_i + i_{j(i)} + r_{k(ji)} + t_{l(i)} + C_m + OC_{im} \\ & + it_{jl(i)} + Ci_{jm(i)} + r_{k(ji)}t_{l(i)} + Cr_{mk(ji)} + Ct_{ml(i)} \\ & + Cit_{mjl(i)} + Crt_{mkl(ji)} + e_{ijklmo} \end{aligned}$$

where y_{ijklmo} is the response value (survival, number of female flowers, or number of male flowers) of the oth individual topgraft in the mth crown position from the lth topgraft clone on the kth ramet of the jth interstock for the ith cooperator, i.e., multiple grafts occurred occasionally for a treatment level; μ is the overall mean; O_i is the fixed effect of the ith cooperator; $i_{j(i)}$ is the random effect of the jth interstock clone nested within cooperator, $\sim\mathrm{NID}(0, \sigma_i^2)$; $r_{k(ji)}$ is the random effect of the kth ramet nested within interstock clone and cooperator, $\sim\mathrm{NID}(0, \sigma_r^2)$; $t_{l(i)}$ is the random effect of the lth topgraft clone nested within cooperator, $\sim\mathrm{NID}(0, \sigma_t^2)$; C_m is the fixed effect of the mth crown position; OC_{im} is the fixed interaction effect of the ith cooperator and the mth crown position; $it_{jl(i)}$ is the random interaction effect of the jth interstock clone nested within cooperator and the lth topgraft clone nested within cooperator, $\sim\mathrm{NID}(0, \sigma_{it}^2)$; $Ci_{jm(i)}$ is the random interaction effect between the mth crown position and the jth interstock clone nested within cooperator, $\sim\mathrm{NID}(0, \sigma_{Ci}^2)$; $r_{k(ji)}t_{l(i)}$ is the random interaction effect between the kth ramet nested within interstock and cooperator and the lth topgraft clone nested within cooperator, $\sim\mathrm{NID}(0, \sigma_{rt}^2)$; $Cr_{mk(ji)}$ is the random interaction effect between the mth crown position and the kth ramet nested within interstock clone and cooperator, $\sim\mathrm{NID}(0, \sigma_{Cr}^2)$; $Ct_{ml(i)}$ is the random interaction effect between the mth crown position and the lth topgraft clone nested within cooperator,

Table 2. First-stage analysis of variance for survival and female and male strobili production using a full model with all of the experimental design factors that were significant at 25% for at least one response variable.

	Response variable (p)		
Model effects	Survival	Female flowering	Male flowering
Fixed effects			
Cooperator	0.0261	0.0122	0.0078
Crown	0.01	<0.0001	0.18
Cooperator × crown	0.192	0.134	ns
Random effects (variance (% of total variation))			
Topgraft clone (topgraft)	5.36	23.67	8.66
Interstock	16.28	12.53	6.70
Ramet	6.00	ns	6.61
Topgraft × interstock	ns	4.10	3.90
Interstock × crown	ns	2.00	11.71
Topgraft × crown	ns	ns	2.90
Topgraft × ramet × crown	11.44	ns	ns
Residual	60.87	57.70	59.30

Note: *p* values are shown for fixed effects and variance components, expressed as a percentage of the total phenotypic variance for random effects. 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). ns, effect not significant at the 0.25 level in the fitted model.

$\sim\mathrm{NID}(0, \sigma^2_{Ct})$; $Cit_{mjl(i)}$ is the random interaction between the *m*th crown position, the *j*th interstock clone nested within cooperator, and the *l*th top graft clone nested within cooperator, $\sim\mathrm{NID}(0, \sigma^2_{Cit})$; $Crt_{mkl(ji)}$ is the random interaction between the *m*th crown position, the *k*th ramet nested within interstock clone and cooperator, and the *l*th top graft clone nested within cooperator, $\sim\mathrm{NID}(0, \sigma^2_{Crt})$; and e_{ijklmo} is the residual, $\sim\mathrm{NID}(0, \sigma^2_e)$.

For each dependent variable, effects were maintained in the model at a 25% significance level using a backward elimination approach. To test the significance of the variance components for the random effects, a Wald test was used (Greene 2000).

In the second stage of the statistical analysis, the effects of the ad hoc variables' geographic direction, branch order, and topgraft chronological age on the response variables (topgraft flowering and survival) were analyzed. Because of the large number of missing values and inconsistency in the levels of these predictor variables among cooperators, the analysis was conducted using a more balanced subset of data for each ad hoc variable.

For geographic direction, the five cooperators that consistently topgrafted in northeast, southeast, northwest and southwest geographic direction orientations composed the subset of the data used in the analysis. For branch order, two subsets were defined. The first subset, called b-order 1, for testing the effect of the three levels of branch order (primary, secondary and tertiary) was formed using data from the three cooperators that topgrafted in all three branch orders (1, 2, and 3). The second subset, called b-order 2, for testing the effect of primary and secondary branch order consisted of the first subset with tertiary data deleted plus data from the four additional cooperators that topgrafted on only branch orders 1 and 2. Finally, for the third predictor, topgraft chronological age, expressed as "selection", there were two discrete classes (backward and forward selection) and the analysis was formed using data from the two cooperators that topgrafted backward and forward selections in sufficient numbers for comparison.

The fitted linear model from the first-stage analysis (experimental design factors) was the base model to which the three new ad hoc 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.

Results and discussion

First-stage analysis, experimental design factors

Topgraft survival

One year after grafting a total of 1861 topgrafts (of 2561, 72.6%), from 200 topgrafted clones out of 209 clones were alive. Significant differences in survival were found among cooperators (Table 2) with a minimum of 46.1% and a maximum of 86.1% survival. Further, the differences in survival among crown positions were significant (p = 0.01). The highest survival was obtained in the mid-top (75.3%) followed by the top position (69.9%) and finally the mid-crown position (67.2%). One of the possible factors associated with lower survival rate in the top compared with the mid-top could be the larger diameter of the interstock branches in the upper crown (apex and first whorl shoots) in relation to the scions, which made proper alignment of the cambia of the scion and interstock difficult.

The scion clone was unimportant for survival. The most important detectable source of variation was interstock clone, which accounted for 16.3% of the total phenotypic variance. Consistent with this result, interstock clone has been reported as an important factor in topgraft survival and flowering in *Pinus taeda* L., *Pinus sylvestris* L., and a hybrid between *Pinus palustris* P. Mill. and *Pinus elliottii* Engelm.

(McKeand and Raley 2000; Almqvist and Ekberg 2001; Lott et al. 2003).

Topgraft flowering

Topgrafting was a very effective tool promoting both female and male strobili 1 year from grafting. While some topgrafts did not flower, the overall mean and maximum number of flowers per live topgraft were 2.52 and 43 female strobili and 1.67 and 59 male strobili. From the 1861 living topgrafts, 53.8% bore strobili; of those, 77% produced only female strobili, 9% produced only male strobili, and 14% produced both male and female strobili. About 54% of the topgrafts with female strobili bore more than three strobili per graft. For economic reasons, this yield can be considered as a minimum number of flowers for a graft to be worthy of bagging for breeding. About 72% of the topgrafts produced more than three male strobili and so were potentially suitable for collection of pollen for breeding. These results manifest the ability of topgrafting to promote sufficient early flowering to enable breeding to start the first year after grafting.

In terms of scion genotypes, the flowering yield was very satisfactory. From the 200 scion clones with live grafts, 168 produced strobili and 54.8%, 1.2%, and 44% of the topgrafted clones produced female, male, and both female and male strobili, respectively. More than 70% of the scion genotypes with female strobili bore more than three female strobili, which makes them potentially suitable for breeding. Male strobili production by scion clone was less prolific with only about 33% of the topgrafted clones producing male strobili having more than three male strobili.

Cooperator effect on flowering

Female and male strobili production showed significant variation among cooperators (Table 2). Whereas for one cooperator there was almost no flowering, the highest mean female and male flowering yields for cooperator were 4.4 and 4.8 strobili per live graft, respectively.

Defined as interstock age (Table 1), the age of the interstock scion at grafting plus the age of the interstock graft (orchard) are factors correlated with crown size and vigor that varied among cooperators and could have affected the rate of cone initiation and the proportion of male–female strobili production (Kozlowski 1971; Greenwood 1994). To assess the correlation between flowering response and interstock age, a simple regression analysis was used. The regression analysis of flowering yield as a function of the by-cooperator means for interstock age showed no significant slope for either flower gender. To have a more general inference about the correlation between flowering responses and interstock age, a pooled regression analysis of flower yield and all individual interstock ages was conducted. In this case, a significant and negative slope of the interstock age parameter for female flowering (p = 0.0001, parameter estimate = –0.0366) was found but with a very low R^2 (0.02). The slope for male flowering was also significant but positive (p = 0.0004, parameter estimate = 0.0004) with an even lower R^2 (0.0089). The very low R^2 values indicate that very little of the variation in flowering is explained by interstock age. Thus, large variation in flowering among cooperator was not the result of interstock age or maturity differences among cooperators but due to additional factors not controlled in this study.

Crown position effect on flowering

Significant differences in strobili production were found among crown positions for female strobili (Table 2). The flower distribution within the crown showed an increasing progression of female strobili per living graft from the lower to the upper crown locations (Fig. 2A). The overall number of female strobili present in the top crown, 3.22 strobili per live graft, was considerably higher than in the mid-top and mid-crown positions with 2.08 and 1.42 strobili per live graft, respectively. For male strobili, the flowering variation among crown positions was not significant (p = 0.19). The highest flowering yields for male strobili (2.07 and 2.01 strobili per living graft) were reached in the top and mid-top positions, respectively. Male strobili production was lowest in the mid-crown with an overall yield of 1.14 male strobili per living graft (Fig. 2A). When expressed as a percentage of total strobili produced, male and female production as a function of crown position showed similar trends (Fig. 2B). Almost 60% of the female strobili and about 50% of the male strobili were borne in the top crown (Fig. 2B).

The higher concentration of female strobili in the upper crown was an expected outcome for slash pine topgrafts given the considerable number of reports describing this pattern in natural flowering in several conifer species (Marquard and Hanover 1984*a*, 1984*b*; Greenwood 1994; Clarke and Malcolm 1998). The fact that male strobili production was lowest in the mid-crown was an unanticipated result, and there is a lack of studies on male flower distribution for topgrafts in pine species that could serve as references.

With respect to material for breeding 1 year after grafting, about 50% of the male flowering topgrafts that bore enough flowers to be collected (more than three strobili per graft) were borne in the top two whorls of the crown (Fig. 2C). For topgrafts with female strobili, this percentage was even higher, 57%. Thus, despite the mid-top having the higher topgrafting survival, a significantly higher concentration of topgrafts with enough female and male strobili for bagging and pollen collection were located in the top. While the upper crown appears to be the most efficient crown location for early production of female and male strobili, branches in the top two whorls might not be abundant enough for large-scale grafting; therefore, topgrafting in both top and mid-top positions is recommended as a more efficient strategy ensuring an adequate supply of flowers for breeding.

There was no significant interaction of cooperator and crown position for male strobili production (Table 2). The near significant cooperator by crown position interaction for female strobili production (p = 0.14) was investigated graphically (Fig. 3) and revealed increased levels of female strobili in the top whorls of the crown across all the cooperators except the one with the lowest overall flower production.

To evaluate strobili promotion efficiency by crown position, a numerical indicator was calculated as the product of the crown position survival rate and the number of strobili per live topgraft. Thus, strobili promotion efficiency represents the number of strobili per grafted scion (Table 3). The sig-

Fig. 2. Female and male strobili production by crown positions. (A) Least square means of female and male strobili production per live graft. (B) Female and male strobili production as a proportion of the total. (C) Proportion of topgrafts that produce enough flowers (more than three strobili) to be bagged and used in breeding. The crown positions are defined as follows: top, the first two whorls; mid-top, about whorl 4; mid-crown, usually about whorl 6.

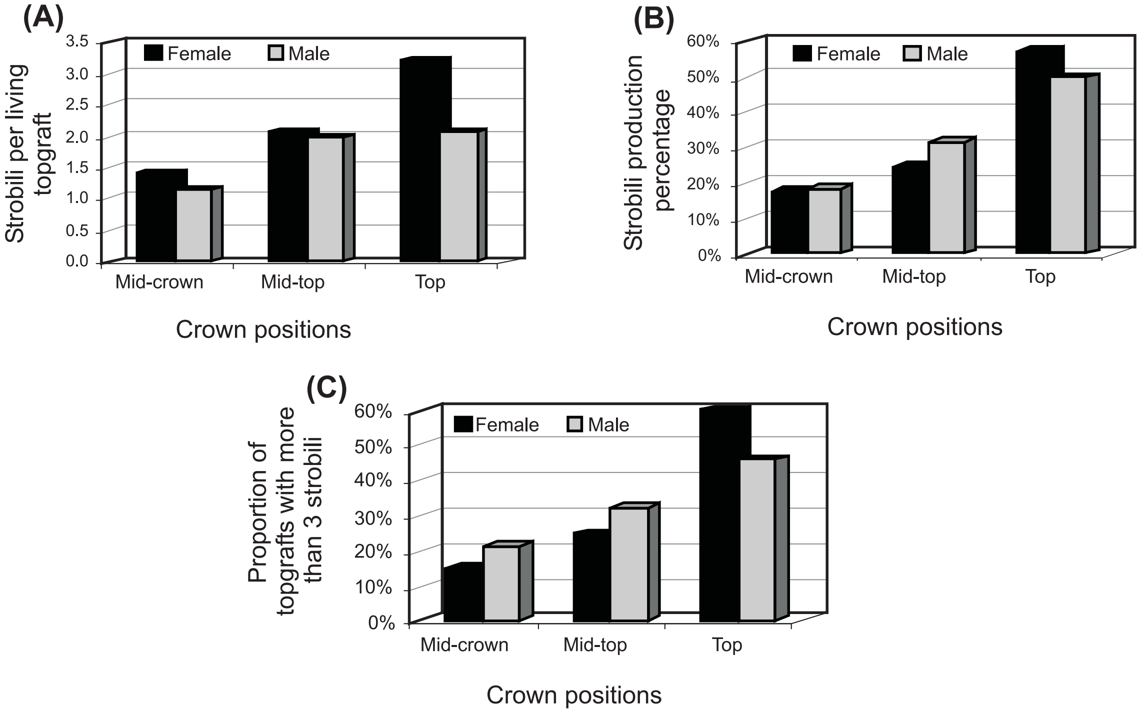
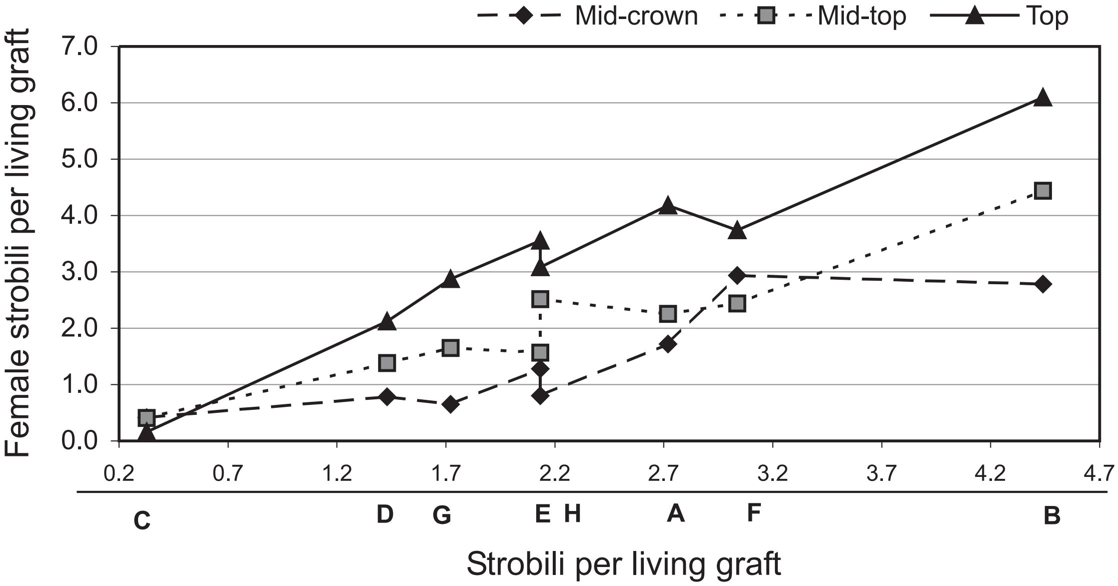


Fig. 3. Least square means for female strobili production for cooperator by crown position interaction. On the *x* axis, the numeric scale of strobili per live graft is displayed. Also on the *x* axis, cooperators (A–H) are sorted and proportionally located, from lower to higher, by the mean female strobili per live topgraft value.



nificantly larger female flowering rate in the top crown position overwhelmed the effect of higher survival of the mid-crown, making the top position the most efficient for promoting female strobili. The higher survival rate of the mid-top made this position the most efficient at producing male strobili.

For breeding practice, the primary concern is to ensure not just high levels but also balanced production of pollen and female flowers for breeding; therefore, allocating higher proportions of topgrafts in the top and mid-top positions represents a less risky and also more efficient scenario for increasing the efficiency of male and female strobili production.

Table 3. Strobili promotion efficiency (SPE) by crown position.

Crown position	Survival rate	Female flowers per live grafts	Male flowers per live grafts	Female SPE	Male SPE	Total SPE
Top	0.70	3.22	2.07	2.26	1.45	3.71
Mid-top	0.75	2.08	2.01	1.57	1.52	3.09
Mid-crown	0.67	1.42	1.15	0.95	0.77	1.73

Note: Crown positions are defined as follows: top, the first two whorls; mid-top, about whorl 4; mid-crown, usually about whorl 6. Efficiency is expressed as flowers per grafted scion and calculated as SPE = crown position survival rate (%) × number of strobili per live topgraft.

Topgraft and interstock clonal effects on flowering

Consistent with topgrafting studies in *P. taeda* and *P. sylvestris* (Gooding et al. 1999; McKeand and Raley 2000; Almqvist and Ekberg 2001), the genetic material, both scion and interstock clones, had large effects on the flowering response. In our study, 23% and 8% of the variation in total female and male flowering, respectively, was due to differences among scion clones (Table 2), the largest detectable source of variation of flower initiation in female strobili and the second largest for male strobili. Clonal differences among interstocks (i.e., seed orchard clones), 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. The effect of the topgraft clone by interstock clone interaction was also less important and accounted for the 4.1% and 3.9% of the total female and male flowering variation, respectively. The ability of interstock clones to promote topgraft flowering is manifest in the data, with several cases of interstock clones being relatively consistent in their good or bad capacity to stimulate topgraft flowering throughout the clones grafted into their crowns. In a similar fashion, for both flower genders, it was repeatedly observed that good and bad flowering scion clones were consistent throughout the interstocks on which they were grafted. Thus, for male and especially for female strobili, strobilus production is related to the specific topgraft clone capability for flowering when topgrafted; however, even a good flowering topgraft clone will have a low flowering rate when grafted into a poor interstock. This result points out the importance of prior identification of interstock clones that consistently promote topgraft 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 *P. taeda* and *P. sylvestris* (Schmidtling 1983; McKeand and Raley 2000; Almqvist and Ekberg 2001), and therefore, the practicality of selecting good interstock clones for their flowering performance has been viewed as not promising (McKeand and Raley 2000; Almqvist and Ekberg 2001). For slash pine topgrafting, this correlation has not been assessed, and factors like interstock age and consistency of seed production could be used to select interstock clones (Powell and White 1994). In addition, topgrafting each scion clone onto multiple interstock clones would ameliorate the risk of having poor flower initiation and poor survival caused by interstock clone.

The effect “ramet nested within interstock” is the clonal replication of the interstock within a given seed orchard; therefore, the ramet effect should capture some of the environmental effects causing variation in survival and flowering among interstock clones (e.g., scion quality, microsite, rootstock differences, grafting time and date, weather, etc.). In consequence, the lack of variation due to ramet within the clone observed in female flowering indicates that most of the female flowering variation among interstock clones is due to genotypic interstock differences. Another important effect, interstock by crown position interaction (Table 2), explained 11.7% of the male flowering phenotypic variation. This interaction implies that the suitability of an interstock clone on promoting male strobili is subject to crown positions.

Second-stage analysis of ad hoc factors

Geographic direction effects on topgrafting responses

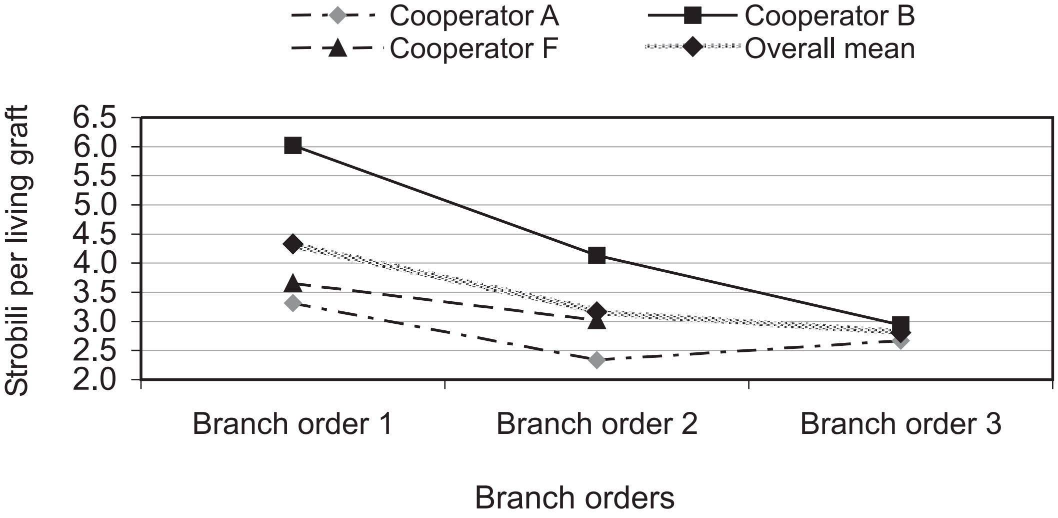
A separate analysis was conduced to test the effect of crown geographic direction on topgrafting responses using a subset of the data composed by the five cooperators that located the topgrafts in the northeast, northwest, southeast, and southwest geographic directions. For all the ad hoc factors, the statistical analysis used the fitted model for the experimental design factors as a base model, and the additional ad hoc effects (main effect and interactions) were added and fitted with a backward elimination approach.

Topgrafting performance in terms of survival and strobili production was not significantly influenced by the geographic direction. The effect of geographic direction was significant only when interacting with topgraft clone and interstock clone effects. This interaction accounted for the 9.6% of the total survival variation and for 6.8% of the male flowering variation, but it did not have a meaningful biological interpretation.

Branch order effects on topgrafting responses

As a main effect, branch order was significant only for female strobili production in the subset b-order 1 ($p = 0.013$), in which the effect of three levels of branch order on the response variables was tested. The overall female strobili yield reached its highest value (4.33 strobili per living graft) in order 1 branches followed by order 2 branches and finally by order 3 branches with overall yields of 3.16 and 2.81 strobili per living graft, respectively. The decreasing female strobili production with higher order branches pattern was relatively consistent among all three cooperators (Fig. 4). This tendency was supported by the nonsignificant level of the cooperator by branch order interaction ($p = 0.19$). Male strobili production was less sensitive to the effect of branch order in either data subset (b-order 1 and b-order 2), and even though there were some significant interactions, they were not biologically meaningful.

Fig. 4. Branch order by cooperator interaction for female strobili production using the subset branch order 1. The subset branch order 1 was formed using data from the three cooperators (A, B, and F) that topgrafted in all three branch orders (1,2, and 3).



A significant interaction between branch order and crown position was found for survival in subset b-order 2 (p = 0.031) The least square mean values of this interaction show that survival in second-order branches was superior in topgrafts located in mid-crown and mid-top, while survival of first-order grafts was higher in the top crown. Also, for survival, the topgraft clone by ramet by branch order interaction exerted an important effect accounting for 18.3% of the total survival variation; however, this three-way interaction did not have a clear biological interpretation.

Scion chronological age (selection) effect on topgrafting responses

Only two cooperators (B and E) topgrafted both backward and forward selections, greatly reducing the amount of data for this variable. Missing cells in the data for several effect combinations resulted in even more reduced and unbalanced data. The effect of scion age analyzed in terms of backward and forward selections was not significant at any level for topgraft survival. For male strobili production, significantly superior (p = 0.03) strobili production was found on backward selections (mean = 1.14 strobili per living graft) versus forward selections (mean = 0.14). The nonsignificant trend noted in this study of higher female production from older scion material is consistent with the literature (Greenwood 1981; Parker et al. 1998). The means for scion age groups were 3.79 strobili per live graft for backward selections versus 2.27 for forward selections.

There was a significant interaction of cooperator by selection age for female strobili production (p = 0.0001) due to the fact that higher female flowering occurred on older scion material for cooperator B, while for cooperator E, the response had the opposite trend. Selection age by crown position interaction for female strobili production was also significant (p = 0.05); however, backward selections were superior for female strobili promotion in all crown positions (Fig. 5).

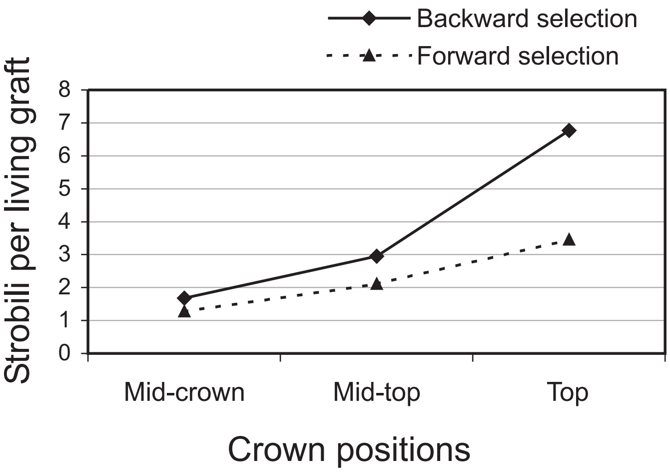
Conclusions

The prolific flowering induced by topgrafting in slash pine 1 year after grafting provided evidence of the operational usefulness of this technique to promote early flowering for breeding. With the effect of scion clone being significant and important for male strobili and especially for female strobili, it is clear that there are different clonal potentials to produce flowers when topgrafted. This fact might compromise the incorporation of poor topgraft flowering clones into the breeding program through topgrafting.

Given the large interstock clone effect on topgraft survival and its moderate effect on topgraft flowering, it is important to identify and select the clones that as interstocks promote good topgraft survival and flowering. The problem of selecting a good interstock clone can be in part overcome by topgrafting selected genotypes onto more interstock clones to ameliorate 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 was in the mid-top followed by the top crown position. Grafting in the top of the crown was highly superior

Fig. 5. Least-squares means for selection age (backward or forward selection) by crown position interaction on female strobili production using a subset of data composed of two cooperators (B and E).



for promoting female strobili followed by the mid-top position. Allocating proportions of topgrafts in the top and mid-top crown is recommended as a more efficient strategy increasing male and female flower production in balanced proportions for breeding.

Among the ad hoc variables, geographic direction showed no significant effect either on topgraft survival or on topgraft strobilus promotion. Branch order was a relevant source of variation only for female strobili production. 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 could also be grafted onto third-order branches. The results showed an effect of scion chronological age on male strobili production and a trend toward increased female strobili production, with chronologically older scions producing more strobili when topgrafted. This higher efficiency of older scions in promoting flowering may represent an added advantage for incorporating backward selections in the breeding population. Further studies using a more balanced and larger data set are recommended to support these results.

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