

SURVIVAL AND PROMOTION OF FEMALE AND MALE STROBILI BY  
TOPGRAFTING IN A THIRD-CYCLE SLASH PINE (*Pinus elliottii* var. *elliottii*)  
BREEDING PROGRAM

By

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by

Alex Mauricio Medina Perez

This thesis is dedicated to my family, especially to my mother Maria whose personal example and character have markedly determined the course of my life. To my sister Jacqueline, whose resolution in a crucial family stage opened a door to our family. To my twin-brother Ariel, who since our conception has been the closest person to me and with whom I share all my memories and present in an unconditional and unique way. To all my relatives from Chile that have looked at our life and academic accomplishments as a source of example and admiration. Finally, I would like to dedicate this work to a person that has been unconditionally close to me during all this process, my girl friend Nathalia.

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Abstract of Thesis Presented to the Graduate School  
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SURVIVAL AND PROMOTION OF FEMALE AND MALE STROBILI BY  
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A study of flowering response to topgrafting was conducted to obtain better understanding and refine the operational use of this technique in the slash pine third-cycle breeding population. 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.

Topgrafting was a very effective tool for promoting both female and male strobili. After one year the overall flowering yield per live topgraft was 2.52 and 1.67 female and male strobili, respectively. The 84% of the live topgraft clones produced strobili, and almost half of them bore both flower sexes. 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. Clonal differences among interstocks accounted for 12.5% of the female and 6.7% of the male strobili variation, and for 16.3% of the topgraft survival variation. This result points out the importance of identification and selection of clones that as interstock promote good topgraft survival and flowering.

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 in promoting female strobili followed by mid-top position. Flowering differences among crown position were not significant for male strobili. 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 in any topgraft response variable. Branch order was a relevant source of variation only for female strobili production with first order branches being significantly superior promoting female strobili, followed by second order 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 for overlapping generations.

## CHAPTER 1 INTRODUCTION

Since the slash pine tree improvement program started in 1953 under the direction of the Cooperative Forest Genetics Research Program (CFGRP), there has been consistent progress in terms of genetic gain, cooperative research and the use of new technology. 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 even shorter time, 10-11 years. To accomplish the goal for generation length, ‘topgrafting’ of the selected clones has been incorporated into the breeding strategy. Topgrafting serves as a tool to accelerate flowering and consequently allow breeding to start as soon as one year after grafting (Bramlett and Burris, 1995; Lott *et al.*, 2003). Given that the topgrafts are made in the existing seed orchards of the cooperative members, they also serve as temporary third generation clone banks. Taking all this into account the incorporation of topgrafting into the third cycle strategy has the potential to accelerate the cycle turnover by as many as 5 years (Gezan *et al.*, 2003).

Topgrafting, also called topworking, is the method of grafting scions from juvenile selections into the crowns of reproductively mature trees, called the interstock. The

resulting ramets consist of three different genotypes: the rootstock of the seed orchard tree which was itself grafted, the interstock which was grafted in the seed orchard and is mature at this point, and the topgraft. The underlying principle of topgrafting is that the fitness, physiological stability, and reproductive competence of the interstock is transferred to the topgraft, while the scion carries the desirable genetic information for breeding (Hartman and Kestler, 1983; Almqvist and Ekberg, 2001). Topgrafting has been used for decades in arboriculture and horticulture and more recently in forestry as a tool for generating seed, updating genetic material, enhancing early fruit production and overcoming specific problems like incompatibility, disease, insect resistance, drought, shade tolerance, and hardiness.

There are numerous reports of topgrafting being a very effective tool for stimulating both female and male strobili in several pine species like loblolly pine (*Pinus taeda*), scots pine (*Pinus sylvestris*), longleaf (*Pinus palustris*), and longleaf x slash pine hybrid (Bramlett *et al.*, 1995; Bramlett and Burris, 1995; Bramlett, 1997; Gooding *et al.*, 1999; Almqvist and Ekberg, 2001). The method has been extensively and successfully applied recently in a loblolly breeding program providing the opportunity to shorten loblolly pine breeding cycles to fewer than 5 years (McKeand and Raley, 2000).

The topgraft clone and interstock clone generally exert important effects in topgraft development and flowering with variation among species. Strong effects of topgraft and interstock clones over male and female flowering were found by Almqvist *et al.* (2001). Working with six mature and seven young clones of *Pinus sylvestris* as interstocks and topgraft, respectively, they also found a significant interaction between interstock and topgraft for female flowering and a strong interstock effect in topgraft survival. McKeand

and Raley, (2000), studying the interstock effect in loblolly pine, found strong effects of the topgraft clone and interstock clone in both female and male strobili initiation. It was also interesting that the flowering fitness of the graft was not related to its interstock capability, meaning that when a good flowering clone is used as interstock, it may or may not stimulate good flowering in the topgrafts. In an earlier study in loblolly pine topgrafting, Schmidting (1983) found that topgraft flowering was significantly affected by interstock and that the previous flowering vigor of a ramet used for interstock was no indicator of the development and flowering of the grafted ramet. The lack of correspondence between flowering of an interstock and its ability to promote flowering in a topgraft makes it difficult to recognize *a priori* good interstock clones for stimulating flowering in topgrafts (McKeand and Raley, 2000); however, once good interstock clones are identified, they can provide a good physical base to use for clone banks and for extensive breeding operations.

A strong influence of crown position on vegetative and reproductive (sexual identity and amount of strobili) development in conifer and broadleaf species has been well documented. Structure, apical control, light interception (affecting light quality and quantity), and physiological activity are some of the factors in flower initiation and development (Parker *et al.*, 1998; Remphrey *et al.*, 2002). In conifers numerous studies have reported significant differences in flowering among crown position (Powell, 1970; Ross, 1983; Parker *et al.*, 1998; Tosh and Powell, 1990; Greenwood, 1994; Kozlowski and Pallardi, 1996; Clarke and Malcolm, 1998). In species of the *Abies* genus, the unisexual female strobili are born typically high on the crown in the upper side of the previous year's twig growth, while male strobili clusters develop lower in the crown

(Young and Young, 1992). Similarly, in species of the genus *Araucaria*, *Picea*, *Larix* and *Pinus*, also monoecious species, the male flowers usually appear lower in the crown than female flowers in young trees; however, as the trees get older and the whole crown branches reach maturity, male and female flowers come closer to each other and usually overlap in the mid-crown (Tosh and Powell, 1991; Young and Young, 1992; Greenwood, 1994; Clarke and Malcolm, 1998). Also significant differences in survival, and female and male flower production have been found among interstock clones and crown positions in *Pinus taeda* and *Pinus sylvestris* topgrafts (McKeand and Raley, 2000; Almqvist and Ekberg, 2001).

Several studies have attempted to elucidate the pattern of strobili and sexual allocation within crown position in conifers. Marquard and Hanover (1984a) studying cone distribution in the crown and the effects of exogenous gibberellin hormone applications in *Picea glauca*, describe the existence of three cone zones: female zone, above whorl 2 producing almost exclusively seed cones; transition zone, from whorl 2 to whorl 3 bearing both male and female strobili; and finally from whorl 3 to 5, a male zone which bears almost exclusively pollen cones. In this study, GA<sub>4/7</sub> treatment increased female and male cone formation in the transition zone, while applications in the male zone induced female strobili and strongly enhanced male cone production. Similarly, in two *larix* species, female strobili were induced in the male cone zone by GA<sub>4/7</sub> applications (Bonnet-Masimbert, 1982). In a separate study, Marquard and Hanover (1984b), in the same specie, found that GA<sub>4/7</sub> was inefficient enhancing strobili production in the upper most crown region. These findings allowed him to suggest the existence of an endogenous gibberellin gradient from lower to upper crown positions and

that necessary endogenous conditions for female flowering in the top crown were already good and were not improved with exogenous GA applications.

In *Picea mariana*, a positive correlation between the age of the trees and the decreasing distribution of the seed cone toward lower crown regions was found (Caron and Powell, 1992). In their results and starting at age 7, the seed cone region was restricted to distal 1-year-old braches, and it systematically expanded toward 5 and even 6-year-old branches while increasing tree age up to 18 years-old. The total production and number of trees bearing seed cones also increased with the age.

In view of these studies, in natural sexual development, seed cone initiation is usually associated with vegetative shoots of moderately vigorous growth, while pollen cones are associated with less vigorous shoots (Powell, 1970; Ross, 1983; Young and Young, 1992). What drives bud sex determination and allocation is likely not to be the shoot vigor itself, but its relationship with endogenous gibberellin (GA) availability for flower initiation uses (Ross 1983). GA biosynthesis and metabolism increase as trees age (Pharis and Kuo, 1977). At the initial development stages of the plant, GAs are mobilized and submitted to vegetative growth, but as trees approach sexual maturity and concentrations of GA exceed vegetative growth demands, shoots in the uppermost whorls begin to initiate seed cones (Ross 1983). As trees, growing rate and shoot vigor start to decline progressively toward lower branches, along with increasing rate of male cones in less vigorous shoots (Ross 1983; Tosh and Powell, 1991; Young and Young, 1992; Clarke and Malcolm, 1998). At this point less endogenous GA is need to satisfy growing requirements and therefore more becomes available for flowering response in a descending gradient from top to lower crown regions (Powell, 1970; Ross, 1983). At the

same time, as trees age and crown size increases, the already settled female zone and uppermost male zone rise; however, because the female flowering capacity decrease in lower branches as the GA concentration and vigor of the shoots also decline (Powell, 1970; Ross, 1983), the female zone is gradually overlapped by the rising male zone and thus, the transition zone arises.

As well as crown region, branch character (branch order) has been reported as a factor affecting production and distribution of seed and pollen cones. Studying the number and distribution of seed and pollen cones of 5, 6 and 7 year old trees of *Larix laricina*, Tosh and Powell (1991) found that most seed cones were born on first and second order branches, while pollen cones were concentrated on long-shoots of second order branches. They also reported that seed and pollen cones typically developed terminally on short-shoot axes and laterally on long-shoots. In crowns of rooted cuttings and mature forest-grown *Picea sitchensis* trees, female strobili were borne mostly on second order branches at the 2<sup>nd</sup> and 3<sup>rd</sup> whorls. This pattern of strobili bearing expanded toward more proximal shoots on upper whorls and more distal shoots on lower whorls as the total cone production increased (Clarke and Malcolm, 1998). In the same study male strobili tended to be borne on less distal shoots than female strobili on third order branches. As interstocks transmit their reproductive suitability to topgrafts, the allocation and distribution of the grafts in the interstock's crown becomes important in order to have enough female and male flowers available for effective breeding.

Maturation or scion chronological age has also been reported as a factor influencing growth and flowering; and hence, it can affect seed production and length of the breeding cycle. Greenwood (1981) assessed the effect of chronological age on strobili production

of loblolly pine finding higher total flower production for chronologically older scion. In a similar study, but working with slash pine clones of different ages (forward and backward selections), Parker (1998) found that chronologically older scions (backward selections) produced slightly more female flowers and had lower vegetative development (fewer branches and less growth) than younger scions (forward selections). Younger scions grew more in both height and diameter and produced more male strobili than older scions. Chronological age was found to have similar effects in growth and reproduction within forward selections clones. In general, chronologically older scion clones flowered sooner and more prolifically than younger clones, and since earlier flowering can shorten the cycle turnover, precocious flowering clones may affect directly the length of the breeding cycle. Also differences in maturity stage of interstock clones can have an important effect on topgraft flowering response (Jayawickrama *et al.*, 1997; Bramlett, 1997).

The CFGRP at the University of Florida is establishing the slash pine third-cycle breeding generation through topgrafting the selected clones, mostly forward selections but also some backward selections, onto sexually-mature, insect-protected seed orchard trees. 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). In the winter of 2003, 2561 topgrafts were established in seed orchards of the cooperative members just after the first round of third-cycle selections were made. These 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 thesis is to understand the effect of the genetic material (interstock

clones and topgraft clones) and the interstock crown position on survival and flowering response in slash pine topgrafting. This study should provide better understanding of topgrafting in slash pine, being an important step in increasing the efficiency of this technique for accelerating flowering and shortening the necessary time for breeding.

The specific objectives of this study are to determine the effects of the following factors on topgraft survival and production of male and female strobili:

1. Genotype of scion and interstock clones;
2. Interstock crown position: top, mid-top and mid-crown;
3. Quadrant of topgrafts: north, south, east, west and their combinations;
4. Branch order of the interstock branch where the topgraft is made: primary, secondary and so on; and
5. Scion chronological age.

## CHAPTER 2 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 (34 backward and 175 forward selections) were grafted in winter 2003 and were used as experimental material for this study. The topgrafts were established in eight slash pine seed orchards (first and second generation) and one slash pine clone bank by CFGRP members. The seed orchards are located in northern Florida, and southern Georgia and Alabama (Table 1). Topgrafting sites were intensively managed with herbaceous weed control, fertilization and insect protection as normal treatments. Healthy ramets with well developed crowns were selected as interstocks. Second-generation CFGRP seed orchards are usually composed of uneven age genetic material (second generation selections and some first generation backward selections). Thus the interstock clones varied in chronological age within a seed orchard. Interstock age also varied widely among orchards, with mean ages ranging from 21 to 72 years (Table 2-1).

Scions were collected in January 2003 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 selections. The chronological age of backward selection scions, although not precisely determined, ranged from 22 to over 80 years old. Three companies topgrafted both backward and forward selections, five companies only forward selections and one company just backward selections (Table 2-1).

Table 2-1. Seed orchard location, number of interstock clones, mean age of interstock clones and number of topgrafts by cooperator for topgrafts made in winter 2005.

Seed orchard location county, state	Number of interstock clones	interstock mean age (years)	Number of top grafts		
			Backward selections	Forward selections	Total
Santa Rosa, FL	10	63	6	338	344
Taylor, FL	10	21	125	220	345
Dooly, GA	7	32	.	146	146
Decatur, GA	5	54	.	463	463
Charlton, GA	11	40	77	384	461
Hamilton, FL	6	41	.	136	136
Tattnall, GA	8	72	.	140	140
Nassau, FL	16	42	.	366	366
Toombs, GA	2	34	160	.	160
	75		368	2193	2561

After scion collection and before grafting, scions were immersed in paraffin at 80°-90° C to protect the scion's bud from physical damage and dehydration. A variation of the modified cleft was the standard grafting method used with the exception of one cooperator that used a regular modified cleft (Figure 2-1). Once grafted, the graft union was wrapped with Parafilm to avoid desiccation. 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 selection. The three crown positions were defined as: top (the first two whorls), mid-top (about whorl 4), and mid-crown (usually about whorl 6). About 3-4 months after grafting, a first survival assessment was recorded and grafts were released by pruning surrounding branches around the graft.

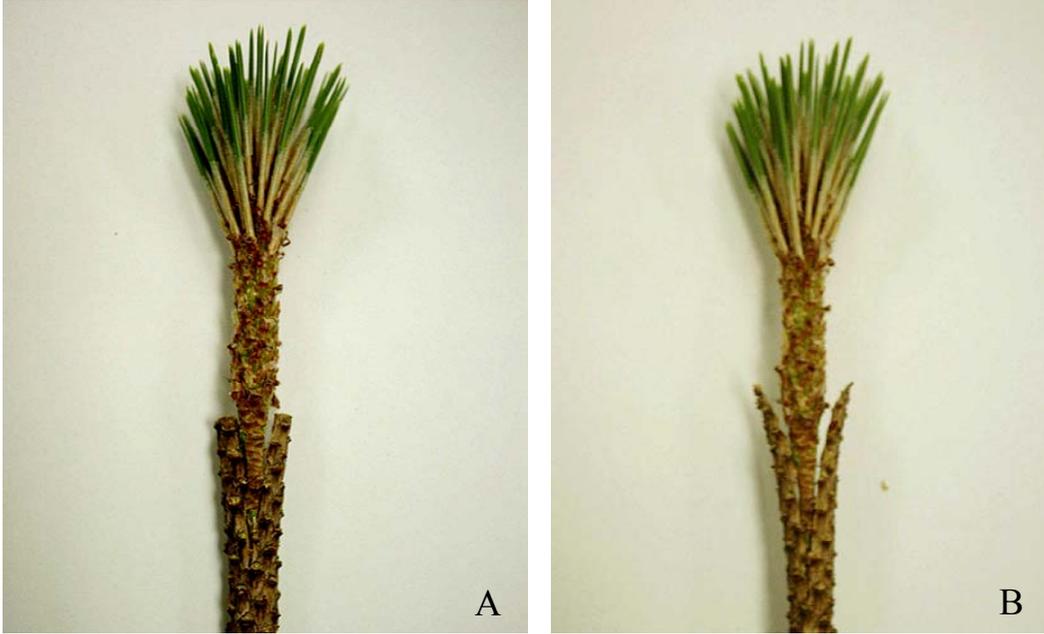


Figure 2-1. Graft method used in slash pine topgrafting. (A) regular modified cleft graft and (B) variant of modified cleft graft.

Survival, and female and male strobili production were measured on topgrafted branches in January of 2004. To relate topgraft survival and flowering with branch character and light exposure, the branch order and the crown quadrant, for each topgraft, were also recorded by some cooperators. 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 (Figure 2-2). For quadrant, the topgraft cardinal point orientation (north, south, east, west) and their combinations (north-east, north-west south-east and south-west) were recorded.

To collect the data, a standard spreadsheet was prepared containing individual topgraft identification, topgraft location (in the seed orchard and on the interstock) and columns to record topgraft survival and flower production. During grafting release the dead topgrafts were cut off by some cooperators and no visual mark was left in the

receptor cut branch, so the identity of the eliminated topgrafts were lost. This situation created problems in the data collection because, after one year from grafting, it was impossible to determine the quadrant and branch order fields for the dead topgrafts. It is important to make clear that branch order and quadrant, although recorded, were adhoc variables, not controlled as part of the true experimental design.

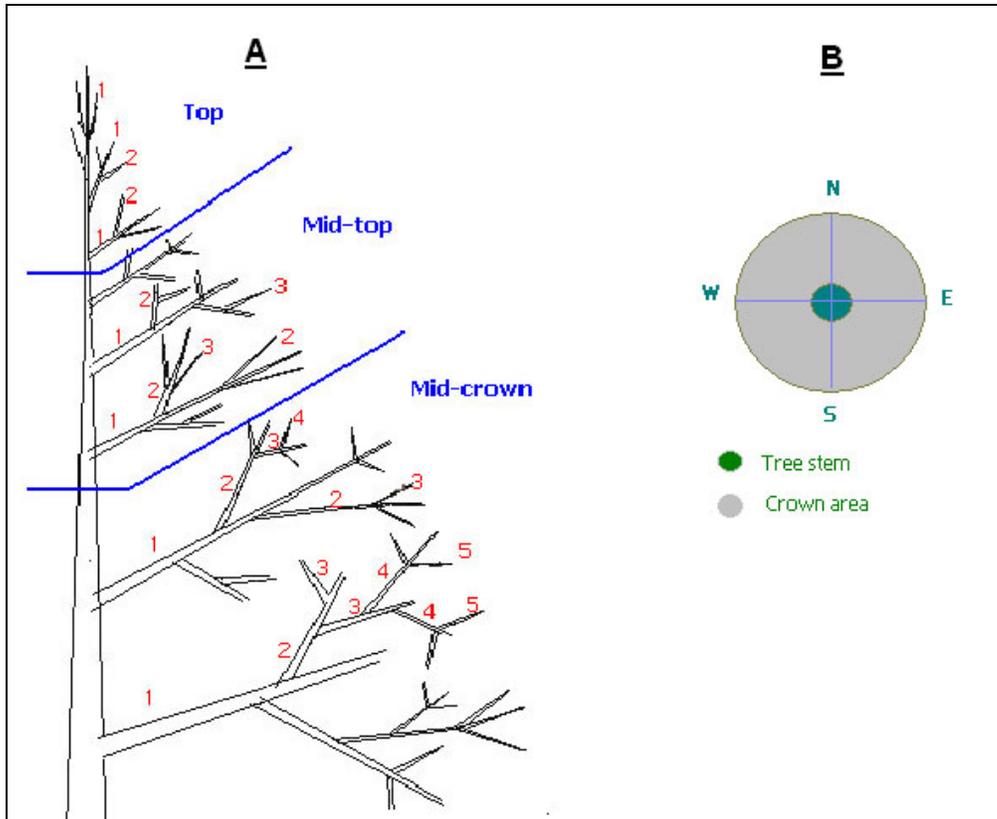


Figure 2-2. Crown categories for slash pine topgrafting. (A) Interstock crown position (Top, Mid-top and Mid-crown) and interstock branch order 1, 2...5. (B) Cardinal directions for topgraft quadrant definition.

Survival, number of female strobili and number of male strobili one year after topgrafting were the response variables of this study. 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 effect on survival, and female and male strobili

production. For strobili production, one of the nine cooperators was left out of the analysis because very few living topgrafts produced either male or female strobili. Topgraft clones and interstock clones were unique for every cooperator, and for a broader level of inference they were considered as random effects in the model.

The initial full linear model was as follow:

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

where

$y_{ijklmo}$  is the response value (survival, number of female flowers or number of male flower) of the  $o^{\text{th}}$  individual topgraft in the  $m^{\text{th}}$  crown position from the  $l^{\text{th}}$  topgraft clone on the  $k^{\text{th}}$  ramet of the  $j^{\text{th}}$  interstock for the  $i^{\text{th}}$  cooperator;

$\mu$  = the overall mean;

$O_i$  = the fixed effect of the  $i^{\text{th}}$  cooperator;

$i_{j(i)}$  = the random effect of the  $j^{\text{th}}$  interstock clone nested within cooperator,  
 $\sim \text{NID}(0, \sigma^2_i)$ ;

$r_{k(ji)}$  = the random effect of the  $k^{\text{th}}$  ramet nested within interstock clone and cooperator,  $\sim \text{NID}(0, \sigma^2_r)$ ;

$t_{l(i)}$  = the random effect of the  $l^{\text{th}}$  topgraft clone nested within cooperator,  
 $\sim \text{NID}(0, \sigma^2_t)$ ;

$C_m$  = the fixed effect of the  $m^{\text{th}}$  crown position;

$OC_{im}$  = the fixed interaction effect of the  $i^{\text{th}}$  cooperator and the  $m^{\text{th}}$  crown position;

- $it_{jl(i)}$  = the random interaction effect of the  $j^{\text{th}}$  interstock clone nested within cooperator and the  $l^{\text{th}}$  topgraft clone nested within cooperator  $\sim \text{NID}(0, \sigma_{it}^2)$ ;
- $Ci_{jm(i)}$  = the random interaction effect between the  $m^{\text{th}}$  crown position and the  $j^{\text{th}}$  interstock clone nested within cooperator,  $\sim \text{NID}(0, \sigma_{Ci}^2)$ ;
- $r_{k(j)l(i)}$  = the random interaction effect between the  $k^{\text{th}}$  ramet nested within interstock and cooperator, and the  $l^{\text{th}}$  topgraft clone nested within cooperator  $\sim \text{NID}(0, \sigma_{rt}^2)$ ;
- $Cr_{mk(ji)}$  = the random interaction effect between the  $m^{\text{th}}$  crown position and the  $k^{\text{th}}$  ramet nested within interstock clone and cooperator,  $\sim \text{NID}(0, \sigma_{Cr}^2)$ ;
- $Ct_{ml(i)}$  = the random interaction effect between the  $m^{\text{th}}$  crown position and the  $l^{\text{th}}$  topgraft clone nested within cooperator,  $\sim \text{NID}(0, \sigma_{Ct}^2)$ ;
- $Cit_{mjl(i)}$  = the random interaction between the  $m^{\text{th}}$  crown position, the  $j^{\text{th}}$  interstock clone nested within cooperator and the  $l^{\text{th}}$  top graft clone nested within cooperator,  $\sim \text{NID}(0, \sigma_{Cit}^2)$ ;
- $Crt_{mkl(ji)}$  = the random interaction between the  $m^{\text{th}}$  crown position, the  $k^{\text{th}}$  ramet nested within interstock clone and cooperator and the  $l^{\text{th}}$  top graft clone nested within cooperator,  $\sim \text{NID}(0, \sigma_{Crt}^2)$ ; and
- $e_{ijklmo}$  = the residual effect,  $\sim \text{NID}(0, \sigma_e^2)$ ;

After the initial analysis using the full linear model, model elements were deleted if they did not meet the significance criteria. For each dependent variable effects were maintained in the model at 25% significant level using a backward elimination approach.

To test the significance of the variance components for the random predictors a Wald Test was used (Greene, 2000).

In the second stage of the statistical analysis, the effects of quadrant, branch order and topgraft chronological age on the response variables (topgraft flowering and survival) were analyzed using a different approach. The large number of missing values and inconsistency in the levels of these three new predictor variables among cooperators, made the analysis of their effects on the response using the whole data set problematic. Analyzing a more balanced subset of the full data set for each variable seemed a better approach for obtaining reliable and interpretable results.

For quadrant, the five cooperators that consistently topgrafted in north-east, south-east, north-west and south-west quadrant orientations composed the subset of the data used in the analysis. For branch order two subsets were defined. The first subset, called border 1, for testing the effect of the three levels of branch order (primary, secondary and tertiary) on the response variables, was formed using data from the three cooperators that topgrafted in all the three branch orders (1, 2 and 3). The second subset, called border 2, for testing the effect of primary and secondary branch order on topgraft flowering and survival, consisted of the first subset plus data from the four additional cooperators that topgrafted on only branch orders one and two. 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 only two cooperators that topgrafted backward and forward selections in sufficient numbers. There was an insufficient range of ages among forward selections (ages 4 to 6) to allow testing the effect of topgraft chronological age within forward selection clones.

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.

## CHAPTER 3 RESULTS AND DISCUSSION

### **First Stage Analysis, Experimental Design Factors**

#### **Topgraft survival**

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 3-1) with a minimum and maximum mean survival of 46.1% and 86.1%, respectively. Also the differences in survival among crown positions were significant ( $p$ -value = 0.01). Higher survival rate was obtained in the mid-top (75.3%), followed by the top (69.9%) and finally by the mid-crown position (67.2%). One of the possible factors associated with lower survival rate in the top compared to the mid-top could be the larger diameter of some interstock branches in the upper crown (apex and first whorl shoots) with respect to the scions, which made proper alignment of the cambiums of the scion and interstock difficult.

The scion topgraft clone did not have an important effect on survival. The most important detectable source of variation was interstock, 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*, *Pinus sylvestris* and in a hybrid between *Pinus palustris* and *Pinus elliottii* (McKeand and Raley, 2000; Almpvist and Ekberg, 2001; Lott *et al.*, 2003).

Table 3-1. 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 <sup>a</sup>	Response variables (p-value)		
	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 <sup>b</sup>
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%

<sup>a</sup> 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).

<sup>b</sup> effect not significant at the 0.25 level in the fitted model.

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 survival variation among interstock clones (e.g., scion quality, micro site, rootstock, grafting time and date, weather, etc.). Given the low variation in survival due to ramet differences, just 6% compared to 16% for interstock, it is reasonable to think that most of the survival variation among interstock clones is due to genotypic differences plus additional external factors like interstock chronological age.

As topgraft survival was highly influenced by interstock genotype, the use of interstocks that yield higher survival of topgrafts is recommended to increase the

efficiency of topgrafting, but the selection criteria should also include the capability of the interstock for promoting topgraft flowering.

Topgrafting was a very effective tool promoting both female and male strobili one year from grafting. 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. From the 1861 living topgrafts, 53.8% bore strobili, of those 77% produced only female strobili, 19% produced only male and 14% produced both male and female strobili. About 54% of the topgrafts with female strobili bore more than 3 strobili per graft (Figure 3-1-A). For economic reasons in breeding, this yield can be considered as a minimum number of flowers for a graft to be worthy of bagging. Male strobili production was distributed with about 72% of the topgrafts producing male strobili having more than 3 strobili, and so potentially suitable for collection. These results manifest the competence of topgrafting for promoting 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 genotypes with live grafts, 168 produced strobili. In this case 54.8, 1.2 and 44% of the topgrafts produced female, male and both (female and male) strobili, respectively (Figure 3-1-B). More than 70% of the scion genotypes with female strobili bore more than 3 female strobili which makes them potentially suitable for breeding. Male strobili production by scion clone was less prolific with about 33% of the genotypes with male strobili having more than 3 male strobili.

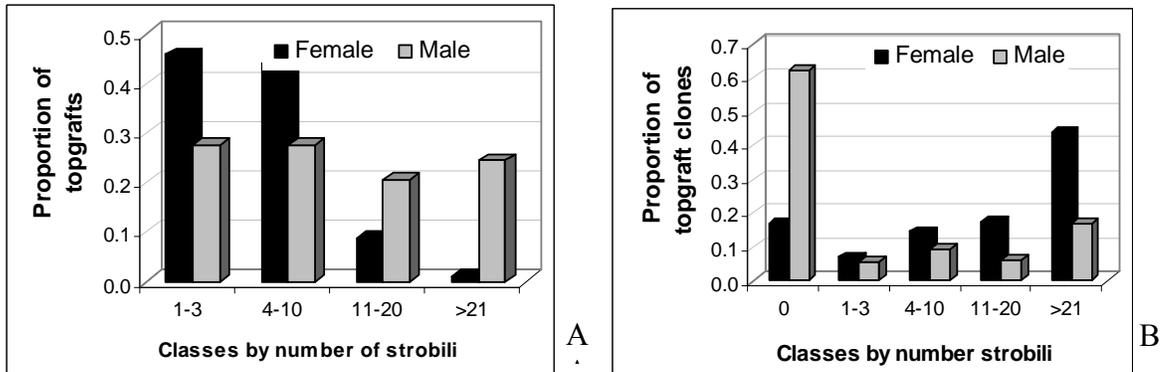


Figure 3-1. Proportion of topgrafts by strobili production classes: (A) Proportion of flowering topgrafts by classes of strobili production; (B) Proportion of scion genotypes by classes of strobili production. In (A), the proportions are with respect to grafts producing flowers, so strobili production classes start at 1. In (B), the strobili production classes start from 0 because the proportions are with respect to all living grafts for scion genotypes.

### Cooperator effect on flowering

Female and male strobili production showed significant variation among cooperators (Table 3-1). Whereas for one cooperator there was almost no flowering, considered as an irregular situation, the highest mean female and male flowering yields for a cooperator were 4.4 and 4.8 strobili per live graft (Figure 3-2). Cooperators B, D, E and H merit special interest because the differences between male and female strobili production were extreme, suggesting that variation in values of endogenous and/or exogenous factors, can cause wide variation in male and female strobili promotion.

Scion collection and manipulation, grafter, soil nutrition, water availability, weather condition and insect protection are some of the factors that may vary among cooperators, that were not controlled in the experiment, and that affect flower initiation and development (Kozłowski, 1971). Interstock maturation and crown size are two factors correlated with the age of the interstock, that may also vary among cooperators, and that can affect the rate of cone initiation and the proportion of male-female strobili production (Kozłowski, 1971; Greenwood, 1994).

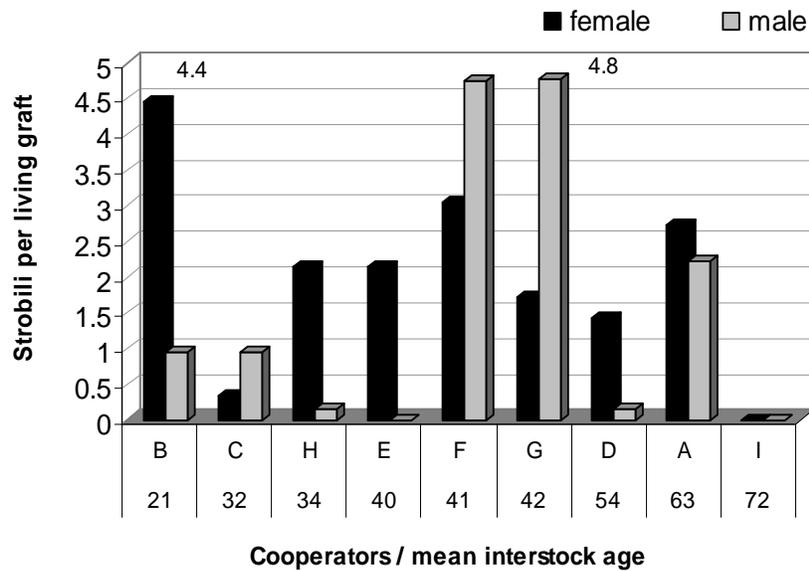


Figure 3-2. Least square means for female and male strobili production by cooperator. Also in x-axis, cooperators (capital letters) are sorted and located, from lower to higher, by the mean interstock age which is displayed below each cooperator.

In Figure 3-2 cooperators are sorted and located in the x-axis, from lower to higher, by their mean interstock age. By looking at the plot we can see that for both flower genders the variation of strobili production per living graft among cooperators did not follow the trend in mean interstock age. This lack of correspondence indicates that factors others than interstock age are determining flowering yield variation among cooperators. To assess the correlation between flowering response and interstock age a simple regression analysis was conducted. The regression analysis of flowering yield as a function of mean interstock age by cooperator showed no significant slope for the age parameter for either flower gender. To have a more general inference about the correlation between flowering responses and interstock age, a pooled regression analysis of flowering 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-value <0.0001, parameter estimate -0.0366) was found; however, a very low correlation

(R-square= 0.02) suggesting that the female flowering yield is barely sensitive to interstock age variation. For male flowering the slope was also significant but positive (p-value = 0.0004; parameter estimate = 0.0004) with an even lower correlation factor (R-squared= 0.0089). Thus, the strong variation of flowering among cooperators is not likely to be a result of interstock age or maturity stage differences among cooperators, but due to additional factors not controlled in this study.

In conifers, the time of cone initiation, although varying within and among species, normally starts early in the season preceding the spring where cones open and pollination occurs (Kozłowski, 1971; Kozłowski and Pallardi, 1996). Initiation time is also influenced by weather, site conditions and seed orchard cultural practices. Female and male strobili differentiate at different times and rates, with male cones initiating first and developing faster (Kozłowski, 1971). Before differentiation and physiological mechanisms due to connecting vascular tissues occurs, the topgraft's wound callus has to heal and new vascular cambium has to form, which can take several weeks (Kozłowski and Pallardi, 1996). In our study topgrafting was carry out from early February to mid March, with more than one month difference between earlier and later grafting date among cooperators. Considering the timing of floral initiation and grafting acclimation, later grafted scions might not have been as receptive for cone initiation as scions grafted earlier in the season; and therefore their flowering yield could have been diminished, especially for male cones which initiate earlier. This timing consideration could explain the low rate of male strobili production of cooperators D and H (Figure 3-2), the two cooperators that started topgrafting later in the season; however, additional studies are necessary for testing this hypothesis.

### **Crown position effect on flowering**

Significant differences in strobili production were found among crown positions for female strobili (Table 3-1). The flower distribution within crown showed a regular progression of female strobili per living graft from lower to upper crown locations. The overall female strobili yield in the top crown, 3.22 strobili per live graft, was considerably higher than the mid-top and mid-crown positions with 2.08 and 1.42 strobili per live graft of yield, respectively. For male strobili, the flowering variation among crown positions was not significant ( $p$ -value= 0.19). The highest flowering yields for male strobili, also showing very similar value (2.07 and 2.01 strobili per living graft), were reached on the top and mid-top positions, respectively. The flowering yield was considerable lower on mid-crown branches with overall yield of 1.14 male strobili per living graft (Figure 3-3A). Almost 60% of the female strobili and about 50% of the male strobili were borne in the top crown (Figure 3-3B).

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 several conifer species (Powell, 1970; Ross, 1983; Marquard and Hanover, 1984a; Marquard and Hanover, 1984b; Tosh and Powell, 1990; Young and Young, 1992; Greenwood, 1994; Kozlowski and Pallardi, 1996; Clarke and Malcolm, 1998); In a different manner that male strobili production reached a lower yield in the mid-crown was an unanticipated result; however, to our knowledge, there is a lack of previous studies on topgrafting flower distribution in pine species that could serve as more comparable references.

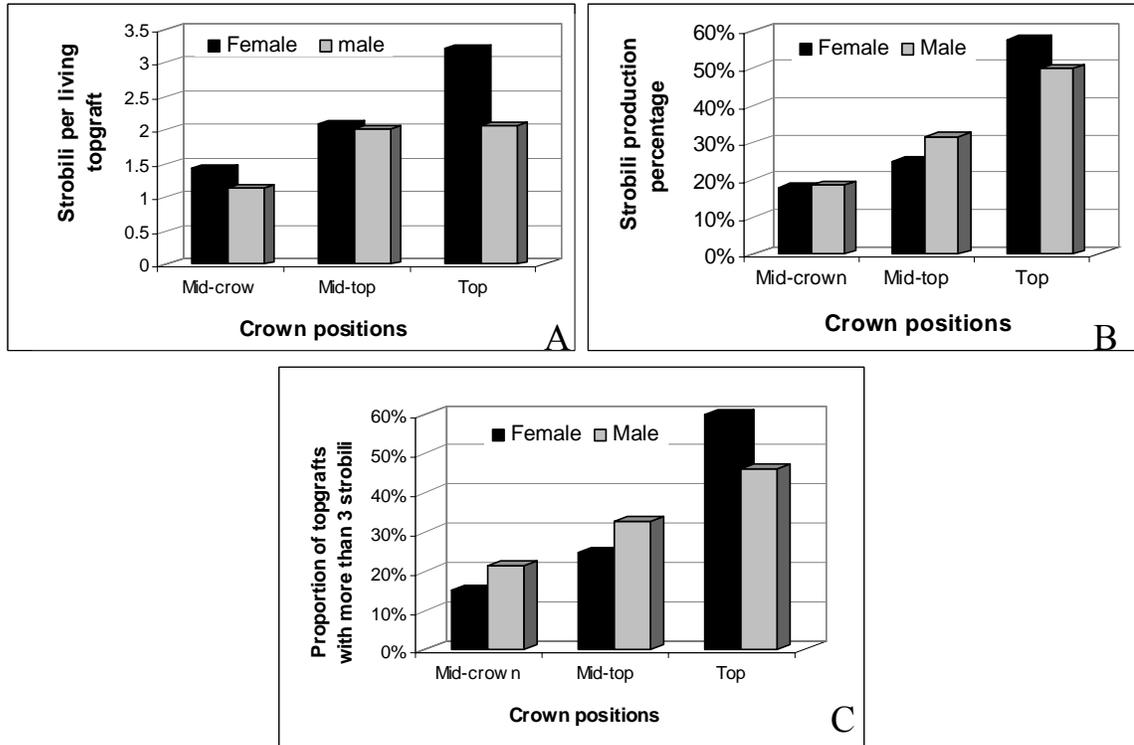


Figure 3-3. Female and male strobili production by crown positions. (A) Least square means of female and male strobili production per live graft, (B) Female and male strobili production as proportion of the total, and (C) Proportion of topgrafts that produce enough flowers ( $> 3$  strobili) to be bagged and used in breeding. The crown positions are defined as: top (the first two whorls), mid-top (about whorl 4), and mid-crown (usually about whorl 6).

With respect to material for breeding after one year from grafting, about 50% of the male flowering topgrafts, that bore enough flowers to be collected ( $> 3$  strobili per graft), were borne in the top 2 whorls of the crown. For topgrafts with female strobili this percentage was even higher, 57% (Figure 3-3 C). 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, respectively, 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 2 whorls might not be abundant enough for large scale crossing; 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 3-1), implying that the pattern of male flower distribution within the crown was consistent among cooperators. The pattern of female flowering distribution within the crown was also consistent among cooperators with some slight non-significant deviation from the main effect (interaction p-value = 0.13). From Figure 3-4, in which cooperators are sorted by their overall female strobili production per live graft on the x-axis, it is possible to ascertain that the pattern of female strobili production within the crown remains relatively constant across cooperators, with increasing levels of production as branches approached the top whorls of the crown. The plot also showed that cooperator C which yielded less than 0.5 strobili per living graft and a total of just 31 female strobili bore in 7 topgrafts, exhibit a different crown position ranking; however, given the reduced data, the results for cooperator C are not meaningful for the general trend. Also the observed lack of parallelism among cooperators was not significant (p-value= 0.14).

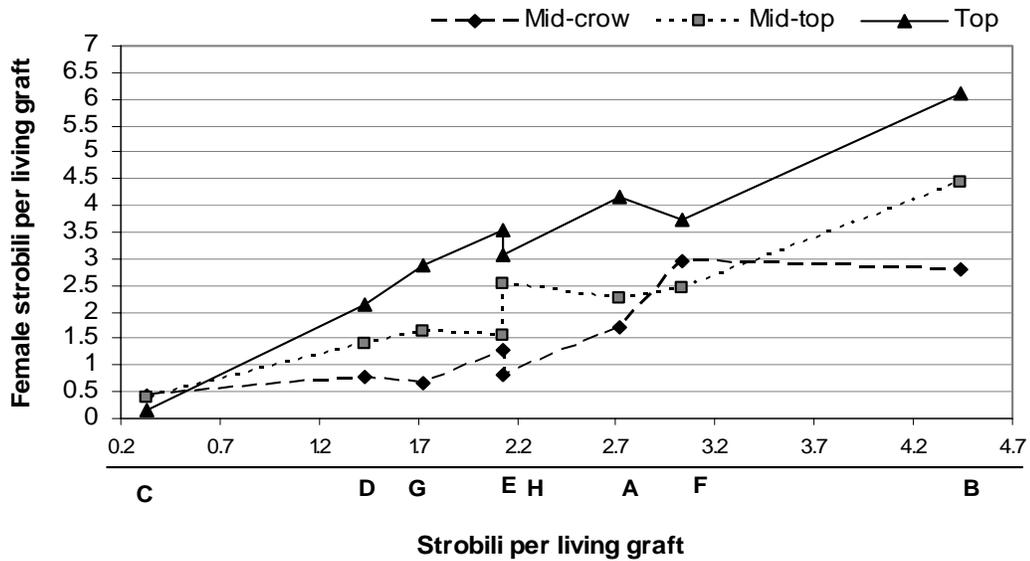


Figure 3-4. Least square means for female strobili production for cooperator by crown position interaction. On the x-axis, the numeric scale of strobili per live graft is displayed. Also on the x-axis, cooperators (capital letters) are sorted and proportionally located, from lower to higher, by the mean female strobili per live topgraft value.

Interstocks transmit part of their reproductive and physiological fitness to the topgraft (Hartman and Kestler, 1983; Almqvist and Ekberg, 2001). Based on this principle, the mechanisms of sexual determination and crown strobili allocation discussed by Powell (1970); Ross (1983); Marquard and Hanover (1984a); and Marquard and Hanover (1984b) previously described, can be at some level, applied to topgrafting flowering response. So, perhaps the gradient of female strobili production from mid-crown to top crown found in our study can be explained as a result of the shoot vigor and endogenous GA (gibberellin acid) gradient from lower to upper crown regions; However, the pattern of higher concentration of male strobili in the top and mid-top crown position respect to mid-crown is not easily explained by the shoot vigor-GA regulatory mechanism and therefore other mechanisms are expected to be involved.

### **Topgraft and interstock clones effect on flowering**

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. In our study more than 23 and 8% of the total female and male flowering variation, respectively, were due to differences in scion clone (Table 3-1), being the largest detectable source of variation of flower initiation in female strobili and the second largest for male strobili. The large influence of topgraft clone on flowering is evidenced when looking at the high variation in the proportion of topgraft clones among classes of strobili production (Figure 3-1B). In a closer look at the data by cooperator, it is observed that some clones exhibit higher flowering potential than others. A clear example is observed in cooperator C in which approximately 32% of the scion clones bore female flowers, with about 71% of the total strobili borne by one scion clone.

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. 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 dissimilar ability of interstock clones to promote topgraft flowering is also observed 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 kept their fitness relatively constant throughout the interstocks on which they were grafted. Thus, for male and especially for female strobili, the cone 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 topgrafted into a bad interstock. This results point out importance of a prior identification of interstock clones that will 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 *Pinus taeda* and *Pinus sylvestris* (Schmidting, 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). For slash pine topgrafting this correlation has not been assessed, and any intent should consider factors like interstock age and number of year of evaluation considering a potential seed production cycle. Powell and White (1994) studying the cone and seed yield of slash pine using data collected from 41 seed orchards, have reported a bumper crop production every 3-4 years in slash pine seed orchards. They also found that during poor cone crop years, all seed orchards consistently reported poor cone yields; however, it was a variation in the years when the different seed orchards reached their top cone production. Thus, when correlating interstocks flowering fitness and their suitability promoting topgraft flower initiation in slash pine, at least two scenarios (bad and good cone crop years) should be taken in consideration and not only an overall interstock yield. If the alternative of selecting good interstock clones by their flowering yield results to be inapplicable, the problem can be in part overcome by topgrafting selected genotypes into more interstocks clones, this ameliorates the risk of having poor flower initiation and poor survival caused by interstock clone.

A final important interaction indicated that in male strobili production interstock by crown position interaction 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 changes in different crown positions. The ramet effect, nested within interstock, had no contribution to the female strobili total variance and accounted for the 6.6% of the male flowering variation. As was seen for topgraft survival, this suggests that the female flowering variation among interstock clones is likely to be due to genotypic interstock differences.

To evaluate strobili promotion efficiency (SPE) 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, SPE represent the number of strobili per grafted scion, the results are presented in the Table 3-2.

Table 3-2. Strobili promotion efficiency (SPE) by crown position. Efficiency is expressed as flowers per grafted scion and calculated as:  $SPE = \text{crown position survival rate (\%)} * \text{number of strobili per live topgraft}$ .

Crown position	Survival rate	Female flowers per live graft	Male flowers per live graft	Female efficiency	Male efficiency	Total efficiency
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

Crown positions are defined as: top (the first two whorls), mid-top (about whorl 4), and mid-crown (usually about whorl 6).

The significantly larger female flowering rate reached in the top crown position overwhelmed the effect of higher survival of the mid-crown, placing top position as the most efficient one for promoting female strobili. In a different way, the higher survival rate of the mid-top placed this position as the most efficient producing male strobili.

For breeding practice the primary concern is to ensure not just high but also a 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 topgraft's male and female strobili production.

### **Second Stage Analysis, of Effects of Adhoc Factors**

#### **Quadrant effect on topgrafting responses**

A separate analysis was conducted to test the effect of quadrant on topgrafting responses using a subset data composed by the 5 cooperators that located the topgrafts in the north east, northwest, southeast and southwest quadrants. As in all the adhoc factors, for quadrant the statistical analysis used the fitted model for the experimental design factors as a base model, to which the additional quadrant effects (main effect and interactions) were added and fitted by the same process in which a full model was fitted with non-significant effects ( $p=0.25$ ) being eliminated in a backward elimination approach. The results of the statistical analysis are shown in the Table 3-3.

Quadrant effect was not significant for female flowering at 25% either as a main effect or as part of an interaction, and therefore the output of that analysis is not reported in Table 3-3. In general quadrant did not have an important effect on either survival or male flowering. For survival, the three way interaction between topgraft clone, interstock clone and quadrant was the only effect causing significant variation, accounting for the 9.6% of the total survival variation; however, it does not have an important biological interpretation.

Table 3-3. Second stage analysis of variance using the data from only 5 cooperators (A, B, C, F and G) to test the effect of quadrant on the response variables: survival and male strobili promotion. P-value for fixed effects and variance components as a percentage of the total phenotypic variance for random effects are shown.

Model for quadrant <sup>a</sup>	Response variables	
	Survival	Male
Cooperator	0.0090	0.069
Crown	< 0.0001	0.17
Cooperator*crown	0.0323	ns <sup>b</sup>
Quadrant	0.7200	0.102
Crown*quadrant	ns	0.09
Random effects	Variance (% of total variation)	
Topgraft	29.60%	10.40%
Interstock	12.00%	ns
Ramet	ns	12.30%
Topgraft*interst	ns	2.30%
Interstock*crown	1.30%	4.70%
Interst*quadrant	2.70%	ns
Topgraft*interstock*quadrant	9.60%	6.38%
Residual	44.56%	63.80%

<sup>a</sup> 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).

<sup>b</sup> effect not significant at 25% of confidence in the fitted model.

For male strobili production quadrant was not significant at  $\alpha=0.05$  at either main effect or at an interaction level. The overall highest male strobili yield was reached when topgrafting in the quadrant south-west; however, this trend was not consistent in all crown positions (Figure 3-5). The quadrant by crown position interaction might be explained by a scenario that associates quadrant with the existence of a predominant wind or sun exposure pattern in the seed orchard.

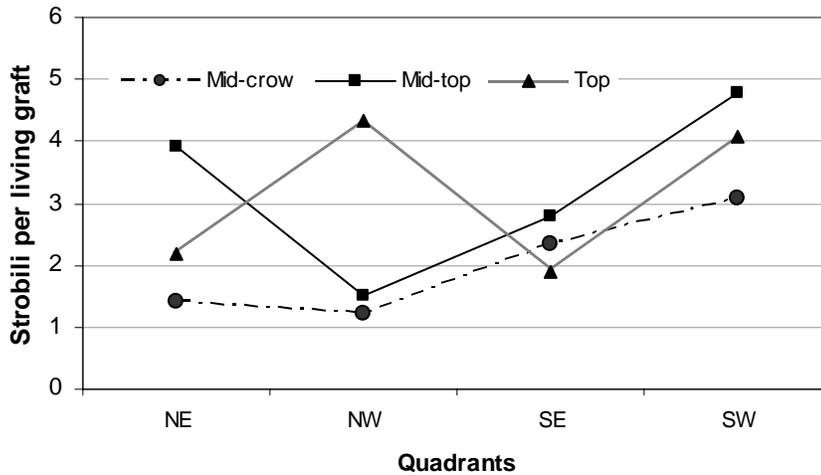


Figure 3-5. Quadrant by crown position interaction for male strobili production. Quadrants are: north-east (NE), north-west (NW), south-east (SE) and south-west (SW). The subset for testing the quadrant effect on the response variables was composed by the 5 cooperators that located their topgrafts in these quadrants.

### Branch order effect on topgrafting responses

No significant effect of branch order either as main effect or as part of an interaction was found for branch order subset border 1 on survival response, at the 25% of significance level, so the data is not shown in Table 3-4. As a main effect branch order was significant only for female strobili production in the subset border 1 (Table 3-4), in which the effect of the three levels of branch order on the response variables were 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 the three cooperators that formed the subset border 1 (Figure 3-6). This tendency is supported by the non-significant level for the cooperator by branch order interaction (p-value 0.19).

Table 3-4. Second stage analysis of variance for testing the effect of branch order (border) on survival, female and male strobili promotion. P-value for fixed effects and variance component as percentage of the total phenotypic variance for random effects are shown. Border 1, corresponds to the first subset data analysis for testing the three levels of branch order effect utilizing data from 3 cooperators (A, B and F). Border 2 represents the second subset data analysis for testing the effect of primary and secondary branch order levels utilizing data from 7 cooperators (A, B, C, D, F, G and H).

Model for branch order <sup>a</sup>	Survival	Female		Male	
	border 2	Border 1	Border 2	border 1	border 2
Cooperator	0.0164	0.31	0.0021	0.0228	0.057
Crown	0.3612	<0.0001	<0.0002	0.071	0.26
Cooperator*crown	ns <sup>b</sup>	Ns	0.238	ns	Ns
Border	0.6736	0.012	0.148	0.18	0.25
Cooperator*border	Ns	0.181	0.1044	0.15	0.107
Crown*border	0.0303	Ns	ns	0.21	Ns
Random effects	Variance components (%)				
Topgraft	Ns	33.4%	31.4%	9.5%	3.4%
Interstock	18.6%	12.3%	12.4%	ns	16.8%
Ramet	4.3%	Ns	ns	ns	2.1%
Topgraft*interstock	Ns	4.2%	6.5%	19.3%	Ns
Interstock*crown	Ns	3.5%	ns	ns	14.4%
Topgraft *crown	Ns	Ns	ns	ns	6.9%
ramet*border	Ns	Ns	ns	6.7%	Ns
Topgraft*interstock*border	Ns	Ns	ns	ns	10.0%
Topgraft*ramet*crown	15.0%	Ns	ns	ns	Ns
Topgraft*ramet*border	18.3%	Ns	ns	ns	Ns
Interstock*crown*border	Ns	Ns	5.0%	ns	Ns
Residual	43.8%	46.5%	45.1%	64.3%	46.0%

<sup>a</sup> predictor variables in the model are: cooperator, crown (crown position), border (branch order 1, 2, 3 of the interstock receptor branch), topgraft (topgraft clone nested within cooperator), interstock (nested within cooperator), and ramet (interstock replication, nested within interstock and cooperator).

Male strobili production was less sensitive to the effect of branch order at either subset data (border 1 and border 2), and even though there were some significant interactions they are not biologically meaningful.

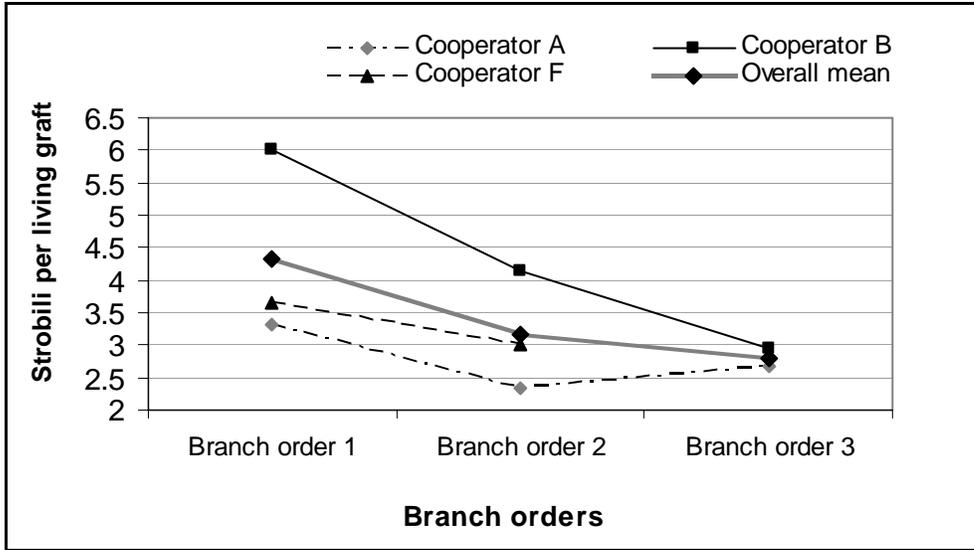


Figure 3-6. Branch order by cooperator interaction for female strobili production using the subset border 1. The subset border 1 was formed using data from the three cooperators (A, B and F) that topgrafted in all the three branch orders (1,2 and 3). Also the overall mean of survival by branch order is displayed in the plot.

A significant interaction between branch order and crown position was found for survival on subset border 2. The plot of the least square means of this interactions (Figure 3-7) shows that survival on second order branches was superior in topgrafts located on mid-crown and mid-top, while survival of terminal shoots grafts (first order) was higher for topgrafts in the top crown. Also for survival, the topgraft by ramet by border interaction exerted an important effect accounting for 18.3% of the total survival variation; however, this three way interaction does not have an important biological interpretation.

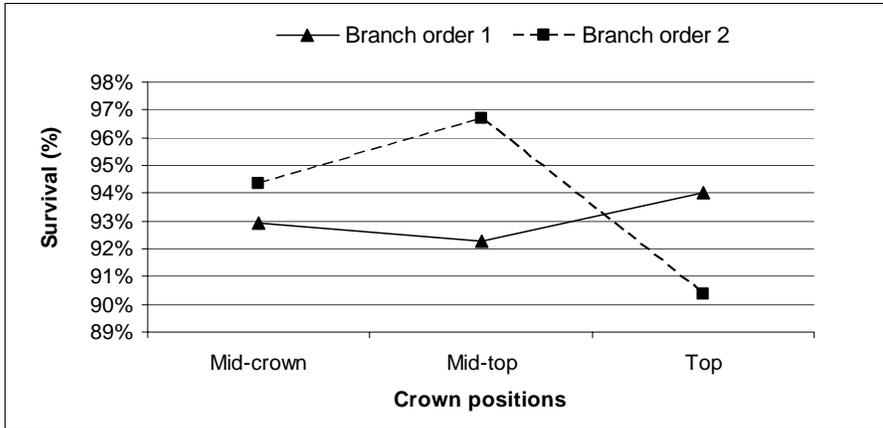


Figure 3-7. Branch order by crown position interaction for survival response using the subset border 2 which represents the second subset data analysis for testing the effect of primary and secondary branch order levels utilizing data from 7 cooperators (A, B, C, D, F, G and H).

### Scion chronological age (selection) effect on topgrafting responses

Only two cooperators (B and E), topgrafted both backward and forward selections which greatly reduced the amount of data for testing the effect of this variable. Missing values in the data at several variable levels resulted in an even more reduced and unbalanced data. The effect of scion age on topgraft responses analyzed in terms of backward and forward scion selections was not significant at any level for topgraft survival and therefore the output of that analysis is not reported. Significant differences between backward and forward selections were found for male and female strobili production (Table 3-5). Although the variation of female strobili among selections was not significant ( $p$ -value=0.1), the overall strobili production per live graft was 1.6 times larger for backward than forward selection with overall means of 3.79 and 2.27 female strobili per live graft, respectively. Male strobili production also reached significantly higher overall yield on older scion material (backward selections) with respect to younger scions (forward selections), with overall means of 1.14 and 0.14 male strobili per living graft for backward and forward selections, respectively.

Table 3-5. Second stage analysis of variance for testing the effect of chronological scion age, expressed in terms of backward and forward selection, on the responses: female and male strobili promotion. Data from two cooperators was used for this analysis. P-value for fixed effects and variance component as percentage of the total phenotypic variance for random effects are shown.

Model for selection <sup>a</sup>	Response variables	
Fixed effect	Female	Male
Cooperator	0.0029	0.15
Crown	<0.0001	<0.0001
Cooperator*crown	0.1939	ns <sup>b</sup>
Selection	0.0906	0.0303
Cooperator*selection	0.0001	0.16
Crown*selection	0.043	ns
Random effects	Variance components (%)	
Topgraft	20.4%	25.0%
Interstock	12.8%	ns
Residual	66.7%	74.9%

<sup>a</sup> predictor variables in the model are: cooperator, crown (crown position), selection (scion chronological age expressed as backward or forward selection), topgraft (topgraft clone nested within cooperator), interstock (nested within cooperator) and ramet (interstock replication, nested within interstock and cooperator).

<sup>b</sup> effect not significant at 25% of confidence in the fitted model.

The significant interaction of cooperator by selection for female strobili production indicates that the superiority of backward selection scions for female strobili production was not consistent among the two cooperators. In Figure 3-8A we can see that female flowering in cooperator E did not follow this trend and that the overall pattern of higher female flowering yield in backward selection is most likely to be a consequence of the very high overall flowering yield of cooperator B on backward selection compared to forward selection.

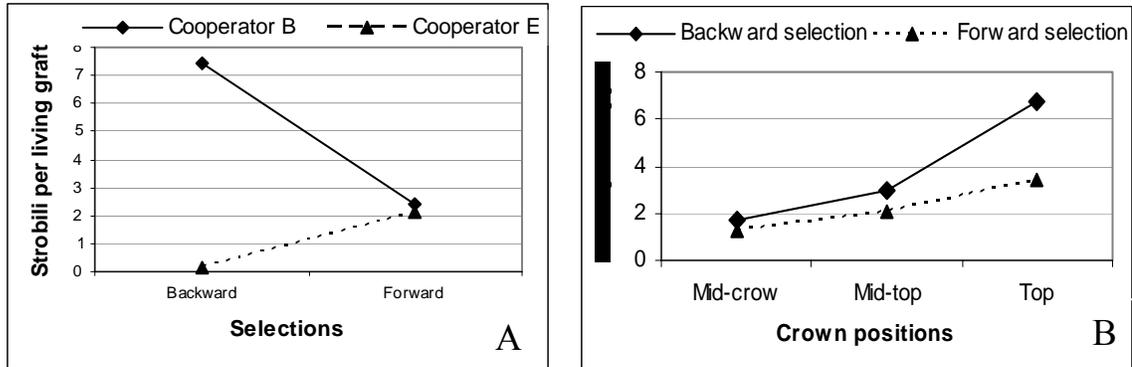


Figure 3-8. Effect of selection type on female strobili production using a subset data composed of two cooperators (B and E). (A) Lsmeans for selection by cooperators interaction. (B) Lsmeans for selection by crown position interaction. Selection corresponds to the scion chronological age expressed as backward or forward selection.

Although selection by crown position interaction for female strobili production was significant at 5%, backward selection kept its superiority promoting female strobili on all crown positions (Figure 3-8B), then the significant interaction only reflects the lack of parallelism between backward and forward selections curves. Higher female strobili production in older scion material respect to juvenile scion seems to be a regular trend in conifer species. In our results the low significant level of the differences of female strobili production among selection types (10%), is probably due to the reduced data which decreased the power to detect differences; nevertheless, there was a large difference in the overall female strobili per live graft.

## CHAPTER 4 CONCLUSIONS

The prolific flowering induced by topgrafting in slash pine one year after grafting provided evidence of the operational usefulness of this technique to promote early flowering for breeding. 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.

With the effect of scion clone being significant and important in male strobili and especially in female strobili, it became clear that there is a different potential among slash pine clones to produce flowers 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.

Given the large interstock 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. 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 interstocks 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 for overlapping generations; and hence, further studies using a more balanced and larger data is recommended to support these results.

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## BIOGRAPHICAL SKETCH

Alex M. Medina Perez was born and raised in Villarrica, IX region, Chile. The imposing forest of *Araucaria* and *Nothofagus* species surrounding his home town impressed on him an awareness and interest for biology and ecology. This curiosity finally led him to study forest engineering at Universidad Austral de Chile in 1993. Working as a research assistant in a breeding program of Chilean native species from 1998 to 2000, he became interested in the forest genetic field. In January 2003, he was enrolled in the School of Forest Resources and Conservation at University of Florida to initiate his graduate studies with a Master of Science focusing in genetic tree improvement. His belief in God, his passion for nature and love for life have always been his inspiration and life purpose.