# Top Grafting Longleaf x Slash Pine F<sub>1</sub> Hybrids on Mature Longleaf and Slash Pine Interstocks

L. H. Lott<sup>1</sup>, L. M. Lott<sup>1</sup>, M. Stine<sup>2</sup>, T. L. Kubisiak<sup>1</sup>, and C. D. Nelson<sup>1</sup>

Abstract: -- Top grafting is used to accelerate the breeding cycle of loblolly pine (Pinus taeda L). Scions, collected from seedlings as young as 1 year from seed, are grafted onto mature interstocks of the same or a related species. Male and female strobili production often begins 1 or 2 years after grafting, thus potentially decreasing the generation time by several years over conventional accelerated breeding methods. We are interested in applying top grafting to our breeding program involving interspecific hybrids, in particular longleaf x slash pine (Pinus palustris Mill. x Pinus elliottii Englem. var. elliottii) hybrids and their backcross generations. Towards this end, we grafted scions from 16 longleaf x slash pine  $F_1$  selections onto two longleaf and two slash pine interstock trees of different genotype. The  $F_1$  selections were 6 years old and none showed any signs of strobili development prior to, or during the experiment. A total of 100 grafts were made on to each interstock species. Scion survival after the second year was significantly higher on slash (72%) pine interstocks than on longleaf pine (18%). However there were no differences in male or female strobili production per living scion between the interstock species. Scions grew longer and produced more branches on slash pine interstocks than they did on longleaf. Given the relatively poor survival of the scions grafted onto longleaf interstocks and the reasonably good strobili production of scions grafted onto slash, we recommend using slash pine as the interstock species for top grafting longleaf x slash F<sub>1</sub> hybrids.

Keywords: Pinus palustris, Pinus elliottii, accelerated breeding, interspecific hybrids, grass stage

# **INTRODUCTION**

Longleaf pine possesses many desirable qualities such as excellent bole form, high naval stores content, moderate to high wood specific gravity, and fusiform rust resistance (USDA 1965). Despite these qualities, an extended phase of juvenile development referred to as the grass stage has limited longleaf pine's use in artificial regeneration programs (Schmidtling and White 1989). The grass stage greatly increases the opportunity for brown spot needle blight infection caused by the fungus *Scirrhia acicola* (Dearn.) Siggers (1944). This disease can greatly prolong the grass stage and, if severe enough, can kill seedlings. Increased seedling mortality, when compared to the other southern pines and the unpredictability of the duration of the grass stage, make planting longleaf pine a risky investment under intensive management systems. Inter-specific hybrids of longleaf pine have shown promise for addressing the problem of delayed

<sup>&</sup>lt;sup>1</sup> USDA Forest Service, Southern Institute of Forest Genetics, 23332 MS Highway 67, Saucier, MS 39574, USA; <sup>2</sup> School of Renewable Natural Resources, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA.

height growth, but pedigree advancement has been greatly hindered by the long juvenile period characteristic of pines.

Top grafting is a technique whereby juvenile scion material is grafted into reproductively mature trees and is being widely used to accelerate the breeding cycle of loblolly pines in seed orchards. This technique has been successfully used to promote early initiation of strobili in loblolly pine (Bramlett and Burris 1995, 1998; McKeand and Raley 2000). Gooding and others (1999) demonstrated that loblolly pine could be top grafted successfully onto loblolly or slash pine interstocks without a penalty in graft success or strobili production. It was also noted that scions grafted onto slash pine grew faster, making bagging (for control pollination) slightly easier and faster. By top grafting, advanced generation selections can be made and grafted in the spring. These grafts often produce strobili that can be pollinated the next spring, and produce cones with a good yield of sound seed in the fall of the following year, thus shortening the breeding cycle to as few as 5 or 6 years.

Our overall goal is to apply top grafting to help accelerate our breeding program involving interspecific hybrids of longleaf x slash pine and their backcross progenies. The specific objectives of this research were to: 1) determine which pine species might serve as a better source of interstock for top grafting longleaf x slash pine  $F_1$  scion material; and 2) test for genotype differences among the  $F_1$  trees used as a source of scion material.

# MATERIALS AND METHODS

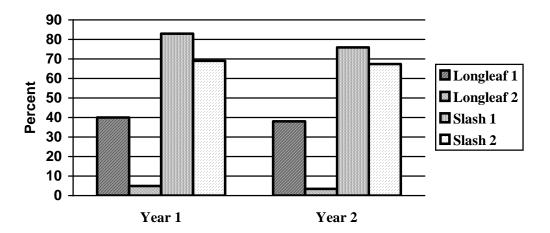
Scions were collected during the week of March 7, 2000, from 16 different longleaf pine x slash pine  $F_1$  hybrids growing in a 6 year-old test planting located on the Harrison Experimental Forest (HEF) in southeast Mississippi. The trees were selected from five different full-sib families. Prior to and during the course of the study there was no evidence of strobili development on any of the F<sub>1</sub> trees. After collection, the scions were divided into two groups and kept moist and cool (4° C) until grafting. In order to accommodate all the grafts, two slash pine trees and two longleaf pine trees (each of different genotype) located in a grafted seed orchard on the HEF were used. The interstock trees were identified as being reproductively mature based on prior observations of cone production, although the age and height of the longleaf interstocks were considerably older and taller, respectively (~30 years and 56.8 feet vs. ~15 years and 49 feet). Scion material was grafted into the upper crown of the interstock trees using a standard cleft and wax grafting method (White and others 1983). All grafting was performed by a single technician (LML) during the week of March 14, 2000. A total of 100 grafts were made onto each of the interstock species.

The following metrics were recorded at both 1 and 2 years post-grafting: scion survival, diameter of the scion approximately 5 cm above the union, length of the scion, number of first order branches, number of female strobili, number of male strobili, and the number of first year cones. Data were analyzed using the PROC MEANS and PROC GLM

procedures in SAS version 8 (SAS 1999). All significance tests were performed at p<0.05.

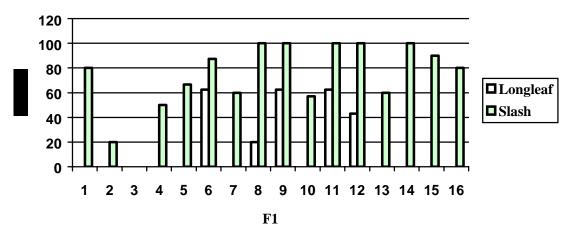
### **RESULTS AND DISCUSSION**

After one year, there were significant differences in survival based upon interstock species with 77% of the scions grafted onto slash pine interstock alive compared to only 20% of those grafted onto longleaf pine (Figure 1). A few additional scions were lost (died and missing tags) over the course of the second year, but these additional losses were not considerable (year two survival percentages were 72% and 18%, respectively). Although we tested only two interstock trees, the specific tree of the interstock species was found to have a significant effect on graft survival. This was especially apparent for longleaf pine. Approximately 40% of the scions grafted onto one of the longleaf pine interstock trees (17 of 42 grafts) were alive after 1 year whereas only 5% of those grafted onto the other (3 of 58 grafts) survived. Differences in scion survival between the slash pine interstocks were not apparent (83% versus 70%).



**Figure 1.** Percent survival of scions grafted onto four different interstocks after 1 and 2 years.

Scion material was collected from a total of 16 different interspecific  $F_1$  trees. The number of scions collected from each  $F_1$  tree ranged from as few as 6 to as many as 20, averaging 12.5. Differences in graft survival were observed among the  $F_1$  genotypes from which scions were collected. The percentage of live scions per  $F_1$  genotype after one year ranged from 0.0% to 87.5%, averaging 41.3% and is shown by interstock species in Figure 2. No significant interstock by  $F_1$  genotype interactions were observed, however it is important to note than only 5 of 16  $F_1$  genotypes survived on longleaf pine interstocks while 15 of 16 survived on slash pine.



**Figure 2.** Percent survival after 1 year for scions collected from 16 different longleaf pine x slash pine  $F_1$  genotypes.

Of those grafts that survived the first year, the length and diameter was significantly greater for those grafted onto slash pine versus those grafted onto longleaf pine. After the first growing season, the scions on slash pine were nearly twice as long as those on longleaf pine (average 58 cm versus 31 cm, respectively). This increased growth continued through the second growing season (average 83 cm versus 42 cm, respectively). The diameter of the scions after the first and second years was also significantly greater on slash pine than on longleaf pine (second year average 2.1 cm versus 1.5 cm, respectively). These results are similar to those reported by Gooding and others (1999) where it was noted that scions grafted onto slash pine grew faster than those grafted onto loblolly pine. The number of branches per living scion in year 2 was also significantly greater for grafts on slash pine (4.2 branches) than for those on longleaf pine (2.1 branches).

Interstock species were not significantly different in terms of the average number of pollen clusters produced per living graft after either the first or second year. In the first year, longleaf pine produced 0.25 pollen clusters per living graft and slash pine produced an average of 0.20. In the second year, the numbers of pollen clusters per living graft increased to 1.71 for longleaf pine and to 2.95 for slash pine. There was however, a significant difference between interstock species in terms of the average number of female strobili produced per living graft. In the first year, the surviving scions on longleaf pine produced on average significantly more female strobili (18 strobili per 20 grafts) than did those on slash pine (29 strobili per 75 grafts). However, this difference was not significant after the second year (20 strobili per 19 grafts for longleaf pine versus 79 strobili per 75 grafts for slash). Thus, slash pine interstock did better in terms of survival and growth of the scion, but by the second year, there was little difference between interstock species in terms of male and female strobili production per living graft.

Although slash pine was found to provide higher graft survival rates in this study, further investigation among and within interstock species is warranted. McKeand and Raley (2000) found significant differences among interstock genotypes of loblolly pine in the number of strobili produced and in this study, examining only two trees per species, we were unable to adequately test this hypothesis. Additionally, the branches on our longleaf pine interstock trees were generally larger in diameter when compared to the  $F_1$  scions. This made aligning the scion and branch difficult, and may have contributed to the lower survival rates on the longleaf interstocks. Finally, it is also likely that the developmental and physiological status of the interstock trees play a critical role in graft success. In the present study, the longleaf pine interstocks were about 15 years older and 7.8 feet taller than the slash and all grafting was completed at the same time of the year. Both age- and height-of-interstock and time-of-year effects need to be evaluated further to insure an efficient implementation of top grafting in longleaf x slash pine backcross breeding.

In conclusion, given the poor survival of the scions grafted onto longleaf pine interstocks and the relatively good survival and strobili production of scions grafted onto slash pine, we recommend slash pine as the interstock species for top grafting longleaf x slash  $F_1$ hybrids. Though, as backcross generations advance, and the scions become more like the recurrent species, we may need to reevaluate the choice of interstock species. By employing the top grafting technique a breeder should be able to substantially reduce generation time, possibly to as few as 5 or 6 years.

### ACKNOWLEDGEMENTS

We gratefully acknowledge Robert Stewart of the Mississippi Forestry Commission for guidance in top grafting techniques and Gay Flurry (SIFG) in helping with data collection.

### LITERATURED CITED

- Bramlett, D. L. and L C. Burris. 1995. Topworking young scions into reproductive mature loblolly pine. In: Proc. 23<sup>rd</sup> South. Forest Tree Improv. Conf., Asheville, NC, pp. 234-241.
- Bramlett, D. L. and L. C. Burris. 1998. Topworking genetic selections to reduce the breeding cycle in loblolly pine. In: Proc. Ninth Biennial South. Silviculture Conf., Clemson, SC, pp. 12-15.
- Gooding, G. D., F. E. Bridgwater, D.L. Bramlett and W.J. Lowe. 1999. Top grafting loblolly pine in the western gulf. In: Proc. 25<sup>th</sup> South. Forest Tree Improv. Conf., New Orleans, LA, pp. 60-66.
- McKeand, S. E. and E. M. Raley. 2000. Interstock effects on strobilus initiation in topgrafted loblolly pine. Forest Genetics 7:179-182.
- SAS Institute Inc., SAS/STAT Users Guide, Version 8, SAS Institute Inc., Cary, NC, 3884 p.
- Schmidtling, R. C. and T. L. White. 1989. Genetics and tree improvement of longleaf pine. USDA-Forest Service, Gen. Tech. Rep. SO-75, 13 p.

- Siggers, P. V. 1944. The brown spot needle blight of pine seedlings. USDA Tech. Bull. No. 870, 36 p.
- USDA Forest Service. 1965. Silvics of forest trees of the United States. USDA-Forest Service, Agriculture Handbook No. 271, 762 p.
- White, G., W. J. Lowe and J. Wright. 1983. Paraffin grafting techniques for loblolly pine. Southern Journal of Applied Forestry 7:116-118.