TIMING OF BAG APPLICATION AND REMOVAL IN CONTROLLED MASS POLLINATION

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INTRODUCTION

Controlled mass pollination (CMP) among outstanding parents is one way to increase genetic gains from traditional wind-pollinated seed orchards, but the economic success of CMP depends on both genetic gains and costs. CMP has been shown to be cost-effective (Bridgwater et al. 1998) even when costs were adjusted for risk (Byram and Bridgwater 1999, These Proceedings). Both of these studies assumed that CMP was 100% effective. That is, there was no pollen contamination during the CMP process that would reduce the expected gains from mating outstanding parents. This assumption is not always met under operational conditions due to variable strobilus development and the limited amount of time to conduct CMP. It is important for producers of CMP seeds to know how much contaminated seed might be produced under operational conditions.

Keywords: Pollen contamination., electrophoretic analysis, strobilus developmental stage.

METHODS AND MATERIALS

Twelve treatment combinations were applied to ramets of two female parents in a grafted loblolly pine seed orchard that was fully reproductive. The first group of treatments examined the effects of applying pollination bags at different female strobilus stages. In three treatments the bags were applied when female strobili were in stages 2, 3, and 4 (Bramlett and O'Gwynn 1980), respectively, no pollen was applied, and bags were removed ten days after maximum receptivity of the female strobili. Thus, any seeds produced from these treatments must have come from contaminating pollen. The second group of three treatments was the same; except that controlled mass pollination was done twice while female strobili were in stages 4.5 to 5.5. In this group of treatments, pollen from the desired parents competed with contaminating pollen that may have entered pollen chambers before bagging. A second group of treatments examined the effects of removing bags at different intervals following pollination. In the first three of these bags were applied at female strobilus stage 2, pollen was applied twice at stages 4.5 to 5.5, and bags were removed immediately, and two and eight days following the second pollination. A last group of two treatments was examined to test various operationally efficient combinations of the variables under study. In each of these the bags were applied at female strobilus stage 3 and bags were removed two days after the last pollination. In one of these, a single pollination was done at maximum female strobilus receptivity (stage 5.0) and in the other, two pollinations were done as before. Pollen from three different male parents was applied to each of the treatment combinations that received artificial pollinations.

Cone and filled seed counts were made for each treatment. Selected treatments for two crosses (81069 x 81056 and 81069 x 71022) that produced sufficient numbers of filled seeds were sent to the National Forest Genetics Electrophoresis Laboratory (NFGEL) in California for paternity analysis to determine the level of contamination for each treatment. Seeds were genotyped at 22 isozyme loci for 1,013 megagametophyte/embryo pairs. Both unambiguous and cryptic contamination levels were estimated. The frequencies of cryptic (ambiguous with regard to male parent) were extremely low (less than 1%) and are not reported herein.

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The level of contamination in one cross (81069 x 71022) was so high (all treatments had 30% to 50% contamination) that we concluded that the pollen source was not pure. Thus, the results from the electophoretic analysis are reported only for a single cross (81069 x 81056).

RESULTS

Examination of the first group of treatments, which received no artificial pollinations, showed that pollen contamination did occur if female strobili were bagged after stage 2 (Table 1). This group of treatments is useful only to illustrate that contamination can occur if bagging is delayed. Since artificial pollinations were not made, contaminating pollen did not have to compete for space in pollen chambers with applied pollen.

Table 1. Numbers of cones / filled seeds per cone for bagging at strobilus stages 2, 3, and 4 with nc pollen applied. Bags were removed 10 days after the first pollination.

Female	Male	Strobilus Developmental Stage When Bagged		
		2	3	4
81028	71037	0	0	2 / 30
	81069	0	0	5 / 21
	81077	0	1 / 0	8 / 18
81069	71022	0	0	0
	81056	0	1 / 26	0
	91039	0	0	0

Even though contaminating pollen may be present before bags are applied, adequate and timely artificial pollination may reduce seeds produced from contaminating pollen to acceptable levels. Paternity analysis revealed that there were no seeds produced from contaminating pollen with two artificial pollinations even when bags were applied at female strobilus stage 4 (Table 2). When bags were removed two days after the second pollination (Treatment 3*) rather than ten days as in the other treatments a small (2%) of contaminant seed was produced.

Table 2. Numbers of cones / filled seeds per cone for bagging at strobilus stages 2, 3 Two pollinations were made at stages 4.5 and 5.5 and bags were removed 10 days after the first pollination. Numbers in parentheses for cross 81069 x 81056 are percentages seeds produced from contaminating pollen.

Female	Male	Strobilus Developmental Stage When Bagged			
		2	3	4	3*
81028	71037	1 / 34	7 / 78	4 / 50	8 / 67
	81069	5 / 34	14 / 34	7 / 40	2 / 33
	81077	0	7 / 0.3	6 / 55	5 / 12
81069	71022	1 / 62	4 / 119	9 / 88	11 / 59
	81056	6 / 62 (0 %)	5 / 57 (0 %)	8 / 43 (0 %)	10 / 65 (2%)
	91039	4 / 45	5 / 41	3 / 66	5 / 106

* Same treatment as 3, but bag was removed 2 days after the second pollination.

The results from the remaining treatments show that it is unwise to remove bags immediately following artificial pollination at maximum receptivity (Treatment 0, Table 3) or to pollinate only once (Treatment 2*).

These two options would be operationally desirable, but resulted in 41% and 66% seeds from contaminating pollen, respectively.

Table 3. Numbers of cones / filled seeds per cone for bag removal 0, 2, or 8 days after the last pollination. Two pollinations were made at stages 4.5 and 5.5 and bags were removed 10 days after the first pollination. Numbers in parentheses for cross 81069 x 81056 are percentages seeds produced from contaminating pollen.

		Strobilus stage at bagging.				
		2			3	
Female	Male	Days after last pollination to bag removal				
		0	2	8	2	2 *
81028	71037	1 / 70	0	1 / 65	8 / 84	4 / 72
	81069	0	0	2 / 45	13 / 52	3 / 9
	81077	2 / 0	0	1 / 2	9 / 3	2 / 6
81069	71022	2 / 58	6 / 100	8 / 64	4 / 84	5 / 104
	81056	5 / 79 (41%)	1 / 24 (0 %)	1 / 36	7 / 122	6 / 185 (66%)
	91039	4 / 40	-	8 / 57	6 / 47	-

* Same treatment as 2, but only one pollination at strobilus stage 5.0.

CONCLUSIONS

Although pollen contamination can occur when female strobili are bagged after developmental stage 3, adequate and timely artificial pollinations can reduce the proportions of contaminant seeds to inconsequential levels. Removing pollination bags sooner than two days following pollination at maximum female strobilus receptivity or pollinating only once resulted in high levels of seeds produced from contaminant pollen.

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MOLECULAR MECHANISMS OF AUXIN ACTION AND RESPONSE IN LOBLOLLY PINE *(PINUS TAEDA* L.)

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In loblolly pine, very little is known about the genes involved in auxin response, their function or their regulation. We are interested in dissecting the molecular mechanisms underlying auxin response that lead into pathways responsible for different traits in pine, including adventitious root formation.

We previously cloned 5 genes from loblolly pine (LPEAs: Loblolly Pine Early Auxin-induced) that belong to a large family of plant genes known as the Aux/IAA genes. We have been pursuing two main lines of research concerning these genes. One is to determine gene function and the other is to assess factors and cis-elements regulating expression.

We have transformed full length cDNAs of three LPEA genes fused to the CMV 35S promoter into tobacco, and observed phenotypic variation in the progeny of some of the transgenic lines. Transformants containing LPEA1 and LPEA5, though variable, show similar phenotypes. The most pronounced phenotype is plants with severely impaired growth and developmental characteristics--very slow growth, low flower set and partial sterility. Most lines transformed with LPEA2, however, did not show any abnormal phenotype, except for one line that displays severe morphological abnormalities--altered leaf shape, slow growth, very dense inflorescences, extended pistil and partial sterility.

We are also trying to assess mechanisms and factors involved in regulation of LPEAs. We have found that maturation state significantly affects expression. In juvenile tissues, LPEAs were more strongly induced by auxin and the transcript levels remained high for a longer time period than in mature tissues. Recently, we isolated a genomic clone corresponding to LPEA1 cDNA . We fused different parts from the promoter region to a GUS reporter gene and transformed them into tobacco. Though results of these experiments are preliminary, we observed that most of the GUS activity is localized in the region of the vascular elements, suggesting tissue specificity of the promoter or higher auxin levels in these regions.

In addition to studying the LPEAs, we are using microarray approach to clone other genes that are induced by auxin. We are now in the process of confirming and further characterizing the induction of candidate genes by auxin.

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