AN OPERATIONAL TRIAL OF SUPPLEMENTAL MASS POLLINATION IN A LOBLOLLY PINE SEED ORCHARD

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Abstract.--Five ramets each of two second-generation loblolly pine seed orchard clones were supplementally mass pollinated (SMP'd) with pollen heterozygous for a rare, electrophoretically detectable marker allele. Pollen was applied once to all strobili clusters on a tree at or shortly before its period of maximum female receptivity. A pole duster was used to apply freshly processed pollen. Four ramets each of the two study clones were reserved as untreated controls to be pollinated by the orchard pollen cloud. SMP success was quantified for each ramet as the proportion of embryos fertilized by marker pollen in a 100-seed sample. Ramets of clone 11-1027 averaged 48% SMP success; those of clone 8-1048 averaged 69%. Levels of the marker allele in 100-seed samples of untreated control ramets were negligible. Differing SMP success rates among ramets of a clone are probably due to the timing of application as well as the quality of the application technique. Supplemental mass pollination appears promising as a pollen management tool in loblolly pine seed orchards.

Keywords: *Pinus taeda* L., supplemental mass pollination, seed orchard, electrophoresis.

INTRODUCTION

Supplemental mass pollination (SMP) is the broadcast application of desired pollen to receptive female strobili that are not isolated from the ambient pollen cloud (Bridgwater and Trew 1981). Pollen is applied with the expectation that a high proportion of ovules in treated strobili will be fertilized by applied pollen rather than by ambient pollen. Implementing SMP as a seed orchard management practice requires efficient pollen collection and processing methods, a reliable means of applying this pollen to large numbers of receptive female strobili, and, most important, a high degree of success at effecting the desired fertilizations. SMP success, the proportion of seed fathered by applied pollen, must be consistently high in operationally generated seedlots for this management technique to be economically justifiable.

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Supplemental mass pollination is not a new idea (Wakeley et al. 1966, Franklin 1971, Woessner and Franklin 1973), but its operational application has been hampered by the lack of a suitable means to quantify success. Phenotypic markers such as hybridity (Wakeley et al. 1966) and rust resistance (Bridgwater et al. 1987) have been used with limited success. Bridgwater and Williams (1983) applied dyed pollen, followed by ovule dissection to determine the proportion of ovules containing dyed pollen grains. But this technique only indicates success at introducing pollen into the ovule. It does not quantify actual fertilization success. Perhaps the best technique for quantifying SMP success is the use of electrophoretically detectable gene markers (Hadders 1984, Wheeler and Jech 1985). Pollen is collected from clones containing a rare marker allele and used to SMP selected female clones whose genotypes facilitate electrophoretic assay of embryos. This technique provides an unequivocal means of determining paternity of SMP-derived seedlots. From this, SMP success can be reliably estimated.

This study was undertaken to estimate the level of SMP success achievable in an operational program. Fresh pollen was collected from a clone containing a rare allele, and applied to ramets of two seed orchard clones. Seed derived from this treatment was collected and assayed electrophoretically to determine levels of SMP success.

METHODS

Study Design

Study trees were selected from a second-generation coastal loblolly pine seed orchard near Charleston, South Carolina established in 1973. Nine ramets each of clones 8-1048 and 11-1027 were randomly selected and numbered from 1 to 9. Ramets 1 to 5 were SMP'd using marker pollen. Ramets 6 to 9 were reserved as open-pollinated controls.

Clones were chosen on the basis of their allozyme genotypes at the malate dehydrogenase 1 (MDH1) locus, as well as their reproductive phenologies. Clones 8-1048 and 11-1027, the female parents, are homozygous for the most common MDH1 allele (relative mobility=100) in the seed orchard population. Their diploid genotype is MDH1-100/100. The male clone is the only one of 58 seed orchard clones heterozygous for a rare fast-migrating allele, with relative mobility of 112. Its diploid genotype is MDH1-100/112. Allele MDH1-112 can be readily detected in embryos using starch gel electrophoresis. The pollen donor sheds pollen before female strobili of 8-1048 and 11-1027 are fully receptive. Female clones were receptive at the peak of seed orchard pollen flight.

Pollen Application

Freshly processed pollen was applied in spring 1985 to all strobili clusters of a tree at or shortly before the majority of female strobili were maximally receptive. Each treatment tree was SMP'd once, except for ramet 3 of clone 11-1027 which was inadvertently treated twice. Application dates are indicated in Table 1 *(see* RESULTS AND DISCUSSION section). All ramets of

a clone were not necessarily treated on the same day because enough processed pollen was not immediately available. Pollen was applied from a self-propelled mobile aerial lift with a Westvaco-designed pole duster, using dry nitrogen as a propellant (10 psi). The pole duster was calibrated to deliver 0.5-1.0 cc of pollen to each strobili cluster.

Electrophoretic Assay of Seed

At least thirty cones were randomly picked from throughout the crown of every SMP'd and open-pollinated tree in fall 1986. Sound seeds were extracted and cleaned, and kept separate by ramet within clone. A random sample of 100 germinated seeds per ramet was selected for assay via starch gel electrophoresis.

Electrophoresis was done using techniques adapted from Conkle et al. (1982). Germinated embryos were dissected from each seed, macerated in a phosphate buffer, and the crude homogenate absorbed onto chromatography paper wicks. Gels were 11% starch. A morpholine citrate (pH 6.1) gel and electrode buffer system was used (Conkle's "D" system). Gels were run at 60 milliamps for 4.5 hours. Gels were stained for MDH, the enzyme system resolving the gene locus containing the pollen marker allele. The diploid genotype of each assayed embryo was determined at the MDH1 gene locus. SMP success was determined as twice the proportion of embryos with diploid genotype MDH1-100/112. Since the marker pollen was heterozygous for MDH1-100 and MDH1-112, one-half of its pollen grains will carry the common allele and one-half will carry the faster-migrating rare allele. Thus it is necessary to double the frequency of MDH1-112 in the assayed progeny to estimate the number of fertilizations attributable to the marker pollen.

The basic response variable of this study is the proportion of fertilizations attributable to applied marker pollen. This proportion will be referred to as "SMP success" (%). The values are reported for each treated and control tree. This SMP success rate is derived from the 100-seed sample assayed from each study tree. The 95% confidence interval, based on the binomial distribution (Table W, Rohlf and Sokal 1969) is reported for each SMP success percentage. SMP success reported for the open-pollinated controls would, of course, be expected to be zero. However, low levels of the marker allele can be expected in the controls due to open pollination by marker pollen seed orchard ramets, or by pollination from sources located outside of the orchard that contain allele MDH1-112.

RESULTS AND DISCUSSION

SMP Success Rate

This study was designed to quantify the levels of success that can be achieved in an operational SMP program. Significant differences in SMP success rates among treated ramets are suggested by non-overlapping confidence intervals. I interpret these differences largely as a response to better application technique as we gained experience applying pollen.

The percentages of open-pollinated embryos fertilized by supplementally applied pollen is summarized in Table 1 by ramet for the two mother clones, 11-1027 and 8-1048. The presence of the marker allele in untreated control ramets is also indicated. Figure 1 presents these data for the SMP'd trees only. SMP success on ramets of 11-1027, the first mother clone to be treated, ranged from 28% to 70% with a mean of 48%. Interestingly, ramet 3, which was treated twice, assayed at 70% SMP success. But ramet 5, which was only treated once, assayed at 66% SMP success. Two applications may not significantly increase SMP success. Bridgwater and Williams (1983) concluded this in their study on loblolly pine. Wheeler and Jech (1986) also observed this in a Douglas-fir seed orchard. SMP success on ramets of 8-1048, which was treated next, ranged from 58% to 78% with a mean of 69% (Table 1 and Figure 1). SMP success rates for 8-1048 ramets are generally higher and less variable than those of 11-1027 treated ramets.

Table 1. Percentage of open-pollinated embryos fertilized by supplementally applied marker pollen on SMP'd trees and percentage apparently fertilized by ambient marker pollen on control trees. Ramet number and application date(s) are noted. 100 randomly selected embryos observed from each ramet.

Clone					
11-1027			8-1048		
SMP success (%)	ramet number	appl. date(s)	SMP success (%)	ramet number	appl. date
SMP'd					
28	1	3/27	58	1	3/29
38	2	3/27	66	2	3/29
70	3	3/28, 3/30	72	3	3/30
40	4	3/28	72	4	3/30
66	5	3/28	78	5	3/30
$\bar{X}$ = 48.4			$\bar{X}$ = 69.2		
Control					
0	6	---	6	6	---
2	7	---	2	7	---
0	8	---	2	8	---
0	9	---	4	9	---
$\bar{X}$ = 0.5			$\bar{X}$ = 3.5		

Because enough processed pollen was not always available, all ramets of a clone were not treated on the same day. However, treatment was completed within two days. Because of the study design it is difficult to ascribe differences in SMP success between treated ramets to a particular cause. However, SMP success rates for ramets 1, 2, and 4 of clone 11-1027 appear to be significantly lower than that attained in the other treated ramets (Figure 1). Figure 2 plots SMP success rate for each treated ramet as a function of the order in which that ramet was treated. The value for ramet 3 of clone 11-1027 is plotted last because this ramet was treated twice. A smooth curve through these points suggests that we were on a learning curve. By the fourth treated ramet, SMP success rate had climbed above 50% and consistently

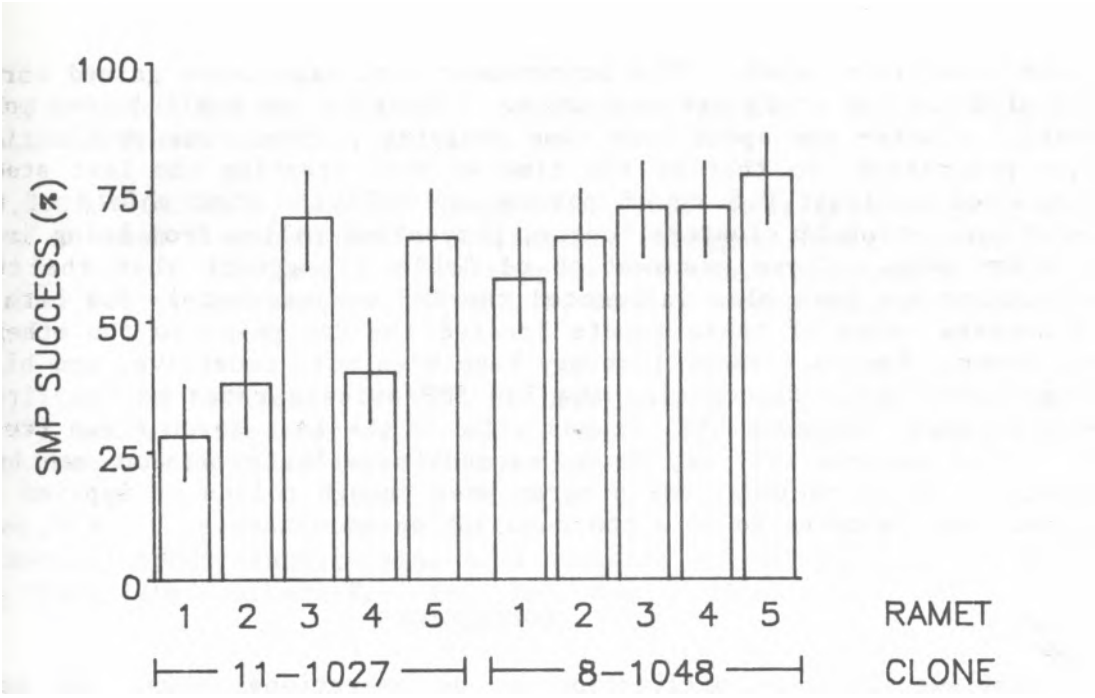

Figure 1. Levels of SMP success in treated ramets (1-5) of clones 11-1027 and 8-1048. Vertical lines represent 95% confidence interval. Ramets were SMP'd with pollen heterozygous for a rare, fast-migrating allele at MDH1 gene locus. Ramets treated once, at or shortly before maximum female receptivity. Ramet 3 of clone 11-1027 treated twice, two days apart.

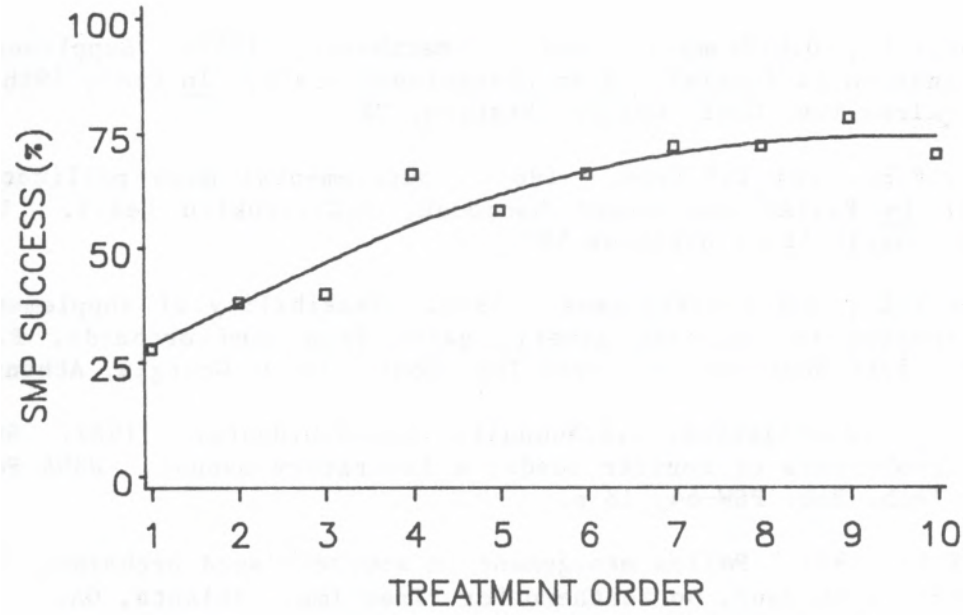

Figure 2. SMP success rates of treated ramets plotted against order of treatment. A smooth curve is drawn through data points to illustrate apparent learning curve. SMP success rate of first three ramets was below 50%. By the fourth ramet, application method had improved so that SMP success rate was consistently above 50%.

stayed above this level. This improvement with experience gained corresponds well with how the study was conducted. Initially, we applied less pollen per strobili cluster and spent less time applying pollen. Our application technique progressed, so that by the time we were treating the last study trees we applied at least 0.5 cc of pollen and held the wind shield of the pole duster over strobili clusters longer, preventing pollen from being immediately blown away. Close examination of Table 1 suggests that the timing of application may have also influenced the SMP success rate. For both clones, SMP success rates of those ramets treated the day prior to the other ramets were lower. Ramets treated last may have been more receptive, and higher SMP success resulted. Discounting the low SMP success rates of the first three trees treated, the mean SMP success rate of the last seven trees treated was 69%. This success rate may be a reasonable estimate of what can be accomplished in an operational SMP program when enough pollen is applied and sufficient time is taken to do a thorough job on each tree.

CONCLUSIONS

Supplemental mass pollination on an operational scale can be accomplished and, when using fresh pollen, success rates as high as 70% should be consistently obtainable. Results from this study and others suggest that one pollen application at or shortly before maximum female receptivity should be sufficient to achieve high fertilization success by the applied pollen. The primary determinants of success are the timing of pollen application and thorough treatment of female strobili clusters.

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