FEASIBILITY OF SUPPLEMENTAL MASS POLLINATION TO INCREASE GENETIC GAINS FROM SEED ORCHARDS

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Abstract.--Dyed pollen was applied to unprotected clusters of female strobili of three clones in a mature, loblolly pine, (Pinus taeda L.), seed orchard. Single and multiple pollinations were made as strobilus receptivity progressed. Dissection of ovules on strobili collected after the pollination season was past revealed that supplemental mass pollination to increase genetic gains is feasible with proper timing and application. The best average percentage of dyed pollen in ovules of the three clones was 92% for multiple pollinations. An average of 86% dyed pollen was introduced into pollen chambers of the three clones with a single application when strobili were at maximum receptivity.

Additional keywords: Supplemental mass pollination, Pinus taeda, genetic gain.

INTRODUCTION

Supplemental mass pollination is the broadcast application of pollen to strobili that are not isolated from wind-borne pollen. Potential benefits from supplemental mass pollination in conventional intraspecific tree improvement programs are increases in the yield of sound seeds and in realized genetic gains. Filled seed yields can be improved in loblolly pine seed orchards under certain conditions (Bridgwater and Bramlett 1982). However, the feasibility of supplemental mass pollination to increase genetic gains has not been demonstrated.

Genetic gains may be increased in established seed orchards through supplemental mass pollination only if supplemental pollen can be introduced into ovules in sufficient numbers to compete with wind-borne pollen.

METHODS

In this study we quantified the numbers and proportions of wind-borne and supplemental pollen grains in pollen chambers of three loblolly pine clones in a mature seed orchard. [2/] The study was conducted during the springs of 1980 and 1981 when the seed orchard was 20 and 21 years from grafting, respectively. Treatments were applied to varying numbers of strobili in clusters on one ramet of each of three clones. Different ramets were used in the two study years.

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[2/] Weyerhaeuser Company's J. P. Weyerhaeuser First Generation Piedmont Seed Orchard near Washington, North Carolina.

Dyed pollen grains were used in supplemental pollinations. These were easily distinguished from wind-borne pollen (which had not been dyed) when ovules were dissected. The procedures used for dissections were essentially those of Matthews and Blalock (1981). Dyed pollen was applied as strobilus receptivity progressed from stage 3 through stage 5 late (Bramlett and O'Gwynn 1981). Four supplemental pollination schedules (treatments) were used in the 1980 study. The first schedule consisted of four applications of dyed pollen at receptivity stages 3, 4, 5 and 5 late. Each of the three remaining treatments began successively one stage later, that is, 4 + 5 + 5 late, 5 + 5 late, and 5 late. Treatments in the 1981 study were done in the same fashion but included one more stage of strobilus development so that treatment schedules were 3 + 4 + 4 late + 5 + 5 late, 4 + 4 late + 5 + 5 late, 4 late + 5 + 5 late, 5 + 5 late and 5 late. Also, in the 1981 study a series of single supplemental pollinations were made at each of the 5 successive stages of strobilus receptivity. One to several clusters of strobili were selected for wind-pollinated checks in each study to quantify the levels of wind-borne pollen.

Pollen applications were made from a plastic squeeze bottle with a small tube extending from the top whereby the pollen spray was directed onto individual strobili. Pollen amounts were not quantified. Dyed pollen was simply applied until it could be seen on all surfaces of the strobilus. To accomplish the delivery of the same quantities of pollen in an operational application of supplemental pollen would probably be uneconomic if not impossible. However, the objective of this trial was to test the feasiblity of supplemental mass pollinations, not its practicality.

Fifteen ovules from each of three cones representing each clone x treatment cell were dissected in the 1980 study. Nine ovules from each of five cones were sampled from each clone x treatment cell in the 1981 study. In both studies, one of the two ovules per scale was dissected. One-third of the scales came from each of the distal, medial, and proximal parts of the strobilus. The numbers of dyed and wind-borne pollen grains were counted for each dissected ovule.

Standard analysis of variance procedures were used to partition the variance among clones, treatments, cones in clones x treatment cells, ovules in cones and the interaction between clones and treatments and to test hypotheses. Duncan's multiple range tests were used to group values not significantly different at the 95% confidence level.

RESULTS AND DISCUSSION

The trait of primary interest, percentage of supplemental (dyed) pollen grains per ovule, differed significantly among clones and treatments in both studies. The percentage of dyed pollen grains theoretically estimated the proportion of ovules that will produce a seed resulting from fertilization by a supplemental pollen grain. We assumed that the wind-borne and supplemental pollen are both 100% viable and there are no differential genetic effects.

Two applications of supplemental pollen (5 + 5 late) beginning at the stage of maximum receptivity (stage 5) was the best, multiple-pollination treatment in both the 1980 and 1981 studies (Tables 1 and 2). Average percentages of dyed pollen for all three clones for treatment 5 + 5 late were 72% and 92% in 1980 and 1981, respectively. This result was unexpected. It seemed reasonable at the outset of the study that treatment schedules which began earlier and thus had more applications of supplemental pollen would have the highest percentages of dyed pollen. Had this been the case, the effects of a single application of pollen at any receptivity stage could have been determined by substraction. It became apparent that this simple system was not in operation upon examination of the results of the 1980 study (Table 1). In fact, the observed trend was the opposite of that which was expected. Thus, we added the series of single applications in 1981 as well as repeated the series of multiple treatments. Again, in the 1981 study, the expected trend for multiple pollinations was not realized. The reasons for this unexpected trend are not known. However, this puzzling result is primarily of academic interest since the simplest, least intensive treatment (5 + 5 late) was best in both years. A single application of supplemental pollen at maximum receptivity was nearly as effective as the best multiple-pollination treatment. We realized 86% dyed pollen in pollen chambers with a single application at strobilus receptivity stage 5. The trend in the series of single-application treatments was as expected. The proportion of dyed pollen grains per ovule increased as the treatments were applied closer and closer to full receptivity. The proportion dropped off markedly just after maximum receptivity (Table 3.)

Table 1.--Results from the 1980 Supplemental Mass Pollination Trial.

Treatment Differences

Supplemental Pollen Applied at Flower Stage(s)	Mean % Supplemental Pollen In Pollen Chambers	Mean Total Numbers (Wind + Supplemental) Pollen In Pollen Chambers
5 + 5 Late	72% a[1/]	6.5 a
4 + 5 + 5 Late	67% ab	6.2 a
3 + 4 + 5 + 5 Late	59% b	5.5 ab
5 Late	30% c	5.2 b
Wind Pollinated Only		4.7 b

Clonal Differences For the Best (5 + 5 Late) Treatment Above

Clone	Mean % Supplemental Pollen In Pollen Chambers
8-118	84% a[1/]
8-107	74% a
8-126	58% b

1/ Means followed by the same letter are not significantly different at the 95% confidence level.

Table 2.--Results from the multiple-pollination treatments in the 1981 Supplemental Mass Pollination Trial.

Differences Among Multiple-Pollination Treatments

Supplemental Pollen Applied at Flower Stage(s)	Mean % Supplemental Pollen In Pollen Chambers	Mean Total Numbers (Wind + Supplemental) Pollen In Pollen Chambers
5 + 5 Late	92% a[1/]	5.4 a
3 + 4 + 4 Late + 5 + 5 Late	84% a	4.9 abc
4 Late + 5 + 5 Late	72% ab	5.4 a
4 + 4 Late + 5 + 5 Late	71% ab	5.2 ab
5 Late	55% b	4.1 bc
Wind Pollinated Only		3.8 c

Clonal Differences For The Best (5 + 5 Late) Treatment Above

Clone	Mean % Supplemental Pollen In Pollen Chambers
8-118	98% a
8-126	93% b
8-107	80% b

1/ Means followed by the same letter are not significantly different at the 95% confidence level.

Table 3.--Results from the single-pollination treatments in the 1981 Supplemental Mass Pollination Trial.

Differences Among Single-Pollination Treatments

Supplemental Pollen Applied at Flower Stage(s)	Mean % Supplemental Pollen In Pollen Chambers	Mean Total Numbers (Wind + Supplemental) Pollen In Pollen Chambers
5	86% a[1/]	5.5 a
4 Late	68% a	5.2 ab
5 Late	55% ab	4.1 bc
4	39% ab	4.7 abc
3	2% b	4.0 c
Wind Pollinated Only		3.8 c

Clonal Differences For The Best (5 + 5 Late) Treatment Above

Clone	Mean % Supplemental
8-118	100% a[1/]
8-126	84% b
8-107	73% b

1/ Means followed by the same letter are not significantly different at the 95% confidence level.

The timing of applications of supplemental pollen was apparently critical. Beginning applications before maximum receptivity (stage 5) reduced the expected proportions of seeds from supplemental pollen in both cumulative and the single application series (Tables 1, 2 and 3). The reduction was even greater when application was made after maximum receptivity had passed (5 late).

The timing of supplemental pollinations is also critical because pollen applied to strobili first has the best chance of producing seeds (Franklin 1974). Thus, if wind-borne pollen arrived before supplemental pollen, the predicted proportions of seeds from supplemental pollination may be overestimated in this experiment. It was not possible in the dissection process to determine whether dyed or wind-borne pollen had a positional advantage on the nucellus. The proportions of seed from supplemental pollen may be overestimated in this experiment for another reason. A comparison of total number of dyed + wind-borne pollen in pollen chambers to the number in wind-pollinated check ovules (Tables 1, 2 and 3) shows that there was always more pollen in pollen chambers that contained dyed pollen. Furthermore, the total number of pollen grains increased as the proportion of dyed pollen increased. This suggested that dyed pollen grains may have been smaller than wind-borne pollen and thus it was possible to put more of them into pollen chambers. Therefore, their proportionate numbers would be greater than if they had not been dyed. The magnitude of this overestimate (if it exists) was probably small. We did not measure pollen grains in this experiment. However, other workers report that the numbers of pollen grains in a volume of pollen dyed in alcohol-soluble dyes is 10% greater than in equal volumes of pollen that had not been dyed (Personal Communications, Dr. M. S. Greenwood, Weyerhaeuser Co., Hot Springs, AR).

The best treatments in the three series should still result in substantially large numbers of seeds from supplemental pollen, even though the porportion of seeds expected from supplemental pollen may have been overestimated. In the best 1980 treatment (5 + 5 late), 24% of the dissected ovules contained only supplemental pollen. Seventy-nine percent of the ovules contained greater than 50% supplemental pollen thus the supplemental pollen had a numerical advantage. The best multiple-application treatment in 1981 (5 + 5 late) had 63% of ovules which contained only supplemental pollen and 87% with more than 50% supplemental pollen. The best single-application treatment (stage 5) had 73% ovules with only dyed pollen and 96% that contained more than 50% dyed pollen.

There were statistically significant differences among the three clones for the best treatment in each of the three treatment series (Tables 1, 2 and 3). There were no meaningful interactions between clones and treatments. In other words, multiple pollinations at stages 5 + 5 late or a single pollination at stage 5 was best (or not significantly poorer than the best treatment) for all three clones in all three treatment series. Clones 8-107 and 8-126 changed ranks in the 5 + 5 late treatment in the two study years. This was probably due to the variance introduced by relying upon a subjective determination of the appropriate strobilus receptivity stage for application.

CONCLUSIONS AND RECOMMENDATIONS

Genetic gains can he increased through supplemental mass pollinations. Success depends upon pollen application when female strobili are at maximum receptivity.

Supplemental pollination was most successful when two successive applications were made at strobilus receptivity stages 5 and 5 late. The best single application was made at stage 5, or maximum receptivity. Earlier or later applications reduced the percentage of supplemental pollen in pollen chambers.

This study clearly demonstrated the importance of precise timing of supplemental pollinations. However, we did not control pollen amounts. Rather, we applied large amounts of supplemental pollen to individual strobili to determine the feasibility of supplemental mass pollinations. If such large amounts of pollen were used in a large-scale operation, the system might not be economical. Further research should be done to quantify success in operational systems.

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