SUPPLEMENTAL MASS POLLINATION IS FEASIBLE ON AN OPERATIONAL SCALE

F. E. Bridgwater, D. L. Bramlett, and F. R. Matthews 1/

Abstract.--A commercially available pole duster with a compressed-air tank for pollen delivery was used to pilot-test supplemental pollination to increase genetic gains in loblolly pine on an operational scale. Two operators working together required an average of 19 minutes to treat each ramet, which were approximately 50 feet tall with an average of 174 clusters of female strobili. Proper timing of applications was critical; those made after peak pollen shed in the seed orchard were not effective.

Additional keywords: *Pinus taeda,* fusiform rust.

Supplemental mass pollination (SMP) has been shown to be effective for increasing genetic gains from loblolly pine (*Pinus taeda* L.) seed orchards (Bridgwater and Williams 1983). They showed that a single, properly timed application of supplemental pollen to strobili not isolated from windborne pollen resulted in an average of 86% selected pollen grains in ovules. However, their study employed destructive sampling to determine the proportion of dyed pollen in ovules and did not directly measure the proportion of seeds arising from supplemental pollen. Furthermore, their experiment was done on a scale much smaller than would be required for an operational SMP program where timing and methods for application would be more difficult. The trial reported here was conducted to test the feasibility of SMP to increase genetic gains using methods and equipment that would be required in an operational program.

A second objective was to determine the time required to do SMP to aid in planning operational SMP programs.

METHODS

One way to increase genetic gains from SMP is to capitalize on good specific combinations. If specific crosses among orchard parents are known to be better in some respect than wind-pollinated seeds from the same parents, then repeating those favorable crosses with SMP will increase the genetic potential of their offspring to the extent that it is successful.

1/ Research Geneticist, Project Leader, and Plant Pathologist, respectively, USDA Forest Service, Southeastern Forest Experiment Station. The authors wish to thank Billy Arnold, the Georgia-Kraft Co., Weyerhaeuser Co., and the North Carolina State University-Industry Tree Improvement Cooperative for their assistance in conducting this study.

Five good specific crosses among seven seed orchard parents were identified in the Briarpatch seed orchard of the Georgia-Kraft Company in Putnam County, Georgia. These crosses were chosen primarily for resistance to fusiform rust (*Cronartium quercuum* (Berk.] Miyabe ex Shirae f. sp. *fusiforme*), and secondarily for good growth rates. Performance was determined by examining performance levels, which are relative scores for all the clones and specific crosses among them (Hatcher et al. 1981). None of the five crosses had been tested in fewer than four progeny tests and all had been evaluated for 8 years in at least one test.

The success of SMP was estimated by comparing the percentage of rust-free seedlings arising from controlled pollinations, SMP, and wind pollinations on ramets of the same genotypes. The difference between the percentages of rust-free seedlings from the controlled-pollinated (CP) and wind-pollinated (WP) seedlings represented the potential for improvement, and the difference between percentages of rust-free seedlings from SMP ramets and from SMP ramets and WP ramets measured the success of SMP. Thus, if CP seedlings were 31% rust free, SMP seedlings were 24% rust free, and WP seedlings 21% rust free, the SMP success would be 30%. The percentage of rust-free seedlings was increased 3% with SMP while the potential improvement was 10% with CP.

At least 1.5 liters of pollen were collected and processed for storage from each of three pollen parents during 1983. Pollen was dried for 24 hours at 39 °C to 5% to 6% moisture content, and frozen in quart jars at -20 °C until application during spring, 1984. In *vitro* pollen viability was determined 1 month before pollen was applied and was found to be 72%, 80%, and 86% for the three pollen parents.

Control pollinations were made by using standard procedures for the southern pines on one ramet of each clone. A second ramet received SMP when a majority of female strobili reached stages 4L-5 (Bramlett and O'Gwynn 1981). Supplemental pollen was applied with a commercially available pole duster illustrated and described in Figure 1. The pole duster and compressed-air tank complete with fittings cost less than $150 when purchased in 1983. A third ramet of each clone far enough removed from the SMP ramet to avoid the possibility of accidental pollination was tagged for the collection of wind-pollinated seeds.

A crew of two applied the supplemental pollen from a bucket truck. One crew member operated the bucket controls and kept track of the progress of pollinations to ensure that most, if not all, strobili received supplemental pollen. The second crew member applied 1 to 1.5 ml of pollen to each cluster of female strobili by simply lowering the windscreen over each cluster and applying two short bursts of compressed air. The pole duster had previously been calibrated by trial and error to apply 0.5 to 0.75 ml pollen per burst of compressed air. This amount of pollen was judged to be sufficient for pollination by applying different amounts of dyed pollen with the pole duster and subsequently dissecting the female strobili to determine pollination effectiveness.

Cones were harvested in the fall of 1985, and seeds were extracted and processed for sowing in the spring of 1986. Both a field study and an

Figure 1. Illustration of the pole duster used in this trial. The pole duster is a 6-foot section of 1/2-inch diameter aluminum tubing with a pollen bottle installed in-line. A 6-inch diameter aluminum funnel served as a windscreen which is placed over a cluster of strobili. A compressed-air supply was fitted to the base of the pollinator where a thumb-operated level controlled the air supply and, thus, pollen emission. An emitter was devised to place over the 1/8-inch copper tubing in the windscreen so that pollen was forced against the sides of the windscreen and onto the strobili from all sides.

artificial inoculation test in a greenhouse were initiated in the spring of 1986. Unfortunately, the field test failed due to a drought, thus only the results of the greenhouse study are reported here.

The inoculum source was aeciospores of the fusiform rust fungus collected in 1974 from loblolly pine galls in Clark County, Georgia. This bulk spore collection was processed and stored according to the procedures outlined by Roncadori and Matthews (1966). Basidiospores used in the pine inoculations were produced by infecting seedlings of northern red oak (*Quercus* *rubra* L.) with the aeciospores. The basidiospores were harvested from the oak leaves and used in a concentrated basidiospore spray (Matthews and Rowan 1972). Pine seeds were germinated, and immediately after emergence were transplanted into flats in lots of 20 each. At 4 weeks of age, the seedlings were passed on a conveyor belt under an aqueous spray of basidiospores. The inoculum contained 50 M spores/ml and was dispersed in 8 ml aliquots on each flat of seedlings as it passed under the spray nozzle. In *vitro* germination of basidiospores immediately prior to inoculation was 85%.

The inoculum was applied to 6 flats of 20 seedlings for most seed lots, but some lots had fewer germinants and, thus, a reduced number of seedlings were available for inoculation. Immediately after inoculation, the seedlings were placed in a mist chamber and held at 20 C for 24 hours. They were then grown in the greenhouse for 6 months before being examined for infection. Seedlings with active rust galls were used to determine the percentage of infected seedlings. The numbers of seedlings used to determine the percentages of rust-free seedlings ranged from 26 to 120, and averaged 107. Seeds from a susceptible check seed lot, #4666-4 from Houston County, Georgia, were used to evaluate inoculation results.

Percentages of rust-free seedlings were analyzed by standard analysis of variance procedures. Both percentages and their transformed values (square root transformation) yielded the same results, therefore percentages are reported here.

RESULTS AND DISCUSSION

SMP Success

SMP was successful when done before peak pollen flight occurred in the seed orchard (Figure 2). The percentage success was 144% and 30% for crosses 556x521 and 110x556, respectively. The percentage of rust-free seedlings in the SMP treatment exceeded the percentage in the CP treatment for the cross 556x521. This probably occurred as the result of sampling errors, which were large due to the small numbers of seedlings in the study. In any event, the differences between CP and SMP treatments were not significantly different at the alpha = 0.10 level. Since there were no significant interactions between treatments and the two crosses made before pollen was shed in the seed orchard, another analysis of variance was made for their pooled data. The average percentage of rust-free seedlings was 26% for SMP and 28% for the CP crosses on the two females receiving SMP before pollen was shed in the seed orchard. That is, the estimate of the average percentage success was 80%. However, the coefficient of variation for the percentage of rust-free seedlings was 44%. Thus, SMP appears to be feasible when operational Trocedures are simulated, but the level of success is still in question.

SMP was not successful on the three females that received treatment after peak pollen shed in the seed orchard (Figure 3). Strobili on these female parents were observed to be in stages 4-4L, the stages just preceding stage 5, or full receptivity, on the morning of the day of peak pollen shed in the seed orchard. If this had truly been an operational program, SMP should have been done at that time in anticipation of pollen shed. Delaying SMP in this trial demonstrated the ineffectiveness of applications after peak pollen shed in the orchard.

There were significant interactions among these three females and treatments, therefore the response of each female was considered individually. Crosses 182x521 and 114x521, respectively, received SMP 24 and 48 hours after the peak of seed orchard pollen shed began. There were no significant differences among the three treatments for 182x521, but for 114x521, the percentage of rust-free seedlings for the CP treatments was significantly less (10%) than either SMP or WP treatments (22% and 24%, respectively). The expected mid-parent value based on wind-pollinated values for this cross was 21%, which suggests that there was negative specific combining ability for resistance to fusiform rust in this experiment. In that case, the proportionate reduction in the percentage of rust-free seedlings with

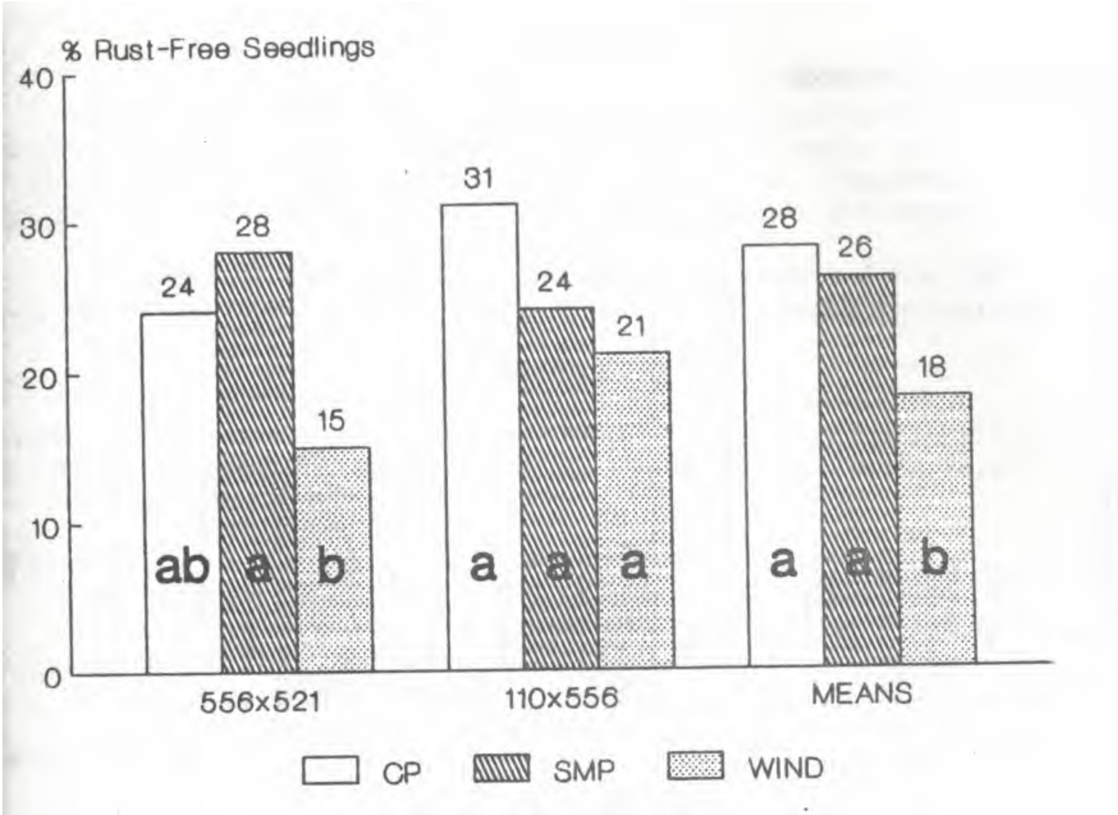

Figure 2. Percentages of rust-free seedlings from controlled-pollinated (CP), supplemental mass pollinated (SMP), and wind-pollinated females when SMP was done before the peak of seed orchard pollen shed occurred. Treatment bars annotated with the same lower case letter were not significantly different at P = 90%.

controlled pollination compared with wind pollination would indicate the level of success from SMP. However, the small difference measured was not significant. The cross 1027x1542 had the greatest difference between the CP and WP treatments and would have offered the best opportunity to quantify the success of SMP. However, there was no significant difference between the percentages of rust-free seedlings with SMP applied after peak seed orchard pollen shed and wind pollination.

Time Required for SMP

The time required to do SMP treatments was surprisingly short and predictable. The numbers of clusters of strobili per ramet and the times required to set up the bucket truck and accomplish the SMP application are given in Table 1.

The linear regression of minutes required for SMP on numbers of clusters of female strobili was Minutes = 10.7 + 0.048 (clusters of strobili) with a standard error of 1.2 minutes. This prediction equation will facilitate planning operational SMP programs.

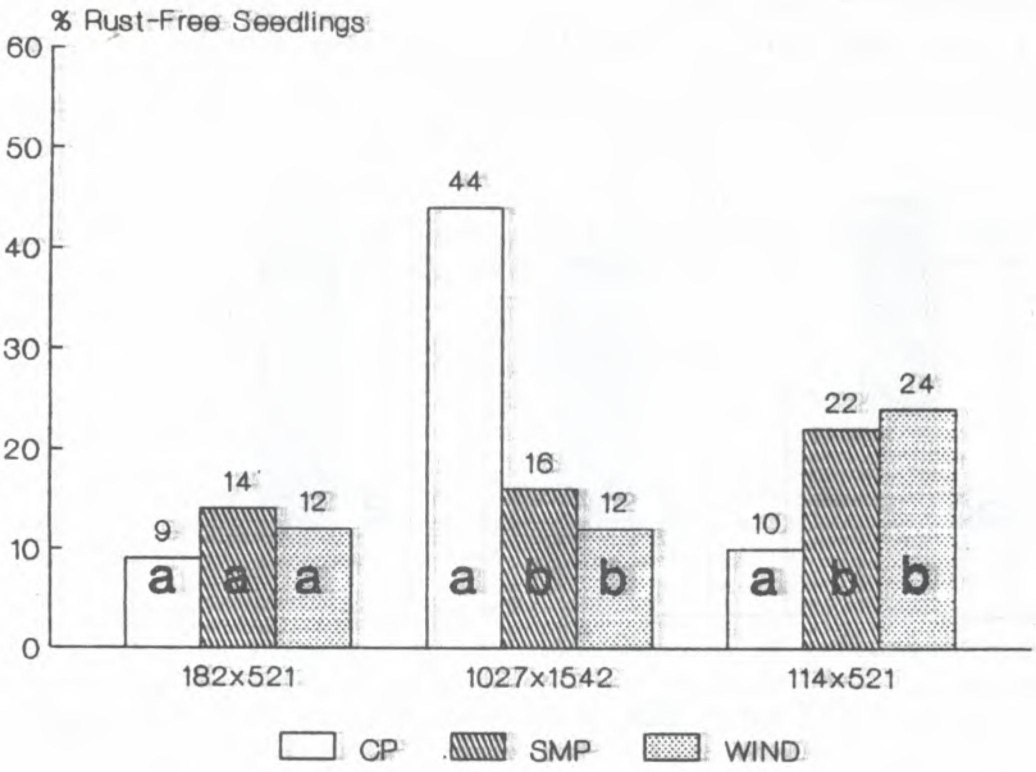

Figure 3. Percentages of rust-free seedlings from controlled-pollinated (CP), supplemental mass pollinated (SMP), and wind-pollinated females when SMP was done after the peak of seed orchard pollen shed occurred. Treatment bars with the same lower case letter were not significantly different at P = 90%.

Table 1.--Number of clusters of strobili and time required per ramet for SMP

Ramet number	Clusters of strobili (No.)	Time for SMP (min.)
1	153	18
2	189	22
3	162	18
4	214	20
5	238	22
6	93	15
Mean	174	19

CONCLUSIONS

Supplemental mass pollination is feasible on an operational scale. Supplemental pollen must be applied to receptive female strobili before peak pollen shed in the seed orchard to ensure success. Supplemental pollen applied after peak seed orchard pollen shed was not effective.

The number of man-hours and the equipment required for SMP put such programs within the reach of every operator who wishes to implement them.

LITERATURE CITED

Bramlett, D. L. and C. H. O'Gwynn. 1981. Controlled pollination. In: Pollen Management Handbook, E. C. Franklin, ed., USDA Forest Service, Agri. Handb. 587. p. 44-51.

Bridgwater, F. E. and C. G. Williams. 1983. Feasibility of supplemental mass pollination to increase genetic gains from seed orchards. In: Proc., 17th Southern Forest Tree Improvement Conf., Athens, Georgia, June 7-9, 1983. National Technical Information Service, Springfield, VA. p. 78-83.

Hatcher, A. V., F. E. Bridgwater and R. J. Weir. 1981. Performance level-a standarized score for progengy test performance. Silvae Genet. 30(6):184-197.

Matthews, F. R. and S. J. Rowan. 1972. An improved method for large-scale inoculations of pine and oak with _Cronartium fusiforme._ Plant Dis. Rep. 56:931-934.

Roncadori, R. W. and F. R. Matthews. 1966. Storage and germination of aeciospores of _Cronartium fusiforme._ Phytopathology 56:1328-1329.