

Hybridizing Pines with Diluted Pollen ^{1/}

R.Z. Callaham ^{2/}

When pollen is limited or expensive to collect, tree breeders should use diluted pollen so long as seed production is not affected. Duffield and I looked into the feasibility of using pollen dilution at the Institute of Forest Genetics at Placerville a number of years ago (Callaham and Duffield, 1961). Our first results were promising. Since then diluted pollens are used occasionally in research breeding at Placerville. The California Region of the U. S. Forest Service uses them in mass-production of pine hybrids. Diluted pollens might be used advantageously in breeding southern pines in seed orchards. This report gives results of the most recent experience at Placerville with diluted pollens.

Our first studies at Placerville showed that viable pollen can be diluted with dead pine pollen without ill effects. Dilutions to 50 percent live pollen did not affect cone set, total seeds per cone, or the proportion of sound seeds. Unresolved were questions of how far can we dilute and what is the effect of using pollen of different species as the diluent. A comprehensive series of interspecific hybridizations was made in 1962 to learn more about possibilities of diluting pine pollen.

PROCEDURE

We were primarily interested in three hybrids having commercial possibilities. These were Pinus attenuata x P. radiata, P. monticola x P. strobus, and the backcross hybrid P. jeffreyi x (P. jeffreyi x P. coulteri). Trees for breeding were selected in natural stands on the Eldorado National Forest near Placerville and in the Institute's arboretum. Mixtures of pollen from 2 or 3 trees of each pollen parent species were prepared and diluted with heat-killed pollen. Pollen lots were used a few days later to pollinate receptive conelets on bagged branches. Each lot was used to pollinate three bags on each of three trees of the seed parent species.

Ten pollen lots were used on each tree. Seven lots contained increasing amounts, 10, 20, 30, 40, 50, 70, and 100 percent, of live pollen of the desired pollen parent. The diluent in this case was dead pollen of the seed parent. A control pollen lot was live pollen of the seed parent species. To determine the influence of the species used as the diluent, pollen of two species known to be genetically incompatible with the seed parent were used separately as 50 percent diluents.

For each cross the cones were harvested in 1963, and the seeds were extracted, winnowed, and counted. Percent of all seeds that were sound was calculated.

RESULTS AND DISCUSSION

Pollen dilution did not affect cone set. About the same proportion of pollinated conelets developed into cones regardless of pollen dilution. This confirms our earlier finding.

However, total seeds per cone was significantly reduced at the greatest dilutions. This contradicts our earlier conclusion which was based on fragmentary data. Analysis of variance showed a significant effect of dilution on number of seeds per cone when data were combined for all nine seed trees. Dilutions having only 10 and 20 percent viable pollen combined significantly fewer, 60 and 59, seeds per cone, but 30, 40, 50, 70 and 100 percent viable pollen resulted in 72, 81, 88, 82, and 81 seeds per cone. These results suggest that dilution

^{1/} Research reported here was done while the author was Leader of the Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, Placerville, California.

^{2/} Chief, Branch of Forest Genetics Research Forest Service, U. S. Department of Agriculture Washington, D. C.

to less than 30 percent live pollen may reduce total seed yield.

Pollen dilution had a significant effect on the proportion of seed that were sound. The sound seed percent varied with the hybrid being produced as follows:

<u>Hybrid</u>	<u>Viabie pollen - percent</u>			
	<u>100</u>	<u>50</u>	<u>20</u>	<u>10</u>
<u>attenuata x radiata</u>	72	68	62	64
<u>monticola x strobis</u>	33	43	25	15
<u>jeffreyi x (j. x el.)</u>	54	52	47	36

The two species involved in the hybrid P. attenuata x radiata are highly compatible, and even 10 percent viable pollen gave high proportions of sound seeds. Pinus monticola and strobis are less compatible, and dilutions containing only 10 percent viable pollen gave few sound seeds. Results for the three P. monticola seed trees were variable, so the reduction was not quite significant at the 5 percent confidence level. A highly significant reduction in proportion of sound seed occurred when dilutions to 10 percent were used to produce P. jeffreyi x (jeffreyi x coulteri). The high degree of genetic incompatibility between the species involved in this hybrid may have influenced the result.

The species of pollen used as diluent at the 50 percent level did not significantly alter total seed yield or proportion of seed that were sound. The average seed yields were as follows:

<u>Seed parent</u>	<u>Diluent species</u>		
	<u>at.</u>	<u>jef.</u>	<u>mon.</u>
<u>attenuata</u>	59	75	68
<u>jeffreyi</u>	148	160	188
<u>monticola</u>	27	43	45

These results suggest that dead pollen of any pine species can be used as a diluent with equal results.

These results show that it is safe to use pollen dilutions having only 30 percent of live pollen. Strictly speaking, the results pertain to interspecific hybridizations, but I believe they can be extrapolated to hybridization within species where highly compatible parents are being crossed. In your southern pine seed orchards excellent sets of sound seeds probably can be obtained with only 10 percent viable pollen in the pollination syringe. You might be able to reduce your "stud fee" by 70 percent or more by diluting valuable pollens.

SUMMARY

Interspecific pine hybridizations at Placerville show the extent to which viable pine pollen can be diluted with dead pollen. Valuable lots of pine pollen can be diluted generally to 30 percent live pollen and in certain cases to 10 percent with no effect on the proportion of seeds that are sound. Dilutions having only 10 or 20 percent viable pollen did produce significantly fewer total seeds per cone. Varying the species of pollen used as diluent did not affect seed production. In breeding southern pines pollen probably can be safely diluted with dead pollen to only 30 percent live pollen without effect on seed production.

LITERATURE CITED

Callaham, R. Z. and J. W. Duffield, 1961. Stretching the pollen supply. Jour. Forestry 50:204-207.