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Hybridizing Pines With Diluted Pollen

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INHALTSVERZEICHNIS

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Hybridizing Pines With Diluted Pollen

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Diluted pollens would have many uses by the tree breeder. Dilutions would be particularly advantageous in making many controlled pollinations with a limited amount of pollen. They also would be useful in artificial mass pollinations of orchards or single trees. Diluted pollens might help overcome troublesome genetic barriers to crossing. Feasibility of using diluted pollens is being studied at the Institute of Forest Genetics at Placerville, California, in an effort to develop improved procedures of hybridizing pines.

Preliminary studies by CALLAHAM and DUFFIELD (1961) indicated that viable pollen could be diluted with an equal amount of dead pine pollen without reducing production of viable seeds. These first studies did not adequately show effects of dilution with large quantities of dead pollen, but did find that seed production dropped as the proportion of diluents was increased. On the other hand, too much viable pollen may inhibit seed set and reduce seed size (TER-AVANESYAN 1959). FOWLER'S (1964) data showed that both cone set and full seeds per cone decreased as dilution of *Pinus resinosa* pollen with *P. koraiensis* increased.

Other studies have shown that proximity of pollen grains to one another affects pollen germination and seed production. DUFFIELD (1954), VISSER (1955), McWILLIAM (1960) and others have shown that pollen germination *in vitro* is stimulated when pollen grains are close to one another, as would occur without dilution. McWILLIAM (1960) also showed that the addition of extracts from dead pollen benefited germination of scattered pollen cultures.

The first studies at Placerville did not investigate the potentially important question of the species of pollen used as diluent. McWILLIAM (1960) found that mixing viable pollen of three pine species had no effect on subsequent germination. He suggested that pollen grains of different species do not have differential effects upon one another during germination. Considerable research has been carried out in Russia on mentor pollens; results suggest that living or dead "foreign" pollens may have specific effects on seed yield and resulting progenies (ARZUMANOVA 1956; KOVARSKII 1956; LEBEDEVA 1960; NESTEROV 1956; POLYAKOV 1955).

Research by VISSER (1955) and by STANLEY and LICHTENBERG (1963) showed that boron stimulates pollen germination *in vitro*. Additions of boron might stimulate pollen growth *in vivo* and facilitate production of difficult hybrids.

A number of questions still needed answers. Therefore, a comprehensive study was started in 1962 at the Institute of Forest Genetics. It involved an adequate series of diluted pollens in the production of three hybrids. Pollens of three species were used as diluents, and boron was added to a few crosses to study its effects.

Procedure

Three hybrids having commercial potential were chosen for study: *Pinus attenuata* × *radiata*, *P. monticola* × *strobus*, and the backcross hybrid, *P. jeffreyi* × *(jeffreyi* × *coulteri)*. These hybrids also were selected because each is separated from the others by strong genetic barriers.

Three trees of each seed parent species were selected for controlled pollination. The *P. monticola* and *P. jeffreyi* trees grow in native stands on the Eldorado National Forest, near Placerville. The *P. attenuata* trees grow in the arboretum at the Institute.

The pollen parents of the hybrids also grow in the arboretum at the Institute. Pollen was collected from two trees of *P. radiata*, three of *P. strobus*, and three F_1 hybrids *P. jeffreyi* × *coulteri*. Pollen for the intraspecies control crosses was collected from two or three trees of each seed parent. Special care was taken to obtain pollen from trees unrelated to the trees actually used as seed parents.

Equal volumes of pollen from each tree were combined in a mix for the pollen species. Mixed pollens to be used as diluents were killed by exposure to 85° C for 16 hours.

Viability of the pollen *in vitro* was determined by the method of RIGHTER (1939). Average germination for the pollen mixes ranged from 50 to 81 percent.

Detailed study of the germinability of the diluted *P. strobus* lots was carried out by Mrs. GERALDINE B. LARSON in September 1962. She germinated pollen on 0.5 percent agar plates at 29° C. Number of germinated grains and length of pollen tubes were determined after 72 hours.

The basic design of each interspecific hybridization involved 10 pollen lots each used to pollinate three bags on a seed tree *(table 1)*. Seven lots contained increasing amounts, 10, 20, 30, 40, 50, 70, and 100 percent, of live pollen mixes of the desired pollen parent species. The diluent was dead pollen of the seed parent species. Fresh and dead pollen were mixed by weight to produce the desired dilution. Relative pollen germinability was disregarded in composing the diluted pollen lots. A control pollen lot to produce an intraspecies cross contained only live pollen of the seed parent species. In two pollen lots, dead pollens of two species known to be genetically incompatible with the seed parent were used as diluents at the 50 percent level.

Finely ground boric acid (H_3BO_3) was added only to the pollen lots containing 10 and 50 percent live pollen of *P. jeffreyi* × *coulteri*. One gram of boric acid was added for each 100 grams of live and dead pollen in the lot. Dilutions containing boric acid were used in pollinating three bags on each of the *P. jeffreyi* seed trees. Dilutions containing boric acid were not used on *P. attenuata* or *P. monticola* seed trees.

All pollen handling and breeding procedures followed those set forth by CUMMING and RIGHTER (1948). Cones were collected and counted when mature, in the fall of 1963. Every seed was removed from each cone. Sound seeds were separated from hollow seeds by winnowing. Total yield of seeds per cone, that is sound plus hollow, proportion of sound seeds, and weight of 40 seeds were determined for each cross.

Seeds were stratified before sowing in the nursery in May 1964. Replicated randomized block designs were used. Mean days to germinate was determined for each cross.

[1]) A report of research conducted while the author was in charge of the Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Placerville, California.

viable pollen resulted in a significant reduction in proportion of sound seed.

Sound seeds per cone, or total seeds per cone multiplied by the proportion of sound seeds, show the actual yield of viable seed after use of diluted pollen. Although the proportion of sound seeds was high, few sound seeds per cone of *P. attenuata* × *radiata* were produced with only 10 percent viable pollen.

Sound seeds	Viable pollen
	(percent)
40	50
34	40
28	30
24	20
12	10

Obviously, not enough viable pollen grains were available to fertilize the ovules as dilution increased. The data for the other two hybrids were similar. From 30 to 40 percent viable pollen seems to be the least that can be used if yields of sound seeds per cone are to be high. This condition may be related to the limited number of pollen grains which can enter each ovule.

Some tree breeders may want the highest possible yields of sound seeds if only little pollen is available. If this is the case, dilution to 10 percent or less viable pollen will give the maximum yield of sound seeds per unit of viable pollen. Certainly many more conelets must be pollinated, but the extra seeds may be worth the extra efforts. For *P. attenuata* × *radiata*, 10 cones pollinated with 10 percent viable pollen would produce 120 sound seeds; 2 cones pollinated with 50 percent viable pollen would produce 80 sound seeds; and 1 cone pollinated with 100 percent viable pollen would produce only 49 sound seeds.

Variation in effect of pollen dilution on sound seed production may be related to the genetic compatibility of the species being hybridized. Parents of *P. attenuata* × *radiata* are highly compatible. The interspecific hybrid in this study was produced as easily as intraspecies hybrids. The high proportion of sound hybrid seeds at extreme pollen dilutions may reflect the high degree of genetic compatibility between the parent species. On the other hand, parents of *P. monticola* ×*strobus* and *P. jeffreyi* × *(jeffreyi* × *coulteri)* are relatively incompatible, and the low proportion of sound hybrid seeds at 10 and 20 percent dilutions may reflect the genetic barriers to crossing these species.

Studies of the effect of pollen dilutions in crosses within a species where genetic incompatibility does not exist would be interesting. Perhaps higher proportions of sound seeds could be produced by extreme dilutions containing 10 percent or less viable pollen if genetic barriers to crossing were absent. Even without precedent research, it seems safe to recommend dilutions containing only 30 or 40 percent viable pollen for intraspecific hybridizations.

Degree of pollen dilution did not affect weight or germination rate of the sound seed. After stratification the average number of days for germination were:

Hybrid	Dilution *(percent)*						
	10	20	30	40	50	70	100
	Number of days						
monticola × *strobus*	20.8	20.2	19.9	20.5	20.2	20.6	20.9
jeffreyi × *(jeffreyi* × *coulteri)*	19.8	20.4	19.9	20.2	19.8	19.5	20.0
attenuata × *radiata*	20.2	19.8	19.7	19.8	19.6	19.5	19.6

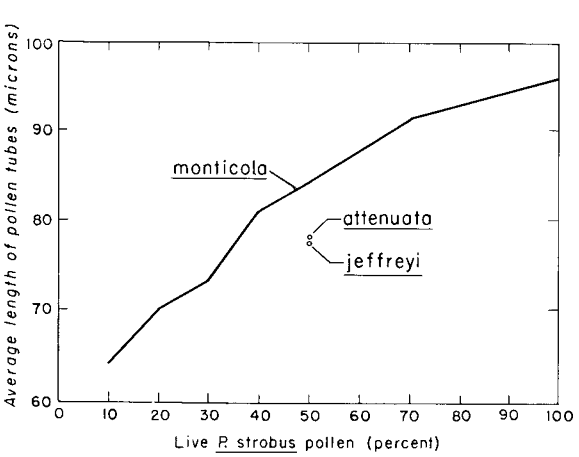

Figure 3. — Length of *Pinus strobus* pollen tubes after 72 hours for pollen lots diluted with dead pollens of *P. monticola*, *P. attenuata*, or *P. jeffreyi*.

Dilution did not influence the number of viable pollen grains that germinated *in vitro*. Mrs. LARSON's study of the *P. strobus* dilution series showed this to be true. Actual germination percent was very close to expected germination percent at all dilutions greater than 10 percent.

The only major deviation was at the dilution containing 10 percent viable pollen. The expected germination of this lot was 9.2 percent based on 92.3 percent germination of the undiluted pollen lot. The actual germination of the 10 percent dilution was 17.7 percent. A chi-square analysis showed the probability of such a deviation to be far less than 0.005. The deviation is meaningless although highly significant in the statistical sense.

The important finding here is that germination was not adversely influenced by dilutions containing up to 90 percent of dead pollen. The scattered live grains in highly diluted pollen lots were not expected to germinate as well as the closely packed grains in undiluted lots. The results provide confirmation to MCWILLIAM's (1960) suggestion that dead pollen grains produce some factor essential to the germination of scattered or isolated live pollen grains.

Dilution did influence the length of pollen tubes in Mrs. LARSON's study *(fig. 3)*. High concentrations of live *P. strobus* pollen resulted in longer pollen tubes. Dilution to 10 percent live pollen plus 90 percent dead *P. monticola* pollen gave tubes only two-thirds as long. Obviously, dead pollen of another species, while providing the germination stimulus, does not provide the stimulus to pollen tube growth. Aggregations of live pollen must produce some factor required for pollen tube extension.

Species as Diluents

The species of pine pollen used as diluent at the 50 percent level did not influence cone set. The data were highly variable. The proportion of strobili maturing after crosses using maternal pollen as the diluent was not significantly different from the proportion of strobili maturing after crosses using nonmaternal species as the diluent. Strobili pollinated and cones produced from nine pollination bags were:

Hybrid	Dead diluent used		
	monticola	*jeffreyi*	*attenuata*
monticola × *strobus*	41:28	36:17	29:23
jeffreyi × *(jeffreyi* × *coulteri)*	17:11	18:16	19:14
attenuata × *radiata*	28:22	33:30	42:35

Table 2. — Mean seed production for three hybrids when dead pollens of three species were used as 50 percent diluents.

Hybrid	Total no. seeds per cone			Percent sound seed		
	Diluent-			Diluent-		
	monticola	*jeffreyi*	*attenuata*	*monticola*	*jeffreyi*	*attenuata*
monticola × *strobus*	45	42	27	42.7	36.0	33.3
jeffreyi × (*jeffreyi* × *coulteri*)	184	160	148	48.7	51.7	49.0
attenuata × *radiata*	68	75	59	69.7	63.7	68.3

Diluent species did not significantly influence total seeds per cone or proportion of seeds that were sound (*table 2*). Data were quite variable, and analyses of variance did not disclose any significant differences in seed production related to species used as diluent. From the available data I can only conclude that the presence of dead pollen of a genetically incompatible species does not favor or disfavor hybrid seed production.

Germination of *P. strobus* pollen *in vitro* was not affected by the species used as diluent at the 50 percent level. When the diluents were *P. monticola, P. jeffreyi,* and *P. attenuata*, average germination was 51.0, 49.3, and 55.0 percent, respectively; and average length of pollen tubes was 84, 78, and 78 microns (*fig. 3*). These results support McWilliam's (1960) conclusion that pine pollens are nonspecific in providing factors required for pollen germination. They also indicate that pine pollens are nonspecific in providing factors required for pollen tube elongation.

Species used as diluent did not influence seed weight or germination rate in the nursery.

Adding Boric Acid

The addition of finely ground boric acid to dilutions containing 10 or 50 percent viable pollen did not have a significant effect on cone set or seed yields (*table 3*). Boric acid did not have a statistically significant effect on total seeds per cone. One of the five seed lots showed a slight decrease in total seed when boron was added. For the four seed lots showing a boron response, the average increase in total seeds associated with boron was 42 percent. Similarly, the yield of sound seeds was not significantly affected by boric acid additions, but boron additions were consistently associated with higher proportions of sound seed.

These results of using boric acid *in vivo* suggest that further boron additions should be used in controlled pollinations. Stanley and Lichtenberg (1963) have shown that the chemical source and concentration of boron added to pollen influence pollen growth. Future studies should determine the effects on cone and seed production of a variety of sources and concentrations of boron.

Controlled Versus Open Pollination

Relatively few data are available to compare results of controlled artificial pollination and open pollination by wind, so results of the check crosses are presented although they do not pertain to the dilution study. Controlled intraspecific crosses were made on the *P. jeffreyi* and *P. monticola* seed trees in the dilution study. A few open-pollinated cones also were collected when cones resulting from these crosses were harvested. Controlled pollination produced more total seeds per cone than open pollination on five of the six seed trees (*fig. 1*), but the difference was not significant at the 5 percent confidence level. Controlled pollination resulted in a significant increase in the proportion of seeds that were sound (*fig. 2*). For all six seed trees, controlled pollination gave 85 percent sound seed, and open pollination gave 71 percent sound seed.

The advantage of controlled pollination is particularly noticeable when one considers sound seeds per cone. For the three *P. jeffreyi* seed trees the advantage was 110 versus 67 sound seeds per cone. For the three *P. monticola* seed trees, it was 53 versus 25 sound seeds per cone. The hand of man outproduced nature about two to one. Of course, these data only pertain to this breeding situation, but the results suggest that average yields of sound seeds from open-pollinated cones should not be used to predict yields from controlled intraspecific crossing.

Table 3. — Effect of adding boric acid (BA) to dilutions containing 10 and 50 percent viable pollen on production of seeds of the hybrid *Pinus jeffreyi* × (*jeffreyi* × *coulteri*).

Seed parent	Dilution	Total seeds per cone		Sound seeds	
		With BA	Without BA	With BA	Without BA
	Percent			*Percent*	
Eld 18-1	10	87	147	44	43
Eld 18-7	10	128	96	37	33
Eld 26-3	10	141	71	42	33
Eld 18-7	50	199	159	51	50
Eld 26-3	50	161	117	54	50

Summary

Diluted pollens would have a number of uses to pine breeders wanting to produce the most seeds with a limited amount of pollen. Effects of dilution were investigated for three interspecific hybrids: *P. monticola* × *strobus, P. jeffreyi* × (*jeffreyi* × *coulteri*), *P. attenuata* × *radiata*. These hybrids have commercial importance and represent different sections of the genus *Pinus*.

The study included dilutions containing 10, 20, 30, 40, 50, 70, and 100 percent viable pollen of the maternal species. Heat-killed pollens of *P. monticola, P. jeffreyi,* and *P. attenuata* also were used as diluents at the 50 percent level. Boric acid as a source of boron was added to a few diluted pollen lots to study its effects on seed production.

Pollen dilution did not influence cone set, but dilutions containing only 10 or 20 percent viable pollen produced significantly fewer total seeds per cone.

The proportion of seeds that were sound was influenced by pollen dilution, but the results varied with the hybrid being produced. Dilutions containing only 10 or 20 percent viable pollen gave as high a proportion of sound seed as 50 or 100 percent viable pollen for the cross between the genetically compatible parents of *P. attenuata* × *radiata*. These extreme dilutions resulted in low proportions of sound seed for crosses between the relatively incompatible parents of *P. jeffreyi* × (*jeffreyi* × *coulteri*) and *P. monticola* × *strobus*.

Pollinations with 30 to 50 percent viable pollen can be expected to produce high yields of sound seeds for all of these interspecific hybrids. Hybridizations within species using as little as 30 percent viable pollen are recommended.

Degree of pollen dilution did not affect weight of sound seeds, germination rate of seeds, or germination of viable

P. strobus pollen grains *in vitro*. Dead pollen grains apparently produced some factor essential to germination of scattered grains, but they did not provide the stimulus to pollen tube growth given by live pollen. Pollen tubes were only two-thirds as long for viable grains in 10 percent dilutions as for those in undiluted viable pollen.

The species of pollen used as diluent at the 50 percent level may influence cone set. More cones were harvested when pollen of the seed parent species was used as diluent.

The diluent species did not significantly influence total seeds per cone, proportion of seeds that were sound, or the germination *in vitro* of *P. strobus* pollen. The presence of dead pollen of a genetically incompatible species does not favor or disfavor hybrid seed production.

Additions of boric acid to dilutions containing 10 or 50 percent viable pollen did not have a significant effect on cone set or seed yield. The results of adding boric acid suggested that boron may increase both total number of seeds per cone and proportion of sound seeds. Further study is needed of the chemical form and concentration of boron in seed production.

Controlled intraspecific hybridizations produced more total seeds per cone, and controlled crosses produced twice as many sound seeds as were produced from open pollination by wind.

Literature Cited

ARZUMANOVA, A. M.: (Using pollen from different genera for hybridization.) Agrobiologiya 1: 52—61 (1956). (In Russian.) — CALLAHAM, R. Z., and DUFFIELD, J. W.: Stretching the pollen supply. Jour. Forestry 59: 204—207 (1961). — CUMMING, W. C., and RIGHTER, F. I.: Methods used to control pollination of pines in the Sierra Nevada of California. U. S. Dept. Agr. Circ. 792, 18 pp. (1948). — DUFFIELD, J. W.: Studies of the extraction, storage, and testing of pine pollen. Z. Forstgenetik 3: 39—45 (1954). — FOWLER, D. P.: Pregermination selection against a deleterious mutant in red pine. Forest Sci. 10: 335—336 (1964). — KOVARSKII, A. E.: The effect of mentor pollen in the self-pollination of corn. Selektsia i semenovodstvo 4: 7—12 (1956). (Transl. from Russian OTS 60—21892.) — LEBEDEVA, Z. V.: The effect of foreign pollination on the depression of self-pollination in maize. Doklady Akad. Nauk SSSR. Biol. Sci. Sect. 126: 488—491 (1960). (Transl.) — MCWILLIAM, J. R.: Pollen germination of *Pinus* as affected by the environment. Forest Sci. 6: 27—39 (1960). — NESTEROV, Y. S.: The role of maternal pollen in intervarietal pollination of the apple. Agrobiologiya 4: 132—135 (1956). (Transl. from Russian OTS 60—21902.) — POLYAKOV, I. M.: (I. V. MICHURIN's doctrines concerning fertilization in the light of new experimental data.) Zhur. Obshch. Biol. 16: 368—382 (1955). (In Russian.) — RIGHTER, F. I.: A simple method of making germination tests of pine pollen. Jour. Forestry 37: 574—576 (1939). — STANLEY, R. G., and LICHTENBERG, E. A.: The effect of various boron compounds on the in vitro germination of pollen. Physiologia Plant. 16: 337—346 (1963). — TER-AVANESYAN, D. V.: The effect of the quantity of pollen used on the inheritance of characters. Indian Jour. Genetics and Plant Breed. 19: 30—35 (1959). — VISSER, T.: Germination and storage of pollen. H. Veenman u. Zonen, Wageningen, Netherlands, 68 pp. (1955).