BREEDING TECHNIQUES FOR WHITE OAK

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Because it is a major species on vast acreages in the Eastern United States, many organizations are considering improvement programs for white oak (Quercus alba L.). Such programs will require information on techniques for breeding, and particularly on extraction and storage of pollen and controlled pollination. This paper describes a series of limited trials in which some techniques for breeding white oak were developed.

Sharp and Chisman (1961) described morphological development of staminate flowers, and Sharp and Sprague (1967) described stages in pistillate flowering, acorn development, and the influence of weather factors on seed yield. Individual white oak trees normally shed their pollen before the female flowers are receptive (Sharp and Chisman 1961; Sharp and Sprague 1967; Irgens-Moller 1955). Maximum receptivity in a variety of oaks pollinated by Piatnitsky (1960) was 3 to 5 days after anthesis of the staminate flowers. Sharp and Sprague (1967) reported that white oak flowers with separate, elongate-reflexed styles and the inner surface of the stigma reddish to reddish-yellow appear to be receptive. Williamson (1966) observed a great deal of acorn abscission between the times of pollination and fertilization.

Controlled pollinations have been reported by Piatnitsky (1960), Ness (1918), Wright (1953), and Schreiner and Duffield (1942). No accounts have been found of controlled pollinating oak flowers in viscose sausage casings, which permit the passage of light, water, and air but not contaminating pollen. Interspecific hybrids from controlled pollinations have been produced by Ness (1918), Schreiner and Duffield (1942), and Piatnitsky (1960).

METHODS

Controlled Pollination

In New Haven, Connecticut, three large, fruitful, open-grown white oaks were chosen, and emerging shoots were emasculated and bagged on May 4 and 5, 1968. By removing most leaves without pistillate inflorescences in their axils it was possible to include several shoots in each bag. The

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number of stems bearing female flowers, as well as the number of inflorescences and flowers per stem, were recorded for each bag. Only flowers with exposed stigmata were tallied. Most inflorescences had one or more spherical bodies which were assumed to be flowers that had not developed normally. These exhibited no evidence of an ovarial opening or stigmatic surface and were often on the extreme distal tips of flower stalks.

Depending on the tree, the peak of anthesis occurred on May 12, 13, or 14. Catkins were collected between May 8 and 13. Pollen was extracted by shaking and sieving after overnight drying in the laboratory. For interim storage, the pollen was refrigerated in closed vials.

Germination was tested 1 week after collection. After 93 hours at 27° C., germination of a polymix of three trees ranged from 18 to 25 percent. In another test, however, only 5 percent of pollen from one of these trees germinated compared to 45 and 64 percent germination from the others. Hence, the polymix probably represented two individuals.

To define the time of receptivity, a different series of five bags on each tree were pollinated every day for 5 days (a total of 25 bags per tree). Because it was uncertain whether every flower of a given inflorescence reached the stage of receptivity at the same time, the efficiency of multiple pollination was also tested. One series of five bags on each tree was pollinated every day for 5 days (a total of five bags per tree). For controls, one **series** of five bags on each tree was left unpollinated and one series of five branches was left unbagged for wind pollination.

Pollinating was done during the morning hours beginning the third day after anthesis. Pollen was applied generously with a brush because it was somewhat sticky.

Tops of the bags were cut away on June 1, about 2 weeks after anthesis. The bases of all bags and twigs proximal to them were sprayed with Bird Tanglefoot, a sticky repellent substance, to discourage birds, squirrels, and other predators. Branches were also sprayed with DDT to eliminate a scale insect infesting one study tree.

Branches were inspected on July 3 and on August 18, before acorn maturity, to determine whether abscission had occurred. On August 19 the branches that still bore developing acorns were enclosed in aluminum screening wire to protect against squirrels and birds and prevent loss of acorns at maturity. By August 1 squirrels had been observed eating green acorns, which had scarcely begun to enlarge. Acorns were collected on September 12, weighed individually, and sown immediately in a mixture of soil, peat, and sand in a greenhouse. Germination was recorded 2 months later.

The relationship between premature abscission and cytological development after controlled and wind pollinations was studied in southern **Mississippi** in 1968. Two trees were control-pollinated by the methods described above, except that pollinations were made with an aspirator. Immature acorns were collected during the growing season, but enough (one-third) matured to compare success of controlled and **wind pollination.**

Gibberellin Effect

The procedure of germinating pollen on artificial media is an important one, because a great deal of effort can be wasted if pollinations are made with inviable pollen. Germination, while not guaranteeing that the pollen is capable of fertilization, does provide some indication of viability. Gibberellins accelerated the rate of germination of pollen of Douglas-fir **[Pseudotsuga menziesii (Mirb.)** Franco] as shown by Ching and Ching (1959) but had no effect on pollen of Pinus mugo in trials reported by Nygaard (1969).

Because Hart (unpublished data) demonstrated that gibberellic acid increased pollen germination in oaks, the effect of GA7 was tested in a factorial experiment with three levels of sucrose, 0, 5, and 10 percent, and four levels of GA7, 0, 100, 200, and 300 ppm, in distilled water. Each treatment was replicated twice. All media were autoclaved. Freshly collected pollen was dusted over a drop of the media on a slide, and incubated as a hanging drop culture in a van Tiegham cell at 27° C. in darkness.

Counts of pollen germination and measurements of pollen tube growth were made approximately 2, 8, 11, 34, 46, and 96 hours after initiation of the trial; by 96 hours, germination and tube growth appeared to have ceased.

Pollen Storage

Storage of pollen for up to 2 months would enable crosses of later-blooming northern trees with pollen from those further south, and storage for 1 year would permit crossing early-blooming trees with pollen of late-blooming trees in any region. Viability can be maintained in many species at particular temperatures and humidities, and freeze-drying has also been successful with some species. Both methods were tried. Viability of stored pollen was tested in vitro (germination on artificial media) and in vivo (actual fertilization as shown by seed set).

Pollen was collected from three trees at the beginning of anthesis. It was extracted by shaking and sieving after drying overnight. After storage for 6 to 8 weeks in the refrigerator, the pollen was divided for tests of the two types of storage methods.

In the first, several relative humidities were tested for storage at 2° C. The humidities were achieved by placing pollen in dessicators

containing saturated salt solutions. The salts were potassium acetate, calcium chloride, calcium nitrate, and sodium chloride. At equilibria the relative humidities above saturated solutions of these salts are 23.5, 40, 65, and 75 percent, respectively. A stirrer whose shaft extended through the top of each dessicator turned a fan to eliminate humidity gradients. Pollen samples from each of the three trees were kept separate and considered as replicates.

Freeze-drying was tested on pollen from a single tree. The experiment included, factorially, two pretreatments, prefrozen or not prefrozen; two methods of storage at -5° C., under nitrogen or under vacuum; and three periods of evacuation, 10, 30, or 60 minutes on the freeze-dry apparatus. In the prefreezing treatment, the temperature of the pollen was lowered to -80° C. for about 15 seconds in an acetone-dry ice bath prior to freeze-drying. Ampules containing pollen were sealed with a propane torch after evacuation and while still attached to the freeze-dryer. For vacuum storage, the ampules were sealed while under the original vacuum; for nitrogen storage, dry nitrogen gas was admitted to the ampules through the freeze-drying manifold.

For the humidity tests germination was scored after 70 days and after 295 days of storage. For the freeze-dry series, germination was counted only after 300 days of storage. The germination medium was 2 percent agar, 5 percent sucrose, and 0.01 percent boric acid. Incubation was in the dark at 22° C. between microculture slides sealed together to prevent dehydration. All materials were autoclaved before dusting the pollen on the medium. After incubation for 18 to 24 hours, germination was scored as the number of grains with pollen tubes longer than the width of the grain. Results were expressed as a percentage of the 100 counts from two randomly selected fields under a 10X microscope. After arc sine transformation, the data were subjected to analysis of variance.

To test the effectiveness of the pollen, it was applied to bagged female flowers on two trees. Techniques were identical to those described earlier. On each tree, 40 branches bearing female flowers were bagged about 4 days before anthesis, on May 10 and 11, 1970. Anthesis occurred on May 15 and 16. Pollinations were made on May 20 and 21, and bags were removed on June 6. Because of the scarcity of stored pollen, every storage treatment was not represented in the pollinations. Controls consisted of pollinations made with fresh pollen of the same tree as that used in the storage trial and of bagged unpollinated branches.

All statistical tests were done at the 0.05 level of probability.

RESULTS AND DISCUSSION

Controlled Pollination

The viscose bags proved effective. No acorns were set on the 12 bagged, unpollinated branches which survived accidents until fall. The percentage of seed sets from controlled pollinations was 23.6 compared to 7 for wind pollinations. Additionally, 2.1 acorns per bag were collected from controlled pollinations but only 0.8 from wind pollinations (table 1).

Table 1.--Results of controlled and wind pollinations in Connecticut, 1968

Item and unit of measure	Controlled pollination tree				Wind pollination tree			
	A	B	C	Total	A	B	C	Total
Branches bagged (No.)	24	27	20	71	3	5	4	12
Branches setting seed (No.)	19	16	9	45	0	3	3	6
Flowers (No.)	271	182	172	625	33	60	33	126
Acorns (No.)	86	43	19	148	0	6	3	9
Seed set (Pct.)	31.7	23.6	11.0	23.6	0	10.0	10.0	7.0
Acorns/branch (No.)	3.6	1.6	1.0	2.1	0	1.2	0.8	0.8

There was no difference in the success of single pollinations made from 3 to 7 days after anthesis (table 2). Apparently the period of receptivity lasts for at least 7 days after anthesis, and pollination at any point during this period should be successful.

Table 2.--Yield of mature acorns by date of pollination and by tree

Days from anthesis to pollination	Tree			
	A	B	C	Mean
	----- Acorns/flower -----			
3	0.33	0.31	0.07	0.25
4	0	.22	.08	.14
5	.26	.24	.13	.23
6	.25	.10	.17	.28
7	.35	.37	0	.28
Mean	.38	.25	.13	

Statistically significant differences were not found between single and multiple pollinations in terms of seed set. However, the number of surviving branches in the multiple-pollination treatment was small, only 11.

Sixty-one percent of the acorns collected germinated within 2 months after sowing. Germinating acorns were also significantly heavier than those that failed to germinate.

Differences among replications (trees) were evident in all analyses. We conclude that breeding parents should be carefully chosen for reproductive ability when controlled pollinating oaks.

Controlled pollination in white oak proved feasible in this study. In fact, controlled pollination resulted in higher seed set and sound seed yield, than wind pollination on all trees investigated. Bagging with screen wire seemed to protect against weeviling, which was noted only in unshielded acorns.

In the study by Williamson (1966), 65 percent of the acorns on white oaks fell prematurely within the first month after pollination. In our studies very few acorns abscissed by July 3, 1968, which was nearly 2 months after pollination. In fact, abscission occurred in only one of 13 bagged, unpollinated branches before July 3; development in the others appeared normal. Complete abscission had occurred on all bagged, unpollinated branches by the time of the next inspection, August 19. Climatic differences between Williamson's study area and New Haven and genetic differences among trees may account for the contrasting results.

Results from the experiment in Mississippi (table 3) were similar to those from Connecticut. Controlled pollinations yielded a higher seed set (6.5 percent) than wind pollinations (1.3 percent).

Table 3.--Results of controlled and wind pollinations in southern Mississippi, 1968

Item and unit of measure	Controlled pollination tree			Wind pollination tree		
	1	2	Total	1	2	Total
Branches bagged (No.)	21	25	46	62	55	117
Branches setting seed (No.)	6	3	9	0	9	9
Flowers (No.)	162	130	292	402	347	749
Acorns (No.)	16	3	19	0	10	10
Seed set (Pct.)	9.9	3.1	6.5	0	2.9	1.3
Acorns/branch (No.)	0.8	0.1	0.4	0	0.2	0.1

The difference in seed set between the two locations may be associated with the length of the growing seasons. The time interval from anthesis to collection is 100 days greater in Mississippi than Connecticut. During this extended period the acorns are subject to additional effects of predators (animals, fungi, and insects) and to stress conditions such as drought.

Better seed sets from controlled than wind pollinations at both locations may indicate that controlled pollination is feasible over the entire north-south range of white oak. Yield/pollination was low in both areas, however, and no attempt was made to cross northern with southern sources.

Gibberellin Effect

Pollen germination was depressed by GA7 in all levels of sucrose. Additionally, neither GA7 nor sucrose alone increased the rate of germination or tube length. It must be concluded, therefore, that GA7 does not improve but, rather, impairs the estimate of pollen viability.

Pollen Storage

The average germination of freshly collected pollen was 34 percent. Germination averaged 31 percent for pollen stored 70 days at 23.5 percent RH; that stored at higher humidities failed to germinate. After 295 days in storage at 23.5 percent RH, germination still averaged nearly 20 percent.

The best freeze-drying treatment combination was prefreezing followed by freeze-drying for 10 minutes and storage under vacuum. After 300 days the average germination achieved with this treatment was 9 percent, which compares poorly to 31.5 percent for pollen from the same tree stored at 2° C. and 23.5 percent RH for 295 days.

None of the unpollinated flowers produced acorns and all abscissed before June 15. Thus, there was no evidence of contamination or apomictic behavior. At least one viable acorn was produced by pollinating with year-old pollen (1) stored at 2° C. and 23.5 percent RH, (2) freeze-dried without prefreezing for 10 minutes and stored in nitrogen gas, (3) prefrozen, freeze-dried for 30 minutes and stored in nitrogen gas, (4) prefrozen, freeze-dried for 10 minutes and stored in vacuum, and (5) prefrozen, freeze-dried for 30 minutes and stored in vacuum (table 4). In this in vivo test of viability for pollen stored 1 year, the freeze-dried pollen produced higher acorn yields than the pollen stored at 2° C. and 23.5 percent RH. The in vitro test predicted the reverse. Though the numbers of pollinations and the seed set were minimal, it was demonstrated that year-old, stored pollen is capable of fertilization. The freeze-drying treatments involving storage in nitrogen appear to be the best and warrant further testing.

Table 4.--Acorns set on white oak flowers pollinated with fresh pollen and with pollen stored under various conditions

Storage conditions	Flowers pollinated	Acorns	Success ratio
	Number	Number	Percent
Not stored (fresh)	152	41	27
2° C., 23.5 percent RH	29	1	3
Freeze-dried 10 min., Nitrogen storage, not prefrozen	38	5	13
Vaccum storage, prefrozen	63	1	2
Freeze-dried 30 min., Nitrogen storage, prefrozen	56	5	9
Vacuum storage, prefrozen	41	1	2

Some abscission of flowers and stalks was noted in all treatments at the time of debagging about 3 weeks after pollination. In the controlled-pollination trials of 1968, very little abscission was observed even 2 months after pollination. Therefore, there may be some year-to-year variation in premature fruit drop.

This is the first known report of successful storage of oak pollen for 1 year. Controlled pollination, again as in 1968, resulted in higher seed set than that commonly reported for wind pollination [e.g., 1 to 5 percent according to Williamson (1966)].

All experiments were exploratory but the results are encouraging. Intraspecific controlled pollination of white oak demonstrated that feasible pollen viability was maintained in storage for 1 year, and pollen viability was assessed by germination on artificial media.

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