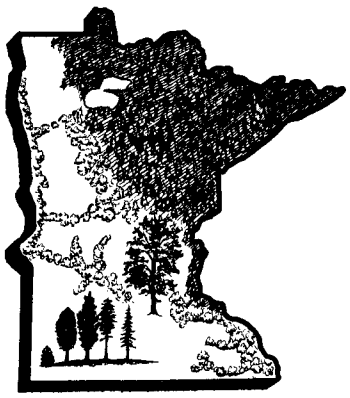

MINNESOTA FORESTRY NOTES

No. 62
October 15, 1957

VACUUM STORAGE OF POLLEN PROVES FEASIBLE[1]

John C. Barber and Donald M. Stewart[2]

There is a need in any tree breeding program for a means of storing pollen a year or more. It is frequently necessary to keep pollen of late blooming species until the following season to make crosses with those which flower early. Pollen can be forced from some species, but for the conifers, forcing has not been successful more than a few days in advance of normal anthesis. A storage method which would permit holding pollen for several years in the case of collections from inaccessible trees or pollen furnished by cooperators would be desirable.

There have been numerous reports in the literature on the storage of pine and spruce pollens (Duffield, 1954; Duffield and Snow, 1941; Johnson, 1943). Pollens of these species have been successfully stored at 50 percent relative humidity and at temperatures of 32° to 39° F. for a year or more. Recently Sato and Muto (1955) mixed Adsol and K_2S with pollen of Alnus and Betula and stored it in viable condition in evacuated containers. K_2S alone reduced germination. Kellerman (1915) vacuum processed citrus pollen which remained viable when shipped to Japan.

The method used in this experiment was that described by Stewart (1956) for storage of spores of Puccinia graminis. This method has increased the storage life of the rust spores from about 4 months to 10 months.

In this study fresh pollen of jack pine (Pinus banksiana Lamb.), white spruce [Picea glauca (Moench.) Voss.], and Norway spruce [Picea abies (L.) Karst.] was dried in glass tubes for two hours under vacuum equivalent to three inches of mercury, the tubes sealed, and stored at 38° F. Pollen alone, and mixed with chlorophyll, hemin, and sodium chelate of iron was so stored. Part of each lot was stored in cotton stoppered bottles at approximately 50% R.H. in the same refrigerator. Germination percentage prior to storage was not determined. Each lot of pollen was collected from several trees. The pollen was refrigerated immediately after extraction and placed under the desired storage conditions within 48 hours.

After preliminary trials with several concentrations of sucrose in distilled water and in 0.75% agar, the hanging-drop technique described by Righter (1939) was used for germination tests. Tests were made in petri dishes at room temperature (70° - 80° F.), each dish containing the combinations of distilled water, 5% and 10% sucrose with pollen from each of the five storage conditions for one species. Each dish contained one replication for one species. Two replications and 50 pollen grains in each drop were counted after 54 hours.

1/ This study was made possible by a grant from the Charles K. Blandin Foundation of Grand Rapids, Minnesota.

2/ Respectively, Research Assistant, School of Forestry, University of Minnesota, and Plant Pathologist, Plant Pest Control Division, Agricultural Research Service, U. S. Department of Agriculture.

Published by the School of Forestry, University of Minnesota, St. Paul 1, Minnesota, cooperating with the Division of Forestry, Minnesota Conservation Department, and Forest Industries of Minnesota

An analysis of variance of the data summarized in Table 1 indicates a highly significant difference (1% level) in germination due to storage treatment. The major portion of this variation is accounted for by the iron chelate treatment. A highly significant interaction was also obtained for the species x storage test. The iron chelate treatments of the spruce pollens probably contributed the major part of the variation to this interaction. A highly significant difference was also noted between species, but this is not interpretable because the initial germination at the time of storage was not known.

Table 1. Percent of pollen germinating at 54 hours.

	Jack Pine			White Spruce			Norway Spruce		
	Concentration of sucrose in solution - Percent								
	0	5	10	0	5	10	0	5	10
Untreated Check	85	92	89	78	79	78	84	80	81
Vacuum only	94	91	97	85	94	86	76	83	70
Vacuum-Chlorophyll	75	72	78	84	85	88	74	88	86
Vacuum-Hemin	76	82	75	85	89	79	67	75	76
Vacuum-Na Fe	73	90	77	20	47	20	57	45	43

In the samples containing the additives, the slight reduction in germination in some cases might be attributable to the additive entering solution. Echols and Mergen (1956) reported that concentrations of an iron chelate complex as high as 250 ppm. reduced germination of slash pine pollen while a concentration of 50 ppm. was beneficial. The inhibition of germination was attributed to the sodium present in the complex. The amount of additive which entered any test drop with the pollen could not be controlled under the conditions of this experiment.

At the end of one year, the percentage germination of pollen stored under vacuum did not differ appreciably from that stored in air in the refrigerator, though there is some indication of slightly higher germination in some vacuum stored lots for each species. However, this experiment does establish that the pollen of the species used can remain viable when stored under vacuum with its coincident drying.

Duffield, J. W.
1954. Studies of extraction, storage, and testing of pine pollen. Z. Forstgenet. 3(2):39-45.

Duffield, John W. and Albert G. Snow, Jr.
1941. Pollen longevity of Pinus strobus and Pinus resinosa as controlled by humidity and temperature. Amer. Jour. Bot. 28(2):175-177.

Echols, R. M. and Francois Mergen
1956. Germination of slash pine pollen in vitro. For. Sci. 2(4):321-327.

Johnson, L. P. V.
1943. The storage and artificial germination of forest tree pollens. Can. Jour. Res. 21(11):332-342.

Kellerman, Maude
1915. Successful long-distance shipment of citrus pollen. Science ns. 42(1081):375-377.

Righter, F. I.
1939. A simple method of making germination tests of pine pollen. Jour. For. 37:574-576.

Sato, Yoshio and Kazuyoshi Muto
1955. On the viability of forest tree pollen. Res. Bull. Col. Exp. For. Hokkaido U. 17(2):966-979.

Stewart, Donald M.
1956. A vacuum drying process for preservation of Puccinia graminis. Phytopath. 46(4):234-235.

Published as Scientific Journal Series Paper No. 3812, of the Minn. Agr. Exp. Sta.