VACUUM STORAGE OF YELLOW-POPLAR POLLEN

by James R. Wilcox'

Vacuum-drying, followed by storage *in vacuo* or in an inert gas, is effective for storing pollen of many species. It permits storage at room environments without rigid controls of either temperature or humidity, an advantage that becomes paramount during long-distance transfers of pollen when critical storage conditions are impossible to maintain. In the study reported here, the objectives were to learn if vacuum storage is effective for yellow-poplar *(Liriodendron tulipifera L.)* pollen and to determine optimum rehydration conditions.

Methods

Yellow-poplar flowers were removed from trees 1 to 2 days prior to anthesis and placed in containers of water. Early in the morning of the day they were expected to shed, they were brought into the laboratory. The gynoecium, sepals, and petals were removed from each flower, and the ring of anthers attached to the stem was placed on a pane of glass. Laboratory temperatures ranged from 68-78° F and humidities from 55-90 percent. By mid-afternoon the anthers had dehisced and the pollen was shaken free by jarring the anthers against the glass. Fifteen to twenty flowers were required to produce 5 milliliters of pollen.

Approximately 5 milliliters of pollen, without pretreatment, were placed in each of fifteen 25-milliliter ampoules that were immediately attached to the ports of a freeze-dryer (King 1961) and dried for 0.5, 2, and 8 hours. Timing started when the vacuum pump had reduced the pressure to 0.05-0.10 mm. of mercury. At the end of each designated time five ampoules were sealed by melting the necks with a torch. All ampoules were stored in the uncontrolled environment of the laboratory. Two check lots of pollen were simply stored in cotton-stoppered bottles, one in the laboratory and one in a refrigerator at 5° C.

The drying process is referred to as auto-freezing by King (1961) and as vacuum-drying by Layne and Hagedorn (1963). Since no prefreezing was done and subfreezing temperatures of samples, as measured by a thermocouple in one ampoule, were not reached, the terminology of Layne and Hagedorn will be used.

The five ampoules representing each drying period were randomly assigned to five storage periods: 0 days (opened immediately), 45 days, and 3, 6, and 12 months. When the ampoules were opened duplicate pollen samples from each were immediately checked for moisture content and germination percent, and additional samples were rehydrated at 25, 50, and 75 percent relative humidity at room temperature and at 5° C for 16 days. Pollen samples were removed from each of the rehydration treatments at 1, 2, 4, 8, and 16 days and checked for germination. Moisture content was based on initial weight and on dry weight following 1 hour's exposure to 100° C. Pollen was germinated on a medium comprised of 1 percent agar and 10 percent sucrose. Germination percentage was computed from a count of 200 grains per sample after 8 hours' incubation in a moist chamber at laboratory temperature. A grain was counted as germinated if the length of the germ tube exceeded the diameter of the grain.

Results

Immediate effects of treatment. — Vacuum-drying decreased the moisture content from 27 to 4.2 percent in the first half-hour, to 2.8 in 2 hours, and to 1.6 percent in 8 hours. This pattern is in general agreement with results reported for birch (Jensen 1964) and pine pollen (Jensen 1964; Ching and Ching 1964).

Germinability declined as drying proceeded. Initial germination of fresh pollen was 83 percent. This decreased to 20, 17, and 7 percent after 0.5, 2, and 8 hours of vacuum-drying. In contrast, King (1959) reported no decrease in viability of loblolly pine pollen immediately following as much as 3 hours of freeze-drying. Jensen (1964) noted a slight decrease in germinability of pine and birch pollen immediately after vacuum treatment, but no decrease in seed set in controlled pollinations.

Effects of storage. — Germinability of all samples decreased as storage time increased (table 1). Most striking was the drop from 89 to 0 percent of the untreated check stored at room temperature for 45 days. Subsequent tests have shown that fresh pollen loses all viability after 10 to 14 days' storage at room temperature.

Undried samples stored at 5° C germinated better than the evacuated samples stored at room temperatures. After 12 months, samples stored at 5° C still averaged 7 percent germination.

Comparisons among vacuum-dried samples, following exposure to optimum rehydration conditions, indicated that 0.5 hour of drying resulted in the highest germination through 6 months' storage. Germination at the end of 12 months varied from 1 to 2 percent for all vacuum-dried treatments. The decrease in germination over time was more rapid than what Jensen (1964) reported for pine and birch pollen stored under similar conditions.

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Table 1.--Germination of controls and vacuum-dried pollen samples following exposure to optimum recovery conditions (in percent)

Storage time (months)	Controls stored at: 25° C	Controls stored at: 5° C	Vacuum-dried for: 30 minutes	Vacuum-dried for: 2 hours	Vacuum-dried for: 8 hours
0	89	77.5	20.5	17.5	7.0
1.5	0	33.5	21.0	2.5	1.5
3	0	33.0	8.5	2.5	2.5
6	0	19.5	6.5	3.0	2.0
12	0	7.0	.5	1.0	.5

Effects of rehydration. — King (1961) has discussed the importance of environment in the rehydration of vacuum-dried samples. He reported that loblolly pine pollen exposed for 48 hours to 60 percent relative humidity at 5° C germinated four times as well as that cultured immediately after opening.

In the present study rehydration increased germination in varying degrees. Results for samples vacuum-dried for 30 minutes are presented in figure 1. At equal relative humidities, samples rehydrated at 5° C consistently germinated better than those rehydrated at room temperature. Within the two temperature regimes, relative humidity affected the germination percent, particularly at 5° C. Samples exposed for 24 hours to 75 percent relative humidity at 5° C germinated three times as well as non-rehydrated samples. Changes in germination percent after 24 hours appear to be a reflection of storage conditions and demonstrate the importance of using the pollen as soon as possible after rehydration. The samples vacuum-dried for 2 and 8 hours and stored for the various times responded in the same way to the different rehydration conditions.

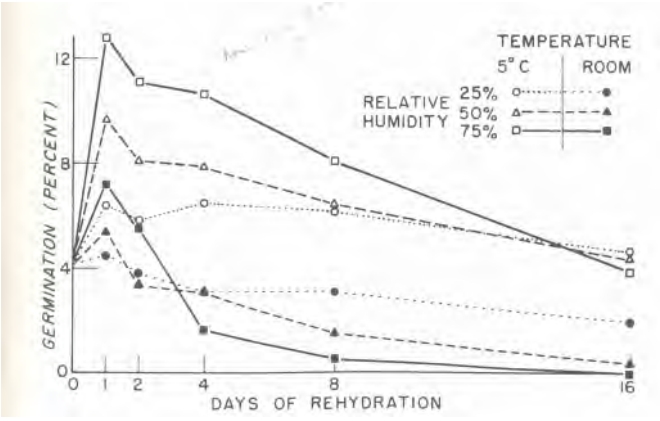

FIGURE 1. — Germination response, following exposure to various rehydration conditions, of pollen vacuum-dried for 30 minutes.

Discussion and Conclusions

While vacuum-drying yellow-poplar pollen permitted storage at room temperature without the rapid and complete loss of viability suffered by undried samples, it nevertheless reduced germinability considerably. Reducing evacuation time or storing dried samples at low temperatures, or both, may alleviate this difficulty and permit shipment of pollen over great distances. Such shipment has been successful for other genera (King 1961, 1965).

Germination of samples dried for 30 minutes was 8.5 percent after 3 months' storage. Although this is a drastic reduction from germinability of fresh pollen, studies with other species (Callaham and Duffield 1961) indicate that it may still be adequate for good seed set from controlled breeding. Pollinations have been made with samples from all the treatments, with checks stored for 1 year, and with fresh pollen.

The relatively high germinability of undried pollen stored at 5° C indicates this is the best storage method of those tested where long-distance transfers are not anticipated.

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