Long-term Storage of Fruit Tree Pollen and Its Application in Breeding

By TOMOYA AKIHAMA,* MITSUO OMURA*
and ITARU KOZAKI**

* Division of Fruit Breeding, Fruit Tree Research Station
** Agriculture, Forestry and Fisheries Research Council Secretariat

It is considered that long-term storage of fruit tree pollen makes hybridizations possible between cultivars differing in flowering time or between plants growing in remote places, with a result of extended utilization of germplasm. In fruit growing, mixed planting of pollinizers at a rate of 10—30% is required to increase productivity in such cases as Japanese pear and apple, or artificial pollinations using the pollen stored for 1 year are practiced. Techniques of long-term storage of pollen may effectively be utilized in these cases.

Research on long-term storage conditions for fruit tree pollen has developed by finding out the optimum conditions for low temperature and low moisture content. King & Hesse (1938) worked with pollen of cherry, plum, apple, quince, etc., Olmo (1942) with grape pollen, and Wakisaka and Hayashi (1966) with Japanese pear pollen.

Recently ultra-low temperature storage has come to be employed for long-term storage of fruit tree pollen, particulary the storage in liquid nitrogen after the freeze-drying of pollen has progressed. Visser (1955) reported that the pollen storage of apple and pear under lower temperature resulted in longer life of pollen, and the storage under low air pressure after freeze-drying was also effective. Kozaki (1975) suggested with peach and pear pollens a possibility of preserving their life for more than 10 years by the storage at —20°C after freeze-drying. Akihama et al. (1978) also investigated in detail the freeze-drying of pollen with a result of firm confidence for long-term storage of pollen.

Freeze-drying of pollen and their life

Germinability of freeze-dried pollen appears to vary markedly, depending on the moisture contents of pollen prior to the freeze-drying, pre-freezing temperature, duration of freeze-drying, and conditions of rehydration, etc.

As given in Table 1, which shows results of Japanese pear pollen, fresh pollen with moisture content of 7.9% gave a high germinability of about 69.5% after the freeze-drying, whereas moistened pollen gave a markedly decreased germinability although its moisture content at the end of the freeze-drying (residual moisture content) was high. This result coincides well that of Ching and Ching (1964). A treatment, pre-freezing, Compared to auto-freezing,

Table 1. Effect of initial moisture content before freeze-drying on germinability and residual moisture content of Japanese pear pollen

Pollen condition	Moistened		Fresh	Dried	
Initial moisture content	31.0%	17.0	7.9	5.6	3.7
Residual moisture content	15.0%	9.8	5.6	4.8	3.6
Germinability after F.-D.*	11.4%	12.8	69.5	71.1	72.4

* Each pollen was pre-freezed at —20°C for 24 hrs, and then freeze-dried (F.-D.) for 30 min

was not apparently effective with Japanese pear, but more or less effective with peach, though not so influential.

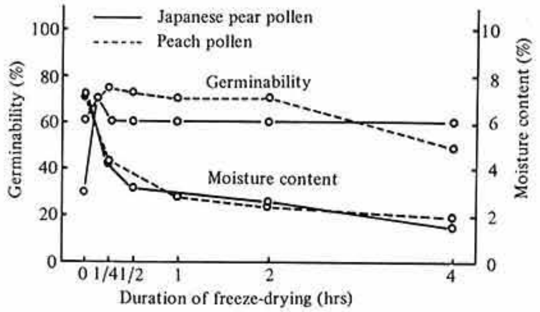

Fig. 1. Effects of freeze-drying duration on residual moisture content and germinability after storage of peach and Japanese pear pollen

As shown in Fig. 1, moisture contents of pollen decreased gradually as the duration of freeze-drying treatment increased, but 1/4 and 1/8 hr durations were most appropriate for peach and Japanese pear pollens, respectively, judged from the germinability after 18 months of storage, although appreciable differences were not observed up to about 2 hrs of duration. This result shows that pollens of peach and Japanese pear are tolerant to long freeze-drying duration as compared with black walnut pollen studied by Hall & Farmer (1964).

To recover the germinability of pollen stored under the dry condition after freeze-drying, it is necessary to consider the conditions of rehydration of pollen. As shown in Figs. 2 and

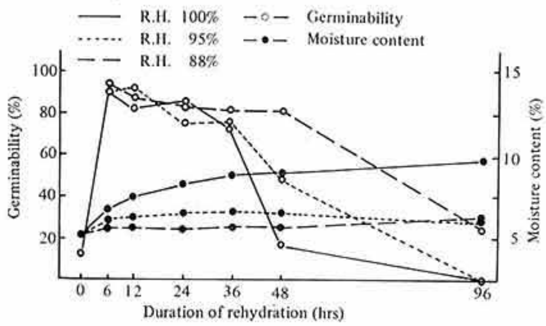

Fig. 2. Changes of moisture content and germinability of freeze-dried peach pollen by rehydration at 20°C in various relative humidity

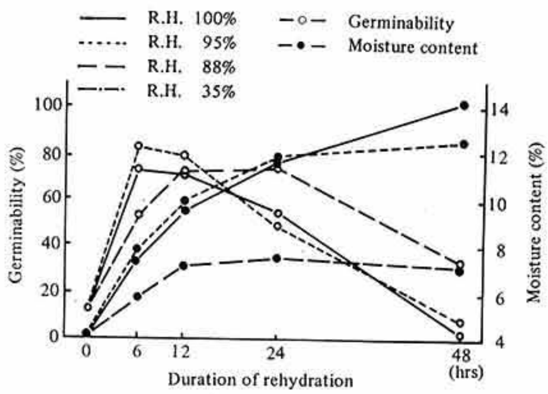

Fig. 3. Changes of moisture content and germinability of freeze-dried Japanese pear pollen by rehydration at 20°C in various relative humidity

3, the germinability was almost recovered after 6 hrs at 20°C, but it decreased rapidly after 48 hrs in the 100% relative humidity plot. It suggests that the rehydration at somewhat low humidity would be desirable to keep the pollen life for longer period after the rehydration.

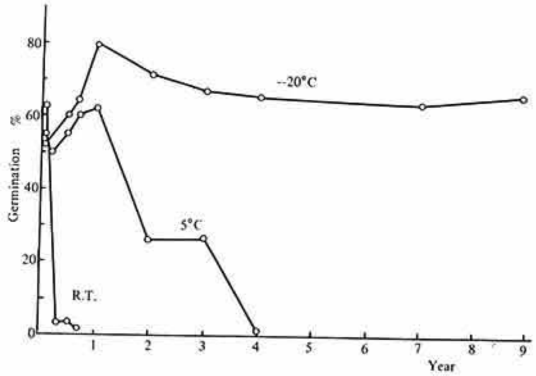

Fig. 4. Germination of freeze-dried peach (Cv. Nishiki) pollen stored under vacuum at −20 °C, 5 °C or room temperature (R.T.)

Fig. 4 shows germination, successively examined, of peach pollen (cultivar Nishiki) that was stored at room temperature, 5°C, or −20°C, after freeze-drying. The pollen lost viability within 1 year at room temperature, and after 4 years at 5°C. On the contrary, when stored at −20°C germinability more than 70% was maintained after 9 years.

Peach and Japanese pear pollen did not show any decrease in germinability when stored for

Table 2. Fruit set by stored Japanese pear (cv. Chojuro) pollen

Pollen	Storage	Germinability	Fruit set % (Seed number) Cultivar of female parent Okusankichi	Bartlett	Yakumo
Fresh	0 year	80%	87% (2.2)	95% (8.3)	90% (6.2)
Freeze-dried	0 year	60	82 (2.6)	68 (7.5)	—
Freeze-dried	3 year at -196°C	84	90 (2.2)	67 (8.7)	86 (6.8)
Freeze-dried	6 years at −20°C	75	76 (2.2)	67 (7.4)	—

more than 4 years at the temperature lower than −20°C under usual dry condition. If the ultra-low temperature storage following the freeze-drying as practiced in this study is employed, almost permanent storage would become possible.

Application of pollen storage to cross-breeding

As it was made clear that pollen life of peach and Japanese pear can be prolonged to long period by adopting low temperature storage associated with freeze-drying, crossing experiments were carried out in the field to know the fertility.

Results of crossing tests with pollen (Japanese pear, cultivar Chōjūrō) stored at low temperature after freeze-drying are shown in Table 2, in which fertility is expressed by fruit set. The pollen stored for 3—6 years in liquid nitrogen (−196°C) or in deep-freezer (−20°C) showed 67—90% of fruit set against three cultivars of female parents, Okusankichi, Bartlett, and Yakumo, indicating no problem in practical use of stored pollen. Table 3 shows fruit set

Table 3. Fruit set by stored peach (cv. Nishiki) pollen

Pollen	Storage	Germinability	Fruit set by self-pollination
Fresh	0 year	90%	30%
Freeze-dried	0 year	73	29
Freeze-dried	9 year at −20°C	82	28

of 28% with pollen (peach cultivar Nishiki) stored for 9 years at −20°C, as compared with 30% for fresh pollen. This result also proves the practicability of stored pollen.

Based on these results, it may be concluded that pollen of Japanese pear and peach can be stored semi-permanently. In Fig. 5, a proce-

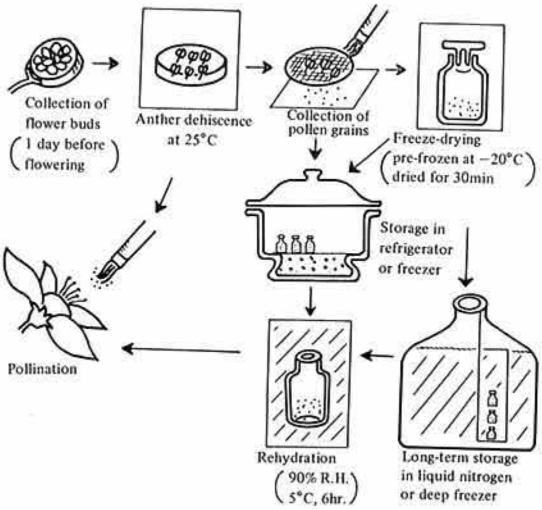

Fig. 5. Method of storage and utilization of fruit tree pollen

dure of the practical method of storage is illustrated. At first, flowers just 1 day prior to anthesis are collected in the field, and anthers gathered are kept in chambers at 25°C for 1 day and night to allow anther dehiscence. Next, the pollen is separated from anthers by sieving. For the usual low temperature storage, pollen thus collected is preserved in glass vials placed in desiccators in refrigerator or freezer. In case when freeze-drying is to be applied, however, pollen is pre-frozen at −20°C, followed by freeze-drying for 30 min, and then stored in glass vials placed in liquid nitrogen. When liquid nitrogen is not available, deep-freezers can be used instead. To use the pollen, thus stored, for pollination, the pollen is subjected to rehydration, using hygrostat, at relative humidity of 90% at 5°C for 6 hrs, considering the simultaneity with expected flowering dates of female parents. The pollen is applied to prebagged flowers using a writing brush.

Based on the success of pollen storage and its use for pollination in Japanese pear and peach, the pollen exchange with foreign countries was initiated in 1977. As the pollen storage requires only small scale facilities (Plate 1), it would not be so difficult to implement so-called pollen bank in future.

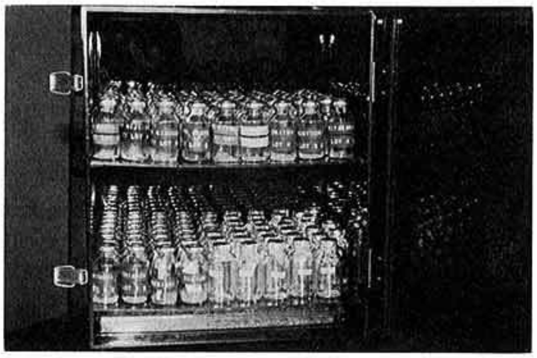

Plate 1. Storage of fruit tree pollen. Freeze-dried pollens are tightly capped in glass vials and stored in a desiccator at ultra-low temperature

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