RESEARCH NOTE

LONG-TERM STORAGE OF TOBACCO POLLEN[1]

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Tobacco pollen was stored for 90 months in four ways. Two storage methods involved use of a desiccant (silica gel) and different storage temperatures and one involved freeze-drying and sealing the pollen in a glass ampoule. In the fourth method pollen was stored at − 17°C without silica gel. All methods kept the pollen at lower than ambient temperatures (5°C or − 17°C) and highly viable for 12 months. The lower temperature methods maintained good pollen viability for 36 months. The freeze-dried method at − 17°C storage maintained the highest percentage viability *in vitro*, but all the low temperature methods preserved pollen viability sufficiently well to produce seed when used in crosses after 90 months of storage. No abnormalities were observed among the seedlings.

Pollen of tobacco (*Nicotiana tabacum* L.) may be stored for pollination of plants when anthesis and stigma receptivity do not coincide. Long term storage of pollen in constant demand for use in plant breeding may be more efficient and economical than production and maintenance of living plants. Tobacco pollen has been stored effectively at low temperatures (0 to − 18°C) and low relative humidity (<5% RH) (2, 6, 7) and has been freeze-dried successfully (6, 7). Pollen of pine, apple, and other fruit and nut trees has been stored at low temperature (− 15°C to − 23°C) for varying lengths of time (3, 4, 5, 8, 9).

A study of the storage of tobacco pollen was initiated in 1969 in an effort to develop a storage method with which pollen can be (a) easily prepared for storage, (b) kept viable for a long period, and (c) maintained with minimal expense or effort. After two years all methods produced viable and functional pollen, but the ability of the pollens to germinate differed (7). Remnant lots of the original pollen were kept under the original conditions and the results of viability tests after seven and one-half years of storage are presented.

MATERIALS AND METHODS

Four methods of pollen storage were tested. Pollen was placed (1) in No. 00 gelatin capsules and stored over silica gel desiccant in a tightly sealed, screw-top glass jar at 5°C; (2) in No. 00 gelatin capsules in a screw-top glass jar and held at − 17°C; (3) in No. 00 gelatin capsules over silica gel in a screw-top glass jar and held at − 17°C; and (4) in ampoules, freeze-dried, and held at − 17°C.

Flowers from the flue-cured cultivar 'NC 95' grown in 1969 in five field replications at the Oxford Tobacco Research Laboratory, Oxford, North Carolina were placed with their basal ends in water until the anthers dehisced. The pollen was allocated at random into four lots, each randomly assigned to a different storage method.

Fully dehisced anthers were removed with forceps so that no filaments remained. In methods 1, 2, and 3 the anthers were placed in a 00 size gelatin capsules, and placed in glass jars. The desiccant in methods 1 and 3 was placed outside the capsules. The desiccant was changed when the relative humidity inside the jar exceeded approximately 20% according to a dye indicator. In method 4 the anthers were placed in 1 ml constricted glass ampoules and immersed in a dry ice-acetone bath (− 78°C) for five minutes. The ampoules were attached to a mechanically refrigerated freeze-dryer unit and the moisture removed under a vacuum of 100 microns (mercury). Twenty minutes after all external frost had disappeared the ampoules were sealed with an air-gas sealing-off torch. In method 1 pollen was stored at 5°C. In methods 2, 3, and 4 pollen was stored at − 17°C.

Viability was determined by germination of the pollen on a boron-sucrose solution as indicated in the earlier report (1). Viability determinations were begun 17 days after pollen preparation (referred to hereafter as initial) and then after 3, 6, 9, 18, 24, 36, and 90 months of storage. After 24 and 90 months of storage the pollen was used in crosses on greenhouse-grown plants. Capsule set and germination of seed from the pollinations were observed.

RESULTS AND DISCUSSION

The germination percentages for the four storage methods are presented graphically in **Fig. 1.** All methods had acceptable germination percentages for 12 months. Methods 2, 3, and 4 gave good germination percentages for 24 months. There was some indication that silica gel was detrimental to viability, as

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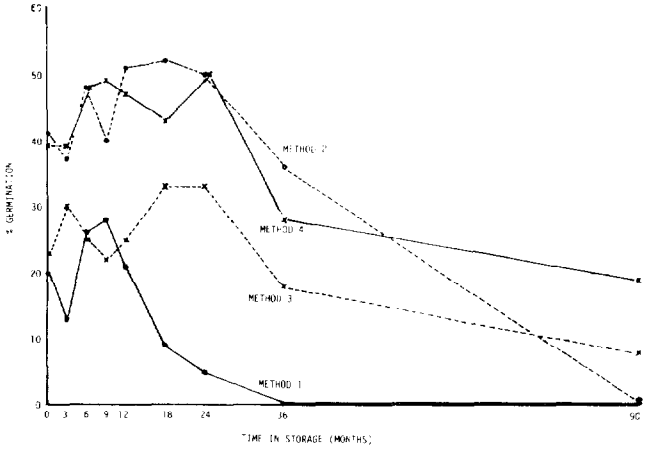

Fig. 1. Germination percentages of tobacco pollen after various periods of time under several methods of storage.

shown by the lower percentages of method 3 compared to method 2. Over the entire period the highest percentage germination resulted from method 4. Method 3 at − 17°C had higher germination percentage than method 1 at 5°C.

Remnant 90-month pollen was used in crosses on greenhouse plants and compared to fresh pollen from greenhouse plants as well as pollen that had been stored for 7 months under conditions of method 2. All lots of pollen caused capsules to set but the capsules formed using pollen from method 1 contained no seed. The percentage seed germination from crosses using the remaining lots of pollen was above 90% with the exception of seed from lot 2 (pollen storage method 2) which was 56%.

These results indicate that tobacco pollen can be stored for periods of twelve months under either of the four methods tested. Methods 2, 3, and 4 may be used for 24 months. Longer storage may be achieved by use of a technique such as method 4 in which the moisture level around the pollen is kept consistently low. Method 3 can also be used for longer-term storage but methods using silica gel require that the silica gel be changed periodically in order to prevent moisture variation. The greatest advantage of methods such as 2 and 3 is that they are easier to prepare and do not require the use of freeze-drying techniques.

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