Research Note No. 3

Pollen Storage in Tobacco

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Table 1. Average percent germination and capsule set of tobacco pollen treated in different ways.

Preservative Treatment	Storage Conditions	Percent Germination and Capsule Set After 7 days	Cap. Set	30 days	Cap. Set	71 days	Cap. Set	388 days	Cap. Set
Dry Ice and Acetone	Laboratory	27(50)[a]	+[b]	13(27)	+			0	—
	Freezer at −18°C							73(68)	+
Liquid Nitrogen Gelatin Capsule	Laboratory Refrigerator	15(50)	+	12(27)	+	4	+	0	—

[a] *Values in parentheses indicate percent germination of fresh pollen from greenhouse used as a check without preservative treatment or storage*

[b] *+ capsule set; — no capsule set*

Methods of storing tobacco pollen (*Nicotiana tabacum L.*) have been reported in the literature. Dean (1) reported that tobacco pollen stored in Petri dishes at 0°C and less than 5 percent relative humidity was viable and had a germination percentage of 21.5 after 136 weeks. Jensen (3) reported germination and seed set for *Nicotiana glutinosa* pollen after storage of 30 months. He freeze-dried the pollen under vacuum and stored it under sealed conditions at 5°C. King (4) reported the results of freeze-drying pollen of several plant species.

Tobacco pollen is collected at Oxford by removing flowers with unopened corollas and placing the basal ends of the flowers in tap water. Approximately 12 hours later the anthers are well dehised and ready for use for crossing or storage. Pollen is routinely stored by placing the dehised anthers into No. 00 gelatin capsules and storing the capsules in a tightly sealed screw-top jar containing a desiccant. The desiccant is changed in the jars whenever an indicator packet shows that the relative humidity exceeds 20 percent. The storage jars are kept in a household refrigerator at 5°C. This method of storage has satisfactorily maintained viability for periods of 36 months but the desiccant must be changed periodically and the percent germination [Dean (2)] of the pollen drops to a rather low level compared to fresh pollen. In spite of the low percentage germination stored pollen provides a full capsule set of viable seed when used in crossing.

An investigation was undertaken at the Oxford Tobacco Research Station, Oxford, North Carolina in 1966 to determine whether pollen of flue-cured tobacco could survive freeze-drying and the effects of storage following this treatment. Pollen was collected from greenhouse plants and after the anthers were well dehised they were placed in 1 ml. constricted glass ampoules. Two methods were used to quick-freeze the pollen. One method involved pouring liquid nitrogen (−195°C) onto the pollen, and the other involved immersing the base of the ampoule in a dry ice-acetone bath (−78°C) for five min-

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utes. Following freezing the ampoules were attached to a mechanically refrigerated freeze-dry unit and the water vapor sublimed off under a vacuum of 100 microns (mercury). The end point was taken as that time when all external frost had disappeared from the ampoule plus 15 minutes. The ampoules were sealed with an air-gas sealing-off torch.

The most useful storage procedure would be to store the sealed ampoules under room conditions with no particular attention to storage environment. This was the storage procedure for most of the material, however, one lot was stored at −18°C in a freezer. The results of the freeze-dried pollen together with fresh pollen and pollen stored in gelatin capsules as checks may be seen in **Table 1.** The results shown in **Table 1** indicate that tobacco pollen can survive freeze-drying with either dry ice and acetone or liquid nitrogen. This is shown by ability of the pollen to germinate *in vitro* and to set seed when used in crossing. The pollen maintained its viability when stored under laboratory conditions for as long as 30 days but not as long as 388 days. However the single lot that was freeze-dried and stored at −18°C did maintain high germinability *in vitro* and set seed capsules after storage of 388 days. Further investigation is planned.

Literature Cited

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