STORAGE OF SUNFLOWER POLLEN

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INTRODUCTION

Plant breeders are often faced with the problem of how to overcome limitations to crossing if the flowering time of the parents do not coincide. Moreover, the maintenance of genetic variability is one of the most crucial tasks of modern plant breeding.

The need to increase world food production gives ever greater importance to co-operation between research teams, but the legal restrictions designed to protect the breeder's variety rights obstruct the free exchange of seed, as do plant hygiene measures.

The quarantine regulations are justified by the fact that certain diseases are carried and transmitted by seed. At present, legal protection is only extended to state registered varieties and lines, yet if co-operation is to be successful there should be an exchange of genes at an early stage of breeding, so that rapid and direct use can be made of the genes in a given crossing programme with the guaranteed priority of the donor. But this is yet to be achieved in practice.

For sunflower, which will be one of the most important food and energy crops of the future, the breeding process has accelerated leading to new possibilities of hybridization. In the course of this work, co-operation with distant research institutions raised the necessity of storing and exchanging pollen.

There is extensive literature on the storage and germination of pollen, dating back to 1913. This is well illustrated by the monograph published by Johri and Vasil (1961). However, sunflower is not included among the plants listed.

In recent years the physiological differences between binucleate and trinucleate pollen grains have been studied (Hoekstra and Bruinsma, 1975; Johri and Shivanna, 1977) and it has been found that trinucleate pollen has a shorter lifespan, since its metabolism is more intense and the stored nutrients are exhausted within a shorter time. The pollen of species belonging to the Compositae is of this type. In investigations carried out by Werfft (1951) the pollen of 16 species, stored at 19—32°C for 21—71 days, retained its germination ability, but the species used were all known to be easy to store. Maize pollen, on the other hand, is extremely difficult to store, but recently Barnabás and Rajki (1976) managed to store it for more than a year by deep-freezing at —196°C without loss of viability.

A single publication is available which makes any mention of sunflower. According to this paper Arnoldova kept sunflower pollen in the laboratory for 380 days and obtained seed setting when the pollen was used for crossing (Doroshenko, 1928). Experience shows that this result cannot be even approximately reproduced.

MATERIAL AND METHOD

The storage experiments were carried out on pollen collected from the Hungarian sunflower variety GK-70. The pollen brush developed at Szeged was used for the large-scale collection of pollen (Lajkó, 1981). The pollen originated from plant individuals grown under field conditions at Kiszombor. Samples were collected in 1977, 1979 and 1981 and were all stored until July 1981 using the deep-freezing method elaborated at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár.

As a first step, the water content of the sieved pollen was determined. If this exceeded 30% on a fresh weight basis, the water content of the pollen was reduced to around 20% by blowing dry air through it.

The water content was calculated by measuring the water loss incurred when 0.1 g samples were dried at 105°C to constant weight.

Pollen dried to a suitable value was put into 2 cm^3 polyethylene ampoules which could be sealed.

Some of the samples were stored in a deep-freezer at —76°C, while the remainder was placed in Dewar vessels containing liquid nitrogen at —196°C.

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In the course of the experiments the ability of the pollen to produce pollen tubes, one of the most reliable signs of viability, was regularly checked. This was necessary in order to obtain information on the probability of successful crossing. Pollen tube growth was carried out in 20—30% saccharose solution containing 0.01% boric acid and 0.001% $CaCl_2 \cdot 2H_2O$. Only samples where the pollen tube growth was satisfactory were used for crossing.

The pollen used in the crosses was stored under the conditions described above for 1 449, 733, 106 or 77 days. Investigations were also made on how the environmental conditions generally ensuing before and after storage (in an exciccator at 5°C above anhydrous $CaCl_2$) influence the fertilization ability of sunflower pollen.

The fertilization ability of the pollen was tested in crosses carried out on inflorescences of the CH—O cytoplasmic male sterile maternal line which were isolated in ethamine bags. In each treatment the number of achenes is the average for 5 inflorescences. Inflorescences where the first six rows were in flower were used in each of the crosses, which were carried out on July 22nd 1981, or after a period of 24,48 or 216 hours from this date in the Kiszombor nursery by brushing on a uniform quantity of pollen.

RESULTS

The experiments were aimed at simplifying the execution of crossing programmes both within a single institute and in co-operation. The elaboration of methods for the collection of a large quantity of pollen and for storing the pollen over long periods facilitated the approach to the question.

Previous experience showed that sunflower pollen retained its fertilization ability for 20—25 days without any significant damage when kept at 4—5°C with a relative humidity of less than 40%. This time period is long enough to allow the pollen to be transported.

In the present experiments a method of storing pollen for longer periods was sought.

In the course of the experiments temperatures of —76°C and —196°C were chosen in the hope that a definite longterm storage temperature could be determined for practical use. When deep-freezing lasted for 106—1 449 days, a temperature of —76°C was used, while —196°C was only applied for the shorter 77 days storage period, since storage in liquid nitrogen is more expensive. This method will only be used if the previous storage conditions give negative results.

The most reliable indication of the successful storage of sunflower pollen proved to be a fertilization ability test, which can be expressed as the number of viable achenes formed in the inflorescence of male sterile sunflowers (Table 1). The data in Table 1 show that even without the use of liquid nitrogen, sunflower pollen can be successfully stored for years at —76°C.

Table 1

Effect of storage period and temperature on the fertilization ability of sunflower pollen.

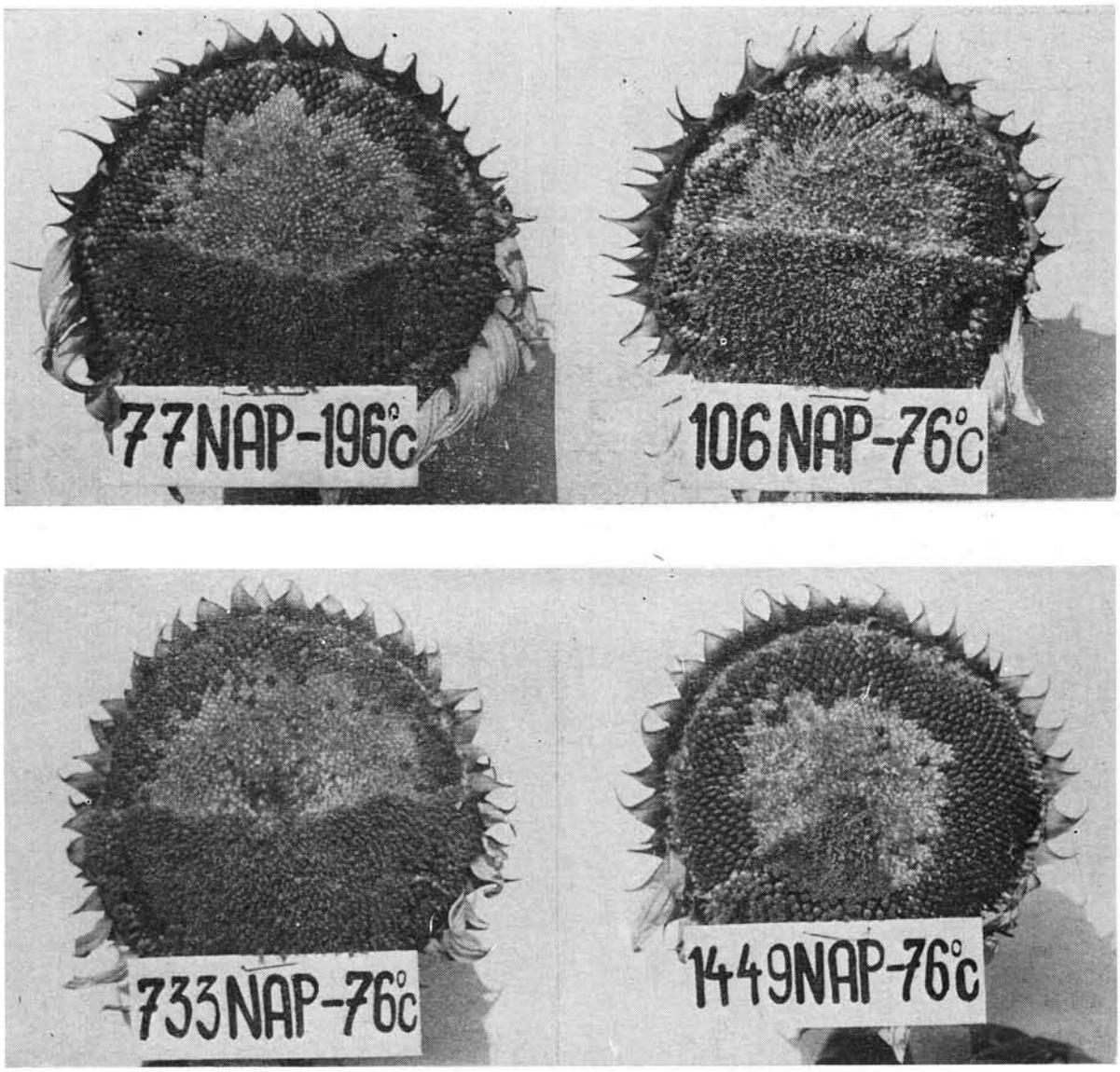
Storage period (days)	No. of achenes/inflorescence	
	Storage temperature : —76°C	Storage temperature : —196°C
77	—	751
106	196	—
733	579	—
1 449	800	—

It can be seen from the table that the long storage period did not reduced the viability of the pollen ; in fact, deep-frozen pollen proved to be more fertile. It would seem that at such low storage temperatures the length of storage has no effect on pollen viability. The differences observed in the experiment can probably be attributed to the effect of variations in the environmental factors during the formation and collection of the pollen, since the various pollen samples were collected at different times.

After storage for years at —76°C or —196°C sunflower pollen can be used by breeders in crossing programmes. Figure 1 gives a visual demonstration of the success of crossing, as after fertilization the dark tones of the normally filled achenes are easily distinguished from the lighter gaps caused by empty grains and from the lighter bands on the control flowers where no pollen was applied.

Investigations were also conducted on a problem which often arises in practice : namely, what effect storage at 5°C either before or after freezing has on the fertilization ability. This situation is met with when the pollen is not collected or utilized for crossing in the same place where it is deep-frozen, thus making it necessary to transport it over a certain distance.

The data of Table 2 indicate that the best results are obtained when the pollen is utilized immediately, but even samples kept at 5°C for 216 hours after deep-freezing could still be used successfully in crossing.



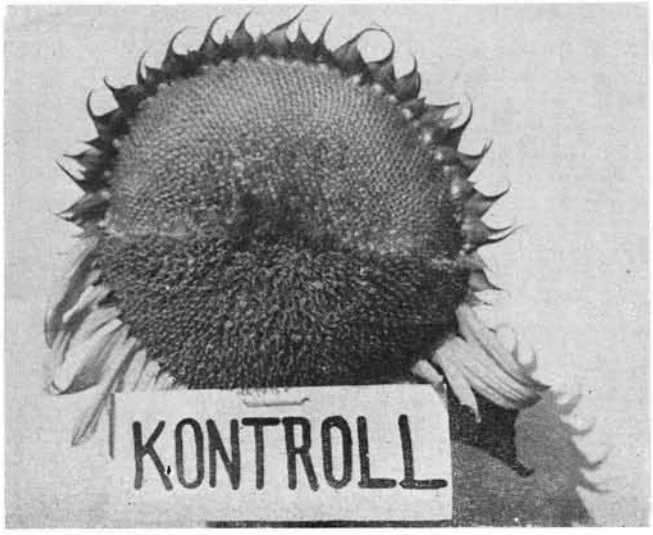


Fig. 1 — Sunflower inflorescences fertilized with pollen deep-frozen for various lengths of time.

Table 2

Correlation between length of storage at 5°C before and after deep-freezing and the fertilization ability of the pollen

Storage period after deep-freezing (hours)	No. of achenes/inflorescence				
	deep-frozen at —196°C for 77 days			deep-frozen at —76°C	
	storage period prior to deep-freezing (hours)			freezing period (days)	
	24	48	72	1 449	733
4	751	778	561	800	579
24	254	930	703	237	176
48	—	260	149	560	236
216	265	467	349	481	287

CONCLUSIONS

1. The experimental results show that sunflower pollen can be stored at —76°C or —196°C and can be successfully used in breeding even after approximately 4 years.

2. Storage for several days at 5°C and low relative humidity prior to freezing has no significant effect on the success of crossing.

3. The possibility of storage at 5°C after deep-freezing facilitates the transportation of the pollen, since even after 9 days 265—467 achenes per inflorescence were obtained for a single crossing, averaged over the treatments.

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LA CONSERVATION DU POLLEN DE TOURNESOL

Résumé

A des températures de —76°C à —196°C, le pollen de la variété-population de tournesol GK-70 garde la fertilité et la viabilité pendant une période assez longue, pouvant atteindre même 4 ans. Si avant la congélation le pollen est gardé pendant quelques jours à 5°C à une humidité relativement réduite, les résultats de la pollinisation n'en sont pas significativement améliorés. Les meilleurs résultats sont obtenus lorsque le pollen est utilisé immédiatement après avoir été congelé, mais il est possible d'obtenir une bonne fécondation avec du pollen exposé à une température de 5°C pour une période plus ou moins longue, même de 216 heures.

CONSERVACIÓN DEL POLENO DE GIRASOL

Resúmen

Fue estudiado el poleno de la variedad — población de girasol GK-70. Los resultados obtenidos han mostrado que éste, a las temperaturas —76°C 0—196°C mantiene su fertilidad y viabilidad durante un largo período de tiempo, hasta cuatro años. La conservación durante algunos días a 5°C y la humedad relativamente reducida, antes de la congelación, no tuvo un efecto significativo sobre los resultados de la polenización.

Los mejores resultados se obtienen cuando el poleno se usa inmediatamente después de la congelación, pero una buena fecundación se puede realisar también con poleno a la vez guardado a 5°C después de la congelación, incluso durante 216 horas.