Storage of Grape Pollen

By H. P. Olmo, *University of California, Davis, Calif.*

A NUMBER of investigators have demonstrated that the longevity of deciduous fruit pollens may be prolonged from 1 to 5 years or possibly more by regulation of the temperature and humidity during storage. Wanner (7) has discussed the earlier European work, previous to 1934, on the germinability of grape pollen as related to longevity. The important conclusions that concern us may be briefly summarized. Grape pollen germinates well as much as a week after collection under the usual laboratory conditions, but no germination has been demonstrated even 6 months later. An exceptional case reported by Lorbeer was a sample (1925) of Rupestris du Lot pollen stored over calcium chloride that gave 25 per cent germination after 13 months, but attempts to repeat this result during the next two years failed. Storage over concentrated sulfuric acid resulted in a very rapid loss in viability, the percentage germination decreasing over one-half in a few days. This loss in viability was associated with a decrease in pollen tube length in vitro. Wanner's recommendation to grape breeders is to employ only fresh pollen, in view of its rapid loss in germinability. In 1937 Nebel and Ruttle (5) reported successful storage of two grape pollens, the Concord and Golden Muscat, which retained their viability after being held for one year at 8 to 10 degrees C and 50 per cent humidity.

A considerable saving in time and effort might be effected if samples could be stored in a "pollen bank" and used as needed. The value of such a method becomes especially apparent if varieties blossom at different periods, if pollen must be obtained from another locality, or if the same variety is used frequently in hybridization. From the standpoint of the grape breeder the successful establishment of a pollen bank would require that the samples retain their viability for at least a year, preferably longer, and that normal seedlings would be obtained from the use of such pollen. It would be an added advantage if the same pollen sample could be used for more than a single season's work.

Methods

Three varieties of *Vitis vinifera* L. were used: the Muscat of Alexandria, Thompson Seedless (Sultanina), and Monukka. Massed pollen samples were obtained June 2, 1938, from 20 clusters of each variety. The clusters were in one-half blossom stage and each was taken from a different vine. The pollen is easily gathered by striking the clusters upon a piece of plate glass. The pollen adheres to the surface and the extraneous flower parts are blown off. Using a razor blade, the pollen is then swept together on the glass surface and finally scraped over the projecting side of the plate into small shell vials or in druggist's gelatin capsules. This method of collection yields a uniform and clean pollen sample.

Four storage temperatures were used: –12 degrees C, 2 degrees C, 10 degrees C, and room temperature (20 degrees C). During the

course of the experiments fluctuations occurred for short time periods, the temperature ranges can be given as −11 to −12 degrees C, 0 to 2 degrees C, 9 to 14 degrees C, and 20 to 27 degrees C. Calculated relative humidities of 25 and 50 per cent were maintained with sulfuric acid-water mixtures, with the exception of −12 degrees C storage where the humidities maintained were approximately 28 per cent and 54 per cent.

Once each year during the pollination season (end of May) the sealed containers were taken from storage. They were left overnight in the laboratory to reach room temperature. The vials of pollen were then removed, taken to the field, and the samples used in pollinating, just as in routine breeding operations. As a test of the fertilization capacity of the pollen, pollinations were usually made on male sterile varieties as did Wanner (7), thus eliminating the need of emasculation or possibility of selfing of the female variety. Pollinations made in 1939 were on the variety Saint Emilion after emasculation, and these results must be considered less reliable owing to the effect of emasculation, age of flowers used, and possibility of some selfing. However, in 1940, 1941, and 1942 male sterile varieties were used. The technique was further refined by placing several of the pollens on portions of the same cluster, 50 to 200 flowers being pollinated with each sample. On the same afternoon, as soon as the pollens were brought back from the field, germination tests were made in 20 per cent sucrose solution, using the hanging drop method. This concentration was found to be optimum for grape pollen by Winkler (8). Three slides were cultured of each sample, 200 to 300 grains were counted on each slide. Only those grains that had tubes at least three times their diameter were considered as germinated. All results are given as the average per cent of germination after 6 hours at 25 to 26 degrees C.

Discussion

The effect of the different storage treatments on the germinability of the pollen is presented in Table I. The most striking result is that all three varieties have shown the greatest longevity at a temperature well below freezing and at the lower humidity of 28 per cent. At the end of four years the Monukka sample has only decreased from 34 to 13 per cent, but the decline in the other two varieties is more marked. The most rapid fall in germinability is shown by the pollen having the highest initial germination, whereas Monukka with the greatest longevity to date had the lowest viability at the start of the experiment. This might indicate that pollens of a high initial reactivity are apt to decline more rapidly in viability. At 2 degrees C the lower humidity appears to add about a year to the longevity of the pollen when compared with 50 per cent humidity. Thus the germination percentage of the sample at 50 per cent humidity at the end of the first year is about that of the 25 per cent sample at the end of the second year. At the temperature of 10 degrees C the pollen remains viable for less than two years under the most favorable humidity. At room temperature, even with controlled humidity, the pollens do not survive a single year.

TABLE I—Per Cent Pollen Germination after Various Storage Treatments

Duration of Storage Period	Temperature and Humidity							
	−12* / 28	−12 / 54	2 / 25	2 / 50	10 / 25	10 / 50	Room / 25	Room / 50
Thompson Seedless								
Initial	44	44	44	44	44	44	44	44
1 year	35	26	6	8	14	<1	0	0
2 years	35	24	7	0	0	0	0	0
3 years	12	2	0	0	0	—	—	—
4 years	9	0	<1	0	—	—	—	—
Muscat of Alexandria								
Initial	57	57	57	57	57	57	57	57
1 year	29	21	16	12	8	<1	0	0
2 years	—	17	8	0	0	0	0	0
3 years	10	1	1	0	0	0	—	—
4 years	6	0	1	0	0	—	—	—
Monukka								
Initial	34	34	34	34	34	34	34	34
1 year	35	31	19	8	8	0	0	<1
2 years	23	26	6	0	0	—	—	—
3 years	—	3	0	<1	0	—	—	—
4 years	21	0	<1	0	0	—	—	—

*The upper number refers to temperature of storage in degrees C, and the lower to the per cent relative humidity.

The results above do not agree entirely with the optimum temperature and humidity conditions that have been reported for deciduous fruit pollens. Nebel (4, 5) has stated the optimum conditions for a number of pollens to be about 2 to 8 eight degrees C maintained in desiccators over sulfuric acid opened every 6 months with no control of the atmosphere and without light. Nebel, however, was judging from the results published by Holman and Brubaker (1), Pfundt (6), and Knowlton (3), and from the fact that he was able to hold pollen at the above-reported "optimum" conditions for a long period of time. Thus a sample of Montmorency cherry was showing 2 per cent germination after 5½ years. As Nebel (4) points out, the optimum humidity might be lower than 50 per cent. However, no temperatures lower than 2 degrees C were employed. King and Hesse (2), with as many as 16 pollens of deciduous fruit trees, gave the optimum conditions after 550 days to approximate 36 degrees F (2 degrees C) and 25 per cent relative humidity (the latter quoted by Nebel (4) as 28 per cent). Their experiments are not yet concluded.

They report storing pollen at 10 degrees F in one of the same storage rooms that was used in the present experiment, but without any control of the humidity. The humidity control even at temperatures below freezing is very important. It is probable that longevity of fruit pollens — unless grape pollen acts in a far different manner — may be further prolonged by storage at temperatures below freezing and perhaps even at lower humidities than we have so far used for grape pollens. This is not an unreasonable supposition, since if pollens are not killed by low temperatures the rate of respiration would be kept lower and the pollen might be expected to have a longer life. In this

regard Knowlton (3) found that Antirrhinum pollen remains viable longest under conditions of low temperature (0 to –17 degrees C).

Some authors (1, 3) have indicated that even though pollen is able to germinate in vitro, fertilization and seed production may fail. Whenever possible the fertilization capacity of stored samples was checked with freshly gathered pollen of the same variety. The data are presented in Table II.

TABLE II—PER CENT SET OF SEEDED BERRIES OBTAINED WITH FRESH AND STORED POLLENS

Date of Pollination	Age of Pollen (Years)	Fresh	−12* / 28	−12 / 54	2 / 25	2 / 50	10 / 25	10 / 50
Sultanina								
May 26, 1939	1	17	8	7	23	3	26	0
May 22, 1940	2	31	37	11	16	0	—	0
May 26, 1941	3	54	57	0	0	1	—	—
May 30, 1942	4	24	24	0	0	—	—	—
Muscat of Alexandria								
May 26, 1939	1	—	3	—	—	—	23	0
May 22, 1940	2	11	25	—	12	8	—	0
May 26, 1941	3	65	48	0	0	1	—	—
May 30, 1942	4	23	22	0	0	0	—	—
Monukka								
May 26, 1939	1	16	12	—	15	13	26	—
May 22, 1940	2	40	41	—	22	0	—	—
May 26, 1941	3	67	59	0	—	0	0	0
May 30, 1942	4	22	16	0	0	0	—	—

*The upper number is temperature in degrees C, the lower the per cent relative humidity.

The berries with seeds were harvested and the set expressed as a percentage of the number of flowers pollinated. These results must be compared with the normal sets obtained with the use of fresh pollen on the same variety for the year in question. One can then compare the pollen germination in vitro as given in Table I with the fertilization capacity of the same sample. In only a single instance has a pollen sample that was considered wholly inviable by germination test resulted in a fertilization; this was a sample of Thompson Seedless stored at 2/50 and used after 3 years. Even here the berry set was only 2 per cent of the set obtained with fresh pollen.

When pollens showing germinations in vitro of 1 to 3 per cent are used in pollination they practically always fail to bring about fertilization. However, pollens producing as few as 6 to 8 per cent of viable grains in culture may produce sets that approach or occasionally exceed the normal sets. Thus the sample of Thompson Seedless 2/25, which after 2 years showed 6 per cent germination produced a set 135 per cent of normal, and the sample of Monukka 10/25 after 1 year in storage produced a set of 162 per cent of normal. By further comparisons of germinability in vitro and fertilization capacity it is obvious that there is practically no correlation between berry set and the relative germinability of pollen, providing the pollen gives more than 4 or 5 per cent germination. Expressed in another way: a pollen sample with a high average germinability might be no more successful in accomplishing fertilization than a pollen sample of medium or low viability. These conclusions would agree with the experiments performed by Wanner (7) who falied to demonstrate that high yields of individual vines were associated with high viability of the pollen.

For practical purposes the grape breeder in making routine crosses should hardly rely on pollen samples of less than 6 per cent germination. It would appear, therefore, (Table I) that under the best storage conditions reported herein it would be risky to expect a pollen sample to remain useful for much more than 4 years.

Under the best storage conditions of this experiment, namely, –12 degrees C and approximately 28 per cent relative humidity, the pollens have retained sufficient viability to enable them to be used just as well as fresh pollens. Thus the percentage berry sets of 4-year-old pollen are practically normal. Seedlings grown from using 3-year-old pollen thus far appear equal in growth and as normal as those arising from pollinations with fresh pollen. It will of course be necessary to bring progenies to fruiting before one can be certain that no genetic changes of consequence have been produced by the aging of such pollen samples.

SUMMARY

Pollen samples of three varieties of *Vitis vinifera* L. were stored under temperatures of –12, 2, 10, and 20 degrees C and confined in relative humidities of 25 and 50 per cent, with the exception of the –12 degrees C treatment held at relative humidities of 28 and 54 per cent.

Pollen longevity was increased most markedly at the lowest temperature, –12 degrees C, and the lowest humidity, 28 per cent. A sample of Monukka pollen with initial germination of 34 per cent showed 21 per cent viability after 4 years in storage.

It has been demonstrated that the fertilization capacity of a given pollen is practically nil if the pollen shows only 0 to 3 per cent germination in vitro. Pollens of 6 per cent or higher viability may give berry sets equivalent to normal. Pollens stored under the best conditions reported and used in pollinations each year gave berry sets equivalent to fresh pollen after 4 years.

The results reported indicate that the supposed optimum conditions of temperature and humidity for the storage of deciduous fruit pollens may be even lower than those so far reported.

It appears entirely feasible to establish a "pollen bank" in which the breeder may use samples each pollination season as needed, then returning them to storage for future use.

Seedlings grown from the use of 3-year-old pollen appear equal in growth and as normal as those hybrids produced with fresh pollen.

LITERATURE CITED

1. HOLMAN, R. M., and BRUBAKER, F. On the longevity of pollen. *Univ. Calif. Pub. in Botany* 13: 179–204. 1926.
2. KING, J. R., and HESSE, C. O. Pollen longevity studies with deciduous fruits. *Proc. Amer. Soc. Hort. Sci.* 36: 310–313. 1939.
3. KNOWLTON, H. E. Studies in pollen, with special reference to longevity. *Cornell Univ. Agr. Exp. Sta. Mem.* 52. 1922.