Studies on the longevity of stored pine pollen (*Pinus silvestris* L. and *Pinus contorta* var. *Murrayana* Engelm.)

Lagringsduglighet hos tallpollen

by

CARIN EKLUNDH EHRENBERG

MEDDELANDEN FRÅN
STATENS SKOGSFORSKNINGSINSTITUT
BAND 49 · NR 7

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Introduction

For the purpose of making crosses between trees flowering at different times of the spring or in different years, pollen sometimes has to be collected one year and not used until the next flowering season or later. Thus it is of importance to store pollen in such a way that the highest possible viability is retained for long periods. The methods used for extracting pollen as well as temperature and humidity conditions during storage can influence the longevity of pollen. In some species, for instance some members of the Graminae, the longevity of the pollen is very short and it looses its germinability within a few hours or a few days. Pine pollen on the contrary is relatively durable and remains viable for months even when stored at room temperature (CUMMING and RIGHTER 1948). Keeping the germinability high during longer periods requires, however, special storage conditions.

The present paper deals with some experiments carried out during the years 1952 to 1959 with pine pollen. The germinability of fresh pollen as well as of pollen stored during one to seven years at different temperatures was examined in laboratory tests. Furthermore three year old pollen was used in crossing experiments on two different occasions.

The methods used here give rise to rather high sampling errors owing to difficulties in carrying out the laboratory tests in exactly the same way every time, to fungi infections in the pollen-water mixture and so on. However, having as an end to give information about the possibilities of storing pine pollen during a longer period with the viability and fertilization capacity intact, the experiments have been considered reliable enough to alow some conclusions.

Material and methods

In 1952 three different pollen samples were harvested and stored. One lot consisted of mixed pollen from several trees of *Pinus contorta* var. *Murrayana* (M), and a second lot consisted of mixed pollen from some *Pinus silvestris* trees (B). The third lot was taken from an individual *Pinus silvestris* tree, Poltava 1 (P). The trees are growing in the park surrounding the Forest Research Institute, Stockholm. The catkins were collected immediately before the release of pollen, placed on a piece of paper at room temperature, and left

there till the pollen had shed from the catkins and could be transferred to glass vials. The glass vials with pollen were kept in a desiccator over silica gel with cobolt indicator at + 4—6° C and a relative humidity of about 0 % for some weeks. After that the pollen was transferred to glass ampoules (28 ml volume), which were closed by melting together the walls of the elongated end of the ampoule. Each kind of pollen was divided into six ampoules, three of which were completely filled with pollen (a), the other three only filled to the half of the volume (b). They were distributed in pairs to three different storage conditions: room temperature, + 4—6° C in a refrigerator (I) and — 18° C in a deep freeze (II). When pollen should be used in germination tests, the ampoules were cautiously opened at the elongated end, a sufficiently large amount of pollen for one test was poured out on the culture medium, and the ampoules were then immediately reclosed and put back in the storage-place.

In June 1954 two new pollen samples of *Pinus silvestris* were included in the experiment, namely pollen from the pines S:21 Dalecarlia, Lat 10°55' and 11: 18, Gästrikland, Lat 60° 56'. The extraction of pollen from the catkins was carried out in the same way as previously described, and the pollen was put into small glass ampoules (3 ml volume) and stored at + 4° C and — 18° C. At the same time the pollen samples from 1952 were transferred from the original big ampoules to several small ones, one such little ampoule being used at every germination or pollination test.

Samples of fresh pollen were dusted on a slide, stained with aceto-carmine and examined for the percentage of "good grains". Only those grains which were evenly and well stained were classified as good.

Different methods of germinating pollen were tried, for instance agar gel or distilled water with various sugar concentrations as substrate. However, distilled water without any sugar gave as good or nearly as good results as regards germination per cent as any of the other media (cf. LIDFORSS 1896, RIGHTER 1939, DENGLER and SCAMONI 1939) and the use of this medium was finally chosen as a standard method. This method was as follows: about half a cm^3 of distilled water was poured into an 8 mm deep, rounded cavity of a thick glass dish, 5 × 5 cm square and 1 cm high. The pollen was dusted as a thin layer on the water surface, and the dish was covered with a thin glass slide which served as a tightly fitting lid. In 1952 and 1953 the pollen was germinated at room temperature, but from 1954 and onwards the dishes were incubated in darkness in a thermostat at about + 30° C, since these culture conditions enhance germination of old pollen. With a pipette, samples were taken from each dish after 24, 48 and 72 hours and in some experiments, after 12 and 96 hours as well. These samples were stained with aceto-carmine and in each case about 200 pollen grains were examined

microscopically to determine the per cent germination. To obtain mean pollen tube lengths, ten tubes were chosen at random in each sample and the contours drawn on a paper with the aid of a camera lucida. The means given for each sample are in units, 1 unit equalling 2.7 μ. The number of pollen tubes with an abnormal form, such as forked or bag-shaped or bursted tubes, was recorded.

In the crossing experiments with fresh and stored pollen the same methods were used as previously described by EHRENBERG and SIMAK (1957). The first crossings were made in 1955. Some grafts, four to nine years old, were used as mother trees. Pollen from the mixed lot of *Pinus silvestris* (B), stored in 1952, and from the pines S: 21 and 11: 18 stored in 1954, were included in the experiment. On the grafts some flowers were left unisolated to allow open pollination. The cones were harvested in November 1956.

The second crossing experiment was carried out in 1957 with pollen from the pines S: 21 and 11: 18 and with fresh pollen from the three pines chosen as mother trees (Poltava 1, Korpilombolo 6 and 7). These pines, about 30 years old, had previously been analysed for their cone- and seed-setting characteristics after crossing and open pollination.

The analysis of the seed was made by the X-ray method described by SIMAK and GUSTAFSSON (1953).

The water content in stored pollen was analysed in 1959. The pollen samples (about 50 mg) were dried to constant weight at room temperature over silica gel under reduced pressure (0.1—0.2 mm Hg).

Results and discussion

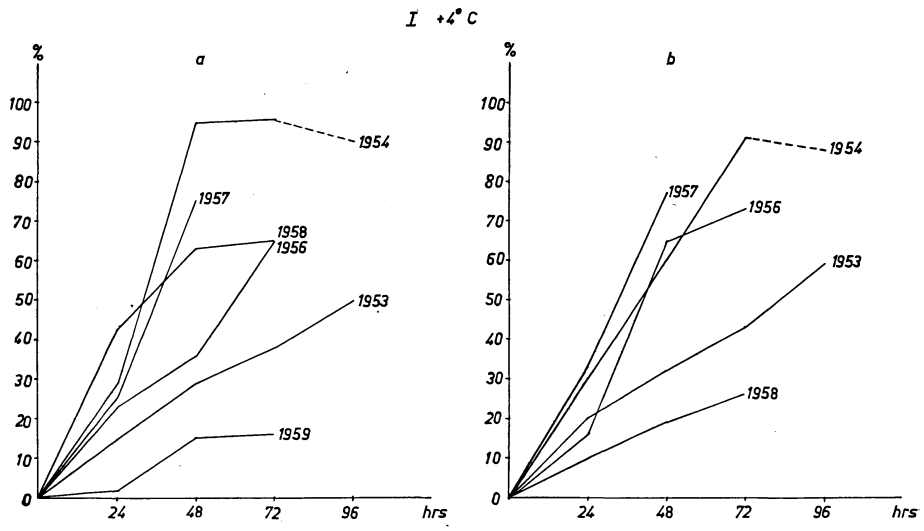
Analysis of fresh pollen

All the trees used in the experiments had normally developed pollen with only a slight variation in size and shape. The percentage of morphologically good pollen grains ranged between 90 and 100. In general there is some variation of pollen quality from year to year in the same tree. From two of the trees, 11: 18 and S: 21, fresh pollen has been analysed in several years, the number of empty or abnormal pollen grains never exceeding ten per cent as seen below.

Percentage of morhologically good pollen

Year	*1950*	*1951*	*1952*	*1953*	*1954*	*1955*	*1958*
11:18	96	98	96	94	95	99	96
S:21	—	—	95	97	96	95	—

Pollen from these two trees has been used in other crossing experiments as well in different years with good results. The germination experiments



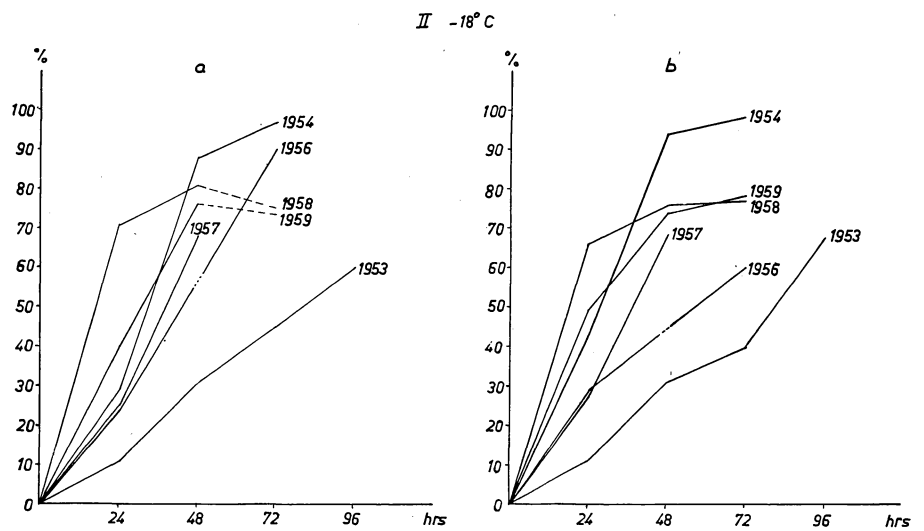


Fig. 1 a. Percentage of germinated pollen grains. Pollen stored 1—7 years.

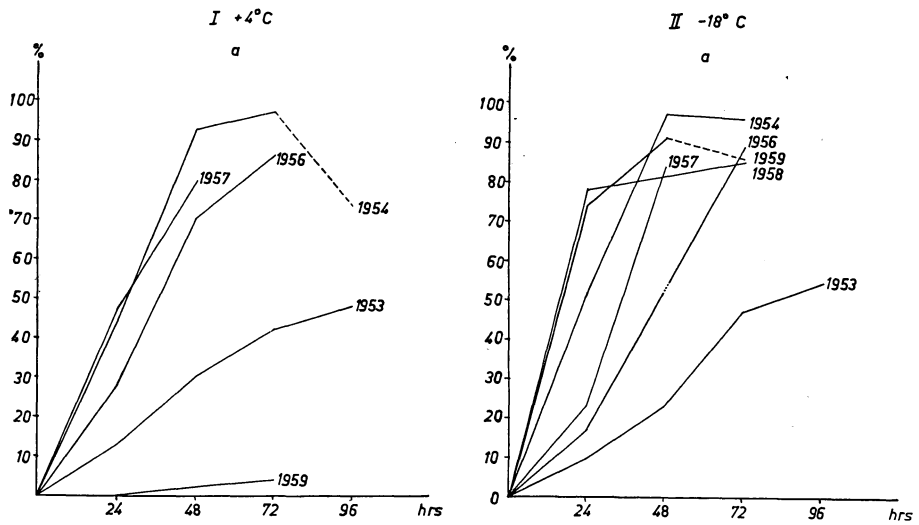


Fig. 1 b. Percentage of germinated pollen grains. Pollen stored 1—7 years.

too, have shown a high per cent of viable pollen. Thus the pollen quality. estimated by the number of morphologically good grains, was considered good in all the pollen samples included in this study.

Germination analysis

In 1952 the fresh pollen samples were tested for their germinability For some unknown reason the pollen of the mixed lot (B) and of the single tree (P) of *Pinus silvestris* used for this test did not germinate at all, but it grew well in 1953 (at room temperature) and still better in 1954 (at +30° C) when the next tests were carried out with new portions of pollen. The results of the germination experiments in different years are given in fig. 1.

The pollen kept at room temperature had already lost its viability entirely after twelve months of storage. Not a single grain showed any tendency to form tubes or even bulges (cf. PFUNDT 1909, PFEIFFER 1936, DENGLER, and SCAMONI 1939). This pollen was then discarded and left out of the later experiments.

The rather low values obtained in 1953 for the germination of all the different kinds of pollen are centainly due to the fact that the germination tests

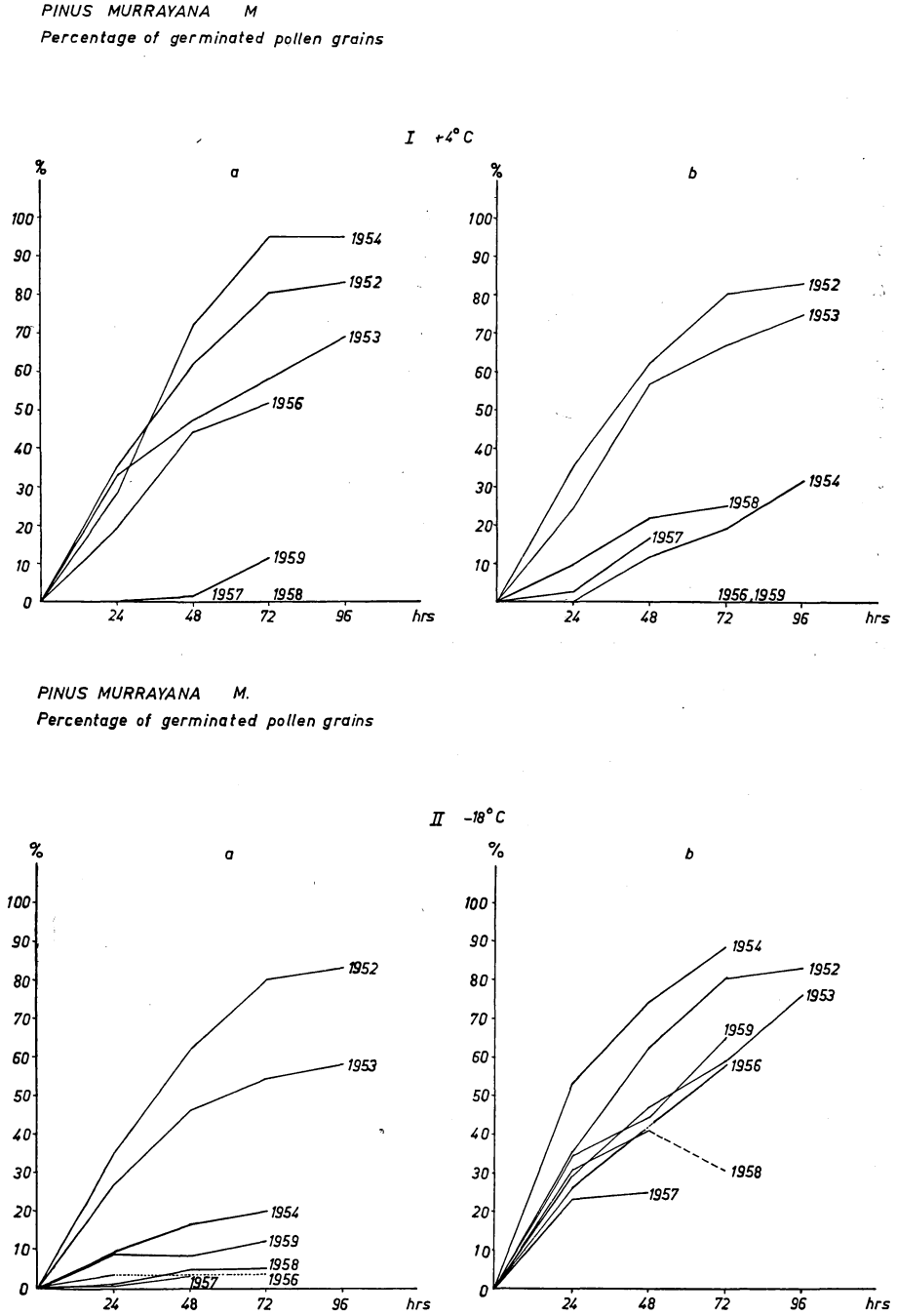
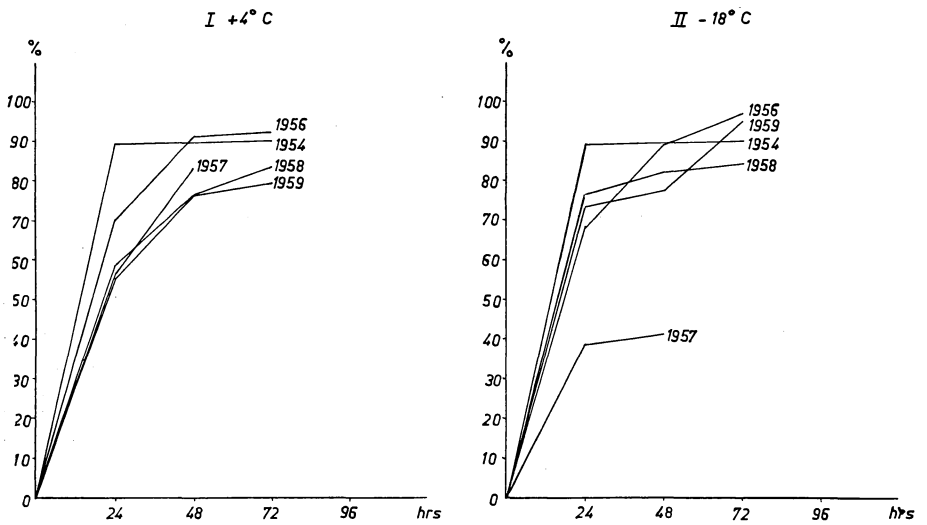


Fig. 1 c. Percentage of germinated pollen grains. Pollen stored 1—7 years.

PINUS SILVESTRIS 11 : 18
Percentage of germinated pollen grains



PINUS SILVESTRIS S : 21
Percentage of germinated pollen grains

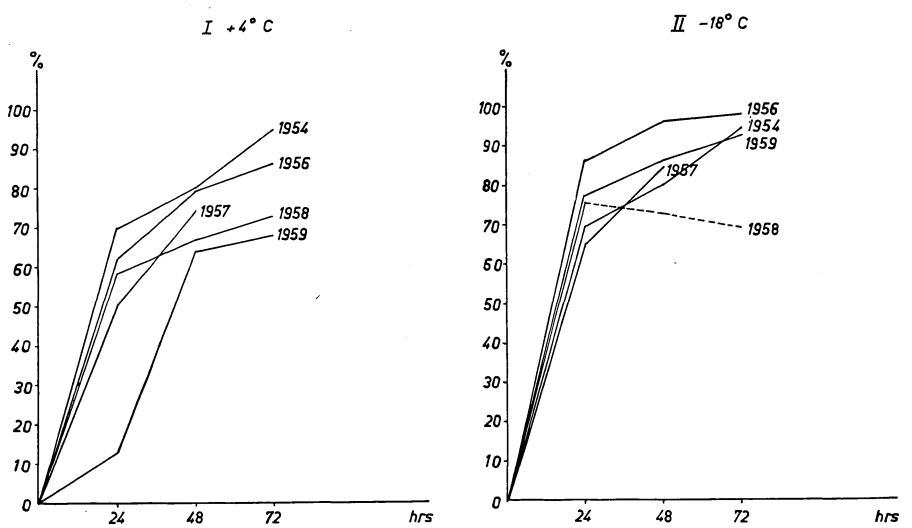


Fig. 1 d. Percentage of germinated pollen grains. Pollen stored 1—5 years.

were carried out at room temperature. The fresh pollen of *Pinus Murrayana*, tested in 1952 in the same culture conditions, germinated in spite of this to over 80 per cent. Thus unfavourable growing conditions seem to affect the fresh pollen less than pollen stored for several months (cf. PFUNDT 1909, BECK and JOLY 1941). An experiment performed in 1956 with two year old pollen, cultured in different temperatures showed differences in time of onset of germination and percentage of germinated grains (Fig. 2). In this case there was still a high germination per cent of pollen stored at + 4° C and at — 18° C, while pollen stored at room temperature showed no viability.

The two different kinds of pollen from *Pinus silvestris*, stored in 1952, germinated well in 1954 after two years of storage. In the following years their viability seemed to decrease slowly. The values in 1958 are about the same as the ones in 1956 and 1957 in all the tests made except in B I b, where the germination per cent was only about 26, probably due to an error in the performance of the test. There were no considerable differences between the apparent effects of the two storage methods up to 1958, when the pollen had been stored for six years, the irregular variation in the sequence of the year-values no doubt being due to sampling errors, but in 1959 such a difference became apparent. The germination per cent of pollen, stored at + 4° C (I a), was then low, while pollen, stored at — 18° C (II), still showed a high number of viable grains.

In *Pinus Murrayana* (M.), on the other hand, the longevity of the pollen seems to be less than in *Pinus silvestris*. 90 per cent or more (1954, I a, II b) two year old pollen grains was still able to germinate, but after four years the germinability decreased strikingly. Pollen stored at — 18° C in half-filled ampoules (II b), seemed to retain its viability longer than pollen stored in other conditions. If there are genetically conditioned differences as to the pollen longevity between the two *Pinus*-species, or if the divergences obtained in this experiment are due to different requirements for storage conditions and culture methods has to be studied further. Usually different species vary widely in this respect (for review of literature see DUFFIELD 1954, ECHOLS and MERGEN 1956).

The pollen samples from the two *Pinus silvestris* trees 11:18 and S:21, collected and stored in 1954, were tested for their germinability when fresh in 1954 and after storage for two to five years. The data obtained were similar to those obtained for the other group of *Pinus silvestris* pollen from 1952: a high percentage of viable grains in two year old pollen and a slight decrease in viability during the following three years. Pollen of 11:18, stored at — 18° C, did not grow well in 1957, but as 85 per cent of the grains germinated in 1958, this failure must have been due to some experimental error. There was one small divergence, though, between the two groups: the pollen from 1954

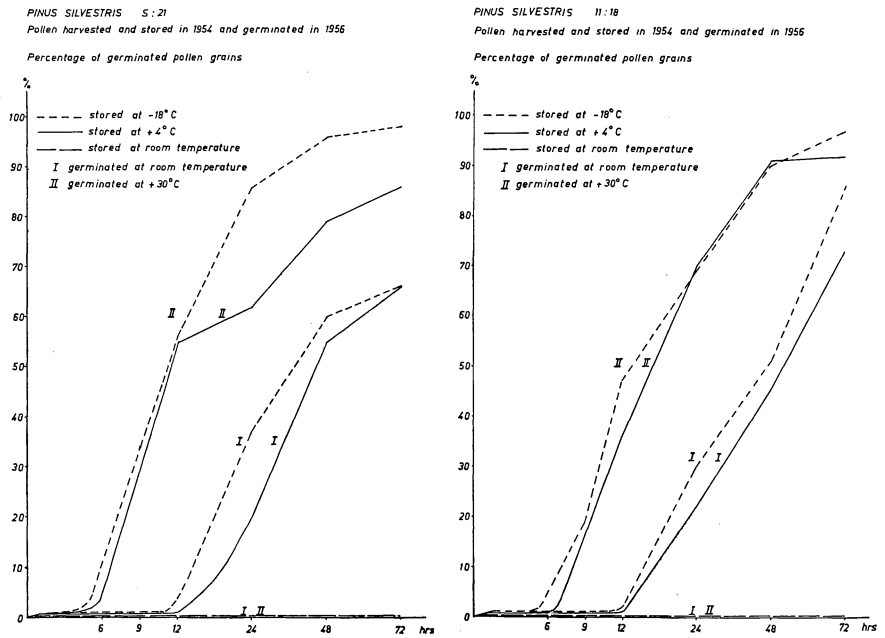


Fig. 2. Percentage of germinated pollen grains in room temperature and at + 30° C. Pollen stored in 2 years.

reached a high percentage of germinated grains already in 24 hours as a rule, while the samples from 1952 generally started germination at a slower pace. This holds true for all the tests in the two groups, cannot be assigned to differences in storage conditions, and is probably due to inherent characters of the different kinds of pollen or to some difference in handling the pollen lots during the collection and drying.

The data obtained in these experiments allow some conclusions about which storage method is the most appropriate for keeping pine-pollen viable for long periods. The results given in fig. 1 do not show any regular superiority of one method over the other during the first six years. After seven years of storage, however, pollen stored at — 18° C, still gave a high germination per cent (except M II a), while pollen, stored at + 4° C, seemed to have a reduced viability. Considering, too, the results obtained in the crossing experiments (see below) the tentative conclusion that pollen stored at — 18° C keeps its viability longer is probably correct.

The length and form of the pollen tubes

In the germination tests, carried out in the years 1957—1959 the length of the pollen tubes were measured and the percentage of abnormal tubes re-

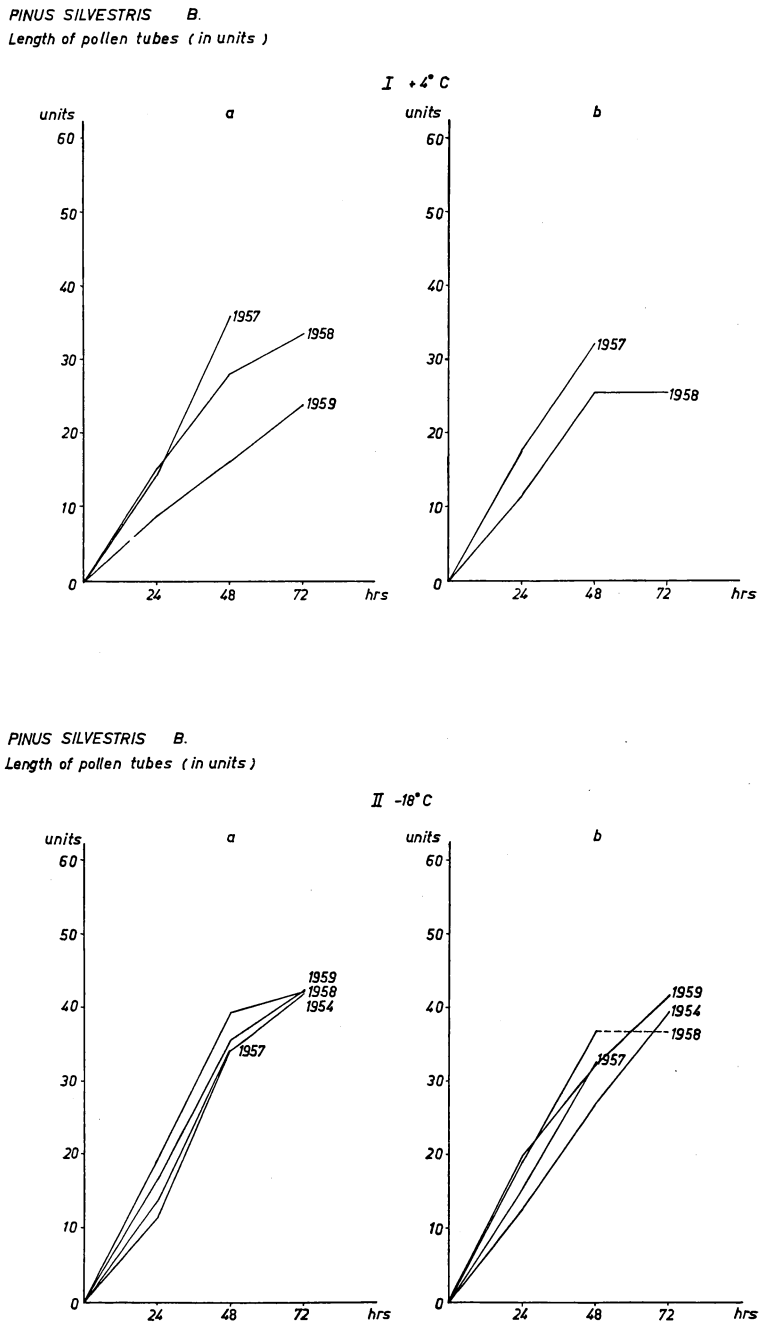


Fig. 3 a. Length of pollen tubes. Pollen stored 2—7 years.

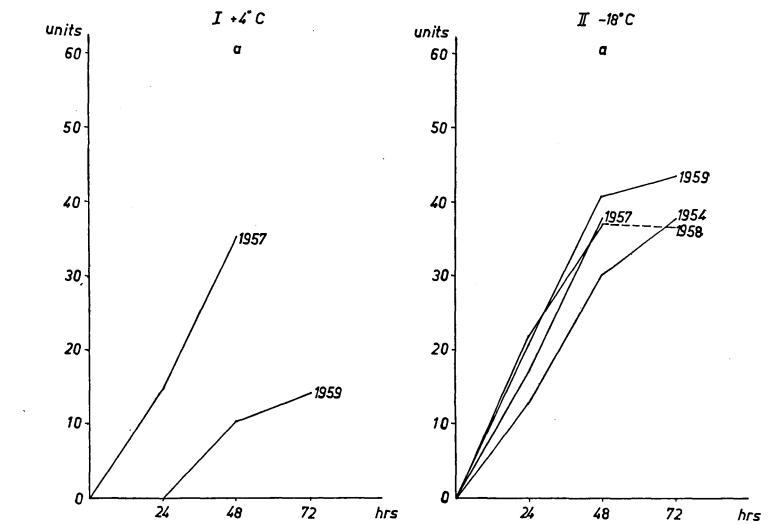


Fig. 3 b. Length of pollen tubes. Pollen stored 2—7 years.

corded. In two cases such measurements were made in 1954 as well (Fig. 3. Table 1 a-e). There was no evidence of less growing capacity of the tubes, when the pollen had been stored during two or more years (cf. TANAKA 1955). For instance the sample B II b (*Pinus silvestris*, mixed pollen) had a mean tube length of 39.4 units after 72 hours in 1954, and of 41.8 units in 1959. The corresponding numbers for sample P II a (*Pinus silvestris*, single tree) were 37.7 and 43.5 units. Nor did any positive differences in the effects of the various storage-methods become apparent, the tube lengths varying rather irregularly from year to year, but there was a slight indication that storage at — 18° C was more favourable for getting long tubes. An interesting fact, however, is that the pollen tubes of the *Pinus Murrayana*-lot in all cases were shorter than those of *Pinus silvestris*. Furthermore the percentage of abnormally shaped pollen tubes was higher, too, in the *Pinus Murrayana* samples (Table 1 c). Such abnormal tubes were rarely seen in fresh pollen. They occurred occasionally in the old pollen samples after 24 hours of germination in nearly all slides but naturally they were more common when the tubes had grown out fully. The tubes were either forked or swollen at the tips or grew out in duplicate from the same grain (Fig. 4-11). Such deformations have been observed previously (see for instance TANAKA 1955, for *Pinus densiflora*), when pollen grains were grown in sucrose solutions, and were attributed to the presence of sucrose

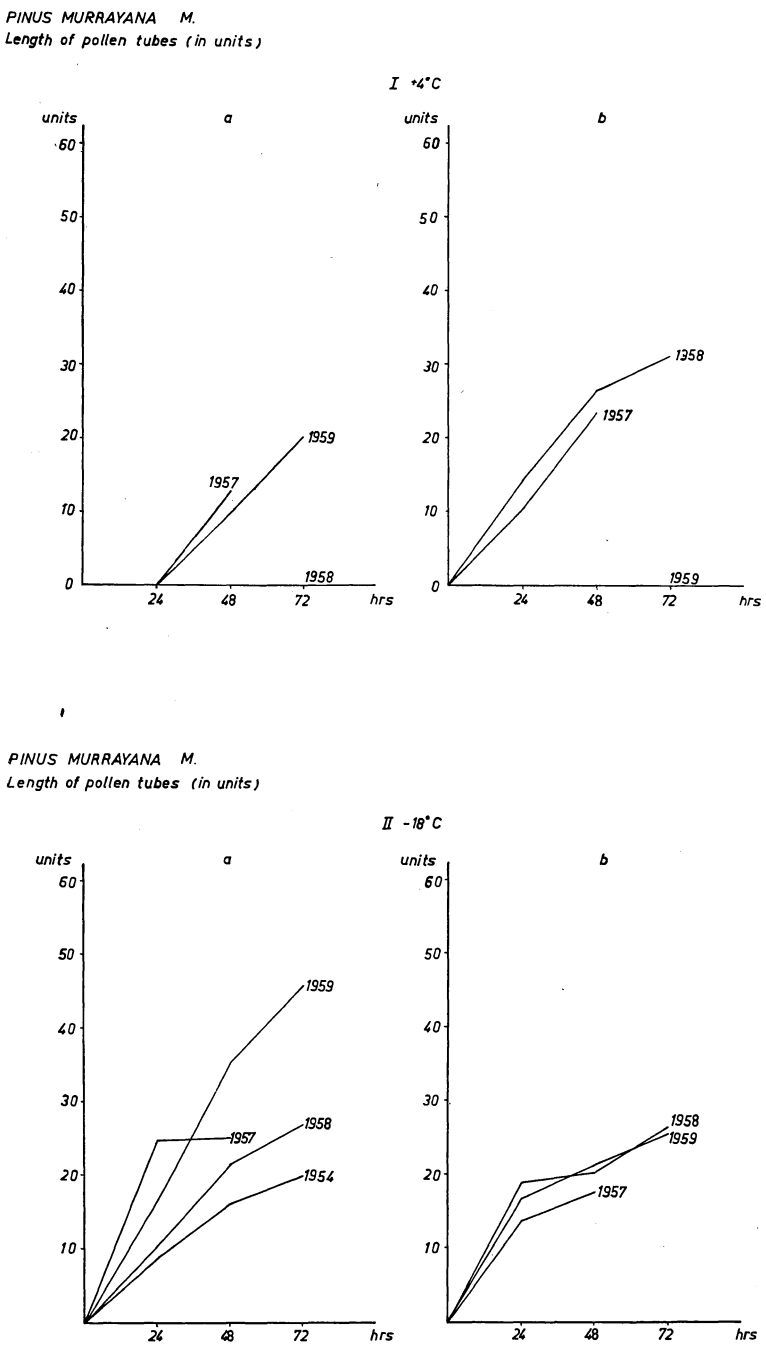


Fig. 3 c. Length of pollen tubes. Pollen stored 5—7 years.

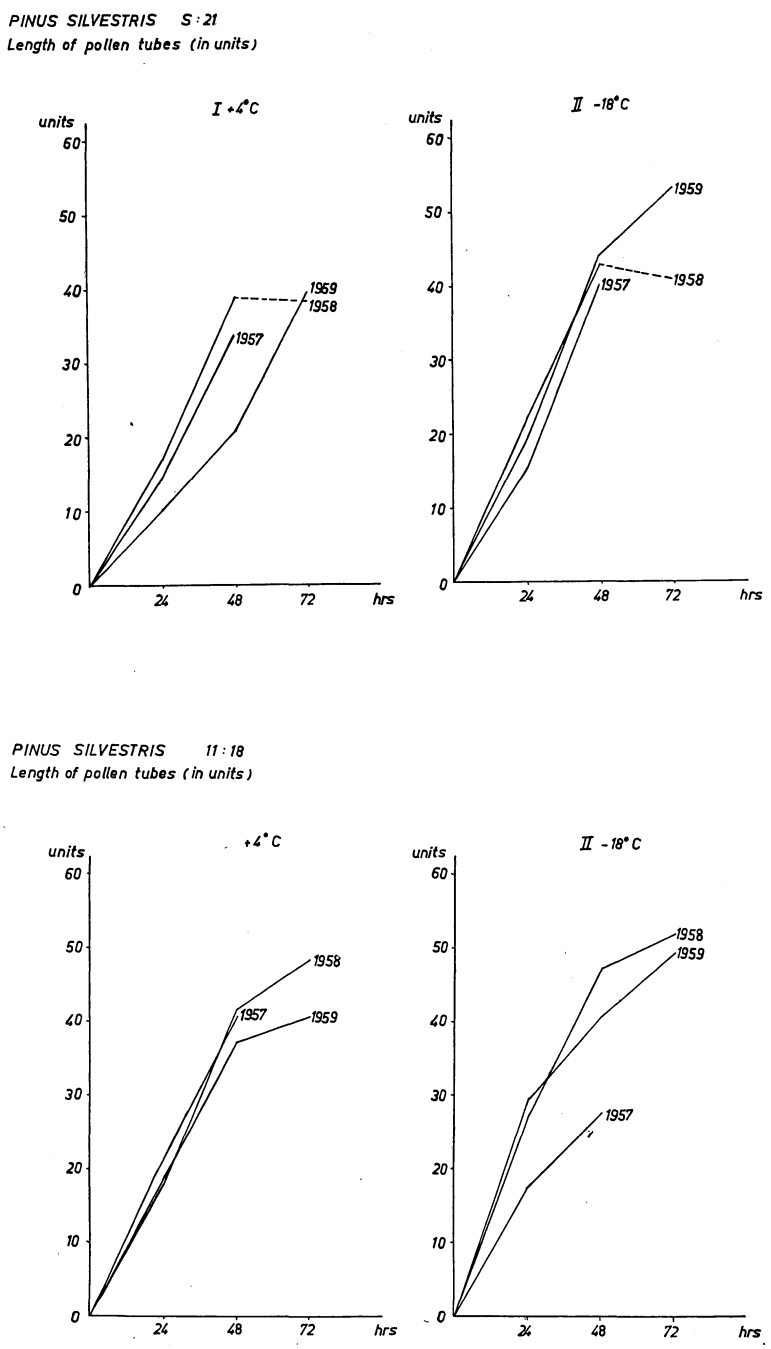


Fig. 3 d. Length of pollen tubes. Pollen stored 3—5 years.

Table 1 a. Pinus silvestris (B). Percentage of normal and irregularly grown pollen tubes after 24, 48 and 72 hours of germination i distilled water at +30° C. Pollen stored in 1952.

Germinated in year	Pollen sample No.	Storage temperature	Percentage of tubes														
			24 hours					48 hours					72 hours				
			normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst
1954	B I a	+ 4°	99	1				97		1	1		99			1	
	b		100					97	1	1	1		98	1		1	
1957	a		100					95	4		1						
	b		99		1			80	11	1	8						
1958	a		90	6	3		1	83	7	5	5		62	15	8	15	
	b		60	18	22			37	10	36	17		27	27	24	12	
1959	a[1]							71	21	8			54	29	10	2	5
1954	a	—18°	96	1	3			87		8	5		87		4	9	
	b		98		2			73		7	20		65	1	5	29	
1957	a		96	4				95	4		1						
	b		99				1	88	10		1	1					
1958	a		89	3	1		7	72	4	1	12	11	66	6		15	13
	b		85	3	1		11	52	5		16	27	54	11	2	18	15
1959	a		93	2	2		1	83	12	1	1	3	80	16	2	1	1
	b		94	2			4	84	10	1	3	2	73	20	3	1	3

[1] Only very short tubes developed after 24 hours.

Table 1 b. Pinus silvestris (P). Percentage of normal and irregularly grown pollen tubes after 24, 48 and 72 hours of germination in distilled water at +30° C. Pollen stored in 1952.

Germinated in year	Pollen sample No.	Storage temperature	Percentage of tubes														
			24 hours					48 hours					72 hours				
			normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst
1954	P I	+ 4°	94	2	4			89	10	1			97	1	1	1	
1957			100					81	7	1	11						
1957	P II	—18°	100					93	6		1						
1958			94	2	2		2	60	13	1	13	13	45	23	3	16	13
1959			99	1				81	13		3	4	67	27	1	4	1

in the culture medium. Since the same abnormalities occur in pollen tubes growing on water, tonicity of the medium cannot be the sole determinant of the mode of tube growth. An explanation worth considering is given by BECK and JOLY 1941. According to them the tubes expand and finally burst at the tips when the supply of nutrients or auxins within the grain, essential for the development of the tube walls, is exhausted. The walls are then distended and finally split.

In this connection it should be mentioned that MARCET (1951) reported a variation of the percentage of deformed pollen tubes, distinctly correlated

Table 1 c. Pinus silvestris (S:21). Percentage of normal and irregularly grown pollen tubes after 24, 48 and 72 hours of germination in distilled water at +30°C. Pollen stored in 1954.

Germinated in year	Pollen sample No.	Storage temperature	Percentage of tubes														
			24 hours					48 hours					72 hours				
			normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst
1957	S: 21 I	+ 4°	100					90	5	4		1					
1958			96		4			71	6	10	13		56	10	13	21	
1959			72	14	13		1	78	16	3	1	2	67	17	3	7	6
1957	S: 21II	—18°	99	1				83	1	3	13						
1958			92				8	59	9	2	11	26	47	7	7	10	29
1959			97		3			65	26	1	9	2	63	32	1	3	1

Table 1 d. Pinus silvestris (11:18). Percentage of normal and irregularly grown pollen tubes after 24, 48 and 72 hours of germination in distilled water at +30°C. Pollen stored in 1954.

Germinated in year	Pollen sample No.	Storage temperature	Percentage of tubes														
			24 hours					48 hours					72 hours				
			normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst
1957	11: 18 I	+ 4°	97	2	1			82	11	7							
1958			93	3	4			69	8	3	12	8	72	12	5	11	2
1959			84	12	1		3	63	29	4	1	3	50	32	7	1	10
1957	11: 18 II	—18°	96	2		2		84	1	2	13						
1958			83	2	2	5	8	60	5	1	7	27	53	20	3	10	14
1959			89	10	1			68	20	4	4	4	52	31	1	1	15

Table 1 e. Pinus Murrayana (M). Percentage of normal and irregularly grown pollen tubes after 24, 48 and 72 hours of germination in distilled water at +30°C. Pollen stored in 1952.

Germinated in year	Pollen sample No.	Storage temperature	Percentage of tubes														
			24 hours					48 hours					72 hours				
			normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst	normal	with bulbs	duplicate	forked	burst
1954	M I a	+ 4°	94	6				81	11	1	7		81	6	3	10	
	b[1]							85	1	10	4		82	7	7	4	
1957	b		92	5	3			81	14		4	1					
1958	a		69	25	5	1		27	63	5	5		8	75	7	10	
	b		62	24	4	8	2	18	35	2	18	27	12	28	7	41	12
1959	a[2]							78.7	21.3				64.5	30.3		5.2	
1957	M II a[3]	—18°	91.2	7.3	1.5			70	24	2	3	1					
	b		91	8		1		47	52	1							
1958	b		82	14	3	1		40	29	10	21		22	30	6	42	
1959	a		79	16	4	1		57	24	9	9	1	32	40	3	24	1
	b		57	33	1	8	1	38	50	4	3	5	15	82	1	1	1

[1] Only very short tubes developed after 24 hours.
[2] Less than 100 tubes examined. Short and badly developed tubes.
[3] Less than 100 tubes examined after 24 hours.

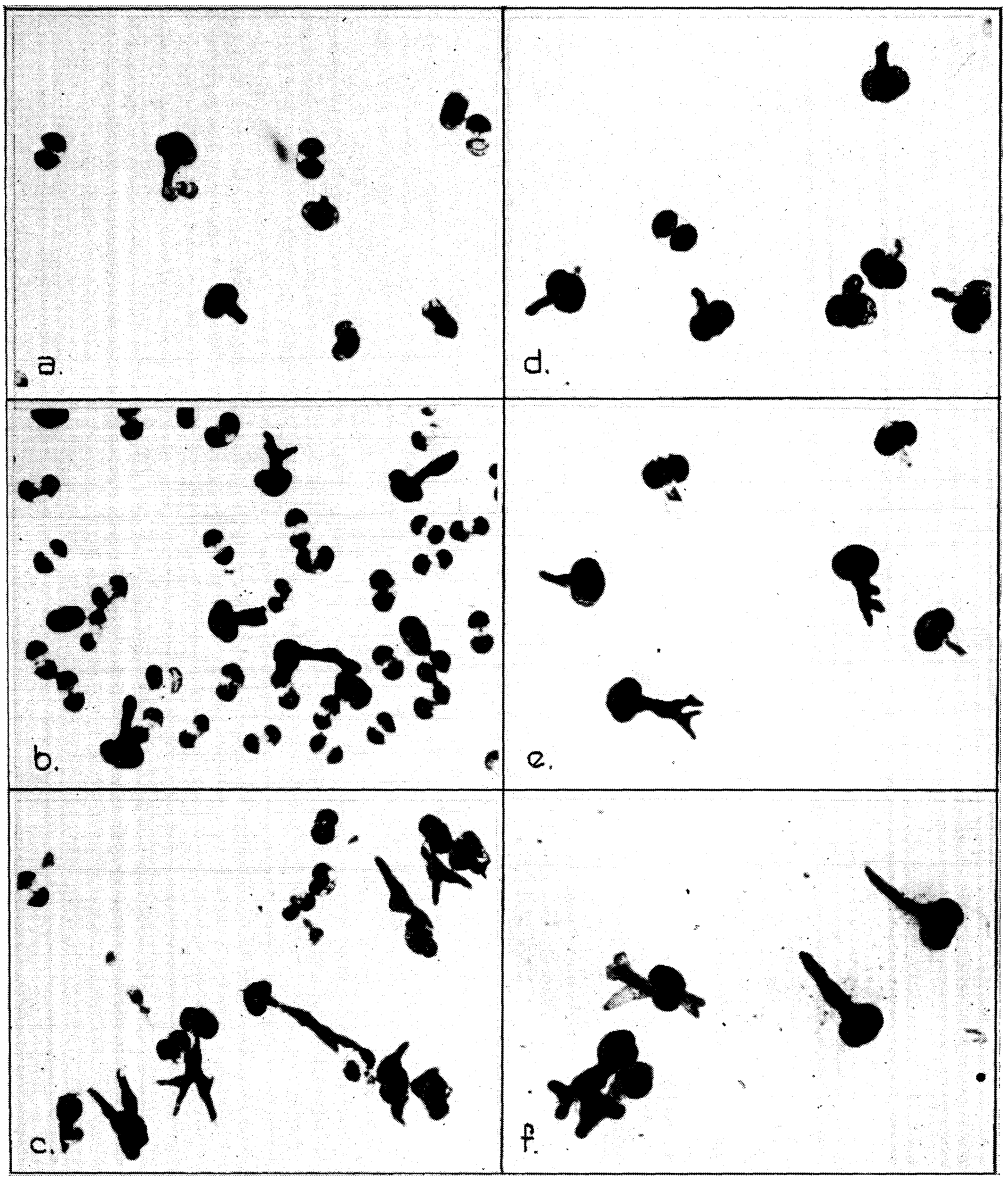


Fig. 4. Pollen of *Pinus Murrayana*, harvested in 1952. a—c) Stored at +4° C (M I), grown 1958 in a) 24 b) 48 c) 72 hours. d—f) Stored at — 18° C (M II), grown 1958 in d) 24 e) 48 f) 72 hours. × ca 90.

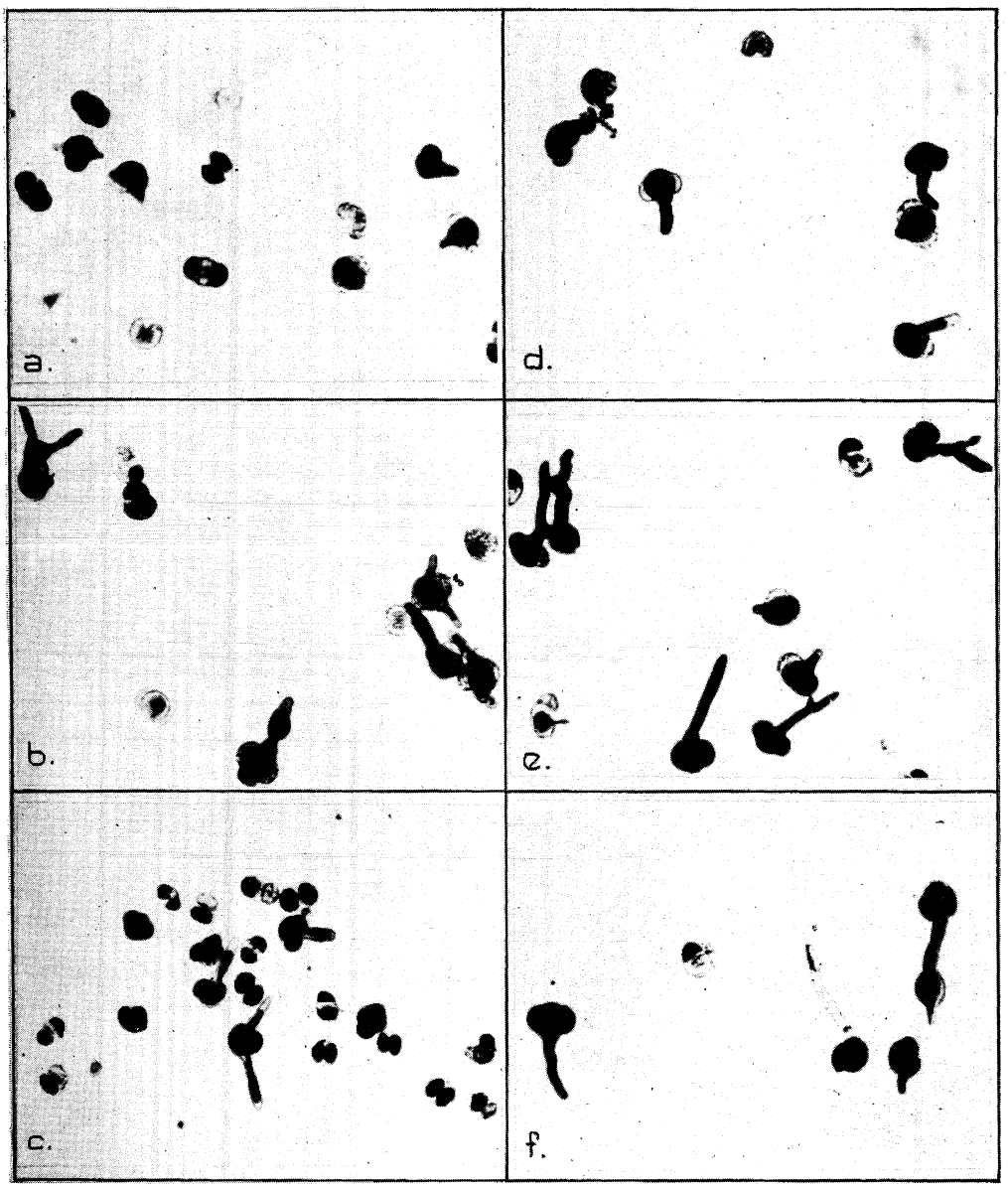


Fig. 5. Pollen of *Pinus silvestris*, harvested in 1952. a—c) Stored at +4° C (B I), grown 1958 in a) 24 b) 48 c) 72 hours. d—f) Stored at — 18° C (B II), grown 1958 in d) 24 e) 48 f) 72 hours. × ca 90.

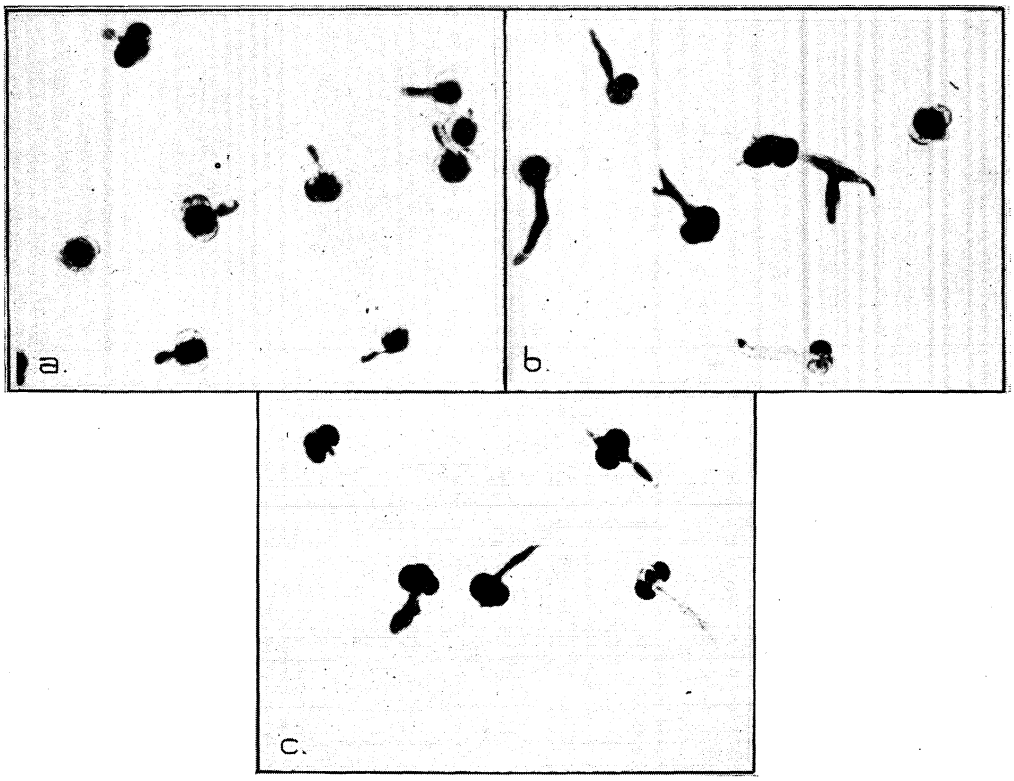


Fig. 6. Pollen of *Pinus silvestris* (S: 21 I), harvested in 1954, stored at +4° C, grown 1958 in a) 24 b) 48 c) 72 hours. × ca 90.

with differences in starch content, between individual trees within the species *Pinus silvestris*. This indicates that there may be not only an interspecific variation in this respect but an intraspecific variation as well (cf. BRINK 1924).

Thus pollen of *Pinus Murrayana* not only seems to loose its germinability in shorter time than *Pinus silvestris* pollen but also to be less adapted to the standard method of germination, used in this experiment. The possibilities remain that other culture media, for instance sugar solutions, might give other results as regards the percentage of abnormal tubes or the germination per cent in *Pinus Murrayana*.

Crossing experiments

The results of the crossing experiments in 1955 and 1957 are summarized in tables 2 and 3. The grafts, originating from 20 different trees and used

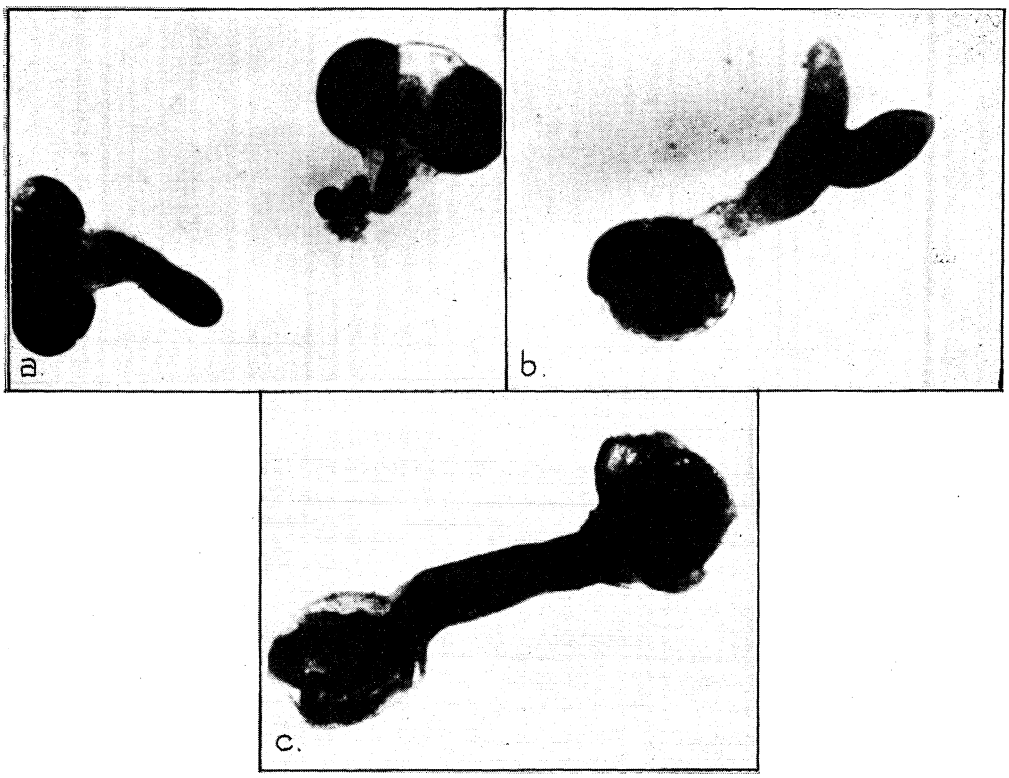


Fig. 7. Pollen of *Pinus silvestris* (11: 18 II), harvested in 1954, stored at — 18° C, grown in 1958 a) 24 hours, one tube bursted b) 48 hours forked tube c) 72 hours, swollen tube end. × ca 400.

as female parents in 1955, had not previously been examined as to their cone- and seed- setting capability. For that reason each kind of pollen was tried on one to six grafts of different origin. Out of the 20 grafts 10 did not render any cones regardless of the kind of pollen used. The remaining 10 cone-bearing grafts originated from eight different mother trees. For comparison cones after open pollination were collected from the same graftings as well.

Pollen of S: 21, both kinds (+4° C and — 18° C), failed altogether to function in this experiment, probably due to inappropriate handling of the pollen or to the grafts chosen being unsuitable (cf. Table 2). The two kinds of mixed pollen, stored at — 18° C for three years (II B, a and b), gave fairly good results as to seed setting and seed quality (57.9 and 61.8 per cent full seeds). Still these values were markedly lower than those obtained after pollination with pollen from 11: 18, — 18° C (II), stored in the same way but only during one year. Here the percentage of full seeds was about the same as after open

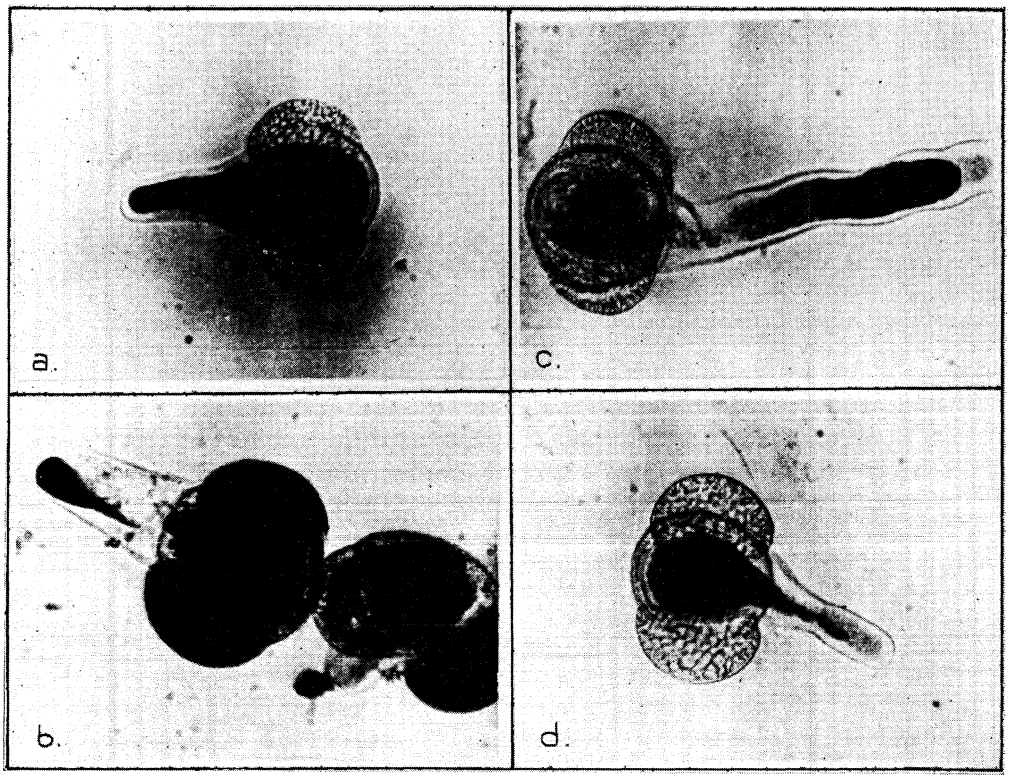


Fig. 8. Pollen of *Pinus silvestris* (B I), harvested in 1952, stored at +4° C, grown 1959 in a–c) 24 d) 48 hours. a) normal tube b) one tube bursted c—d) plasma irregularities. × ca 400.

air pollination, the percentage of seeds in embryo class III, though, being nearly twice as high.

When, on the other hand, pollen of 11:18, stored at + 4 °C (I) was used, there was a remarkable decrease in seed yield. The amount of full seeds was only 14.3 per cent, all seeds belonging to embryo class IV. One well developed cone in this crossing had no seeds at all (parthenocony, cf. EHRENBERG and SIMAK 1957).

The five control flowers (isolated but not pollinated conelets) did not develop into cones. Thus the presence of pollen seems to stimulate cone development even when no fertilization occurs (cf. PLYM FORSHELL 1953, EHRENBERG and SIMAK 1957).

Unfortunately only two lots of pollen stored at +4° C were included in this experiment, one of which failed entirely. The indication that storing pollen at +4° C was less favourable than the other storage method at —18° C was, however, substantiated by the results obtained in the following experiment.

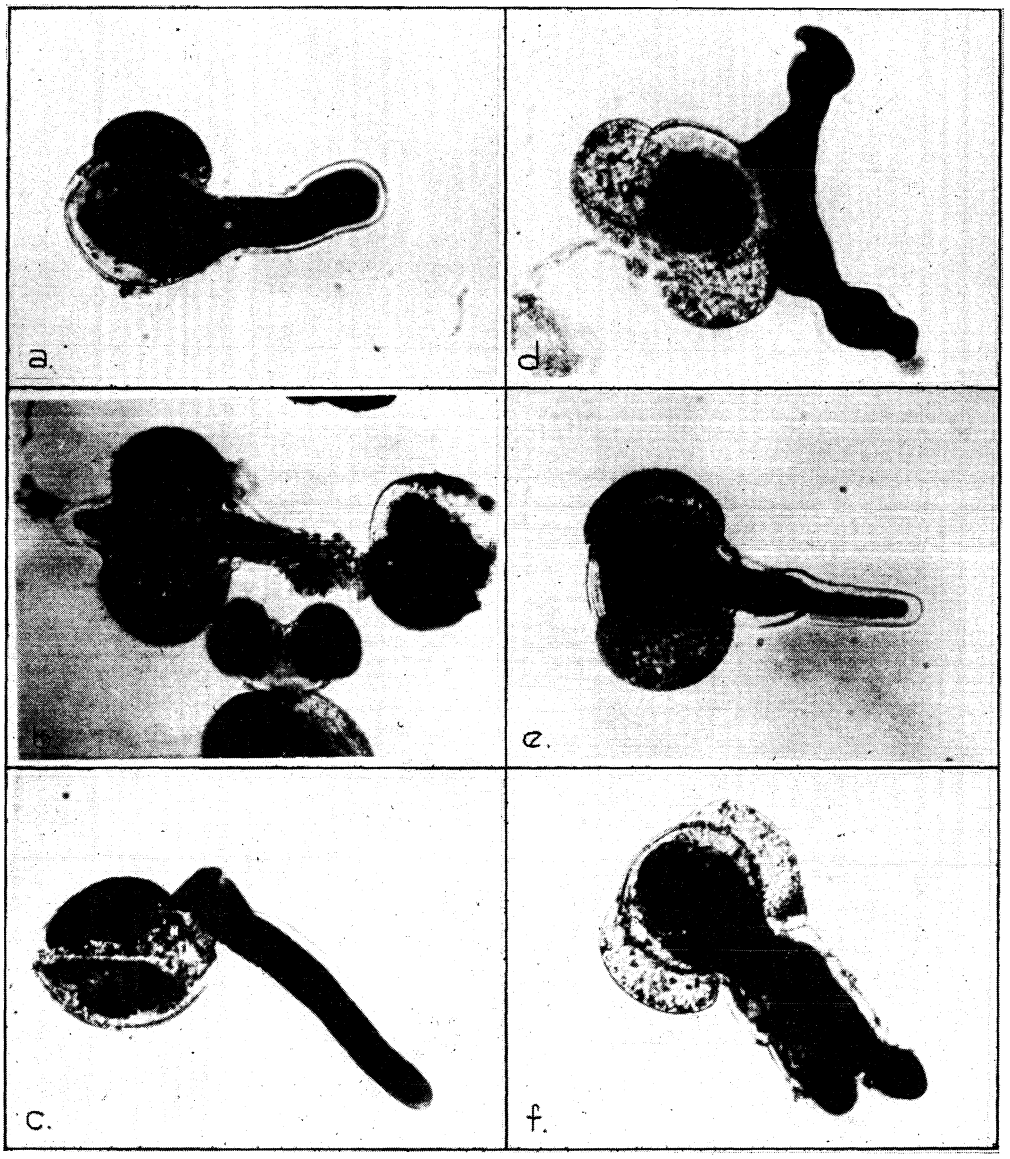


Fig. 9. Pollen of *Pinus Murrayana* (M I), harvested in 1952, stored at +4° C, grown 1959 in a—b) 24 c—d) 48 e—f) 72 hours. a) pollen tube slightly swollen at the end b) duplicate tubes, one of them bursted c) tube abnormally bent, plasma well developed d) forked tube, swollen at the ends e) the plasma only fills out the basic part of the tube f) two tubes growing parallely. × ca 400.

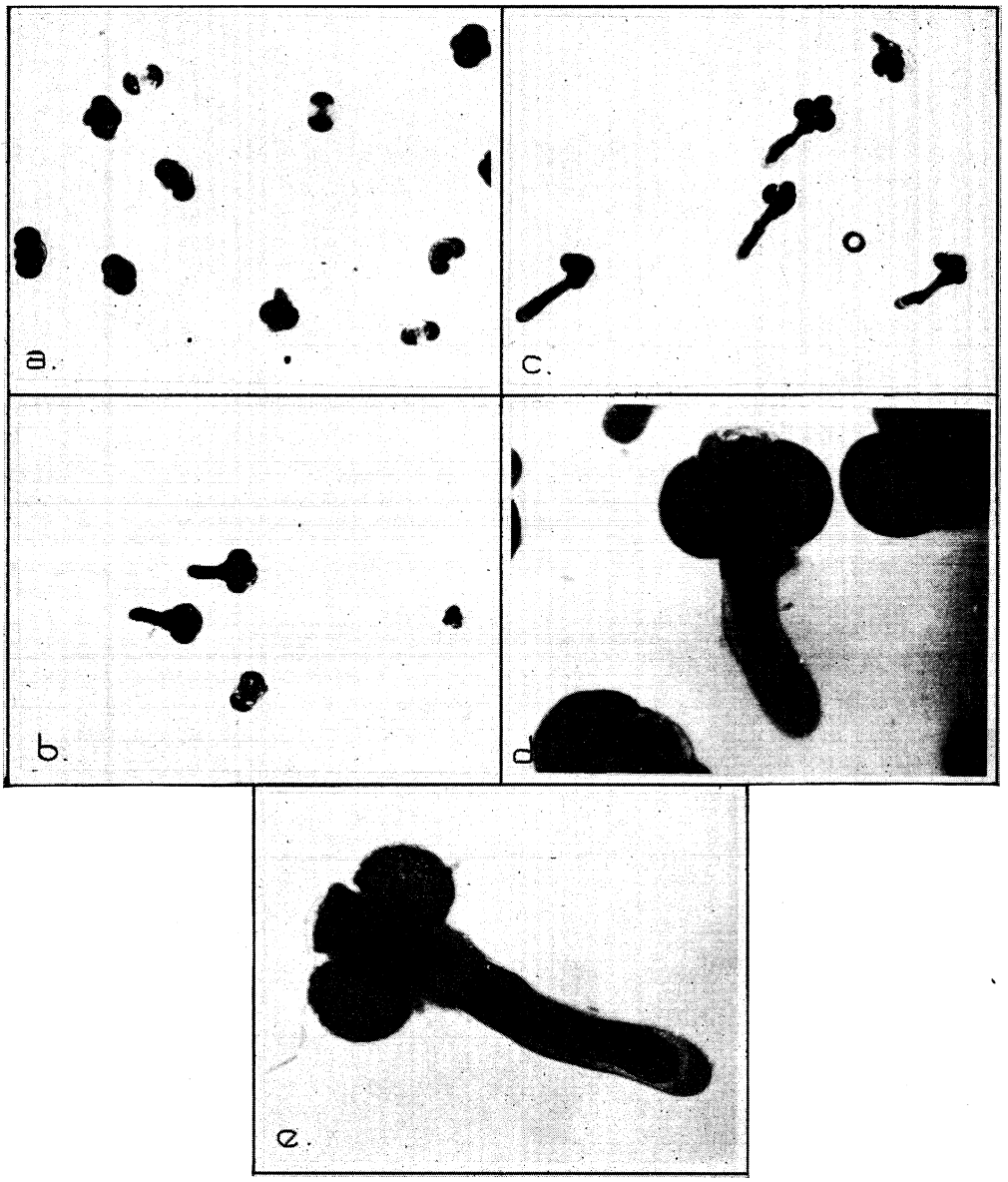


Fig. 10. *Pinus silvestris* (11: 18). Fresh pollen, grown 1956 in a) 24 b) 48 c) 72 d) 48 e) 72 hours. a—c) × ca 90, d—e) × ca 400.

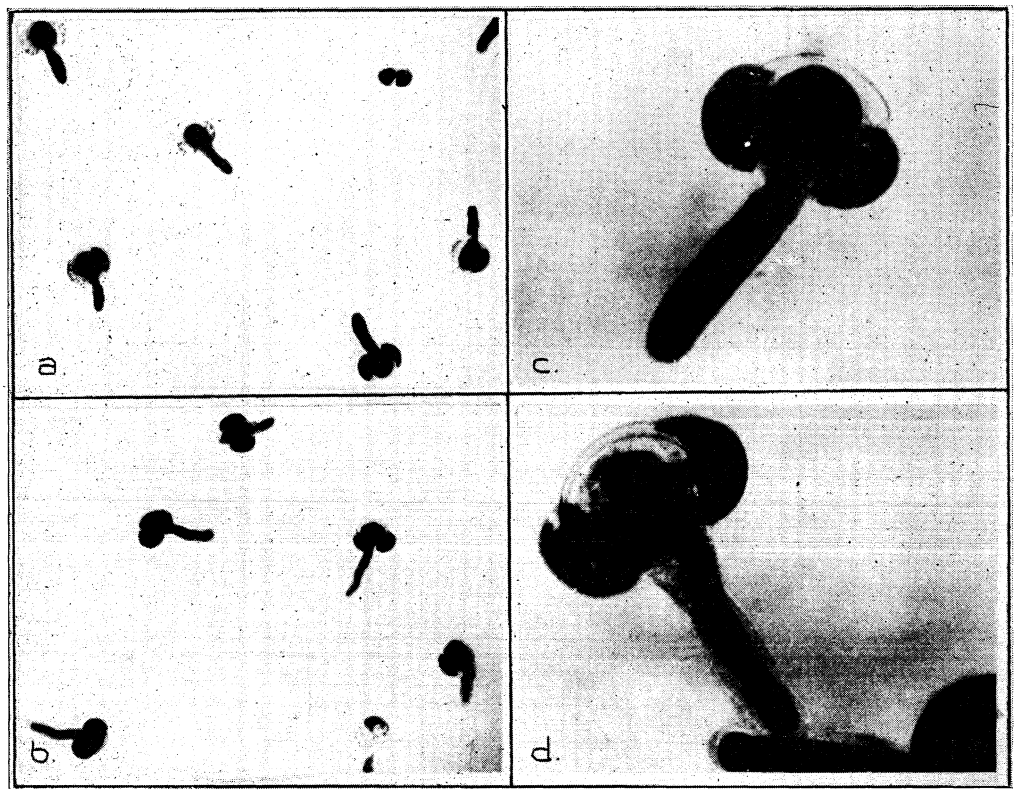


Fig. 11. *Pinus silvestris* (Poltava 1). Fresh pollen, grown 1957 in a) 24 b) 48 c) 24 d) 48 hours. a—b) × ca 90, c—d) × ca 400.

The crosses were repeated in 1957 with pollen of 11:18 and S:21, both storage methods (I and II), and then on a larger scale and on full grown trees. The results are summarized in table 3. In the cases where + 4° C pollen was used, cones with only empty seeds developed in four cases, while in two the pollination produced no result. The —18° C pollen, on the other hand, seemed to be viable still, but the cone and total seed yield as well as the number of full seeds per cone and the percentage of full seeds were lower than in crosses where fresh pollen was used. The quality of the seeds obtained after pollination with stored pollen was, on the whole, equal to that of seeds developed after pollination with fresh pollen. In the special cross Korpilombolo 6×11:18, both + 4° C and — 18° C, no cones developed. Nor did open pollination yield any cones in this tree as was also the case in the tree Korpilombolo 7. One control flower out of 26 developed into a small cone without seeds or seed wings. In crosses other than the above mentioned the length of the obtained

Table 2. *Pinus silvestris*. Pollination with stored pollen 1955.

No. of grafts ♀	♂	Year of storage	Storage temperature	Number of		Number of seeds		Number of seeds per cone		Per cent seeds in embryo classes					Per cent full seeds
				fls.	cones	tot.	full	tot.	full	o	I	II	III	IV	
5	control	—	—	5	0										
6	11: 18	1954	+ 4°	6	3	21	3	7	1	85.7				14.3	14.3
4	11: 18	1954	—18°	6	1	37	32	37	32	13.5			8.1	78.4	86.5
3	S: 21	1954	+ 4°	6	0										
1	S: 21	1954	—18°	4	0										
2	B	1952	—18°a	6	2	19	11	9.5	5.5	42.1		5.3	5.3	47.4	57.9
4	B	1952	—18°b	6	3	55	34	18.3	11.3	38.2		1.8	3.6	56.4	61.8
5	o.p.	—	—		9	277	256	30.8	28.4	7.6		0.4	3.9	88.1	92.4

Table 3. *Pinus silvestris*. Pollination with stored pollen 1957.

♀	♂	Storage		Number of		Per cent cones	Number of seeds		Number of seeds per cone		Per cent seeds in embryo classes					Per cent full seeds	Mean cone length mm	Number of seedless wings
		year	temp.	fls.	cones		tot.	full	tot.	full	o	I	II	III	IV			
Poltava 1	control a	—	—	10	0	0												
	control b	—	—	11	1	0.9	0											
	11: 18	1954	+ 4°	34	3	8.8	20	0	6.7	0						0	41.3	19
	11: 18	1954	—18°	35	1	2.9	13	6	13	6	53.8		7.7	7.7	30.8	46.2	35.7	8
	S: 21	1954	+ 4°	35	2	5.7	4	0	2	0	100.0					0	42.6	18
	S: 21	1954	—18°	32	4	12.5	63	23	16	5.8	63.5		1.6	7.9	27.0	36.5	41.7	21
	Korpilombolo 6	—	—	46	14	30.4	214	135	15.3	9.6	36.9		1.9	8.4	52.8	63.1	41.9	49
	o. p.	—	—	39	17	43.6	299	213	17.6	12.6	29.1	0.3	2.3	9.4	59.2	71.2	42.8	36
																	$\bar{m}=41.0$ $\pm$ 0.577	
Korpilombolo 6	control	—	—	2	0	0												
	11: 18	1954	+ 4°	3	0	0												
	11: 18	1954	—18°	3	0	0												
	S: 21	1954	+ 4°	3	1	33.3	17	0	17	0	100.0					0	44.8	32
	S: 21	1954	—18°	3	1	33.3	33	8	33	8	75.8			3.0	21.2	24.2	46.7	1
	Poltava 1	—	—	3	1	33.3	43	33	43	33	23.3		2.3	16.3	58.1	76.7	50.8	8
	o. p.	—	—	2	0	0												
																	$\bar{m}=47.4$ $\pm$ 0.177	
Korpilombolo 7	control	—	—	3	0	0												
	11: 18	1954	+ 4°	3	0	0												
	11: 18	1954	—18°	4	3	75.0	63	50	21	16.7	20.6		1.6	3.2	74.6	79.4	37.5	8
	S: 21	1954	+ 4°	3	1	33.3	8	0	8	0	100.0					0	33.0	12
	S: 21	1954	—18°	5	2	40.0	52	20	26	10	61.5		3.8	3.8	30.8	38.5	30.5	2
	Poltava 1	—	—	3	3	100.0	85	82	28.3	27.3	3.5			23.5	72.9	96.5	35.3	10
	o. p.	—	—	18	0	0												
																	$\bar{m}=34.6$ $\pm$ 1.277	

Table 4 a. Water content in pollen stored at +4°C during five and seven years.

Pollen sample		Year of storage	Water content per cent	Water content $\overline{m}$
P. silvestris	S: 21	1954	5.97 } 5.59	
	S: 21		5.21 }	*P. silvestris* 1954: 6.27 %
	11: 18	1954	6.51 } 6.88	
	11: 18		7.25 }	
	B a	1952	6.38	
	B b	1952	6.14	*P. silvestris* 1952: 6.14 %
	P a	1952	5.92	
P. Murrayana	M a	1952	6.81	*P. Murrayana* 1952: 7.55 %
	M b	1952	8.30	

Table 4 b. Water content in pollen stored at —18°C during five and seven years.

Pollen sample		Year of storage	Water content per cent	Water content $\overline{m}$
P. silvestris	S: 21	1954	5.53	
	11: 18	1954	5.31	*P. silvestris* 1954: 5.42
	B a	1952	6.39	
	B b	1952	(4.23)[1]	*P. silvestris* 1952: 6.43
	P a	1952	6.47	
	M a	1952	5.14	
P. Murrayana	M b	1952	8.74	*P. Murrayana* 1952: 6.94

[1] Uncertain value.

cones varied slightly from tree to tree, Korpilombolo 6 having the longest cones ($\overline{m}$ = 47.4 ± 0,177 mm) and Korpilombolo 7 the smallest ones ($\overline{m}$ = 34.6 ± 1,277 mm), the variation between the trees being greater than within the trees (Table 3). This is in accordance with the results obtained by PLYM FORSHELL (1953) in her studies of the variation of the cone in Scots pine. The cone size is characteristic of the mother tree and only slightly modified by environmental factors.

The water content in stored pollen

An analysis of the water content in stored pollen was made in 1959, including each kind of pollen stored in different conditions. Although the experiments were carried out with very little material and only repeated once except with 11: 18 and S: 21, because of scarceness of pollen, the results of the ex-

periments may be given here (Table 4 a and b). The water content in the different pollen samples, which ranged between 5.1 and 8.7 %, cannot be significantly influenced by the water content in the air of the small closed ampoules. The amount of water in this volume of air (3 ml) must be very low as compared with that in the pollen (weight of samples about 200 mg). Thus, 1 ml water-saturated air contains 0.017 mg of water at + 20° C and 0.001 mg at —20° C (Handbook of Chemistry and Physics 1955, 37 Ed. p. 2296).

Hence, the water content of the samples is practically only determined by the handling of pollen prior to storing.

Information on the moisture content of fresh pine pollen is scarce. Previous informations available given for *Pinus silvestris*, state 7,5—7,9 % water in fresh pollen (Research Department, AB Kabi, Stockholm, Sweden, ELSER und GANZMÜLLER 1930). The present experiments only concern pollen with slightly lower moisture content and nothing can be said about the effect of variation of the humidity factor.

Conclusions

As many authors have pointed out (a.o. DUFFIELD 1954, CUMMING and RIGHTER 1948) viable pollen is not necessarily fertile or, otherwise expressed, the capability of germination *in vitro* does not vouch for good results *in vivo*. In some experiments with *Picea* and *Alnus* species carried out by the present author (unpubl.), irradiated pollen (10, 30 and 90 kr) germinated to over 50 % *in vitro*. When used in crossing experiments, the pollen given the highest dose did not lead to seed formation. Nevertheless testing pollen under laboratory conditions gives some information about the state of a pollen sample. If no germination occurs, it probably contains few or no fertile pollen grains (cf. however ROEMER 1914, BRINK 1924, NEBEL and RUTTLE 1936, PFEIFFER 1939, BECK and JOLY 1941). For properly judging pollen quality with regard to its fertilization capacity, pollination experiments should be carried out as well. Hitherto no laboratory tests have been found to be absolutely reliable as proof of the viability of pollen *in vivo*.

There is a general agreement on the fact that pollen of widely different species remains viable for a longer period when stored at low temperatures (0° C to + 5° C) than when stored at room temperature. Still lower storage temperatures (— 5° C to —17° C) have been tried too, and with good results. Very low humidity (0—10 %) or as high as 75 % and more seem to be harmful regardless of the temperatures used. Usually a humidity percentage ranging from 30—75 % is recommended, the most favourable one for several species being 50 % (cf. a.o. NEBEL and RUTTLE 1936). The right combination of temperature and relative humidity is, however, of even greater importance for the retention of the viability of pollen.

JOHNSON (1943) working with five different pine species, found 50—75 % relative humidity at +2° C to be the best storage conditions. DUFFIELD and SNOW (1941) in their studies of pollen longevity of *Pinus strobus* and *Pinus resinosa* established 50 % relative humidity at 0° C to +4° C as being a suitable combination. Good results were obtained by MARCET (1951) by storing pollen of *Pinus silvestris* in 50 % humidity at +3° C for 20 months. These conclusions are drawn from data obtained in laboratory tests.

The results reported in the present paper substantiate the statements concerning the beneficial effect of cold storage on the retention of viability by pollen as measured by germination precentage on culture media. In this respect both the temperatures used here, +4° C and —18° C, gave about the same results, when the storage period did not exceed six years. When measured by fertilization capability on the contrary, there was a distinct difference between the effects of the two storage conditions already after three years, —18° C being the most favourable one.

Summary

Pollen of *Pinus silvestris* and *Pinus contorta* var. *Murrayana* was stored at room temperature, at +4° C and at —18° C, and was tested for germinability after various number of years of storage. The pollen was cultured on distilled water in darkness in a thermostat at about 30° C.

Crossing experiments were carried out on two different occasions with three-year-old pollen.

The pollen stored at room temperature had already lost its viability after twelve months.

The pollen of *Pinus silvestris* germinated well after two years of storage at the lower temperatures. In the following years the germinability decreased slowly but was still high after six years. Differences between the effects of the two storage methods (+4° and —18° C) became apparent when the pollen had been stored for seven years. Then the germination per cent of pollen stored at +4° was low, while pollen stored at —18° C still retained a high number of viable grains.

In *Pinus Murrayana* the longevity of pollen seemed to be lower than in *Pinus silvestris*. Two-year-old pollen germinated 90 per cent or more, but after four years the germinability decreased strikingly. Pollen stored at —18° C retained its viability longer than pollen stored in other conditions.

The length of the pollen tubes were measured and the percentage of abnormal tubes were recorded in some of the germination tests. There was no evidence of reduced growing capacity of the tubes after the pollen had been stored for two years or more. Nor did any positive differences of the effects on tube growth of the various storage methods become apparent, but there was a slight indication that storage at —18° C was more favourable for the production of long tubes. In

general the tubes of *Pinus Murrayana* pollen were shorter than those of *Pinus silvestris*. The percentage of abnormally shaped tubes was higher in the *Pinus Murrayana* samples.

In the crossing experiments with three-year- old pollen, the fertilization capability of pollen stored at —18° C was distinctly greater than that of pollen stored at +4° C.

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Sammanfattning

Lagringsduglighet hos tallpollen

Hos pollen av *Pinus silvestris* och *Pinus contorta* var. *Murrayana*, lagrat i slutna glasampuller vid tre olika temperaturer, undersöktes groningsförmågan efter ett till sju års lagringstid. Pollenet förvarades dels i rumstemperatur, dels vid + 4° C i kylskåp och dels vid —18° C i frysbox. Groningarna utfördes på destillerat vatten i glasskålar med lock, insatta i värmeskåp vid +30° C.

Pollen, lagrat i *rumstemperatur*, hade redan efter 12 månader förlorat sin vitalitet.

Hos pollen av *Pinus silvestris*, lagrat vid +4° C, bibehölls groningsförmågan intakt under två års tid (ca 90 % grodda korn) för att under de följande fyra åren långsamt avtaga. Efter sju års lagring grodde endast ca 20 % av pollenkornen. Vid förvaring i frysbox däremot, sjönk groningsförmågan långsammare och var ännu efter sju år uppe i ca 80 %. Den gynnsamma effekten på pollenets livslängd av lagring vid låg temperatur, — 18° C, var tydligt påvisbar.

Groningsförmågan hos *Pinus Murrayana*-pollen avtog hastigare än hos *Pinus silvestris*-pollen. Fyra-årigt pollen, förvarat vid +4° C, grodde med ca 50 %. Efter ytterligare tre års lagring grodde endast ett fåtal pollenkorn. Den lägsta lagringstemperaturen, —18° C, var fördelaktigare för bibehållande av pollenvitaliteten än den högre temperaturen.

Korsningsförsök med tre år gammalt pollen av *Pinus silvestris* utfördes under två blomningssäsonger. Som moderträd användes dels unga ympar, dels fullvuxna träd med riklig blomning och god kott- och frösättning. Pollen, förvarat vid + 4° C, hade låg eller ingen fertilitet, medan den andra gruppen pollen, förvarat vid —18° C, fortfarande var funktionsdugligt. I korsningsförsök med detta pollen erhölls dock lägre procent matat frö än i parallella korsningar med färskt pollen. Graden av fertilitet hos tallpollen kan icke med säkerhet fastställas genom groningsförsök i laboratorium.

Pollenslangarnas tillväxt och form undersöktes i vissa försöksserier. Tillväxten, bedömd efter pollenslangens längd efter 24, 48 och 72 timmars groning, var oberoende av lagringstidens längd men påverkades av lagringstemperaturen. Pollenslangarna hos *Pinus Murrayana*-pollen var i allmänhet kortare och procenten pollenslangar, som missbildats eller brustit, högre än hos *Pinus silvestris*.

Vattenhalten i 5 och 7 år gammalt pollen bestämdes och befanns vara något lägre än den som anges för färskt pollen av *Pinus silvestris* (7,5—7,9 %). Inga försök med att variera luftfuktigheten under förvaringstiden har utförts.