Studies of Extraction, Storage, and Testing of Pine Pollen

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Introduction

This report assembles the results of a number of srnall exploratory studies on the extraction, storage, and viability testing of pollen of several species of pines. These studies indicate clearly the need for more knowledge of the physiology of pollen — particularly of the relation between atmospheric humidity at the time of pollen shedding and the subsequent reactions of the pollen to environmental conditions.

1. The Importance of Extraction Humidity and its Control

The importance of humidity of the air in which dehiscence occurs has long been recognized. LIDFORSS (1899) allowed flowers of a number of angiosperm species to shed pollen under various humidity levels, and found that, in general, pollen shed at the higher humidities was more germinable and more resistant to moistening injury than that shed at lower humidities.

On March 18, 1946, the writer set up a small test to determine the effects of extraction humidity on germinability and storage properties of pollen of *Pinus canariensis.* Unopened catkins were collected from branch tips from which some pollen had already been shed. All material was taken from a single tree. Equal numbers of catkins were placed in each of four cylindrical wire baskets set in funnels which led into small shell vials. Each assembly was placed in a glass jar in which relative humidity was maintained at 10 percent, 25 percent, or 75 percent by sulfuric acid solutions. All jars were then placed in a constant temperature cabinet at 22° C. On March 28, the extractors were removed from the constant temperature cabinet and shaken vigorously until no more pollen fell into the vials. The yield of pollen and the change in length of catkins during the 10-day extraction period were:

Extraction humidity	Pollen yield (cc.)	Change in length of Catkins (Percent)
10%	.67	−10
25%	.77	0
50%	1.00	+50
75%	.29	+75

After the catkins from the 75 percent relative humidity extractor had been exposed to the relatively dry air of the laboratory for a few minutes, they shed about 0.7 cc. of pollen, which, however, was not used in the subsequent tests of germinabilily and storage.

These findings appear relatively easy to interpret on the basis that pollen shedding is the resultant of two processes: The biological process of growth and elongation of the catkin axis and the physical process of rupture of the sporangia. The growth of the catkin axis requires water, which may be furnished by the vascular system to catkins on the tree or by a humid atmosphere to catkins in an extractor. Rupture of the sporangia requires relative desiccation of the Sporangium walls by a rather low humidity (50 percent or less in this test). This interpretation is similar to the one arrived at by SCAMONI (1938), who, however, used catkin-bearing branches of P. *silvestris* rather than detached catkins in his experiments. Very likely, in nature, elongation of the catkin axis and rupture of sporangia, occur simultaneously, the first process supported by moisture supplied through the vascular system and the second promoted by high evaporation associated with sunshine, wind, and high temperatures (SCAMONI, 1938). It appears quite difficult to reproduce these conditions in any practical type of extractor, but there is probably a good chance of bringing about these two processes in sequence, by adopting an extraction schedule under which the catkin axes are allowed to elongate at a relative humidity of about 50 percent and a temperature of 25° to 30° C., followed by reduction of humidity to 10 percent or 25 percent to cause rupture of sporangia.

So much for the mechanics of pollen extraction. What of the effects of extraction humidity on the viability of the pollen? To answer this question, the pollen of P. *canariensis* extracted on March 28, 1946, was, on the same day, subdivided into nine lots for each extraction humidity, and stored at 0°, 10°, and 22° C. and at 10 percent, 25 percent, and 50 percent relative humidity.

All 36 samples were tested by the vapor method[2]) on November 12, 1946, two replications being set up. Fifty grains of each treatment were counted in each replication, making a sample of 100 grains per treatment. The results are shown in Table 1.

Table 1
Germination of *Pinus canariensis* pollen stored 229 days

Storage conditions	Percent germination when extraction humidity was —			
	10%	25%	50%	75%
Temperature 0° C.				
10% humidity	67	68	68	52
25% humidity	78	62	72	19
50% humidity	76	78	68	21
temperature 10° C.				
10% humidity	61	70	82	18
25% humidity	76	68	71	4
50% humidity	64	89	33	3
Temperature 22° C.				
10% humidity	55	83	57	13
25% humidity	55	39	40	0
50% humidity	5	7	0	0

The results of this test may be summarized as follows: Extraction at 75 percent relative humidity is disadvantageous regardless of how the pollen is stored subsequently. It should be noted, however, that the pollen extracted at 75 percent relative humidity was handled in a room with a relative humidity of 30 percent to 50 percent when the pollen was segregated into lots for the storage test. These fluctuations in humidity, although brief, may have influenced the germinability of certain lots.

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[2]) Pollen testing methods are described in a subsequent section of this paper.

During the course of the germination counts, certain observations were made on the appearance, tube length, forking, and bursting of each lot. Every sample of pollen extracted at 75 percent relative humidity had a large majority of grains which floated with bladders up and had two or three large vacuoles generally arranged along the short axis of these grains when viewed from above. This was also true of the lot extracted at 50 percent and stored at 22° C. and 50 percent. In general, pollen extracted at 10 percent or 25 percent and stored at 0° C. or 10° C. showed a high proportion of cytoplasmic extrusion both from germinated and ungerminated grains. There was less of this bursting in the lots extracted at 50 percent and virtually none in those extracted at 75 percent.

Only by experiment can these findings with respect to germinability of pollen extracted under various humidity levels be related to the ability of such pollen to effect fertilization — particularly after storage of one year or more. Nevertheless, it seems clear that variations in extraction humidity such as those used in this experiment produce effects which are certainly worth investigating further, especially since variations of equal magnitude undoubtedly occur between routine extractions of the pollen used in breeding. Therefore, it should be worthwhile to develop an extraction technique which will afford fairly accurate and individual control of the extraction humidity (and temperature) of each lot of pollen. Such a technique will not only be useful in conducting experiments to determine optimum extraction conditions, but also, after these are determined, it will be necessary in routine breeding operations. It is quite possible that once pollen extraction is better standardized and species requirements understood, many species crosses which have failed in the past may be found possible. At any rate, it seems clear that standardization of humidity and temperature at which pollen is extracted must precede any careful work on pollen storage and barriers to species crosses.

2. Pollen Storage

In 1945, samples of pollen of seven species of pines were placed in storage by Dr. N. T. Mirov. Four levels of temperature (0°, 5°, 10° and 22° C.) and four levels of relative humidity (10%, 25%, 50% and 75%) were used. The dates of collecting and storing of the pollen samples are as follows:

Species	Date collected	Date stored
P. radiata D. Don	—	February 24
P. canariensis Smith	April 10	April 18
P. taeda L.	April 11	April 18
P. echinata Mill.	May 3	May 10
P. ponderosa Laws.	May 24	July 2
P. strobus L.	May 24	July 2
P. lambertiana Dougl.	July 11	August 8

In 1946, the germinability of these samples was determined by dusting pollen from the storage vials onto marked spots inside a Petri dish bottom. All sixteen storage conditions were tested in a single dish, and two dishes were set up for each species. The samples from each storage condition were arranged in randomly-selected Latin squares. Each Petri dish bottom so set up was then inverted into a Petri dish cover with enough water in it to cover the bottom and provide a water seal, so that the air in the dish was kept saturated with water vapor. The dishes were then incubated at 25° to 28° C. for 48 to 72 hours. Germination percents were determined from 100-grain samples, that is, the sums of two determinations of 50 grains each (Table 2).

Table 2
Pollen germinability — seven pine species

Species	Date of test	Days stored	Storage temp. °C.	Relative humidity in storage			
				10%	25%	50%	75%
				Percent germinated			
P. radiata	May 22, 1946	391	0	2	78	67	1
			5	3	73	56	0
			10	33	69	11	0
			22	10	24	0	0
P. canariensis	May 22, 1946	338	0	61	62	70	30
			5	68	61	60	4
			10	65	57	51	0
			22	65	36	0	—
P. taeda	May 22, 1946	338	0	39	36	30	7
			5	30	33	33	0
			10	35	30	58	0
			22	41	46	0	0
P. echinata	Oct. 15, 1946	523	0	86	82	98	60
			5	91	83	90	4
			10	82	83	94	0
			22	88	67	0	0
P. ponderosa	Sept. 10, 1946	435	0	71	68	71	0
			5	60	58	51	0
			10	70	61	16	0
			22	70	57	0	0
P. strobus	Oct. 15, 1946	470	0	85	82	83	13
			5	88	91	81	0
			10	88	69	36	0
			22	74	66	0	0
P. lambertiana	Sept. 10, 1946	398	0	9	18	11	0
			5	7	36	28	0
			10	16	27	6	0
			22	31	5	0	0

In two respects these tests are quite consistent and conclusive. First, storage at 75 percent relative humidity is undesirable, regardless of temperature. Secondly, in all species, storage at 50 percent relative humidity and 22° C. is undesirable, and in most species, this is true of storage at 25 percent relative humidity and 22° C. In one respect the species tested seem to fall into two groups. *P. radiata* and *P. lambertiana* pollen stored at 10 percent relative humidity and at 0° and 5° C. showed very low germinability, whereas this effect was not found in the other five species tested. In order to determine whether this effect was real and not due to experimental errors, a repeat test was made, October 18, 1946, with pollen of *P. radiata* (Table 3).

Table 3
Germination percentages — *Pinus radiata* pollen stored for 540 days

Temperature °C.	Germination when relative humidity in storage was					
	10%		25%		50%	
	No.[1])	%[2])	No.[1])	%[2])	No.[1])	%[2])
0	0		18		22	
	0		16		23	
	1	1	24	78	21	85
	0		20		19	
5	0		22		24	
	0		moldy		19	
	7	7	22	84	20	80
	0		19		17	
10	0		21		14	
	7		23		8	
	14	34	25	90	10	41
	13		21		9	

[1]) Number germinated in a 25-grain sample.
[2]) Totals or germination percent.

Results of this test fully bear out the indications that *P. radiata* pollen is sensitive to low-humidity, low-temperature storage. It may be asked whether this is a matter of actual species differences or is an effect of differences in extracting and handling the pollen samples before they were stored. Although some of the data in Table 1 make it appear that low extraction humidity makes pollen more sensitive to low-humidity storage, yet several repeat tests with samples extracted and stored at low humidities failed to give consistent results. Therefore, this question must remain open for the moment.

In discussing storage requirements, the results of the present work should be related to previous findings. DUFFIELD and SNOW (1941) found that storage at 0° or 4° C. and 50 percent relative humidity was most favorable for pollen of *P. strobus* and *P. resinosa*. The present work is in general agreement with these findings, and supplements them to the limited extent that it shows that if the optimum humidity is greater than 50 percent it certainly is not as great as 75 percent. In this respect the present work differs from the findings of JOHNSON (1943), who reported good germination for several pine and spruce species after 12 months' storage at 2° C. and 75 percent relative humidity. In fact, he reported this condition to be the optimum for one lot of *P. strobus* and one of *Picea glauca*. This is an important difference, and the reason for it should be investigated. Another of JOHNSON's findings is in agreement with the present study, namely that, "There is considerable specific difference in tolerance of low humidity storage at both 2° C. and room temperature". However, it should be remembered that presumed species and tree differences in JOHNSON's work, as well as the present one, might in reality be traceable to extraction differences. It is interesting to note that JOHNSON states, "All material was stored for several days over anhydrous calcium chloride prior to testing", but does not specify extraction techniques. This preliminary desiccation of the pollen might well account for the intolerance to low humidity storage of most of his samples, although, as previously noted, this question is still open. In the study by DUFFIELD and SNOW (1941), pollen was extracted by allowing catkins to open and shed on sheets of paper spread out on tables in rooms with a relative humidity of probably 25 percent to 40 percent, although this was not stated in their paper. How these conditions compare with those in the extractors used at the Institute of Forest Genetics, (CUMMING and RIGHTER, 1948) would be difficult to state, but probably extraction in an open room occurs at a lower humidity than in a funnel-and-bag extractor.

Another factor which should be kept in mind in comparing JOHNSON's work with that reported here is that different germination techniques were used. JOHNSON sowed his pollen directly from the desiccators onto a moist agar medium, whereas in the present tests, vapor germination was used. The significance of this difference in procedure will be discussed later.

Storage at 25 percent relative humidity appears to be indicated by the present and previous studies on the basis that this level of humidity has not been found injurious to pollen of any pine species at low temperatures, and is definitely preferable to 50 percent relative humidity when it is necessary, because of limited cold storage space, to store pollen at or near room temperature.

3. Pollen Testing

a. Importance of pollen testing

By testing of pollen is meant the determination of its viability by means of artificial germination tests. It should be recognized that testing pollen by means of artificial germination is quite different from similar tests of seeds. Our knowledge of the growth requirements of seedlings is usually sufficient to enable us to predict from the results of a germination test, the number of plants that may be derived from a given lot of seed. With pollen, the situation is quite different. The relationships between the pollen grain and the intended maternal tissues are so complex and little-known — especially in the gymnosperms, that artificial germination tests cannot be relied upon to give positive information as to the fertilizing potential of a sample of pollen. However, it seems reasonable to assume provisionally that pollen which cannot be made to germinate by usual means or which, on germination, shows unusually poor tube growth, is likely to be ineffective in causing fertilization. Herein lies the present practical value of pollen testing as a routine procedure in breeding operations. Perhaps the coordination of pollination and germination tests will enable us, in the future, to make positive as well as negative predictions from germination tests. At any rate, pollen extracted by prevailing methods is often enough found to be non-germinable to fully justify routine testing if only to avoid waste of time and materials which results when non-functional pollen is used in breeding operations. An even more cogent reason for routine testing is the likelihood of drawing incorrect conclusions from the failure of certain attempted crosses if non-functional pollen is used unwittingly.

b. Methods of pollen testing

Three main methods of germinating pollen have been used. They are the hanging drop, agar gel, and vapor methods. The hanging drop method has been described by RIGHTER (1939) and the agar gel method by DUFFIELD and SNOW (1941). The vapor method is a modification of a technique described by VON WALDERDORFF (1924), who used dried 10 percent gelatin solution on slides as a substrate for the pollen. Moisture was supplied to substrate and pollen by placing drops of sodium chloride solutions of various vapor pressures near the pollen. As used by the writer, this method is simplified to the extent of omitting the gelatin and providing water vapor by the addition of distilled water to the bottom of the culture dish.

The details of the method are as follows: A rubber stamp consisting of a number of circles of 5 mm. inside diameter arranged in rows and columns to form a rectangle as in the method described by RIGHTER (1939) is used to stamp rings of vaseline into the inside of the bottom (smaller part) of a Petri dish. These rings serve to localize the pollen, which is dusted on the dry glass surfaces within each ring. In order to achieve an even dusting, the pollen is picked up on the end of a wood applicator 1 mm. in diameter, which is then tapped over the upper end of a vertical glass tube about 60 mm. long and 5 mm. inside diameter. The tube is thrust through the hole in a square plate of transparent plastic large enough so that whatever part of the dish is being dusted, the whole dish is protected from air currents and air-borne contamination. A clean glass tube and a fresh applicator are used for each lot of pollen. When all the pollen

samples have been dusted into the dish bottom, it is inverted into the larger member of the dish, which has enough water in it to provide an air lock. Incubation is at 26° C. to 28° C. This method may be readily transformed into a hanging drop method by adding a drop of distilled water to each ring before adding the pollen. Two difficulties are often encountered in setting up vapor germination cultures. First, the pollen is likely to roll down the sides of the glass tube rather than falling freely through the air, resulting in a crescent-shaped deposit of pollen at one side of the ring. Second, under some conditions, the pollen, the glass tube, or both, may pick up a charge, so that when the glass tube is withdrawn after the dusting, the pollen flies all over the Petri dish bottom. This can generally be avoided by withdrawing the tube slowly.

In relation to the vapor germination method, it is interesting to note that KÜHLWEIN (1937) reported that he was unable to germinate pollen of *Pinus silvestris* or *P. montana* on clean glass in a saturated atmosphere, while gymnosperm pollens with swelling layers, such as *Taxus, Chamaecyparis, Thuya,* and *Juniperus* germinated well under these conditions. In the present work, some vapor cultures have had so much condensation on the glass on which the pollen grains were lying that they were for all practical purposes hanging drop cultures — with this important difference: the moistening of the pollen was gradual. However, many of the cultures have been true vapor cultures, the only visible water being on the surfaces of the pollen grains and the immediately adjacent glass. In fact, the optical properties of such cultures are such that, in order to make germination counts readily, condensation of a large amount of water within the rings is induced by heating the bottom of the culture for a few minutes. It is hard to reconcile this finding with KÜHLWEIN's (1937), failure to germinate pine pollen on glass in saturated air.

c. Relation of testing method to extraction, storage, and species

For the purposes of this discussion, the methods mentioned above may be grouped into those in which the pollen is transferred directly from storage to a wet germination medium and the vapor method by which the pollen is gradually moistened and finally wetted in an atmosphere saturated with water vapor. The results achieved by these two general methods (Table 4) may be quite similar or very different depending on species and conditions of extraction and storage.

Table 4
Germination of pollen of four pine species tested by drop and by vapor methods

Species	Germination method	Storage humidity[1]	
		10%	50%
		Percent germination[2]	
P. canariensis	Drop	60	70
	Vapor	72	84
P. lambertiana	Drop	23	73
	Vapor	66	70
P. ponderosa	Drop	28	89
	Vapor	84	92
P. radiata	Drop	16	80
	Vapor	8	78

[1]) Storage temperature 0° C. for each lot.
[2]) Each figure is based on 4 samples of 50 grains each.

Results of a test presented in Table 4 suggest that for *P. lambertiana* and *P. ponderosa* the hanging drop method is much more suitable for revealing the results of storage at different humidities. Whether storage of pollen of these species at low humidity would have an unfavorable effect on fertilization comparable to that on germination in distilled water remains to be tested. In the case of *P. radiata,* storage at low humidity appears to depress germinability whether the drop or the vapor method is used. The drop method appears to give better germination with pollen stored at low humidity, and, if significant, this is an interesting reversal of the relations found in *P. lambertiana* and *P. ponderosa.* Finally, neither storage humidity nor germination method appears to affect the germinability of *P. canariensis* pollen stored at 0° C. to nearly the striking extent found with the other three species. However, this difference may possibly be traced back to conditions of extraction. To test this possibility an experiment was set up with the same lots of pollen used in the *P. canariensis* extraction humidity experiment.

Table 5
Germination of *Pinus canariensis* pollen tested by drop and by vapor methods

Storage		Germination method	Extraction humidity	
Temp.	Humidity		10%	50%
			Percent germination[1]	
0° C.	10%	Drop	72	51
		Vapor	78	72
	50%	Drop	72	69
		Vapor	79	79
22° C.	10%	Drop	67	9
		Vapor	74	77
	50%	Drop	34	0
		Vapor	22	0

[1]) Each figure is based on 4 samples of 50 grains each.

Results of this experiment (Table 5) suggest that low-humidity extraction not only made *P. canariensis* pollen more resistant to storage at high temperature and high humidity, but made it indifferent to germination method as well. Again, the question of which germination method would lead to the correct evaluation of the pollen for use in breeding must remain unanswered for the present. However, the two experiments just reported leave little doubt that as a laboratory procedure for revealing humidity treatment effects, the hanging drop method is far more sensitive than the vapor method.

The findings of DUFFIELD and SNOW (1941), of JOHNSON (1943) and of the present study suggest that pollen of some conifer species is sensitive to low humidity storage. It has been shown that extraction humidity may affect storage responses of *P. canariensis* pollen, and now it appears that the evaluation of these responses depends on germination procedures. These results therefore indicate that conclusions regarding species differences in storage responses can be regarded as tentative and that their confirmation awaits standardization not only of extraction methods, but of germination techniques and probably, in the end, of pollination tests as well. If hanging-drop or other wet-medium tests are used, the problem of securing a definite relation between the mass of the medium and that of the pollen sown on it may be of great importance. At present, the hanging drop method gives highly variable results — probably traceable to variations in density of sowing. Some of these may be eliminated by increasing

the precision of the suspension and pipetting method of setting up hanging-drop cultures which has already been developed by RIGHTER (1939). POHL (1937a) obtained quite uniform results in pollen grain counts with the use of water suspension methods.

d. Relation of testing methods to previous work on moisture relations of pollen

Several investigators have reported beneficial effects from humidifying pollen before placing it on a wet germination medium (RUTTLE and NEBEL, 1937; PFEIFFER, 1939). DUFFIELD and SNOW (1941) showed that this effect was more marked in the case of pollen of *Pinus strobus* and *P. resinosa* stored at low humidities. The vapor method used in the present studies is a logical development in view of these findings. Nevertheless, it is not clear that the higher germinability revealed when pollen is humidified before wetting is any more indicative of the value of the pollen for breeding than is the somewhat lower value determined by wet-medium tests without preliminary humidification. It must be emphasized that laboratory germination tests should as soon as possible be correlated with pollination tests of samples of pollen stored under various conditions. Certain difficulties in running comprehensive tests of this nature should be recognized at the outset. As DUFFIELD and SNOW pointed out: "Tests of the ability of pollen to function in vivo — conclusively demonstrate pollen viability if the results are positive. Negative results of pollination tests are fully significant only when the reproductive behavior of the plants used is well known." Such tests may be "confounded by biological factors such as incompatibilities and abortions since little is known concerning these factors" in the pines.

e. Possible relations between moisture history of pollen and response to growth substances

Although laboratory tests of pollen germinability may be of uncertain value for practical breeding operations, they may prove to be powerful tools for increasing our understanding of the physiology of pollen. Scattered bits of evidence suggest that the great significance of moisture history for the germination requirements of pollen may be in some way related to auxins. Although pine pollen has been shown to be a rich source of auxin (GUSTAFSON, 1937), the work of SMITH (1939), who found that pollen of *P. austriaca* germinated in a 1 ppm. solution of indole-3 acetic acid but not in his controls, suggests that under certain conditions the auxin in pine pollen may be inactivated or unavailable. Many other investigators working with pollen of many pine species have found it readily germinable under a wide range of conditions, and presumably, in most cases, without externally supplied auxin. Thus it seems possible that SMITH's sample of pollen had been treated in some manner which caused it to require auxin for germination. BRINK (1924) found that clumped pollen grains put out tubes at a more rapid rate than grains isolated on the germination medium. This appears to be consistent with the findings of KUHN (1937), who discusses at length the requirements of some pollens for specific substances which may be secreted by the pistil or carried by the pollen itself in minute quantities and shows that pollens of this type may be germinated only on small amounts of semi-solid media which will not, by leaching and dilution, reduce the substance contributed by the pollen below an effective concentration. LIDFORSS (1899) found that the injury to pollen caused by wetting and subsequent drying was less if the grains were clumped. SMITH (1942) suggests that this may not have been so much an effect of retarded drying as a matter of reduced aeration possibly affecting oxidation and inactivation of auxins.

f. Possibilities of developing laboratory criteria other than germinability

So far, the whole discussion has dealt with germinability of pollen. As has been suggested, this property cannot be considered to be, in itself, a reliable index of fertilizing ability. Probably what germinability reveals is that a certain percentage of grains is able to cross a certain functional threshold. No doubt, fertilization is some distance beyond this threshold, but germination tests give little or no information as to how much of this distance the germinated grains could cover in nature. In practice, at the Institute, this lack has been recognized, and an effort made to compensate for it by several means. These consist of (1) noting the time elapsed between set-up of test and germination counting, (2) noting length of pollen tubes as a multiple of grain diameter, (3) estimating the percentage of burst grains. It is probably fair to state that the latter two types of data are the results of rapid ocular estimates and that it would be unsound to base critical comparisons of pollen lots on them except in cases of unusually large differences. In such cases these differences would probably be reflected in germination percentages.

For experiments designed to evaluate precisely extraction and storage conditions, it should be possible to develop measurements which would give more information on the fertilizing potential of pollen than the enumerations largely relied on up to present. It would seem that determination of the rate of pollen-tube elongation should be a relatively simple matter, and that once this rate is determined, it might be shown to have a rather simple relation to fertilizing potential. While the latter proposition is very likely sound, the accurate determination of pollen-tube elongation rates depends on refinements of technique which have yet to be attained. One example of an attempt to determine elongation rates will illustrate this. When the germination percentages for *P. radiata* shown in Table 2 were determined, measurements of pollen-tube lengths were also made. These, together with germination percentages, are shown in Table 6. In this table, the germination percentage based on a sample of 50 grains is given for each plate as is pollen-tube length, each value being based on 10 measurements.

Table 6
Germination and pollen tube lengths[1]) of *P. radiata* pollen stored 391 days

Storage temperature		Storage humidity					
		25%			50%		
		Plate A	Plate B	Mean	Plate A	Plate B	Mean
0° C.	Germ.Percent	80	76	78	66	68	67
	Tube Length	22.2	33.2	27.7	19.2	26.6	22.9
5° C.	Germ.Percent	80	66	73	48	64	56
	Tube Length	25.3	32.4	28.9	17.4	24.6	21.0
10° C.	Germ.Percent	68	70	69	8	14	11
	Tube Length	19.3	34.8	27.1	21.4	23.6	18.0

[1]) Pollen tube lengths given in ocular micrometer units. One unit equals 6.7 micra.

It will be noted that germination percents of the A and B plates do not differ consistently, and that the A and B plate percents are rather close to their means (Table 6). In the case of pollen-tube length, however, it is clear that for every storage condition under which germination occurred in both plates, the growth rate in plate B was higher than in plate A. This no doubt means that temperature was slightly higher in plate B than in plate A during germination and tube growth. In this particular test, the plates were placed side-by-side on the top shelf of a seed germinating incubator running at 25° C. The uniformity of conditions within the incubator appears to have left something to be desired, but sufficient uniformity could probably be attained only in an aircirculating incubator with very precise thermostatic controls. At any rate, it is clear that tube elongation is more sensitive to incubation conditions than is germination percent. Analysis of variance of the pollen tube lengths shows that the superiority of the B plate series is highly significant, and further, that storage at 25 percent relative humidity results in more rapid pollen-tube growth than storage at 50 percent relative humidity (high significance). There was no significant difference in the effect of the three rather low temperatures on pollen-tube growth. Scanning Table 6, it appears that an evaluation of the storage methods by pollen-tube growth would differ little from one based on germination percent — both measures indicate that storage at 25 percent relative humidity is somewhat superior to storage at 50 percent relative humidity. So far, it seems that little gain in information has been achieved after a considerable increase in labor. Perhaps germination percent and pollen-tube growth are highly and positively correlated. This possibility should be studied further for if it proves to be fact, there would be little utility in estimating pollen-tube growth, at least for routine testing.

Another aspect of pollen cultures which may be related to the fertilizing potential of pollen is the amount of bursting or cytoplasmic extrusion by pollen-grains and pollen-tubes. This is at present noted in routine tests. Bursting of pollen-tubes is a phenomenon which has been shown to be related to a wide variety of conditions of pollen extraction Lidforss (1899) and of storage and germination techniques Kühlwein (1937).

In the extraction experiment with pollen of *P. canariensis* (Table 1) each germination count was accompanied by a rating as to the amount of bursting. Practically all samples extracted at 10 percent or 25 percent relative humidity showed bursting regardless of subsequent storage, while very few of the samples extracted at 50 percent relative humidity showed bursting.

In the storage and germination test reported in Table 4, similar notes were taken on amount of bursting. The most striking result was that all samples of *P. lambertiana* pollen stored at 0° C. and 10 percent relative humidity and germinated by the vapor method showed a high percent of bursting, while little bursting was found among samples of this species stored at 10 percent relative humidity and germinated in a drop or stored at 50 percent relative humidity and germinated by either method.

It therefore appears that bursting is influenced by a number of factors not yet understood or not controlled by our present techniques. Perhaps when our pollen handling techniques are further standardized, studies of bursting will be of some value.

Two other morphological observations were made during the present study. Pollen which has been stored at 75 percent relative humidity appears to float differently from pollen stored at lower humidities. Generally, pollen stored at 10 percent to 50 percent relative humidity (except that stored at 22° C. and 50 percent relative humidity) floats with the bladders more or less to one side, while that stored at 75 percent relative humidity floats with the bladders up so that when viewed from above, each grain is symmetrical along two axes. One is immediately struck by the symmetrical and uniform appearance of such a lot of pollen when one views it under the microscope in a hanging drop. In the extraction experiment reported in Table 1, it was noted that all pollen extracted at 75 percent relative humidity, regardless of subsequent storage, showed a very high percentage of grains which floated with bladders up, and in addition had two or three large vacuoles in the central part of the grain between the bladders. These two visible criteria may be of some value in detecting promptly lots of pollen which have been subjected to unfavorable extraction or storage conditions. How soon pollen takes on these aspects after being placed in a drop has not yet been determined.

During the storage experiments reported in Table 2 it was noted, especially when making the tests of *P. ponderosa* and *P. lambertiana,* that pollen stored at 75 percent relative humidity and at 50 percent relative humidity and 22° C. took up water from the saturated atmosphere of the culture dish very much more rapidly than did the other samples of pollen. In this particular test, the incubation temperature was between 20° C. and 25° C. and no condensation occurred in the tops of the dishes. The low humidity samples appeared quite dry to the naked eye after two days, at which time condensation was quite noticeable on the high humidity samples. None of this latter pollen germinated, but uniformly presented the symmetrical „bladders up" appearance.

Conclusions

The principal conclusion which can be drawn from these studies is that pine pollen, although notoriously more long-lived than many angiosperm pollens, is quite sensitive to its temperature and moisture environment. This implies, for further studies, that temperature and humidity should be under control during extraction in order to obtain pollen which will give reproducible results in storage studies. Recent studies of conifer seeds have pointed to the same conclusion as regards seed storage.

Aside from the purely technical implications for future empirical studies of pollen handling, the present report indicates that many basic aspects of pollen physiology and biochemistry need exploration.

Zusammenfassung

Titel der Arbeit: *Untersuchungen über Extraktion, Aufbewahrung und Prüfung von Kiefernpollen.* — Ergebnisse mehrerer Einzeluntersuchungen, die die Extraktion, die Aufbewahrung und die Prüfung der Lebensfähigkeit von Pollen verschiedener Kiefernarten zum Gegenstand hatten, werden zusammengefaßt. Sie sollen zugleich den Ausgangspunkt für spätere Arbeiten über die Pollenphysiologie bilden.

Den Einfluß der Feuchtigkeit während der Pollen-Extraktion behandeln die ersten Versuche mit *Pinus cana-*

riensis. Im Verlauf einer 10tägigen Extraktionsperiode bei 50% Luftfeuchtigkeit erbringen die Kätzchen den höchsten Pollenertrag, gleichzeitig verlängern sich ihre Achsen um 50%. Bei 10%iger Luftfeuchtigkeit verschlechtert sich das Ergebnis, und die Achse verkürzt sich um 10%. Die Kätzchen in 75%iger Luftfeuchtigkeit schütten den größten Teil des Pollens erst nach Abbruch des Versuches im trockenen Labor-Klima. Zwei Erscheinungen lassen sich beim Pollinierungsvorgang trennen: 1. der biologische Prozeß der Kätzchenverlängerung und 2. der physikalische Prozeß des Reißens der Mikrosporangien. — Die Abhängigkeit der Lebensfähigkeit des Pollens von der Höhe der Extraktionsfeuchtigkeit beurteilt ein Pollenaufbewahrungsversuch von 229 Tagen mit variierten Feuchtigkeits- und Temperaturverhältnissen in jeweils 2 Wiederholungen. Jede Probenahme umfaßt dabei 50 Pollenkörner, also 100 bei jedem Aufbewahrungsbeispiel (s. Tab. 1). 75%ige relative Luftfeuchtigkeit bei der Extraktion des Pollens schadet der Keimfähigkeit bei jedem Aufbewahrungsklima. Höhere Keimfähigkeit wird in allen Fällen bei geringerer Extraktionsfeuchtigkeit erzielt. Die Nachprüfung der erhaltenen Zahlen im Befruchtungsexperiment muß später erfolgen. Ebenso muß eine weitere Vervollkommnung der Extraktionstechnik erreicht werden.

Der Klärung von Zusammenhängen zwischen den Aufbewahrungsbedingungen und der Keimfähigkeit des Pollens dienen andere Experimente mit 7 verschiedenen Kiefernarten (Aufbewahrungszeit etwa 1 Jahr und mehr). Vier Temperatur-Varianten (0°, 5°, 10°, 22° C) mit je vier verschiedenen Graden relativer Feuchtigkeit (10%, 25%, 50%, 75%) werden bei allen Arten verglichen (Tab. 2). 75%ige relative Feuchtigkeit bei der Aufbewahrung ist auf jeder Temperaturstufe unerwünscht; 50% ist ebenso unzweckmäßig, wenn bei 22° C aufbewahrt wird; bei den meisten Arten auch 25% bei 22° C. Eine andere Tendenz zeigen dagegen *P. radiata* und *P. lambertiana*, die bei 10% und 0° bzw. 5° C die schlechtesten Keimergebnisse bringen. Offen bleibt dabei, ob für dieses entgegengesetzte Verhalten womöglich die niedrige Extraktionsfeuchtigkeit bei der Pollengewinnung verantwortlich sein kann. — Als allgemeines Ergebnis ist feststellbar, daß der Pollen aller Kiefernarten bei 25% relativer Feuchtigkeit und niedrigen Temperaturen ohne Schaden aufbewahrt werden kann und daß dieses Verfahren einer Aufbewahrung bei 50% und Zimmertemperatur vorzuziehen ist.

Die Pollenprüfung mit Hilfe künstlicher Keimteste wird kritisch beurteilt. In ihrer bisherigen Form sagen sie nicht ohne Einschränkung über die Befruchtungsfähigkeit des Pollens aus, und sie müssen künftig mit Ergebnissen von kontrollierten Bestäubungsversuchen verglichen werden. Zur Pollenkeimung im Laboratorium wird eine Dampfmethode (vapor method) angewendet. Die gewünschte Dampfspannung erzeugt man durch Zusatz von destilliertem Wasser auf den Boden der Kulturschale. Die Keimung erfolgt bei 26° bis 28° C. Die Methode selbst ist von RIGHTER (1939) beschrieben worden. In der Tab. 4 stehen die mit dieser Methode erzielten Keimerfolge den Ergebnissen der Pollenkeimung im „hängenden Tropfen" gegenüber. Die dortigen Zahlengruppen unterscheiden sich je nach Objekt voneinander und lassen keine allgemeine Beziehung zum Aufbewahrungsklima des Pollens erkennen. U. a. hat eine niedrige Extraktionsfeuchtigkeit bei dem Pollen von *P. canariensis* eine hohe Resistenz gegen die Einflüsse hoher Temperatur und Feuchtigkeit bei der Pollenaufbewahrung zur Folge, unabhängig von der verwendeten Keimmethode. Die wichtige Frage nach der besten Keimmethodik für Züchtungszwecke kann durch den Vergleich beider Versuche nicht beantwortet werden. Womöglich scheint in manchen Fällen die Tropfen-Methode vorteilhafter zu sein. Die Ergebnisse aller Einzeluntersuchungen lassen es ganz allgemein zweifelhaft erscheinen, daß den Laboratoriumsprüfungen der Pollenkeimfähigkeit für Züchtungszwecke überhaupt ein Wert beizumessen ist. Dagegen können sie aber den Ansatzpunkt für eine weitere Erforschung der Pollenphysiologie darstellen. In diesem Zusammenhang werden einige Literaturdaten besprochen. — Außer der Pollenkeimfähigkeit lassen sich auch andere Kriteria zur laboratoriumsmäßigen Pollenbeurteilung heranziehen. Die Messung der Pollenschlauchlängen erweist sich dazu als mögliche Grundlage. Nach der Aufbewahrungszeit von 391 Tagen variieren z. B. die mittleren Pollenschlauchlängen von *P. radiata* in positiver Korrelation mit den Keimprozenten der Versuchsglieder (Tab. 6). Als weitere Beurteilungsmomente können auch Beobachtungen über den Umfang des Platzens von Pollenschläuchen oder des Austrittes von Plasma aus den Pollenkörnern Verwendung finden. Versuche mit *P. canariensis* und *P. lambertiana* zeigen allerdings, daß das Platzen der Pollenschläuche von mehreren, heute noch unbekannten Faktoren abhängt.

Die Erfahrungen mit dem von Natur aus langlebigen Kiefernpollen verdeutlichen in allen Fällen andererseits seine große Empfindlichkeit gegen Temperatur und Feuchtigkeit. Beiden Faktoren muß deshalb besonders bei der Pollenextraktion große Beachtung geschenkt werden. Nur solche Aufbewahrungsversuche haben einen Wert, deren Ergebnisse sich reproduzieren lassen. Dies ist jedoch erst dann erreichbar, wenn das Gebiet der Pollen-Physiologie und der Pollen-Biochemie neu bearbeitet wird.

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