FACTORS AFFECTING POLLEN GERMINATION AND LONGEVITY IN HOPS, HUMULUS LUPULUS L.

by

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INTRODUCTION

The common hop, Humulus lupulus L., a member of the Urticaceae family, is a perennial, dioecious, herbacious plant with staminate and pistillate inflorescences borne on separate plants. The dried inflorescences (called strobiles) of the female plant constitute the hops of commerce. The male hop plant is of no commercial value but is necessary in a hop breeding program.

A morphological description of hop pollen is characteristic of other species in the Urticaceae family, in that the spheroid grain is simple, smooth-textured, and void of germinal furrows. This simplicity in structure, coupled with a prominent aspidate feature, is evident in most wind-pollinated species (42, p. 382-384). Variation in pollen size is prominent within genotypes with diameters ranging from 17 to 24 μ and averaging 21 microns (7, p. 57-59). Generally, hop pollen grains have only three germ pores, each surrounded with a conspicuous subexineous thickening, but four and five pores are not uncommon in some genotypes (Figure 1).

A successful hop breeding program occasionally requires crossing genotypes having wide differences in maturity. Such

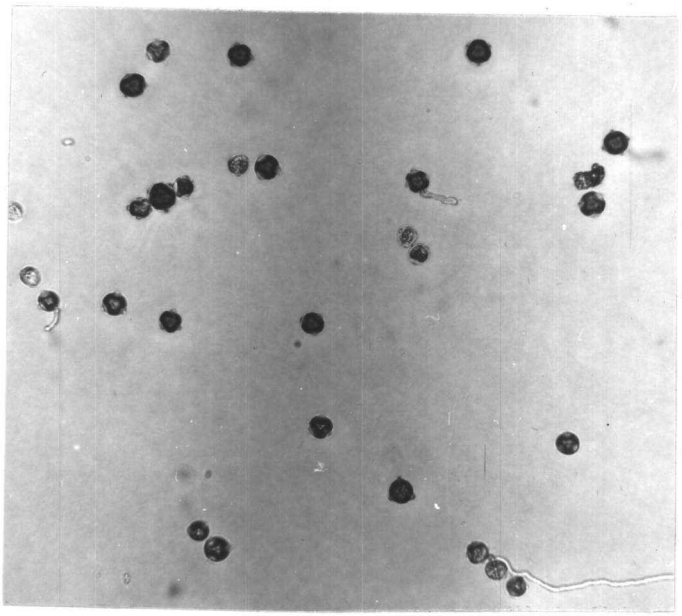

Figure 1. Pollen grains showing variability in size and number of germ pores (X 300).

crosses are not always feasible because male plants may not be shedding pollen at the time stigmas of the female are receptive. Disharmony in time of flowering of two hop clones can be partially compensated for by altering pruning and training times for the male and female plant (8, p. 27-29, 44-45). Fore and Sather (9, vol. 10, p. 21, 25) noted that the pollen shedding periods for different male genotypes varied from four to 52 days, with an average period of 25 days. Stigmas of female inflorescences were found to be receptive to pollen for one to two weeks. However, only a limited number of stigmas within an inflorescence are receptive at one time during the flowering period. The abbreviated period in which a sufficient

amount of pollen is available for fertilization still presents a problem.

Methods are available to store viable pollen of some species; therefore, the difference in time of flowering of two parents does not restrict specific crosses in a breeding program. Proper storage conditions for maintaining viability must be determined for each species to enable the breeder to cross early females with late males. Stored pollen provides the use of germ plasm in other countries and is not subject to quarantine regulations. Use of stored pollen also affords opportunity to establish pollen banks to provide pollen of genotypes which are no longer available.

Knowledge of pollen physiology is essential to explain breeding behavior in a species and to provide a basis for storage procedures. This study was initiated to determine the effects of controlled storage conditions on hop pollen viability and longevity. Storage conditions tested were limited to those which could be easily controlled in a hop breeding program. Nutrient-medium was used as a technique to test pollen longevity during storage and to determine chemical and physiological requirements for pollen germination. Amounts of seed set following controlled pollinations were used as a test of this technique.

REVIEW OF LITERATURE

Specific crosses are often unsuccessful in spite of all of the precautions taken during pollination; and, unless sterility is the main cause, failure of seed setting may be due to slow growth of pollen tubes or their degeneration in the styles. Swaminathan (40, vol. 176, p. 888) obtained viable seeds from crossing Solanum pinnatisectum with other species of Solanum by removing the stigma and part of the style and placing a drop of sugar-agar medium on the cut end of the style.

Normally, pollen grains do not germinate in water, but aqueous solutions of sucrose produce good results (42, vol. 47, p. 239). Most workers state that germination in water alone is usually poor and pollen tubes are short. Pollen of some plants may germinate easily over a wide range of conditions, while in others the requirements may be very exacting. In most cases, the length of the pollen tube obtained in vitro is significantly shorter than that in vivo.

Pollen grains are highly sensitive to temperature and humidity, and the effect of these factors on pollen germination and tube growth can be readily ascertained. However, pollen grains also show great variability when they are collected from different anthers of a flower or even from the same anther (19, vol. 32, p. 463),

(42, vol. 47, p. 239), and (24, vol. 85, p. 136-137). Brink (6, vol. 11, p. 422-428), Visser (43, vol. 55, p. 36-37), and Brewbacker and Majumder (3, vol. 48, p. 458-459) noted that approximately an equal number of pollen grains have to be spread evenly on the medium to obtain comparable results. Workers report that, in spite of precautions to eliminate all known variables, results usually are not repeatable even under apparently similar conditions.

Autotrophic plants or plant tissues which contain chlorophyll are capable of synthesizing their own metabolites for growth and development. Organisms which do not contain chlorophyll become parasites or saprophytes. Generally, pollen does not contain chlorophyll (26, p. 161-162) and is largely dependent on nutrition from external sources. Fresh pollen contains some reserve food which may be utilized for germination and pollen tube growth, but it is not adequate for the extensive tube elongation which occurs in vivo. Utilization of externally acquired nutrients by pollen growing in vitro and in vivo has been a controversial issue among workers. Anthony and Harlan (1, vol. 18, p. 526, 535) and Visser (43, vol. 55, p. 6, 7, 52) believe that supplied sugars play only an osmotic role, and pollen tubes in vivo do not get nutrients or sugar from the style. Brink (5, vol. 11, p. 227-228), O'Kelley (31, vol. 42, p. 325-326) (30, vol. 44, p. 242-243), and Vasil (42, vol. 47, p. 245) stated

that exogenous nutrition, besides having an osmotic role, supplied food material for elongating tubes either in a medium or in the style. O'Kelley (31, vol. 29, p. 325) germinated pollen of Tecoma radicans with carbon-14 labeled sugars and found 72 per cent of CO_2 respired by pollen tubes came from the sucrose. This evidence apparently disproves the endogenous nutrition theory, but it could also be explained merely as a "luxury consumption" of externally supplied carbohydrate.

Normally, a lower sucrose concentration is required for fresh pollen germination than for stored pollen (43, vol. 55, p. 61). This may be attributed to a difference in the permeability or osmotic concentration of fresh and stored pollen. Visser (43, vol. 55, p. 45-46) showed that sensitivity of pollen to boron increases with age. It is generally assumed that stored pollen, which becomes dry and shriveled, germinates poorly or not at all. However, pollen germinates better if it is exposed to higher humidities before germination and supplied with a higher concentration of sucrose than required by fresh pollen. Therefore, possible causes of loss of viability may be desiccation, utilization of food reserves, or inactivation of enzymes, causing failure of metabolic processes responsible for germination. Knowlton (23, p. 786) studied the correlation between aging of pollen and loss of moisture, respiration, food depletion,

and reduction in the quantity of certain enzymes and found them to be unimportant factors influencing longevity. Maximum germination of pollen and tube length were obtained between 20 and 30 degrees C. (22, vol. 35, p. 594), and the Q 10 was approximately 2 (43, vol. 55, p. 33). Visser (43, vol. 55, p. 32) reported pollen-tube bursting and abnormal tube growth with high temperatures.

Studies on the effect of boric acid on pollen indicate that concentrations of 100 to 150 ppm are favorable for germination and higher concentrations are inhibitory (42, vol. 47, p. 243). Additional studies indicated that pollen is generally low in boron content, and this deficiency is made up by boron from the style. Gauch and Duggar (11, vol. 28, p. 457-458) showed that boron combined with sugar to form an ionizable sugar-borate complex which was readily translocated as compared with non-ionizable sugar molecules. There are three functions of boron in pollen germination and tube growth, as stated by Vasil (42, vol. 47, p. 246): (1) It promotes absorption and metabolism of sugars; (2) it increases oxygen uptake; and (3) it is involved in synthesis of peptic materials in elongating pollen tube walls.

The influence of hydrogen-ion concentration on metabolism of plants is generally understood, but the theories of pH on germination of pollen are conflicting. Optimum germination of Lathyrus

odoratus occurred at pH 7.0, and the zone for germination was very narrow (4, vol. 12, p. 160-161). The possibility of hydrogen-ion concentration having a direct effect on respiration of reserve food in the pollen was proposed.

In 1922, Knowlton (23, p. 769) stored pollen of Antirrhinum at five temperatures and concluded that the lower the temperature the longer the viability. Later, Holman and Brubacker (14, vol. 13, p. 182-187) tested the viability of 52 species at 17 to 22 degrees C. and at 0, 27, 63, and 92 per cent relative humidity and found that storage at low humidities tripled, on the average, the pollen longevity of most species.

Pollen of numerous species has been reported to retain longevity for one year or longer when stored at 0 to 10 degrees C. and 10 to 40 per cent relative humidities. These conditions were successful in the works of Nebel and Ruttle (29, vol. 14, p. 356), King and Hesse (21, vol. 36, p. 312), and Nebel (28, vol. 37, p. 131) on pollen of several fruit trees; Pfeiffer (34, vol. 8, p. 147-148), (36, vol. 9, p. 202-203), (35, vol. 13, p. 284-289) on Amaryllis, Cinchona, and Lillium; Olmo (32, vol. 41, p. 220) on Vitis; and Stone, et. al., (39, vol. 42, p. 308) on Pistacia.

The pollen of Gramineae is extremely short-lived in contrast to pollen of the above plants, and the range of humidity (10 to 40 per

cent) which is favorable to most other pollen is harmful. This is evident from the work of Knowlton (23, p. 780) and Jones and Newell (16, vol. 40, p. 201) on corn, in that the maximum longevity of corn was obtained at 50 to 80 per cent relative humidity. It was concluded that desiccation was one of the important factors which caused death of pollen during storage. Pfeiffer (34, vol. 8, p. 147) noted a close correlation between longevity of pollen and the moisture content of the air with different maxima and minima for pollen from different plants. According to Holman and Brubacker (14, vol. 13, p. 185-186), the longevity of air-dried pollen never did exceed that of pollen stored over low humidities. A relative humidity of 27.2 per cent was much better than high humidities and slightly better than 0.005 per cent humidity. Investigations by Visser (43, vol. 55, p. 57-60) indicated that pollen of most crop plants retained maximum viability at 10 to 40 per cent relative humidity and at low temperature. Pollen stored at low humidities remained viable for long periods, but at high humidities, it was attacked by bacteria and fungi. Germination of Cinchona pollen was increased when transferred to a more favorable temperature and humidity following storage at room temperature and dry atmosphere (35, vol. 13, p. 291-292), (33, vol. 18, p. 154), (34, vol. 8, p. 355).

Knowlton (23, p. 768) stated that Pollen of Antirrhinum remained viable longest at -18 to -30 degrees C. The pollen of Lilium and Amaryllis (34, vol. 8, p. 147), (36, vol. 9, p. 209-210), date (9, vol. 35, p. 93), and grape (32, vol. 32, p. 220) was stored for long periods at deep-freeze temperatures without being adversely affected. It appeared that an optimum moisture condition was specific for pollen longevity, whereas low temperature was necessary to retard respiration; hence, it was possible to maintain longevity within a range of low temperatures.

Conditions other than temperature and humidity affect the viability of pollen. Early work by Kellerman (18, vol. 43, p. 376) showed that pollen of citrus retained a high percentage of germination when it was predried and shipped under vacuum. Pollen of Lilium (34, vol. 8, p. 145), apple and pear (43, vol. 55, p. 53-54) stored under reduced air pressure retained viability. On the contrary, pollen grains of Antirrhinum (23, p. 770) and Cinchona (35, vol. 13, p. 287) retained viability longer at normal than at reduced air pressure. Knowlton (23, p. 769) also found that a high concentration of CO_2 increased longevity, whereas an atmosphere of pure oxygen was less favorable. It was assumed that storage of pollen at reduced pressure resulted in an increased CO_2 concentration from respiration. Pollen of Pinus taeda remained viable for 99 days when

sealed in nitrogen gas following freeze-dry treatment and storage in an uncontrolled environment (20, vol. 86, p. 385). Hanson (13, vol. 1, p. 116) stated that alfalfa pollen, which was vacuum-dried and stored at zero degrees F. or room temperature, maintained high viability for 183 days.

A major source of difficulty in the storage of pollen is erratic germination of stored samples. Pollen which fails to germinate in one or two previous tests may give a high percentage of germination in subsequent tests. In other cases, the percentage of germination after storage for a few days may be higher than that of fresh pollen (14, vol. 13, p. 186-187). Nebel and Ruttle (29, vol. 14, p. 357) stated that these variations may be due to lack of uniformity in the sample, but that other unknown factors may also be involved.

Kearney and Harrison (17, vol. 44, p. 194, 207) described that bursting of pollen in nutrient solutions as "pseudo germination." Percentage of viability calculated on the basis of "ejection" was confirmed by field pollination and percentage of seed set. This evidence would indicate that stored pollen which failed to germinate in vitro, or only burst and formed protrusions, may sometimes be viable and give satisfactory seed set when used for pollination.

Pollen which failed to germinate in vitro but produced a satisfactory fruit set in vivo was reported by Knowlton (23, p. 766-767).

Occasionally, there was little or no fruit set, even when pollen germinated *in vitro*. Failure of germination *in vitro* was probably due to deficiencies caused during storage which were later compensated by stigmatic and stylar tissues at time of germination. This view was supported by work on *Pistacia* (39, vol. 32, p. 312), grape (32, vol. 41, p. 222), tobacco (12, vol. 62, p. 12-13), and tomato (43, vol. 55, p. 57) where pollen displayed low germination, but fruit set was satisfactory.

Potato pollen stored at -30 degrees F, gave no germination in culture media, but effected seed set when used for field pollination (19, vol. 32, p. 462). Thus, absence of germination *in vitro* does not necessarily imply that the stored pollen is dead or too weak for field use. Holman and Brubacker (14, vol. 13, p. 78-79), also believe that pollen which is incapable of germinating in artificial media may be stimulated by some substances in the stigma to form a tube and, perhaps under favorable conditions, bring about fertilization. Knowlton (23, p. 771) showed that *Antirrhinum* pollen, which remained viable 670 days, did not germinate in artificial media after 180 days unless a piece of stigma were placed in the medium. Visser (43, vol. 55, p. 37) stated that apple and pear pollen, containing 98 to 99 per cent dead pollen, caused moderate fruit sets, provided large amounts of pollen were used for pollination.

From the foregoing account, it is obvious that, under low temperature and humidity, pollen usually retains viability for long periods, although requirements may vary from species to species. Generally, near-freezing temperature and 25 to 50 per cent relative humidity are suitable for prolonging longevity. The factors responsible for loss of viability during storage are not fully understood, and the problem of stored pollen giving fruit set comparable to that of fresh pollen remains unsettled.

The earliest work reported on the study of hop longevity and viability was by Winge (38, vol. 11, p. 29-30), who collected hop pollen shed from inflorescences stored in the laboratory. Three days later, it was capable of fertilizing the female plant, but failed to germinate on artificial culture. Limited germination and pollen tube growth was observed with excised styles placed in the water medium. Holubinsky and Rybatschenko (15, vol. 27, p. 846-848) showed pollen to remain viable for a few days without controlling temperature and humidity and demonstrated the ability of hop pollen to germinate on agar-sucrose culture. Mori (27, vol. 5, p. 37-42) and Puri and Brooks (37, p. 19) reported successful germination of pollen on agar medium containing 0.4 molar concentration of sucrose. Only in the last few years has successful germination of hop pollen been accomplished and, at present, no longevity studies have been reported.

MATERIALS AND METHODS

Pollen collected from several male genotypes in the hop breeding block located on the College East Farm was used in this study. The male genotypes investigated represented a wide range of origins. Selection of individual genotypes was governed by date of anthesis and pollen yield.

Hop pollen shedding is positively associated with temperature and negatively associated with relative humidity. Mori (27, vol. 5, p. 37-42) indicated that the optimum time to collect pollen to obtain maximum viability was during the morning from anthers which had dehisced for a short time. In western Oregon, shedding begins about 7 to 9 a.m., and reaches a maximum about 11 a.m. to 2 p.m. on clear days during flowering (7, p. 49-52). Therefore, collection was made near noon each day.

Pollen collection from each genotype was accomplished by covering six to 10 flowering branches with parchment bags secured to the stem with paper clips. Bags were placed on the branches during early morning and removed 28 to 30 hours later during the period of maximum pollen shedding. During the process, florets were also broken from the staminate panicle and collected along with the pollen.

A composite sample was made of pollen, foreign material, and florets from each genotype. Each pollen composite was screened through a Tyler standard screen of 100 mesh having 0.147-millimeter openings. The 100-mesh screen did not exclude all the resinous material borne in the furrows of the anthers, and this contaminate was found to be detrimental to hop viability. Later pollen collections were screened through a 200-mesh screen , with openings of 0.074 millimeters, which separated the resin glands from the pollen. The amount of screened pollen collected from each plant was five to 10 milliliters by volume. Viability was then determined by germination on an artificial medium. Each sample was apportioned at random to each respective experiment and placed under storage conditions.

In certain instances, quantities of pollen were held in cotton-stoppered glass vials at a storage temperature of 0 degrees C. and 20 per cent relative humidity until they were placed under the experimental conditions 24 to 72 hours later. Previous tests indicated that this procedure did not materially reduce pollen viability. Viability tests were conducted after temporary storage in these instances, and the results were used as the initial germination percentages.

The five relative humidities reported in these tests were maintained over saturated aqueous salt solutions (25, p. 1420-1422) contained in gallon glass jars sealed with wax-lined screw type covers. Humidity control in these desiccators was obtained by the use of chemicals as follows:

Desiccator	Chemical	% R.H. (20°C.)
1	$NaHSO_4.H_2O$	52
2	KCNS	47
3	$ZnNO_3.6H_2O$	42
4	$CaCl_2.6H_2O$	32.3
5	$ZnCl_2.1\text{-}1/2H_2O$	10

All relative humidities were calculated at 20 degrees C., and no adjustments were made for variations in humidity due to fluctuations of temperature or to periodic opening of the desiccators. Pollen from each genotype was placed into glass vials lightly stoppered with cotton and stored in desiccators at 0 degrees C. in a household electric refrigerator.

Samples for storage under low levels of oxygen and water vapor were prepared by a vacuum-drying technique. Six partitioned pollen samples, approximately 0.25 cc by volume, from each genotype were placed into ampules made from glass tubes, eight millimeters in diameter, four inches in length, drawn to a small capillary at the mid-point, and closed at one end. A quick-frozen pretreatment was given some samples. This involved placing the

closed ends of the ampules, containing pollen, into an acetone-dry-ice mixture (-60°C.) for 15 minutes and following immediately with vacuum-drying. Other storage treatments included vacuum-drying without prior quick-freezing. Vacuum-drying was obtained by the use of a Welch vacuum pump. Pressure was determined five minutes after the test was started by means of a Todd Universal Vacuum Gauge, of the 3-scale McLeod type. All treatments were vacuum-dried for one hour at a vacuum pressure of 1 μ of Hg. One genotype was also dried at a pressure of 250 microns. The individual tubes were sealed under vacuum by fusing the tube constriction before the pump was stopped. One lot of three ampules was placed in the freezing compartment of the refrigerator (-13°C.), and a duplicate lot was stored in an office cabinet with normal fluctuating room temperatures.

Vacuum-dried pollen was tested for viability after six months, and pollen stored in desiccators under controlled humidities and temperature was tested three, five, and six months after initial storage. Since pollen longevity studies are of a continuing nature, additional germination tests and field pollinations will be conducted.

The culture medium used for pollen germination was a 16 per cent sucrose and 1 per cent agar mixture adjusted to pH 5.2, as indicated by a Beckman battery-operated pH meter. The agar was

dissolved in distilled water which was preheated to 100 degrees C. The solution was retained in an electric oven at 100 degrees C. for 15 to 20 minutes, at which time the agar was completely dissolved. Sucrose was added to the heated agar-water solution and stirred until dissolved. The medium was cooled to 40 degrees C. and adjusted to pH 5.2, with N/20 hydrochloric acid and sodium hydroxide. A thin layer of the liquid medium was placed on a microscope slide with an eyedropper and allowed to solidify.

Pollen was dusted uniformly over the solidified medium on the slide with a cotton swab on a toothpick. The slides were placed on wet paper towels in a plastic container, covered, and incubated in thermostatically controlled chambers. The chambers were equipped with fluorescent lighting of low intensity (less than 200 foot-candle) and controlled temperature of 20 degrees C. for eight hours and 30 degrees C. for 16 hours. The chambers were illuminated only during the 30-degree C. temperature period.

The slides were examined after 20 to 24 hours under a Bausch and Lomb binocular microscope at 300 magnification. Germination percentages were determined by counting 100 pollen grains in each of two random samples for each treatment. Twenty-five randomly selected pollen tubes were measured with an ocular micrometer to determine the maximum and minimum lengths of the tubes in the treatment.

Controlled pollinations, with stored pollen, were conducted on female genotypes located in the breeding block in the College Hop Yard. Pollinating procedure was identical to the technique used in the hop breeding program located at Corvallis, Oregon. Three previously bagged flowering laterals were pollinated on each genotype at the time of optimum stigma receptiveness. The composite of mature female inflorescences, from the three pollinated laterals, was picked by hand, and total cone number was determined. The cones were dried, threshed, and cleaned to obtain only sound seeds. A total seed count was made from each successful cross and, following a vernalization period, the seeds were planted in a greenhouse for observations.

Analyses of variance were made on the data from two stored pollen germination studies. Both experiments were of factorial design, but due to an inequality in number of observations, a one-way classification of analysis of variance was used in one study. A multiple range test, computed from standard error, was used for testing the homogeneity of treatment means in each study.

RESULTS AND DISCUSSION

Germination of Hop Pollen on Artificial Media

Genotypic differences in pollen germination and tube growth displayed on artificial culture are given in Table 1.

Table 1. Genotypic Differences in Germination and Tube Growth of Fresh Pollen on Agar-Sucrose Media.

Genotype	Pollen Germination (%)	Length of Pollen Tube (μ)		Distribution of Germ Pores per Grain (%)		
		Range	Average	3	4	5
221-2	16	198-546	306	100.0	-	-
218-2	23	159-555	370	96.8	3.2	-
219-2	35	207-581	370	90.5	9.5	-
103	1	85-338	178	81.5	17.5	1.0
123	23	203-615	364	98.8	1.2	-
106-S	37	104-270	128	100.0	-	-

Abnormal pollen grains appeared to be deficient in their capacity to germinate and ability to produce long pollen tubes. Variation in germ pore number may be a factor in unsuccessful fertilization, since grains with other than three pores rarely germinated on

artificial culture. Abnormal grains which did germinate produced short pollen tubes and other abnormalities (Figure 2).

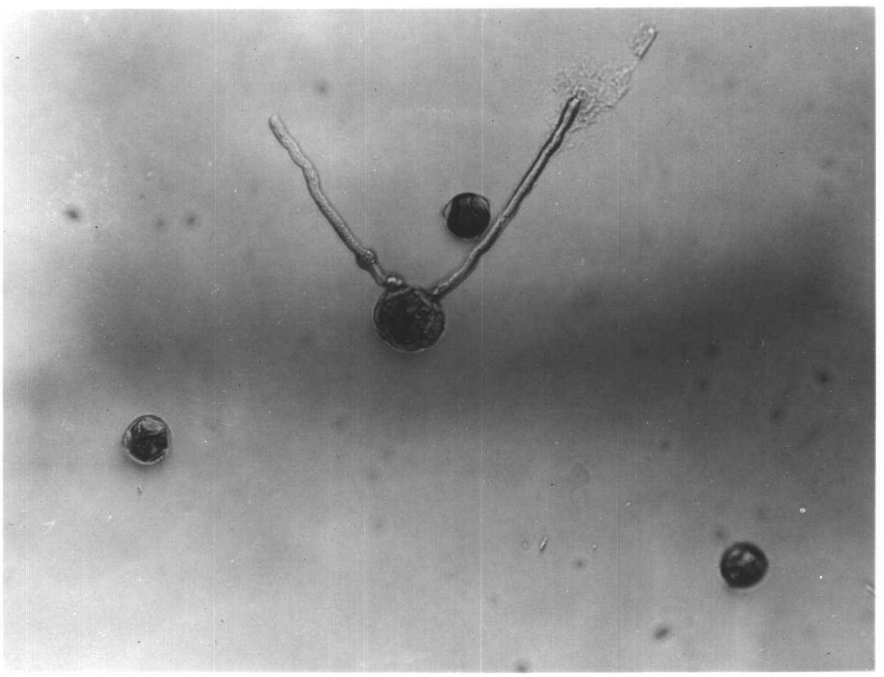

Figure 2. Two tubes produced by one pollen grain with five germ pores. (X 400).

Hop pollen grains are binucleate, having a small generative nucleus and a larger vegetative nucleus. Division of the generative nucleus in the tubes was not noted in hop pollen germinated in vitro (Figure 3).

Successful storage of pollen grains from hundreds of genera has been recorded (14, vol. 13, p. 179-294), but it is apparent that

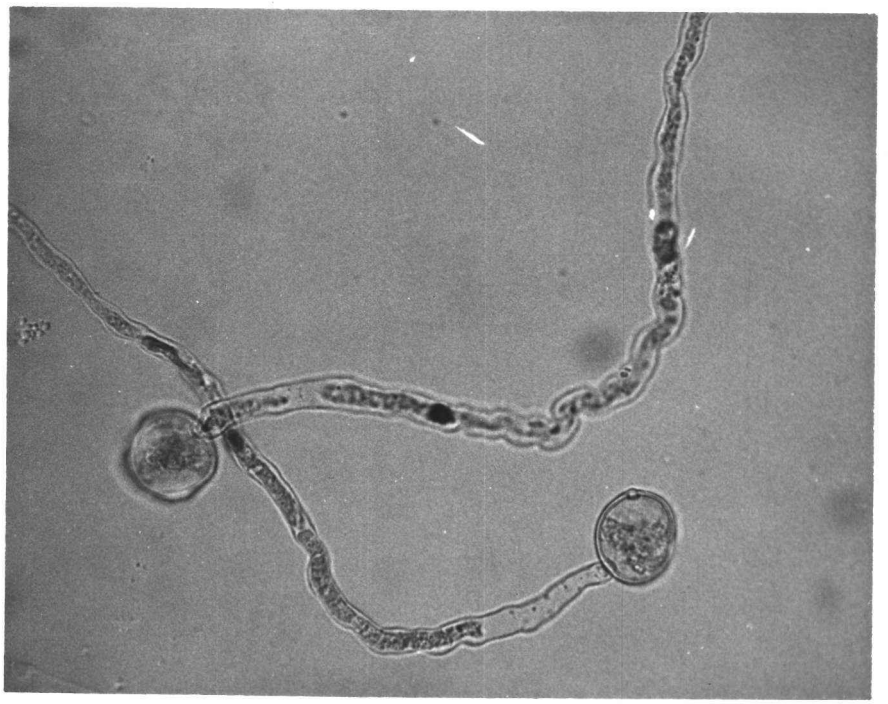

Figure 3. Germinated pollen grains with vegetative nucleus and darkly stained generative nucleus (X 300 enlarged).

most of these genera are of the binucleate pollen grain type. Trinucleate grains (such as those of Gramineae) rarely can be stored for more than a few days and are difficult to germinate in vitro (2, vol. 19, p. 126-127).

Verification of a "pollen growth factor" (3, vol. 48, p. 457-464), related to second mitotic division of the generative nucleus, could be an important advancement in understanding germinating ability and tube growth of pollen. The growth factor noted was

produced in binucleate pollen until the generative nucleus divided. It appeared that tube elongation was regulated by the time interval between initial germination and second mitotic division. The short life and inability of trinucleate pollen to germinate on artificial media may be related to limited availability of this factor.

Factors Affecting Germination of Hop Pollen

Fresh hop pollen germinated with equal success on 16 or 12 per cent sucrose. Germination percentages of stored hop pollen (genotype 125) as related to sucrose concentration in the medium are shown in Table 2.

Table 2. Per Cent Germination of Stored Hop Pollen on One Per Cent Agar Medium, Adjusted to pH 5.2, with Different Concentrations of Sucrose.

% Sucrose	0	2	4	6	8	10	12	14	16	18
% Germination	5	5	10	11	7	14	43	31	32	23
Avg. Tube Length (μ)	38	43	85	79	63	96	390	263	321	120

The pollen used was stored three months under 32 per cent relative humidity at 0 degrees C. The highest germination percentage was obtained with 12 per cent sucrose. This concentration appeared to be more optimum than a higher concentration as contrasted to fresh pollen germination.

Pollen tubes resulting from germination on low sucrose concentration ruptured at the tip during the 24-hour period of germination. Hence, pollen tube measurements represent the length of the intact tube and not the potential elongation if tube burst had not occurred. Tube rupturing was evident at sucrose concentrations of zero to 10 per cent, and this may indicate an osmotic role of sucrose. This would imply that the particular pollen tested had an 0.3 to 0.4 M osmotic concentration and media with lower values resulted in tube bursting due to increased turgor pressure. Visser (43, vol. 55, p. 44) stated that "pseudo germination," rupturing, or ejection at the germ pore was an indication of germination. In this study, a pollen grain was recorded as being viable only if the pollen tube exceeded five microns in length. "Pseudo germination" was not recorded, but was noted as being present in most of the tests conducted and, occasionally, equalled the number of germinated pollen.

Few studies have been conducted on the effect of hydrogen-ion concentration on pollen germination. Pollen of many species can tolerate a wide range of hydrogen-ion concentrations, whereas only few species are specific to a narrow zone for germination (4, vol. 12, p. 149-187). It appeared that hop pollen germinative ability was inhibited in a basic or neutral medium (Table 3). Results showed that the optimum pH for hop pollen germination was

between 4.7 and 5.5. Limited tolerance to pH was displayed when a pH change of one unit from the optimum resulted in reduced germination.

Table 3. Per Cent Germination of Stored Hop Pollen on One Per Cent Agar and Twelve Per Cent Sucrose, with Different Concentrations of Hydrogen Ions.

pH	3.7	4.3	4.7	5.2	5.5	6.1	6.8	7.2
% Germination	0	7	23	33	22	10	2	0

Boron, as boric acid, was detrimental to pollen germination at concentrations of 20 to 600 ppm. Extrusions occurred in media high in boron, but tubes developed at 20 to 50 ppm. Pollen germinated on media containing five ppm of gibberellin as potassium gibberellate displayed an increased pollen tube growth, but germination percentage was not affected. Media which contained both boron and gibberellin resulted in poor, if any, germination.

Procedures used in the germination of pollen on artificial media imply that only sucrose concentration, boron, hormones, vitamins, or amino acids are critical. The liquid constituent of the medium is only rarely considered or indicated in pollen germination studies, but it is assumed to be distilled water. Pollen from some species will germinate in water, and hop pollen is a member

of this group; but rarely has pollen germination been recorded with de-ionized distilled water.

Hop pollen failed to germinate on medium prepared with four different sources of water obtained from stills, three having condenser water discharges of less than 60 degrees C., and one having a discharge of 90 degrees C. Hundreds of culture slides, with water from low discharge temperature stills, were examined for pollen germination, and in no cases did the pollen germinate. Successful germination was obtained only with distilled water condensed at a temperature of 90 degrees C., with a Barnstead stainless steel still.

The limitation of pollen germination to one distilled water source was probably related to conditions of distillation. This water was condensed at a higher than normal temperature, and excess water vapor that escaped at the end of the condenser probably removed volatile organic constituents which were toxic to pollen.

Water condensed at a high temperature from the Barnstead still was redistilled via a glass-still apparatus and provided a means for comparing double-distilled with single-distilled water. Germination percentages using media with two types of distilled water are listed in Table 4. Germination percentages were comparable for fresh media containing two types of distilled water, but an increased germination percentage was obtained with a three-day-old medium containing

double-distilled water. The aged-media factor affected only pollen stored in low humidities and was more effective at 20 per cent relative humidity. Brewbacker and Majumder (3, vol. 48, p. 458-459) found a decrease in fresh pollen germination using media prepared the day before and related this to a change in pH. Stored media in the present study had pH determinations conducted prior to usage, and pH was found to remain unchanged for five days.

Table 4. Per Cent Germination of Pollen of Genotype 125, Stored at Different Relative Humidities, on Media Containing Two Types of Distilled Water.

Type of Water in Media[1/]	% Relative Humidity in Storage					
	10	20	32	42	47	52
Single-Distilled Water						
Fresh	0	3	8	7	4	4
Double-Distilled Water						
Fresh	0	2	8	4	4	3
3-Day-Old [2/]	0	35	22	5	7	1

1/ Medium contained 12 per cent sucrose, 1 per cent agar, and adjusted to pH 5.2.

2/ Excess medium from initial test was stored for three days in a glass beaker at 40 degrees C.

Results of additional viability tests using single- and double-distilled water are listed in Table 5. Treatments also included glass cover slips, which were placed over the medium after the

Table 5. Per Cent Germination of Pollen of Genotype 125, Stored at Two Different Relative Humidities, on Fresh and Two-Day-Old Media Containing Two Types of Distilled Water.

Germination Media [1]	Storage Condition at 0°C. 20 % pH	32 % pH
Single-Distilled Water		
Fresh	12b [3]	6b
Fresh + Cover [2]	17b	25a
2-Day-Old + Cover	36a	29a
Double-Distilled Water		
Fresh	10b	12b
Fresh + Cover	30a	18ab
2-Day-Old + Cover	36a	29a

1/ All media contained 12 per cent sucrose, 1 per cent agar, and adjusted to pH 5.2.

2/ A glass cover slip was placed on the medium after pollen was applied.

3/ Treatment means with the same alphabetical letter are not considered significantly different at the 5 per cent level.

slides were dusted with pollen. These data indicate that an old medium was more favorable for germination than a fresh medium, but cover slips used on a fresh medium tended to compensate for differences in promotion of germination.

Freshly prepared media apparently were not adequate for testing maximum viability of pollen stored under low humidities. Media, contained in a covered glass beaker and stored for two or three

days in a 40-degree C. water bath, provided an optimum culture for pollen germination. Comparable pollen germination was obtained with fresh media only after a glass cover slip was placed in contact with pollen and solidified culture.

The chemical effects of the two methods in promoting pollen germination could be identical. Possibly a chemical change at the solution-glass interface contributed a synergistic effect necessary for germination of stored pollen. The chemical change (or factor) was probably sub-optimum in fresh media, but optimum after two days' storage in a warm water bath. Pollen failed to germinate on media maintained longer than five days. The increased germination under a glass cover slip, on fresh media, could be attributed to the same chemical change, except that the optimum level developed within a shorter period of time. The glass cover technique enabled pollen grains to immediately utilize any beneficial effect arising at the solution-glass interface; whereas, with old medium, maintained as a liquid, the beneficial effect had to reach an optimum level throughout the medium.

It is suggested that boron formed an ionized boron-sucrose complex which enhanced translocation and absorption of sucrose. The glass cover slips utilized in this study were made of Corning Brand Glass, Number 0211, which is a soft type glass. Boron is a

chemical constituent of this type glass and is believed to be the beneficial "factor" in pollen germination. Complex formation of an additional diffused growth factor (3, vol. 48, p. 457-464) may have resulted in germination of adjacent pollen grains.

The boron-sucrose ion may not be necessary for germination of pollen stored at high humidities, since the grains are higher in moisture and exhibit an increased mobility of essentials for growth. There was a direct relation between duration of storage and response to the factor, and it would appear this response was related to decreased permeability of aged pollen. Other factors, such as anerobiosis and high CO_2 concentration, may be significant, but an explanation that the response is probably due to factors which influence sucrose uptake is more compatible with present knowledge of pollen chemistry and physiology.

Factors Affecting Longevity of Stored Pollen

Pollen from six genotypes was included in the storage longevity study, but results of four were omitted due to the presence of resin glands. The four samples had been screened through a 100-mesh screen, which failed to separate large resin glands from the pollen. Stored pollen-resin gland mixtures became clumpy after short duration as a result of resinous excretion from ruptured glands.

Glands also supported growth of fungi which further contributed to losses of viability in these samples. Subsequent pollen collections were screened through a 200-mesh screen for desired purity. Results of pollen longevity stored under controlled temperature and humidities are listed in Table 6.

Table 6. Germination Percentages of Hop Pollen Stored Under Different Relative Humidities at 0° C.

Genotype	Per Cent Relative Humidity in Storage	Months in Storage							
		0	3		5		6		
125									
	52	76	4	c	8	a	1	e	
	47	76	1	c	7	a	20	c	
	42	76	0	c	2	a	19	c	
	32	76	25	a	18	a	29	b	
	20	76	12	b	12	a	36	a	
	10	76	0	c	0	a	0	e	
G-2034									
	52	30	1	c	2	a	2	e	
	47	30	2	c	14	a	9	d	
	42	30	4	c	14	a	11	d	
	32	30	4	c	6	a	6	de	
	20	30	2	c	9	a	18	c	
	10	30	0	c	0	a	0	e	

Treatment means, at each storage period, with the same alphabetical letter, are not considered different at the 5 per cent level.

Pollen germinations were conducted on 16 per cent sucrose media at the end of three months' storage. The high viability noted at the end of six months' storage would indicate the germinations

conducted at the 3-month period did not represent maximum viability. Since a 16 per cent sucrose medium was optimum for fresh pollen (37, p. 19), the inadequacy of the same medium with stored pollen points to a change in germination requirements of stored pollen. Subsequent tests indicated that the media for stored pollen should be changed to achieve more valid tests of viability. The culture used after five months' storage consisted of 12 per cent sucrose, and at the end of six months, the glass cover technique was added.

Hop pollen showed a substantial reduction in germination at the end of three months' storage under all humidities. A relative humidity of 20 per cent was significant in maintaining longevity of pollen, but higher moisture conditions resulted in decreasing pollen viability. Genotype 125 did not germinate after three months when stored at 42 per cent relative humidity; otherwise, pollen from both genotypes retained viability at intermediate humidities. A moisture-genotype interaction was evident at each storage date, but both genotypes responded alike to both low and high humidities after six months' storage. Storage at very low humidity was fatal, even within a short period. Pollen stored at 10 per cent relative humidity had a sticky appearance, and individual grains adhered together, forming a solid mass. Clumping of pollen is usually related with high humidity, but in this case, the adhesive material

responsible apparently was exuded from the grain. Exudation could result as a post-mortem effect from a change in chemical structure of the protoplasm in the grain, such as going from a sol to gel state.

Pollen was also stored under reduced pressure with very low levels of oxygen at either room or freezing temperatures. In addition to initial germination percentages, viabilities were determined on pollen which had been treated for storage. Germination of pollen 24 hours after evacuation was poor in all cases, except that pollen of genotype 425-1 germinated 16 per cent. Ampules from each treatment were opened after six months' storage, and the pollen was tested for germination with negative results. The ampules were left open and stored for three days under an atmosphere of 100 per cent relative humidity at zero degrees C. Germination percentages obtained under these conditions are listed in Table 7. A rehumidification period of longer duration did not increase germination percentages. High moisture conditions were believed to benefit pollen grain permeability and enhance translocation of sucrose and other diffusable factors. Pollen tube elongation was comparable to that of fresh pollen, averaging 350 μ. Genotype 125 produced pollen tubes which were longer than those observed in previous tests. These tubes consistently grew from 540 μ to 720 μ in length.

Table 7. Germination Percentages of Pollen of Different Genotypes Subjected to Different Treatments and Stored for Six Months.

Genotype	Germination Percentage of Fresh Pollen	Germination Percentage After 6 Mos.		
		Quick-Freeze Vacuum Dry (1 μ)	Vacuum Dry (1 μ)	Vacuum Dry(250 μ)
		Storage at Room Temperature		
125	45	5	6	-
421	10	2	4	8
425-1	10	10	-	-
G-2034	6	0	-	-
		Storage at -12 Degrees C.		
125	45	11	-	7
421	10	0	6	9
425-1	10	4	-	-
G-2034	6	0	32	-

Pollen of G-2034 and 421 did not survive the quick-freezing and vacuum-drying treatment. These two genotypes are genetically related, in that G-2034 is a male plant from a progeny of which 421 was the male parent. Both genotypes displayed a large number of shrunken and collapsed pollen grains, which was not evident with pollen of other genotypes stored under reduced pressure. Another similarity was the absence of extrusions, which are especially noticeable in stored pollen which produces few pollen tubes. Therefore, if extrusions are an indication of viability, the pollen of these two genotypes was nonviable.

Pollen of 421 stored under reduced pressure, without prior quick-freezing, appeared to remain viable at room temperature. Pollen of G-2034 subjected to vacuum-drying appeared to remain viable at -12 degrees C. Vacuum-drying at either one or 250 microns of pressure appeared to retain pollen viability.

Only limited data were obtained on the influence of reduced storage pressure on hop pollen viability, due to the time-consuming procedure. Originally, it was thought necessary to quick-freeze a sample prior to vacuum-dry, but data obtained on four genotypes indicated that an extremely low temperature was detrimental to pollen viability.

Controlled Pollinations With Stored Pollen

Hop pollen collected in 1960 was stored in 2-ounce, screw-cap jars at 3 degrees C. without controlled humidity. The pollen was maintained under these conditions for 12 months and utilized in field pollinations. Germination tests were conducted on culture medium (16 per cent sucrose, 1 per cent agar at pH 5.2) prior to attempting pollinations.

Seed sets of only two to four seeds per cone are not uncommon from field pollinations using fresh pollen. On this basis, stored pollen from five genotypes could be regarded as being equal in viability to fresh pollen, although pollen from four of the five genotypes

failed to germinate on artificial medium (Table 8). Different females pollinated with pollen from the same genotypes produced inconsistent seed sets. Pollen from genotype 527-1 failed to germinate on artificial medium, yet resulted in 0.1 to 4.1 seeds per cone when used in pollinating three different females.

Table 8. Viability of Stored Hop Pollen as Determined by Seed Set with Field Pollinations and Germination on Culture Medium.

Cross		No. Cones	No.	No. Seeds	Per Cent of Pollen Germinated on
Male	Female	Harvested	Seeds	Per Cone	Artificial Media
221-1					2
	X 416	167	6	0.0	
221-2					0
	X 203	44	52	1.2	
	X 209	96	120	1.2	
	X 210	22	9	0.4	
	X 402	53	107	2.0	
	X 505	35	39	1.1	
318-1,2					0
	X 203	37	22	0.6	
	X 210	44	38	0.9	
321-1					0
	X 210	98	56	0.6	
	X 404	90	206	3.3	
418-1,2					2
	X 203	24	38	1.6	
524-2					0
	X 322	43	66	1.5	
526-4					2
	X 322	38	226	6.0	
527-1					0
	X 210	24	2	0.1	
	X 314	95	276	2.9	
	X 25-S	28	114	4.1	

The lack of correlation between pollen germination on sugar-agar culture and seed set from field pollination indicates that viability was not determined precisely. There may be two reasons for this: (1) The difference between in vitro and in vivo measures of viability may be due to degradation of endogenous growth factors in pollen and their subsequent replenishment by the stigma and style of the female; and (2) unsuccessful pollinations may be caused by female unreceptiveness or other incompatabilities rather than lack of pollen viability.

Workers are not in accord as to a reliable test of pollen viability. Staining techniques (such as acetocarmine) have been used in the past and replaced by in vitro and in vivo methods. Most workers favor either germination tests on culture or field pollinations to determine pollen viability, but few employ both techniques; yet both groups express concern over variability in their results. Difficulty of controlled pollination undoubtedly limits the use of in vivo testing of pollen viability with many species. However, employment of both methods results in confirmation of one method against the other. Both methods can be easily applied with hop plants since the species is dioecious. Female plants can be bagged to exclude unwanted pollen; pollen collection is easy; and pollination techniques can be easily performed.

SUMMARY AND CONCLUSIONS

1. Presence of abnormal pollen grains within genotypes indicated variable development during sporogenesis.
2. Percentage of pollen germination and tube elongation were positively associated. Relation between germination and sucrose concentration was represented by an optimum curve. Bursting of pollen tubes was negatively associated with the osmotic concentration of agar-sucrose media.
3. Distilled water condensed at a low temperature apparently contained factors (presumably organic) which were detrimental to pollen germination.
4. Gibberellic acid and boron, in combination, reduced the germinability of pollen tested on agar-sucrose media. Gibberellic acid increased tube elongation, but not germination.
5. Glass cover slips and two- or three-day-old media significantly increased germinative ability of pollen. Both phenomena are believed due to complex of boron and an unknown substance having germination promoting properties.
6. Longevity of hop pollen was increased under low humidity conditions. Pollen stored one year at 3 degrees C. with uncontrolled humidity was effective in field pollinations. Stored

pollen appeared to be more specific in requirements for germination than fresh pollen.

7. Pollen longevity was maintained under reduced pressure at either room or below freezing temperature. Lyophilization was detrimental to hop viability.

8. Pollen stored at reduced pressure germinated only after rehumidification.

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