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A Study of Factors Affecting the Germination of Alfalfa and Safflower Pollen

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A STUDY OF FACTORS AFFECTING THE GERMINATION OF ALFALFA AND SAFFLOWER POLLEN

by

Ling Lin

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Ling Lin

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INTRODUCTION

Pollen grains serve a very important role in the life cycle of flowering plants, in that they are involved in the transmission of inherited characteristics from generation to generation. Because of their small size the study of pollen grains has been necessarily associated with the development of the microscope. Not until the middle of the seventeenth century, when Hooke gave the world the compound microscope, was an instrument available with sufficient power to adequately reveal the shapes of pollen grains. However, two of Hooke's contemporaries, Malpighi and Grew, who used a simple microscope, are generally recognized as the co-founders of pollen grain morphology.

Nearly a century later Kolreuter discovered that pollen grains possess two distinct coats and observed that in some species the outer coat is elastic and often has spines and other sculptures. He also noticed that when pollen grains get wet the inner coat protrudes through apertures in the outer coat.

The mechanics of fertilization in plants were discussed by several researchers during the middle 1700's and early 1800's. It was found that pollen grains germinated after making contact with the stigma, and

subsequently, pollen tubes grew down through the styles and into the ovules.

In the middle of the eighteenth century Gleichen suggested that pollen contained spermatozoa and had to burst in order to discharge them and effect fertilization. Needham in about 1740 discovered that many pollen grains, upon being brought into contact with water, expand, extruding papillae at their pores, and eventually burst, discharging their contents which appeared to consist of a viscous fluid charged with granules. He believed these granules to be the fertilizing materials which when discharged on the stigma, made their way through channels in the style to the ovules.

Turpin in 1820 was the first to draw attention to the formation of the pollen tube. He stated that the pollen wall consists of two layers which he called the "exhymenium" and the "endhymenium". He concluded that when a pollen grain is placed in water the outer membrane ruptures and the inner membrane expands, forming a thin-walled intestine-like tube bearing its exceedingly small granules. He assumed that these granules were endowed with independent movement.

In an attempt to find out how pollen tubes arising from the small pollen grains are able to traverse long styles, Amici, in approximately 1830, discovered that the stylar tissue provides nourishment for the growth of pollen tubes. His findings suggested that pollen might be induced to germinate and grow on artificial media of the proper nutrients were present.

A knowledge of pollen grain structure and physiology is often very useful to a plant breeder, whose work involves artificial pollination. Crosses are frequently desired between plants whose flowering periods do not coincide. Pollen storage is one possible means by which such a cross might be effected. In situations involving pollen storage periodic assays of pollen viability are necessary. One of the simplest tests of pollen viability involves the germination of pollen on artificial media.

Many of the difficulties encountered in obtaining inter-specific and intergeneric hybrids are traceable to problems involving pollen germination or pollen tube growth. Pollen may fail to germinate on foreign stigmas; the growth of the pollen tube may be too slow to reach the ovule in time to effect fertilization; or the pollen tubes may burst in the styles. In some instances these barriers to fertilization have been overcome by altering the stigmas or styles physically or by influencing the rate of pollen germination and pollen tube growth by manipulating environmental conditions or by using certain chemicals.

Attempts to artificially germinate the pollen of different plant species have met with varying success. In the family Gramineae, for example, pollen has generally proved very difficult to germinate, whereas pollen from many members of the cucurbit and legume families germinates quite readily. In the present study two species, representing opposite extremes in pollen germinability under artificial conditions, were compared under varying environmental conditions and on a wide variety of media.

REVIEW OF LITERATURE

Since Von Mahl first observed that pollen of some plants would form tubes in moist air, numerous attempts have been made to germinate pollen on artificial media (5). Pollen of many species germinates readily on a wide variety of media. With other species, however, the requirements for pollen germination are quite exact, and with a number of species only negative results have been reported.

Artificial media studies

Numerous workers have studied the chemical make-up of pollen grains, as well as that of stylar and stigmatic tissue, as a possible approach to the formulation of effective artificial germination media. According to a review by Brink (5):

> Behrens (1875) concluded from the results of his microchemical studies that the slimy substances of the stigma contain amuloid materials, sugar, and probably other carbohydrates. The anatomical investigations of Dalmer (1880) revealed that reserve food materials are freely distributed in the tissues leading to the ovules. Green (1894) and Kirkwood (1906) discovered that the stylar tissue of the lily was filled with minute granules of starch which were gradually hydrolyzed to sugar, indicating the presence of diastase and invertase. They concluded that the food material does not extend to the stigma, and that the reserve material in the style is intended for the growing pollen tube. Anderson and kulp analyzed maize pollen and found that the principal reserve materials were carbyhydrates in the forms of starch and sucrose. The percentages of starch and sucrose in the pollen of different varieties were reported as

	Yellow Dent (Leaming)	White Flint (Luce's Favorite)	Pop Corn
Starch	11.07	19.04	18.03
Sucrose	9.09	2.97	14.18

Paton (23) analyzed pollen from 18 species, among which were pines, lily, maples, rye and corn, for twelve different enzymes. Invertase, catalase and reductase were found in all 18 species. Pepsin, trypsin, erepsin and lipase were present in the pollen of several species. Cytase was found in six of them.

Sugars appear to play an important role in pollen germination and tube growth, both under natural conditions and on artificial media. O'Kelley (21) has suggested that sugars serve to regulate the osmotic pressure of the germination media as well as to furnish an energy source for pollen germination.

Adams (1) conducted pollen germination studies with apple pollen, using cane sugar solutions ranging from 5 to 50 percent. Pollen was taken from an anther which had recently dehisced. The grains were dusted on a clean slide, a drop of cane sugar solution was placed on the slide and was stirred with a needle to distribute the pollen grains uniformly. The pollen germinated very rapidly if the liquid medium was spread in a very thin layer so that oxygen was readily available to the pollen.

Martin (20) studied the germination of clover pollen and found that a sugar solution containing agar of gelatin resulted in less bursting of pollen tubes than did the liquid medium. He found that two to five grams of agar or

gelatin added to a 0.731 volume-normal sucrose solution constituted the best medium for pollen germination of Irifolium hybridium and T. repens. Sucrose, dextrose, maltose, lactose and arbinose were all tested. Sucrose proved most satisfactory. He concluded that the only function of sugar in the germination of T. hybridium pollen was the controlling of water supply.

Martin (20) mentioned that a German breeder was unable to germinate the pollen of Ornithogalum ecklonii and some species of grasses, except on stigmas.

Effects of pollen germination stimulators

1. Plant auxins as stimulators of pollen germination and pollen tube growth.

Ching (10) reported that the germination and tube growth of Doublas fir pollen are proportionally increased with increasing concentrations of gibberellic acid in the culture medium. The potassium salt of gibberellic acid was used at concentrations of 10, 110 and 1000 ppm. Gibberellin had its greatest effect on pollen which had been collected under hot, dry conditions (90^{o} F. and 44 percent relative humidity). It had much less effect on the germination and tube growth of pollen which had been collected under hot, humid conditions.

Chandler (9) observed variable results following the addition of gibberellin to pollen germination media. He found that pollen from nine species did not germinate on the control medium and that germination was

not affected by the addition of gibberellic acid. Pollen from 10 species germinated on a wide variety of media, but germination was greatly inhibited by the addition of gibberellic acid. Pollen which did germinate formed abnormal tubes, e. g. , excessive coiling, enlarged tips and exuding cytoplasm. Pollen from seven species germinated on the control medium and showed a marked increase in tube length when gibberellic acid was added.

The effect of gibberellic acid on the growth of pollen tubes was studied and compared with the effect of indole acetic acid by Kato (16). Pollen of Lilium longiflorum was used in this study. A sugar-agar medium, containing two percent agar and eight percent sucrose, was used, and gibberellic acid or IAA was added in concentrations ranging from .05 to 200 mg/liter. The test medium was adjusted to pH 6.4-7.0 and the temperature was maintained at 25^{o} - 28^{o} C. Best results were obtained with the addition of gibberellic acid at a concentration of 50mg/liter. Pollen tube growth on this medium was five times that observed on the control medium. Elongation of pollen tubes in the gibberellic acid medium was stright and smooth and appeared more normal than pollen tube alongation in the medium containing IAA. At IAA concentrations of 5-200mg/liter, tips of the pollen tubes were swollen and stunted. These eventually burst. It was therefore concluded that IAA was a growth inhibitor rather than a growth stimulator to pollen grains of some species.

Smith (28) studied pollen germination in Tradescantia canadiculata, T. occidentallis, Polygonatum commutation, Lathyrus odoratus and Pinus

Austriaca. He used a sucrose medium to which he added indole-3-acetic acid. His results indicated that the addition of IAA increased both the rate and the percentage of pollen germination, and that the rate of pollen tube growth was also stimulated.

Loo and Hwant (19) reported that IAA at a concentration of 10^{-6}M increased the germination of Antirrhinum majus L. pollen approximately 20 percent over the controls. Elongation of pollen tubes in the IAA medium was less rapid than in the control medium, however. In concentrations higher than 10^{-6}M many pollen tubes swelled and burst. They also found that this acid inhibits the germination of Nicotiana tabacum L. pollen.

2. Boric acid as a stimulator.

Pollen itself is generally deficient in boron, and this deficiency is met by comparatively high levels of boron in stigmatic and stylar tissues.

Vasil (32) obtained 62 percent germination of Pennisetum typhoideum pollen in a 30 percent sucrose solution. With the addition of 78 percent boric acid, pollen germination increased to 78 percent and tubes were about double the size of the former ones. Concentrations of boric acid higher than 0.02 percent showed toxic effects, reducing the germination rate to less than five percent and resulting in abnormally short tubes. In other experiments involving pollen from members of the Cruciferae, Cucurbitaceae, Gramineae, Leguminoseae and Solanceae families, Vasil (31) observed that additions of 100-150 ppm of boric acid to the medium frequently stimulated pollen germination and pollen tube development. The stimulatory effect of boron on pollen germination, according to Vasil's conception, is due to increased

absorption; increased translocation and metabolism of sugars because of the formation of sugar-borate complexes; increased oxygen uptake; and to its role in the synthesis of pectic materials for the all of the actively growing pollen tubes.

Stanley and Loewas (30) found that the germination and tube growth of pear pollen were almost totally dependent on an exogenous source of boron. The optimum concentration appeared to be 10 ug/ml. At boron concentrations above 38 ug/ml, tube growth fell off sharply. They also observed a 10 percent increase in the germination of pine pollen in the presence of optimal boron levels.

O'kelley (22) utilized radioactive sucrose, glucose, and fructose in studying the effects of various levels of boron on pollen germination. He observed that O_2 uptake and sugar absorption by pollen grains were stimulated by the addition of small amounts of boron to the sugar solutions. However, if the concentration of boric acid was raised higher than 100ppm, O_2 uptake appeared to be slightly inhibited.

It has been suggested that boric acid affects the pH of the medium and also restricts the population of micro-organisms in the cultures, but boric acid is a weak acid and does not bring about any noticeable change in the pH of the medium at the concentrations used in pollen germination.

3. Dicarboxylic acids as stimulators of pollen germination.

A Russian plant breeder, Petrochenko (24), studied pollen germination in apples, grapes, tomatoes, cucumbers, and melons. Germination

was carried out on a medium consisting of 15 percent sucrose and one percent agar, to which was added various concentrations of succinic, fumeric, and adipic acid. The concentrations ranged from 0.01 to 0.0001M. His results indicated that concentrations of succinic and adipic acids from 0.00p to 0.0001M stimulated pollen germination and tube growth in apples, grapes, cucumbers, and watermelons. Tomato and watermelon pollen failed to germinate in the straight sugar-agar solution. Grape pollen (Chaush variety) germinated in the sucrose-agar medium only after being held at 5^{o} - 7^{o} C for 24 hours just prior to plating, with the temperature subsequently being raised to 25^{o} C. Petrochenko concluded that dicarboxylic acids at low concentrations stimulate pollen germination and at high concentrations (higher than 0.01M) inhibit pollen germination.

4. Vitamin B as a stimulator.

Schopfer (25) reported that many types of pollen grains are rich in growth factors, especially in thiamine and some of its components. He found that additions of thiamine increased the germination percentage of Carica quercifolea pollen on artificial media. It is possible that thiamine may turn out to be the active principle which is responsible for the increased growth of pollen tubes when yeast extract is added to the germination medium.

Cooper (12) worked with pollen germination in Carica papaya and reported that lactoflavin (a natural product of B_2) and ascorbic acid were

effective in increasing germination. The natural lactoflavin preparation was found to contain boron, which, as has been pointed out, is essential to the germination of many kinds of pollen. The effect of a synthetic preparation of lactoflavin was only about half as great as that of the natural product. Consequently, it was assumed that the increase in germination brought about by the addition of natural lactoflavin was due to the combined effect of boron and lactoflavin. Cooper also listed thiamine, indole-3 acetic acid and nicotinic acid as being effective in increasing pollen germination.

Dandliker and his co-workers (14) added crystalline vitamin B_1 to the sugar-agar pollen germination medium. They observed a significant increase, over the control, in the germination of ortando pollen, when 100ug B_1 per cc was added.

5. Mineral elements as stimulators.

Brewbaker and Kwack (4), in formulating a medium for pollen germination studies, found that additions of calcium were necessary for optimum germination. They concluded that calcium could not be successfully replaced in their germination medium by magnesium or potassium. Higginbotham (15) also reporetd calcium to have a beneficial effect in his pollen germination studies. Couey and Smith (13) observed that a combination of calcium and magnesium was more effective in germination media than either element alone.

Brink (6) studied pollen germination and pollen tube growth in sweet peas and in Nicotiana. He found that $MgCl_2$ was highly toxic to sweet pea pollen, but in trace amounts, actually stimulated germination and tube growth in Nicotiana. He further observed that additions of $CaCl_2$ to the germination medium stimulated sweet pea pollen but inhibited Nicotiana pollen.

Environmental effects on pollen germination

Anthony and Harlan (2), studying the germination of barley pollen, observed that pollen left exposed to free air for only two minutes lost moisture and began to shrink. The shrunken pollen was capable of germination if its moisture content had not reached a dangerously low level. However, pollen which had been exposed to free air for 10 minutes, and which, consequently, was severely dessicated and shrunken, had lost its germinative properties completely. They considered temperature also to be a critical factor in pollen germination and viability. They found that barley pollen retained a high level of viability after 24 hours when held at 10^{o} C.

In pollen germination studies conducted by Buckholz and Blakeslee (8) temperature variations were regarded as their greatest source of error. They found that pollen tube growth in many plant species is very temperature-sensitive. In Datura stramonium, for example, they observed that tube growth fell off rapidly on either side of 92^{o} F.

The relationship of temperature to alfalfa pollen tube growth has been studied by Sexsmith and Fryer (27). They found that alfalfa pollen tubes grow more rapidly as the temperature increases from 70° to 100° G.

Lehman and Puri (18) studied the effects of temperature on the germination of hand-collected and bee-collected alfalfa pollen. Pollen germinated best at 86° F. Pollen germination and tube growth were better for hand-collected pollen than for bee-collected pollen.

Using Newton Pippin apple pollen, Adams (1) found 13° C. to be optimum for germination and that best results were obtained by plating the pollen near 1:00 p, m, No differences in pollen tube growth were noted when the studies were carried out in the dark vs. light.

Smith (29) found 25° C. to be optimum for the germination and tube growth of Antirrhinum and Bryophyllum dalgremmontianum pollen. Only negligible growth took place at lower temperatures, and a broadening and bloating of the distal portion of the tubes was evident at higher temperatures.

Time of pollen collection

Wodehouse (33) found that it was best to collect pollen for germination from the first flowers to open rather than from those that bloom toward th end of the flowering season. The late flowers are usually less vigorous and are likely to be infested with insects. For most grasses,

collecting work should usually be done in the early morning. Selected flowers should be placed in the shedding pans as quickly as possible. Air-dried pollen will gradually deteriorate, but the rate of deterioration may be greatly reduced by completely dessicating and enclosing it in air-tight containers.

Vasil (31) worked on pollen germination within the Cucurbitaceae family. He obtained best results by collecting pollen from plants soon after anther dehiscence, which usually takes place early in the morning.

Ching (11) found that pine pollen could best be collected around eight o'clock in the morning.

Martin (20) was able to obtain viable pollen of Trifolium pratense for his germination tests anytime between the hours of 9 a. m. and 3 p. m.

MATERIALS AND METHODS

Sources, methods and times of pollen collection

The variety of safflower (Carthamus tinctorius) used in this study was 'Gila', and the alfalfa (Medicago sativa) used was an unnamed breeding line obtained from Dr. M. W. Pedersen, A.R.S. U.S.D.A., Logan, Utah.

Field collections of pollen were made by clipping new-blooming heads of safflower and racemes of alfalfa, inserting the stems in a bottle containing tap water, and bringing them into the laboratory where the actual pollen collection took place. Pollen collection in the greenhouse was made from intact flowers. The pollen was collected with a small camelhair brush.

Collections were made at 8 a.m., 12 noon and 5 p.m. in order to determine the best time of day for pollen collection.

Plating and counting procedures

The pollen was dusted on media in petri dishes. Except for the temperature studies, wherein a rather wide range of temperatures was used, the petri dishes were held at room temperature (approximately 75°-78° F.) in the laboratory. Each treatment was replicated five times, and five microscopic fields were counted for each replication. The average

for the five fields was used as the germination percentage for each replication.

Germination was checked at 30-minute intervals following plating to follow the progress of germination. The final counts were made two hours after plating.

Media used

Both solid and liquid media were used in these studies, with the percentage of agar varying from 0 to 4. Distilled water was used in the preparation of all media.

A number of sugars were tested as media constituents. These included sucrose, glucose, fructose, raffinose, dextrose and lactose. Concentrations ranged from 5 to 50 percent.

Boric, adipic, fumeric, succinic, indole acetic and gibberellic acids, in various concentrations from 1 to 1000 ppm., were tested for their possible effects on pollen germination and tube growth.

Various concentrations of the mineral elements calcium, potassium and magnesium and the vitamins thiamine and lactoflavin were also examined for their possible effects on the germination of alfalfa and safflower pollen. For the safflower studies a basic liquid medium containing 40 percent sucrose was used. To this the various acids, minerals and vitamins were added. With alfalfa, the basic medium consisted of 10 percent sucrose and two percent agar.

Longevity and temperature studies

In order to determine how long alfalfa and safflower pollen will remain viable when exposed to air-drying at room temperature in the laboratory, pollen was collected and subsequently plated at 2-hour intervals for periods up to eight hours.

Alfalfa pollen was exposed to temperatures of 55, 70, 80, 95 and 102 degrees Fahrenheit during germination to determine its optimum germination temperature.

RESULTS AND DISCUSSION

Time of pollen collection

Both safflower and alfalfa pollen were collected in the morning (8 a. m.), at noon, and in the later afternoon (5 p. m.) in an effort to determine if the time of collection affects germination on artificial media. Tables 1 and 2 show the data and their statistical analysis for alfalfa pollen. Highly significant differences were eivent among the three pollen collection times, with morning being best and evening the poorest.

Table 1. Percent germination of alfalfa pollen collected at three times during the day

Time	Replications					Average
	1	2	3	4	5	
Morning	80.2	81.2	78.8	79.2	78.0	79.5
Noon	68.0	70.0	68.8	66.8	69.0	68.5
Evening	42.8	48.0	45.2	46.2	38.0	44.0

Safflower pollen did not germinate on sugar-agar at all, so the effect of time of collections on the germination of safflower pollen was not determined.

Table 2. Analysis of variance of the effect of different time of pollen collection on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	34.13	8.54	1.52
Times	2	3292.31	1646.15	350.99**
Error	8	37.51	4.69	
Total	14	3363.94		

** Significant at .01 level

Pollen longevity

Safflower and alfalfa pollen was collected at 8 a.m. and exposed to air-drying in the laboratory, at room temperature, for periods of 2, 4, 6 and 8 hours. At the end of each time interval the pollen was plated on a sucrose-agar medium.

Safflower pollen exposed to open air for two hours in the laboratory shrivelled badly and failed to germinate at all. The effect of time was less drastic in the case of alfalfa pollen; nonetheless, pollen viability had dropped to zero at the end of eight hours (Figure 1). After a four-hour exposure to open air in the laboratory, viability of alfalfa pollen had been reduced 50 percent. The data and their analysis are contained in Tables 3 and 4.

Table 3. Percent germination of alfalfa pollen exposed to air-drying for periods of 2, 4, 6, and 8 hours

Rep.	Time				
	8 a.m.	10 a.m. (check)	Noon	2 p.m.	4 p.m.
1	80.6	54.8	42.0	15.6	0
2	81.0	54.2	40.2	18.2	0
3	78.0	55.6	42.0	19.6	0
4	82.4	55.8	39.6	16.4	0
5	80.4	56.8	38.8	20.0	0
Avg.	80.5	55.4	40.5	18.0	0

L.S.D. (.05) = 2.4

Table 4. Analysis of variance of the effect of time on the viability of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	1.46	0.37	0.12
Time intervals	3	10336.08	3445.36	1152.0**
Error	12	35.82	2.99	
Total	19	10373.36		

** Significant at the .01 level

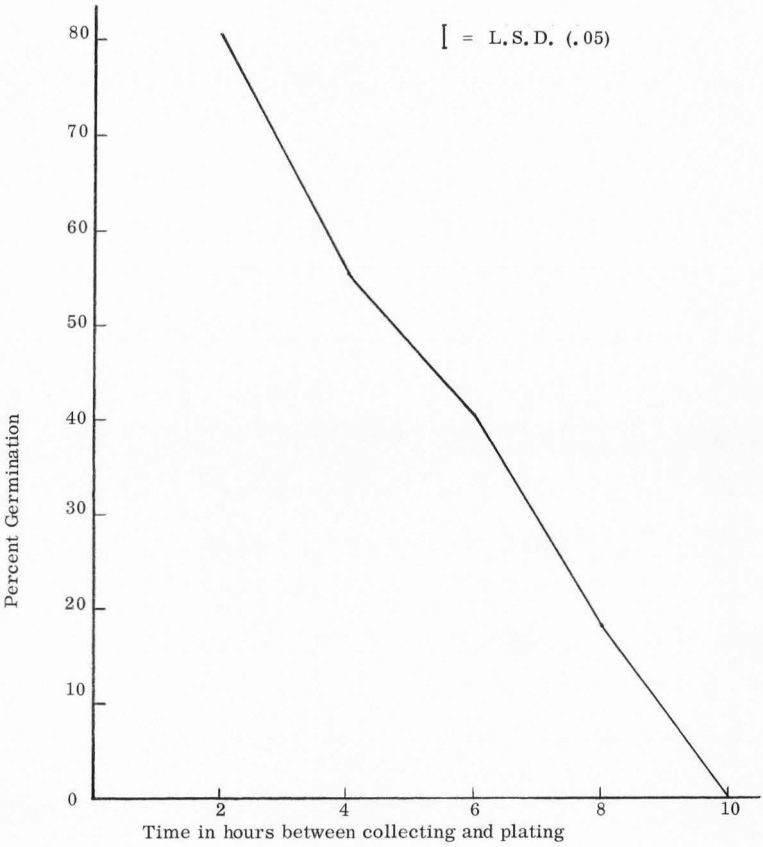


Figure 1. The effect of time on the viability of alfalfa pollen exposed to open air on the laboratory

Artificial media studies

Effect of various sugars on pollen germination. The results in this phase of the study were essentially negative, as far as safflower pollen is concerned. Sucrose, glucose, fructose, maltose, lactose and raffinose were tested as both solid and liquid germination media in concentrations ranging from 0 to 50 percent. With only one exception, no germination of safflower pollen was obtained. The sole exception occurred in liquid medium containing 35-40 percent sucrose. In this plate several pollen grains exhibited three short tube "buds" (Figure 2-B). In subsequent studies, wherein certain acids and growth-stimulating compounds were combined with 30-40 percent sucrose, considerable germination (though somewhat abnormal) of safflower pollen was observed. These results will be considered in later sections.

Profuse germination of alfalfa pollen occurred on all sugars and at all concentrations used. Germination of alfalfa pollen even took place on a wet glass slide. Although good germination was noted with all sugars and at all concentrations, it appeared that 10-20 percent sucrose in 2 percent agar constituted the ideal medium.

Effect of certain growth regulators on pollen germination and pollen tube growth

Inasmuch as gibberellic and idole-acetic acids have been reported to stimulate pollen germination in a number of species, it was hoped that they might exhibit this same effect with safflower pollen. No germination

Figure 2. Variations in the germination and subsequent tube growth of safflower pollen treated with various chemicals and plated on both solid and liquid media. (Magnification = 600X for B and 150X for all others.)

A. Safflower pollen exuding cytoplasm on the solid sucrose-agar medium.

B. A safflower pollen grain showing pollen tube "buds" in a 40 percent sucrose solution.

C. Twisted pollen tubes (safflower) in a 40 percent sucrose solution containing 250 ppm of boric acid.

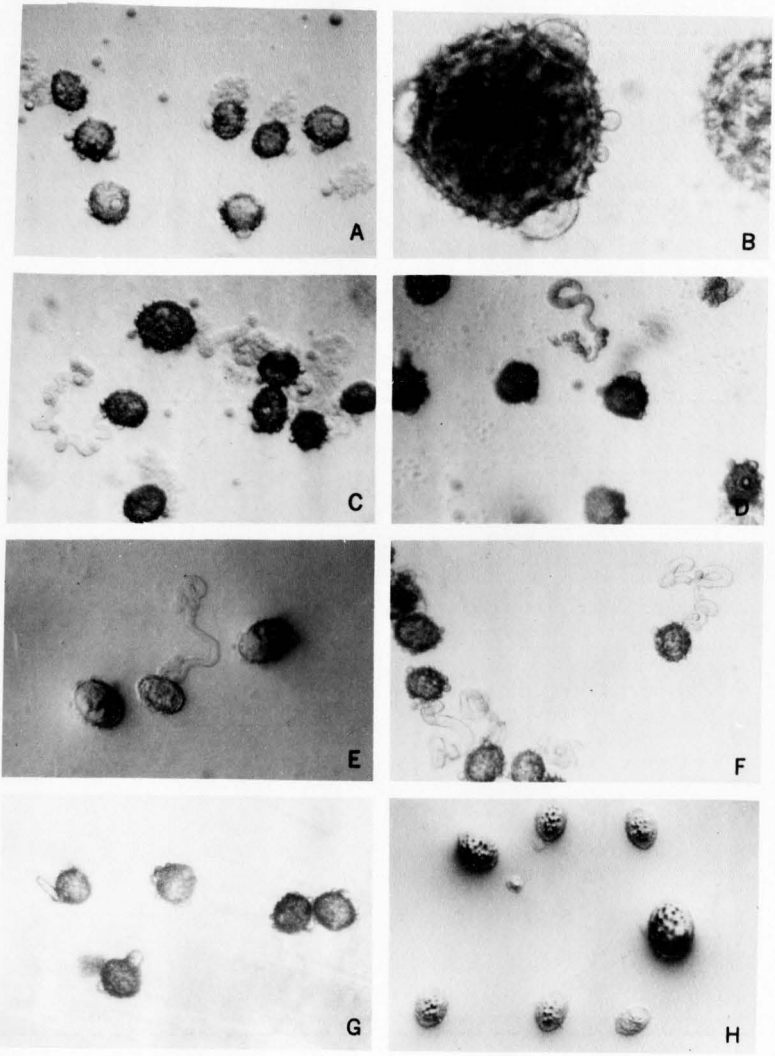
D. Twisted, broken pollen tubes (safflower) on artificial media after setting in laboratory for several hours.

E. Safflower pollen germinated in a 40 percent sucrose-solution containing 500 ppm of calcium.

F. Safflower pollen germinated in a 40 percent sucrose solution containing 300 ppm of thiamine.

G. Short pollen tubes on pollen grains removed from safflower stigmas.

H. Shrunken safflower pollen grains which had been exposed to an hour of air-drying in the laboratory.


A
B
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H

Figure 3. Variations in the germination and subsequent tube growth of alfalfa pollen treated with various chemicals and plated on a sucrose-agar medium. (Magnification = 150X.)

A. Alfalfa pollen germinated on the control medium containing 10 percent sucrose and 2 percent agar.

B. Alfalfa pollen germinated on a medium containing 50 ppm of gibberellic.acid.

C. Alfalfa pollen germinated on a medium containing 250 ppm of lactoflavin.

D. Alfalfa pollen germinated on a medium containing 180 ppm of boric acid.

E. Alfalfa pollen germinated on a medium containing 500 ppm of boric acid.

F. Alfalfa pollen germinated on a medium containing 100 ppm of succinic acid.

G. Alfalfa pollen germinated on a medium containing 200 ppm of of succinic acid.

H. Alfalfa pollen germinated on a medium containing 10 ppm of fumeric acid.

I. Alfalfa pollen germinated on a medium containing 50 ppm of fumeric acid.

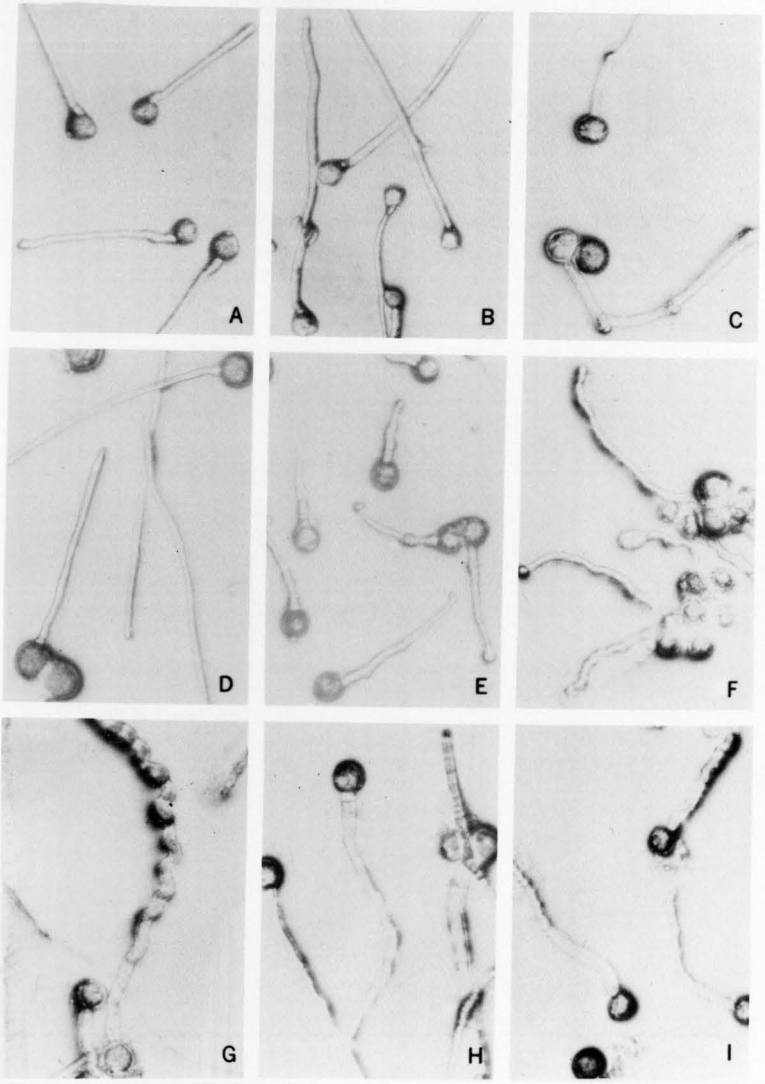
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Figure 3 J. Alfalfa pollen germinated on the control medium containing 10 percent sucrose and 2 percent agar.

K. Alfalfa pollen germinated on a medium containing 50 ppm of adipic acid.

L. Alfalfa pollen germinated on the contol medium and held at 60 70° F.

M. Alfalfa pollen germinated on a medium containing 10 ppm of potassium.

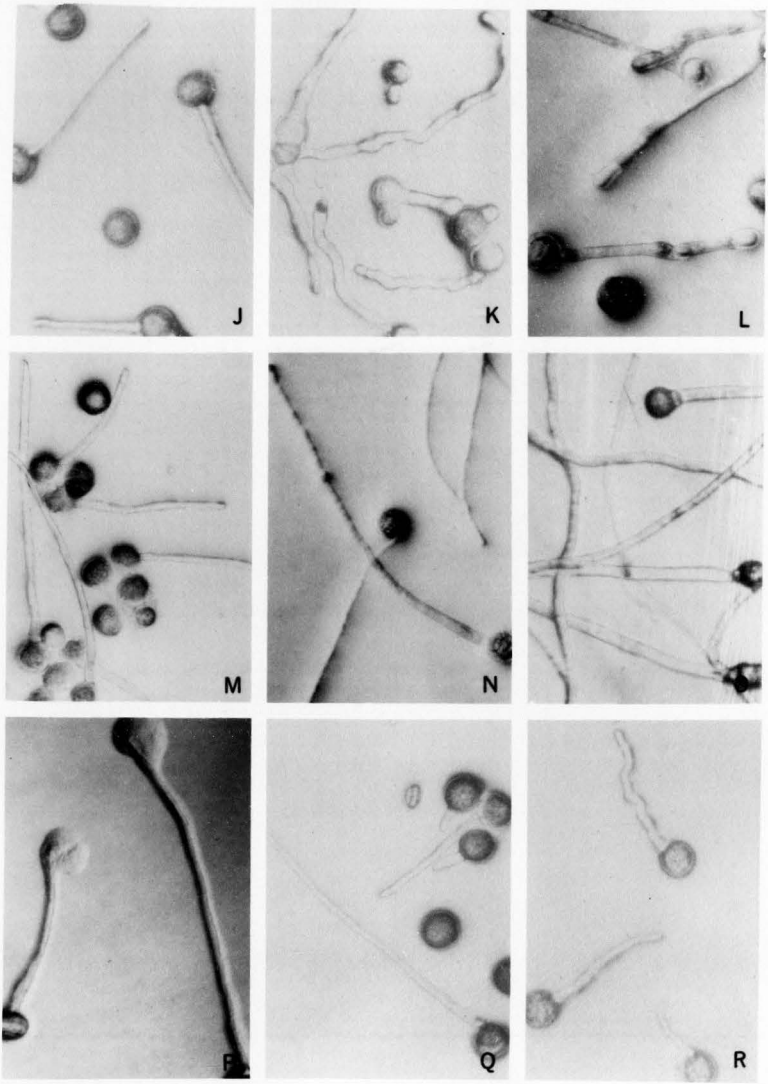
N. Alfalfa pollen germinated on a medium containing 50 ppm of potassium.

O. Alfalfa pollen germinated on a medium containing the lower concentrations of calcium (10-100 ppm).

P. Alfalfa pollen germinated on a medium containing the higher concentrations of calcium (500-1000 ppm).

Q. Alfalfa pollen germinated on a medium containing the lower concentrations of thiamine hydrochloride (50-100 ppm).

R. Alfalfa pollen germinated on a medium containing the higher concentrations of thiamine hydrochloride (500-1000 ppm).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was observed, however, in response to additions of either compound to the basic sucrose medium.

By way of contrast, both gibberellic acid and IAA had significant effects on the germination of alfalfa pollen when these compounds were added to a basic medium containing 10 percent sucrose in 2 percent agar. Tables 5 through 8 contain the germination data and their analysis. Highly significant germination differences were evident in response to the 10 levels of gibberellic acid and the six levels of IAA used. The germination of alfalfa pollen was apparently stimulated slightly by very low concentrations of gibberellic acid, but was depressed somewhat at concentrations above 300 ppm.

All concentrations of IAA reduced the germination of alfalfa pollen. Germination in hte medium containing 200 ppm was less than 1/10 that observed in the "0" check.

A graphic representation of the effects of gibberellic acid and IAA on the germination of alfalfa pollen is given in Figures 4 and 5.

Effect of boric acid on pollen germination

Because boron has been shown to be conspicuously present in stigmatic tissue and boric acid has been reported to stimulate pollen germination in certain plant species, it was included in the present study.

The data in Table 9 indicates that boric acid has a definite stimulatory effect on the germination of safflower pollen.

Table 5. Percent germination of alfalfa pollen on a medium containing various concentrations of gibberellic acid

Rep.	Concentration of gibberellic acid (ppm)									
	0	10	50	100	200	300	400	500	600	1000
1	74.8	79.2	89.8	83.6	75.6	71.2	68.8	67.8	70.8	68.8
2	75.4	78.0	90.6	82.2	79.6	72.8	67.6	71.6	70.0	71.0
3	78.4	76.4	88.2	82.0	76.4	70.0	72.4	68.0	68.2	70.2
4	79.6	79.6	89.2	81.2	75.2	68.8	69.0	69.4	66.8	68.8
5	78.0	77.2	92.8	78.2	74.8	69.0	68.2	74.0	67.8	67.0
Avg.	78.0	78.1	90.1	81.4	76.3	70.4	69.2	70.2	68.7	69.2

L.S.D. (.05) = 2.4

Table 6. Analysis of variance of the effect of various gibberellic acid concentrations on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	9.68	2.84	0.83
Concentrations	9	2209.95	245.55	71.18**
Error	36	122.99	3.42	
Total	49	2342.99		

** Significant at the .01 level.

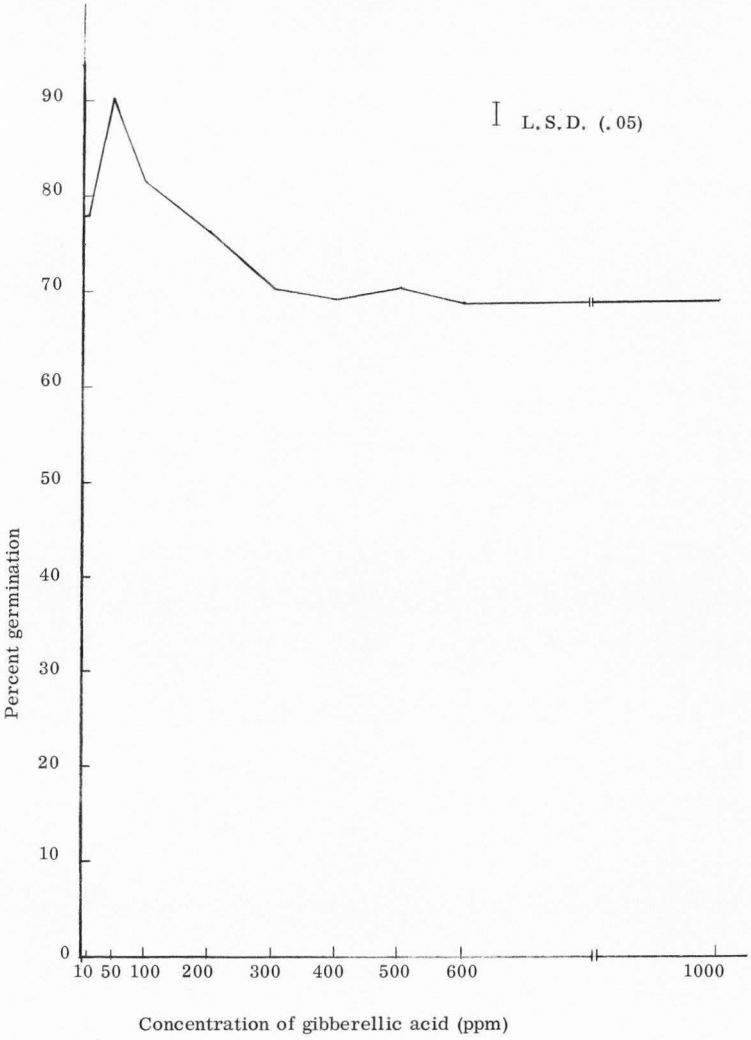

Figure 4. The effect of various concentrations of gibberellic acid on the germination of alfalfa pollen

Table 7. Percent germination of alfalfa pollen on a medium containing various concentrations of idole acetic acid

Rep.	Concentration of indole acetic acid (ppm)					
	0	1	10	50	100	200
1	78.8	68.8	66.8	48.2	34.2	6.0
2	80.4	67.6	67.0	52.0	38.0	8.4
3	79.8	70.2	64.2	48.8	38.2	9.2
4	77.8	69.4	65.0	50.6	36.8	6.8
5	79.0	68.4	64.6	51.6	34.6	7.0
Avg.	79.2	68.9	65.5	50.2	36.4	7.5

L.S.D. (.05)

Table 8. Analysis of variance of the effect of various IAA concentrations on the germination of alfalfa pollen.

Source of Cariation	Degrees of freedom	Sums of squares	Mean squares	F
Replication	4	11.95	2.99	1.28
Concentrations	5	17159.80	3431.96	1472.94**
Error	20	46.56	2.33	
Total	29	17218.31		

** Significant at the .01 level.

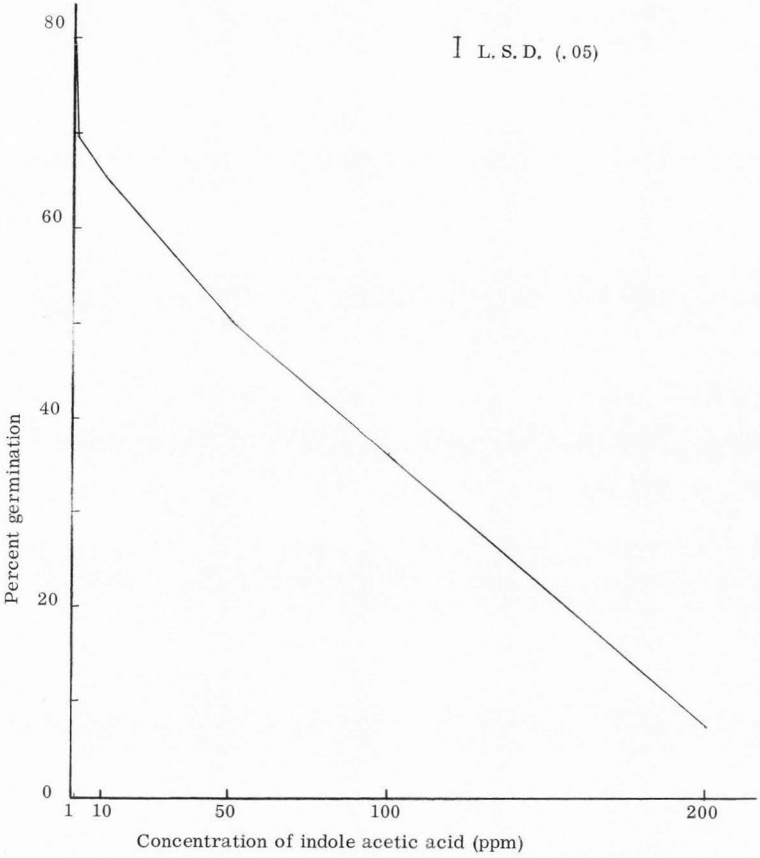

Figure 5. The effect of various concentrations of indole acetic acid on the germination of alfalfa pollen

Table 9. Percent germination of safflower pollen on a medium containing various concentrations of boric acid

Rep.	Concentration of boric acid (ppm)						
	0	50	100	150	200	250	300
1	0	4.0	11.2	10.8	16.0	36.6	4.0
2	0	0.2	10.0	14.2	18.8	30.8	7.2
3	0	2.4	8.6	13.8	17.0	34.2	8.0
4	0	2.0	8.2	11.6	17.0	34.8	6.8
5	0	0	9.8	14.0	18.2	35.6	7.8
Avg.	0	1.7	9.6	12.9	17.4	34.4	6.8

L.S.D. (.05) = 2.4

Germination improved with increments of boric acid up to 250 ppm, and then dropped off rapidly. Germination appeared somewhat abnormal, with twisted pollen tubes which frequently burst (Figure 2-C); however, no truly "normal" pollen tubes were observed with safflower pollen in response to any of the treatments or media used in this thesis study. The analysis of the data and their graphic representation are shown in Table 10 and Figure 6, respectively.

Boric acid in concentrations up to 300 ppm also increased the germination of alfalfa pollen, although not to the extent that it did with safflower pollen (Table 11). The analysis of variance and a graph showing the

Table 10. Analysis of variance of the effect of various boric acid concentrations on the germination of safflower pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	2.75	0.69	0.24
Concentrations	5	3258.15	651.63	222.40**
Error	20	58.53	2.93	
Total	29	3319.43		

** Significant at the .01 level

Table 11. Percent germination of alfalfa pollen on a medium containing various concentrations of boric acid

Rep.	Concentration of boric acid (ppm)							
	0	10	50	100	200	300	400	500
1	78.4	82.4	81.6	89.2	84.8	80.6	58.0	40.8
2	79.2	78.2	84.4	86.8	82.0	80.2	55.6	36.2
3	78.0	79.6	80.8	89.6	82.2	81.6	56.4	42.0
4	77.2	80.0	81.8	88.8	82.4	78.2	56.8	38.2
5	78.0	79.8	82.0	88.0	84.0	80.8	56.0	36.0
Avg.	78.2	80.0	82.1	88.5	83.1	80.3	56.6	38.6

L. S. D. (.05) = 0.6

effect of boric acid on the germination of alfalfa pollen are presented in Table 12 and Figure 7. At the higher concentrations of boric acid the pollen tubes were shorter than normal and exhibited a slight curling and knobbiness (Figure 3-E).

Table 12. . Analysis of variance of the effect of various boric acid concentrations on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	15.66	3.92	2.07
Concentrations	7	10012.70	1430.39	756.83**
Error	28	53.03	1.89	
Total	39	10091.39		

** Significant at the .01 level

Effect of certain dicarboxylic acids on pollen germination

A number of organic acids have been reported to have a stimulatory effect on pollen germination (27). Consequently, several of these were examined for their possible effect in increasing the germination of safflower and alfalfa pollen.

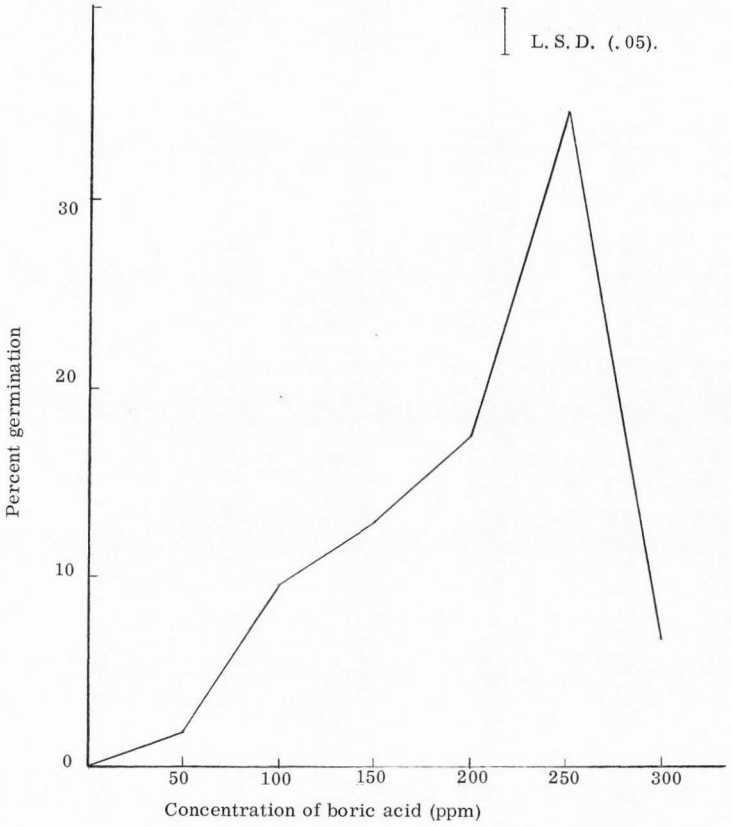

Figure 6. The effect of various concentrations of boric acid on the germination of safflower pollen.

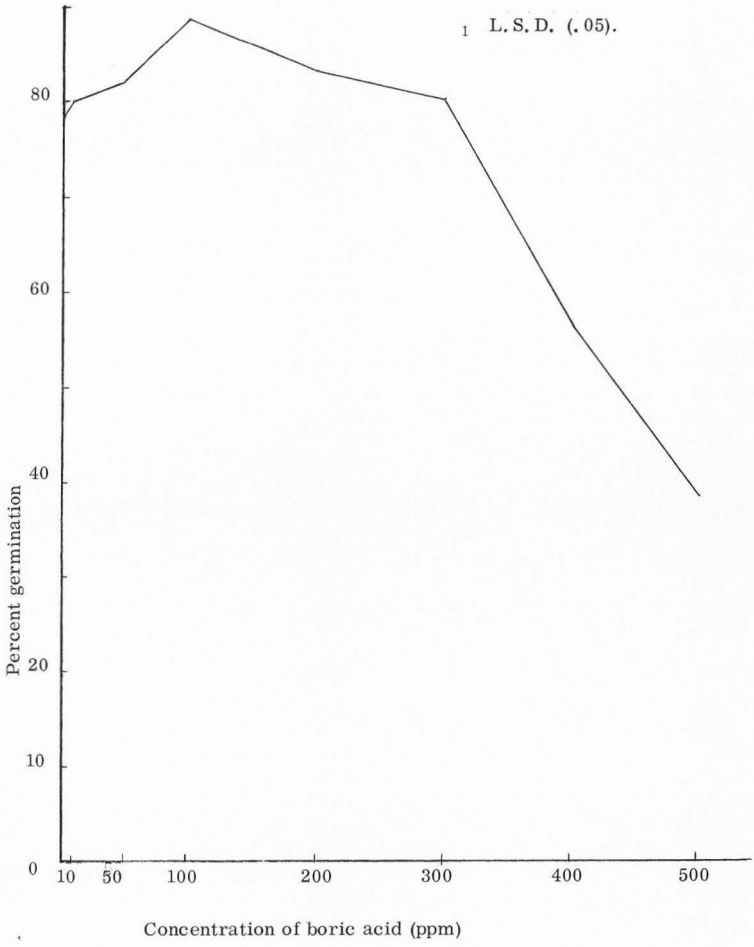

Figure 7. The effect of various concentrations of boric acid on the germination of alfalfa pollen.

Fumeric acid. Table 13 and 14 show the germination data, and their statistical analysis, for safflower pollen on a 40 percent sucrose medium to which had been added various concentrations of fumeric acid. All concentrations tested resulted in better germination than that exhibited by the check. However, approximately 250 ppm appeared to be the most effective concentration. The results are shown graphically in Figure 8. As was the case with the boric acid treatments, pollen tubes were abnormal on the fumeric acid medium, e.g., tubes were twisted and frequently burst.

Alfalfa pollen showed a slight increase in germination at very low concentrations of fumeric acid (10 ppm); however, at 100 ppm germination was sharply curtailed (Table 15). An analysis of the data is given in Table 16. The data are plotted in Figure 9. Compared to the control plates, pollen tubes were thicker and showed more coiling on media containing fumeric acid (Figures 3H and I).

Succinic acid. The effect of succinic acid on the germination of safflower pollen can be seen in Table 17 and Figure 10. Concentrations in the range of 250-300 ppm effectively increased germination; however, pollen tube growth was somewhat abnormal. An analysis of the germination data for safflower pollen on a medium containing succinic acid is presented in Table 18.

The germination of alfalfa pollen on a medium containing 10 ppm of succinic acid fell slightly below that observed on the control medium; however, when the concentration was increased to 50 ppm, germination

Table 13. Percent germination of safflower pollen on a medium containing various concentrations of fumeric acid

Rep.	Concentration of fumeric acid (ppm)				
1	0	18.2	32.4	20.4	8.0
2	0	20.4	34.0	27.2	7.8
3	0	19.2	35.2	25.0	11.2
4	0	19.4	33.6	26.2	9.0
5	0	18.8	33.2	25.8	9.2
Avg.	0	19.2	38.7	24.9	9.0

L.S.D. (.05) = 1.6

Table 14. Analysis of variance of the effect of various fumeric acid concentrations on the germination of safflower pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	21.01	5.25	2.97
Concentrations	3	1602.07	534.02	301.70**
Error	12	21.28	1.77	
Total	19	1644.36		

**Significant at the .01 level

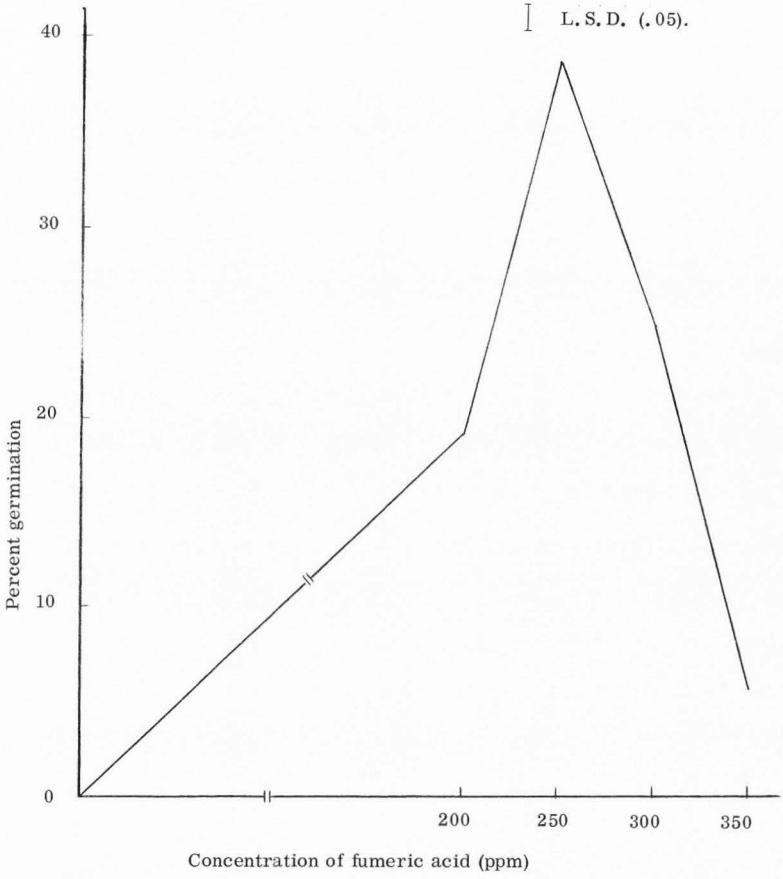

Figure 8. The effect of various concentrations of fumeric acid on the germination of safflower pollen

Table 15. Percent germination of alfalfa pollen on a medium containing various concentrations of fumeric acid

Rep.	Concentration of fumeric acid				
1	78.0	86.6	74.4	5.2	0
2	79.8	88.4	73.2	2.4	0
3	80.2	84.8	72.4	4.0	0
4	78.8	86.0	73.4	3.8	0
5	78.6	88.0	74.0	2.8	0
Avg.	79.1	86.8	73.5	3.6	0

L.S.D. (.05) = 1.8

Table 16. Analysis of variance of the effect of various fumeric acid concentrations on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	1.63	0.41	0.25
Concentrations	3	2201.41	7400.47	4540.17**
Error	12	19.60	1.63	
Total	19	22221.64		

** Significant at the .01 level.

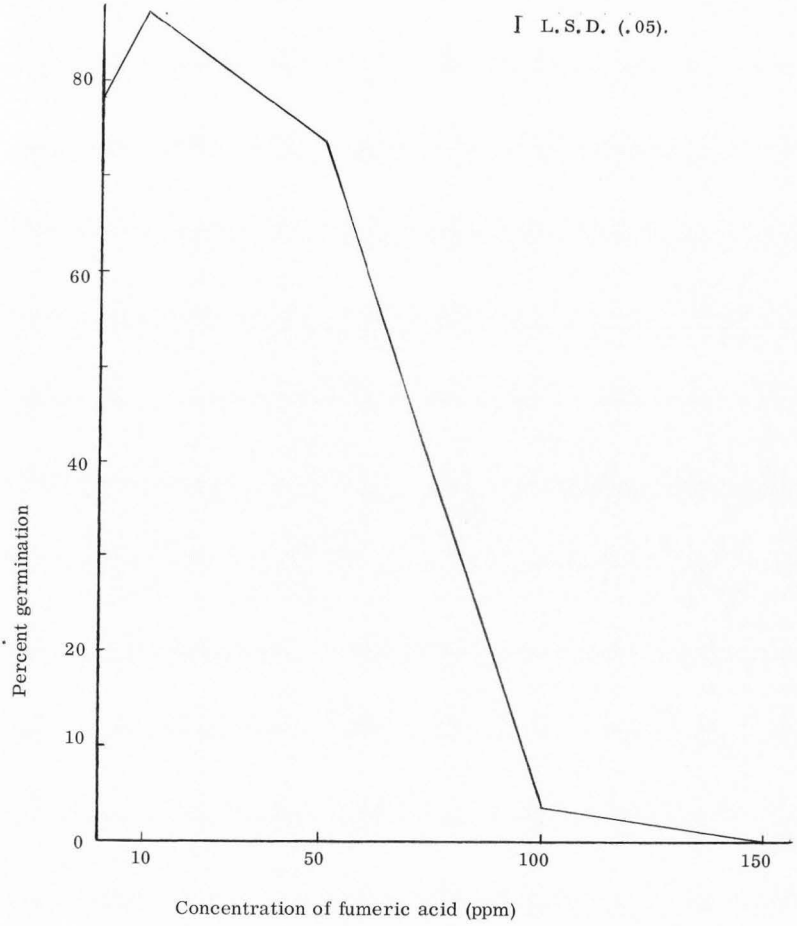

Figure 9. The effect of various concentrations of fumeric acid on the germination of alfalfa pollen

Table 17. Percent germination of safflower pollen on a medium containing various concentrations of succinic acid

Rep.	Concentration of succinic acid (ppm)				
	0	200	250	300	350
1	0	1.2	35.2	36.8	0
2	0	3.0	28.8	37.7	2.2
3	0	0	29.0	40.0	0
4	0	5.0	38.0	35.2	5.0
5	0	4.4	26.8	41.2	3.2
Avg.	0	2.7	32.4	38.1	2.1

L.S.D. (.05) = 4.4

Table 18. Analysis of variance of the effect of various succinic acid concentrations on the germination of safflower pollen

Source of variation	Degrees of Freedom	Sums of squares	Mean squares	F
Replications	4	29.61	7.40	0.73
Concentrations	3	5370.37	1790.12	176.02**
Error	12	122.01	10.17	
Total	19	5521.99		

** Significant at the .01 level.

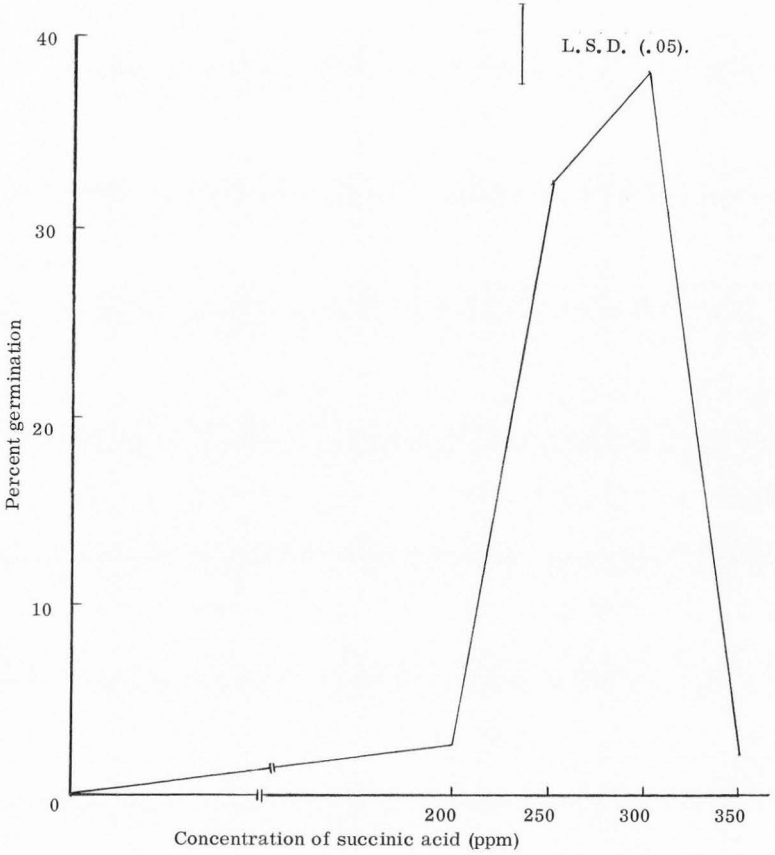

Figure 10. The effect of various concentrations of succinic acid on the germination of safflower pollen

significantly exceeded the control (Figure 11). Germination fell off again slightly at 100 ppm, and dropped sharply at 200 ppm of succinic acid. The germination data and their analysis are presented in Table 19 and 20. It was observed that pollen tubes growing on media containing over 100 ppm of succinic acid were shorter and generally more abnormal than pollen tubes on media containing 10 and 50 ppm. Photomicrographs of the abnormal germination of alfalfa pollen on media containing succinic acid are shown in Figures 3-F and 3-G.

Adipic acid. Adipic acid was added to the basic sucrose medium in concentrations ranging up to 400 ppm. Except for a low level of germination (12 percent) at about 200 ppm, safflower pollen failed to germinate on media containing adipic acid.

Adipic acid also failed to stimulate the germination of alfalfa pollen. It did not inhibit germination at low concentrations (50 ppm), but at concentrations of 100 ppm and above, germination fell off sharply (Table 21). The data are plotted graphically in Figure 12, and their statistical analysis is presented in Table 22. Alfalfa pollen tubes growing on media containing adipic acid showed many of the same abnormalities exhibited by the pollen tubes on succinic acid media (Figure 3-K).

Table 19. Percent germination of alfalfa pollen on a medium containing various concentrations of succinic acid

Rep.	Concentration of succinic acid (ppm)				
	0	10	50	100	200
1	79.2	62.4	78.6	60.0	22.4
2	80.2	67.6	84.8	62.4	18.4
3	78.4	68.0	86.0	60.8	15.2
4	77.8	64.4	85.6	62.4	11.6
5	79.0	66.8	79.8	61.8	18.8
Avg.	78.9	65.8	83.0	61.5	17.3

L.S.D.(.05) = 4.0

Table 20. Analysis of varinace of the effect of various succinic acid concentrations on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	17.72	4.43	0.49
Concentrations	4	13690.12	3422.53	376.93**
Error	16	145.25	9.08	
Total	24	13853.09		

**Significant at the .01 level

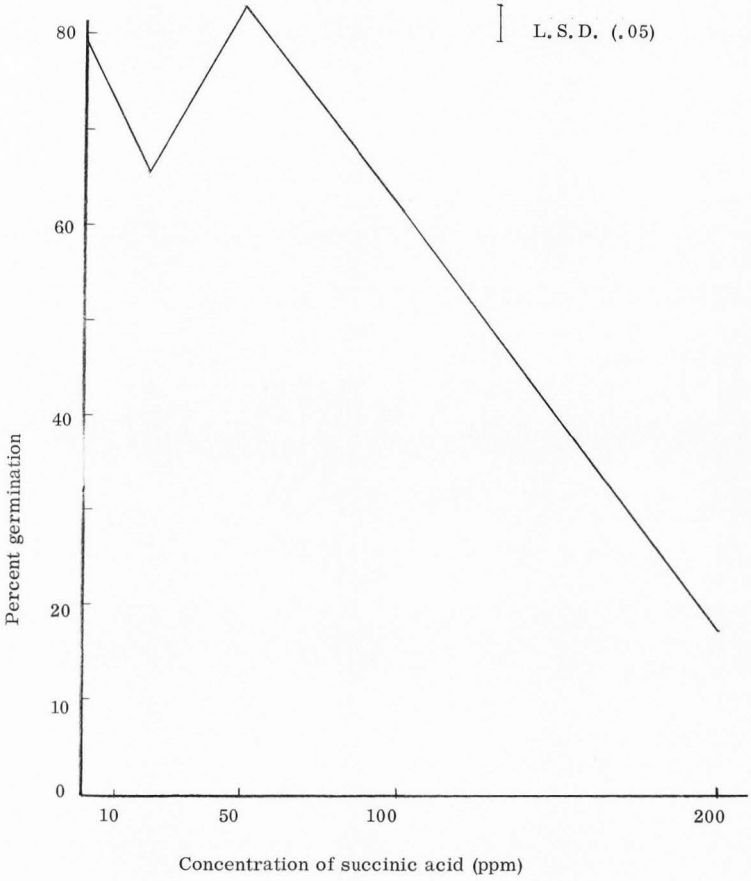

Figure 11. The effect of various concentrations of succinic acid on the germination of alfalfa pollen

Table 21. Percent germination of alfalfa pollen on a medium containing various concentrations of adipic acid.

Rep.	Concentrations of adipic acid (ppm)					
	0	50	100	200	300	400
1	77.6	76.8	56.0	43.6	31.2	3.2
2	78.4	77.2	52.0	35.6	32.0	6.0
3	83.2	78.0	53.6	34.4	31.2	7.2
4	80.0	81.6	51.6	38.0	30.4	4.8
5	79.0	77.6	56.4	36.4	32.8	3.8
Avg.	79.7	78.2	53.9	37.6	31.5	5.0

L.S.D. (.05) = 3.2

Table 22. Analysis of variance of the effect of various adinic acid concentrations of the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	4.64	1.16	0.19
Concentrations	5	20905.91	4151.18	702.72**
Error	20	118.98	5.95	
Total	29	21029.53		

** Significant at the .01 level

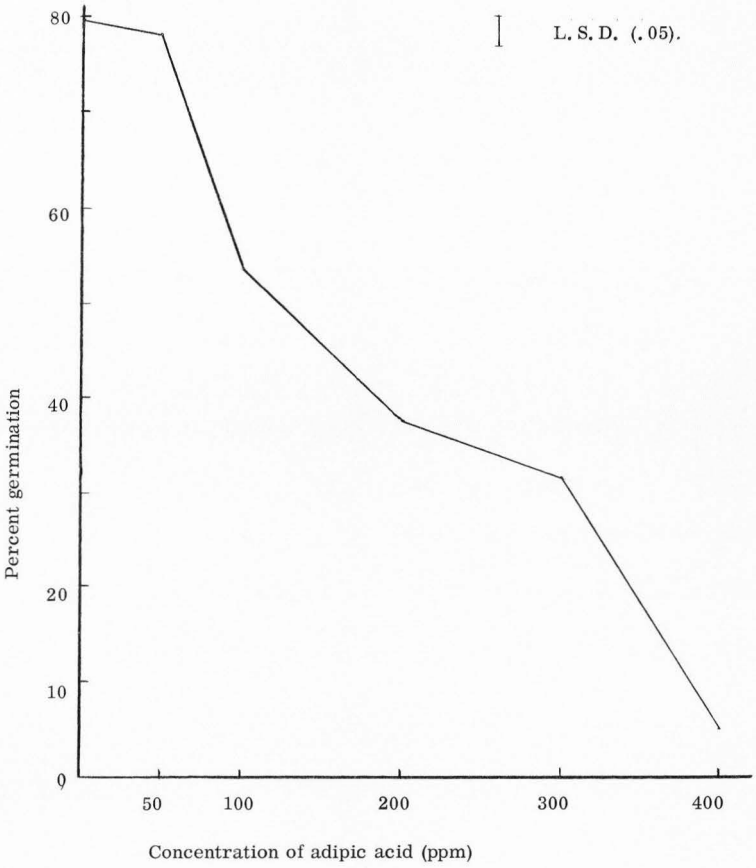

Figure 12. The effect of various concentrations of adipic acid on the germination of alfalfa pollen

Effect of certain vitamins on pollen germination

In recent years it has been reported that the beneficial effects of yeast extracts on the growth of pollen and fungus spores in vitro were due, in part, to certain of the B vitamins. Consequently, several of these vitamins were used in the present study.

Thiamine. The addition of thaimine hydrochloride to the basic germination medium had a stimulatory effect on safflower pollen (Figure 13). Three hundred ppm appeared to be optimum, resulting in nearly 35 percent germination (Table 23). Considerable abnormal pollen growth, e. g., twisted, stubby and broken tubes, (Figure 2-F) was observed, however. The data are analyzed in Table 24.

A slightly, but significant, increase in the germination of alfalfa pollen was noted when 50 and 100 ppm of thiamine hydrochloride were added to the medium (Table 25). At concentrations above 100 ppm the germination percentage gradually declined (Figure 14). Pollen ubes were longer than normal at concentrations from 100 to 200 ppm; and they were shorter than normal on media containing over 300 ppm of thiamine hydrochloride Figures 3-Q & 3-R). The effect of thiamine onthe germination and growth of alfalfa pollen was very similar to the effect of gibberellic acid and boron.

The analysis of the data concerning the effect of thiamine on the germination of alfalfa pollen is given in Table 26.

Table 23. Percent germination of safflower pollen on a medium containing various concentrations of thiamine hydrochloride

Rep.	Concentration of thiamine hydrochloride (ppm)			
	0	200	300	400
1	0	20.2	39.2	12.0
2	0	18.0	28.0	7.8
3	0	14.4	30.2	8.0
4	0	16.0	37.2	9.8
5	0	15.2	39.8	7.6
Average	0	16.8	34.9	9.0

L.S.D. (.05) = 6.4

Table 24. Analysis of variance of the effect of various concentrations of thiamine hydrochloride on the germination of safflower pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	78.87	19.72	1.03
Concentrations	2	1759.40	879.70	45.87**
Error	8	153.47	19.18	
Total	14	1991.74		

** Significant at the .01 level

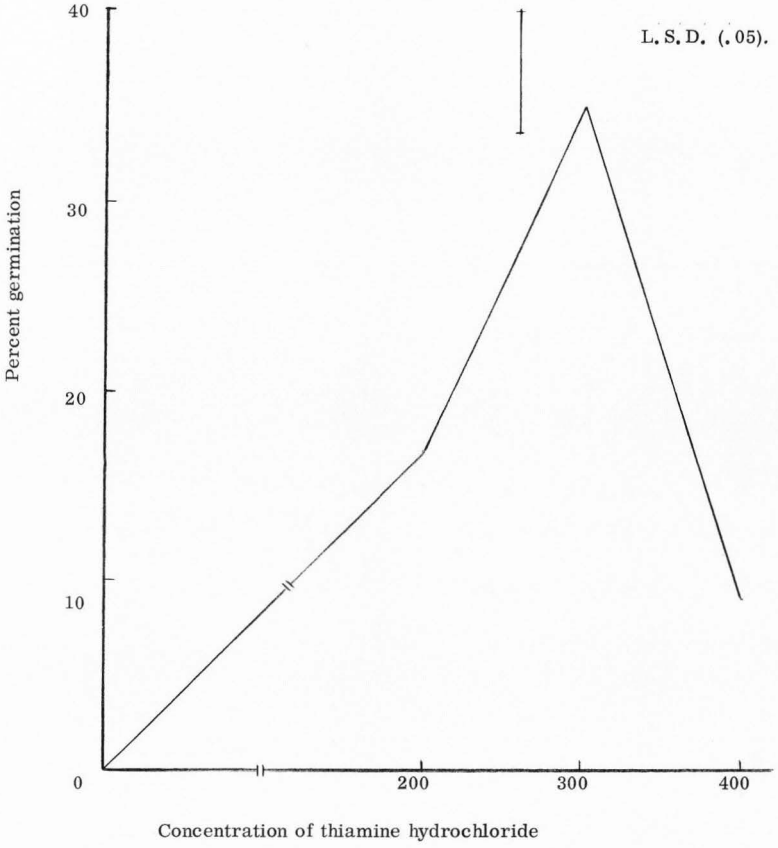

Figure 13. The effect of various concentrations of thiamine hydrochloride on the germination of safflower pollen

Table 25. Percent germination of alfalfa pollen on a medium containing various concentrations of thiamine hydrochloride

Rep.	Concentrations of thiamine hydrochloride (ppm)								
	0	50	100	200	300	400	500	600	1000
1	79.2	82.0	83.2	70.4	68.2	54.8	52.8	44.4	33.2
2	78.2	80.0	85.6	72.0	70.8	52.4	52.8	40.8	32.0
3	76.8	81.6	84.4	74.8	71.4	54.0	50.8	36.4	34.8
4	79.8	87.6	86.0	72.8	74.0	59.2	51.6	40.2	31.6
5	80.6	83.2	82.8	71.2	69.8	63.2	54.0	41.2	32.0
Avg.	78.9	82.9	84.4	72.2	70.8	56.7	52.4	40.6	32.7

L.S.D. (.05) = 3.2

Table 26. Analysis of variance of the effect of various concentrations of thiamine hydrochloride on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	30.05	7.51	1.18
Concentrations	8	14107.12	1763.39	278.14**
Error	32	202.98	6.34	
Total	44	14340.15		

** Significant at the .01 level

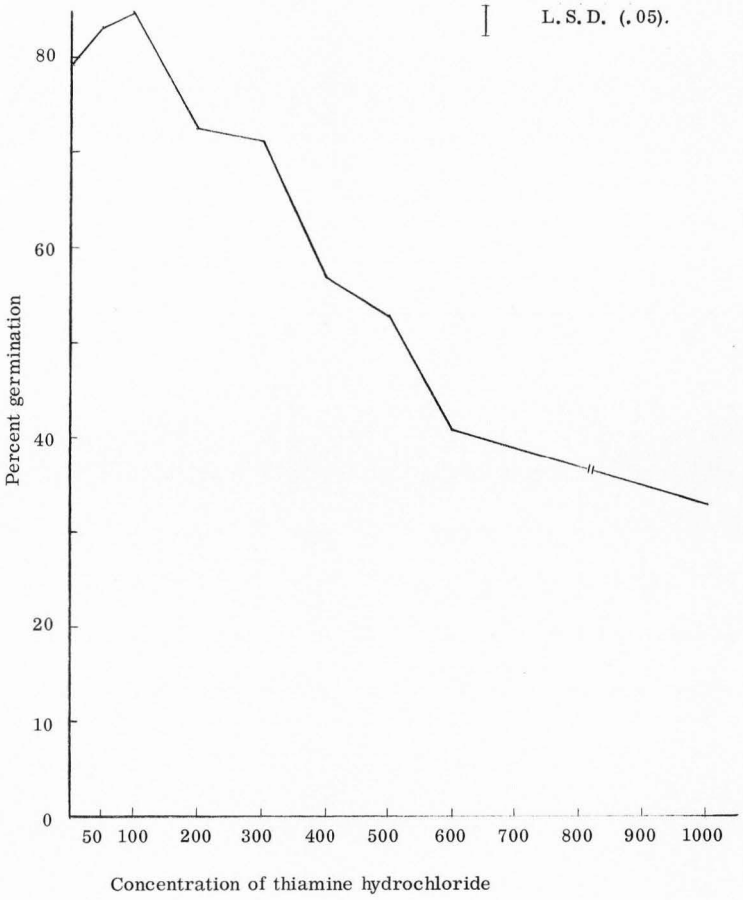

Figure 14. The effect of various concentrations of thiamine hydrochloride on the germination of alfalfa pollen

Lactoflavin. Although lactoflavin has been reported to increase pollen germination in certain species, it failed to stimulate the germination of safflower and alfalfa pollen in this study. Safflower pollen failed to germinate at all when placed in media containing additions of lactoflavin. Alfalfa pollen germinated to a limited extent on such media, but the tubes were all abnormal (Figure 3-C).

Effect of various mineral elements on pollen germination

Less than one percent, by weight, of the protoplasm in plant cells is made up of mineral elements; however, they serve a very important role in plant metabolism. Calcium is present in plant tissue in larger amounts than any other mineral present.

Inasmuch as various workers have reported increased pollen germination following the addition of certain mineral elements to germination media, calcium, magnesium and potassium were tested for their effect on the germination of safflower and alfalfa pollen.

Calcium. The addition of several hundred ppm of calcium to the basic sucrose medium, significantly increased the germination of safflower pollen (Table 27 and 28). Over 1/3 of the pollen grains plated on media containing 500 ppm of calcium germinated; however, the subsequent pollen tube growth was abnormal (Figure 2-E). When the concentration was raised to 700 ppm, germination was reduced to less than three percent. The data are shown graphically in Figure 15.

Table 27. Percent germination of safflower pollen on a medium containing various concentrations of calcium

Rep	Concentration of calcium (ppm)					
	0	300	400	500	600	700
1	0	1.2	22.4	39.2	12.0	3.0
2	0	0	18.0	36.0	7.8	2.4
3	0	2.8	16.8	40.2	9.2	1.8
4	0	1.6	17.2	34.6	10.4	2.2
5	0	3.0	20.0	38.2	8.8	3.2
Average	0	1.3	18.9	37.6	9.6	2.5

L.S.D. (.05) = 2.0

Table 28. Analysis of variance of the effect of various concentrations of calcium on the germination of safflower pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	24.11	6.03	2.64
Concentrations	4	4421.15	1105.29	484.78**
Error	16	36.50	2.28	
Total	24	4481.76		

** Significant at the .01 level

Alfalfa pollen reacted quite differently to additions of calcium. In no instance did added calcium significantly increase the germination of alfalfa pollen (Table 29). Instead, germination began falling off at concentrations of calcium above 100 ppm, gradually declining to slightly less than 50 percent of the check at a concentration of 1000 (Figure 16). The analysis is shown in Table 30. Alfalfa pollen tubes growing on a medium containing the higher concentrations of calcium were shorter, thicker and showed more bursting of tips than did tubes on the control medium (Figure 3-P).

Magnesium. Safflower pollen failed to respond to additions of magnesium to the germination medium. Magnesium in concentrations of 50 ppm or less neigher stimulated nor inhibited the germination of alfalfa pollen (Table 31). Germination dropped off rapidly with the addition of 100 ppm, however (Figure 17). The data are analyzed in Table 32.

Potassium. Potassium added to the germination medium appeared to increase the germination of alfalfa pollen slightly at a concentration of 50 ppm (Figure 18). At concentrations above this level, however germination fell significantly below the check (Table 33). An analysis of the data is presented in Table 34. Pollen tubes growing on media containing potassium were abnormally thin and knobby (Figure 3-M).

Safflower pollen failed to germinate on media containing additions of potassium.

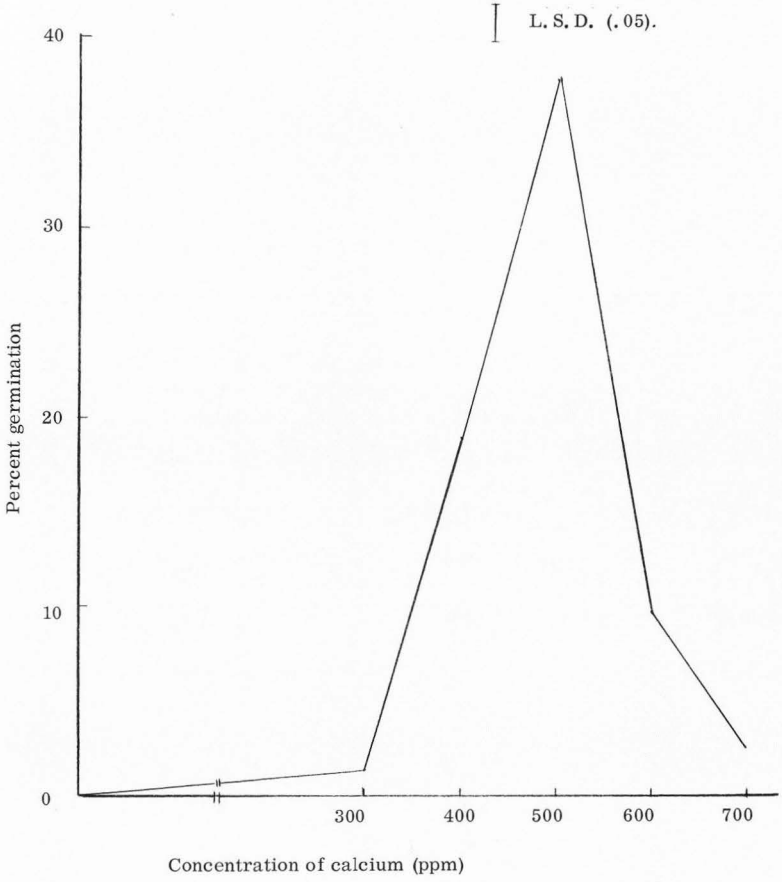

Figure 15. The effect of various concentrations of calcium on the germination pf safflower pollen

Table 29. Percent germination of alfalfa pollen on a medium containing various concentrations of calcium

Rep.	Concentration of calcium (ppm)								
	0	10	50	100	200	300	400	500	1000
1	78.7	80.0	82.0	76.0	70.8	70.8	64.4	60.0	35.6
2	79.2	78.0	80.0	78.2	74.2	68.2	63.6	58.0	32.4
3	80.2	79.6	79.0	80.0	72.8	71.2	66.8	61.6	37.6
4	77.8	80.4	78.8	74.6	70.0	70.2	70.0	61.2	35.6
5	80.4	83.6	78.2	74.8	76.2	72.4	63.6	56.8	34.2
Avg.	79.3	80.3	79.6	76.7	72.8	70.6	65.7	59.5	35.1

L.S.D. (.05) = 2.6

Table 30. Analysis of variance of the effect of various concentrations of calcium on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	16.74	4.19	1.01
Concentrations	8	8369.41	1046.18	253.31**
Error	32	132.14	4.13	
Total	44	8518.29		

** Significant at the .01 level

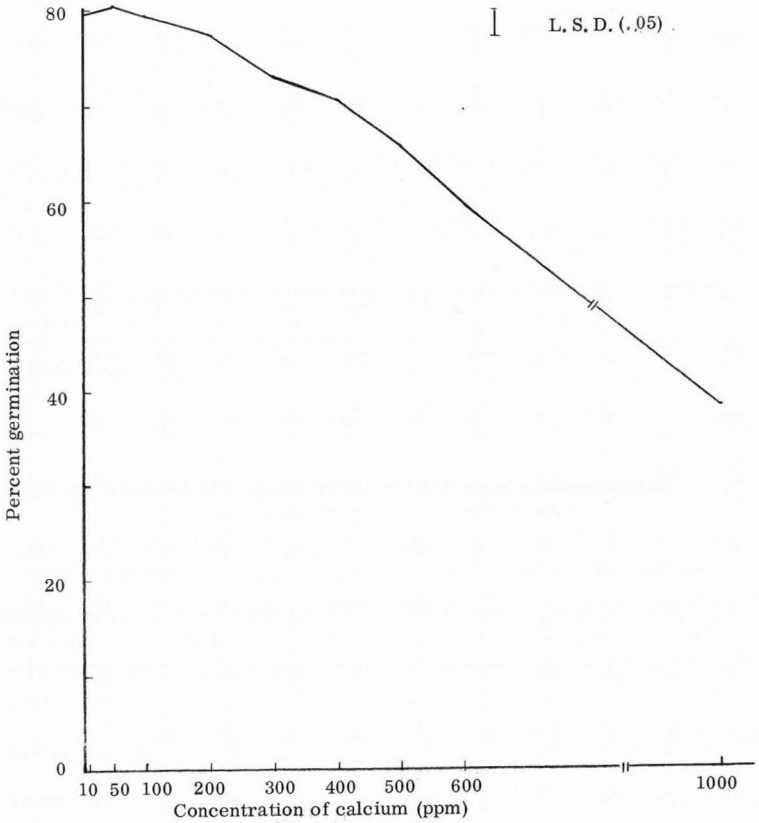

Figure 16. The effect of various concentrations of calcium on the germination of alfalfa pollen

Table 31. Percent germination of alfalfa pollen on a medium containing various concentrations of magnesium

Rep.	Concentration of magnesium (ppm)			
1	78.2	79.8	78.8	24.2
2	80.2	76.6	82.2	24.2
3	81.6	78.4	79.8	26.4
4	79.8	80.8	82.6	28.0
5	78.6	77.8	81.8	30.2
Average	79.7	78.7	81.0	27.7

L.S.D. (.05) = 2.9

Table 32. Analysis of variance of the effect of various concentrations of magnesium on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	14.85	3.71	0.83
Concentrations	3	10185.25	3395.08	756.14**
Error	12	53.86	4.49	
Total	19	10253.96		

** Significant at .01 level

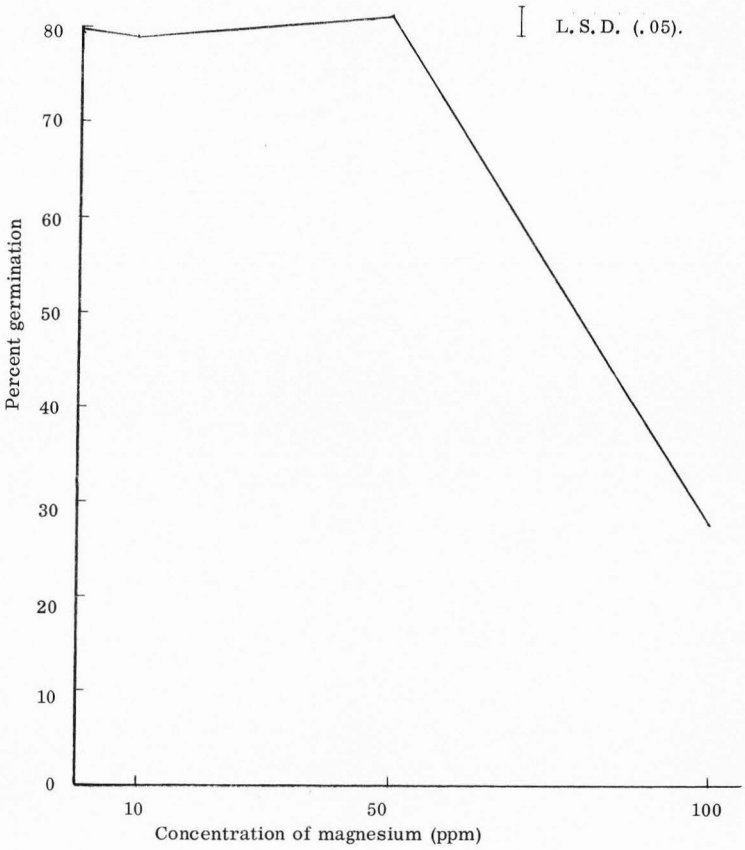

Figure 17. The effect of various concentrations of magnesium on the germination of alfalfa pollen

Table 33. Percent germination of alfalfa pollen on a medium containing various concentrations of potassium

Rep.	Concentration of potassium (ppm)						
1	80.2	78.8	81.2	61.4	54.0	44.4	16.2
2	79.0	78.0	82.8	59.4	50.8	46.8	18.2
3	82.0	80.2	86.4	58.2	52.0	42.0	15.0
4	80.8	78.8	84.4	62.0	49.8	43.0	16.0
5	79.8	81.2	82.4	59.2	51.2	46.2	17.2
Avg.	80.4	79.4	83.4	60.0	51.6	44.5	16.5

L.S.D. (.05) = 2.2

Table 34. Analysis of variance of the effect of various concentrations of potassium on the germination of alfalfa pollen

Source of variations	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	0.54	0.13	0.04
Concentrations	6	17702.10	2950.35	1003.52**
Error	24	70.62	2.94	
Total	34	17773.26		

** Significant at the .01 level

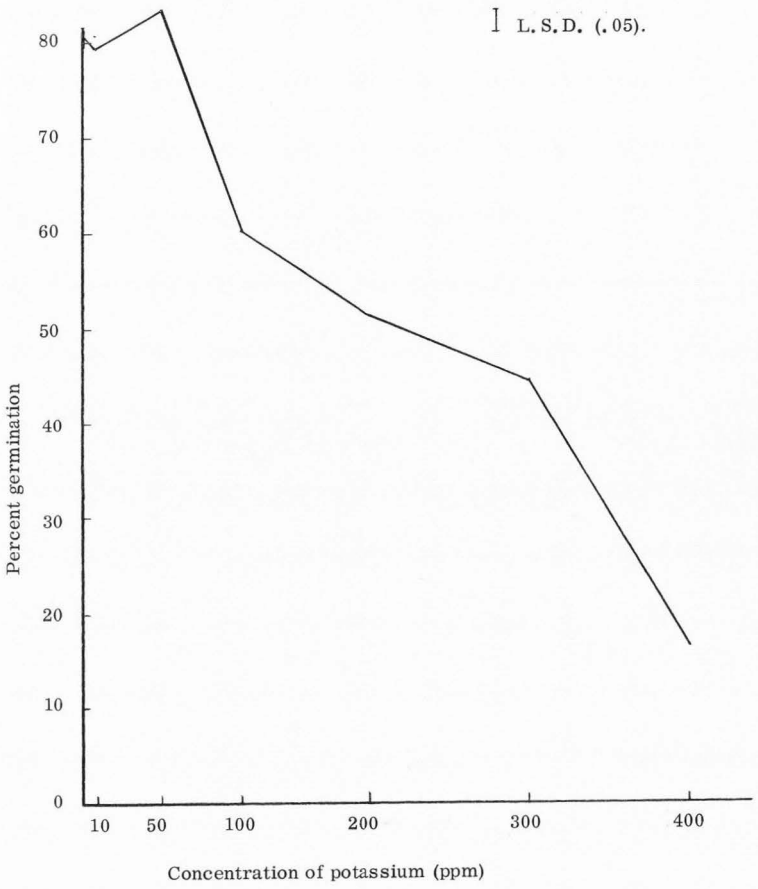

Figure 18. The effect of various concentrations of potassium on the germination of alfalfa pollen

Effect of temperature on pollen germination

Inasmuch as safflower pollen can be germinated only with difficulty, and then only to a limited degree, under artificial conditions, only alfalfa pollen was used in the temperature study. Alfalfa pollen was plated on the basic sucrose-agar medium and held at temperatures of 55, 70, 80, 95 and 102 degrees Fahrenheit. The data are presented in Table 35. Alfalfa pollen failed to germinate at 55° F., reached its maximum germination at 80-95° F., and declined to 60 percent germination at 102° F. The analysis of variance is given in Table 36. At temperatures below 70, the pollen tubes were broader, shorter and knobbier than normal (Figure 3-L).

Table 35. Percent germination of alfalfa pollen at various temperatures

Rep.	Temperature (F°)				
	55°	70°	80°	95°	102°
1	0	54.2	79.6	78.8	61.6
2	0	56.0	82.0	77.6	57.6
3	0	56.8	80.8	79.2	64.0
4	0	54.0	78.8	78.8	62.8
5	0	56.8	75.6	80.0	56.8
Total	0	55.6	79.4	78.9	60.6

** Significant at the .01 level

Table 36. Analysis of variance of the effect of various temperatures on the germination of alfalfa pollen

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Replications	4	14.17	3.54	0.57
Temperatures	3	2280.70	760.23	121.64**
Error	12	74.94	6.25	
Total	19	2369.81		

** Significant at the .01 level

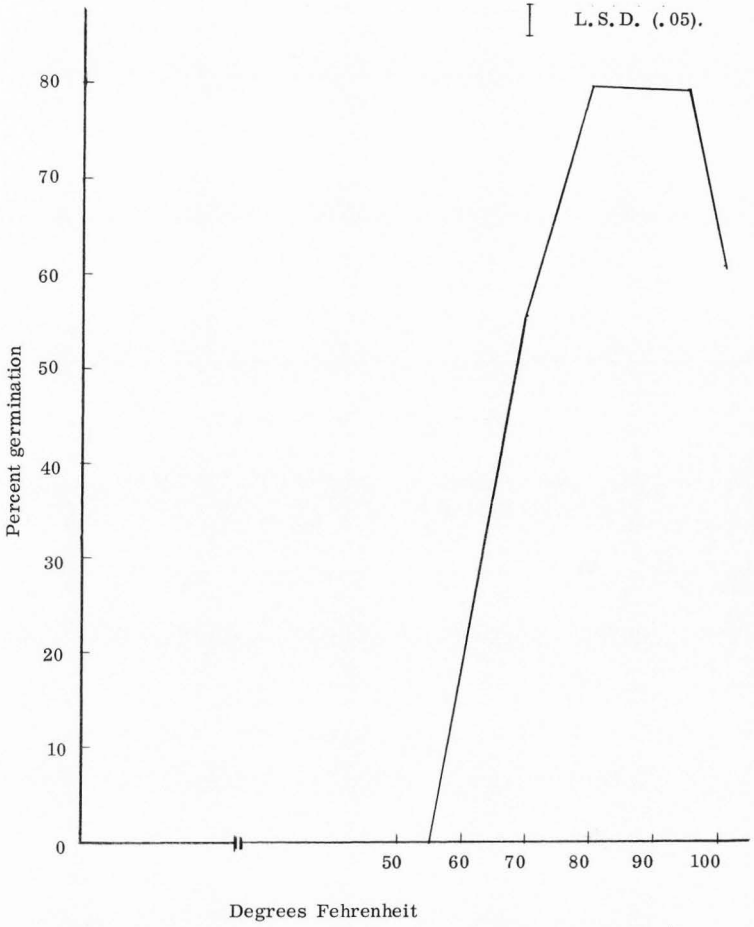

Figure 19. The effect of various temperatures on the germination of alfalfa pollen

SUMMARY AND CONCLUSIONS

Safflower

1. Safflower pollen is one of the most difficult types of pollen to germinate on artificial medium. In the present study it failed to germinate on any kind of straight sugar-agar media.
2. Germination occurred in media containing 40 percent sucrose to which additions of any of the following were made: boric acid, fumeric acid, adipic acid, succinic acid, thiamine, and calcium.
3. Although some germination took place in these media, the percent germination was relatively low and all pollen tubes were abnormal.
4. Liquid media proved superior to solid media for safflower pollen

Alfalfa

1. Alfalfa pollen germinated readily on almost all media used in this study.
2. A medium consisting of 10 percent sucrose and two percent agar provided the most satisfactory basic medium.
3. The best germination was obtained when additions of calcium, thiamine or gibberellic acid were made to this basic medium.
4. These additions resulted in abnormal pollen tubes, however, if their concentration exceeded approximately 1000 ppm.

5. The best time for pollen collection appeared to be around 8 a. m. The optimum germination temperature was approximately 80^{o} F.

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