EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON POLLEN GERMINATION OF GOSSYPIUM

by

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ABSTRACT

Twenty varieties of Gossypium were tested for pollen germination on an artificial medium composed of 100 ml. of distilled water, 3.5 g. bacto-agar, 26 g. sucrose, 70 mg. manganous sulfate, 40 mg. calcium nitrate, and 40 mg. boric acid. The mean percentage of pollen germination ranged from 6.8 to 18.0, with overall mean of 11.9 percent. There were significant differences among varieties in which Rilcot 90, with mean germination of 18.0 percent, was highest in the group. There were also significant differences among dates with August 6 to August 17 showing the highest germinations. Some temperatures and relative humidities at certain lag periods were highly correlated with percent germination of the varieties tested, but no single day showed a high correlation of temperature and/or relative humidity with all twenty varieties.

The study on the determination of the best time of the day to expect maximum pollen germination indicated that variety has an influence. Super Okra Leaf showed maximum germination at 7:00 A.M. while Deltapine 16 and Pima S-4 indicated 10:00 A.M. to be very favorable for pollen germination. Pima S-4 consistently showed low germinations on the media throughout the experimental period.

CHAPTER I

INTRODUCTION

As our world of science develops more and more every day, it becomes quite necessary for modern scientific workers to have a broader knowledge of science, use more sophisticated techniques, and to understand our environment. They must acquire basic knowledge that can be used to develop new techniques and approaches in order to avoid existing and future problems that could jeopardize human interests. The scientists are challenged to improve and/or change the present practices that no longer can be exploited advantageously. With more scientific exploration comes an enlarged viewpoint on the part of research workers toward their environment and this, in turn, leads to more avenues of approach that man could safely and profitably explore with outstanding benefits.

The Malthus theory of population explosion proposed many centuries ago, and the frightening idea that people will have to struggle to survive on the face of the earth in the near future is not only invalid but is very short-sighted and unrealistic. Progress made every now and then in all phases of scientific endeavor builds up to make a real significant success towards overcoming our obstacles in life. Even studies on very small things like pollen

grains, which seem to be negligible, could contribute significantly if properly carried out and then utilized.

Pollen germination studies could be quite useful in many fields of cotton research including the breeding behavior of the crop, interspecific hybridizations, male-sterility studies, evolutionary and statistical genetics, fertility and reproductive physiology, pollination and fruit-set relations, cytology and cyto-genetics, storage and longevity studies, pesticidal and fungicidal influences on pollen germination, and ecological factors affecting pollination and yield (16). A rapid and reliable *in vitro* method of pollen grain germination of cotton would make studies of the preceding problems indeed feasible. Research workers in cotton have had discouraging results for a long time in attempting to germinate cotton pollen grains on artificial media. Now that two practical methods are available, namely, the Bronckers and Taylor *in vitro* methods of pollen germination of *Gossypium*, experiments may be designed to solve some of the problems involving cotton pollen. The Bronckers method, used and improved by Miravalle (16), has been reported reliable. The Taylor method, the easiest and fastest method most recently devised, seems to be quite successful with *G. hirsutum* and was selected to be used in this study. Taylor (22) has reported absolute failure using his method with *G. barbadense*; however, this study primarily involves *G. hirsutum*.

Many varieties developed for Arizona and other environments in the United States of America are produced in Tucson for various purposes. It should be obvious by now that tests on pollen germination in cotton would be very fascinating and useful and many breeding programs could evolve and progress made from the application of the results. Many varieties bred for different climatic conditions and purposes, but grown in Tucson mainly for experimental uses, have been deliberately included in this trial. Improved methods and techniques could in the future, facilitate germination of all types of Gossypium pollen and this, in turn, should challenge more research workers to investigate fields where pollen germination is a prerequisite.

Even though the Taylor method has been devised and this present research has been carried out in the United States, there is no reason to limit such type of program to this country alone for similar studies could be applicable in all cotton-growing areas of the world. This is, primarily, because the growth medium or technique could be easily prepared and germination completed in the laboratory with relatively inexpensive facilities and, hence, lend itself to widespread use.

CHAPTER II

REVIEW OF LITERATURE

Several attempts to germinate cotton pollen grains have proven to be frustrating; however, some progress towards developing proper media for cotton pollen germination has been reported from time to time. Quite recently some promising methods have been discovered. Detailed studies regarding the effect of temperature and/or relative humidity on cotton pollen germinations are practically unavailable in the literature. A review of the techniques used and results obtained primarily with cotton pollen is presented. Some techniques used to germinate pollen grains of other species will also be discussed since they have some application to the present study.

Four methods of determining cotton pollen viability have been devised. The first method tried was an *in vitro* germination technique which proved to be a failure to early research workers. As a result, research was shifted to other means in which pollen-bursting as an index of viability as well as *in situ* methods of germination were explored. Very recently, the use of tetrazolium salts as vital pollen stains was reported. Surprisingly, some scientists have lately returned to the oldest system of *in vitro* germination and have developed substantially successful media.

As early as 1923, Kearney (8), and in 1924 and 1932 Kearney and Harrison (9, 10), being resigned to the failure of *in vitro* methods, tested cotton pollen viability as a percentage of pollen grains exploding in five percent solution of sugar and in distilled water. The pollen grains immediately explode as soon as the sugar solution or the distilled water is applied. In this method, the abnormally small and abnormally large pollen grains failed to burst with no possible explanation given.

In 1929, Banerji (4) reported difficulties in cotton pollen germination using several methods proven successful with other crops. He was not successful with the Badami (1922-23) castor oil, aqueous solutions of cane sugar and glucose, agar plus sucrose, gelatin plus sucrose, moist parchment paper, or moist chamber methods. He reported, however, successful germination with inconsistent results, using dry watch glass suspended over water in a covered petri dish.

In 1930, Shibuya (19) reported success in germinating pollen grains of cotton using agar with sucrose method in which he moistened his dry medium with water before inverting the prepared slides of pollen over the agar with sucrose medium. The most favorable concentrations of media developed were 35 percent sucrose with 5 percent agar and 40 percent sucrose with 4 percent agar. Using the

latter concentration, he obtained the highest percentage of germination at 25°C.

In 1938, Iyengar (7) described a method of germinating cotton pollen and two tracing methods of detecting pollen tubes *in situ*. The two tracing methods used were (a) dissecting away the outer cortex of the style and staining the central strand of connecting tissue, and (b) embedding and sectioning the pistil. The former has been reported as the most preferable technique because the pollen tubes could be easily traced starting from the grain to the tip of the tube.

In 1961, Bronckers (5) developed a promising technique of *in vitro* germination of cotton pollen grains which was accepted by many people as reliable. He obtained as high as 82 percent germination. Bronckers' method will be described in detail under Miravalle's work because it is one of the significant developments in the studies of cotton pollen germination.

In 1962, Vasil (23) pointed out that it is really difficult to germinate cotton pollen grains since practically all burst when placed in water or low concentration sucrose solutions.

In 1963, Klyukvina (11) tested many methods in attempting to develop a good *in vitro* method of cotton pollen germination. He used sucrose solutions of different concentrations, agar-agar solutions with sucrose, agar-agar

with a castor oil and cotton oil, cotton oil refined and crude, castor and cedar oil, and simple germination on a clean glass. Moisture, light and temperature conditions were also varied. Refined castor oil method gave him 80-100 percent germination.

In 1964, Sarvella (18) reported work with vital-stain testing of pollen viability in cotton. Using several procedures, she tested various stains in different concentrations and media. Criteria used in studying the stains were speed of reaction and spurting of pollen grain contents. The different lines of cotton used were (a) G. hirsutum, (b) Asiatic cotton, (c) partial male-steriles ms-1 ms-1 and ms-3 ms-3, (d) totally male-sterile ms-2 ms-2, (e) monosomics M6 and M4, (f) interspecific hybrid (B_1D_1) and (g) a haploid. Tetrazolium red and 2,3,5-triphenyl tetrazolium chloride gave very promising results with the former staining faster than the latter. Spurting of the contents of the pollen grains was effectively controlled by mixing 1 part of stain with 5 parts of a 60 percent sucrose solution.

Results of the staining experiment indicated that all of the pollen grains of G. hirsutum, Asiatic cottons, and monosomics were well stained; some of the pollen grains of the partial male-sterile line were stained; while the pollen grains of the completely male sterile, the interspecific hybrid, and the haploid lines did not stain.

The interspecific hybrid and the haploid were both sterile lines. This technique definitely separated the potentially poor pollen group from the potential fertiles. Similar staining results were also obtained with 0.01 percent crystal violet mixed with sucrose in the same ratio as the tetrazolium chloride except that the crystal violet took longer to stain the pollen grains.

In the same year, Aslam, Brown and Kohel (2) reported their studies dealing with evaluation of seven tetrazolium salts as vital pollen stains in cottons of G. hirsutum. Results were similar to those obtained by Sarvella (18), and of the seven salts used only 2,3,5-triphenyl tetrazolium chloride and tetrazolium red gave positive results. Again, they confirmed that 60 percent sucrose solution should be added to the tetrazolium salts to avoid rupturing of pollen grains.

Alexander (1) reported his work on differential staining of aborted and nonaborted pollen using a single staining solution. This staining technique was generally applicable to most genera and pollen grains studied included those having thin-wall, thick-wall, and being dehisced and non-dehisced. Some modification in procedures were made. As in the case of Gossypium, having thick-walled and sticky pollen, he first removed the sticky material by fixing non-dehisced but mature anthers in Carnoy's fixative

and then proceeded to his staining procedure after releasing the pollen grains from the anthers. Aborted pollen grains stained green while the walls of the nonaborted ones stained green and their protoplasm red.

In 1965, Miravalle (16), using Bronckers' method, reported his work on germination of cotton pollen. Since this in vitro method seems to be one of the most useful methods it would be worthwhile to outline the procedure used and mention the results he obtained.

In this method, half a filter paper is placed in a 15 cm. diameter petri dish and 50 mg. of acenaphthene scattered on the filter paper. About 15 drops of distilled water is then added to the acenaphthene on the filter paper. Pollen grains are then piled at the edge of a microscope slide and placed in the petri dish with the pile of pollen grains close to the edge of the filter paper. The petri dish is closed and put in a germinator at 25°C. Germination is completed within 24 hours. A drop of lactophenol is then added to the pollen grains to scatter them out and a stain is finally added before making germination counts under a microscope. Using a stock of Acala 4-42, Miravalle obtained pollen germination ranging from 35 to 73 percent. The author himself has used Bronckers' method in Tucson, Arizona and observed substantial germination, but finally decided to shift to the Taylor method which he considered to be more advantageous for reasons that will be explained later.

As recent as 1971, McDonald (15) reported work on cotton pollen germination *in vitro* using the Bronckers method. He compared four sources of pollen for germination, namely, (a) several commercial varieties, (b) fertile B-line of *G. anomalum*, (c) dehisced A-line of *G. anomalum*, and also (d) an undehisced, but dissected A-line. The commercial varieties gave the highest germination percentages, and Deltapine M-8, experimental variety used as a B-line, averaged as high as 50.8 percent. The B-line gave higher germination than the A-lines, while the dissected A-line was superior to the dehisced A-line in which case McDonald suspected exposure to the environment to be a possible cause for the decline in germination percent.

Taylor (22) tested several media for germination of cotton and reported that one gave fairly good results. Even though this method has not produced results significantly different than the best of earlier methods, it does have certain merits as compared to others, such as being simple to use and rapid for evaluation, making it more useful as a research tool.

The medium consists of agar, sucrose, manganous sulfate, calcium nitrate, and boric acid. Taylor reported 10-64 percent germination with overall mean of 30 percent with several varieties of *G. hirsutum*. According to Taylor, Pima cotton (*G. barbadense*) failed to germinate on this

medium, but with no explanation given. Further details of this method will be given in another section.

In a number of species, techniques for pollen germination have been developed which are similar in many respects to the techniques used for cotton pollen. Those techniques considered pertinent to the present study are reviewed below.

Bair and Loomis (3) developed a very fast method of germinating maize pollen grains which gave them as high as 90 percent germination. Their medium, containing 0.7 percent agar and 15 percent sucrose, gave the highest germination at 23°C. and 90 percent relative humidity.

Pfahler (17) presented a simple but fast way of germinating rye pollen grains. The medium used consisted of 100 ml. distilled water, 3.5 g. of bacto-agar, 25 g. sucrose and 20 mg. of boric acid. He obtained a mean germination of 39.1 percent which was not particularly high, but possibly real.

Studying the rates of germination and tube growth of stored and fresh alfalfa pollen, Lehman and Puri (13) got a maximum of 90 percent germination in a reasonably short time using a medium consisting of 100 ml. distilled water, 20 g. sucrose, and 1.5 g. bacto-agar. Germination was accomplished in darkness at 30 $\pm$ 1°C.

Straley and Melton (21) reported that temperature during plant growth and development had a significant influence on *in vitro* germination and tube length of alfalfa pollen.

In a brief article on germination of oat pollen on artificial media, Wallace and Karbassi (24) reported obtaining as high as 80 percent germination in five minutes from the time of seeding the pollen on the medium. Aging the medium 2 to 10 days prior to application of pollen grains proved to be necessary for improving germination and reducing bursting of the pollen grains.

Maun, Teare, and Canode (14) presented their study on artificial cultures used to germinate pollen grains of Kentucky Bluegrass. They observed good germination and growth in a medium composed of 13 percent sucrose, 0.75 percent agar-agar, 10 ppm. of calcium nitrate, and 5 ppm. boric acid in an incubator set at about 21°C. and 90 percent relative humidity in complete darkness.

Layne and Hagedorn (12), studying the effect of boron and agar on germination of pea pollen in sucrose media, confirmed that sucrose is quite necessary as an external source of energy to the germinating pollen grains. The best medium that gave them about 50 percent germination was composed of 10 ppm. boron and 20 percent sucrose; boron inhibited pollen germination when agar was present in the medium.

With tests run to determine the optimum hydrogen ion concentration and environmental temperature for *in vitro* germination of *Hibiscus rosa-sinensis* pollen, Cochis (6) got a maximum of 40 percent germination at pH of 6.5 to 7.0 and a maximum of 60 percent germination at temperatures of 21°C. to 29.5°C. The medium in his case contained 1 percent shredded agar (USP) and 40 percent sucrose.

A summary of methods of pollen germination suggests that percentages of 40 to 60 percent, regardless of the method or source of pollen, are to be expected. This report does not attempt a comprehensive study of all the factors that may be concerned in pollen germination, but using the best germination method that is available, to answer such questions as (a) optimum time to expect maximum germination, (b) length of viability period, (c) varietial differences, etc.

CHAPTER III

MATERIALS AND METHODS

Germination Medium

Taylor's method (22) has been found very attractive for use in this study mainly for its simplicity and speed of germination. Detailed discussion on why Taylor's method was selected over Bronckers' method will be given later, even though the latter method gives a greater percent germination. The components of Taylor's medium and the steps for its preparation are given below:

100 ml. distilled water
3.5 bacto-agar
25 g. sucrose
70 mg. manganous sulfate
40 mg. calcium nitrate
40 mg. boric acid

Mix the above ingredients together and autoclave at 15 pounds pressure for five minutes.

Pour into petri dishes to a depth of 4 to 7 mm. and cover the petri dishes as soon as possible to avoid any contamination.

It has been found necessary to age plates for at least 24 hours at refrigeration

temperature (5°C.) mainly to avoid sinking of pollen grains into the medium.

Let the stored plates reach room temperature before seeding with pollen.

Store for three hours at 30°C. and observe under microscope (staining unnecessary).

Source of Pollen Grains

A total of 20 varieties were used in one experiment and three varieties in another trial. All varieties were planted in experimental plots at the Campbell Avenue Farm of The University of Arizona in Tucson, Arizona. Proper management including insecticide sprays, cultivations and irrigation were given as required. The first experiment dealt with varietal differences and environmental influences on pollen grain germination in cotton, with special emphasis on temperature and relative humidity. The following 20 varieties were included in this experiment:

Paymaster 266
Lankart 3840
Blanco 3363
Dunn 56C
Dunn 119
Rilcot 90
Rilcot Stripper-Cala S
Blightmaster
Lockett 4789A
Quapaw
Coker 201
Stoneville 213
Deltapine 16
Acala 1517-D
6608-182-5 (AZ Sel)

6702-102-3 (AZ Sel)
6704-12-6 (AZ Sel)
L-48-15-118 (AZ Sel)
LE-1-70 (TX Sel)
Rowden

The second experiment was designed to study any possible differences in germination of pollen grains collected and seeded at different times of the day. Here, Deltapine 16 and a Super Okra Leaf, both G. hirsutum varieties, and Pima S-4, a G. barbadense variety, were used even though Pima S-4 had been reported previously as its germination being unsuccessful on this medium.

Seeding, Germination and Counting Procedures

After the media in stored plates had reached room temperature, the plates were taken to the farm for inoculation with pollen grains so there would be a minimum of opportunity for dessication before seeding. Flowers were selected at random from various parts of the plant and detached for pollen inoculation. Adequate numbers of pollen grains were dusted into the plates by tapping the flowers with the fingers. Maximum care was given to avoid unnecessarily heavy seeding or piling of pollen that would result in inadequate embedding on the medium and difficulty in counting under the microscope. Two different times of collection were carried out in the experimental procedure--one consisted of collecting the pollen at 930 hours, twice

per week, starting July 27, 1971 (first bloom), and the second consisted of collection of pollen every hour from 700 hours to 1400 hours, once each week, starting July 26 1971. The plates were covered immediately after inoculation and taken to the Cotton Fiber Laboratory of The University of Arizona where the germination tests were conducted. The plates were placed in the germinator upside down to avoid pollen bursting caused by drops of condensed vapor that could settle on them if in an upright position. The germinator was set at 30°C. A pan of water was placed inside the germinator in order to maintain the relative humidity near 100 percent. The lights in the room were on 24 hours a day throughout the experimental period and, therefore, germination was in the light as the germinator had glass sides. The laboratory had constant room temperature of 21°C. and relative humidity of 65 percent.

Germination counts were made using a dissecting microscope three hours after the time the plates were placed in the germinator. Pollen counts were made in five random microscope fields per plate and percent germination calculated. Pollen grains were considered germinated when their pollen tubes were longer than two times the diameter of the pollen grains.

Weather Data

As the environmental effect on germination of pollen was part of the study, it was found necessary to collect weather data from the farm where the plant material was grown. The effects of maximum temperature, minimum temperature, maximum relative humidity, and minimum relative humidity on pollen germination beginning 25 days prior to pollen dehiscense were studied. The daily weather data beginning with a 25-day lag period were recorded and tested for correlation with germination percentage.

Experimental Design and Statistical Analysis of Data

The first experiment (varieties over blooming period) was treated as a randomized block design with varieties as treatments and dates as blocks. The second experiment (viability over time-of-day sequence) was treated as split-plot design with varieties as the main plots, hours as the sub-plots, and dates as replications. Statistical analysis was carried out as outlined by Steel and Torrie (20) to identify any significant differences between varieties, dates and hours. A computerized correlation analysis was done between daily maximum temperature and variety, minimum temperature and variety, maximum relative humidity and variety, and minimum relative humidity and variety,

relating germinations to time lag periods of 1 to 25 days. The form of analysis of variance for each experiment is given below in Tables 1 and 2.

Table 1. Analysis of Variance Between Variety Differences in Germination Percentages over Blooming Period, First Experiment.

Source of Variation	df		
Blocks (dates)	r-1	=	12
Treatments (varieties)	t-1	=	19
Error	(r-1) (t-1)	=	228
Total	rt-1	=	259

Table 2. Analysis of Variance for Germination Percentages Based on Time of Day Sequence, Using Three Varieties, Second Experiment.

Source of Variation	df		
Replicates	r-1	=	7
Varieties	t-1	=	2
Error (a)	(t-1) (r-1)	=	14
Hours	h-1	=	7
Varieties x hours	(t-1) (h-1)	=	14
Error (b)	t(r-1) (h-1)	=	147
Total	thr-1	=	191

CHAPTER IV

RESULTS AND DISCUSSION

Artificial Media for Pollen Germination

Review of literature reveals several methods of germinating pollen grains of cotton on artificial media have been tried. With the exception of the Bronckers' and Taylor's methods, no method has been satisfactory or reasonably repeatable and it has proven difficult to germinate cotton pollen grains on the artificial media. Taylor's method, the latest medium developed, was selected to be used in this project because of the following advantages it has over Bronckers' method:

1. It is a very fast method; it takes about three hours to complete germination provided that proper temperature and humidity are supplied and maintained and, therefore, is useful in time sequence studies. Bronckers' method takes about 24 hours for complete germination.

2. Longer pollen tubes are produced; therefore the problem of differentiating between germinated and ungerminated pollen grains is drastically minimized.

3. It is very simple to handle and requires a minimum of laboratory work. No staining of germinated pollen grains is necessary. Unlike Taylor's method, the Bronckers

method requires staining of the germinated pollen grains before they can be clearly seen under the microscope (22).

The specific function of each component of the medium used in this project could not be definitely ascertained; nevertheless, sucrose regulates diffusion rate and serves as external energy source (17). Sucrose has been an important constituent of *in vitro* media used in germinating pollen grains of many species (3, 13, 17, 22). Agar controls the degree of embedding of the pollen grains on the surface of the medium and by doing so regulates the amount of oxygen and solution that could be absorbed by the pollen grains (3). This is due to the fact that the percentage of agar included can alter the physical properties of the medium (17). By making the surface as the researcher desires it, Boron is known to have a substantial influence in increasing percent germination and length of pollen tube (17). Boric acid is essential for *in vitro* pollen germination; and according to Pfahler (17) no rye pollen grain would germinate in the absence of boric acid from his medium. Even though calcium nitrate is known to increase *in vitro* pollen germination and pollen tube growth in many species, Pfahler (17) reported that addition of calcium nitrate to his medium significantly reduced pollen tube length of rye but did not alter percent pollen germination. Manganous sulfate is given as one of the essential constituents of

Taylor's best artificial medium even though the specific function was not established (22).

Selection of Varieties

The first experiment included 20 varieties and was designed to find any varietal differences in germination as well as any possible responses to environmental factors, mainly temperature and relative humidity. It was designed in such a way that it covers representatives of all types of cultivated cottons grown in the United States. It contained varieties developed for the High Plains, irrigated West (Acala types), rainbelt, and for stripper harvesting. The planting date was April 23, 1971. Table 3 represents the list of the varieties as well as their classifications, based on the area of origin.

In the second experiment in which the primary goal was to investigate any differences in the amount of germination of cotton pollen grains collected at different times of the day, three morphologically different varieties were selected. Two G. hirsutum varieties, one with normal and the other one with Super Okra Leaf, were selected basically because of the differences in leaf shape and their possible effect on environment. Since the leaf area is much smaller in the Super Okra Leaf than the normal leaves, more air circulation, sunshine penetration, and less humidity were expected around the plant; therefore, these factors were

Table 3. Selected Cotton Varieties and Environmental Areas of Adaptation in the United States.

Variety	Environmental Area or Type
Paymaster 266	High Plains Variety (Short season - cool temperature)
Lankart 3840	High Plains Variety
Dunn 56C	"
Dunn 119	"
Blightmaster	"
Lockett 4789A	"
Quapaw	"
Rilcot 90	"
Blanco 3363	"
Coker 201	Rainbelt Variety
Stoneville 213	"
Deltapine 16	"
Rowden	Rainbelt Variety (Broad genetic base)
LE-1-70	"
1517-D	Acala Type (High temperature, high elevation)
6608-182-5	Acala Type (High temperature, low elevation)
6702-102-3	Acala Type
6704-12-6	"
L-48-15-118	"
Rilcot Stripper Cala S	Stripper Type

suspected as possible causes of some variations in pollen germination. The third variety, a G. barbadense, was expected to germinate less in this medium as compared to the G. hirsutum varieties, and it was included to ascertain this fact.

Pollen Germination Results

Some plates were deliberately removed from the germinator after the first half-hour to examine any pollen germinations. It was found that many pollen grains had germinated by this time; however, to insure the maximum germination possible and thus give uniformity to counts, the plates were kept in the germinator for three hours. There was no difficulty in differentiating the germinated from the ungerminated pollen grains (Figure 1). As observed, the pollen tubes were sufficiently long to assure that germination had occurred but were not necessarily of sufficient length to be equivalent to the length of the stigma. This apparent difference in length should be of little concern for two reasons; (a) the pollen tubes grew on artificial media for only a three-hour period which is probably a much shorter time than would be required to complete the fertilization process in a flower, and (b) once the pollen tube has germinated and penetrated the surface of the stigma, the pollen tube development might be concerned only with the developing tip of the tube in the mother tissue. If the

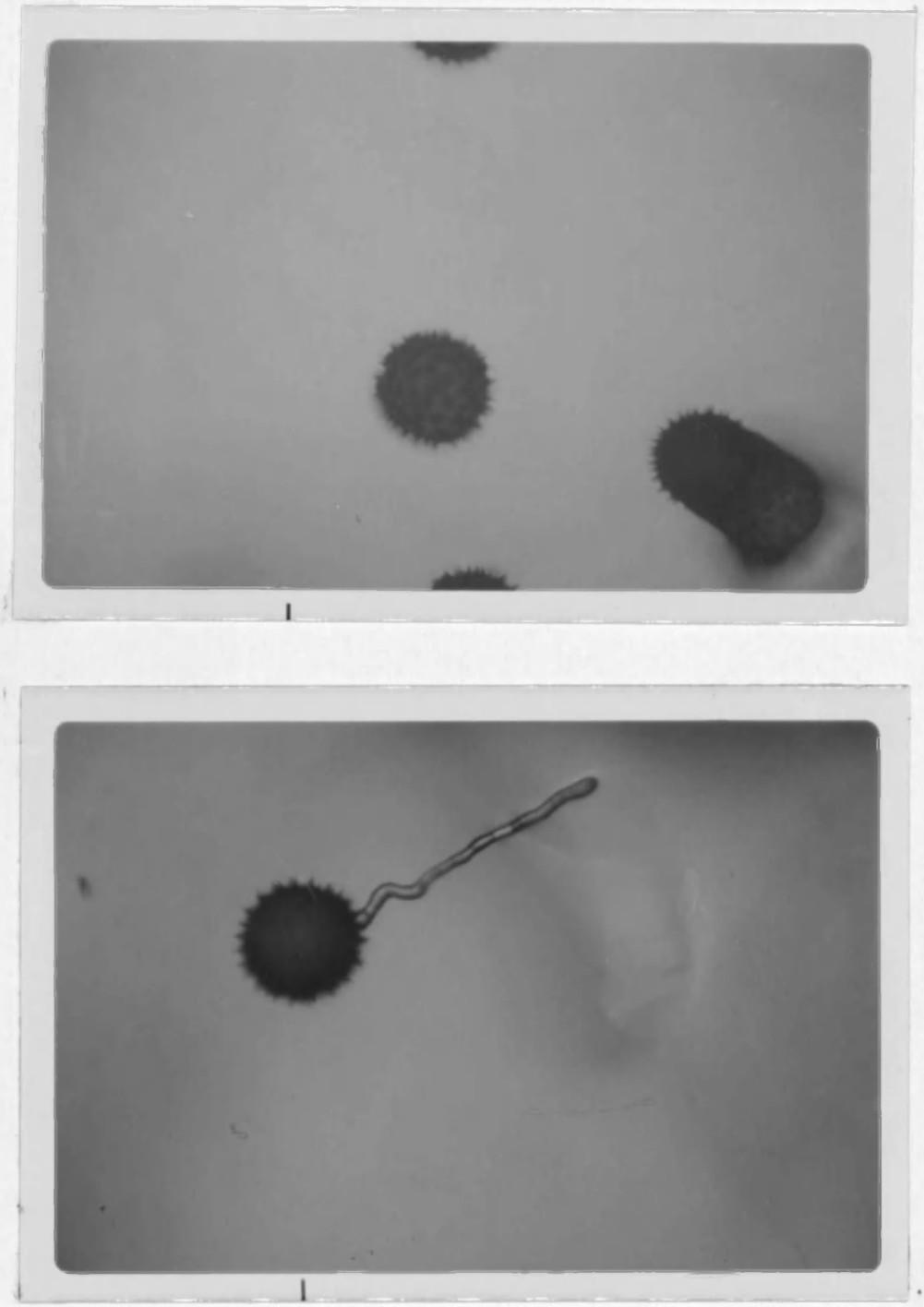

Figure 1. Pollen Grain Before Germination (top), and After Germination (bottom), but Before the End of the Tube Ruptures.

artificial media could totally duplicate the stigmatic tissue of a flower, then the developing pollen tube would probably be within the media rather than on the surface and there would be no rupturing and release of exudate, as shown in Figure 2, from a pollen tube developed on the artificial media used.

The experiment in which 20 varieties were included was started on July 27, 1971 and data were accumulated twice per week until September 10. This experiment was primarily designed to answer questions on the presence or absence of any varietal differences in pollen germination under changing environmental conditions. This consisted of differences in temperature and/or relative humidity at a certain lag period on the percent pollen germination and assessing any particular lag period having highest correlation with percent germination in all varieties. Based on previous weather history during the summer months in Arizona, the expected high should have been 45°C. and this was expected to be reflected in the germination percentages based on a 20-25 day lag period. Arizona did not experience the extremes in temperature during this study. The data were statistically analyzed as randomized complete block design according to Steel and Torrie (20). Results of statistical analysis of data showed highly significant differences among varieties as well as among different dates.

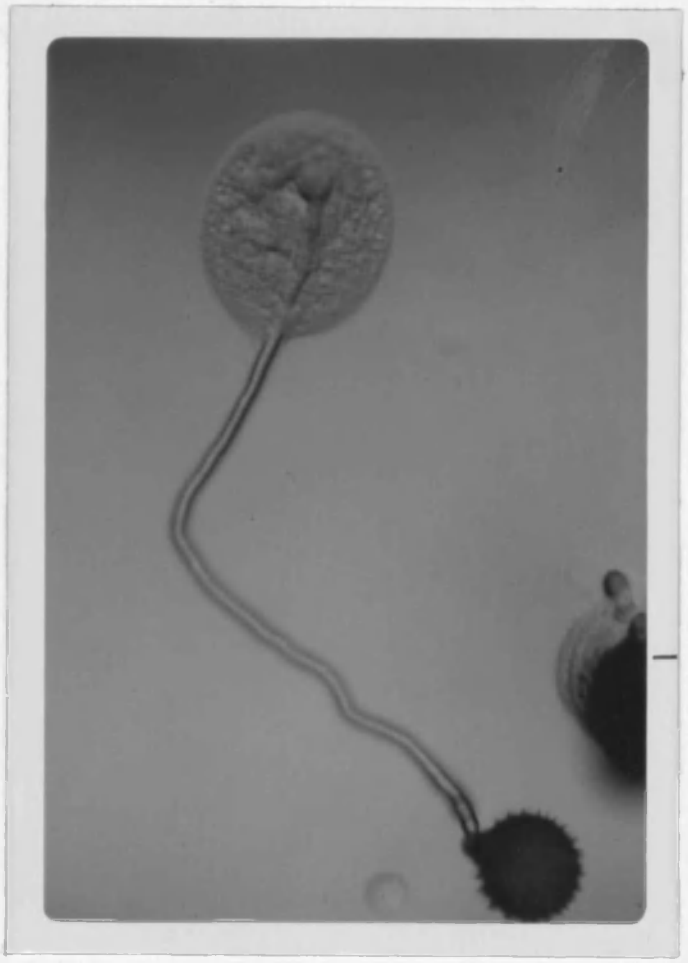

Figure 2. Pollen Grain after Germination and Rupturing of the End of the Pollen Tube Releasing Some Exudate.

Even though the germinations obtained were generally low, ranging from 0 percent to 39.7 percent, there is a very clear indication that varietal difference plays an important role. Varietal germination means overall dates ranged from 6.8 percent to 18.0 percent (Table 4). The varietal mean germinations indicate that Rilcot 90 with 18.0 percent was the highest ranking, Lankart 3840 with 16.6 percent in the second place, and L-48-15-118 with 16.0 percent in the third place. If we examine the individual readings rather than means, Lankart 3840 with 39.7 percent germination on August 6 ranked the highest, Lockett 4789A with 33.0 percent germination on August 13 ranked second, and Rilcot 90 with 32.2 percent germination on August 6 ranked third. Considering all varieties at all dates, an overall mean germination of 11.9 percent was obtained (Appendix I).

The mean germination percentages of all 20 varieties at the 13 different dates studied ranged from 6.7 to 19.3 percent (Figure 3). The highest mean germination was obtained on August 13. In addition, the data clearly indicated that for most of the varieties studied, germination was highest between August 6 and August 17, inclusive. Provided repeatability of this information could be secured, the data suggest that it would be quite beneficial from the point of pollen germination to plan the planting date for any variety in such a way that the maximum flowering would

Table 4. Mean Germination Percentage for Twenty Varieties During Blooming Period, July 27 to September 10, Campbell Avenue Farm, Tucson, Arizona, 1971.

Variety	Percent	Variety	Percent
Rilcot 90	18.0	Blightmaster	10.6
Lankart	16.6	1517-D	10.5
L-48-15-118	16.0	Stoneville	10.5
LE-1-70	15.9	6702-102-3	10.4
6704-12-6	14.9	Dunn 56C	10.3
Lockett 4789A	14.9	Coker 201	9.1
Blanco 3363	13.2	Paymaster 266	8.6
Rilcot Stripper Cala S	12.2	Rowden	7.9
6608-182-5	12.0	Deltapine 16	7.3
Dunn 119	11.8	Quapaw	6.8
Mean	11.9		
LSD .05	± 4.47		
LSD .01	± 5.87		

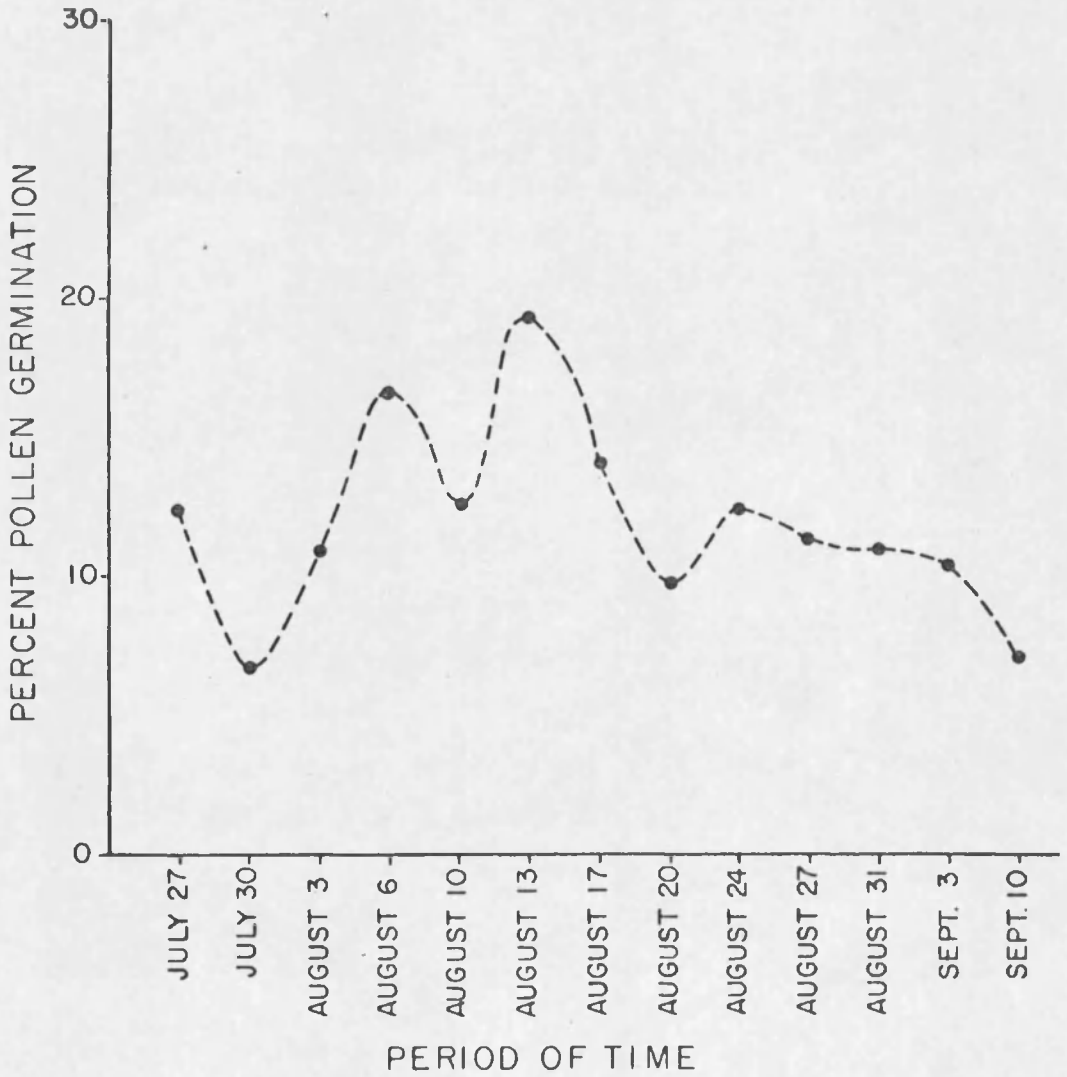

Figure 3. Mean Percentage Pollen Germination of Twenty Varieties During Blooming Period, Campbell Avenue Farm, Tucson, Arizona, 1971.

occur within those days. Such a planting date would be apropos with environmental conditions as characterized 1971; however, in a growing season that experiences higher maximum temperatures, the planting date probably should be set to shift the flowering date to an earlier period. Figure 4 gives the number of varieties with highest percent germinations versus the dates, with largest concentration around August 13.

The big differences observed between germinations at different dates could be due to the age of the plants or some environmental effects, or both. Information dealing with age of plant as one of the factors influencing cotton pollen germination could not be found in the literature. Temperature and relative humidity are reported to have a definite effect (15). Temperature and relative humidity data were gathered from the same area of the farm where the varieties were grown (Appendices II and III). Computerized correlation studies were run between varieties vs. maximum temperature, varieties vs. minimum temperature, varieties vs. maximum relative humidity and varieties vs. minimum relative humidity starting from 1 day up to 25 days lag. In each case, no single lag period showed the highest correlation that could account for all the varieties. Different varieties showed different responses to these environmental factors at different lag periods, but this could be expected because each variety has been designed for a given area of the

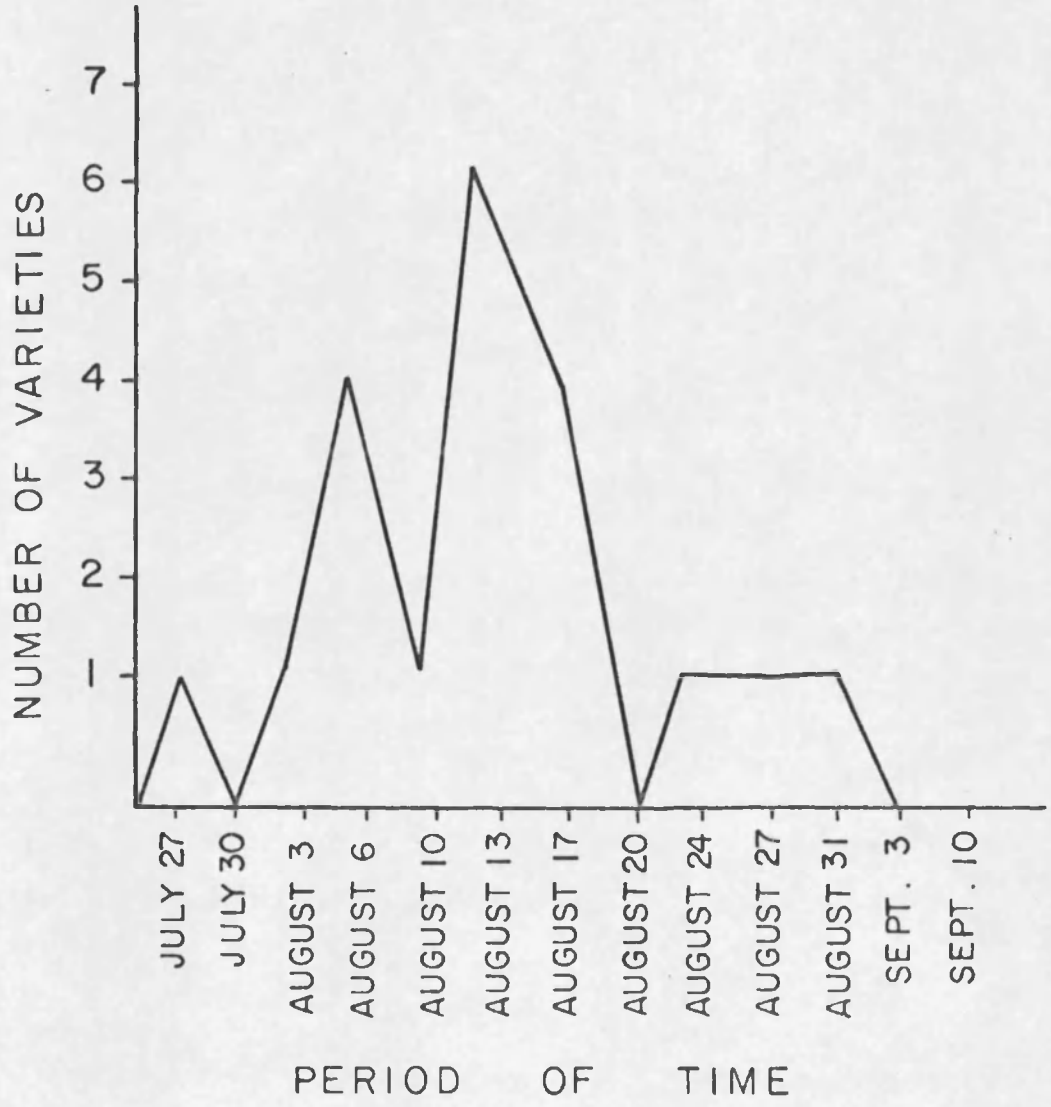

Figure 4. Distribution of Varieties by Number with Highest Percent Germinations by Dates, Campbell Avenue Farm, Tucson, Arizona, 1971.

country and selection pressure, inadvertently or intentionally, applied for that temperature and humidity environment. For example, the Acalas were selected for high temperatures and long season and the rainbelt varieties for lower maximum temperatures and short season. The results may appear to be very ambiguous and, therefore, no single definite conclusion could be given pertaining to environmental influence, but this does not rule out the essential role played by it if each variety is considered in the context of its breeding program.

The second experiment dealing with pollen germination of three varieties at different hours of the day, starting July 26, 1971, was conducted once per week until September 10, 1971. Results of this experiment indicate that there is a highly significant difference between hours, varieties, and dates (Table 5 and Figure 5). Both Deltapine 16 and Pima S-4 reached their highest mean germinations of 12.1 percent and 6.5 percent, respectively, at 1000 hours in the morning. Super Okra Leaf reached its highest mean germination of 14.3 percent at 700 hours in the morning. This is most probably due to the fact that the Super Okra Leaf had more aeration and less moisture around the flowers, and more sunshine penetration of the plant, resulting perhaps in dehiscence of anthers earlier in the morning, releasing an abundance of matured pollen grains.

Table 5. Hourly Percentage Pollen Germination, Mean Three Varieties, Campbell Avenue Farm, Tucson, Arizona, 1971.

Variety	Hour of Collection								Variety $\bar{X}$
	700	800	900	1000	1100	1200	1300	1400	
Deltapine 16	8.5	8.6	10.8	12.1	9.3	5.0	5.9	4.9	8.1
Super Okra Leaf	14.3	11.6	11.0	9.5	8.5	5.8	3.0	3.0	8.4
Pima S-4	3.0	3.9	6.4	6.5	4.5	3.5	1.1	3.4	4.1
$\bar{X}$ (Overall)	8.6	8.0	9.4	9.4	7.4	4.8	3.3	3.8	6.8
$\bar{X}$ (Exclude Pima S-4)	11.4	10.1	10.9	10.8	8.9	5.4	4.5	3.9	8.2

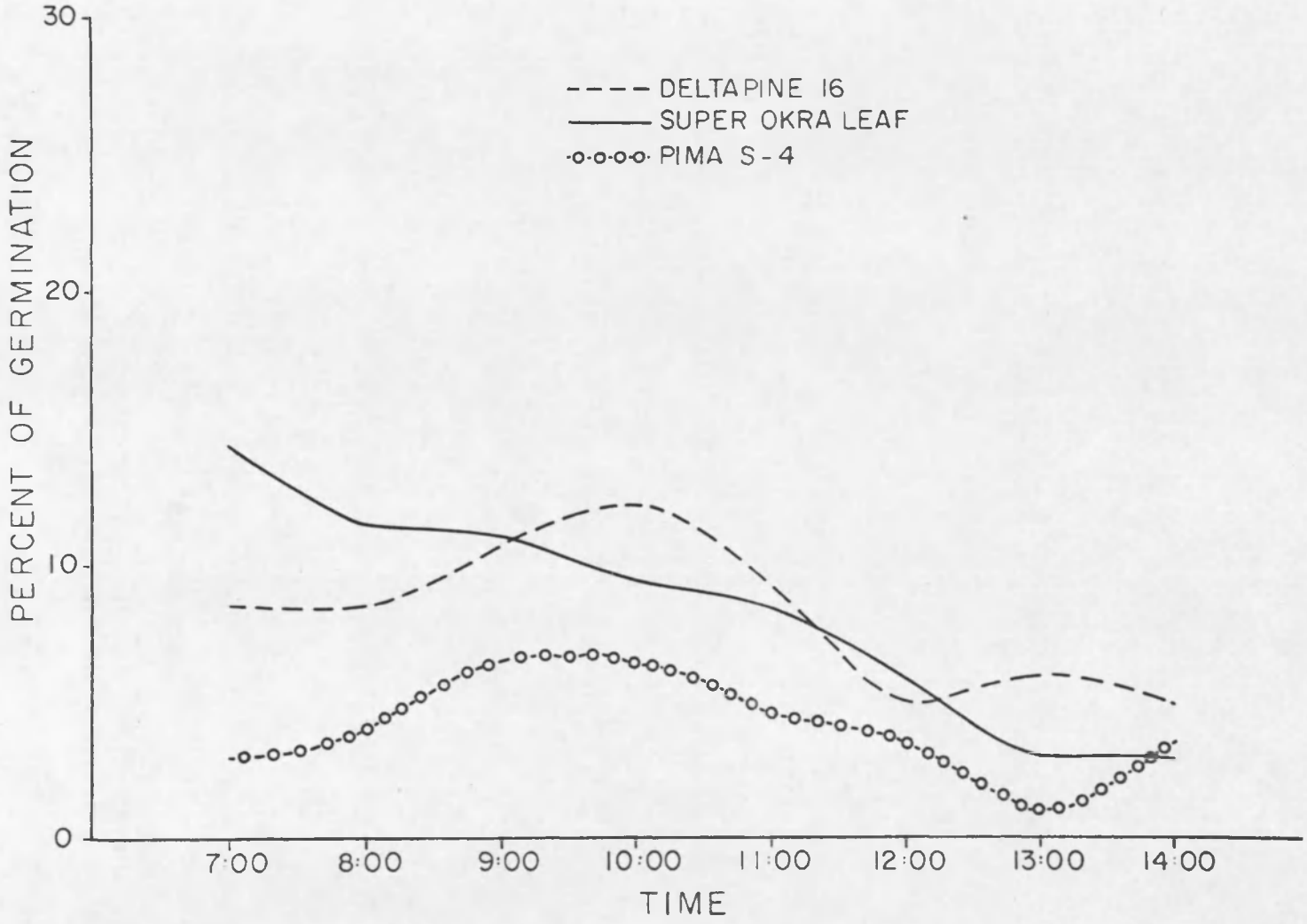

Figure 5. Hourly Percentage Pollen Germination, Three Varieties, Campbell Avenue Farm, Tucson, Arizona, 1971.

The hypothesis of pollen germination being related to light, aeration, humidity, etc., is further substantiated by the fact that Deltapine 16 and Pima S-4 were slower in dehiscing pollen and do have larger leaves. The leaf area probably conserves more moisture around the plant, causes less aeration and less sunshine penetration, and might result in delayed maturity of pollen grains and also delayed rupturing of the anthers to release the pollen grains. Super Okra Leaf was found to have the highest amount of germination with an overall mean germination of 8.4 percent. Deltapine 16, with overall mean germination of 8.1 percent, was second while Pima S-4 with overall mean germination of 4.1 percent was the least in the group (Figure 6). American Pima (Pima S-4) does not seem to properly respond to this germination medium and, in fact, Taylor (22), after failing to germinate pollen grains of American Pima on his artificial medium, termed his attempt as frustrating.

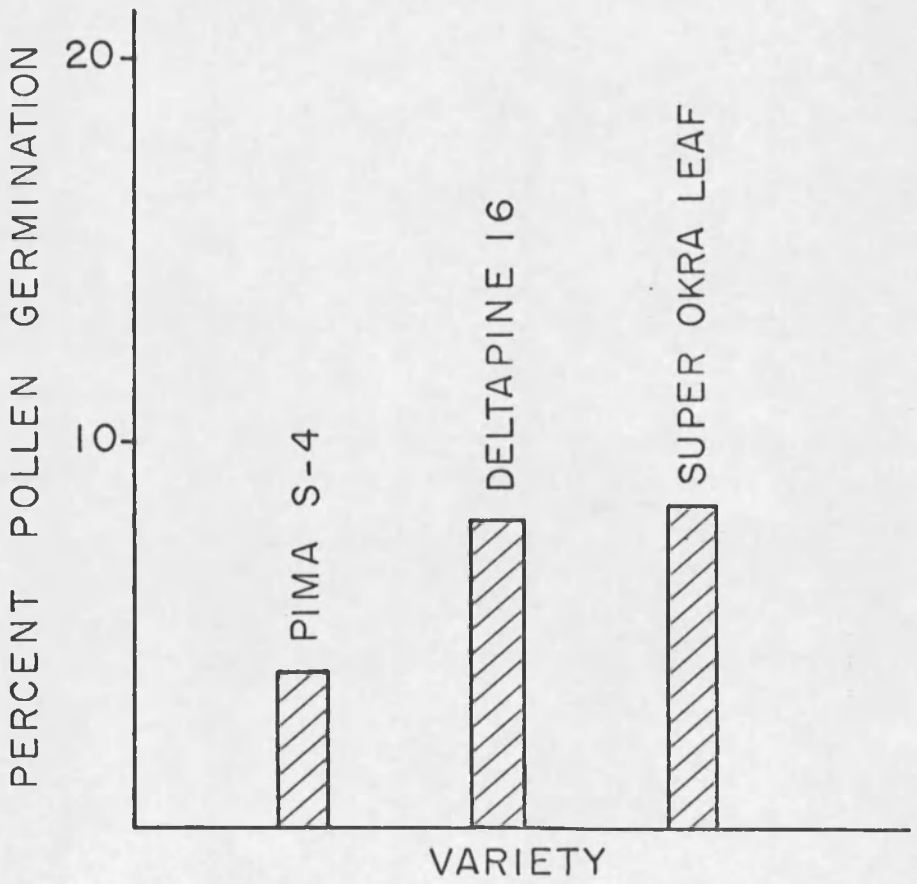

Figure 6. Relative Mean Percentage of Pollen Germination of Three Varieties, Campbell Avenue Farm, Tucson, Arizona, 1971.

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

This study dealt with two separate experiments on *in vitro* germination of pollen grains of *Gossypium*. Both experiments were carried out simultaneously in the summer of 1971 at The University of Arizona, Tucson, Arizona. The first experiment, including 20 varieties, was designed to study the varietal differences in pollen germinations as well as the effect of temperature and relative humidity on pollen germination. Highly significant differences between varieties were found with Rilcot 90 having the highest percentage pollen grain germination on the average. Even though it seemed obvious that environment affected pollen germination, as indicated by highly significant differences among dates, it has been difficult to pin-point a particular date that would indicate highest correlation with all 20 varieties, but this could be expected when the diverse origin of the varieties is considered.

Relating the pollen grain germination percentages to groups based on origins would indicate from the data of this study that the Acala types as a group germinated the best and the rainbelt varieties averaged less (2.8 percent).

The surprising observation is to note that the High Plains types seem to be distributed from highest to lowest. This apparent incongruency is possibly related to the fact that cotton varieties are developed for specific areas and selection pressure was unknowingly applied. For example, 1517-D is an Acala developed in New Mexico at elevations equivalent to the High Plains area of the cotton-belt, and 6702-102-3 (Arizona Selection) is the result of a complex cross including parental lines that were very early and has been grown a relatively short time in the high temperature zone of the Western United States. What genetic response to temperature should be expected? The High Plains varieties are probably most diverse in genotypic architecture of any group and, therefore, most diverse in response to the Arizona environment.

A second point to consider in evaluating whether or not germination percentages are low is, "What percentage of the pollen grains are actually viable?" Researchers have no doubt assumed that an abundance of pollen insured a good crop; however, an abundance of pollen is not necessarily related to abundance of fertile pollen. If 8.2 percent of pollen produced in a flower were fertile, then this is more than adequate to fertilize the 28-32 ovules in a cotton boll. A major problem in cotton production that tends to give credence to the question, "Is pollen really fertile?"

is to try to explain the so-called "summer cutout." Traditionally, researchers have suggested that the plant has a full fruit load and physiologically stops setting more; however, this hypothesis is weak because one plant of a genetically stable variety may have 10 bolls when the "cut-out" occurs and another have 30 bolls. A simpler and equally plausible explanation could be that the pollen became inviable at that period and fertilization of ovules was not affected. This hypothesis is further supported by the fact that high yields occur in years with relatively low summer temperature--or temperature below that critical for pollen survival.

The second experiment was designed to pin-point the time of the day in which dehisced pollen grains would show maximum germination. Two of the three varieties used indicated 1000 hours to be the most favorable time while the third variety showed 700 hours to be the optimum time for pollen germination. It is interesting to note that two of the varieties have normal leaves while the third one, which had the earliest germination period, was a Super Okra type. Pima cotton (G. barbadense) was extremely low in germination on this medium.

There is every reason to believe that germination of pollen grain on any given day for any variety could be altered slightly based on the micro-climate of the flower

and not solely on the effects produced at the time or near differentiation of the pollen-bearing structure. For example, the maximum daily temperature 18 to 20 days prior to bloom may have been such that only 50 percent of the pollen in a bloom should have been inviable but on the day of bloom at dehiscence time, the flower could have been exposed to excessive wind, rain, sunlight, etc., which could have further reduced the viable pollen percentage and this changed by the hour. In a variety such as Pima S-4, the reverse may occur and with the deterioration of some product on the pollen grain, germination percentages could increase. There is no data to support when fertilization of a Pima S-4 flower occurs relative to a Deltapine, but this study does show that Pima S-4 was uniformly viable over a longer period than the G. hirsutum varieties.

Suggestions and Conclusions

There is no doubt that information on cotton pollen grain germination and behavior would be extremely important to cotton breeders. The breeder could use such information in developing varieties that would maximize yields insofar as fertilization affects yield. He could plan his planting date to fit the flowering stage for maximum shed of fertile pollen into a certain period of time in a conducive environment. He could avoid use of insecticides, herbicides, and fungicides that would interfere with the biological

processes and normal functioning of pollen grains. All these programs could be based on the development of a more reliable *in vitro* method of pollen germination.

Eventually, a more satisfactory procedure or medium may be found for pollen germination but the Taylor method, attractive and satisfactory in the writer's judgment, has been employed here to conduct studies on varietal differences and environmental effects determined by *in vitro* pollen germination of *Gossypium*. At least three concrete conclusions could be made from the results obtained in this project:

1. There is a clear indication that the rate of pollen germination in cotton is inherent in a variety.

2. Temperature and relative humidity have an effect on pollen germination even though no single day could be isolated as having the highest correlation with all cultivars used.

3. Cotton pollen grains seem to germinate best early, by 10:00 o'clock in the morning, the exact time depending on variety.

While this study is a step forward in pin-pointing some problems relative to pollen germination in cotton, suggestions for future research include:

1. Refine the medium to increase germination percentages if possible.

2. Study effects of clumping of the grains on the medium.

3. Produce pollen under controlled conditions to independently isolate temperature and humidity effects on pollen maturation.

4. Correlate germination techniques with tetrazolium studies, pollen bursting, starch accumulation, etc.

5. Apply this information to a controlled breeding program.

6. Include undehisced pollen grains in germination series, etc.

APPENDIX I

PERCENTAGE GERMINATIONS OF SELECTED VARIETIES BY DATES, CAMPBELL AVENUE FARM, TUCSON, ARIZONA, 1971

Variety	Dates													Mean
	7/25	7/30	8/3	8/6	8/10	8/13	8/17	8/20	8/24	8/27	8/31	9/3	9/10	
Paymaster 266	5.9	2.4	3.7	9.6	8.3	12.4	19.1	2.7	8.5	7.0	7.5	13.4	11.7	8.6
Lankart 3840	18.9	5.4	26.2	39.7	25.7	25.2	16.9	14.7	13.8	25.2	8.7	0	0	16.6
Blanco 3363	5.2	13.3	8.0	11.0	20.0	15.3	27.5	14.6	22.0	12.3	21.4	0	0	13.2
Dunn 56C	14.4	14.7	10.0	7.7	14.4	16.9	18.9	9.9	11.0	10.6	9.8	5.9	0	10.3
Dunn 119	11.2	8.2	13.9	13.8	7.6	28.1	0	11.1	19.1	0	11.4	17.1	11.7	11.7
Rilcot Stripper Cala S	11.8	13.3	9.4	31.5	6.9	20.6	15.3	5.8	3.8	7.7	15.7	6.7	9.6	12.2
Blightmaster	10.0	3.8	11.0	13.7	6.2	17.3	11.6	8.2	10.2	6.4	18.3	6.3	12.8	10.6
Lockett 4789A	14.3	5.0	9.6	23.7	16.3	33.0	16.5	16.5	6.3	8.4	16.2	15.4	12.4	14.9
Quapaw	8.9	4.5	3.8	0	5.6	10.0	9.4	9.9	10.0	13.6	0	13.3	0	6.8
Rilcot 90	19.2	3.2	10.3	32.2	21.2	24.4	15.2	23.1	24.6	15.0	22.4	18.3	5.1	18.0
Coker 201	7.1	5.3	11.6	8.7	11.4	14.8	5.4	10.7	10.1	11.8	6.4	5.4	9.2	9.1
Stoneville 213	20.6	7.2	7.1	13.1	12.6	25.0	14.5	14.9	1.3	7.9	0	12.2	0	10.5
Deltapine 16	13.5	8.0	4.2	3.7	5.2	18.8	14.2	4.5	8.8	8.0	0	3.2	3.5	7.3
1517-D	17.4	2.1	4.0	13.1	14.3	17.1	9.8	5.0	17.3	13.1	1.1	14.6	7.3	10.5
6608-182-5	14.5	5.3	14.8	16.5	18.8	10.0	0	11.3	18.5	12.2	14.7	13.9	5.0	12.0
6702-102-3	5.9	6.0	11.5	23.9	0	16.0	16.5	10.6	0	13.4	9.6	13.6	8.0	10.4
6704-12-6	12.4	6.7	12.2	18.3	21.8	19.2	12.3	4.3	29.0	14.7	18.1	17.5	9.4	14.4
L-48-15-118	11.6	12.4	20.1	19.2	20.4	29.2	18.0	10.0	8.6	14.2	13.9	15.0	15.1	16.0
LE-1-70	9.3	11.5	11.9	20.0	12.8	19.0	28.3	5.5	24.8	22.5	17.0	15.9	3.7	15.9
Rowden	13.1	5.7	13.5	11.4	1.4	12.6	11.3	2.7	0	9.1	10.2	0	11.5	7.4
Mean	12.26		10.84		12.55		14.09		12.39		11.00		7.10	
		6.70		16.64		19.25		9.80		11.41		10.41		

APPENDIX II

TEMPERATURE DATA AT THE CAMPBELL AVENUE FARM
THE UNIVERSITY OF ARIZONA
TUCSON, ARIZONA--1971

Date	Temperature °F. Maximum	Minimum	Date	Temperature °F. Maximum	Minimum
7-1-71	102	74	7-19-71	105	75
7-2-71	100	73	7-20-71	106	77
7-3-71	100	73	7-21-71	102	76
7-4-71	101	69	7-22-71	100	73
7-5-71	100	70	7-23-71	98	70
7-6-71	99	64	7-24-71	97	69
7-7-71	102	62	7-25-71	102	74
7-8-71	106	64	7-26-71	101	74
7-9-71	101	78	7-27-71	103	72
7-10-71	100	73	7-28-71	103	76
7-11-71	102	73	7-29-71	98	75
7-12-71	100	72	7-30-71	100	74
7-13-71	93	74	7-31-71	89	73
7-14-71	103	68	8-1-71	99	73
7-15-71	104	69	8-2-71	104	74
7-16-71	106	79	8-3-71	103	77
7-17-71	108	72	8-4-71	101	76
7-18-71	110	76	8-5-71	100	76

Date	Temperature °F. Maximum	Minimum	Date	Temperature °F. Maximum	Minimum
8-6-71	100	68	8-29-71	100	71
8-7-71	98	68	8-30-71	91	69
8-8-71	99	69	8-31-71	93	71
8-9-71	99	71	9-1-71	96	72
8-10-71	99	73	9-2-71	96	69
8-11-71	93	68	9-3-71	97	72
8-12-71	100	71	9-4-71	91	73
8-13-71	93	71	9-5-71	100	69
8-14-71	99	69	9-6-71	100	72
8-15-71	99	69	9-7-71	98	73
8-16-71	96	69	9-8-71	99	69
8-17-71	88	65	9-9-71	91	69
8-18-71	90	67	9-10-71	91	69
8-19-71	89	69			
8-20-71	88	68			
8-21-71	90	67			
8-22-71	92	70			
8-23-71	97	71			
8-24-71	93	72			
8-25-71	90	71			
8-26-71	92	69			
8-27-71	86	69			
8-28-71	86	69			

APPENDIX III

RELATIVE HUMIDITY DATA AT THE CAMPBELL AVENUE FARM,
THE UNIVERSITY OF ARIZONA
TUCSON, ARIZONA--1971

Date	Relative Humidity % Maximum	Minimum	Date	Relative Humidity % Maximum	Minimum
7-1-71	51	19	7-19-71	38	17
7-2-71	52	21	7-20-71	55	19
7-3-71	53	20	7-21-71	68	26
7-4-71	50	21	7-22-71	72	23
7-5-71	44	21	7-23-71	85	26
7-6-71	46	19	7-24-71	85	22
7-7-71	44	18	7-25-71	70	22
7-8-71	38	16	7-26-71	80	20
7-9-71	36	20	7-27-71	84	16
7-10-71	76	20	7-28-71	76	18
7-11-71	69	18	7-29-71	76	24
7-12-71	56	20	7-30-71	68	20
7-13-71	57	29	7-31-71	69	22
7-14-71	70	18	8-1-71	70	20
7-15-71	52	16	8-2-71	58	18
7-16-71	41	18	8-3-71	53	20
7-17-71	47	14	8-4-71	54	24
7-18-71	46	11	8-5-71	68	26

Date	Relative Humidity % Maximum	Minimum	Date	Relative Humidity % Maximum	Minimum
8-6-71	84	26	8-29-71	82	30
8-7-71	82	18	8-30-71	81	27
8-8-71	82	35	8-31-71	82	29
8-9-71	69	18	9-1-71	82	20
8-10-71	84	21	9-2-71	83	23
8-11-71	82	22	9-3-71	82	22
8-12-71	81	16	9-4-71	74	24
8-13-71	83	20	9-5-71	82	18
8-14-71	73	16	9-6-71	74	22
8-15-71	81	18	9-7-71	80	26
8-16-71	84	22	9-8-71	85	22
8-17-71	84	35	9-9-71	80	24
8-18-71	83	30	9-10-71	84	30
8-19-71	83	34			
8-20-71	83	32			
8-21-71	84	30			
8-22-71	81	28			
8-23-71	83	21			
8-24-71	82	32			
8-25-71	82	40			
8-26-71	82	26			
8-27-71	82	40			
8-28-71	80	30			

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