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GERMINATION OF GUAYULE (PARTHENIUM ARGENTATUM GRAY) POLLEN ON AN ARTIFICIAL MEDIUM

The University of Arizona M.S. 1982

GERMINATION OF GUAYULE (*PARTHENIUM ARGENTATUM* GRAY) POLLEN ON AN ARTIFICIAL MEDIUM

by

Irina Vekcha-Thielo

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THE UNIVERSITY OF ARIZONA

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STATEMENT BY AUTHOR

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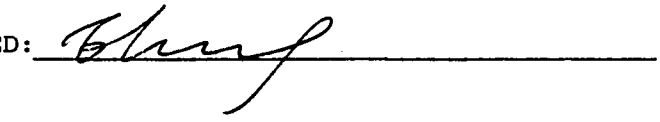
SIGNED: 

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D.T. Ray — August 5, 1982

D. T. RAY — Date

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ABSTRACT

The pollen of five diploid and two tetraploid guayule (Parthenium argentatum Gray) plants was cultured on nutrient media. It was observed that guayule pollen is released as tetrads of spores which will not germinate on the media unless they are separated into individual pollen grains. For each plant, the results of the germination tests varied with the day and the time of day of the pollen collection.

Pollen was cultured on media containing 0 to 30 percent concentrations of either sucrose or mannitol. The 25 percent sucrose yielded the best results. Mannitol had no influence on pollen germination. An addition of the mineral elements B, Ca, and K, and the growth-promoting substances, gibberellic acid and auxin, increased the germination percentages of the pollen of some plants.

The variability in the pollen size, characterized by the coefficient of variation of the pollen diameter, of the two tetraploid and some diploid plants was very high.

CHAPTER 1

INTRODUCTION

Guayule, Parthenium argentatum Gray, is a potential rubber crop in the American Southwest. The forms actually available for commercial exploitation are represented by diploid, triploid and tetraploid populations, which have been subjected to little selection. However, there are currently projects striving to improve the germplasm through intra- and interspecific hybridization.

Reproduction in guayule is very complex, making guayule breeding difficult. Triploid and tetraploid plants are predominantly facultative apomicts. Thus, improvement by crossing is difficult, but favorable forms can be easily fixed. The apomictic reproduction in guayule involves either the development of an embryo from generative or somatic cells without reduction and fertilization, or from reduced generative cell without fertilization, and/or from fertilized nonreduced generative cell. However, in all these cases, pollination is necessary for the development of the endosperm.

Only diploid plants reproduce predominantly sexually. The complexity in breeding of diploids is connected with their self-incompatibility and, hence, their heterozygosity and heterogeneity.

In addition to the reproductive complexity, a major problem in guayule breeding is its low seed viability, usually less than 50 percent

(Lloid, 1911). This is complicated by the viability of the seed varying between seed lots collected at different periods of the year.

In order to explain and, hopefully, overcome the difficulties in guayule reproduction, the development of the female and male gametophytes and their interaction during pollination and fertilization must be studied. This work was designed to study the pollen viability of diploid and tetraploid guayule plants in vitro and in vivo.

Different staining techniques for pollen viability were tried unsuccessfully. These methods included the use of iodine, acetocarmine, tetrazolium chloride and fluorescein diacetate (Gurr, 1965; Heslop-Harrison and Heslop-Harrison, 1970). Our efforts were concentrated on pollen germination in vitro. The absence of knowledge on the nutritional requirements for the in vitro germination of guayule pollen led to the inclusion in our objectives of the study of the influence of different substances on the pollen in vitro.

CHAPTER 2

LITERATURE REVIEW

Reproductive Processes in Guayule

Guayule is a very complex species in respect to its reproductive processes. A polyploid series of diploid (2n = 2x = 36), triploid (2n = 3x = 54) and tetraploid (2n = 4x = 72) exists among natural populations and commercial strains of this species (Rollins, 1950). Bergner (1946) found a profusion of the aneuploid plants in the natural diploid and tetraploid populations of guayule. The highest chromosome number reported by her was 144, which was found among offspring of a hexaploid plant (2n = 6x = 108). She pointed out that probably every number between 36 and 144 could be found if enough individual plants were examined.

The diploids reproduce sexually and are self-incompatible, while the triploids and tetraploids are facultative apomicts, that is they are predominantly apomicts but sometimes reproduce sexually (Essau, 1946; Gerstel and Riner, 1950). Essau showed that where apomixis is the predominant form of reproduction, the megaspore mother cell (MMC) does not form tetrads but develops directly into a uninucleate embryo sac with the same number of chromosomes as in the somatic cells. She found that the apomixis in guayule mainly involves generative apospory; that is, development of an embryo sac from the MMC either without meiosis, or with abnormal meiosis, but without spore formation. In addition, somatic

apospory, the development of an embryo sac from the somatic cell without meiosis and spore formation, also occurs.

According to Powers and Rollins (1945), although the embryo is initiated in the apomicts without fertilization, the endosperm develops only after pollination. Thus, pollination is necessary for the formation of viable seed. This phenomenon of apomictic development of the egg with the aid of pollination, but without fertilization of the egg cell (autonomous embryo development), is termed unreduced pseudogamy.

Studying the morphological characteristics of diploid, triploid and tetraploid populations of guayule, Bergner (1946) found that the diploid populations are characterized by morphological diversity within the different collections, while the various triploid and tetraploid strains are fairly uniform among themselves. The morphological diversity of the diploids is a consequence of amphimixis and cross-pollination, since they are largely self-incompatible, while the uniformity of the polyploid populations is a consequence of apomictic reproduction. She found that in some instances facultative apomixis led to the establishment of clones of aneuploid triploid and tetraploid combinations. She also observed a profusion of aneuploid triploids having from 43 to 56 chromosomes among the progeny of diploids when they were used as the seed parent in crosses with triploid or tetraploid plants.

There are individual plants which deviate from the usual type characteristics of the triploid and tetraploid plants and commercial strains in which they occur. Three types of morphological variants have been distinguished: haploids (polyhaploids), the so-called off-type

normals and aberrants. Essau (1946) has shown that normal meiosis may occur in plants which are predominantly aposporous. Therefore, because meiosis has occurred, some eggs with the reduced number of chromosomes are produced. However, the stimulus for apomictic development is still present, and the egg in the reduced condition and without fertilization gives rise to polyhaploids, a number of which have been found in tetraploid populations (Bergner, 1946).

If reduced egg cells are fertilized, they give rise to the off-type normal plants which may or may not have the same number of chromosomes as the apomictically produced plants in the same culture. The variability in the chromosome number is the result of the varying number contained in the egg nucleus plus the varying number brought in by the pollen grains (Bergner, 1946).

A small percentage of nonreduced eggs in tetraploids may be fertilized with either 18 or 36 chromosome pollen from the male parent, giving rise to pentaploid (2n = 5x = 90) or hexaploid (2n = 6x = 108) aberrant progeny, which reproduces apomictically (Powers, 1945; Bergner, 1946).

Difficulties in Determining Chromosome Numbers in Guayule

Bergner (1946) pointed out that it is difficult to state exactly how much aneuploidy exists among the diploid, triploid and tetraploid collections of guayule because of the additional supernumerary B or miniature chromosomes that occur in some guayule plants. They are "out of rhythm" with the regular chromosomes of the complement and are not constant in number, even in progeny of a single plant. There is but a

slight difference in length between the smallest biarmed A chromosome and a miniature, so a trisomic univalent might be counted as a miniature.

Rollins (1950) considers miniatures as genetically inert. Bergner (1946) pointed out that if the postulation that the miniatures owe their origin to the breakage of biarmed chromosomes is currect, they are duplicate portions of chromosomes and the plants in which they occur are aneuploid to that extent.

Chromosome Behavior in Pollen Mother Cells

Meiosis in pollen mother cells of the diploid, triploid and tetraploid plants of guayule was studied by Bergner (1946). Meiosis in pollen mother cells of the diploid plants was mostly regular. Irregularities during meiosis affecting the quality of pollen as asynapsis and partial or complete failure of cytokinesis were found.

In triploids, trivalents or bivalents plus univalents, and in tetraploid, quadrivalents, trivalents, bivalents and univalents were found at metaphase I. There was the tendency toward equal distribution of the chromosomes in anaphase I in triploids and tetraploids; however, this was more pronounced in the tetraploids.

Pollen Viability

A study of pollen viability in the diploid, triploid and tetraploid collections of guayule was done by Powers and Gardner (1945) and by Bergner (1946). In both studies, the collections of the same level of ploidy differed in average amount of aborted pollen. Powers and Gardner (1945) found that the average percentage of aborted pollen in the diploid

and tetraploid collections was rather low, ranging from 15 to 29 percent in the diploids and 16 to 24 percent in the tetraploids. The average percentage of aborted pollen in the triploid collections (23 to 34 percent) was somewhat greater than in either the diploids or the tetraploids.

Studying the same diploid collections, Bergner (1946) reported higher percentages of aborted pollen grains, in some instances three times higher, than the percentages reported by Powers and Gardner (1945). She considered that the pollen abortion in diploids may be due to either aneuploidy, inversions or meiotic irregularities, and the higher percentage of aborted pollen in triploids due to their frequent meiotic irregularities.

There was no correlation found between the amount of aborted pollen and the number of miniature chromosomes (Bergner, 1946).

Studying the morphology of the pollen grains, Bergner (1946) found that in diploid plants, some of the aborted grains were compound, consisting of the tetrad of incompletely separated spores, frequently of unequal size. Other large grains resulted from dyad instead of tetrad formation and the giant grains of the pollen mother cells were also found. Pollen of triploid plants was distinguished from that of diploids by the variable size of the viable grains rather than by the percentage of the aborted pollen grains. Pollen of tetraploid plants was distinguished from that of diploid plants by the larger size of the viable grains and from that of triploid plants by the more uniform size of the grains. In addition to the pollen size, some other characteristics of the plants,

such as the size of the fruit and the type of trichomes on the leaf, were found to correlate positively with the level of ploidy (Rollins, 1975).

The study of the variance of the percentage of aborted pollen grains by Powers and Gardner (1945) revealed that guayule possesses considerable genetic variability for this characteristic. The variability of the diploid collections was great, 10-17 times larger than the mean of the percentages of aborted pollen grains, and much greater than that of the triploid and tetraploid collections. The triploid collections possessed the smallest variability. The difference in the size of the variance was mainly attributed by the authors to the method of reproduction, i.e., sexual versus apomictic.

Pollen Germination in Vitro. General Aspects

Germination in vitro is a standard test of pollen viability. There is presently no knowledge of the techniques for in vitro germination of guayule pollen. The accumulated information about the in vitro germination of the pollen of other species presents considerable interest.

In vitro pollen germination has been studied since the beginning of the last century. The pollen of some plants can germinate in water, but the percentage and extent of pollen tube growth is considerably less than when the same pollen is cultured in an aqueous solution of sugar. In most cases, sugar of some kind is necessary for germination (Stanley and Linskens, 1974). Some pollen species require the addition of accessory substances, such as boron (B), calcium (Ca) and potassium (K). Some

require very complex medium, and for many pollen species an appropriate medium has not be found (Johri and Vasil, 1961). Some of the pollen may germinate easily under a wide range of conditions, while others germinate only under a strict set of conditions (Linskens, 1964). Different species of pollen have different demands, and sometimes these even vary within the same species (Stanley and Linskens, 1974). Cucumis melo (Tanaka and Mukai, 1955) has been found to require a different medium for the optimal germination of diploid and tetraploid pollen.

There are several in vitro germination methods but the most frequently used are either the hanging drop technique or the agar or gelatin method (Stanley and Linskens, 1974). The hanging drop technique, which was not used in this study, involves a drop of media containing pollen inserted in a circular chamber and suspended over water.

In the case of the agar or gelatin method, the pollen is put on the solid medium. The agar supplies moisture at a constant relative humidity, and various carbohydrates or other pollen growth substances can be readily incorporated in it. The thickness of the agar, as well as the agar and sugar concentrations, affect moisture availability and germination. Aerobic conditions are very good on the surface of the agar plates.

As a rule, the length of the pollen tube obtained in vitro is significantly shorter than that in vivo (Johri and Vasil, 1961). Rosen and Gawlik (1966) found that the structure of the pollen tube of lily pollen grown in vitro is similar to that growing in incompatible pistils. The authors postulated that compatible tubes undergo a transition from

autotrophic to heterotrophic nutrition using the substances of the pistil, while the tubes in incompatible pistils are unable to make this transition and are arrested in their growth by the exhaustion of endogenous substances.

Certain physiological differences between binucleate and trinucleate pollen, including the difference in pollen germination in vitro, have been reported. Binucleate pollen is not usually difficult to germinate in vitro, whereas trinucleate pollen, including grass pollen, germinates with considerable difficulty (Brewbaker and Majumder, 1961). Kirby and Smith (1974) explained this difference by the metabolic requirements for mitosis of the generative cell which occurs prior to shedding in trinucleate pollen. This mitotic activity apparently deprives the pollen protoplast of substances essential for the germination in vitro.

Many workers pointed out great variability in the results of in vitro germination studies. Pollen grains are highly sensitive to external factors, particularly to temperature and humidity, so the effect of these factors can be ascertained (Johri and Vasil, 1961). Time of collection, season of the year and method of incoulation can also influence germination (Stanley and Linskens, 1974). Pollen germination shows great variability when it is collected from different anthers of a flower or even from the same anther (Vasil, 1960; Johri and Vasil, 1961). The concentration of pollen can also affect the capacity of pollen to grow. A high pollen concentration can inhibit germination, but generally a certain minimum concentration of pollen grains must be placed in a given

volume of medium to attain maximum germination (Stanley and Linskens, 1974). This density or population effect has been explained by the diffusion of some substances from the grains in the early stages of germination, so the effective concentrations of these substances occur only when the pollen grains are aggregated. Some authors consider calcium (Kwack and Brewbaker, 1961), while others consider the growth-promoting substances (Ariyasu, 1959) as the diffused substance.

Role of Sugar in Pollen Germination

Since the beginning of this century, much attention has been devoted to the question of the role of sugars, particularly sucrose, in pollen germination and pollen tube growth in vitro and in vivo.

There is general unanimity that the externally supplied sugar is necessary to control the osmotic pressure of the medium during the germination of pollen. An appropriate similarity of the osmotic concentration of the medium with that of pollen is considered as a prerequisite for good germination (Johri and Vasil, 1961).

The occurrence of the conversion of starch into sugars or vice versa in pollen grains during germination was shown by Iwanami (1959). These conversions regulate the osmotic pressure of the pollen in relation to the osmotic pressure of the medium or the tissues of the stigma and style.

There is generally, however, no consensus of opinion on the nutritional role of sugars in pollen germination. One school believes that the externally supplied sugar has only an osmotic role and is not utilized by the tubes for any nutritional purposes. The other school

points out that apart from having an osmotic role, the externally supplied sugar in the medium or in the style serves as a nutritional material for the growing tubes (Johri and Vasil, 1961).

The supporters of the endogenous nutrition of pollen believe that even in vivo the tubes do not get any nourishment from the style. They point out that many pollen species may germinate readily and produce tubes of considerable length in water.

The evidence for the exogenous nutrition is the fact that with the increase of sucrose concentration in the medium, the length of the tubes of many pollen species increases. As Brink (1924) pointed out, the volume of the pollen tubes grown in vitro usually so greatly exceeds the volume of the pollen grains that it may be reasonably supposed that the sugar of the medium has been drawn up in the development of the tubes.

Substantial support for the theory of exogenous nutrition in vitro was provided by the work of O'Kelley (1955) on Tecoma. He observed the incorporation of the labeled sugar from the medium into the growing pollen tubes. Kroh and Helsper (1974), by means of labeling experiments, presented evidence for the flow of material from the style into the pollen during pollen germination in vivo.

The bursting of pollen grains and tubes has been observed during the artificial cultivation of pollen. There are different opinions concerning the causes of bursting. Vasil (1961) found that bursting is inversely related to the osmotic concentration of the medium, so he considers it as an osmotic phenomenon. A number of authors, mentioned in the review of Johri and Vasil (1961), believe that bursting cannot be an

osmotic phenomenon since they did not find the distinct relationship between the number of bursted pollen grains and the concentration of sugar in the medium.

Role of Boron, Calcium, and Potassium in Pollen Germination

It has been shown by numerous experiments that the addition of boron in the form of boric acid stimulates pollen germination and tube elongation of many pollen species (Johri and Vasil, 1961). The effect of boron on pollen germination and tube growth far surpasses the effect produced by any of the known hormones, vitamins, or chemicals. This is perhaps due, as suggested by Johri and Vasil (1961), to the fact that pollen grains initially contain adequate quantities of growth-promoting substances but are usually deficient in boron. Thus, the addition of boron to the nutrient medium greatly improves pollen germination and tube growth.

In other pollen species, boron either has no effect or inhibits pollen tube growth. In these plants, the natural level of boron appears to be adequate and, therefore, any additional supply proves ineffective or inhibitory. As Johri and Vasil (1961) mentioned in their review, Schmueker showed on Nymphae pollen that the stigmatic secretion contains appreciable quantities of boric acid and that the pollen grains require the same concentration of boric acid in the medium for successful germination.

The role of boron in pollen germination was summarized by Vasil (1960) as follows:

1) Boron promotes absorption and metabolism of sugars by promoting sugar-borate complexes,
2) boron increases oxygen uptake,
3) boron is involved in the synthesis of pectic materials for the wall of the actively elongating pollen tubes.

The stimulatory role of calcium ions on pollen germination has been observed in many pollen species; however, its role varies from one species to another (Rosen, 1968). Binding of calcium ion to the pectic regions of the tube wall decreases its permeability and increases its rigidity (Kwack and Kim, 1967). Mascarenhas and Machlis (1964) found that the calcium gradient in the pistil acts as a chemotactant for pollen tube growth, and de Bruyn (1966) showed that the calcium ion eliminates the inhibitory effect caused by many substances.

In some cases, boron and calcium alone do not stimulate pollen germination but their combination can promote germination. Pfahler (1967) showed that an addition to the medium of boron or calcium alone decreases the percentage of germination of corn pollen, but their combination greatly promotes germination.

Although not as much is known about the role of potassium ion, it has been shown to accelerate pollen germination and tube growth (Kwack and MacDonald, 1965).

Role of Growth-Promoting Substances in Pollen Germination

The addition of gibberellic acid at low concentrations to the medium stimulates tube growth and increases percent of germination in vitro in some pollen species (Johri and Vasil, 1961). In addition,

auxins have been shown to promote pollen germination in vitro (Vasil, 1960). It appears that the maintenance of a critical level of gibberellic acid is particularly essential for normal pollen germination and growth. Barendse, Rodrigues Pereira, Berkens, Driessen, Van Eyden-Emons and Linskens (1970) suggested that great losses of gibberellic acid from the pollen in the first hour of germination may influence their capacity to continue germination at a high rate. It may be that the gibberellic acid influences the metabolism of the female tissue where the pollen germinates. In some species, the addition of gibberellic acid may overcome the natural decline during the first hours of germination and thus influence further germination and growth.

CHAPTER 3

MATERIAL AND METHOD

Material

In October 1981 seven diploid and thirteen tetraploid guayule plants were transferred into a glass house on the University of Arizona campus. The diploids had been originally growing outdoors in pots, and the tetraploids were transplanted from the University of Arizona Casa Grande Highway Experiment Station. The plants did not flower until January 1982. Generally, the research was limited by the small amount of pollen available. To facilitate the study, in April three more diploid plants were transferred from the greenhouse at the Campbell Avenue Farm to the campus glasshouse.

The diploid plants which yielded enough pollen for evaluation were numbered N1, N9, N115-48, N115-49, and N115-52. The last three plants were those included in the study in April. The tetraploid plants yielding enough pollen were numbered N1 and N2. All the diploid plants were open-pollinated progeny of diploid ($2n = 2x = 36$) progenitor plants. The plants were analyzed for ploidy level by Dr. D. T. Ray.

The tetraploid plants were open-pollinated progeny from the tetraploid germplasm line N 575. Both tetraploid plants were analyzed to have 73 A chromosomes with plant N1 having one additional miniature chromosome.

Design of the Experiment

The initial objective was to characterize each plant as to the percentage of the germinated pollen grains on different culture media. The pollen germination would then be compared within the same ploidy level, and between the diploids and tetraploids. However, during the course of the experiment, the design was subjected to several modifications.

The study of the effect of different substances in the culture media on the germination of guayule pollen comprised three parts:

1. The influence of sucrose on pollen germination. The treatments included concentrations of 5, 10, 15, 20, 25, and 30 percent sucrose. The media containing the same concentrations of mannitol were used to determine whether sucrose plays a nutritional role, as well as an osmotic role, in pollen germination in vitro.

2. The influence of the three mineral elements, boron, calcium, and potassium, on pollen germination. The following concentrations were used: H_3BO_3 at 0, 1, 10, and 100 ppm; $CaCl_2 \cdot 2H_2O$ at 0, 300, and 600 ppm; and KNO_3 at 0, 10, and 100 ppm; and all 36 possible combinations of these three elements in their respective concentrations.

3. The influence of the growth-promoting substances gibberellic acid (GA) and napthaleneacetic acid (NAA) at 0.1, 1, and 5 ppm.

Distilled water was used as a control in part 1, and the 25% sucrose medium was the control in parts 2 and 3. Four repetitions of each treatment were performed on the agar media.

The small amount of pollen produced by each plant allowed only one repetition of each treatment per day. This added the effect of days into the experimental design.

The inoculation was made in the morning just after the dehiscence of the anthers. The pollen was collected in Petri dishes from the plants by shaking the flowers, then immediately taken to the laboratory where the pollen was placed on the agar plates under the sterile hood with the aid of a brush.

This method yielded very low (3 percent) but very uniform pollen germination for all plants and all treatments. It was suspected that the method of inoculation was affecting the results, thus a new method was implemented. The inoculation was made in the greenhouse by shaking the pollen from the flowers directly onto the culture medium.

Microscopic observation of the pollen showed that the inoculation consisted of two fractions: 1) complexes containing a number of pollen grains, and 2) dispersed pollen grains. The complexes consisted of tetrads of pollen grains held together very tightly, whereas the dispersed pollen consisted only of single pollen grains.

In general, this method yielded a much higher percentage of germinated grains than the first method of inoculation. It was observed that the pollen complexes did not produce pollen tubes on any media, while the pollen in the form of the single grains did germinate. This observation is not in agreement with the results reported by Stanley and Linskens (1974).

A piece of soft paper was utilized to disperse the complexes of spores over the surface of the medium after inoculation. Thus, only single pollen grains were obtained, many of which started to germinate immediately. Once the spores were dispersed, they germinated as easily as the single pollen grains.

Even utilizing the new inoculation method, the results of the germination tests showed great variability between repetitions. In order to obtain a more precise evaluation of each treatment, the number of repetitions was increased. With more repetitions, it was observed that there was a certain percentage of germination for each treatment that could not be exceeded. However, any number smaller than that could be easily obtained, and the very small percentage of germination, near zero percent, was often found in every treatment. It appeared that other environmental factors were preventing the percentage of germination from reaching the maximum for each treatment.

In order to define the source of this variability, pollen germination was studied over the period of a day for several days. The pollen was placed on 25 percent sucrose medium at one hour intervals from 900 to 1300. Four repetitions of each treatment were made and each repetition included pollen from different inflorescences. It was observed that the percentage of germination of the pollen from the same plant on the same medium was different on different days and at different hours.

In order to reduce the variability of the results between repetitions of each treatment, the pollen should be collected at the same

time of one day. Since there was not enough pollen to evaluate all the repetitions of each treatment at one time on one day, each part of the experiment was evaluated as blocks. That is, the control and all four repetitions of all the treatments of the block were performed on one day at the same time of the day. Each block was then analyzed separately with the treatment being compared with their own control. For some plants, in order to establish whether the reaction of the pollen from a particular plant on the different substances of the culture medium was the same on different days, the same experiment was repeated on different days.

Preparation of the Medium

The media was prepared by mixing all the ingredients together, regulating the pH between 6.5 and 7.0, 5 percent agar was added and then autoclaving at 240°F/15 psi for 20 minutes. Under the sterile hood, the solutions were poured in the Petri dishes to a depth of 2-4 mm and allowed to cool to room temperature. After the media had reached room temperature, the covers were placed on the Petri dishes and refrigerated. The media plates containing mannitol were stored at room temperature. Under the refrigeration the media containing 10 or more percent of mannitol produced many crystals and it became impossible to use them. The plates were used for inoculation not earlier than 24 hours after the Petri dishes were covered, and up to 14 days.

The aging of the plates for at least 24 hours was necessary to create good conditions for pollen germination. Taylor (1971) observed that if this is not done, the pollen grains tend either to sink into the

medium where they will not germinate or to take up excessive moisture and rupture. We found that this is also true for guayule pollen.

Just before the inoculation, the Petri dishes were opened under the sterile hood for 5-10 minutes to allow the media to become warm and the moisture on the surface of the media to evaporate. It was observed that if the surface of the media was covered with a film of water, the germination was very poor.

Immediately after the inoculation, the plates were covered and kept at room temperature, and almost immediately the pollen tubes started to appear. In an hour the germination was completed, and the results were scored.

All four repetitions were made on a single plate. For each repetition, random counts of 200 grains were made at a magnification of 100X to determine the percent of germination. The pollen grain was considered germinated when it produced a tube one or more pollen diameters in length. On some media, besides the pollen grains producing pollen tubes, ruptured pollen grains and tubes were observed. The ruptured pollen grains have also been observed during the germination of pollen of other species (Johri and Vasil, 1961; Taylor, 1971). Those grains were considered as germinated if following rotation short tubes were visible.

During the research it was observed that the pollen from some plants contained great numbers of small and, probably, abnormal pollen grains. In order to describe the variability in pollen size, the average diameter of pollen was measured for each plant.

The pollen was collected on the 25 percent sucrose medium, dispersed with a piece of paper, and the diameter of a random sample of 400 pollen grains for each plant was measured at the magnification of 160X using the screw micrometer piece. When put on the medium, the pollen grains swell and become round compared to the non-treated elliptic pollen grains. When placed in water, they become even larger and completely round. In this experiment, only the largest diameter of the pollen grains was measured. The germination percentage of the same pollen which was measured for pollen diameter was recorded.

During the study of the influence of different factors on pollen germination, only in the case of the diploid plant N9, the influence of B, Ca, and K on pollen germination could be performed as a single experiment. For the other diploid plants, the lack of sufficient quantities of pollen required the study to be divided into two experiments. The first, studied the influence of the simultaneous addition of B and Ca, including all the combinations of B and Ca with no K, and the second, the combinations including K, and studying the influence of the simultaneous addition of B, Ca, and K.

In all the experiments, which studied the influence of different factors on pollen germination, the design used was considered to be a one-two-three or four factorial, completely randomized block, although the nature of the experimental material did not permit true randomization. The only possible measure to avoid the bias of the results was the use of the pollen from different inflorescences for each repetition.

For statistical analysis, the inverse sine transformation was applied to the percentage data (Snedecor, 1956). If the variable consists of the percentages, the distribution tends to be binomial in form. The inverse sine transformation weights more heavily the small percentages which have small variance and, thus, normalizes the distribution. In the discussion, only the original data are referred to.

An analysis of variance was performed on the transformed percentage data. For all the experiments with significant F values, except those studying the influence of the concentration of sucrose and mannitol on pollen germination, mean separation was made utilizing the Duncan's Multiple Range test. In the case of the experiments studying the influence of the concentration of sucrose and mannitol on pollen germination, the logic of the experiment suggested comparison of the mean germination percentages arranged according to the corresponding concentrations of sucrose or mannitol. Therefore, in this case, mean separation was made using the Scheffe' test (Hicks, 1973; Little and Hill, 1978).

In the experiments studying the variability of the pollen diameter, the mean, variance, and coefficient of variation of each sample were calculated.

CHAPTER 4

RESULTS AND DISCUSSION

Pollen Germination

The germination of guayule pollen is unique inasmuch as it appears that the pollen is released as tetrads of pollen grains that will not germinate on the culture medium until they are dispersed. For germination, therefore, the pollen must be dispersed into the single grain form. Then germination of the single grains starts almost immediately. Probably, the released pollen is germinable to some degree, but it appears that the membrane covering the tetrad of pollen grains prevents the germination of the pollen on the medium. This may also be true for the pollen germination in vivo. Possibly, this unusual feature is related to the adaptation of this plant to insect pollination. The tetrad complexes are too heavy to be transported by the air movement, thus insects are essential for the pollen transport, and the contact with the insect would release pollen for germination. These features, together with the self-incompatibility of the diploids, facilitate cross-pollination.

The Influence of the Day and Hour of Testing on Pollen Germination

The influence of a particular day and the time of the day of the inoculation on the pollen germination of the three dpiloid and two tetraploid plants are presented in Tables 1-5 and A.1-A.5, Appendix A.

Table 1. Mean separation of the germination percentages of the pollen of the diploid guayule plant N9 tested during 5 days at 900, 1000, 1100, 1200, and 1300 each day.

a) Day

Tmt	4.27	4.21	4.28	5.4	4.26	5.3
O	21.8	18.5	13.6	11.9	10.2	7.1
T	26.1	22.1	19.8	18.8	15.6	13.0

b) Hours

Tmt	1000	1100	900	1200	1300
O	23.9	20.2	14.5	9.6	1.0
T	28.5	25.8	21.9	16.3	3.9

c) Day x Hour Interactions

Tmt	4.21/1000	4.27/1000	4.27/1100	4.21/1100	4.28/1100	4.27/1200
O	44.7	40.0	33.3	25.6	23.4	23.4
T	41.7	39.2	35.2	30.4	28.8	28.7

4.26/900	4.28/1000	5.4/1100	5.4/1200	4.21/900	5.3/900	4.26/1100
22.4	21.1	18.7	17.8	17.3	16.8	16.4
27.8	27.2	25.6	25.0	24.5	24.2	23.8

5.4/1000	4.28/900	5.3/1000	4.26/1000	4.27/900	4.28/1200	4.21/1200
16.0	15.2	11.0	10.6	9.3	7.4	4.8
23.6	22.9	19.2	19.0	17.6	15.6	12.3

5.4/900	5.3/1100	4.27/1300	5.3/1200	5.3/1300	4.26/1200	5.4/1300
6.2	4.0	3.0	2.8	10.7	1.6	1.0
12.3	11.2	9.8	9.5	7.2	6.8	5.7

4.28/1300	4.21/1300	4.26/1300
0.8	0.2	0.1
4.8	1.6	0.8

Tmt = treatment
O = mean of the original data
T = mean of the transformed data

Table 2. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-48 tested during 4 days at 900, 1000, 1100, and 1200 each day.

Mean Separation

a) Days

Tmt	4.28	4.27	4.26	5.4	5.3
O	16.5	16.4	11.4	6.2	5.0
T	23.7	22.9	18.9	13.1	12.0

b) Hours

Tmt	1100	1000	1200	900
O	13.0	12.3	11.2	7.8
T	20.1	19.9	16.7	15.8

c) Day x Hour Interactions

Tmt	4.27/1200	4.28/1000	4.26/1100	4.28/900	4.28/1100	5.4/1100
O	33.5	24.9	23.0	16.9	14.8	13.7
T	34.9	29.9	28.6	24.2	22.6	21.6

4.27/1000	4.27/1100	4.26/1200	5.3/1000	4.28/1200	4.27/900	5.4/1000
12.6	12.0	11.0	10.9	9.4	7.4	7.0
20.8	20.2	19.3	19.2	17.9	15.8	15.2

4.26/1000	4.26/900	5.4/900	5.3/1100	5.4/1200	5.3/1200
6.1	5.6	2.9	1.7	1.1	1.0
14.3	13.6	9.8	7.4	5.9	5.6

Table 3. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-49 tested during 8 days at 900, 1000, 1100, 1200, and 1300 each day.

Mean Separation

a) Days

Tmt	5.19	4.28	4.27	5.16	5.15	5.3	5.4	4.26
O	33.3	17.1	7.8	9.1	9.4	5.4	1.8	0.7
T	34.6	22.9	20.1	14.8	13.8	9.2	5.4	3.1

b) Hours

Tmt	1000	900	1100	1200	1300
O	18.4	15.0	16.2	6.5	3.2
T	22.5	19.4	19.2	10.4	5.9

c) Day x Hour Interactions

Tmt	5.19/1100	4.27/900	5.15/1000	5.9/1200	4.28/1100	4.28/1000
O	80.0	39.0	34.6	29.6	24.8	24.5
T	63.5	38.4	36.0	32.7	29.8	29.6

5.16/1000	5.19/1300	5.19/900	5.3/1000	4.28/900	4.27/1000	4.28/1200
24.1	21.6	20.6	12.4	19.0	17.5	15.3
29.1	27.7	26.6	26.0	25.7	24.4	22.9

5.19/1000	4.27/1100	5.16/900	5.15/900	5.16/1100	5.4/1000	4.27/1200
14.7	12.2	13.4	9.7	6.0	4.4	4.2
22.4	20.2	20.0	18.0	14.0	11.7	11.6

5.4/900	5.3/900	4.26/1100	5.15/1100	4.28/1300	5.3/1100	4.27/1300
4.0	13.3	2.8	1.8	1.7	1.2	1.4
11.4	10.4	9.4	7.6	6.7	6.4	6.1

5.15/1200	4.26/900	5.16/1300	5.3/1200	5.4/1100	5.15/1300	5.4/1300
0.9	0.8	0.6	0.5	0.4	0.2	0.1
5.4	4.6	4.3	3.4	3.2	1.2	0.9

4.26/1000	4.26/1200	4.26/1300	5.3/1300	5.4/1200
0.1	0	0	0	0
0.8	0.6	0	0	0

Table 4. Mean separation of the germination percentages of the pollen of the tetraploid guayule plant N1 tested during 4 days at 900, 1000, 1100, and 1200 each day.

a) Days

Tmt	4.26	4.28	5.3	5.4
O	7.4	4.1	2.3	0.8
T	15.0	11.5	6.6	3.8

b) Day x Hour Interactions

Tmt	4.26/1100	4.26/1200	4.26/1000	4.28/900	5.3/900	4.28/1200
O	12.7	8.4	6.5	6.6	7.1	4.4
20.8	16.8	14.8	14.8	13.8	12.1	10.3

4.28/1100	5.4/1000	4.28/1000	4.26/900	5.3/1000	5.3/1100	5.4/900
3.2	2.3	2.3	1.9	0.9	1.0	0.8
10.3	9.2	8.6	7.8	5.4	5.3	5.0

5.4/1100	5.3/1200	5.4/1200
0.3	0.2	0
2.3	1.6	0

Table 5. Mean separation of the germination percentages of the pollen of the tetraploid guayule plant N2 tested during 4 days at 900, 1000, 1100, and 1200 each day.

a) Days

Tmt	4.26	4.27	5.3	5.4
O	2.5	1.9	0.6	0.3
T	6.0	4.2	2.6	1.4

b) Hours

Tmt	900	1000	1100	1200
O	3.4	1.4	0.3	0.1
T	8.6	4.0	1.3	0.2

c) Day x Hour Interactions

Tmt	4.27/900	4.26/1000	4.26/900	5.3/900	5.4/1100	5.3/1000
O	7.5	5.6	4.3	2.0	1.1	0.2
T	15.8	12.3	11.0	7.6	4.4	1.9

5.4/1000	4.27/1000	4.27/1000	5.3/1200	4.26/1200	4.27/1100	4.27/1200
0.1	0.1	0.1	0.1	0	0	0
1.0	0.9	0.8	0.8	0	0	0

5.3/1100	5.4/900	5.4/1200
0	0	0
0	0	0

The precision of the experiments was rather high for this kind of experiment: the coefficients of variation 28.1, 27.6, and 29.3 for the diploid plants N9 (Appendix A, Table A.1), N115-48 (Appendix A, Table A.2), and N115-49 (Appendix A, Table A.3) respectively, and 15.1 and 76.8 for the tetraploid plants N1 (Appendix A, Table A.4) and N2 (Appendix A, Table A.5) respectively were observed. The analysis of variance indicates the statistical significance of the influence of the day and day x hour interactions for all the plants, and the influence of the hour for all but the tetraploid N1 plant (Appendix A, Tables A.1-A.5).

In general, the results of the pollen germination were rather poor. The highest mean of the day, 33.3 percent, was found in the diploid plant N115-49 on May 19 (Table 3). The other diploid plants, N115-48 and N9, had 16.5 percent and 21.8 percent respectively, on April 28 and April 27 respectively (Tables 1 and 2). The average pollen germination was poorer for the tetraploids tested than for the diploids. The highest mean germination percentages for a day were 71.4 percent and 2.5 percent for tetraploid plants N1 and N2 respectively, both on April 26 (Tables 4 and 5).

Comparing the performance of the pollen tested on the same day, we see that on the average on April 27 the performance of the pollen of the diploids was statistically better than on April 26, May 3 and 4 (Tables 1-3). The performance of the pollen of the tetraploids was significantly better on April 26 than on May 3 and 4 (Tables 4 and 5).

Generally, the diploids had the best germination results at 1000, with the 900 results of the diploid N115-49 not being statistically different from the 1000 results. Also, the 1000 results of the diploids N9 and N115-48 were not statistically different from the 1000 results. However, the 1100 results of the diploid N115-49 were significantly less than the 1000 results (Tables 1-3).

The 1200 inoculations germinated significantly more poorly than the 900, 1000, and 1100 inoculations in the case of the diploids N9 and N115-49. In the case of the diploid N115-48, they were statistically poorer only compared to the 1100 inoculations. For diploids N9 and n115-49, the 1300 inoculations gave the lowest germination percentage. Thus, the best germination percentages for the diploids, N115-48, N115-49, and N9, were 13.0 percent, 18.4 percent, and 23.9 percent respectively at 1100 and the last two at 1000 respectively (Tables 1-3).

In the case of the tetraploid N2, the 900 inoculations germinated statistically higher than the 1000, 1100, and 1200 inoculations, but still very poorly, with the highest mean of 3.4 percent (Table 5).

For all five studied plants, the interactions of day x hour were significant at the 1 percent level (Appendix A, Tables A.1-A.5). The maximum germination percentages found in this experiment were 80.0 percent, 44.7 percent, and 33.5 percent for the diploid plants N115-49, N9, and N115-48, respectively, and 12.7 percent and 7.5 percent for the tetraploid plants N1 and N2 (Tables 1-5).

The dependence of the pollen germination on the day and the time of the day when the pollen was tested probably reflects the high

sensitivity of the guayule pollen to external conditions. The day x hour interactions show that only during certain hours and on certain days are the external conditions in the greenhouse adequate for pollen germination. The periods when the pollen showed the maximum germination percentage were different for each plant. This probably reflects the great genetic diversity found in guayule. The response of the pollen to different external conditions would help to explain the differences in seed set, naturally and artificially obtained by guayule workers at different times of the year (Ray, personal communication).

The difference in pollen germination, in vitro, between tests conducted early and late in the season and between tests conducted on cloudy and sunny days, as well as the influence of the hour of the day, were reported by other authors (Vasil, 1960; Johri and Vasil, 1961; Stanley and Linskens, 1974). The quite unpredictable results obtained in this study point out that more work is needed to define these parameters precisely in guayule.

The Role of Sucrose in Pollen Germination

The influence of different concentrations of sucrose and mannitol on the pollen germination in two diploid plants and one tetraploid plant was studied and the results presented in Table 6. In all the studied plants, the results of the germination of pollen on the media containing different concentrations of mannitol were not statistically different from that of the control containing only distilled water. Various concentrations of sucrose produced results statistically higher than the control.

Table 6. Summary of the results of three experiments of the study of the germination percentages of the pollen of two diploid and one tetraploid plant of guayule tested on the culture media containing different concentrations of sucrose or mannitol. Mean separation using the Scheffe's test.

% of substance	Plant/Date of Testing					
	diploid N9 4.23		diploid N115-49 4.15		tetraploid N1 4.21	
	O	T	O	T	O	T
Mannitol						
0	0.1	1.1	0.8	4.9	0.1	0.9
5	0.1	0.8	0.6	4.2	0.1	1.2
10	0.6	3.2	1.3	6.4	0.7	4.0
15	1.2	3.5	0.7	3.8	1.3	6.2
20	0.5	2.8	0.8	4.2	1.1	6.0
25	1.5	5.6	1.0	4.8	0.7	4.1
30	0	0.6	0.2	1.6	0.1	1.0
Sucrose						
5	0.9	4.5	4.7	12.4	0.9	5.3
10	3.9	11.4	8.5	16.9	4.0	11.4
15	10.4	18.7	15.5	23.1	5.2	13.2
20	26.6	30.5	21.0	27.2	14.8	22.4
25	49.9	45.0	7.2	24.4	22.1	27.6
30	5.4	13.2	1.1	5.8	2.2	4.8
Variance	14.9		6.2		8.5	
CV (%)	35.7		23.7		33.4	

Absence of the positive influence of any concentration of mannitol on pollen germination allows us to suppose that the sucrose in the medium serves as a nutrition source for the growth of pollen tubes in vitro.

In the diploid N9, the highest germination percentage, 49.9 percent, was obtained on the medium containing 25 percent sucrose (Table 6). The results of all the other treatments were statistically poorer than that obtained with 25 percent sucrose.

In the diploid N115-49, the highest germination percentage, 21.0 percent, was obtained on the 20 percent sucrose medium. However, this was not statistically different from the percentage germination obtained on the 15 and 25 percent sucrose media, and these results were statistically higher than the percentage germinations obtained on the other treatments (Table 6).

In the tetraploid plant N1, the highest germination percentage, 22.1 percent, was obtained on the 25 percent medium, but was not statistically different from the results obtained on the 20 percent sucrose medium. The results of the other treatments were statistically poorer, with the 5 percent sucrose medium not being statistically different from the control (Table 6).

In this study, the 25 percent medium produced the best results. We can expect that at this concentration the osmotic equilibrium between the content of the pollen grains of guayule and the medium is established, a condition necessary for good pollen germination in vitro.

Influence of Mineral Elements B, Ca, and K on Pollen Germination

The effect of the three mineral elements, B, Ca, and K, on the pollen germination in vitro of the four diploid and the two tetraploid plants was studied (Tables 7-14, Appendix A Tables A.6-A.14).

An increase in the concentration of B from 0 to 10 ppm did not produce a statistically significant increase in the pollen germination in diploid N115-49. However, at 100 ppm B the germination percentage was statistically higher, 23.2 percent (Table 9).

In the tetraploid N2, with the increase of the concentration of B from 0 to 1, 10, and 100 ppm, the germination percentages first increased from 2.2 to 6.5 percent, then dropped to 0.5 percent and, finally, increased to 3.1 percent, with all the results being statistically different from one another (Table 11). Due to the small absolute values of these germination percentages, we attribute the statistical significance of the results to the sample error.

At 300 ppm Ca the germination percentage of the diploid plant N115-48 was statistically higher than that of the control containing only distilled water (Table 8).

Generally, although the interactions of B x Ca, B x K, and Ca x K were statistically significant for some plants, the increase in the germination percentages by the addition of these combinations compared to the control medium was not very high. The germination percentages produced by these combinations never surpassed 33 percent (Tables 7-14).

Table 7. Mean separation of the germination percentages of the pollen of the diploid guayule plant N9 tested during 2 days on the media containing different concentrations of Ca, B, and K.

a) B x K Interactions

Tmt	100x0						0x0		
O	32.4	27.0	27.9	24.4	23.8	23.4	19.4	19.4	23.4
T	33.2	30.0	29.3	29.2	27.5	26.6	25.7	25.6	24.5

18.7	19.6	16.0
24.3	23.4	22.4

b) B x Ca x K Interactions

Tmt									
O	40.0	39.7	39.4	38.2	37.0	33.6	33.0	36.6	38.4
T	39.1	39.0	38.2	37.9	36.7	34.6	34.2	24.0	33.6

29.1	27.6	31.1	23.8	23.0	22.6	21.8	18.4	19.8	19.1
32.4	31.5	30.9	28.8	28.4	28.2	27.3	25.1	24.9	24.5

13.0	24.2	17.0	19.5	14.9	13.1	13.0	12.8	12.1	18.2
24.5	24.0	23.7	23.3	21.6	21.1	21.1	20.5	20.3	19.5

				0x300x10	1x600x0
14.0	13.6	15.0	28.6	8.4	4.0
19.4	19.3	17.2	17.0	15.5	9.6

Table 8. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-48 tested during 2 days on the media containing different concentrations of B and Ca.

a) Days

Tmt	4.28	4.26
O	28.4	7.9
T	31.5	15.1

b) Ca

Tmt	300	600	0
O	23.7	17.1	13.7
T	26.9	21.9	21.2

c) B x Ca Interactions

Tmt	100x600	100x300	0x300	10x300	100x0	1x0		0x0
O	30.9	30.2	30.7	20.4	19.0	14.8	16.7	10.0
T	32.0	31.2	31.0	25.9	25.6	22.6	19.7	18.9

13.1	10.7	11.1	10.0
18.3	18.4	18.2	18.2

Table 9. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-49 tested during 4 days on the media containing different concentrations of B and Ca.

a) B

Tmt	100	10	0	1
O	23.2	20.4	18.8	12.1
T	27.9	26.2	22.2	17.0

b) Ca

Tmt	300	0	600
O	21.0	19.0	15.7
T	24.7	24.3	21.4

c) B x Ca Interactions

Tmt	100x600	100x0	10x0	10x300	0x300	0x0		
O	26.3	24.5	22.5	21.6	25.5	20.6	18.8	17.0
T	30.3	29.3	28.1	26.5	26.4	25.4	24.1	24.0

1x300	0x600	1x600	1x0
25.5	10.2	9.3	8.6
22.0	16.6	14.6	14.5

Table 10. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-52 tested during 4 days on the media containing different concentrations of B and Ca.

a) Ca

Tmt	0	300	600
O	7.2	4.7	4.7
T	14.1	10.3	10.9

b) B x Ca Interactions

Tmt	10x0			0x0			
O	10.9	7.0	6.4	6.0	5.6	5.2	5.2	4.3
T	17.8	14.0	13.2	13.0	13.0	11.2	10.7	10.2

0x300	10x600	0x600	
5.2	3.8	2.4	2.3
9.2	8.5	7.4	7.0

Table 11. Mean separation of the germination percentages of the pollen of the tetraploid guayule plant N2 tested on the media containing different concentrations of B and Ca.

a) B

Tmt	1	100	0	10
O	6.5	3.1	2.2	0.5
T	14.2	8.6	5.5	2.3

b) Ca

Tmt	0	300	600
O	4.2	3.0	2.2
T	9.9	8.3	4.8

c) B x Ca Interactions

Tmt	0x0			100x300	1x0	10x300	10x600
O	8.8	7.3	6.8	3.9	5.4	4.0	1.2	0.1
T	17.2	15.6	15.0	13.5	11.4	10.4	5.8	1.0

100x600	0x300	0x600	10x0
0.1	0.1	0	0
1.0	0.9	0	0

Table 12. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-48 tested on the media containing different concentrations of B, Ca, and K.

a) B x K Interactions

Tmt	10x100			0x10			
O	23.0	22.9	19.0	14.4	17.2	15.0	12.8
T	28.5	28.0	25.3	21.6	20.8	17.8	17.2

10.9
17.0

b) Ca x K interactions

Tmt	10x0	100x300	100x600	100x0	10x600	10x300
O	23.8	21.9	15.0	14.0	14.8	9.6
T	28.8	27.5	20.9	20.7	19.8	15.3

c) B x Ca x K Interactions

Tmt							
O	35.6	29.3	27.2	25.2	24.5	23.3	22.5
T	36.5	32.8	31.4	30.0	29.6	28.7	28.0

				0x0x10			
22.2	20.8	18.5	18.3	18.0	18.3	16.2	15.8
28.0	27.1	25.4	25.2	25.1	24.2	23.5	23.4

		0x300x10	100x0x100	0x600x10	10x600x10	1x300x10
10.4	9.0	6.9	6.8	1.4	0.5	0
18.8	17.4	14.5	12.4	6.6	2.0	0

Table 13. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-49 tested during 2 days on the media containing different concentrations of B, Ca, and K.

a) K

Tmt	100	10
O	19.1	17.6
T	24.4	21.9

b) Ca x K Interactions

Tmt	300x100			0x10		
O	22.9	20.0	18.6	18.5	15.9	14.1
T	27.0	25.2	24.6	22.7	21.6	18.3

c) B x Ca x K Interactions

Tmt	1x0x10	0x0x100	0x0x10				
0	35.2	22.1	14.0	12.3	11.4	11.0	10.2
T	36.3	28.0	17.6	14.7	13.0	12.8	12.0
8.9	8.8	8.8	8.3	8.2	8.0	8.1	7.8
11.7	11.5	11.4	10.1	10.0	9.7	9.6	9.0
6.8	6.0	5.7	5.3	5.1	3.8	3.4	2.8
8.4	7.3	7.2	7.1	7.0	6.4	5.8	4.4
2.0	1.6						
3.7	3.6						

Table 14. Mean separation of the germination percentages of the pollen of the diploid guayule plant N115-52 tested during 2 days on the media containing different concentrations of B, Ca, and K.

a) Day

Tmt	4.20	4.21
O	7.1	3.0
T	12.7	8.5

b) Ca

Tmt	600	0	300
O	5.6	5.6	3.3
T	12.5	10.1	9.0

c) B x K Interactions

Tmt	1x10						0x0	
O	8.0	6.1	6.0	4.6	4.0	3.7	2.9	3.6
T	13.8	12.1	12.1	11.1	10.5	9.2	8.3	7.5

d) B x Ca x K Interactions

Tmt	1x0x10	1x600x100	1x600x10	0x0x100	10x600x10	0x600x10
O	11.6	8.0	9.7	8.8	4.6	4.9
T	18.0	16.5	15.5	15.1	12.2	12.1

0x600x100	10x0x100	10x300x10	100x300x10	10x0x10	100x300x100	1x300x100
4.3	6.6	3.5	4.4	5.8	2.6	5.4
11.7	11.6	10.7	10.5	10.4	10.4	10.3

100x600x10	0x300x100	1x0x100				
5.4	4.8	4.8	6.0	2.6	2.4	2.2
9.8	9.4	9.3	8.4	7.9	7.7	7.4

0x0x10		
2.4	2.2	1.3
7.3	5.5	2.2

The interactions of B x Ca x K were statistically significant for all the diploid plants. In the case of the diploids N115-49 and N115-52 some of the B x Ca x K combinations produced results better than the control containing no B and Ca and 10 ppm K (Tables 13 and 14). In the case of the diploids N9 and N115-48, none of the combinations were statistically better than the control, while some of them were statistically poorer (Tables 7 and 12).

In the case of the diploid N115-49, the best combinations were 1 x 0 x 10 and 0 x 0 x 100, producing 35.2 percent and 22.1 percent pollen germination, while the control produced 14.0 percent (Table 13). For the diploid N115-52, the best combinations were 1 x 0 x 10, 1 x 600 x 100, 1 x 600 x 10, and 0 x 0 x 100, producing 11.6 percent, 8.0 percent, 9.7 percent, and 8.0 percent respectively, while the control 0 x 0 x 10 produced 2.4 percent pollen germination (Table 14).

Influence of Growth-Promoting Substances on Pollen Germination

The results of the study of the influence of the growth-promoting substances on the germination of pollen of four diploid and two tetraploid plants are presented in Table 15. Except for the diploid N115-49, some concentrations of gibberellic acid (GA) and auxin (NAA) statistically increased the germination percentages. However, this was different for each plant tested.

The results of this study and the study of the influence of the mineral elements on pollen germination showed that some of these substances can increase the germination percentages of the diploid and

Table 15. Summary of six experiments of the study of the germination percentages of the pollen for 4 diploid and 2 tetraploid guayule plants tested on the media containing different concentrations of auxin (NAA) and gibberellic acid (GA).

Plant	Number of Days of Testing	Type of Mean	Control (water)	NAA, ppm 0.1	NAA, ppm 1.0	NAA, ppm 5.0	GA, ppm 0.1	GA, ppm 1.0	GA, ppm 5.0
diploid	3	O	16.0	26.1	23.8	32.5	30.1	31.3	49.3
N9		T	24.5	30.4	28.6	34.7	21.2	33.1	44.2**
diploid	3	O	10.2	13.1	11.5	18.5	17.7	13.5	18.0
N115-49		T	16.4	19.2	22.4	22.5	25.1	20.0	23.9
diploid	2	O	13.5	14.5	15.2	23.2	18.6	14.0	14.8
N115-48		T	18.6	14.5	21.8	28.2**	23.6	21.4	20.7
diploid	2	O	6.9	7.5	6.5	18.9	8.9	12.0	5.6
N115-52		T	15.4	14.9	13.4	24.1**	18.3	20.7**	12.7
tetraploid	2	O	8.5	9.7	5.1	13.9	16.8	14.1	34.5
N1		T	16.5	17.8	12.9	21.0	23.0	21.3	34.1**
tetraploid	1	O	1.4	3.6	2.6	10.4	1.2	11.7	6.3
N2		T	6.5	10.7**	9.1	18.8**	6.1	20.0**	14.5**

**Significantly different from the control at the 1% level.

tetraploid plants; however, the response of the pollen in each studied plant was different.

In diploid N115-49, the maximum germination percentage of 80.0 percent was obtained on the 25 percent sucrose medium (Table 3). This high value can be attributed at least partly to the fact that the study of the influence of the day and the hour of the day on pollen germination of the diploid N115-49 lasted longer than that of the other plants. Therefore, there was a greater chance to perform a germination test during exceptionally good environmental conditions.

For diploid N115-48, the maximum germination percentage, 56.2 percent, was obtained on the 0 x 10 x 10 medium; diploid N9, 64.6 percent on the 5 ppm GA medium; tetraploid N1, 61.8 percent on the 5 ppm GA medium; and tetraploid N2, 11.7 percent on the 1 ppm GA medium.

By inspecting the results, it appears that the pollen of the guayule plants responds individually to the addition of the studied substances. In addition, it appears that when the external conditions are good, it is possible to obtain high germination percentages even on the sucrose medium without any other additions. What those conditions are, how they affect pollen germination, and which stage of the development of the pollen is affected remain to be determined.

The release of the guayule pollen in the form of nongerminable tetrads of pollen grains, as well as the high variability in the results of the germination tests with the day and the hour of testing, are the key problems in the in vitro germination of guayule pollen. The occurrence of these phenomena precludes for the present the discussion of

the questions posed earlier in the research, that is, the inference of the pollen viability from the germination percentages, comparison of the pollen germination of the diploids with that of the tetraploids, and the influence of different substances on pollen germination.

Pollen Diameter

The results of the study of the pollen diameter and the germination percentages of the pollen for the five diploid and the two tetraploid guayule plants are given in Table 16.

The coefficient of variation of the pollen diameters of the tetraploids was very large. Among the diploids, some of the plants also had large coefficients of variation for pollen diameter, while the others had more uniform size. Generally, high variability in pollen diameter followed a decrease in pollen germination.

The variability in diameter observed by Bergner (1946) and in this study can be attributed to irregularities of meiosis in tetraploid and some diploid guayule plants. The abnormal pollen grains with the unbalanced genomes are, probably, not able to absorb water from the culture medium and, therefore, do not swell, while the normal pollen grains greatly increase in volume on the culture medium.

Table 16. The means, variances and coefficients of variation of the diameters of 400 pollen grains and the germination percentages of the pollen tested on the 25 percent sucrose medium.

Plant N	Pollen grain diameter, $\bar{X}$	S^2	CV	Germination, %
dipl. 115-48	21.3	3.3	15.4	16.5
dipl. 9	21.8	1.9	8.7	13.7
dipl. 115-49	21.3	2.5	11.7	8.2
dipl. 115-52	22.6	5.7	25.2	3.2
dipl. 1	22.7	8.7	38.2	1.2
tetr. 1	28.1	9.1	32.3	4.3
tetr. 2	28.1	14.0	49.8	0.3

CHAPTER 5

CONCLUSIONS

The pollen of diploid and tetraploid guayule plants was observed to be released as tetrads of spores, which would not germinate on the culture medium unless dispersed into single pollen grains.

The germination of the pollen of the diploid and tetraploid guayule plants on the culture medium was different on different days and in different periods of the day. During some periods of some days, the germination of pollen on the 25 percent sucrose medium was unusually high while the average germination percentages of the days and hours were rather low. This, probably, reflects the high sensitivity to the external conditions of the guayule pollen.

The germination of the pollen of both diploids and tetraploids depends upon the concentration of sucrose in the media, but is independent of the concentration of mannitol. The 25 percent sucrose media produced the highest germination percentages. The independence of the germination percentage of diploid and tetraploid guayule pollen on the concentration of mannitol is considered proof that sucrose plays a nutritional role, as well as an osmotic role, in pollen germination.

The mineral elements, B, Ca, and K, and the growth-promoting substances, gibberellic acid (GA) and auxin (NAA), were shown to increase pollen germination of both diploid and tetraploid plants; however, each plant responded individually.

The variability of the pollen diameters of the tetraploids is very high. In the case of the diploids, the variability of the pollen diameters in some plants was high, while that of the others was rather low. Generally, the higher the variability, the lower the germination percentages.

APPENDIX A

Table A.1. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N9 tested during 5 days at 900, 1000, 1100, 1200, and 1300 each day.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	119	15451.2				
Days	5	2167.4	433.4	14.6**	2.3	3.2
Hours	4	9029.2	2257.3	7.6**	2.5	3.5
D x H	20	1313.5	656.8	22.1**	1.7	2.1
Error	99	2941.1	29.7			

CV = 28.1%

Table A.2. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-48 tested during 4 days at 900, 1000, 1100, and 1200 each day.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	79	5085.2				
Days	4	1868.1	467.0	18.7**	2.5	3.7
Hours	3	282.6	94.2	8.8*	2.8	4.1
D x H	12	1436.5	119.7	4.8**	1.9	2.5
Error	60	1498.0	25.0			

CV = 27.6%

Table A.3. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-49 tested during 8 days at 900, 1000, 1100, 1200, and 1300 each day.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	159	31246.7				
Days	7	14757.6	2108.2	10.2**	2.1	2.9
Hours	4	6267.0	1566.8	76.0**	2.5	3.6
D x H	28	8573.9	306.2	14.9**	1.7	2.1
Error	80	1648.2	20.6			

CV = 29.3%.

Table A.4. Analysis of variance of the germination percentages of the pollen of the tetraploid guayule plant N1 tested during 4 days at 900, 1000, 1100, and 1200 each day.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	63	2458.7				
Days	3	1191.8	397.3	53.0**	2.8	4.2
Hours	3	65.3	21.8	2.2	2.8	4.2
D x H	9	839.8	98.3	12.4**	2.1	2.8
Error	48	361.8	7.5			

CV = 15.1%

Table A.5. Analysis of variance of the germination percentages of the pollen of the tetraploid guayule plant N2 tested during 4 days at 900, 1000, 1100, and 1200 each day.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	63	1985.5				
Days	3	196.0	65.3	8.8**	2.8	4.2
Hours	3	675.1	225.0	30.4**	2.8	4.2
D x H	9	758.0	84.2	11.4**	2.1	2.8

CV = 76.7%

Table A.6. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N9 tested during 2 days on the media containing different concentrations of calcium chloride (Ca), boric acid (B), and potassium nitrate (K).

Source of Variation	df	SS	MS	Observed F	Required F 5%	Required F 1%
Total	287	46854.3				
Subplot		7528.9				
Day	1	48.1	48.1	0.1		
B	3	792.2	264.1	0.6		
Error	3	1286.7	428.9			
Ca	2	346.4	173.2	0.6	4.5	8.6
Ca x B	6	2664.8	444.1	1.49	3.6	6.4
Error	8	2390.7	298.8			
K	2	1354.4	62.7	0.6	3.0	4.7
K x CA	4	6921.0	1730.2	1.8	2.4	3.4
K x B	6	2476.7	412.8	4.3**	2.1	2.9
K x Ca x B	12	6884.9	2444.3	2.56**	1.8	2.3
Error	240	22907.4	95.4			

CV = 37.1%

Table A.7. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-48 tested during 2 days on the media containing different concentrations of B and Ca.

Source of Variation	df	SS	MS	Observed F	Required F 5%	Required F 1%
Total	95	11801.8				
Day	1	6455.0	6455.0	21.2*	10.1	34.1
B	3	1333.8	444.6	1.5	9.3	29.5
Error	3	915.1	305.0			
Ca	2	631.7	315.8	50.9**	3.1	4.9
B x Ca	6	1956.6	327.6	52.8**	2.2	3.0
Error	80	500.6	6.2			

CV = 16.5%

Table A.8. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-49 tested during 4 days on the media containing different concentrations of B and Ca.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	191	23676.1				
Day	3	6414.2	2138.1	1.78	3.9	7.9
B	3	3306.3	1102.1	9.2**	3.9	7.9
Error	9	1078.2	119.8			
Ca	2	419.2	209.6	32.2**	3.0	4.7
B x Ca	6	1595.1	265.8	40.9**	2..	2.9
Error	168	10863.1	6.5			

CV = 10.9%

Table A.9. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-52 tested during 4 days on the media containing different concentrations of B and Ca.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	191	8637.8				
Day	3	820.7	273.6	3.2	3.9	7.0
B	3	256.7	85.6	1.0	3.9	7.0
Error	9	773.6	86.0	9.4		
Ca	2	529.1	264.6	12.1**	3.0	4.7
B x Ca	8	709.0	118.2	5.4**	2.1	2.9
Error	168	3685.5	21.9			

CV = 37.1%

Table A.10. Analysis of variance of the germination percentages of the pollen of the tetraploid guayule plant N1 tested on the media containing different concentrations of B and Ca.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	47	3554.4				
B	3	325.6	108.5	1.58	2.9	4.4
Ca	2	80.8	40.4	0.5	3.3	5.2
B x Ca	6	676.1	112.7	1.64	2.4	3.4
Error	36	2471.9	68.7			

CV = 33.4%

Table A.11. Analysis of variance of the germination percentages of the pollen of the tetraploid guayule plant N2 tested on the media containing different concentrations of B and Ca.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	47	2246.5				
B	3	929.5	309.8	67.3**	2.9	4.4
Ca	2	214.9	107.4	23.2**	3.3	5.2
B x Ca	6	934.6	155.7	33.8**	2.4	3.4
Error	36	167.5	4.6			

CV = 10.0%

Table A.12. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-48 tested on the media containing different concentrations of B, Ca, and K.

Source of Variation	df	SS	MS	Observed F	Required F 5%	1%
Total	95	12608.2				
B	3	349.5	116.5	1.0	4.8	9.8
Ca	2	340.7	170.4	1.5	5.1	10.9
Error	6	690.2	115.0			
K	1	71.6	716.0	1.2	4.0	7.0
B x K	3	1336.0	445.3	7.2**	2.7	4.1
K x Ca	2	1647.2	823.6	13.3**	3.1	4.9
B x Ca x K	6	3721.0	620.6	10.0**	2.2	3.1
Error	72	4452.0	61.8			

CV = 35.4%

Table A.13. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-49 tested during 2 days on the media containing different concentrations of B, Ca, and K.

Source of Variation	df	SS	MS	Observed F	Required F 5%	Required F 1%
Total	191	24400.9				
Day	1	1329.3	1329.3	8.5	10.1	34.1
B	3	73.5	24.5	0.2	9.3	29.5
Error	3	468.7	156.2			
Ca	2	318.2	159.1	0.3	4.5	8.6
B x Ca	6	3750.6	625.1	1.2	3.6	6.4
Error	8	4128.2	516.0			
K	1	293.5	293.5	7.5**	3.9	6.7
K x Ca	2	918.6	459.3	11.8**	3.0	4.7
B x K	3	22.5	7.5	0.2	2.6	3.9
B x Ca x K	6	6664.8	1110.8	28.5**	2.1	2.9
Error	156	6433.0	39.0			

CV = 26.8%

Table A.14. Analysis of variance of the germination percentages of the pollen of the diploid guayule plant N115-52 tested during 2 days on the media containing different concentations of B, Ca, and K.

Source of Variation	df	SS	MS	Observed F	Required F 5%	Required F 1%
Total	191	9934.2				
Day	1	840.4	840.4	11.0*	10.1	34.1
B	3	396.8	132.3	1.7	9.3	29.5
Error	3	229.9	76.6			
Ca	2	406.2	203.1	4.8*	4.5	8.6
Ca x B	6	638.3	106.4	2.5	3.6	6.4
Error	8	336.2	42.0			
K	1	30.0	30.0	0.8	3.9	6.7
K x Ca	2	5.8	2.9	0.1	3.0	3.7
B x K	3	326.0	108.7	2.7*	2.6	3.9
B x Ca x K	6	542.3	90.4	2.3*	2.1	2.9
Error	156	6182.3	39.6			

CV = 58%

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