

POLLEN-TUBE STUDIES IN *GOSSYPIUM*¹

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(With Plate III and Twenty-six Text-figures)

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I. INTRODUCTION

AMICI (1824) first discovered pollen tubes on the stigma of *Portulaca oleracea*. Since that time the behaviour of pollen tubes has been studied morphologically, physiologically and genetically by many workers. It is beyond the scope of the present work to review all the literature on this

¹ Part II of thesis approved for the M.Sc. degree of the University of London.

vast subject. In general, pollen tubes have been studied by the following three methods: (1) Under *vitro* conditions: by germinating the pollen grains in artificial cultures, such as water (von Mohl, 1834), sugar solutions (Schleiden, 1849), agar-agar (Mangin, 1886), agar and sugar (Pfundt, 1909), and other media. Martin (1913), Brink (1924*a*), Gotoh (1931) and a host of others have all used these methods. (2) Under *vivo* conditions: by tracing the pollen tubes in the pistil either by dissection (Buchholz & Blakeslee in *Datura*, 1927*a, b*), or by embedding the pistil in paraffin wax, cutting sections and reconstructing the tubes from the sections (East & Park on *Nicotiana*, 1917). (3) By indirect methods: pollinating the flowers and excising the stigma and style at different intervals after pollination, and leaving the flowers to set. By calculating the number of flowers set, the number of seeds developed and examining the nature of the resulting plants, the behaviour of the pollen tubes can be inferred. Kakizaki (1930) in *Brassica oleracea*, Kearney & Harrison (1924, 1932) in cotton, and others have contributed a good deal of information on the behaviour of the pollen tubes by this method. By studying the behaviour of the pollen tubes, the causes of self- and cross-incompatibilities met with in plants have been explained.

Our knowledge of the behaviour of the pollen tubes in a large number of plants is rendered difficult for the following reasons. In the first place, in many plants, as in rice (Gotoh, 1931), cotton (Banerji, 1929), etc., it is difficult to germinate the pollen grains artificially. Even if success is obtained, the behaviour of the tubes grown under such conditions does not give a true picture of the conditions existing in the natural medium. Brink (1927), in his studies on the pollen tubes of *Linaria*, comes to the conclusion that "the behaviour of the pollen tube *in vitro* is an uncertain index of its potentialities with respect to development in its natural medium". He further finds that the growth is often quite different from that in the style of the plant. He finally states "this conclusion does not apply to germination which for many species at least appears to proceed in parallel fashion *in vivo* and *vitro*". East & Park (1918) in *Nicotiana* come to similar conclusions. Secondly, the pistils of many plants are not suitable for dissection for the purpose of studying the pollen-tube behaviour directly on the pistil itself. The length of the style and the quantity of tissue outside the conducting tissue to be dissected are important considerations in work of this kind. The method of embedding the pistil in wax and cutting sections to trace the course of the tube is tedious and is not suitable for quantitative work. Thirdly, studying the behaviour of the pollen tubes by the indirect method is also rendered

difficult, as numerous external causes affect the setting of flowers (especially in crops like cotton) and the germination of the seeds.

In the case of plants where it is possible to study the behaviour of the pollen tubes by several methods, one method acting as a check upon the other, the results obtained should be very reliable. Such a procedure has been adopted by Buchholz & Blakeslee (1930) in *Datura*. In the case of cotton it has been the experience of previous workers that the germination of the pollen *in vitro* is not very satisfactory. Badami (1922) obtained partial success by using cold-drawn castor oil for germination as a medium. Banerji (1929) tried various cultures and methods, and he finally concludes that the only requirement for the successful germination of cotton pollen is a careful control of moisture. Kearney (1923) quotes the work of Longley, who grew pollen tubes on the silks of corn and found that great care must be taken to keep the surface not too moist. Shibuya (1930) succeeded in growing pollen tubes by using 5% agar and 35% sucrose kept at 25° C. Banerji (1929) and Kearney (1923) also tried this method. No serious attempt has been made in cotton to trace the pollen tubes in the pistil by dissection. A systematic study of the behaviour of the pollen tubes, employing the method of dissection and observation of tubes *in situ* brings to light a large number of points which could not be made clear by other methods. The results obtained by making use of all three methods have also been compared wherever possible.

II. MATERIAL

The following varieties were grown in pots, in the greenhouse at the Courtauld Genetical Laboratory, Regent's Park, London, during the summer of 1937:

(1) Strain no. 1027 A.L.F. (*G. herbaceum* Linn.). Supplied by the Cotton Breeder, South Gujarat, Surat, India.

(2) Strain no. V 434 (*G. neglectum* Toderö). Supplied by the Cotton Breeder, Central Provinces, India.

(3) Strain no. 546 (*G. herbaceum* Linn.). Supplied by the Cotton Specialist, Coimbatore, South India.

(4) Strain nos. 43 F and 47 F (*G. hirsutum* Mill.). Supplied by the Cotton Research Botanist, Lyallpur, The Punjab, India.

(5) *G. barbadense* Ratto. Supplied by the Curator, Kew Gardens, London.

(6) Strain no. K 2779 (*G. arboreum* L.). Supplied by Vavilov, Russia.

I am highly indebted to the above officers for the supply of seeds. The seeds were sown in March, and flowers appeared by the end of May, in strains nos. 546, V 434, 47 F, K 2779 and *G. barbadense*. Great difficulty was experienced in getting a large number of flowers consistently. The few flowers that were obtained each day were used for dissection and examination of pollen tubes.

III. METHODS

The course of the pollen tubes in the pistil has been traced by two methods: (1) by dissecting away the outer cortex of the style and staining the central strand of connecting tissue; (2) by embedding and sectioning the pistil. The former is more desirable, as the course of all the tubes could be traced from the grain to the tip. Some of the dissection methods tried by previous workers are outlined below. Watkins (1925), Buchholz & Blakeslee (1927*a, b*), Newman (1929), Stout (1931*b*), Nebel (1931), Ayyangar (1931), and Anderson & Sax (1934) have detailed various methods for studying the behaviour of the pollen tubes and their nuclei.

Catcheside in Bolles Lee (1937) and Maheshwari & Wulff (1937) have detailed the various methods of staining pollen tubes.

In the present investigation the main object was to make the method suitable for quantitative studies. As the pistil of cotton has a fairly thick stigma and style and can stand dissection, the method adopted by Buchholz & Blakeslee (1927*a, b*) was tried. Smears of stigma stained with acetocarmine (Anderson & Sax, 1934) did not give satisfactory results. Stout's method (1931*b*) also was not successful. The method finally used is as follows:

The pollinated flowers were collected from the plants and scalded in hot water (70° C.) for 5-6 min. If the object of the experiment is to trace the pollen tubes in the stigma only, no dissection is necessary and the portion of the style not required is cut off. The stigma is next placed either in 1% aqueous magenta or in cotton blue lactophenol. After a few hours it is transferred to lactic acid if magenta is used or to lactophenol if cotton blue is used, and left overnight. On the next day, the material is changed to fresh lactic acid or lactophenol according to the stain used, and it is next placed on a slide using lactic acid or lactophenol as medium. A thick cover-glass (no. 1) is placed on the stigma and gently pressed to spread the tissue. The pollen tubes take a purple colour with magenta and blue colour with cotton blue, while the tissues of the stigma will be almost colourless. The slide is kept in a covered place for some days

before sealing, which was done by using solutions of dammar in chloroform and xylol (Semmens, 1937). Dammar in chloroform was first painted with a brush round the edge of the cover-glass. A final coat is given of dammar in xylol. Glycerine jelly can also be used.

If the pollen tubes have to be traced in the style, dissection of the outer cortical tissue is necessary. This operation is difficult. The length to be dissected depends upon the variety of cotton, as some have long styles and fairly thick cortex, while others have a very short style. The vascular tissue does not offer a great obstacle in this case as there is not much in the style (Text-fig. 4).

If the object of the experiment is to study the entry of the tubes into the cavity of the ovary, the matter is simpler. At a suitable time after pollination the ovary is collected and its outer wall removed. The ovules inside the cavity are also removed and the micropylar ends of the ovules examined under a microscope for the pollen tubes. The placental tissue is removed from the cavity of the ovary with the help of a scalpel and needles, and stained either with cotton blue in lactophenol or with magenta (1% aqueous) and gently teased and mounted in lactophenol if the former stain is used, or lactic acid if the latter. To a trained eye, staining is not necessary to make out the pollen tubes running on the placental tissue in the ovary.

Of the two stains mentioned above, cotton blue in lactophenol is the more desirable, as the differentiation is better and does not fade; but the differentiation is improved after some days. With magenta, the tubes are more transparent, and the contents, such as callose plugs, etc., could be seen clearly. But the stain fades after some time.

By the present method the rate of growth of the pollen tubes could be determined, either by measuring the lengths of the tubes in the style and stigma or by excising the stigma and style at various intervals after pollination and examining the placental region for pollen tubes after 24–26 hr. in the ovary. Instead of excision of style and stigma, the ovary may be collected at different periods after pollination and killed in acetic alcohol.

Though the present method is identical with that of Buchholz & Blakeslee, it differs in one respect. The material after scalding in hot water should not be preserved in formalin and alcohol. When this was done the whole tissue took a deep colour in subsequent staining, and it is hard to differentiate the pollen tubes from the papillate cells of the stigma.

IV. STRUCTURE OF THE PISTIL

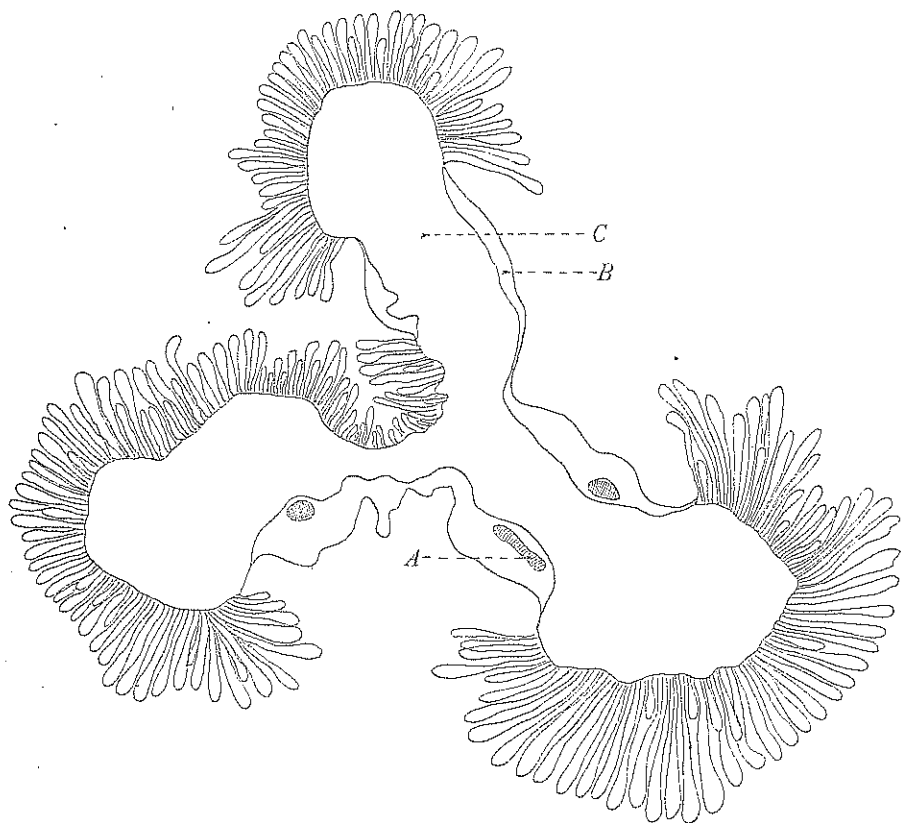
This paper is limited to a study of the course of the pollen tubes in the pistil. No attempt has been made to interpret the structure of the pistil. Gore (1935) and Saunders (1936) give an account of the morphology of the cotton flower. Balls (1905) and Gore (1932) describe the female gametophyte. Beaumont (1927), in his studies on apple, discusses the various views held regarding the conducting tissue through which the pollen tubes pass.

From the point of view of the pollen tube behaviour, the pistils of Angiosperms can be classified under three heads: (1) Solid: the style contains an outer cortex and an inner core of conducting tissue made up of thin-walled elongated cells, as in *Datura* (Buchholz & Blakeslee, 1927*b*). (2) Hollow, as in *Hemerocallis* (Stout, 1921), *Pontederia* (Smith, 1898), *Erythronium* (Schaffner, 1901), *Fagopyrum* (Mahonoy, 1935), etc., in which the style is hollow and the cavity lined by glandular cells. The pollen tubes run free in this cavity and are nourished by the glandular cells. (3) An intermediate condition is seen in *Campanula* (Barnes, 1885) and *Juglans* (Navashin, 1900), where the pollen tubes pass down the styler tissue around the tube. In cotton the pistil is solid and the tubes run in the central conducting tissue as in *Datura* (Text-figs. 2-5). The pistil of cotton has a distinct ovary made up of 3-5 locules, a style of varying length, and a stigma with 3-5 lobes. The length of the stigma also varies with the varieties. The number of lobes in the stigma and the number of locules in the ovary are highly correlated.

The stigma is made up of a central parenchymatous conducting tissue bounded on the outside by the epidermal papillate cells. As we go down the stigma, the cortical cells gradually invade between the epidermal cells and the conducting tissue so that the latter becomes limited to the central area (Text-figs. 1-3). The vascular tissue lies in the cortical area outside the conducting tissue. In certain varieties (as in strain no. K 2779), besides the epidermal papillate cells unicellular hairs arising from the epidermis are also present. The presence of such hairs often serves to identify the strains. Hairs may also arise on the stigma under certain pathological conditions.

The papillate cells of the stigma appear peculiarly bulged and assume various shapes. This bulging appears to be natural. If fresh stigmas are cut and the sections stained with Sudan III containing a little KOH, the bulges are brought out clearly. The papillate cells contain starch granules and oil globules. Starch was tested by iodine

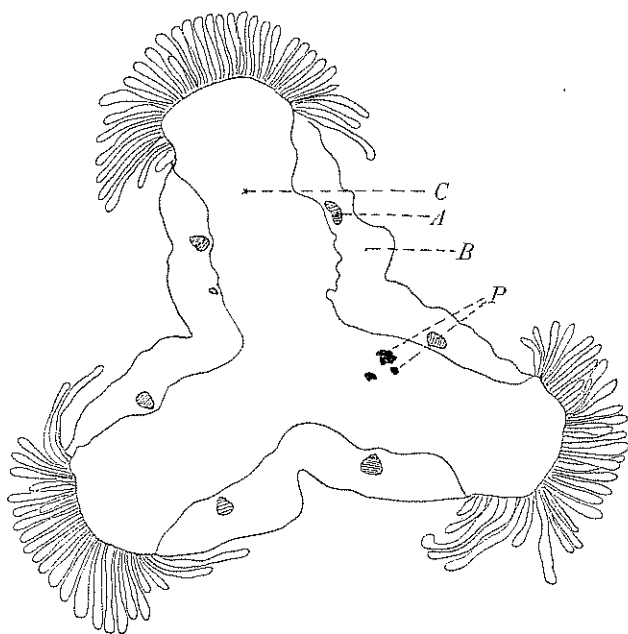
solution with chloral hydrate. The starch was stained bluish violet-brown. In safranin and gentian violet preparations the starch granules were stained red and violet respectively. The oil globules were tested by Sudan III. Seeds of *Ricinus* and *Gossypium* were used for comparison, grades of pink to orange-yellow colours being obtained. The cell walls of



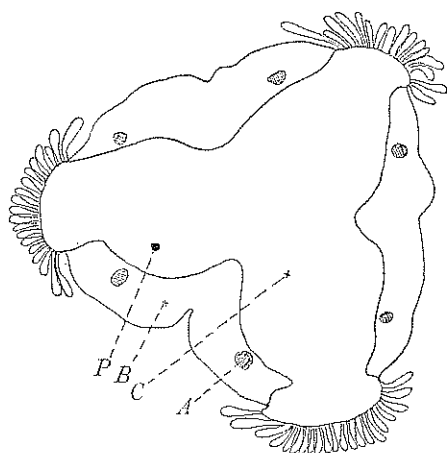
Text-fig. 1.

the papillate cells appear stratified, and pore-like areas are seen (Text-fig. 8). Stout & Chandler (1933), in *Hemerocallis*, report the presence of protuberances in the papillate cells of the stigma. According to them these cells are covered by a layer of mucilage and serve to supply moisture to the germinating pollen grains. Martin (1913) in *Trifolium* says that the exposed portions of the papillate cells of the stigma show a heavy cutinized wall.

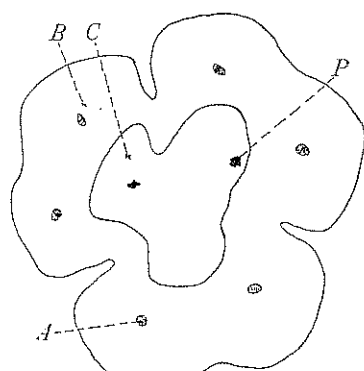
A careful microchemical study is necessary to find out the exact nature of the cell wall. The papillate cells in the present case appear to



Text-fig. 2.



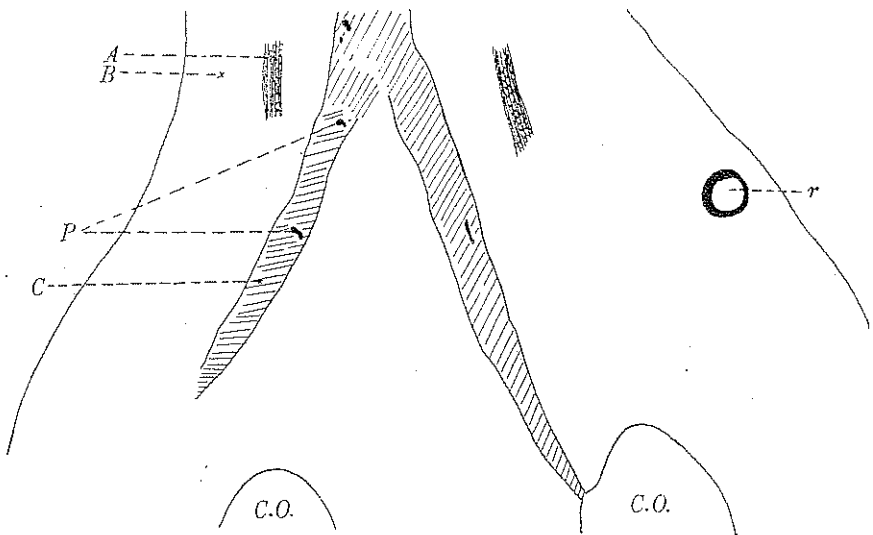
Text-fig. 3.



Text-fig. 4.

have some influence on the germination of the pollen grain. The experiences of Banerji (1929), Longley (quoted from Kearney, 1923) and Shibuya (1930) have shown that for germination of cotton pollen a

careful control of moisture is necessary. Martin (1913), in his studies on alfalfa, comes to the conclusion that the requirement for germination of the pollen depends upon a certain ratio between the moisture delivered by the stigma and the moisture of the air surrounding the stigma. These observations show that the nature of the stigma in the case of cotton also should be such that it must have a contrivance to regulate the moisture for the proper germination of the pollen grains. Mucilaginous layers have the property of storing moisture. The mucilage swells in water, is insoluble in alcohols and is stained with methylene blue and gentian violet. All these properties are seen in the case of cotton. In all probability the cell walls are mucilaginous.



Text-fig. 5.

Ziegenspeck's (1926) observation on the stigmas of *Alopecurus* and *Phleum* is interesting in this connexion. According to him the stigmatic cells of these grasses have pores on their walls and are loaded with starch. These pores form the portals for the entry of the pollen tubes, the openings being called "Amylumfenster". In the present case the tubes pass between the papillate cells (Text-fig. 9, Plate III, fig. 1).

Starch is present in all the regions of the stigma. Gore (1932) also records the presence of starch in the papillate cells of the stigma and in the cells of the style of this plant. The starch granules were of different sizes and may be either simple or compound, being made up of several granules. They are in general bigger than those of the pollen grains. The

difference in shape of the starch granules in the pollen grains has been taken advantage of by some to identify the nature of the pollen tubes in the pistil when a mixture of pollen grains having different shapes of starch granules are pollinated on to a pistil. In *Oenothera*, Renner (1919) found two kinds of pollen grains. The starch granules of *Oe. Lamarckiana* pollen are fusiform, while those of *Oe. muricata* are smaller and round. Further, by examining the pollen tubes of *gracilis* pollen (*Lamarckiana* × *muricata*) growing down a *Lamarckiana* style, he found that the *velans* type with fusiform starch granules grew much more rapidly than the *curvans* type having round starch granules. Gates (1928) says that the small fusiform starch grains are found only in the pollen grains and tubes. The starch grains in the stigma cells are always spherical and larger. But the structure of the starch grains in the haploid pollen is influenced by the sporophyte in which it develops. Thus the *rubens* pollen of *Oe. biennis* and the *gaudens* pollen of *Oe. Lamarckiana* have fusiform starch grains, but the *rubens* pollen found in *Oe. rigida* (*muricata* × *biennis*) and *Oe. laeta* (*muricata* × *Lamarckiana*) is spherical. "This again shows an influence of the cytoplasm on the phenotypic expression of the haplont genotype." In the case of cotton, an examination of several species showed no differences in shape either in the pollen grain or in the pistil. Lang (1937), working with *Gossypium punctatum*, shows that the pollen grains contain uniform tiny spherical starch granules. In the present investigation it is seen that the starch granules differ in size and may be either simple or compound.

Besides starch, oil globules are present both in the stigma and pollen grains. Fehling's test gave a negative reaction for reducing sugars with extracts taken both from the stigma and the pollen grains. Similar agreement in the reserves of the pollen grain and the stigmatic tissue has been recorded by others. Martin (1913), in *Trifolium*, found the absence of starch and the presence of fat in the form of emulsion both in the tissue of the style and in the pollen grain.

It is interesting to note that calcium oxalate crystals are seen throughout the tract of the pollen tubes in the pistil. They are in the form of druses. It is not known how far this substance is useful for pollen-tube growth in the present case. The role of calcium salts on the sweet-pea pollen tube has been emphasized by Brink (1924*b*). In concentrations of 0.02–0.002 *M*, the growth of the tube is markedly enhanced. $MgCl_2$ has similar action on *Nicotiana*. According to him these salts do not affect the wall of the pollen tube; presumably they act on the protoplast itself. Their beneficial effect is related to the changes effected in the

permeability of the protoplast, and it does not act as a true nutrient. Haberlandt (1914) takes the view that the calcium oxalate deposited in the crystal sacs represents deposits of excretory products. Oxalic acid is formed by a variety of metabolic activities in plants (particularly in connexion with protein synthesis). This substance is poisonous to plants and is rendered innocuous by combination with calcium to form the insoluble calcium oxalate. In certain cases the calcium deposits are redissolved and once more taken into the metabolic cycle. This process, observed by a number of workers, takes place when there is a deficiency of calcium in the plant.

In the style (Text-fig. 4) the cortical tissue occupies a much greater area than in the stigma, the conducting tissue being limited to the centre. The vascular strands run outside the conducting tissue in the cortex and may be in six, eight or ten groups depending upon the number of loculi in the ovary. The conducting tissue branches in the upper part of the ovary into as many tracts as there are locules in the ovary (Text-fig. 5). In each locule there are two placentae on which the pollen tubes run. The placentation is axile and the ovules are anatropous. In the placental tissue starch, oil and calcium oxalate crystals are seen.

V. THE COURSE OF THE POLLEN TUBES IN THE PISTIL

The pollen grains after deposition on the stigma commence to germinate. Biourge (1892) first saw that the pollen tubes are the result of the evagination of the intine of the pollen grain. The number of pores on the grain in cotton has been investigated by Trought (1930) and for Malvaceae in general by Lang (1937). The tubes may emerge through any of these pores. No special secretions favouring the growth of the pollen tubes were seen exuding from the stigma. A similar observation has been made by Kearney (1923) in this plant.

As regards the time taken for the germination of the pollen grain after deposition on the stigma, Kearney (1923) states "the further assumption is made, although proof is lacking, that germination began as soon as pollen reached the stigma". To investigate this point eight flowers were pollinated at 11.45 a.m. and killed after 1 hr. The stigmas were scalded in hot water and stained by the method already described. In four cases the pollen grains had put forth tubes. The greatest length of the tube obtained for this interval was 720 μ . In the artificial cultures (35% sugar, 4% agar, at 35° C.) tubes were seen to be put forth 15-20 min. after starting. This time of germination on the stigma was, however, not

always constant, and seemed to vary with numerous environmental factors. The present observations, however, show that at least in some varieties under favourable conditions of temperature and humidity the pollen grains begin to germinate as soon as they reach the stigma. The variation in the germinating capacity of the different species and in reciprocal crosses could not be followed in the present investigation owing to the scarcity of flowers. In apple, Beaumont (1927) says that the pollen grains begin to germinate under favourable conditions within a few hours after pollination. Thus the interval between pollination and germination on the stigma varies widely in different plants.

The pollen tubes during their growth seem to make use of the reserves of the grain. In the pollen tubes grown on artificial media the contents in some cases were found to be fat. The contents of the pollen tubes running in the ovary is sometimes fat, sometimes starch and sometimes both. This may be due to the fact that in the grain both fat and starch are present. All the starch is utilized and only the fat globules are left. Renner (1919) says, that in *Oenothera* mature living pollen grains are always packed with starch, which in the active grains is gradually diminished by respiration and by transformation into fat. Gore (1932) in cotton, reports the presence of starch in the pollen tubes in the style and ovule. In Text-fig. 10 the migration of the reserve substances is shown. The contents move towards the tip where active metabolism is taking place. Brink (1924*a*) made similar observations regarding the migration of the reserve substances in the pollen tubes of *Vinca*. Here the reserves are fatty. In another paper (1924*b*) he states "the rapidity with which the fatty or reserve materials contributed by the pollen grain are digested in the growing tube on artificial media shows that the carbon requirement of the gametophyte is comparatively high. This becomes intelligible when the relative extent of the wall of the tube is considered. The carbon supply may be the most important element in the nutrition of the microgametophyte."

Another point regarding the germination of the pollen on the stigma in cotton is whether the grain can put forth tubes in the basal region of the stigma. Kearney (1923) has pointed out by excision methods that the base of the stigma in cotton is less favourable than the apex for the germination of the pollen and infers from his experiments that "the differences between the two halves of the stigma is responsible for the inferior fertilisation from basally deposited pollen". In the present investigation a large number of preparations were examined, but in no case did the basal region show pollen tubes or grains, which confirms

the inferences of the above authors. The exact cause of this failure of germination requires further investigation.

The tubes, after passing through the papillate cells of the stigma, bend down sharply into the conducting tracts of the stigma, style and ovary (Text-figs. 2-7; Pl. III, fig. 2), and in the cavity of the ovary run on the placental tissue (Pl. III, fig. 3). Some of the tubes may also enter its substance. In some cases tubes were seen to run to the base of the ovary and curve again in the opposite direction. Some of the tubes run on the funiculus from the placental region and enter the micropyle of an ovule. Gore (1932) reports that the pollen tubes often run from the funiculus to the base of the ovule and travel up the wall of the ovule to enter the micropyle. Tubes often cross from one placenta to the other. Similar observations have been made by Ayyangar (1931). The tubes starting from one lobe of the stigma need not necessarily reach the corresponding loculus. Beaumont (1927) finds in apple that tubes which enter one locule may pass through the suture of the carpel into the central cavity. From there tubes may pass through the suture of other carpels and fertilize the ovules in them. A continuous conducting tissue between the carpels is not essential, since Knight (1917) in apple and Dorsey (1919) in plums state that pollen tubes grow beyond the ovules to the base of the carpel and even enter the pedicel of the flower.

Beaumont (1927) emphasizes the need of the stigmas for the germination of the pollen in apple. That they are essential to pollination, pollen germination and tube growth, is shown by the failure of fertilization when all the stigmas were removed from the flowers. He experienced the difficulty of rapid drying and oxidation of the cut stub of the style. He doubts the validity of the results of Namikawa (1923), who obtained a high percentage of set from pollinated destigmatized flowers. He cut the styles off close to the base and pollinated immediately. Miyoshi (1894) pollinated the cut end of the style of *Digitalis purpurea* and found that some tubes appeared at the stigma end.

In the present study some flowers were destigmatized and immediately pollinated and protected by sterilized cotton wool and tissue paper to prevent the cut style end from rapidly drying up. The flowers were examined for pollen tubes, after 24 hr., by dissecting the ovary and examining the placental region. No tubes were seen. In another set of flowers, the style was cut close to the ovary and pollinated immediately on the top of the ovary. No tubes were seen in the placental region after 24 hr. These tests show that in the case of cotton also the pollen grains do not put forth tubes readily when pollinated on the cut ends of the

style or on the top of the ovary. From this failure, the conclusion that the stigma is necessary for pollen germination on the pistil cannot be drawn, for Buchholz *et al.* (1932), in their "splicing" experiments on *Datura*, have emphasized the great care to be taken in performing the operation as quickly as possible and supplying the requisite humidity for germination. East & Park (1918), from their experiences on *Nicotiana*, say that it is possible to obtain pollen-tube penetration followed by fertilization in a decapitated style by the use of proper germinating medium.

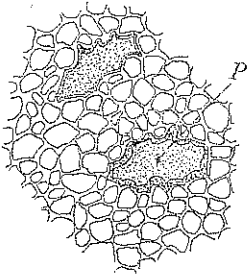
VI. BEHAVIOUR OF THE POLLEN TUBES IN THE STIGMA AND STYLE

As already stated, pollen tubes may be emitted from the grain through several pores. Emission of several tubes from the same grain was seen both *in vitro* and *in vivo*. Andronescu (1915) used the term "pseudogermination" in his studies on *Zea*, and similar behaviour was noticed in cotton. By pseudogermination is meant the sudden ejection of the contents of the pollen grain when it is placed in water or in dilute sugar solution. Kearney & Harrison (1932) describe this phenomenon in cotton as "ejection". These authors in their tests on pollen viability utilized this phenomenon, for it was seen that neither the extraordinarily big nor very small grains burst when placed in water. Gotoh (1931) in his studies on *Hyacinthus* says that in 8-chromosome types one tube per grain is common, while in the types having 12 chromosomes, two tubes per grain are common. In the present investigation an examination of the pollen grain of the diploid Asiatic and the tetraploid American cottons regarding the number of tubes per grain *in vivo* showed the following results:

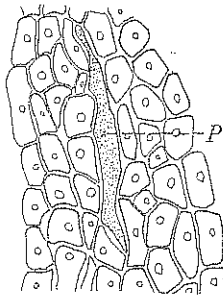
Type of cotton	% of grains with two tubes
<i>G. hirsutum</i> , 47 F (4 <i>n</i>)	68
<i>G. herbaceum</i> , (546) (2 <i>n</i>)	25

Development of two or more tubes per grain has been recorded in a number of plants: *Hibiscus trionum* (Guignard, 1904), *Ulmus americana* (Shattuck, 1905), *Hyacinthus* (Gotoh, 1931), Malvaceae in general (Lang, 1937).

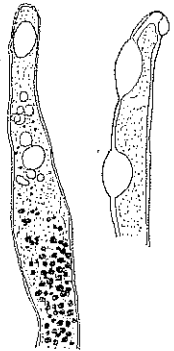
The nuclear migrations in the pollen tubes could not be investigated intensively in the present study. Gotoh (1931), in *Hyacinthus*, observed that the tubes into which the nuclei migrated made normal growth and the other tubes ceased to grow sooner or later. Sometimes two tubes were seen to grow to equal length. The various aberrations in the movement of the nuclei into the tube have been studied by Brink (1924*a*) in *Scilla in vitro*. He says, regarding the nuclear movements that the behaviour



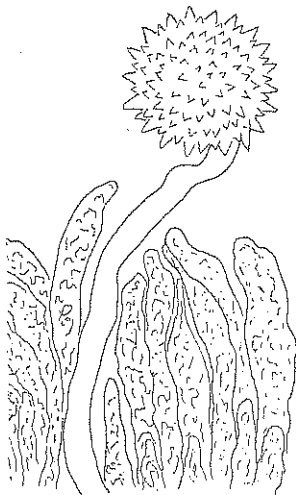
Text-fig. 6.



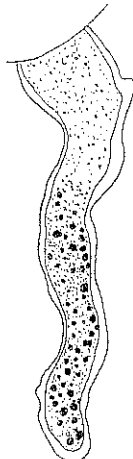
Text-fig. 7.



Text-fig. 8.



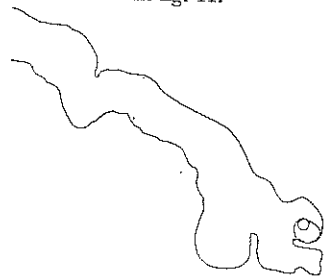
Text-fig. 9.



Text-fig. 10.



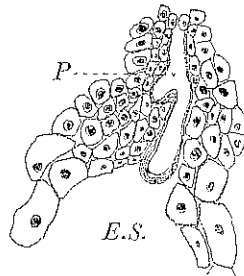
Text-fig. 11.



Text-fig. 12.



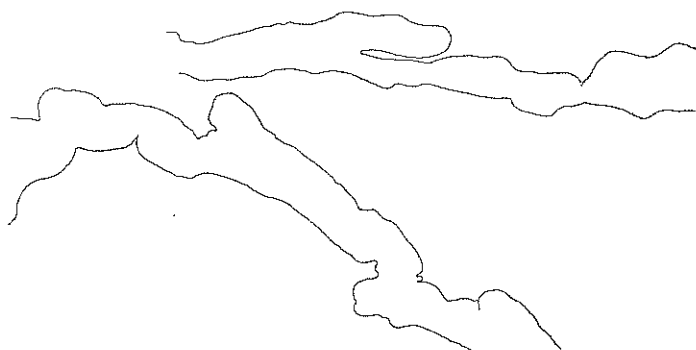
Text-fig. 13.



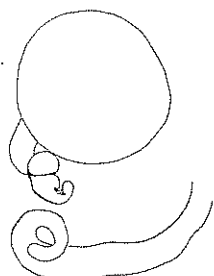
Text-fig. 14.



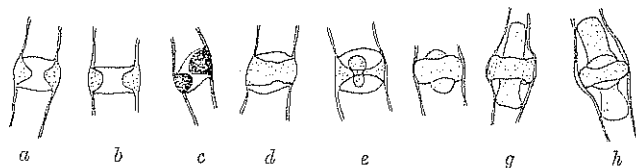
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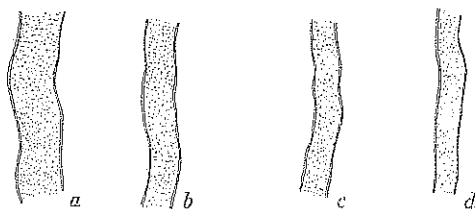
Text-fig. 16.



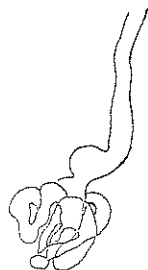
Text-fig. 17.



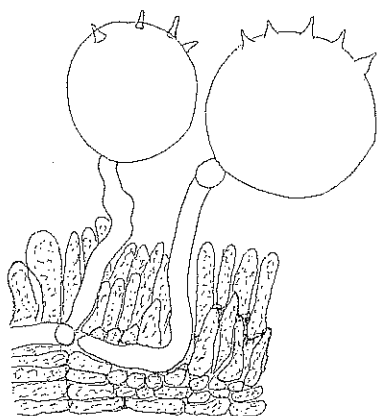
Text-fig. 19.



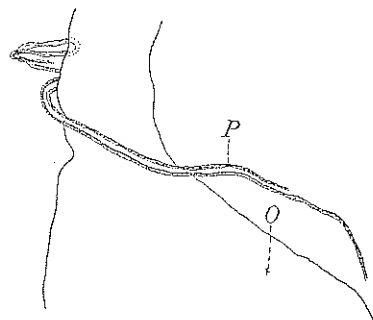
Text-fig. 20.



Text-fig. 18.



Text-fig. 21.



Text-fig. 22.

is similar *in vitro* and *in vivo*. Though it was not possible to observe the migration of the nuclei in the present case, in some of the preparations several points regarding the tube and generative nuclei were noted. In one preparation made by embedding the style in paraffin wax and staining the sections with gentian violet and Bismarck brown, a tube with a dilated end was seen (Text-fig. 11). This contains two vermiform bodies and a number of small fragments. This case resembles very much the figures of Anderson & Sax (1934) in *Tradescantia*, where the pollen tube is shown with a dilated end containing the vermiform male nuclei and a tube nucleus. In all probability the bodies seen by me are the two male nuclei and the tube nucleus degenerating. In this case the tube must have ceased to grow, as indicated by the dilated end and the nuclei at the tip. Examination of more slides showed the presence of such nuclei at the tip of the tube. In another preparation made by dissection, caught in one of the processes of the pollen tube running in the style, a nucleus-like body was seen. This appears to be the tube nucleus (Text-fig. 12). Gotoh (1931) records cases where the nuclei have been caught by the callose plugs. Text-fig. 13 shows the enlarged end of the tube and the tube nucleus at a distance probably disintegrating. Vermiform male nuclei in the pollen tubes have been seen in *Helianthus* (Navashin, 1900), *Iris* (Guignard, 1899), *Scilla* (Brink, 1924a), *Tradescantia* (Anderson & Sax, 1934), etc.

The wall of the pollen tube shows characteristic lamellae (Text-fig. 14). Ishikawa (1918) made a study of the apical portion of the pollen tube of *Oenothera*. The wall was found to contain cellulose and pectic substance. After the ejection of the contents, the wall of the apical portion of the pollen tube was lined with some peculiar substance which is probably of a mucilaginous nature.

The surface of the tubes when passing through the conducting tracts of the pistil, placenta and mucellus, is folded, but when running superficially on the substratum, as in the loose papillate cells of the stigma, on the placental tissue or on agar plates, it is smooth (Text-fig. 9 and Pl. III, fig. 3). In the former case the wall of the tube puts forth processes during growth (Text-figs. 15, 16). These processes may be short or long. In one case the process was abnormally long and formed practically a branch (Pl. III, fig. 4). Branching of the pollen tube has been recorded not uncommonly both in angiosperms and gymnosperms. Coulter & Chamberlain (1904) conclude that the branching is doubtless a common phenomenon, probably associated with the rhizoidal habit, but free branching seems to be characteristic of chalazogamic forms. In cotton,

the entrance of the pollen tube is through the micropyle, but in one case a pollen tube was seen invading the base of the ovule. Renner in *Oenothera* (1919) found that the pollen tubes grown on cultures of various solutions containing NaCl or cane sugar were frequently much branched and abnormal. In the present case, the branching tendency was found to be less in the rapidly growing tubes but very pronounced in the slow-growing ones.

The tip of the tube is often slightly dilated owing to the density of cytoplasm and food materials. In some cases the tubes showed rhizoidal processes (Text-fig. 12). Often the tips were abnormally swollen (Text-figs. 11, 13). Buchholz & Blakeslee (1927*b*), in *Datura*, found that a large number of slow-growing tubes had swollen tips, which tended to burst. Pollen tubes of *Datura meteloides* grow normally in the styles of *D. Stramonium*, but in the reciprocal cross the tubes tended to swell and burst. Similar results were seen in *D. Stramonium* pollinated by its tetraploid mutant. On the other hand, Anderson & Sax (1934) say that in *Tradescantia* the ends of the pollen tubes assumed various shapes, sometimes swollen, often of the same diameter as the tube itself, and sometimes pointed. "On the whole there seemed to be an association between pointed ends and incompatible matings although apparent exceptions were noted." In the case of cotton the tip is normally slightly swollen, but abnormally swollen tips are invariably the slow-growing ones.

The pollen tubes showed various types of coiling during their growth in the pistil. The coils may be either at the tip of the tube or in the middle region, and may start either very early and close to the grain (Text-fig. 17), or very late as in the cavity of the ovary (Text-fig. 18). Such coiled tubes were seen in large numbers in the case of tetraploid pollen placed on the pistil of a diploid variety. According to Stout (1931*b*), in *Brassica pekinensis*, coiling of the pollen tubes is the cause of self-sterility. He saw coiled tubes in all the regions of the pistil. The number of coiled tubes and their distribution in the various regions thus give an index of the degree of incompatibility between the types crossed. Brink (1934) found in *Melilotus officinalis* that in the case of incompatible self-pollinations fewer grains germinate and only a few of these enter the style. The rest curl among the papillate cells of the stigma.

VII. GROWTH RATE OF THE POLLEN TUBES IN THE PISTIL

In the present investigation it was not possible to determine the time taken for the pollen tubes to traverse the various regions of the pistil. It may be mentioned, however, that in a large number of cases examined under the conditions of London, in the greenhouse, with a given temperature and humidity, the pollen tubes did not pass beyond the region of the stigma when pollinated between 10 a.m. and 12 noon (depending upon the time of bursting of the anthers), and killed at 6 p.m. of the same day. On the next day, at 10 a.m., when the ovary was examined in the placental region, some of the quicker growing tubes had already entered this region and even entered the ovules. In some preliminary experiments made by me in India, at Coimbatore, with *Gossypium hirsutum* (strain Co. 2) during summer, by pollinating flowers at 10 a.m. and excising the various regions of the pistil after various intervals and examining for pollen tubes in the cavity of the ovary, the results were as follows:

Region of the pistil traversed	No. of hours taken
Stigma	6-7
Mid-region of the style	9-10
Full length of the style	12-13
Mean length of the style, 24.7 mm.	

The pollen tube reached the cavity of the ovary as early as 3 a.m. on the next day when the flowers were pollinated at 10 a.m., on the date of flower opening. Banerji (1929), working with the same material and in the same locality (Coimbatore), determined the rate of growth of the pollen tube by excising the style close to the ovary and taking counts of the number of bolls set and the number of seeds developed per locule. At 10 hr. he obtained 2% of bolls set, with a mean number of 2.9 seeds per locule. At 12 hr. he obtained 30% of bolls set with a mean of 4.1 seeds per locule. He concludes that the time taken to traverse the style is 10-12 hr. from pollination. Thus the results of my experiments and that of the above worker agree, barring slight differences produced by environmental causes. The fact that only 4.1 seeds have developed out of the mean of 8.35 seeds per locule in this strain shows that only the quickest tubes have had time to pass the excision region within the above intervals and is also an indication of differential growth rate of pollen tubes even within a pure strain.

Balls (1905) states for Egyptian cottons, that the pollen tubes were seen in the embryo sac after 30 hr. from the time of pollination. Kearney (1923) estimates a growth rate of 1.75 mm. per hour in the case of Pima cotton. The lengths of the fifteen pollen tubes determined after 9½ hr.

from the time of pollination in the case of *G. hirsutum* (47 F), when self-pollinated on an emasculated flower under greenhouse conditions in London, were found to be as follows: length in mm. 1.60, 1.20, 0.80, 0.63, 0.56, 0.49, 0.40, 0.40, 0.31, 0.30, 0.28, 0.27, 0.26, 0.25 and 0.19.

Thus pollen tubes of very different length were obtained. In general, differential growth rate of the pollen tubes may result from several causes. Intensive work on this subject has been done by various workers and only a few of the papers will be cited.

Differences in the chromosomal constitution of the pollen grains may bring about differential growth rate of the pollen tubes. Blakeslee (1928) has shown such behaviour in *Datura*, and Heribert-Nilsson (1911) found that the interval between pollination and fertilization when pollen of *Oenothera Lamarckiana* and the tetraploid *gigas* is placed on the stigma of *Oe. Lamarckiana* is about 19 hr. for *Oe. Lamarckiana* and 21 hr. for *Oe. gigas*.

Differential growth rate is also influenced by genetic causes. As shown by Jones (1920, 1922) and Brink & Burnham (1927) in maize, Buchholz & Blakeslee (1936) in their studies on the effect of radium treatment in *Datura* come to the conclusion that the pollen tube abnormalities in irradiated plants are mutational. They have described five abnormalities in pollen-tube growth. Up to 1931 the authors have recognized more than 40 genes affecting pollen tube growth.

Self-sterility is due in many cases to slow growth of the pollen tubes. East & Park (1918) on *Nicotiana*, Anderson & Sax (1934) on *Tradescantia*, and others have described the various factors inhibiting the growth of the pollen tube in self-pollination. Crane (1926) in plums, cherries and other fruit trees finds that self- and cross-incompatibilities are due to retarded pollen tube growth. Brieger (1927) also came to similar conclusions. The phenomenon of certation, observed by Sirks (1926) in *Datura*, is ascribed to differential pollen-tube growth rate. The term "certation" was introduced by Heribert-Nilsson (1920) to designate the competition between the pollen tubes in his studies on *Oenothera*.

In cotton, differential length of the pollen tubes in a self-pollinated flower of a pure strain may be due to differences in the germination of the pollen grains. When a mixture of pollen grains of diploid Asiatic types (as *herbaceum* and *indicum*) are placed on the stigma of either *herbaceum* or *indicum*, the differential growth rate may also be caused by genetic differences in the determiners for pollen tube growth. The question of self-sterility does not seem to arise in this crop. The species within the diploid as well as those within the tetraploid series freely cross. In the

present investigation, when the pollen of a diploid species is placed on the stigma of a tetraploid species, and vice versa, a large number of pollen tubes were seen freely entering the cavity of the ovary and even the ovules, though a large number showed such abnormalities as swollen tips, etc. Zaitzev (1923), Skovsted (1934) and others obtained successfully interspecific hybrids between the Asiatic and American cottons. Feng (1935), in his studies on species hybrids of Asiatic and American species, says that the low percentage of hybrids obtained in these crosses was not due to retarded pollen-tube growth but probably to some incompatibilities of the gametes.

VIII. CALLOSE PLUGS

Since the pollen tubes in cotton have to traverse a great length of the style and also the cavity of the ovary, before they enter the ovules, the advancing tips of the tubes are shut off from the older portions of the tube by the formation of callose plugs. The formation of these plugs was fully described by Strasburger in 1878 (cited by Gotoh, 1931) in *Gloxinia* and by Elfving in 1879. The plugs are seen very clearly in the dissected material. In the tubes grown on artificial medium, as well as *in vivo*, the regions of the plugs could be made out by the external grooves caused by the growth of the callose rings, from around the wall of the inside of the tube (Text-fig. 13).

The various stages in the development of the plugs are illustrated (Text-fig. 19). They appear to grow centripetally, and after the completion of the partition across the tube lateral processes are formed on either side of the partition. Sometimes the process is developed on one side only. The greatest length of this process is about 44μ , which is about 3-4 times the diameter of the tube. In *Thea Japonica*, var. *Spartiana*, Gotoh (1931) found a plug of 90μ in one case. Secondary plugs form between the primary plugs and at later stages it is hard to distinguish them. Other work on these plugs is cited by Gotoh. The first plug is formed at a distance from the grain which ranges from 160 to 300μ . In *Antirrhinum majus*, Gotoh (1931) records a variation from 174 to 522μ , *in vitro*. In cotton, the distances between the successive plugs were measured in pollen tubes in the style, and were found to be more or less regular within a tube but varied from tube to tube as seen below in three cases:

Pollen tube	Distances in μ between the callose plugs
1	250—200—214—213
2	426—450—525—438
3	288—263

The function of these plugs has been described by Brink (1924 *a*). They seem to shut off the developing tip from the rest of the tube, thus limiting the region from which the vegetative cells absorb nutritive materials from the less exhausted portions of the style. In his experiments on *Vinca*, Brink (1924) found that when the pollen tube is cut off after the callose plug formation, the growth of the tip still continued and the type of growth curve did not alter.

Newman (1929) in *Acacia* traced the pollen tubes by following the callose plugs by staining with orange G and gentian violet. Gold plugs in violet tubes were obtained. In staining reactions he says that the plugs appear to be cellulosic and not much different from the walls. But from their irregular appearance, pitted nature and differential staining he concludes the possibility that they also contain some waste material. The ability to take a differential stain is due to the thickness of the wall. Ishikawa (1918), in his studies on *Oenothera*, says that callose is present in the upper part of the tube as the main component of the plugs.

IX. THE WIDTH OF THE POLLEN TUBE AND ITS RELATION TO THE SIZE OF THE POLLEN GRAIN

Kearney & Harrison (1924) refer to unpublished work of Dr Longley showing that the pollen tubes from the smaller sized pollen grains of upland cotton (*Gossypium hirsutum*) are about half the diameter of the tubes formed by the larger grains of Pima cotton (*G. barbadense*). This raises two questions. First, how far the size of the tube is a varietal feature. Secondly, whether there is any correlation in a species between the size of the grain and the width of the tube given off by it. As already pointed out, the diameter of the tube is uniform during the earlier part of its growth in the stigmatic papillate cells, and again later in the cavity of the ovary, when it runs on the surface of the placental tissue. To answer the first question, pollen tubes running on the placental tissue were killed in chromic-acetic-formalin and preserved in 70% alcohol. They were then brought down gradually to water, then to pure lactic acid for examination. Only those tubes that had been disturbed least by fixatives were taken into consideration. The pollen tube diameters of the three Asiatic and the three American species of cotton were measured. The results are tabulated below, and camera lucida drawings of one tube belonging to the mode of four species where a large number of readings were available are illustrated (Text-fig. 20) for comparison.

From the table it is seen that the pollen tubes of the tetraploids are in general wider than those of the diploids. Minor differences are also seen

between the species in both the groups. The pollen tubes of *G. barbadense* are significantly bigger than those of *G. arboreum*. The difference in the size of the pollen tubes, where they could be easily measured at the region of the placenta, can be applied in identifying the pollen tubes of different varieties and in deciding the pollen tube competition when grains showing such different sized tubes are mixed and applied to the pistil of a given variety. To quote one instance in cotton, Kearney & Harrison (1924), by their indirect investigations, have come to the conclusion that when a mixture of self and foreign pollen is applied to the pistil of a given type, a greater percentage of homozygotes are produced in the resulting progeny and the cause of this type of selective fertilization is due to "pollen antagonism", whereby the germination of the foreign pollen is hindered in the presence of self-pollen on account of some inhibiting reaction set up by the self-pollen. This question will be dealt with later. The

TABLE I

× 6 eyepiece, 1/6 in. objective. Diameter of the pollen tubes in degrees of the ocular micrometer

Species	× 6 eyepiece, 1/6 in. objective. Diameter of the pollen tubes in degrees of the ocular micrometer																Total	Mean		
	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88			92	96
<i>G. arboreum</i>	1	8	4	13	19	10	5	1	1	62	43
<i>G. herbaceum</i>			2	15	21	22	15	4	1	80	46
<i>G. neglectum</i>			2	1	1	11	9	2	26	52
<i>G. hirsutum</i>				2	2	13	22	36	6	4	2	87	56	
Egyptian cotton					3	5	7	4	6	.	3	28	57	
<i>G. barbadense</i> Kew						11	12	7	26	17	7	.	4	8	4	.	1	97	66	

identification of the pollen tubes in the ovary could not be taken up in the present investigation for want of material.

To answer the second question, the diameter of the grain and of the pollen tube given off by it were measured in strain no. 47 F (*G. hirsutum*). No significant correlation was found between the two characters. Thus the size of the tube appears to be a varietal feature. At the same time, the pollen grains of the tetraploids are in general bigger than those of the diploids, as shown by Banerji's (1929) measurements.

Similar differences in size were also seen by me (Table II). Thus the polyploid species have larger pollen grains and tubes.

X. POLLEN ANTAGONISM IN COTTON

As already pointed out, Kearney & Harrison (1924) came to the conclusion that in the case of cotton, when a mixture of self and foreign pollen is applied to the pistil of given species, self-fertilization was preponderant. In an open-pollinated flower a large percentage of the seeds formed were found to be homozygotes, though the flower is well adapted

for cross-pollination. The authors conclude that the cause of homozygosity in the resulting progeny is "pollen antagonism", whereby the presence of like pollen in some way prevents the germination or subsequent development of many of the pollen tubes of the foreign strain when both kinds are present on the stigma. The further assumption is made, "in spite of this unfavourable condition, some of the unlike pollen grains are able to accomplish fertilization, possibly because they are more resistant, possibly because they happened to be so placed as to avoid the tracts of the stigmatic tissue with the like pollen". The authors based their results on indirect methods: (1) by excising the styles and stigmas and taking counts of bolls set and seeds developed; (2) by collecting seeds from the bolls and examining the plants formed by the seeds in the upper and lower halves of the capsule on the assumption that the seeds of the upper halves are fertilized by the faster growing pollen tubes and those of the lower halves by the slower growing tubes.

By the present method of examining the pollen tubes directly on the stigma and also as it is easy to see the pollen tubes entering the ovule, these assumptions could be verified. In the present investigation, observations were limited to two points. In the first place an attempt was made to find out if by placing a mixture of pollen grains of different sizes as *G. herbaceum* and *G. barbadense* on the stigma of *G. herbaceum* both could germinate. Secondly, observations were made to find out whether there is any regular order in the entry of the pollen tubes into the ovules according to their position in the locule.

(a) *The behaviour of the mixture of self and foreign pollen grains as regards germination on the pistil*

Kearney & Harrison (1924) assume from their experiments that the degree of antagonism is proportional to the degree of consanguinity between the pollens mixed, and come to the same conclusions as Jones (1928) in maize, in his studies on selective fertilization. In the present investigation, pollen of widely different groups, as Asiatic and American types, were taken as materials for mixture, so that the size of the grain could be utilized for identification. The pollen grains of Asiatic cottons used in this investigation are yellow and smaller. In the American group the *hirsutum* cotton has a creamy white pollen of larger diameter. According to Kearney & Harrison (1924), the chances of germination of the extremely small and extraordinarily big grains in a given variety are remote, as they fail to burst in water. Hence in a mixture the pollen tubes starting from the larger sized grains must be from the American

groups and the smaller sized from the Asiatic ones. The colour of the grain cannot be utilized as a reliable guide, as it is hard to make out the two kinds of pollen in treated material. The results are given in Table II.

TABLE II

Diameter of the grains from which the pollen tubes were given off, in degrees of ocular micrometer (6 ocular and 1/6 objective)

Nature of the pollen	62	66	70	74	78	82	86	90	94	98	Total
Pollen of 546 only (<i>G. herbaceum</i>)	.	7	14	16	10	.	3	.	.	.	50
43 F (<i>G. hirsutum</i>)	.	.	.	3	14	14	13	4	2	.	50
546 and 43 F	.	1	5	5	4	12	1	1	1	.	30
546 and <i>G. barbadense</i>	.	1	3	9	1	2	5	4	4	.	29
<i>G. neglectum</i> and <i>G. barbadense</i>	2	7	14	17	11	10	13	19	9	1	103

These show that when a mixture of two different pollens is applied both of them germinate. Text-fig. 21 shows the pollen tubes of *G. herbaceum* and *G. barbadense* growing side by side on the stigma of *G. herbaceum*.

(b) Order of entry of the pollen tubes into the ovules according to their position in the locule

The estimation of the pollen-tube growth rate has been based, as already pointed out, on examination of the plants raised from the upper and lower halves of the loculus separately. It is assumed that the faster growing tubes pollinated the ovules in the upper portion of the capsule and the slower growing tubes those in the lower portion. Such a behaviour has been reported by Correns (1918) in *Melandrium* and by Buchholz & Blakeslee (1930) in *Datura*. To test such an assumption by direct observation in cotton, ovules were examined for pollen-tube entry strictly according to their position in the locule from pistils pollinated at 10 a.m. on the date of flower opening, and killed after 25½ hr. The placental region also was examined to make sure of the entry of the pollen tubes into the cavity of the ovary. The results are given in Table III.

TABLE III

Variety	Position of the seeds in the locule									No. of locules examined
	1	2	3	4	5	6	7	8	9	
<i>G. hirsutum</i>	.	3	1	.	1	2	1	.	.	24
Egyptian variety	3	1	2	1	2	2	1	.	.	25
Total	3	4	3	1	3	4	2	.	.	49

Of the forty-nine locules examined, in only twenty cases had the pollen tubes entered one or two ovules, though in all cases examined a

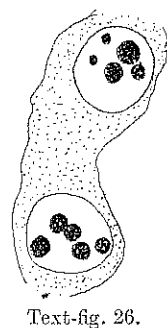
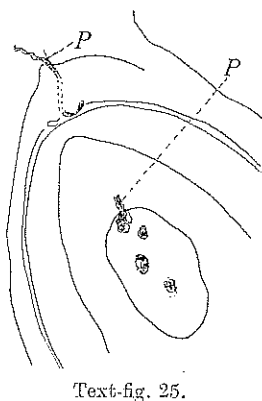
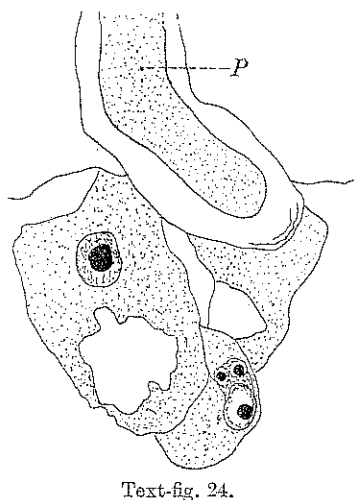
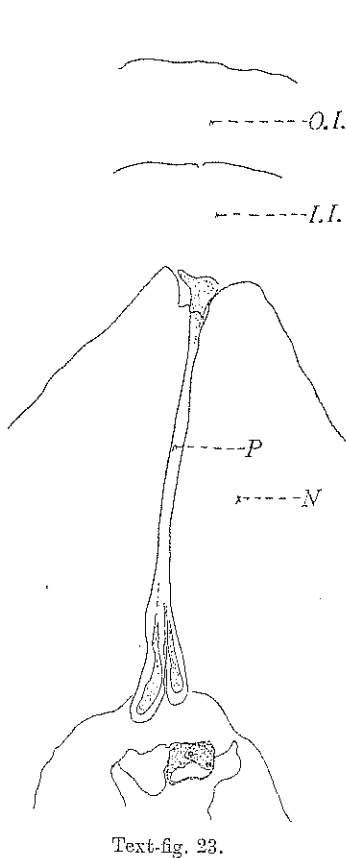
large number of tubes had entered the placental tissue. In the total column, the position of the twenty ovules in which the tubes had entered is seen to be distributed throughout the locule, excepting the last two positions. Thus in the present case, the earliest tubes need not enter always the uppermost ovules. The order of entry does not seem to depend upon the position of the ovule in the locule. Ramanathan & Ayyangar (1932) also state that the ovules nearest the stigma need not necessarily be the first to receive the pollen tubes. According to these workers in one of the varieties of *G. hirsutum*, in a large number of cases, the first tubes seem to enter the ovules of the third position. In the present case, such a mode was not obtained (the readings, however, are few). If we tentatively class the first four ovules as belonging to the upper half and the remaining to the lower half, of the twenty cases in which the tubes have entered, eleven ovules belong to the upper half and nine to the lower half. These few results suggest that the upper ovules are little if any more likely to be fertilized than the lower ones. Kearney & Harrison (1924, 1932), in their experiments, failed to find any difference in the proportion of hybrids from the two halves of the capsule.

XI. NUMBER OF POLLEN TUBES ENTERING AN OVULE

In about 24 % of the ovules examined, more than one pollen tube was found to attack the same ovule. As many as four tubes may enter one ovule. These could be examined best under water after removing the ovules from the placenta, where the tubes are seen floating. In the preparations, made permanent, it is hard to show all the tubes as they are delicate and curl up on the surface of the ovules. In Text-fig. 22, the entry of three tubes into an ovule is shown. Ayyangar (1931) shows a photograph of a cotton ovule in which two tubes are entering. He does not, however, give the frequency of such behaviour nor the fate of the extra tubes in the ovule. Entry of several tubes into an ovule has been reported in other plants also: in *Ulmus americana* (Shattuck, 1905), *Myricaria germanica* (Frisendahl, 1912), *Juglans nigra* (Navashin & Finn, 1913), as many as five tubes to an ovule, *Xyris indica* (Weinzieher, 1914), *Myosurus minimus* (Tschernoyarow, 1915), *Oenothera* (Ishikawa, 1918), *Acacia Baileyana* (Newman, 1934), *Fagopyrum* (Mahony, 1935), and other plants.

XII. THE COURSE AND BEHAVIOUR OF THE POLLEN TUBES IN THE OVULE

For this study a large number of ovules were cut and stained with safranin. The pollen tube enters the micropyle, passing between the two integuments. It penetrates the massive nucellus (Text-fig. 23), and the tip of the tube projects into the embryo sac (Text-figs. 23-5). If the



opening of the two integuments is in the same line the course of the tube is straight. Frequently they do not coincide, and the course is zigzag. The tube sometimes puts forth processes during its passage down the integuments and nucellus (Text-fig. 14). Extensive branching of the tubes and anastomosing has been reported in *Ulmus* (Shattuck, 1905), *Hibiscus trionum* (Guignard, 1904) and other plants. In some cases the

processes are seen to invade the spaces between the two integuments. The migration of the nuclei could not be seen.

Attempts were made to follow the behaviour of the extra pollen tubes in the ovule. In one case two ends of the tubes were seen projecting into the embryo sac, of which one was slightly in advance of the other (Text-fig. 23). It is hard to say whether this is a result of the entry of two tubes or the branches of a single tube. In another case, ends of two tubes, one in advance at the embryo sac and the other between the integuments, were seen (Text-fig. 25). It looks as though only one of the tubes enters the embryo sac and functions in the act of double fertilization. The examination of the number of nucleoli in the endosperm nucleus and the fertilized egg did not show any evidences of polyspermy. In one case, two nuclei with five nucleoli in each were seen (Text-fig. 26). It is hard to interpret this situation as the size of the nucleoli varied. In one nucleus there were three big nucleoli and two small. In the other all the five were of the same size and equal in size to the big nucleoli of the other nucleus. The egg cell was not well preserved in this case.

The pollen tubes are seen to persist at the micropylar end for some days after fertilization. Such behaviour has been reported by Newman (1934) in *Acacia Beilejana*, where the tube is delicate at first, but hardens after fertilization and is easily seen projecting from the nucellus. The micropyle is formed round the pollen tube by the growth of the integuments, which takes place after fertilization. In the case of cotton, the integuments are formed before fertilization and the tube passes between them.

XIII. DISCUSSION

(a) *Relation of the pollen tubes to the conducting tissue*

The study of pollen tubes has produced considerable speculation on several points. Morphologically, the course of the pollen tubes in the pistil has been disputed. They pass through a definite tract in the pistil (Buchholz & Blakeslee, 1927 *b*, in *Datura*), to which the term "conducting tissue" is applied. Beaumont (1927) discusses the various views held regarding the "conducting tissue", and states that in the apple, the tubes pass definitely down this tract. In the ovary they may pass from one chamber to the next where there is no conducting tissue. Namikawa (1923) holds the view that the pollen tubes grow in the outer cortical tissue, from his experiments on the pollination of the destigmatized pistil in apple. Beaumont (1927), however, failed to see such behaviour in this plant. Newman (1934), in *Acacia*, says that the path of the tube in the

style is through no specialized tissue. In cotton the tubes, after passing down the style, run on the placenta and funiculus and so enter the ovule. The presence of a large quantity of nutritive material in the pistil, similar to that of the pollen grain reserves, and the processes put forth by the tubes when in contact with the tissues of the pistil, point to the conclusion that the tubes have an intimate relationship with the tissues they are traversing. Brink & Burnham (1927), in maize, hold the view that for the latter part of the growth of the pollen tube in the style, the tube gets its food material from the conducting tissue.

The relationship between the haploid pollen tubes and the diploid conducting tissue is controlled genetically and is responsible for the various cases of self- and cross-incompatibilities in plants. Anything that upsets the development of the pollen tubes causes incompatibility. The pollen tube may grow slowly and fail to reach the ovule in time. Such failure has been reported in a number of plants by many workers. Alam (1936) finds that self-incompatibility in *Eruca sativa* is due to the presence of an inhibiting substance or reaction which is absent in the buds 2 days before they open, makes itself felt 1 day prior to opening and is most active on the day of flower opening, after which its activity gradually begins to wane. In the present investigation the slow growing tubes are either abnormally swollen or coiled. Incompatibility may also occur where pollen tube growth is normal. Other causes may operate here to bring about failure of fusion of gametes.

In cotton, there is full compatibility between the species of the same chromosome constitution, viz. Asiatic with Asiatic or American with American or Egyptian groups. That the incompatibility is not due to pollen tube behaviour is seen by the fact that in the crosses the tubes travel down the style with an equal rapidity and even enter the ovules. The incapacity to form seeds may be due to failure of union of the gametes or subsequent development of the zygote. Feng's conclusion on this matter has already been pointed out.

(b) *Selective fertilization*

When a mixture of pollen grains of compatible types is dusted on to the stigma, it is often noticed that the resulting seeds and the plants derived from them belong to one of the types. This phenomenon has been reported in a large number of plants by various workers. It is due to differential growth rate of the pollen tubes, which has been observed directly by some and inferred from breeding behaviour by others. The several causes that bring about differential growth rate have already

been pointed out, as has also the type of selective fertilization described by Kearney & Harrison (1932) in cotton.

Kearney & Harrison (1932) conclude that the cause of the type of selective fertilization occurring in cotton is the development by the self-pollen of some inhibiting substance which prevents the germination or subsequent growth of the tubes. In the present investigation it has been pointed out that both types of pollen are able to germinate and the subsequent behaviour of the tubes appears to cause the uniformity of the resulting offspring. The direct observation that the pollen tubes have different diameters, as K 2779 (*G. arboreum*) and V 434 (*G. neglectum*), may help to solve this problem. Brink & Burnham (1927) have shown that the genes in waxy and sugary maize pollen control the initial growth of the pollen tubes.

The estimation of the speed of pollen tube growth rate by the examination of seeds from the upper and lower parts of the locule is not altogether satisfactory in cotton, as the ovules nearest the stigmatic end need not be the first to receive the pollen tubes. The entry of several pollen tubes into the same ovule and the invariable development of a normal seed with no indication of polyspermy probably indicate that the ovule may also exercise a selective action.

The selective action on the male gametophytes may take place throughout their course from germination to fertilization. Such behaviour has been shown by Stout & Chandler (1933) in *Hemerocallis*. Here there are three distinct types of pollen tube behaviour in self-incompatibility: (1) The reactions may be complete in the upper portions of the style. In this case the tubes travel slowly and only advance to a short distance in the styler canal. (2) In many cases of incompatibility the pollen tubes arrive early at the entrance of the ovary, but there is then an inhibition in the advance of the tubes. In such cases it appears that the tubes are reacting to secretions from the ovary. In certain cases the tubes may linger or remain stationary for several hours and then proceed into the ovary. (3) There are cases of almost complete self-incompatibility in which the pollen tubes proceed directly into the ovary without delay and yet seeds are seldom formed. In this condition the reactions of incompatibility involve either (a) the pollen tubes and the secretions of the ovary or possibly of the ovules or of the egg apparatus, or (b) the relation of the sperm and egg apparatus, or (c) the abortion of the young embryos. Reactions of incompatibility after the pollen tubes reach the ovary are to be recognized as a well-defined type of behaviour. This condition has been noted in other plants, as in apples (Cooper, 1929) and

for certain individuals of head cabbage (Sasoka, 1928). In the cyclic incompatibility of plants of *Brassica pekinensis* (Stout, 1931) powerful reactions may operate in the first flowers to open, which produce coiling of pollen tubes, but in certain late flowers the tubes may enter the ovary and yet no seeds or only a few seeds result. Yasuda (1932) concludes in *Petunia* that the substances which inhibit pollen tube growth are mainly secreted in the ovary and then move to the upper part of the stigma. In *Hemerocallis* the reactions of incompatibility may be complete in the upper part of the style in a few cases, but as a rule they are expressed at the entrance to the ovary or within the ovary.

(c) *Polyplody and pollen-tube behaviour*

The relation between polyplody and pollen tube behaviour has been attacked in several ways. A positive correlation has been established between the grain size and pollen tube length *in vitro* by Brink (1927) in two species of *Linaria* and the F_1 and F_2 hybrids between them. The larger grains in F_2 tend to give longer tubes than the smaller ones. Gotoh (1931), in *Hyacinthus*, found that with the increase of number of chromosomes there was a greater rate of growth. In forms having 8 chromosomes, the average length of the tube for a given time was 246μ , while in forms with 12 chromosomes the average length of the tube was 331μ . On the other hand, in *Trifolium* he failed to find such a relation. He concludes that the regular relation between the growth of the tube and the number of chromosomes as seen in *Hyacinthus* is characteristic of autopolyploidy, and the behaviour in *Trifolium* is a result of allopolyploidy. Buchholz & Blakeslee (1927b) found that of the two kinds of pollen of the globe mutant *Datura* ($2n=1$), having respectively n and $(n+1)$ chromosomes, the pollen tubes of the first produced a forward skew, while those of the latter produced a backward skew.

(d) *Polyspermy*

The entry of several pollen tubes into the ovule in cotton has raised several interesting points. Is there any evidence of polyspermy? What is the behaviour of the extra tubes in the ovule? Weinzieher (1914), in *Lychnis Flos-cuculi*, found two pollen tubes pour their contents into the embryo sac. The further behaviour is not mentioned. Navashin & Finn (1913), in *Juglans*, report two or three sets of male nuclei, often found ejected in a single embryo sac, but not two sperms fusing with the egg. The same was observed by Tschernoyarow (1915). Polyspermy could also arise with the entry of more than two male nuclei into an embryo sac.

Chamberlain (1897), in *Libium aurantiacum*, found three male nuclei in the pollen grain. Strasburger (1878) found in the pollen tubes of *Ornithogalum* and of *Scilla nutans* the production of four male nuclei. A case of the fusion of the egg nucleus with two male nuclei has been reported by Ishikawa (1918) in *Oenothera*. Newman (1934), in *Acacia*, found two pollen tubes penetrating one embryo sac. There was never any suggestion in his preparation of any additional male nuclei associated with the egg. Mahony (1935) found two tubes at different levels in the ovule.

In cotton it is not known whether extra male nuclei enter the sac. In one case two tubes were seen entering, but the egg apparatus was not well preserved for critical study. No evidence of polyspermy could be obtained. The extra tubes, as already mentioned, were seen at different distances down the micropyle. It is possible in these cases that no male nuclei could have entered into the embryo sac from such tubes. It looks as though the male nuclei of one tube only enters the embryo sac and functions in the act of double fertilization.

XIV. SUMMARY

1. Artificial germination of cotton pollen is difficult, and the study of the pollen tube behaviour by the excision method is not very satisfactory. The direct observation of the pollen tubes in the pistil helps to clarify certain points which cannot be analysed by other methods.

2. Several methods devised to observe the pollen tubes in the pistil are described, and also a method suitable for quantitative studies of pollen-tube growth for cotton.

3. The course of the pollen tube in the pistil is through a definite "conducting tissue" in the stigma, style and ovary. The pollen tube then runs on the surface of the placenta and ultimately enters an ovule through the micropyle.

4. The pollen grains commence to germinate almost immediately after they are deposited on the stigma. They do not germinate well in the basal areas of the stigma. During germination the pollen grains put forth several tubes. The frequency of two tubes per grain is greater in the case of tetraploid American types than in the diploid Asiatic types.

5. The pollen tubes form branch-like processes in the style. They are absent from the pollen tubes grown *in vitro* and from the parts of the tubes travelling in the loose papillate cells of the stigma and on the placental tissue of the ovary.

6. Abnormalities of the pollen tubes, such as swollen tips and coiling of the tubes, were seen in the case of pollinations of American types on the

Asiatic forms. Coiling was seen in the region of the stigma and of the ovary. The development of callose plugs is described. The width of the pollen tube is not correlated with the grain size of the pollen. But $2n$ Asiatic types in general have smaller diameter than the $4n$ American types. The pollen grains of the tetraploids are in general bigger than the grains of the diploids.

7. The type of incompatibility between the Asiatic and American cotton is not due to differential pollen tube growth but may be due to gametic incompatibility. When a mixture of $2n$ and $4n$ pollen grains is applied to the pistil of either type, both of them germinate. The fertilization of the ovules by the self pollen in the mixture appears to depend upon the subsequent behaviour of the tubes.

8. No definite order of entry by the pollen tubes into the ovule according to their position in the locule was found. The first ovules to receive the pollen tubes in the locule need not necessarily be the ones nearest to the stigma.

9. Two to four pollen tubes were seen entering an ovule, but there was no evidence of polyspermy either with reference to the egg or endosperm. The tube persists at the micropyle for some time after fertilization.

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EXPLANATION OF TEXT-FIGURES

- All figures were drawn at the bench level with the aid of a camera lucida, excepting those in Pl. III, which are photomicrographs. The initial magnification is given against each figure. Text-figs. 1-4 are reduced to 1/4 the initial magnification; and the rest to 1/3.
- Text-fig. 1. Transverse section of the upper region of the stigma showing the conducting tissue (C), outer cortical tissue (B), vascular bundles (A) and papillate cells. $\times 150$.
- Text-fig. 2. Transverse section of the middle region of the stigma. Note the conducting tissue, restricted to the centre and pollen tubes (P) running in this tissue. $\times 150$.
- Text-fig. 3. Transverse section of the lower region of the stigma. $\times 150$.
- Text-fig. 4. Transverse section of the style showing the conducting tissue in the centre and the pollen tubes in this tissue. $\times 150$.
- Text-fig. 5. Longitudinal section of the upper part of the ovary showing the conducting tissue branching into as many parts as there are locules in the ovary. C.O. cavity of the ovary; τ , resin canal. Note the pollen tubes running in the conducting tissue. $\times 90$.
- Text-fig. 6. Transverse section of the style showing a part of the conducting tissue in which the pollen tubes are invading. Note the processes put forth by the tubes. $\times 720$.
- Text-fig. 7. Longitudinal section of the upper part of the ovary showing a part of the conducting tissue and the pollen tube running at this region. $\times 720$.
- Text-fig. 8. Papillate cells of the stigma showing the peculiar bulging and pore-like areas in the wall. Starch grains are shown in one of the cells. $\times 75$.
- Text-fig. 9. Pollen tube running between the papillate cells (dissected material). $\times 220$.

- Text-fig. 10. Pollen tube grown on artificial medium (35% sugar, 4% agar at 35° C.) showing the migration of reserves to the tip of the tube from the grain. × 1440.
- Text-fig. 11. A dilated end of the tube showing two generative nuclei and the tube nucleus disintegrating (paraffin section). × 750.
- Text-fig. 12. Tip of the pollen tube showing a rhizoidal-like process and a nucleus (?) in one of the processes (dissected material). × 1440.
- Text-fig. 13. Dilated end of the tube. In the tube nucleus which was higher up in the same tube is shown (dissected material). × 225.
- Text-fig. 14. Entry of the pollen tube into the embryo sac (*E.S.*). Note the lamellate nature of the wall of the pollen tube and a process of the tube. × 220.
- Text-fig. 15. Pollen tube in the style. Note the processes put forth by the tube (dissected material). × 150.
- Text-fig. 16. Same as Text-fig. 15, at a higher magnification. × 1440.
- Text-fig. 17. Coiled pollen tubes at the region of the stigma. In one coiling is very close to the pollen grain and in the other at the tip of the tube (dissected material). × 225.
- Text-fig. 18. Coiled pollen tubes in the cavity of the ovary (dissected material). × 225.
- Text-fig. 19. Successive stages in the development of the callose plugs (dissected material). × 720.
- Text-fig. 20. Pollen tubes at the region of the placenta. *a* = *G. barbadense*; *b* = *G. hirsutum*; *c* = *G. herbaceum*; *d* = *G. arboreum*. × 220.
- Text-fig. 21. Pollen grains of *herbaceum* (small) and of *barbadense* (large) germinating side by side and the pollen tubes running between the papillate cells (dissected material). × 220.
- Text-fig. 22. Three pollen tubes attacking the same ovule (*O*). × 30.
- Text-fig. 23. Pollen tube (*P*) entering the embryo sac. Note the two ends of the tube. × 225.
- Text-fig. 24. Pollen tube entering the embryo sac and attacking one of the synergids. Note the thick wall of the tube. × 1260.
- Text-fig. 25. Pollen tubes in the ovule. Note the tip of one near the embryo sac and the other curling between the integuments. × 90.
- Text-fig. 26. First division of the endosperm nucleus showing five nucleoli in each nucleus. In one nucleus all the five nucleoli are of the same size. In the other they are of different sizes. × 1440. (Paraffin section.)

EXPLANATION OF PLATE III

- Fig. 1. Pollen tubes running between the papillate cells of the stigma. × 150.
- Fig. 2. Pollen tubes at the upper region of the style.
- Fig. 3. Pollen tubes in the cavity of the ovary, × 150, running on the placental tissue. Two tubes drawn out from the tissue for clarity, × 710.
- Fig. 4. Branching of the pollen tube running in the substance of the placental tissue. × 710.



Fig. 1.



Fig. 2.

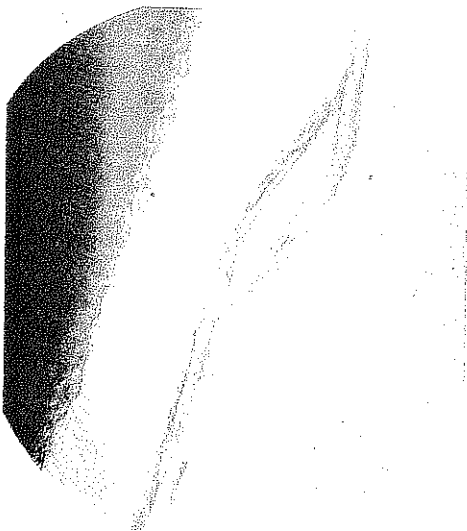


Fig. 3.

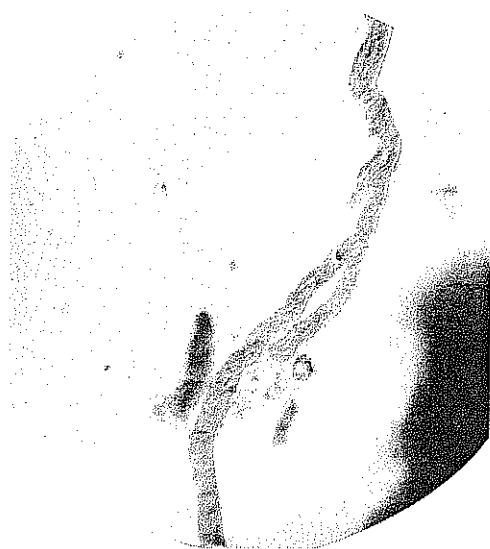


Fig. 4.