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Histological Studies on Five Male-Sterile Strains of Upland Cotton[1]

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ABSTRACT

Microsporogenesis and pollen development in one cytoplasmic male-sterile and four genetic male-sterile strains of Upland cotton (*Gossypium hirsutum* L.) were studied in comparison with a male-fertile variety.

In the cytoplasmically male-sterile Upland cotton with the *G. harknessii* cytoplasm, there was disorganization of tapetal cells and coalescence of pollen mother cells (PMC's) during the premeiotic stages.

In the Ms_4 dominant genetic male-sterile anthers, degeneration of microsporocytes was observed before or shortly after the initiation of prophase I. The tapetal cells were reduced in size in some of the anthers.

In another dominant genetic male-sterile, Ms_7, meiosis appeared normal in the majority of the PMC's. The abortion of microspores was noticed during formation of the pollen wall. There was no observable malfunctioning of the tapetum.

In the double recessive ms_5ms_8 male-sterile anthers, vacuolation of developing microspores led to their degeneration. Nutrient deficiency of several kinds might have been the cause of vacuolation and abortion of pollen.

In Rhyne's double recessive male-sterile anthers, premature degeneration of the tapetum caused the abortion of pollen in most locules. In a few locules viable pollen was produced but the anthers were nondehiscent. Seeds were produced when pollen from the indehiscent anthers was released by crushing and dusted on other male-sterile plants.

Additional index words: Cytoplasmic male-sterility, Genetic male sterility, Microsporogenesis, Anther, Tapetum, Pollen mother cells.

MALE sterility in cotton (*Gossypuim* sp.) is of interest because of its potential practical value to the cotton breeder in producing hybrid cotton. In Upland cotton male sterility can be produced by any one of several genes: ms_1 (11), ms_2 (22), ms_3 (12), Ms_4 (1), ms_5ms_8 (24), Ms_7 (25), and nondehiscent anther (21). Meyer and Meyer (15, 16) have reported a form of cytoplasmic genetic male sterility developed from certain interspecific crosses in *Gossypium*.

Relatively little is known concerning the ontogeny that leads to pollen abortion in male-sterile strains of Upland cotton. The present work was undertaken to compare microsporogenesis and pollen development in five male-sterile strains of Upland cotton (*G. hirsutum*) with a fertile strain.

MATERIALS AND METHODS

Flower buds at various stages of development were collected from the five male-sterile strains listed in Table 1 and from the male-fertile 'Coker 201' variety growing in the greenhouse during the winter of 1972-73. The buds were fixed in Randolph's Craf (10). The material was dehydrated with ethanol and tertiary-butyl alcohol series and embedded in paraffin (10). Cross sections 8 to 12 μ thick were cut and stained with Heidenhain's iron-alum hematoxylin (10). The photomicrographs were taken on panchromatic film using blue filters.

RESULTS

Microsporogenesis and Pollen Development in Male-Fertile Cotton

In the anther primordium the archesporium arises from localized areas of the subepidermal layers. The cells of the archesporium are divided by a periclinal

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Table 1. List of male-sterile strains used in histological studies.

Male-sterile character	Gross morphology
Cytoplasmic male-sterile with G. harknessii Brandegee cytoplasm*	Flowers smaller in size; anthers rudimentary with no pollen.
Dominant genetic male-sterile, Ms_4	Flowers smaller in size and their petals tending to only partially open; anthers rudimentary with no pollen.
Dominant genetic male-sterile. Ms_7	Flowers near normal in size; anthers approximately one-half normal size with no viable pollen.
Double recessive genetic male-sterile, ms_5 ms_6	Flowers near normal in size; anthers reduced in size with no viable pollen.
Rhyne's male-sterile†	Flowers normal in size; anthers near normal size, non-dehiscent; stigma usually crooked; pollen is non-viable or imperfect.

* Cytoplasmic male-sterile stocks of Upland cotton, G. hirsutum with G. harknessi cytoplasm were developed and supplied through the courtesy of Vesta G. Meyer, Geneticist, Delta Branch, Miss. Agr. Exp. Sta. † Rhyne and Rhyne (21) reported that indehiscent-anthers (male-sterility) was controlled by two pairs of recessive genes. Rhyne's male-sterile plants produced viable pollen under some environmental conditions.

wall into an outer layer of primary parietal cells and an inner primary sporogenous tissue. The parietal cells pass through further division and form cell layers which compose an endothecium, a middle layer, and the tapetum. The tapetal cells are full of dense cytoplasm, and at the beginning of meiosis in the pollen mother cells (PMC's), the tapetal nuclei may also undergo some division. The tapetal cells may be uninucleate or binucleate. Generally, at the time of seperation of microspores from the tetrads the tapetum begins to degenerate.

The primary sporogenous cells become polyhedral or subglobose in shape. Their cytoplasm is dense, and there are no vacuoles. The sporogenous cells function directly as PMC's or microsporocytes. Meiosis produces tetrads and the cell walls arise simultaneously between four nuclei. The microspores are usually arranged in a tetrahedral or isobilateral fashion. The primary common membrane dissolves, and the microspores separate.

Each microspore, as it grows, develops a thick exine and thin intine. Spines develop as membrane protuberances on the exine. Germ pores may be seen at certain points within the membrane of the exine. The nuclei of microspores divide and form the generative and vegetative nuclei. The pollen grains are considered to be mature and ripe for pollination when they reach the binucleate stage.

Pollen Abortion in Male-Sterile Stocks

Cytoplasmic Male-Sterile Strains. In the anthers of the cytoplasmic male-sterile strain with *G. harknessii* cytoplasm, there was normal differentiation of anther walls. In the majority of cases the sporogenous tissue collapsed during the premeiotic stages of development. Figure 1 shows the anthers with a mass of dark, misshapen necrotic tissue instead of sporogenous cells. There was disorganization of the tapetum and coalescence of the microsporocytes. Occasionally, a few PMC's were formed in some locules, but these cells degenerated during early stages of prophase. Meiosis did not progress beyond mid-prophase in the cytoplasmic male-sterile anthers.

Dominant Genetic Male-Sterile Strains, Ms_4. The early development and differentiation of the Ms_4 male-sterile cotton strains were similar to that of Coker 201, the fertile variety. Initial degeneration of the meiocytes was observed during the premeiotic stages. Some PMC's were seen initiating meiosis; however, they had collapsed by midprophase (Fig. 2). The dark area among the PMC's in Fig. 2 indicates a mass of dead tissue. The development of the tapetum appeared to be normal. However, in some locules the size of tapetal cells was reduced when compared with those in the fertile anthers. In the Ms_4 sterile anthers meiosis did not proceed beyond prophase I.

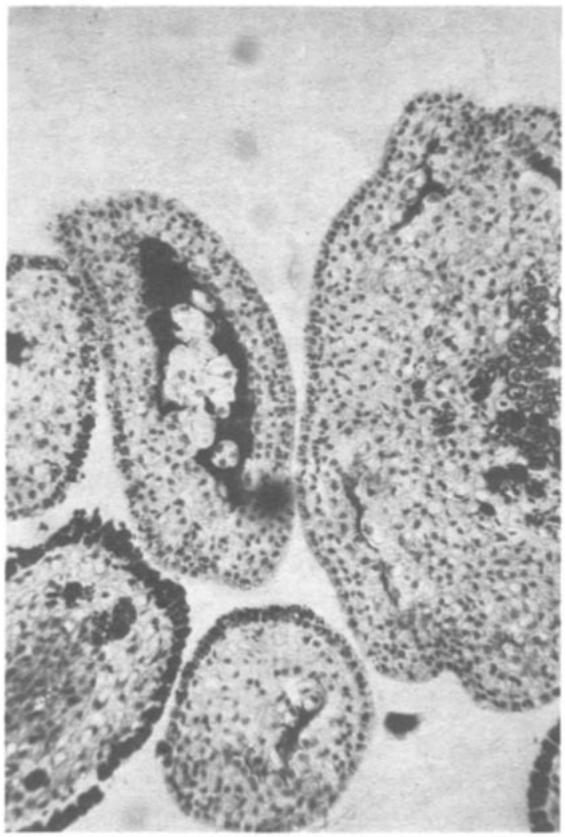

Fig. 1. Transverse section of anther of cytoplasmic male-sterile plant at premeiotic stage showing a mass of darkly stained necrotic tissue in place of sporogenous cells; ca. 45×.

Dominant Genetic Male-Sterile Stock, Ms_7. The premeiotic stages of anther development and differentiation in the Ms_7 strain were similar to those in the male-fertile variety. Degeneration of PMC's in this strain was observed at different stages of meiosis. In some locules, a black mass of dead tissue was seen during premeiotic stages. Degeneration was also observed during the dyad and tetrad stages. In the majority of the locules, normal meiosis occured. In such cases, the microspores degenerated during development of the pollen wall. The developing pollen grains were shrivelled and shrunken (Fig. 3), losing their contents and finally disintegrating. The tapetal cells behaved normally and did not appear to be the cause of male-sterility.

Double Recessive Genetic Male-Sterile Stocks, ms_5ms_6. Anther development and microsporogenesis in the male-sterile ms_5ms_6 strains appeared normal.

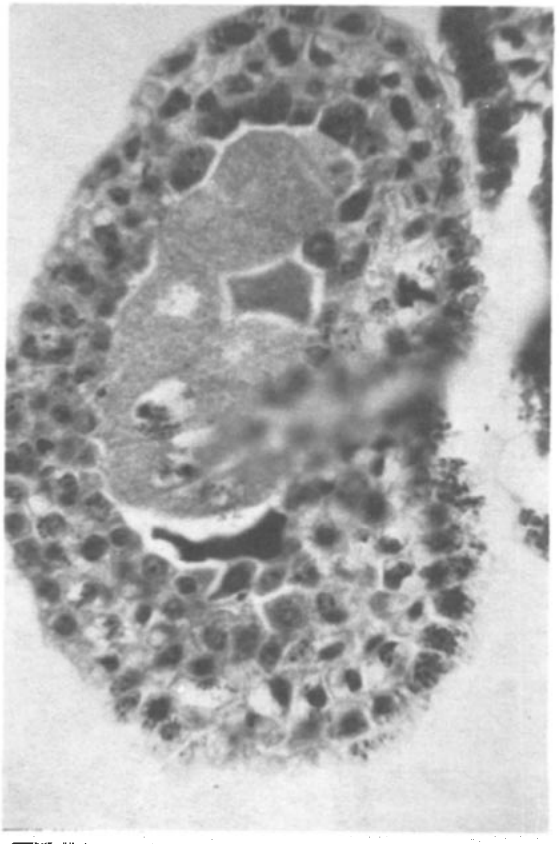

Fig. 2. Transverse section of anther of Ms_4 male-sterile plant showing the PMC's at early prophase stage. The degenerating PMC's and tapetal cells are deeply stained; ca. 129×.

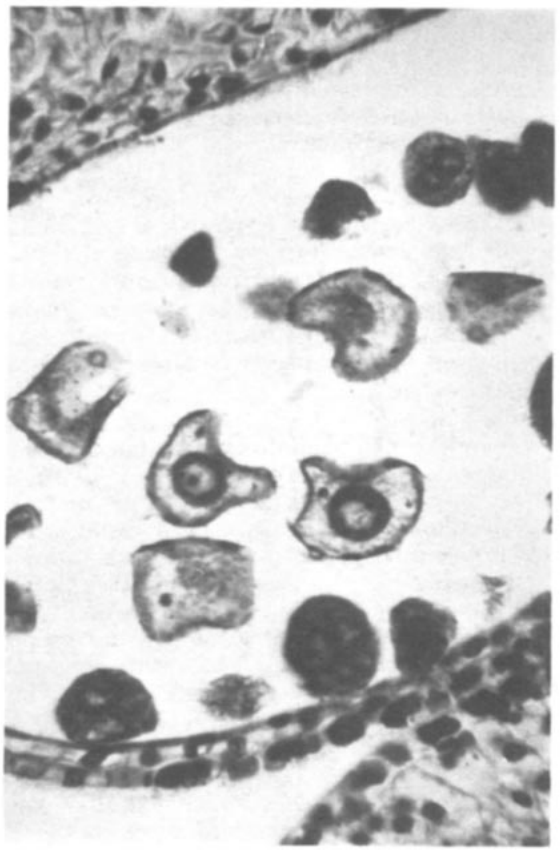

Fig. 3. Transverse section of anther of Ms_7 showing shrivelled and degenerating microspores; ca. 129×.

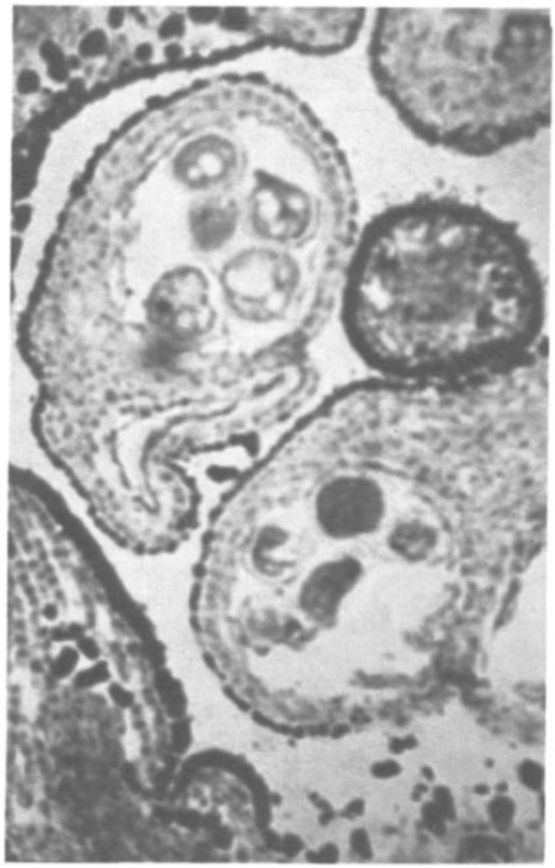

Fig. 4. Transverse section of anther of ms_5ms_6 male-sterile plant showing the development of vacuoles in the microspores; ca. 45×.

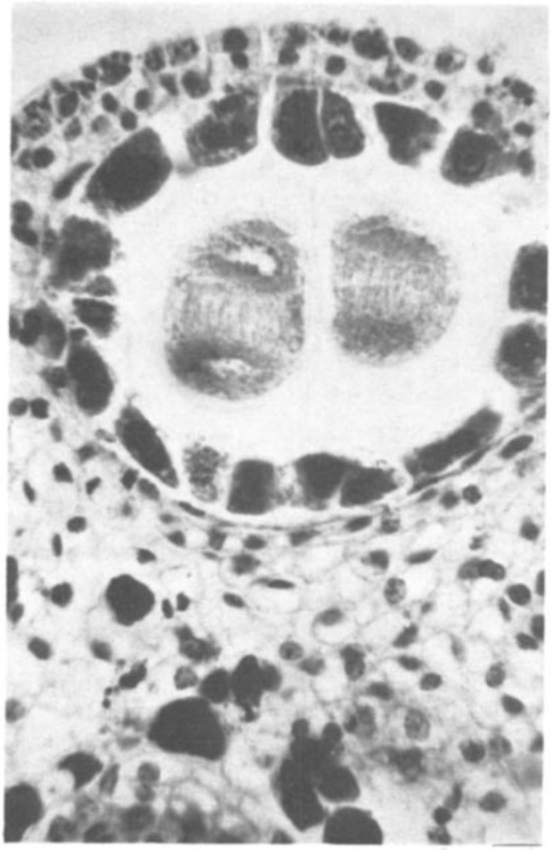

Fig. 5. Transverse section of anther showing PMC's at dyad stage. Tapetal cells are uni- and binucleate; ca. 129×.

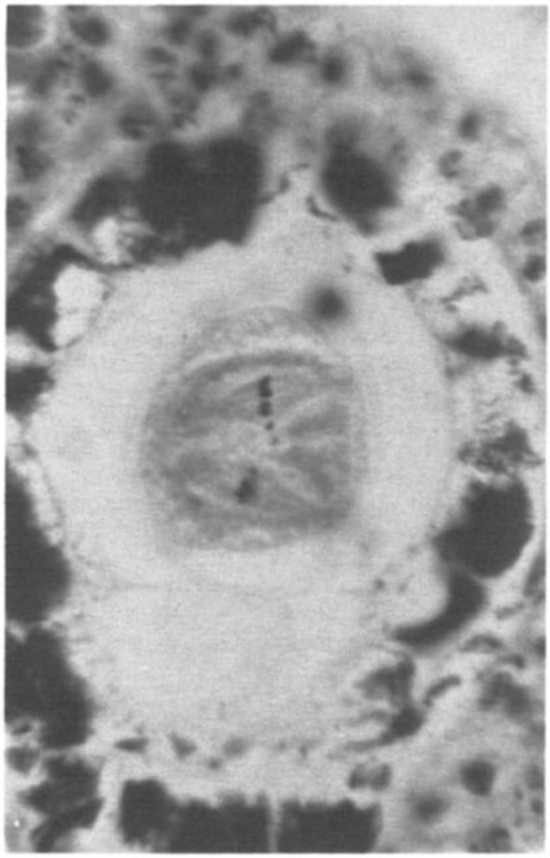

Fig. 6. Transverse section of anther showing degeneration of tapetal cells at anaphase II stage of PMC's; ca. 141×.

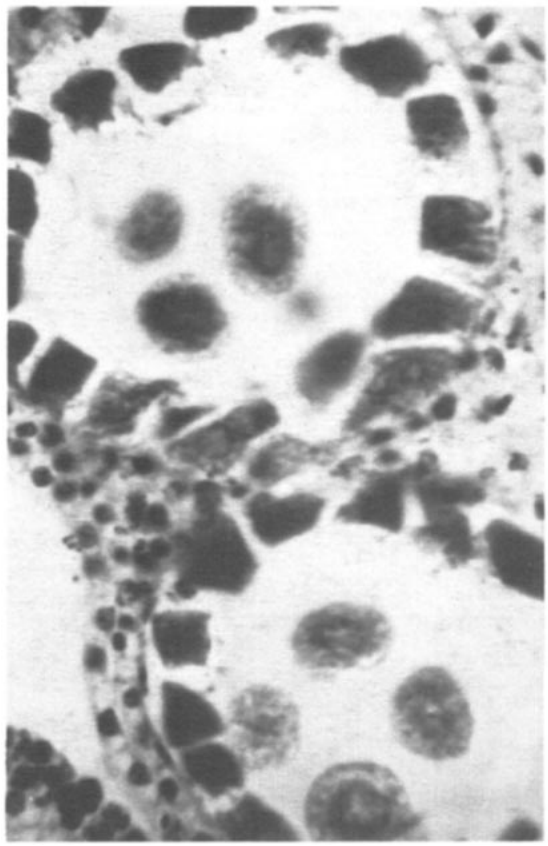

Fig. 7. Transverse section of anther showing tetrads and disintegrating tapetal layer; ca 129×.

There was regular tetrad formation and separation of microspores. Vacuoles were formed during development and maturation of the male gametophyte (Fig. 4). The vacuoles increased in size, crushing the chromatin material. Anther sacs appeared empty at the time of anthesis. The behavior of the tapetum was similar to that of the fertile variety.

Rhyne's Male-Sterile Stocks. The development of the anthers and microsporogenesis in Rhyne's male-sterile strain was normal except in the behavior of the tapetal layer. At meiosis, the tapetal cells were normal until the formation of dyads (Fig. 5). Later, in the majority of the anthers the tapetal cells appear as a black mass, indicating death of the cells. The dead tissue starts disintegrating as meiosis of the PMC's advances (Fig. 6). In such cases the microspores degenerated shortly after their separation from the tetrad mother wall.

In some locules, the tapetal cells appeared to be normal until the formation of the tetrads (Fig. 7), and then started degenerating. As microspore development progressed, the tapetal cells dissolved completely. Figure 8 illustrates the disintegration of dead tissue of the tapetum and the dissolution of pollen grains. Pollen grains were shrivelled; they degenerated completely during or after formation of the pollen wall. However, in a few locules some pollen grains developed normally (Fig. 9) and produced viable gametes. In such cases the majority of the anthers did not dehisce. Seeds were produced when

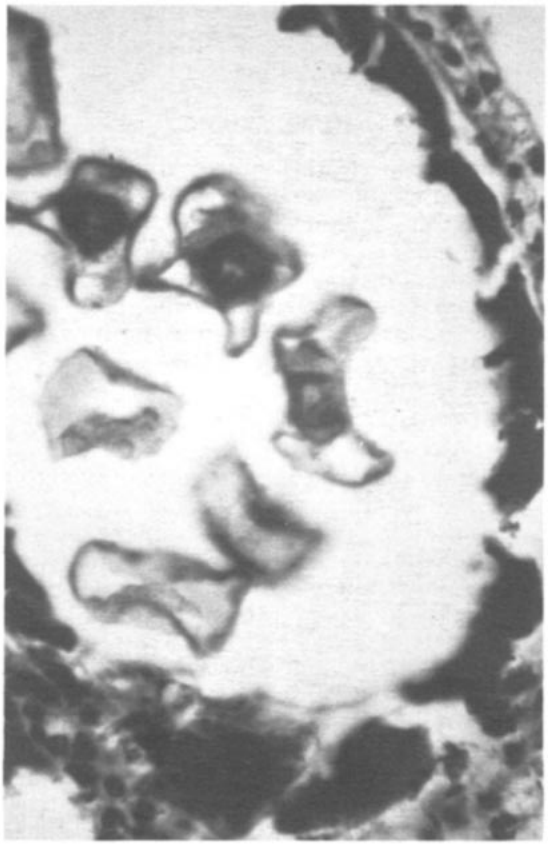

Fig. 8. Transverse section of anther showing shrivelled microspores and degenerating tapetal cells; ca. 129×.

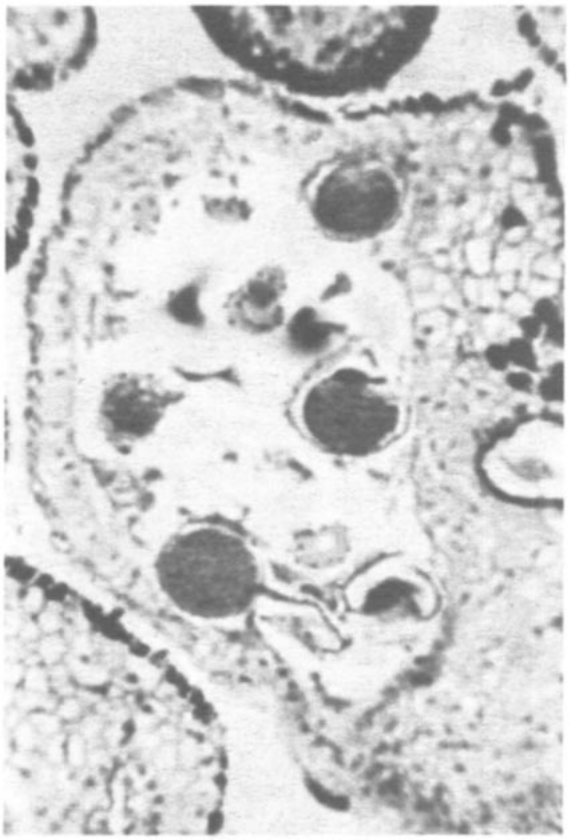

Fig. 9. Transverse section of anther showing both degenerating and good pollen grains; ca. 129×.

such anthers were crushed and the pollen dusted on the stigma of male-sterile lines.

DISCUSSION

Previous to this work, cytological studies on male-sterile cotton strains were limited to the microscopic examination of squash preparations. The findings reported in this paper deal with the probable mechanism involved, or the events leading to the abortion of pollen in one cytoplasmic male-sterile and four genetic male-sterile Upland cotton strains.

Observations on pollen development in the male-fertile Coker 201 were in complete agreement with previous reports (2, 4).

Anther development in five male-sterile strains was similar to that in a normal cotton variety during the archesporial differentiation. The first observable sign of variation occured in the sporogenous tissue of the cytoplasmic male-sterile anthers. In the majority of the locules, there was disorganization of tapetal cells and coalescence of the microsporocytes during the premeiotic stages. Several workers have related the abortion of pollen which has either genetic, cytoplasmic, or environmental basis to malfunction of the tapetum (17, 18, 20, 23). The tapetum plays a vital role in normal pollen development (14). Moss and Heslop-Harrison (17) reported that it is not known what constituents are transferred or how specific a role the tapetum plays in directing events in sporogenous tissue. Abnormalities in tapetal cells supposedly lead to some form of starvation of meiocytes. In the anthers of the cytoplasmic male-sterile cotton strains, disorganization of the tapetal layer might be associated with starvation and eventual death of the microsporocytes before or during the early stages of prophase I.

Studies of histological aspects of pollen abortion in cytoplasmic male sterility in various crops have indicated that degeneration of microspores occurs after tetrad formation and during microspore development. Evaluation of some cytoplasmic male-sterile lines has shown that under certain environmental conditions the sterile plants tend to produce viable pollen, which is not acceptable in hybrid seed production. In cytoplasmic male-sterile Upland cotton with *G. harknessii* cytoplasm, degeneration of microsporocytes is seen very early in the development of pollen. These results indicate that there is little probability of viable pollen being produced in this cytoplasmic male-sterile strain under any climatic conditions. Therefore, the problem of male-sterile cotton lines becoming fertile in the hybrid seed production field should not be encountered.

In the Ms_4 genetic male-sterile anthers, the abortion of PMC's was observed before or shortly after the initiation of prophase I. In some locules these tapetal cells were reduced in size. Allison and Fisher (1) and Weaver and Ashley (25) found no recognizable development of sporogenous cells in Ms_4 genetic male-sterile strains. The failure of PMC's during the premeiotic or early stages of prophase was reported in other crops (3, 8, 18, 23). Moss and Heslop-Harrison (17) have indicated that in maize (*Zea mays* L.) not all pollen sterility could be related to cytologically observable malfunctioning of the tapetum. They found that the particular tapetal defect involved was not the detectable type and that whatever influence disrupted normal development affected the PMC's but not the tapetum. The reduced size of the tapetum in some anther locules of the Ms_4 male-sterile strain might not be detectable in all the anthers, but the tapetal malfunction as indicated by Moss and Heslop-Harrison (17) might be causing the sterility in the Ms_4 genetic male-sterile strain.

In the majority of anthers of the dominant genetic male-sterile strain, Ms_7, abortion was observed at the time of differentiation of the pollen wall. These results support the observations of Weaver and Ashley (25) who reported that there was apparently regular meiosis in the Ms_7 genetic male-sterile strain.

In the Ms_7 anthers it appeared that there were no noticeable abnormalities in the tapetal cells. Degeneration of microspores, during or after the pollen wall differentiation, that does not involve the tapetal cells was reported in wheat (*Triticum* sp.) (9) and in rice (*Oryza* sp.) (6). The development of microspores, according to several authors (7, 13), requires large amounts of nutrients for growth and differentiation. Lack of supply or inability of developing microspores to absorb the nutrients might be responsible for the pollen abortion in this particular type of male sterility.

In the ms_5ms_6 pollen-sterile anthers, the development of vacuoles in the microspores during their maturation period led to the abortion of pollen.

Degeneration of male gametophytes due to the development of vacuoles was reported in male-sterile sweet peas (*Pisum* sp.) (5) and onions (*Allium* sp.) (19). Childers (5) ascribed such vacuolations to nutrient deficiencies. In ms_5ms_6 pollen-sterile anthers pollen abortion might have resulted from such nutrient deficiencies.

In Rhyne's double recessive male-sterile strain abortion of pollen was induced by the premature degeneration of the tapetum which affected the development of microspores. The degeneration of the tapetal cells appeared during meiosis II in some locules while in others the degeneration was noticed at the tetrad stage. Pollen development was rarely completed. However, in a few anthers there was apparently normal development of some viable pollen.

The premature degeneration of the tapetum and resulting failure of nutrient supply appear to be responsible for pollen abortion in Rhyne's male-sterile anthers. In male-sterile plants of several species, the tapetum was observed to be related to pollen abortion. However, there remains a possibility that some factor(s) in male-sterile anthers causes the simultaneous and parallel degeneration of tapetum and sporogenous cells.

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