Anther and pollen development in male fertile intermediate wheatgrass and in cytoplasmic male sterile plants derived from wheat X wheatgrass hybrids
by Bruce Alexander Young

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Genetics
Montana State University
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Abstract:
Light and electron microscopy were used to compare anther and pollen development in male fertile intermediate wheatgrass and in cytoplasmic male sterile and partially male sterile plants derived from durum wheat X wheatgrass hybrids. The male sterile and partially sterile plants were from the third substitution backcross to the amphidiploid hybrid, Triticum durum Desf. X Agropyron intermedium (Host) Beauv., A. intermedium being the recurrent pollen parent. The male fertile plants were from the variety 'Oahe' intermediate wheat-grass.

Anther, pollen, and tapetum development in fertile plants and partially sterile plants was similar to that described for other grasses.

Most of the male sterile plants were consistent from year to year in the type and percentage of sterility, even though some were meiotically very irregular. This was taken as further indication of a cytoplasmic male sterility mechanism operating in this material. However, no specific cytological evidence was found in any of the male sterile or partially male sterile plants which would definitely indicate the cause of the sterility in the backcross material.

A variety of sterility types was observed in the backcross material. An anther containing empty, collapsed microspores was the most common type. Pollen wall (exine) malformations were most commonly found in plants with a high degree of meiotic instability, and all pollen grains which had exine abnormalities were sterile. That exine malformations may inhibit the transfer of essential nutrients from a functioning tapetum to the developing microspore was thought a possibility worthy of further investigation. Meiotic division errors which produced joined microspores or microspores with common, malformed exines were also seen in one meiotically unstable, male sterile plant.

One 'Oahe' plant exhibited a high degree of male sterility during one year of the investigation. Empty microspores with malformed exines were the characteristic sterility type. Unusually high seasonal temperatures during meiosis was thought to be the primary cause of the pollen sterility.

Tapetal cell disturbances were only consistently observed in one male sterile plant. As the tapetum degenerated, the cells failed to form the typical plasmodium through breakage of the tapetal radial walls.

Tapetal orbicular wall deformities were observed in two plants, one male sterile backcross plant, and one 'Oahe' plant the same year it was highly male sterile.

At dehiscence another male sterile plant released only late vacuolate microspores. Development had apparently ceased at that premitotic, pre-engorging stage. In most of the male sterile plants, an occasional microsporangium had an enlarged, vacuolated middle layer which appressed all inner layers to a dark degenerated mass.
The failure of a stomium to form was seen in only one male sterile plant. Meiotically unstable, male sterile and partially male sterile plants had anthers considerably shorter than anthers from male fertile and meiotically stable, partially male sterile backcross plants.

In addition to the cytoplasmic male sterility mechanism, the consequences of several other, as yet unidentified, environmentally sensitive factors were thought to be partially responsible for the variety of sterility types observed in this material.
ANTHER AND POLLEN DEVELOPMENT IN MALE FERTILE INTERMEDIATE WHEATGRASS AND IN CYTOPLASMIC MALE STERILE PLANTS DERIVED FROM WHEAT X WHEATGRASS HYBRIDS

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

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In addition to the cytoplasmic male sterility mechanism, the consequences of several other, as yet unidentified, environmentally sensitive factors were thought to be partially responsible for the variety of sterility types observed in this material.
INTRODUCTION

Few doubt the importance of cytoplasmic male sterility (CMS) as a tool in the production of hybrid cultivars, insofar as these cultivars often exhibit "hybrid vigor" and are useful economically. Cytoplasmic male sterility (i.e., male sterility which is due to factors transmitted only through the female, often modified by nuclear genes) has been reported in or incorporated by various means into scores of species, many of which are important agronomic seed crops (ex. maize, Zea mays L.; sorghum, Sorghum bicolor Moench.; wheat, Triticum aestivum L.; and rice, Oryza sativa L.). However, on the whole, exploitation of CMS is forage crop species has been limited, the commercial use of pearl millet, Pennisetum glaucum (L.) R.Br., in India being the exception. The potential usefulness of other hybrid forage types (cultivars or even species), however, cannot be minimized and the use of CMS in their production is certainly one alternative which should be considered seriously at the start of any breeding program.

Intermediate wheatgrass, Agropyron intermedium (Host) Beauv., is one such forage grass which deserves to be investigated for potential heterosis. It is a cool season, rhizomatous grass, fairly long lived, which does well under limited moisture and has important potential as a range species and for revegetation of depleted grazing land. At Montana State University cytoplasmic male sterility is being incorporated into intermediate wheatgrass toward the goal of exploring
potential hybrid vigor in this species. The incorporation is proceed­
ing via "substitution backcrossing" (SB) in the manner of Kihara
(1951). The genome of one species, in this case *Agropyron intermedium*,
is effectively substituted through backcrossing into the cytoplasm of
another species (*Triticum durum* Desf.), the resulting interaction be­
tween nucleus and cytoplasm producing the male sterility.

Almost everyone who has been involved with cytoplasmic male
sterility, not matter how it was produced (or discovered) has launched
some investigation into its cause. Practically all have been cytologi­
cal developmental studies involving the anther, especially focusing on
two cell types, the tapetal cells and the sporogenous cells. This
would seem to be a logical approach to an investigation into the cause
of cytoplasmic male sterility. However, the difficulty lies in the
fact that practically all investigators are using the same technique,
namely paraffin sections with rather harsh preparation procedures. If
indeed fine distinctions need to be made to detect differences between
male-fertile and male-sterile anthers, and if the timing of various de­
velopmental changes is critical to deduce probable causes of CMS, then
paraffin sections coupled with harsh fixatives will not allow enough
resolution to observe these subtle changes. This assumes, of course,
that differences can be detected visually and these differences might
reflect causes of male sterility.
Since intermediate wheatgrass is a new species with regards to the incorporation of cytoplasmic male sterility, and a cytological investigation into the cause of CMS has not been made in this or any other wheatgrass species, it was felt that some redundancy in the overall approach would be necessary. However, the developmental changes would be observed at the ultrastructural level with an electron microscope, and these observations would be correlated with light microscope observable sections. These would be cut from plastic blocks to a thickness of 1 μm compared to the standard 10 μm with paraffin sections. The reduction in section thickness would greatly improve the resolution and the quality of the sections. In addition, other characteristics such as anther length and spikelet number would be observed and compared between male-fertile and male-sterile plants.

The observation of pollen and tapetum development at the ultrastructural level is not a novel idea. Studies of normal development have been made since the 1950's. However, to my knowledge only three such investigations have been reported so far in CMS species and none in genetic male sterile species. All three publications have reported different cytological findings, and two of the studies have been rather limited. Hence, it was deemed worthwhile to continue such investigations in CMS intermediate wheatgrass, since cytological investigations at the ultrastructural level in CMS species have not been exhaustive.
with respect to gains received as might be considered the case with studies using standard paraffin techniques.

There follows a description of developmental changes in the anthers of male-fertile intermediate wheatgrass versus changes in the anthers of male-sterile intermediate wheatgrass with special emphasis on tapetal and sporogenous cells.
LITERATURE REVIEW

Edwardson (1970) has reviewed most of the literature involving cytoplasmic male sterility through the end of the 1960's. He concentrates for the most part on the breeding aspects of CMS with particular emphasis on fertility restoration genes, for the majority of the CMS literature is devoted to this important economic aspect. He does, however, make occasional mention of cytological behavior in anther tissue. Edwardson divides CMS species into four categories according to the source of the cytoplasmic male sterility. These categories are CMS species which are derived from either 1) intergeneric crosses, 2) interspecific crosses, 3) intraspecific crosses, or from 4) those CMS plants that apparently have arisen spontaneously. The plant material concerned with in this thesis was derived from an intergeneric cross, *Triticum durum* X *Agropyron intermedium*.

Laser and Lersten (1972) in their review of microsporogenesis in CMS angiosperms have determined that of the CMS species they reviewed as to cytological behavior, 10% were from intergeneric crosses, 16% were from interspecific crosses, 18% were from intraspecific crosses, and the vast majority, 56%, were from those CMS species having arisen spontaneously. To give an overall picture of the quantity of literature involving CMS they also report, at their count, CMS being investigated in one aspect or another in 140 species of angiosperms involving 47 genera from 20 families. Of the monocots, grasses
(Gramineae) have been giving the most attention (16 out of 18 monocot species studied), while the dicots with 20 species from 17 genera and 11 families have been studied more diversely. Out of 62 cytological studies they review, only 2 involve electron microscopy, both of these being grass species, *Sorghum bicolor* and *Triticum aestivum*.

One gets the impression that Laser and Lersten feel a certain frustration with the conflicting terminology which has been used when dealing with microsporogenesis and a disappointment at the imprecision of the techniques so far utilized, if indeed the techniques used are reported at all. For the most part the techniques utilized, namely paraffin sections, by their very nature have allowed only vague descriptions of cytoplasmic and wall changes in aborting pollen. In addition, changes induced by the fixative, any fixative, might mimic changes induced by the sterility. Fixative artifacts are a problem no matter what technique one uses, but usually at the ultrastructural level more of these induced changes can be recognized as such. In their publication Laser and Lersten have consolidated the data from 62 CMS cytological studies reassigning currently acceptable terminology where necessary.

The literature surrounding cytological investigations with CMS has, of course, been directed toward making some statement about the cause of cytoplasmic male sterility. These causes have been reported to involve the tapetum, vascular disturbances and deformities, the
timing of callose dissolution, and the possible role of viruses. Natural lines of inquiry relating to these purported causes have included the following: the induction of sterility through the application of chemicals; sterility involving meiotic disturbances; the origin of the pollen grain wall and whether its formation is under sporophytic or gametophytic control; natural or environmental sterility; and changes in anther number and gross anther morphology. That specialized layer of cells in the anther, the tapetum, is universally believed to play a necessary role in the normal development of pollen. Most researchers believe there is a transfer of nutrients from the tapetum to the developing microspore, although direct proof for this nutrient transfer is still lacking. With this tapetum involvement acknowledged, it is logical to look toward a malfunctioning tapetum as being involved in the production of CMS at least in some CMS species.

Tapetal cells can be divided into two distinct types (Davis, 1966), amoeboid and secretory. The periplasmodial or amoeboid type is the least common, that of Tradescantia bracteata being a well-studied example. Most commonly the tapetal cells retain their integrity until a time varying from meiosis I to the quartet stage. The tapetal radial walls then break down, and long fingers of tapetal cytoplasm infiltrate between the PMC's eventually engulfing them so the PMC's appear encased in a vacuole of tapetal cytoplasm. Further pollen development takes place while enveloped in the tapetal cytoplasm with the tapetal
cytoplasm apparently going through a reorganization as pollen development proceeds. However, this reorganization is not a degenerative process (Mepham and Lane, 1969b), and the tapetal cytoplasm retains its functional integrity until just before anthesis. Mepham and Lane (1969b) do not view pollen growth nutrients as being produced by the tapetum, but say that such nutrients may indeed pass through the tapetal cytoplasm. The tapetal cell would then function in a regulatory role. They also conjecture that membrane continuities could exist between the tapetal cytoplasm and the pollen grain which would aid in any transfer of substances between the two cytoplasms.

The secretory tapetum is the most common and the most widely studied with the majority of known cytoplasmic male sterile species having this type of tapetum. The individual tapetal cells maintain their integral walls and exhibit increased metabolic activity until sometime between quartet stage and the first pollen mitotic division. Unlike the periplasmodial tapetum, the secretory tapetal cells then exhibit wall and organelle changes characteristic of senescing or degenerating cells. All trace of tapetal cytoplasm is usually gone by the second pollen mitotic division. The discussion in this thesis will be primarily concerned with those species having a secretory tapetum.

With this background there follows a review of the work involving pollen abortion, tapetal behavior and other pertinent
characteristics in CMS species. In an early study Rogers and Edwardson (1952) working with CMS corn described the sterile pollen as being shriveled and notable by the absence of starch. The male sterile anthers were usually shrunken and nondehiscent with meiosis appearing normal in the imbreds studied. No mention was made, however, of tapetal behavior.

Using cytoplasmic male sterile Triticum species produced by an initial intergeneric cross with Aegilops followed by substitution backcrossing, Fukasawa (1956) reported pollen abortion as occurring at about the first pollen mitotic division. Although most of the aborted pollen had only one nucleus, occasional grains were observed which contained two or three nuclei. Some aborted pollen grains showed microchromatin bodies at the second mitotic division if they developed to that stage, and some grains completed lacked cytoplasm. With respect to tapetal development, he found no differences between the fertile and sterile anthers. Normal, fertile spikes kept in the dark for one week at the time of the first pollen mitosis demonstrated division failures similar to those observed in the male sterile plants. Male sterile anthers were smaller than the fertiles, and approximately 13% of them were malformed, having three loculi instead of the normal four. Fukasawa attributed the immediate reason for the sterility to a failure of the pollen nucleus to divide properly at the first mitotic division.
However, Fukawawa (1956) felt that the cause must in part lie in some factor other than a direct involvement of a malfunctioning tapetum though he conceded, somewhat contradictorily, that there could be a failure of nutrient transfer through the tapetum to the pollen. He theorized that a failure of the pollen itself to synthesize required substances, the failure of nutrient synthesis in some other part of the anther or plant, or an unknown environmental factor of some kind could be responsible for the sterility.

Singh and Hadley (1961) working with CMS sorghum found that most of the sterile pollen did not develop past the uninucleate stage. However, occasional aborted pollen grains were seen which had two or three nuclei. In the fertile material the tapetal cells started to degenerate toward the end of meiosis. However, the sterile tapetum persisted until "fairly mature" pollen was formed. In addition, the tapetal nuclei of the sterile anthers divided endomitotically to produce multinucleate cells, above the binucleate state for normal tapetal cells. The sterile tapetum did not appear to stain as deeply in the later stages of pollen development as did the degenerating tapetum of the fertile anthers. This suggested to the authors that the tapetum was deficient in some substance(s) important for the normal development of sorghum pollen.

Zenkteler (1962) observed that in carrots (Daucus carota L.) while tapetal cells in fertile anthers degenerate after microspore...
formation, in CMS anthers the tapetal cells and nuclei increase in size and form a plasmodium. Later the size of the tapetal nuclei decreases and the microspores degenerate. Meiotic irregularities involving inversions and the associated anaphase bridges were observed in the male sterile material. Male sterile anthers were shrunken, indehiscent and dark brown in color.

Zenkteler (1962) did observe two types of pollen abortion in fertile plants. Sometimes small uninucleate microspores with a thickened exine and shrunken cytoplasm were produced, and occasionally, globular shaped pollen with shrunken cytoplasm and no nuclei were found. One partially sterile plant was studied which produced white anthers and brown anthers. The sterility in the white anthers averaged 28% while the pollen in the brown anthers exhibited a higher percentage of sterility (70%). No evidence of meiotic irregularities was observed.

A nondehiscent anther character with a breeding behavior similar to cytoplasmic male sterility but not as reliable was studied in sorghum by Webster and Singh (1964). The authors reported that in normal anthers the tapetum starts to degenerate at the onset of meiosis while in the nondehiscents, which had anthers of normal size, the tapetum persists until after the free microspore stage, but still degenerates completely by anther dehiscence. They observed that the endothecium was not well-developed in normal fertile anthers. In the mature anther
only the epidermis remained with its inner and tangential walls slightly lignified and outer wall cuticularized. The nondehiscent had a well-developed endothecium with fibrous thickenings between the individual cells in addition to a thick-walled epidermis. The authors attributed the nondehiscent character to the well-developed endothecium and epidermis and the fact that the septum between adjacent loculi didn't disintegrate.

In CMS flax (Dubey and Singh, 1965) pollen development was normal to the quartet stage. Thereafter, the time of abortion varied, often within the same line. Sometimes the microspores degenerated before they were released from the quartet. More often they showed normal development, including wall development through the late microspore stage, but pollen mitosis failed to occur and the microspores aborted at the uninucleate stage. However, occasionally abortion didn't occur until the pollen had fully matured.

In contrast to the normal flax tapetum which degenerates soon after the quartet stage, the tapetum in CMS flax anthers persisted until a later stage in pollen development. The authors felt that in the normal anthers there was a utilization of tapetal products soon after the microspores had separated from the quartet. While in sterile anthers the persisting tapetum failed to supply the necessary nutrients essential to proper pollen development.
Filion and Christie (1966) reported CMS in a clone of orchard grass that in some cases exhibited sporocyte degeneration at the beginning of meiosis, but more often there was a degeneration of microspores soon after their release from the quartet. They found that the tapetum degenerated precipitously at the early microspore stage in contrast to the more progressive degeneration shown by fertile anthers. Meiosis was observed to be fairly normal with a very low incidence of univalents and laggards. The anthers were small, dark green and nondehiscent. They would eventually dehisce but only under higher temperatures 3 to 5 days after anthesis. The endothelial cells failed to elongate and did not develop fibrous bands.

In another study involving CMS sorghum, Brooks, Brooks and Chien (1966) found that sterile tapetum in some cases enlarged and persisted to the microspore prepollen (uninucleate) stage, the stage of pollen abortion, and that the tapetal width at this stage showed variations not seen in the fertiles. They also presented some data which they thought might indicate that the sterility was under gametophytic control. The heterozygous genotype, Ms ms, in S-cytoplasm gave 42.9% male sterility while the homozygous genotype, ms ms, in S-cytoplasm gave 88.8% male sterility.

Working with CMS hexaploid wheat with Aegilops ovata L. cytoplasm, Chauhan and Singh (1966) observed that three types of sterility were found in each spikelet; however, only one type occurred in a
single floret. (1) The tapetum degenerated before the sporocytes separated in meiosis I and abortion of the pollen mother cells quickly followed. Eventually only a noncutinized epidermis was left in a collapsed anther. (2) The tapetum did not degenerate until the exine was formed. However, the pollen grains lacked a pore, nucleus and cytoplasm. (3) The tapetal walls broke down and a plasmodium was formed prior to meiosis. The PMC walls then degenerated and the cytoplasm coalesced with the tapetal periplasmodium. The intermingled mass of tapetal and sporogenous cytoplasms then gradually disappeared.

Joppa, McNeal and Welsh (1966) have reported that in CMS wheat \(T.\) aestivum) with a normal meiosis, the tapetum failed to degenerate and persisted up to anthesis. There was an absence of starch in the aborted pollen, and the time of abortion or cessation of development seemed to occur sometime after the first pollen mitosis. The number of plastids with starch granules increased rapidly following meiosis in both male fertile and male sterile anthers; however, fewer were formed in male sterile anthers. After the first pollen mitosis, the starch granules rapidly disappeared from the male fertile tapetal cells but did not from the persisting tapetum in the CMS anthers. The sterile anthers were shriveled and nondehiscent. Poor vascular development also was observed in the stamens of the CMS lines. The authors felt that the decreased starch production by the CMS tapetum and the lack of starch storage in the maturing microspores might be explained by a
reduced solute transport into the stamens as a result of poor vascular development.

In CMS sudangrass (Alan and Sandal, 1967) meiosis was normal in both fertiles and steriles. The tapetal cells began to degenerate during meiosis in fertile anthers, but in the sterile anthers the tapetum persisted until after the maturation of the microspores. The tapetal nuclei also divided repetitively producing multinucleate cells compared to the binucleate condition in fertile anthers. There was no indication given as to when the pollen grains aborted, but from the photos it seemed to occur at a time approaching the first mitotic division.

Pakendorf (1970) investigated male sterility in the legume, *Lupinus mutabilis* Sweet. The source of the sterility (genetic, cytoplasmic, or environmental) was not discussed although it appears to be cytoplasmic in nature, probably having arisen spontaneously. The PMC's in sterile anthers developed normally through the second meiotic division, but they failed to separate from the quartet and subsequently degenerated. The tapetum persisted and remained intact until very late. The endothecium tended to enlarge as the quartets degenerated. Prior to meiosis, fertile anthers were bright yellow while sterile anthers were a dull green. At dehiscence fertile anthers were orange while the steriles were brown and shriveled.

A different effect has been noted in CMS sweet pepper, *Capsicum annuum* L. (Novak, 1971). The tapetal cells hypertrophied and the
PMC's underwent an irregular meiosis. Eventually at telophase II both the tapetum and the PMC's degenerated into a central mass of tissue. In partially sterile plants, tapetal development was the same as in the fertiles through meiosis. However, the tapetal cells then persisted beyond the time when tapetal cells in fertile anthers had usually degenerated. In the partially sterile plants, the pollen aborted at the uninucleate stage of development before the first pollen mitosis. Novak says that "the secretory function of the tapetum is disturbed which influences the desynchronization of the meiosis of the male gametophyte in pollen sterile plants."

Typical anther and tapetal behavior for the secretory type of tapetum was observed in fertile *Phaseolus atropurpureus* (Pritchard and Hutton, 1972). The tapetum gradually degenerated after the microspores were released from the quartets. The middle anther layer also degenerated and the endothecium developed thickened walls. In CMS anthers, the endothecium failed to develop thickened walls and the middle layer persisted, sometimes increasing in size. The degeneration of the tapetum occurred at the same time as in the fertile anthers, but the nuclei were more persistent and the tapetal cytoplasm was reduced to a deeply staining mass which was not absorbed. The microspores after release from the quartets developed to a thin-walled stage but were irregular in shape. Finally they clumped together and degenerated.
In their cytological investigations of CMS wheat with *Triticum timopheevi* cytoplasm, Rai and Stoskopf (1974) found that pollen abortion could occur during premeiotic, meiotic or postmeiotic developmental stages. If the sporocytes degenerated premeiotically, the tapetal cells hypertrophied and showed an abnormal vacuolization of the cytoplasm. Abortion during meiosis began with a sudden dissolution of the tapetal cell walls releasing the cytoplasmic contents into the anther locule. Degeneration of the PMC's quickly followed. It is interesting to note that in areas of the locule where the tapetal cell walls did not suddenly dissolve the adjacent PMC's did not degenerate. Most of the pollen abortion occurred postmeiotically. In these types the tapetum persisted longer than in fertile anthers either in cellular or plasmodial form.

Rai and Stoskopf (1974) observed that cytoplasmic male sterile plants occasionally produced trilocular anthers. Most of the trilocular anthers developed at an early stage of anther differentiation, but some became trilocular later by the fusion of two of the loculi. Pollen development in these anthers was no different from that in normal anthers with four loculi.

Other anther characteristics noted by Rai and Stoskopf (1974) included the absence of endothecial wall banding (thickenings) in the steriles, occasional asynchronous development of the locules within an anther and the normal development of vascular tissue in CMS anthers.
Similar anthers and pollen abnormalities have been found in genetic male sterile species. Kaul and Singh (1966) observed a microspore degeneration while still in the quartet in two types of barley, while in their six row counterparts pollen degeneration occurred in the free microspore stage. There was also a difference in tapetal behavior between the two types. In the two row barleys the tapetal cells enlarged, became vacuolate, and then collapsed after the microspores degenerated. While in the six row types the tapetum exhibited only moderate radial enlargement before degeneration. In addition, the authors noted a failure of fibrous thickenings to develop in the endothecial cells. This they felt was the reason the sterile anthers did not dehisce.

Roath (1971) investigated morphological development in three genetic male sterile lines of barley (ms 6, ms 7, and ms 8) and the normal variety, 'Betzes'. Two of the mutants, ms 6 and ms 8, had sterile pollen that, in general, failed to differ from fertile pollen in appearance. The latter mutant, ms 8, was found to be somewhat variable with the sterile pollen sometimes appearing shriveled. Degeneration and the formation of a sporogenous plasmodium occurred during the free microspore stage in ms 7. Some separation of the tapetal cells, taken as an indication of early tapetal breakdown, was observed premeiotically in ms 6 and ms 8. However, continued degeneration was not observed during meiosis. At the quartet stage ms 8 exhibited more
tapetal degeneration than the normals. This earlier degeneration subse-
sequently slowed down and by pollen mitosis a large amount of tapetum
was still evident, while in the normals and ms 6 the tapetal tissue
had almost disappeared. In ms 7, the tapetum enlarged (at the quartet
stage), became vacuolate and degenerated.

An anthesis fertile anthers and anthers of ms 6 had two lobes,
while ms 7 and ms 8 had four lobes. In fertile anthers the endothecium
had generally disappeared by anthesis, while it persisted in the an-
thers of ms 7. Overall, Roath (1971) found sterile anthers to be
shorter and narrower than fertile anthers. When stained with tetrazo-
lium chloride, acetocarmine, and potassium iodide, the mutants gave
variable reactions, between stains and between environments (green-
house vs field).

Investigating five different genetic male sterile lines of bar-
ley, Mian et al. (1974) noted three different tapetal behaviors. In
one instance the tapetal cells persisted and failed to degenerate. In
a second variation, the tapetum suddenly collapsed after the free
microspore stage. In the third type of behavior (ms 18), there was a
failure of tapetal karyokinesis resulting, the authors felt, in an
early collapse of the tapetum. Usually the microspores began to degen-
erate soon after a normal meiosis and were completely deformed by the
first pollen mitosis. However, in one line (ms 10, 'Vantage') the
microspores began to abort midway between the end of meiosis and the first mitotic division.

There have been only three published accounts of electron microscopy having been used to study cytoplasmic male sterility. These three studies have been with wheat (DeVries and Ie, 1970), sorghum (Warmke and Overman, 1972; Overman and Warmke, 1972), and pepper (Horner and Rogers, 1974). A brief description of their findings follows relating to the previously described literature.

Male sterile plants derived from three backcrosses to CMS Bison wheat (timopheevi cytoplasm) were used in the investigation by DeVries and Ie (1970). They found no essential difference between the tapetal cells of the fertile and sterile anthers. The sterile pollen had a lobed appearance and very few organelles in the cytoplasm. Little was said regarding the exact time of pollen abortion, although it was certainly after early formation of the pollen exine.

The tapetum in fertile sorghum behaved in a typical fashion for secretory tapeta (Warmke and Overman, 1972; Overman and Warmke, 1972). After the microspores were released from the quartets, the tapetum gradually degenerated, reducing in thickness until it eventually disappeared. However, in the sterile anthers various irregularities were observed. Often during late meiosis the tapetum began to enlarge becoming vacuolate and persisting beyond the usual time of tapetal degeneration. Many of the tapetal radial walls ruptured producing a
partial "intratapetal syncytium" (plasmodium). Sometimes after the microspores were released from the quartets the tapetal cells became highly vacuolate and elongated radially compressing the degenerating microspores into a small locular area. Occasionally all the tapetal radial walls broke down forming a syncytium that almost entirely filled the locular cavity. The tapetal nuclei were often seen clustered together in the cytoplasm.

Warmke and Overman (1972) observed an early breakdown of callose before the onset of meiosis leaving the sporocytes without a callose of primary cell wall. Many fused to form giant polyploid cells, but most of the cells successfully underwent meiosis retaining their individuality. The resulting dyads or quartets, however, often failed to separate. In any case the microspores, fused or not, failed to develop beyond the uninucleate state.

In their investigations with CMS pepper (Capsicum annuum L.), Horner and Rogers (1974) found that the tapetum, starting before meiosis, exhibited increased vacuolization and hypertrophy until by quartet stage the sporocytes had been appressed to a small area in the center of the locule. Callose was secreted around the PMC's; however, the PMC primary wall failed to dissolve and remained intact throughout meiosis. The last developmental event which was observed before the collapse and degeneration of the sporocytes was the formation of a primexine around each member of the quartet.
Laser and Lersten (1972) in their review of cytological expression of CMS found that of the twenty papers describing tapetal behavior in monocots, 25% reported a tapetum that degenerated too rapidly while the majority of the rest reported a tapetum which retained its integrity throughout pollen development or at least persisted until pollen abortion. By their tally, most of the microspores aborted between quartet formation and the first pollen mitosis with only 10% of the studies reporting microspore abnormalities before abortion. They found that of those species which showed abortion before the quartet stage, most were dicots, while the majority of those species that aborted after the second pollen mitosis were monocots.

Since the scheme of normal pollen ontogeny has not been entirely elucidated, though not for want of trying, most of the work surrounding pollen ultrastructure has not concentrated on male sterility and the fine structural abnormalities associated with pollen abortion. During the late 1950's, publications surfaced describing the ultrastructure of pollen walls. These grew into developmental studies ever increasing in scope and the decade of the 1960's is replete with painstaking, elaborate descriptions of pollen development. Although the gross details, especially of wall (exine) development have been agreed upon, there are still numerous points of conflict. For instance, what substances from what organelles contribute to the formation of the pollen wall and tapetal membrane? What does the tapetum contribute to
overall pollen development and at what times does the transfer of substances occur? Is the formation of the exine (pollen wall) controlled from the microspore or does the tapetum have primary influence over exine patterning?

Only recently (1970's) have papers appeared relating to the comparative electron microscopy of male fertile and male sterile species. Continued work in this area, plus associated histochemical studies, will probably provide more answer to questions about normal pollen development than would research involving normal plants alone.

The tripartite structure of the mature pollen wall or exine has long been recognized (Heslop-Harrison, 1963a, 1971). However, authors have used varying terminology when discussing the various layers. Erdtman (1970) has tried to consolidate the synonyms offering preferential terminology. Diagram A illustrates the pollen exine and presents some of the differing terminology, indicating those terms preferred for intermediate wheatgrass. This is fairly consistent with that expressed by Christensen et al. (1972) in sorghum. Also shown in Diagram A is the morphology of the mature tapetal membrane (orbicular wall) with its interconnecting reticulum of Ubisch bodies (orbicles) after the cytoplasm of the tapetal cells has degenerated.

What factors or structures are thought to contribute to the development of the exine? During prophase I of meiosis the PMC's secrete around themselves a special wall composed of callose, a $\beta$-1,3 glucan
Diagram A. Schematic representation of a mature pollen wall (left) and a mature tapetal orbicular wall (right). Preferential terminology is in bold print. Alternative terminology is in parentheses. pi = pollen plasmalemma; s = sporopollenin coat on outer tangential tapetal surface; f = fibrillar component of orbicular wall; ch (ex) = exine channels; ch (in) = intine channels; ch (u) = Ubisch body channels. Magnification approximately X 17,550.
The callose is thought to be secreted by dictyosonés (Echlin and Godwin, 1968b). The callose wall persists through the quartet stage, and all researchers so far have agreed that at least the early elements of the pollen wall are formed while the microspores are encased in the callose of the quartet.

After quartet formation a fibrillar layer termed primexine by Heslop-Harrison (1964) is formed between the plasmalemma and callose wall. This layer is thought to be cellulosic on the basis of its staining and removal by cellulase (Heslop-Harrison, 1968a). While the microspores are still in the quartet, pro-bacula appear in the primexine as radially directed rods associated as groups of lamellae (Heslop-Harrison, 1968b). The material of the pro-bacula is probably lipoprotein and is termed protosporopollenin by Heslop-Harrison, since it is not acetytolysis resistant as is sporopollenin, the principal structural material of the mature exine. The tectum is formed by expansion of the heads of the pro-bacula and tangential growth of the lamellae until the adjacent pro-bacular heads fuse. The lamellae are coated with sporopollenin as development proceeds and usually the lamellated appearance disappears as the exine matures (Heslop-Harrison, 1971). In sorghum (Christensen et al, 1972) lamellated pro-bacula were not found. They used the term bacula to describe the first initials of the exine, since they had the same solid appearance as the mature bacula (collumellae in their terminology).
Tubular fragments of endoplasmic reticulum were found oriented perpendicular to the plasmalemma in pro-bacular areas in *Silene pendula* (Heslop-Harrison, 1963b, in Heslop-Harrison, 1971) and *Zea mays* (Skvarla and Larson, 1966). Skvarla and Larson also found evaginations of endoplasmic reticulum into the plasmalemma which they felt established a template for wall patterning. In *Silene pendula*, *Cannabis sativa* (Heslop-Harrison, 1963) and *Helleborus foetidus* L. (Echlin and Godwin, 1968b) sheets of endoplasmic reticulum were found lying beneath the plasmalemma in the areas which were to form the furrows, and primexine was not deposited in these regions.

Dickinson (1970) working with *Lilium longiflorum* found the same sheets of endoplasmic reticulum, and theorized that the primexine was derived from dictyosome vesicles since in the furrow regions where primexine was absent the endoplasmic reticulum was always interposed between the plasmalemma and the dictyosome vesicles. Heslop-Harrison (1968b) and Dickinson and Heslop-Harrison (1968) have seen in *Lilium* ribosome-like particles clustered around evaginations of the plasmalemma at the base of the pro-bacula. Dickinson (1970) regards these ribosome-like particles as possibly being responsible for the synthesis of intrabacular components.

Nexine development can proceed with or without the deposition of sporopollenin on lamellae as in the bacula. In *Anthurium* Rowley and Southworth (1967) have seen lamellar elements arising next to the
plasmalemma. Sporopollenin accumulated on the two faces of the membrane-like lamellae, leaving an electron lucent area in the middle, and then the lamellae were pressed tangentially outward to form the nexine-2. A similar deposition of sporopollenin around lamellae arising from the cytoplasm was reported in Helleborus by Echlin and Godwin (1969). These trilamellar elements were termed tapes and white lines. Sengupta and Rowley (1974) isolated these tapes (lamellar structures) from the exine of Lycopodium clavatum L. using 2-aminoethanol, high temperature and pressure. After staining with toluidine blue they thought the tapes might be composed of two filaments joined by poly-basic molecules. The filaments, they felt, might not be composed of sporopollenin since the filaments were more resistant to heat and pressure than is sporopollenin. In Zea (Skvarla and Larson, 1966) and sorghum (Christensen et al, 1972) the development of the nexine proceeded without any appearance of lamellar structures.

Exine channels with a diameter of 10-100 μm often have been seen, especially in the Gramineae (Skvarla and Larson, 1966; DeVries and Ie, 1970; Christensen et al, 1972). These channels have been seen to transverse both the tectum and the foot layer (nexine) but with only occasional exception (Rowley et al, 1959) channels have not been seen in the mature columellae (bacula).

Ubisch bodies (orbicles) which form on the surface of the tape-tal cells bear a remarkable resemblance to the exine patterning of the
pollen grain and they have been found to form at the same time and rate as the pollen exine. Rowley et al. (1959) reported that in Poa L. both the exine and the Ubisch bodies are covered with spinules; in Degeneria both are smooth and in Cryptomeria (Pinaceae) both are granulate. Just what significance there is to the similarity between Ubisch patterning and exine patterning remains unknown. Although it may be noted that both the tapetum and the sporogenous cells develop from the same hypodermal tissue of the archesporium (Davis, 1966).

So far Ubisch bodies have been found to form on the secretory type of tapetum only. However, by no means have they been found in all species with secretory tapeta (Echlin and Godwin, 1968a; Horner and Rogers, 1974).

Ubisch bodies, like the exine, are composed primarily of sporopollenin which is deposited gradually on precursor structures. These structures have been termed pro-orbicles and appear as lipid-like spheroids of medium electron density within the cytoplasm of the tapetal cells. Pro-orbicles have been found in several species, among them sorghum (Overman and Wormke, 1972), Pinus (Tourn.) L. (Willemse, 1971), Lilium (Heslop-Harrison and Dickinson, 1969) and Helleborus (Echlin and Godwin, 1968a). Echlin and Godwin have given the most complete account of Ubisch body formation to date. Each pro-orbicle (pro-Ubisch body) was limited by a membrane from which ribosomes projected radially. The ribosomes were seen to be associated with
endoplasmic reticulum, which seemed to be continuous with the pro-orbicle limiting membrane. Willemse (1971) also mentioned an association between pro-orbicles, ribosomes and endoplasmic reticulum although not in as fine detail, while Heslop-Harrison and Dickinson (1969) failed to see any such association at all.

The pro-orbicle spheroids are extruded through the surface probably by the fusing of the plasmalemma with the pro-orbicle limiting membranes (Echlin and Godwin, 1968a) and are coated with sporopollenin. Both Echlin and Godwin and Willemse have seen thin "white lines" of unit membrane dimension in the outer portions of the sporopollenin in the Ubisch bodies.

In those species that have large channels in the pollen exine, channels of similar size are found in the Ubisch bodies, usually radiating from the pro-orbicular area outward (DeVries and Ie, 1970; Christensen et al., 1972; Skvarla and Larson, 1966).

In grasses as the Ubisch bodies mature, a reticulum of sporopollenin is formed interconnecting the orbicles. This reticulum forms part of the so-called tapetal membrane which is the only evidence of the tapetal cells that is present at dehiscence. The tapetal membrane is really not a membrane at all since all trace of the tapetal cytoplasm, cell walls and plasmalemma is gone, but as Banerjee and Bargoorn (1971) and Banerjee (1967) have discovered, consists of Ubisch bodies, a stranded layer (reticulum), a fenestrated backing layer and
microrods on the outer periclinal surface. They worked with carbon replicas of acetolyzed tapetal membranes, and except for the microrods their findings agree with the mature tapetal membranes seen by thin-sectioning (Christensen et al, 1972). The reticulum or stranded layer is often reduced and disorganized at anther maturity (Banerjee, 1967). Instead of tapetal membrane the term orbicular wall is often used synonymously.

What is sporopollenin and where is it formed? Sporopollenin has been generally thought of as an acetolysis resistant polymer of mono- and dicarboxylic fatty acids of a high molecular weight \((C_{90}H_{144}O_{27})\) (Echlin, 1968). It is a very mechanically resistant substance and chemically resistant in a non-oxidative environment. However, it is unsaturated and readily undergoes oxidation under appropriate conditions (Brooks and Shaw, 1971). Brooks and Shaw (1968, 1971) synthesized an unsaturated polymer which was almost indistinguishable from the sporopollenin of Lilium henryii. It was obtained from the copolymerization of several carotenoids (ex. \(\beta\)-carotene) with oxygen and also from the copolymerization of a carotenoid-carotenoid ester extract from Lilium with oxygen. Saying that pro-orbicles consist primarily of carotenoids and carotenoid esters, they theorized that when the pro-orbicles are extruded through the plasmalemma they "come into contact with a catalyst and a source of oxygen." Monomers would repeatedly diffuse to the surface of the pro-orbicles and
polymerize. The carotenoid pro-orbicles could also make contact with the microspore and transfer polymerized or partially polymerized sporopollenin to the exine template.

Heslop-Harrison (1968c) does not think that other carotenoid globules in the tapetum, as measured by their absorbance at 450 nm contribute to sporopollenin synthesis, since synthesis was found to be completed before the globules were released from the tapetal cells. He says that although colored carotenoids do not appear to be involved in sporopollenin synthesis, colorless precursors of related molecules could be.

Working with Allium, Risueno et al. (1969) found a very electron dense material accumulating in the tapetal endoplasmic reticulum which they considered to be the nucleus of the Ubisch bodies, although they did not bear any resemblance to the pro-orbicles found by other workers. They reported that the Ubisch bodies grew in size by the lamellar apposition of membranes, and eventually were released to the surface from the interconnecting endoplasmic reticulum which opened into the anther locules.

Gabara (1974) described a similar situation in the microspores of Berberis vulgaris L. At the second meiotic telophase small electron dense globules were found sometimes associated with the ends of short cisternae of rough endoplasmic reticulum. At quartet formation similar globules were seen associated with the inner and outer surfaces
of the plasmalemma. Garbara hesitated to call this material primexine and suggested further investigations to determine the composition of the globules and whether they may be sporopollenin.

Gupta and Nanda (1972) investigated the histochemistry of the tapetal membrane in several taxa; (Pyrostega venusta (Ker-Gawl) Meirs; Tecoma stans (Linn.) H.B. & K.; Cereus; Helianthus annuus Linn.; Hibiscus rosa-sinensis Linn.; and Ephedra foliata Boiss.). They found that in both taxa with the secretory type of tapetum and those with amoeboïd type, the tapetal membrane consisted of an acetolysis resistant component, (probably sporopollenin) and insoluble polysaccharides (callose, pectin and a cellulose-like material).

Recently some thought has been given to the role of glycocalyces in exine and orbicle formation. Bennett (1963) proposed the term glycocalyx as a term to describe the extracellular, sugary (polysaccharide) coating attached to the outer surface of the plasmalemma in many plant and animal cells, which functions in binding or excluding certain ions and particles from the cell. In plants as reviewed by Roland (1973), the glycocalyx is primarily composed of acid polysaccharides, and he suggested that the highly polymerized polysaccharides are concentrated in Golgi vesicles which contact the plasmalemma and release their contents into the cell wall.

Rowley and Skvarla (1974) demonstrated the presence of acid polysaccharides (staining with PTA in chromic acid) around the inner
spheroidal surface (core) of the Ubisch bodies in *Phleum pratense* L. and also on the tapetal membrane at the base of the Ubisch bodies. They noted that the Ubisch bodies were formed while adjacent to the tapetal plasmalemma and its glycocalyx. Also the limiting membranes of the pro-Ubisch bodies were derived from the plasmalemma and its associated glycocalyx. Rowley and Skvarla interpreted their results as being indicative of a control of sporopollenin deposition through a plasmalemma-glycocalyx system. Rowley (1973) and Rowley and Skvarla (1975) found a deposition of sporopollenin and formation of the exine channels in *Epilobium* L. and the elaborate exine patterning in *Canna* around a glycocalyx network, probably a mucopolysaccharide.

Much earlier Waterkeyn and Bienfait (1970) removed the callose walls from microspores which were laying down early exine elements, and found reverse impressions of the exine in the callose. This they felt was evidence to indicate that the callose wall acted as a template or mold for the formation of the exine.

Risueno et al (1969) felt that the Ubisch bodies are deposited on the surface of the microspore, and that exine deposition might be explained by the Ubisch bodies rolling over the surface of the microspore depositing the lamellae that compose the Ubisch bodies.

Skvarla and Larson (1966) hypothesized that Ubisch bodies might be produced by the accumulation of sporopollenin on membranes from
aborting microspores which are usually present even in fairly fertile anthers.

Godwin (1968a) reported that sporopollenin deposition takes place on spherical pro-orbicles and that it may be under the control of surface membranes which originate from the tapetal endoplasmic reticulum from which the pro-orbicles seem to originate.

Mepham and Lane (1968, 1969a) believed that a "lipid exudate" (pro-sporopollenin) was secreted through the exine to the developing surface and hardened upon oxidation. They found this lipid exudate between the bacula of the exine in Tradescantia and noted that there was a lack of this substance in aborted pollen grains.

What has been reported regarding the passage of nutrient materials from the tapetum to the microspore? Flynn (1971) said that after release from the quartets, more material must enter the developing microspore than goes out in order to account for the great increase in cell volume, and this is most likely to come from the tapetum. Heslop-Harrison (1972) reported that in culturing sporophytes from haploid spores, results were obtained only when early free microspores were used (after callose dissolution and before substantial exine development).

Following tritiated thymidine from the tapetum to the microspore, Takats (1962) found that the label in the tapetum disappeared just before the first pollen mitosis and was found on the microspore
exine, but there was no evidence of incorporation into the dividing nuclei. Takats felt that pollen DNA synthesis is dependent on a non-tapetal precursor pool. In certain grasses Rowley (1962) and Banerjee and Barghoorn (1971) have observed strands of material running between the spinules of the exine and those of the Ubisch bodies which they felt might represent the transfer of substances involved in pollen wall formation. Chambers and Godwin (1961) described a continuity between the tapetal cytoplasm (via funnels in the tectum) and the void in the pollen exine in *Tilia platyphyllos*.

Pieces of tapetal endoplasmic reticulum were seen in the channels of the endexine by Mepham and Lane (1968) in *Tradescantia* (Rupp.) L. a species having the plasmodial type of tapetum. Neither Mepham and Lane (1968) nor Godwin (1968a) have found any evidence that sporopollenin is synthesized in the tapetum and transferred to the exine. However, they did not discount the possibility that sporopollenin precursors might be liberated from the disorganizing tapetum and move to the microspore.

Rowley et al (1970) and Flynn (1971) found several features of pollen walls which they thought might explain the uptake of nutrients by the microspores. In *Algiceras* they found tufts of microtubules (10nm in diameter) associated with a glycocalyx extending from the surface of the exine to the tapetal cells. They hypothesized a similarity between the tubules and the fuzzy coats (acid polysaccharides)
of animal cells which traps material at the cell surface to be engulfed through pinocytosis. Plasmalemma evaginations which penetrate the exine of *Epilobium montanum* early microspores form the basis for the channels (50-300nm in diameter) seen in the mature pollen wall. Wall tubules (7-18nm) were seen in *Nuphar* extending from the nexine-2 to the spinules of the tectum. The diameter of the tubules could be increased under conditions of low divalent cation concentration and decreased under high divalent cation (CaCl₂) concentration. Thus, the tubules may act as a pump, selectively transporting materials across the exine. They also found that at least one substance, β,lanthanum nitrate, can pass through the exine material of *Epilobium* in addition to the channels.

Beer (1911) published an early description of the callose wall that surrounds the PMC's early in meiosis. Heslop-Harrison (1964) felt that the callose wall acts as a "molecular filter" that allowed some nutrients to pass into the PMC but prevented large molecules from entering and disrupting the essential autonomous development of the PMC. It would follow that dissolution of the callose after quartet formation is necessary for most nutrients to pass into the young microspore to facilitate its development. Heslop-Harrison and Mackenzie (1967) found that a thymidine label entered *Lilium* PMC's during leptotene-zygotene before the callose wall was formed, but PMC's after being invested in callose failed to incorporate the label.
After release from the quartets, the young microspores readily incorporated the label. Glucose has been incorporated into the callose wall (a β,1-3 glucan) (Southworth, 1971) as has colloidal iron (Rowley and Dunbar, manuscript in publication cited by Southworth, 1971).

Irregularities in callose behavior have been implicated in the production of abortive microspores. In normal fertile 'Wheatland' sorghum Damon (1961) found that in about 5% of the meiocytes, the PMC walls break down before or during meiosis, the contents intermingle and the callose walls reform, producing multiploid sporocytes. In cytoplasmic male-sterile cultivars derived from Wheatland, the same abnormality was found only in a much higher percentage.

A normal meiosis proceeding to the quartet stage was found to be rare in a line of CMS petunias (Frankel et al, 1969). They reported a higher callose activity from prophase I to the quartet stage in CMS anthers than in fertile anthers, and felt that the early callose activity might contribute to abnormal cytokinesis. In related research Izhar and Frankel (1971) found that until late quartet stage locular pH remains ~7.0. A sudden drop to pH 6.0 results in optimum callose activity and the callose wall is degraded. In one CMS petunia line the pH was always found to be low, the callose wall dissolved early and PMC abortion occurred early. Another CMS line had delayed callose activity, and abortion occurred during the early free microspore stage. In a genetic male-sterile petunia line, the locular pH
remained high, the callose failed to dissolve and the microspores aborted while still in the quartet. Thus, by influencing callose behavior through callase activity, an abnormal locular pH resulted in aborted microspores.

Mepham and Lane (1969) have reported that prior to callose dissolution, numerous small electron lucent vesicles near dictyosomes were seen fusing with the microspore plasmalemma. They felt that these vesicles might contain callase, the enzyme required to degrade the callose special wall.

Warmke and Overman (1972) working with CMS sorghum found that the callose wall separated from the PMC's early in meiosis and formed an amorphous mass in the center of the locule. The PMC's tended to fuse and produce multiploid sporocytes. The involvement of callose in occasional male sterility in otherwise normal plants was noted by Nanda and Gupta (1974). They reported that in those locules where the tapetal cells become hypertrophied, the quartets lacked a callose wall and eventually shriveled. They ascribed this to early callose dissolution due to excessive callase produced by the hypertrophied tapetum, although they offered no substantive evidence.

Is exine patterning under gametophytic or sporophytic control? If sporophytic, is the tapetum responsible or does the pre-division pollen mother cell exert primary control? Most all researchers who have observed pollen ultrastructure now believe that the tapetum does
not exert primary control over exine patterning. All have seen exine
initiates appear while the microspores were still encased in callose;
and believed that the callose acted as an effective barrier to direct
tapetal involvement (Ehrlich, 1958; Heslop-Harrison, 1968c, Godwin,
1968b; Dickinson, 1970, Rogers and Harris, 1969; Horner and Beltz,
1970). Mepham and Lane (1968, 1969) have gone so far as to report
that in *Tradescantia* the exine proper develops while in the quartet
stage, and that the exine is entirely a product of the microspore, and
the tapetum makes no contribution to its development. Usually the
bulk of exine deposition occurs after the microspores are liberated
from the callose walls.

The involvement of the haploid spore genome in the synthesis of
the exine has been somewhat discounted. Godwin (1968b) said that the
segregation of different exine types in the same quartet has never
been recorded. Rogers and Harris (1969) working with triploid *Linum*
hybrids found normal exine and pore in minispores with incomplete
cromosome complements. The thickness of the exine was directly
related to the amount of cytoplasm received by the spore. These re-
sults are in agreement with the feelings of Heslop-Harrison (1968b)
and Rowley and Skvarla (1975) who suggested that information programmed
in the cytoplasm of the PMC before meiosis and carried through to the
spores via the cytoplasm or plasmalemma might be responsible for exine
patterning. Heslop-Harrison (1968b) described multimembraned bodies
containing mitochondria and spherosomes appearing in the early meiotic PMC. The number of those bodies reached a maximum late in meiotic prophase I, the contents being dispersed after quartet formation. He regarded these as possible carriers of selected portions of PMC cytoplasm protected from the meiotic pre-division reorganization, which could supply microspores with enzymes and structural proteins necessary in the early life of the spore.

Further support for the theory that the information required for exine formation is transcribed early in the development of the PMC has been given by the work of Heslop-Harrison (1971) and Dover (1972). Heslop-Harrison centrifuged Lilium anthers and found division errors, aperture abnormalities, and exine reticulum rearrangements associated with centrifugation during diakinesis, metaphase I-telophase II, and the early quartet stage, respectively. Colchicine treatment, predictably produced many division failures, but in addition it often grossly affected exine patterning even in the absence of division errors. Some meiocytes which failed to divide due to centrifugation or colchicine treatment, developed a normal exine patterning.

The establishment of polarity early in development has been reported by Dover (1972) and Christensen and Horner (1974). Dover applied colchicine to plants of Triticum aestivum at varying stages of development. Anthers exposed to 0.5% of colchicine just after the last premeiotic mitosis and before the tapetal nuclear division
produced uninuclear monads (failure of all meiotic cell divisions resulting in only one cell) which were poreless. Colchicine applied just before prophase I resulted in monads with four pores randomly positioned. Normal pore positioning was determined to be at the polar ends of the second meiotic division spindles. A dilute solution of colchicine (.01%) applied before prophase I produced multipolar first division spindles. The result was either a large monad with many random pores (more than four) or a polyad (many cells failing to separate) with a pore at the polar end of each cell. Dover thought that the number of pores might be dependent on the number of spindle-pole determinants whether expressed as spindle poles or not. The spindle axis if expressed would determine the placement of the pore. Colchicine applied soon after the last premeiotic mitosis was found to induce asynapsis at metaphase I.

Dover (1972) also found that in *Triticum aestivum* plants with alien *Aegilops mutica* chromosomes quadrinucleate monads were formed with normal positioning of the pores. The first meiotic division wall had formed but not the second. Normal spindle orientation was observed. *Aegilops mutica* x *Triticum aestivum* hybrids produced cells with micronuclei that often divided at the second meiotic division producing micro-pollen grains which contained a pore. PMC's with micronuclei which divided at anaphase I, but failed to form separate micrograins, produced pollen with 1 to 3 pores.
Poddubnaya-Arnol'di (1962) observed pollen abnormalities associated with division errors in wheat-Agropyron amphidiploids. A failure of the first meiotic division followed by a normal second division resulted in microspore dyads, each nucleus having a diploid number of chromosomes. Pollen grains joined by common walls in groups of twos or fours, along with dwarf, giant, and multiporate pollen grains, were also found in these hybrids.

Christensen and Horner (1974) have hypothesized that it might be the asymmetric distribution of callose around the PMC's which establishes the polarity necessary for proper pore and spindle placement. In sorghum as in most grasses, callose is deposited as a wedge from the center of the locule outward with the side of the meiocytes appressed to the tapetum receiving the last deposition and least amount of callose.

Meiotic disturbances have been described in a number of sterile species. Kinoshita and Nagao (1968) reported normal meiotic behavior in diploid CMS sugar beets, but found an abnormal distribution of chromosomes in tetraploids regardless of their sterility. They observed an abnormal hypertrophy of the tapetum in both CMS diploids and tetraploids. The same type of tapetal growth was associated with a male-sterile gene, although the hypertrophy was not as profound.

Novak and Betlach (1970) found meiotic disturbances in 68-82% of male sterile (CMS) sweet pepper plants but only in 2% of fertile
plants. Anaphase bridges, laggards and micronuclei were common with an occasional trivalent or quadrivalent. The chromosome number was found to be n=12 in all the plants tested. The authors thought that the sterility was caused by the meiotic irregularities, possibly determined by the interaction of nuclear genes with the S-cytoplasm. Within anthers Novak and Betlach observed an asynchronous development of the microspores. Most of the meiocytes aborted during meiosis according to Novak et al (1971). While at dehiscence a normal pepper anther would produce about 500 pollen grains, the sterile anthers produced 30 or less, most of which were nonviable as determined by tetrazolium chloride testing.

Burson and Bennett (1971) observed that some Paspalum species had a low pollen stainability associated with meiotic abnormalities. However, the stainability was usually irregular, some meiotically normal species having a low stainability also. No correlation could be made between meiotic behavior and stainable pollen. An apomictic species, Paspalum proliferum, had an irregular meiosis (5\(^{\text{II}}\) and 40\(^{\text{I}}\)) and a very low pollen fertility (9.4%) and seed set (6%) (Burson, 1975).

Anthers from colchicine-induced CMS sorghum anthers exhibited a breakdown of PMC walls resulting in polyploid and aneuploid cells (Ericksen and Ross, 1963a,b). Cytoplasmic strands were seen connecting the meiocytes to the tapetal cells in sterile anthers and some
tapetal hypertrophy was also observed. Ericksen and Ross thought that the cytoplasmic strands influenced the movement of nutrients from the tapetum to the PMC's causing the failure in PMC wall formation. Male sterile pollen was shrunken and irregular, but there was no mention of the time of abortion.

Temperature and photoperiod have also been known to influence pollen fertility. Stephens (1937) reported that the year 1936 was very hot and dry producing withered sterile anthers in otherwise normal sorghum flowers. A temperature sensitive period from the quartet stage to the first pollen mitosis was noted in onions by Barham and Munger (1950). Below 70°F no viable appearing pollen was formed in male sterile anthers, but a temperature of 70-80°F during the critical period resulted in the production of a small percentage (<1%) of viable appearing pollen as measured by its germination on agar.

Lyubimova (1962) investigating fertility in wheat–Agropyron hybrids, found many more meiotic disturbances on hot (35-37°C) and dry days than was usual for the hybrids. Numerous univalents and micronuclei, chromatid bridges, and associations of five or six instead of tetrads were found. The increase in abortive pollen under these conditions was attributed to the meiotic irregularities. The wheat and Agropyron parental types failed to demonstrate such meiotic irregularities on hot and dry days.
Peterson (1958) in CMS *Capsicum* found that the male sterility was somewhat dependent upon high temperatures. Plants which were completely sterile in August expressed 20-30% fertility in late October. During the winter in the greenhouse these plants had near normal pollen production and could be self-fertilized. The progeny were completely sterile when planted in the field.

Meyer (1966) described a positive correlation between the percentage of sterile cotton anthers and the maximum temperature 15-16 days before anthesis. Also the relative humidity 22-23 days before anthesis was positively correlated with the number of anthers which developed in the flower.

Floral abnormalities (meiotic and tapetal disturbances) produced by growing maize in 8 hour (short) days could be alleviated by night interruptions at low light intensities (Moss and Heslop-Harrison, 1968).

Kaul (1970) felt that high temperatures and photoperiod were responsible for the meiotic irregularities (including polyploidy) and persisting tapetum which resulted in pollen sterility in *Tabernaemontana cornaria*.

Jain (1960) found that high temperatures (33-2°C) for 86 hours before sampling induced neo-centric activity in the chromosome ends of *Lolium perenne* at metaphase I. The chromosomes were consequently
stretched between the poles, breakage occurred and no viable pollen
gains were formed from the deficient meiocytes.

Cool temperatures at the young microspore stage of development
increased male and female sterility in rice (Lin and Peterson, 1975).
The percent sterility was found to increase directly with the time of
cool temperature exposure.

Kaul and Singh (1967a) found that application of sodium 2,3-
chloroisobutyrate on fenu-greek (Trigonella foenum-graecum L.) re-
sulted in a tapetum which enlarged at the quartet stage and pushed the
young microspores to the center of the locule. Tapetal degeneration
followed the abortion of the microspores. The authors attributed the
sterility to starvation of the microspores and the mechanical pressure
exerted by the hypertrophied tapetal cells.

Treating Allium plants with maleic hydrazide in concentrations
above .01% resulted in complete male sterility caused by the early de-
generation of the sporogenous tissue (Kaul and Singh, 1967b).

Application of sodium 1-(p-chlorophenyl) 1,2-dihydro-4, 6-
dimethyl-2-oxonicotinate several days before meiosis induced complete
male sterility in barley (Wang and Lund, 1975). Female fertility was
reduced to 40%. The tapetum in the sterile anthers persisted through
the free microspore stage. Reduced anther length in the treated
plants was also noted.
Grogan and Sarvella (1964) and Sarvella and Grogan (1965) investigated variation in plant morphology in CMS, restored and normal lines of maize. They found that the stalk lengths above the ear, the length of the tassel culm and the lengths of the internodes above and below the ear generally were reduced in the steriles. The internode above the ear, however, varied somewhat with genotype and environment. Sheath lengths, the number of internodes and the length of the stalk below the ear remained as in the normals. Restored versions had a high genotype and environment influence but usually the number of internodes above the ear and the length of the sheaths were reduced. Sarvella and Grogan (1965) found that where internode shortening below the ear in the sterile or restored plants was noted early in development, at maturity the internode length could be normal or even longer.

Sarvella, Grogan, and Myhre (1975) could find no difference in leaf width, epidermal thickness, vascular bundle width, leaf thickness, stomatal opening, or stomatal number between normal, CMS and restored versions of maize.

Meyer and Meyer (1964) and Meyer (1965, 1972) observed that, in interspecific hybrids of cotton, the overall number of anthers and the number of sterile anthers produced varied significantly from one genotype cytoplasm combination to another. In general diploid cytoplasms tended to reduce the number of anthers.
Both Joppa et al (1966) and Prakasa Rao and Jain (1975) observed disorganized, poor vascular development in CMS *Triticum aestivium*. Prakasa Rao and Jain thought that the effect of fertility restoration genes was to restore normal development of the vascular tissue in the filament.

The transmission of an asexual factor for cytoplasmic male sterility across a graft union was reported by Edwardson and Corbett (1961) and Curtis (1967). The progeny of normal scions grafted to male sterile plants showed a substantial percentage of male sterility in generations through the $F_3$ (after grafting).

Edwardson (1962) found electron dense inclusions in root tip cells of CMS corn which were absent from root tips of maintainer lines. Similar inclusions were found in the tapetal cells of both maintainer and CMS lines. Edwardson, however, didn't believe that these inclusions could be viruses because "it seems improbable that only the male-sterile root tips would be infected."
MATERIALS AND METHODS

Selection of Experimental Plant Material

Plant material was chosen from the third substitution backcross generation (SB$_3$) to the amphidiploid hybrid *Triticum durum* x *Agropyron intermedium* with *A. intermedium* being the recurrent pollen parent. This is termed a substitution backcross in that the nucleus of the recurrent pollen parent, in this case intermediate wheatgrass (*A. intermedium*), is effectively being substituted into the cytoplasm of durum wheat (*T. durum*) (Kihara, 1951).

Fifteen hundred individual third substitution backcross generation (SB$_3$) plants were established in 1971 in a nursery at the Montana Agricultural Experiment Station research farm west of Bozeman. These plants were investigated for percent male sterility using aceto-carmine as a fertility indicator. In 1972 spikes from plants which had been identified as male sterile the previous summer were collected and fixed in Farmer's solution (3:1, ethanol:acetic acid). Anthers were dissected from the spikes and standard squash preparations were made of PMC's following macerating and staining the anthers in 1-5% acetocarmine (Smith, 1947).

The number of univalents and bivalents was recorded for each cell where the chromosomes could be clearly distinguished (stages such as diakinesis and anaphase I). In addition, the number of univalents
present at metaphase I, seen as lying off the metaphase plate, was recorded.

Once meiotic data had been collected from the SB\textsubscript{3} generation, the plants were grouped according to their level of male sterility and meiotic stability. For each plant, using the cells where all chromosomes could be counted, the sum of the following criteria was used as an indicator of the meiotic stability and normality of the plant (normal with respect to the number of chromosomes in intermediate wheatgrass): the average number of univalents, the standard error of the number of univalents, the deviation of the average number of bivalents from the expected number of 21 (Schulz-Schaeffer, 1972), and the standard error of the number of bivalents. A stable, normal plant would then have low standard errors, very few univalents, and nearly 21 bivalents, while an unstable, abnormal plant would have high standard errors, many univalents, and a large deviation from the expected 21 bivalents. These data along with the sterility percentages for each plant are shown in Table I. The plants are referred to, as they will be throughout the thesis, by a three unit pedigree number.

For cytological and morphological investigation during the summer of 1974, twenty plants were selected from those listed in Table I. Twenty plants were selected in order to place four plants into each of the following five categories: those plants which were 1) meiotically
Table I. Meiotic data of one SB\(_5\) and 32 SB\(_3\) plants including the criteria for selecting plants for cytological study

<table>
<thead>
<tr>
<th>Plant Number</th>
<th>Cells in</th>
<th>Univalents</th>
<th>Bivalents</th>
<th>Four Criteria</th>
<th>Male Sterility(%)</th>
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<td>Avg. No.</td>
<td>Avg. from 21</td>
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<td>1-9-79</td>
<td>2.9 130</td>
<td>2.4 26</td>
<td>-1.1</td>
<td>0.12</td>
<td>8</td>
</tr>
<tr>
<td>2-16-50</td>
<td>2.9 17</td>
<td>2.3 29</td>
<td>-1.0</td>
<td>0.44</td>
<td>7</td>
</tr>
<tr>
<td>2-17-41</td>
<td>2.1 44</td>
<td>2.2 86</td>
<td>-1.2</td>
<td>0.25</td>
<td>4</td>
</tr>
<tr>
<td>2-17-14</td>
<td>2.4 54</td>
<td>2.5 50</td>
<td>-1.0</td>
<td>1.00</td>
<td>2</td>
</tr>
<tr>
<td>1-2-49</td>
<td>3.8 111</td>
<td>1.0 45</td>
<td>-3.0</td>
<td>0.84</td>
<td>4</td>
</tr>
<tr>
<td>2-16-37</td>
<td>6.2 34</td>
<td>5.1 77</td>
<td>-0.4</td>
<td>0.17</td>
<td>14</td>
</tr>
<tr>
<td>2-23-39</td>
<td>4.2 40</td>
<td>6.0 100</td>
<td>-1.5</td>
<td>0.50</td>
<td>2</td>
</tr>
<tr>
<td>2-17-20</td>
<td>9.0 44</td>
<td>6.4 78</td>
<td>-1.8</td>
<td>0.42</td>
<td>33</td>
</tr>
<tr>
<td>29-1-27</td>
<td>5.7 47</td>
<td>6.6 1.72</td>
<td>-1.9</td>
<td>0.91</td>
<td>8</td>
</tr>
<tr>
<td>2-22-48</td>
<td>7.4 132</td>
<td>11.9 1.77</td>
<td>-4.5</td>
<td>1.09</td>
<td>21</td>
</tr>
</tbody>
</table>

* Percentage male sterility determined in 1973 or previous to 1973
# Percentage male sterility determined in 1975
† Percentage male sterility determined in 1974
stable, normal and male fertile; 2) meiotically stable, normal and partially male sterile; 3) meiotically stable, normal and male sterile; 4) meiotically unstable, abnormal and male sterile; and 5) meiotically unstable, abnormal and partially male sterile.

The four breeder's genotypes of the synthetic variety 'Oahe', hereafter noted as Oahe 1, Oahe 2, Oahe 3, and Oahe 4, were used as examples of meiotically stable and fertile intermediate wheatgrass (category 1 = control) although meiosis was not investigated in these four clones. One plant was selected at random from each of the four isolated Oahe nurseries in which the Oahe breeder's genotypes were maintained. The partially sterile plants with the lowest total of the four criteria were placed in category 2, meiotically stable and partially sterile. The male sterile plants with the lowest four criteria total were placed in category 3. The male sterile plants with the highest criteria total were placed in category 4, meiotically unstable and male sterile.

A four criteria total of 2.0 was designated as the upper limit for placing a plant in a meiotically stable category. A plant having a four criteria total greater than 2.0 would be assigned to a meiotically unstable category. No existing plants which had been investigated meiotically were found to be meiotically unstable and partially sterile. Therefore, four $SB_3$ progeny were selected at random from the $SB_2$ parent which exhibited the most meiotic instability as measured by
the sum of the four criteria. These plants were tentatively placed in category 5, pending a determination of their meiotic behavior and sterility during the summer of 1974. The SB₂ data from which this selection was made is shown in Table II. As can be seen from Table I, three of the four plants did indeed fit category 5, unstable and partially sterile. SB₃-2-16-88 failed to flower and no meiotic data could be collected from this plant.

The plants were initially assigned to categories on the basis of sterility counts retrieved from records previous to 1973. Some of the plants which previous counts had indicated as being totally male sterile were found actually to be only partially male sterile when 1973 sterility percentages were consulted. Consequently, the original composition of the categories was altered. In addition, two plants, SB₃-1-9-79 and SB₅-19-79-31-14, were added to categories 4 and 5, respectively, in 1975. SB₃-1-9-79 was a plant which had maintained male sterility (greater than 98%) over four years and also had high female fertility (38 seeds per spike), but had not been investigated meiotically. The meiotic data collected for this plant in 1975 along with the data of one of its progeny, SB₅-1-9-79-31-14, have been included in Table I. The final composition of the categories is given by Table III. Only one category, unfortunately perhaps the most important category, suffered from the rearrangement. Category 3 was left with
Table II. Meiotic data from the SB\textsubscript{2} generation

<table>
<thead>
<tr>
<th>Plant Number</th>
<th>Deviation of Average from 21</th>
<th>(s_x)</th>
<th>Average number</th>
<th>(s_x)</th>
<th>Criteria total</th>
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<tr>
<td>SB\textsubscript{2}-1</td>
<td>-5.58</td>
<td>.67</td>
<td>16.80</td>
<td>1.10</td>
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<tr>
<td>1-2</td>
<td>.13</td>
<td>.37</td>
<td>4.61</td>
<td>.41</td>
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</tr>
<tr>
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<td>-3.23</td>
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<td>12.00</td>
<td>1.00</td>
<td>17.04</td>
</tr>
<tr>
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<td>.10</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
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<td>8.81</td>
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<td>.97</td>
<td>19.20</td>
<td>1.56</td>
<td>28.50*</td>
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<td>-3.63</td>
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<td>12.57</td>
<td>.78</td>
<td>17.52</td>
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<td>15.37</td>
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<td>20.93</td>
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<td>.45</td>
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<td>2.30</td>
<td>.45</td>
<td>4.73</td>
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<td>4.13</td>
<td>.33</td>
<td>5.10</td>
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<td>7.78</td>
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<td>.60</td>
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</tr>
<tr>
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<td>.56</td>
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</tr>
<tr>
<td>24-2</td>
<td>-2.31</td>
<td>.14</td>
<td>.93</td>
<td>.10</td>
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<td>1.24</td>
<td>4.00</td>
<td>1.76</td>
<td>8.66</td>
</tr>
<tr>
<td>33-1</td>
<td>-1.70</td>
<td>.42</td>
<td>3.30</td>
<td>.86</td>
<td>6.28</td>
</tr>
</tbody>
</table>

* SB\textsubscript{3} progeny from this plant were selected for cytological investigation
Table III. Classification of plants for cytological study according to meiotic behavior and % male sterility

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Plant No.</th>
<th>Four Criteria total**</th>
<th>% Male Sterility 1975*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>meiotically stable and normal and male fertile</td>
<td>Oahe 1</td>
<td>--</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oahe 2</td>
<td>--</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oahe 3</td>
<td>--</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oahe 4</td>
<td>--</td>
<td>5.4</td>
</tr>
<tr>
<td>2</td>
<td>meiotically stable, normal, and partially male sterile</td>
<td>SB$_3$ - 24-2-12</td>
<td>0.0</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24-2-7</td>
<td>.35</td>
<td>8.9</td>
</tr>
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<td>24-2-55</td>
<td>.70</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
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</tr>
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<td>2-17-68</td>
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<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>meiotically stable, normal, and male sterile</td>
<td>SB$_3$ - 2-17-83</td>
<td>1.43</td>
<td>99.9</td>
</tr>
<tr>
<td>4</td>
<td>meiotically unstable abnormal, and male sterile</td>
<td>SB$_3$ - 2-22-68</td>
<td>3.00</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>2-17-20</td>
<td>9.40</td>
<td>100.0</td>
</tr>
<tr>
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<td>2-22-48</td>
<td>19.26</td>
<td>87.9</td>
</tr>
<tr>
<td>5</td>
<td>meiotically unstable abnormal, and partially male sterile</td>
<td>SB$_3$ - 2-16-2</td>
<td>2.16</td>
<td>16.3*</td>
</tr>
<tr>
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<td></td>
<td>SB$_3$ - 1-9-79-31-14</td>
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</tr>
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<td></td>
<td>SB$_3$ - 2-16-42</td>
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<td>6.0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2-16-88</td>
<td>--</td>
<td>87.3</td>
</tr>
</tbody>
</table>

* Average of acetocarmine and IKI sterility percentages
+ Sterility in 1974. In 1975 this plant produced only papery anthers containing no pollen
** Sum of the four meiotic criteria of Table I
only one plant. The other categories each still contained four or
more plants.

The categories were established to account for as much vari­
ation as was possible in a cytological investigation of this type.
To minimize one source of environmental variation (location), the
original 20 plants were transplanted from their rather widespread
locations in the large SB$_3$ and Oahe nurseries to a small strip at the
group of another nursery. Cytological data were collected from this
"transplant" nursery. The two plants added in 1975, SB$_3$-1-9-79 and
SB$_5$-1-9-79-31-14, were investigated in their original locations in two
separate nurseries.

Sample Size

Two hundred and forty anthers were sampled during the summer of
1974. Approximately 43% of these failed to give useful information as
a result of fixation and processing difficulties. About 400 anthers
were sampled during the summer of 1975. Information was obtainable
from only about 55% due to similar technical problems. Duplication of
stages reduced the number actually thin-sectioned to 15% in 1975.

Originally eight stages, concentrating on those following the
first meiotic division were to be studied from each plant. These in­
cluded metaphase I, interkinesis, metaphase II, quartet, early uninu­
nucleate microspore, later uninucleate microspore, binucleate pollen,
and mature trinucleate pollen. From a review of the literature these were the stages where differences were most likely to be found. As a result of the fixation difficulties encountered, and some duplication of stages, identical stages could not be compared between all individual plants. Within each plant category, however, there was enough overlap to obtain an adequate continuum of the eight stages of development which were of most interest, namely later meiotic stages and stages following the release of the microspores from the quartets. In addition, some stages earlier than metaphase I were sufficiently represented to allow comparison between categories of plants.

Sample Collection and Processing (1974)

In the field whole spikes were placed in screw top culture tubes containing cold 5% glutaraldehyde in 0.1M potassium phosphate buffer, pH 7.3. The culture tubes were kept in ice water in a cold chest and transported to the laboratory for dissection of the spikes. Individual florets were excised and one of the three anthers was squashed in acetocarmine for stage determination. As much as possible only basal or secondary florets were sampled, and spikelets near the ends of the spike were avoided. Upper florets and peripheral spikelets are more likely to be sterile and not representative of typical behavior. If a sample of the particular stage of development was needed, the other two anthers were placed in a one dram vial of 5%
glutaraldehyde. One of the anthers was cut in half to be processed for electron microscopy. The other was originally intended to be used for paraffin sectioning. However, as will be discussed, a method of sampling plastic thick sections from the same anther processed for electron microscopy was actually used to compare findings at the light and ultrastructural levels. The third anther, then, was never utilized.

The anthers were fixed for 17-46 hours in glutaraldehyde at 4°C in a refrigerator. Following three 20 minute buffer rinses (0.1M potassium phosphate, pH 7.3) the anthers were placed in phosphate buffered 1% OsO₄ to be fixed 2-1/2 to 3-1/2 hours at 4°C. After osmium fixation, processing continued using the following schedule:

1. Three 20 minute buffer rinses.

2. Dehydration in acetone, ten minutes per step.
   a) 10% acetone in water
   b) 30% acetone in water
   c) 50% acetone in water
   d) 70% acetone in water. Held anthers at this stage for 3 days to 3 weeks at 4°C.
   e) 90% acetone in water
   f) 100% acetone

3. Two 10-minute steps in propylene oxide.

4. Infiltration with Spurr's low viscosity embedding medium (Spurr, 1969).
   a) 50% Spurr's in propylene oxide, 45 minutes on a rotary shaker.
   b) 75% Spurr's, shake 45 minutes.
5. Each anther was placed in a conical BEEM capsule (size 00) and filled with fresh Spurr's embedding plastic.

6. The plastic was cured overnight (15-18 hours) at 70°C.

Sample Collection and Processing (1975)

Anthers were dissected from spikes in the field and placed in vials of 5% glutaraldehyde in 0.05M potassium phosphate buffer, pH 7.1, without determining the meiotic stage. The spikes were purposely oversampled in order to insure an adequate number of representative stages. The stage of development was later ascertained by thick sectioning the embedded anthers. About one-half of the anthers sampled were cut in half, the rest were left intact. In the field the vials were kept in ice water until they were returned to the laboratory where fixation of the samples in glutaraldehyde continued for 19-94 hours at 4°C. Following three 20-minute buffer rinses, the anthers were post-fixed in phosphate buffered 2% OsO₄ for 4-6 hours at 4°C. Then the anthers were again rinsed three times in buffer, dehydrated in acetone (at 70% they were held 4-14 days at 4°C) to propylene oxide, and transferred to 50% Spurr's embedding medium in propylene oxide.

The anthers were infiltrated overnight under a vacuum, then transferred to 75% Spurr's in propylene oxide, placed on a rotary
shaker for one hour, and then infiltrated under a vacuum for 7 more hours. The above routine was repeated twice more with increasing concentrations of Spurr's embedding medium. Finally, from one to three anthers were transferred to each conical BEEM capsule filled with fresh Spurr's, and the plastic was cured overnight (16-20 hours) at 65°C. An extended infiltration schedule was used in 1975, since 50% of the anthers sampled had not been cut in half, a procedure which facilitates infiltration but sometimes damages the tissue.

Thick Sectioning and Staining for Light Microscopy

The plastic blocks were removed from the capsules and trimmed with a razor blade so that the exposed face was at the approximate longitudinal center of the anther. From 8-10 sections one micron thick were taken from each block using glass knives and a Reichert Omu2 ultramicrotome. The sections were lifted from the trough with a hair loop, transferred to a drop of water on a microslide coated with ARG adhesive (Jensen, 1962), and dried on a hot plate at 75°C. After the sections had thoroughly dried, they were covered with a drop of 1% toluidine blue 0 in 0.05M borate buffer, pH 9.0 (Mayhew and Carroll, 1974) and were allowed to stain for 25-30 minutes on a hot plate at 75°C. Sections from anthers collected in 1975 and fixed using a buffer of a different molarity and pH were stained only 5 minutes.
The excess stain was washed off with distilled water. After differentiating the metachromatic stain for two minutes in 95% ethanol followed by two minutes in absolute ethanol, the slides were transferred to xylene for five minutes. Coverglasses were then mounted using 'Namount' mounting medium.

In 1974 the blocks had been trimmed so that only one locule was sectioned. All blocks were thick sectioned (1 μm) and also thin sectioned for electron microscopy. In 1975 the blocks were initially trimmed so that the entire anther cross section could be thick sectioned. After viewing the thick sections, the blocks of interest for electron microscopy were retrimmed to an area representing only one selected locule.

Photographs of thick sections were taken on 35 mm Panatomic-X film using a Wild automatic photomicroscope. A yellow filter was used to enhance the contrast.

Thin Sectioning and Staining for Electron Microscopy

About 20-30 thin sections (60-90 nm) were taken from each block with a Dupont diamond knife using a Reichert OmU2 ultramicrotome. The sections were expanded with toluene vapors and affixed to 300 mesh copper grids. Uranyl acetate followed by lead citrate (Reynold's, 1963) was used to stain the sections. The grids were floated, sections down, on a drop of filtered 6% uranyl acetate in 50% methanol.
and stained for 20-30 minutes. The grids were then washed with de-
creasing concentrations of methanol (50%, 30%, 10% and distilled
water) from squeeze bottles and allowed to dry. A drop of Reynold's
lead citrate was placed on a piece of dental wax in a covered petri
dish containing NaOH pellets. The grids were floated on the drop and
the sections allowed to stain for 3-5 minutes. The grids were then
washed with a stream of 0.02N NaOH followed by a stream of distilled
deonized water from squeeze bottles. After drying the grids were
viewed using a Zeiss EM-9S2 electron microscope, and photographs were
taken on Kodak electron microscope film #4489.

All printing was done on either Kodak Ektamatic SC or Agfa-
Gavaert Brovira photographic paper.

Paraffin Sectioning for Light Microscopy

Usually pollen development is synchronous within any one anther
microsporangium (Vasil, 1967). However, in species with unusually
long anthers there often is a developmental gradient from the bottom
to the top of the anther (Bennett, 1973). To give an indication if
there was any such developmental gradient present in intermediate
wheatgrass with anthers up to 8 mm in length, and if the development
varied substantially from one anther to another within a floret, a
limited study was undertaken using safranin-fast green stained serial
sections from paraffin embedded anthers. In 1975 the three anthers
from basal florets were sampled in two plants, one fertile (Oahe 3) and one sterile (SB^2-17-20). For each plant the basal florets were sampled from a spikelet in the middle of the spike, and from one toward each end of the spike.

The anthers were fixed in Craf V of the Nawaschin series of fixatives (Sass, 1958). An ethanol series into n-butanol with two hour steps was used to dehydrate the anthers (10%, 30%, 50%, 70%, 90%, 100%, 100%, 33% ethanol with n-butanol, 66% n-butanol, 100%, 100%). The anthers were then infiltrated with paraffin and embedded in BEEM capsules (50% paraffin with n-butanol, 75%, 100% paraffin with two hour steps, 100% paraffin overnight). The blocks were trimmed with a razor blade and serial sections 10 μm thick were taken through the entire anther using an AO-920 rotary microtome. The sections were affixed to adhesive coated microslides at 40°C after expanding them on a large drop of 6% formalin.

Staining followed this safranin-fast green schedule:

1. Xylene, 10 minutes
2. 1:1 tertiary butanol:xylene, 10 minutes
3. 95% ethanol, 10 minutes
4. 70% ethanol, 10 minutes
5. Safranin 0 (1% in 55% ethanol + 10 drops 1N NaOH), 24 hours
6. Distilled water, 15 seconds
7. Distilled water, 15 seconds
8. 70% ethanol + 5 drops glacial acetic acid, 10 seconds
9. 95% ethanol, 15 seconds
10. Absolute ethanol, 15 seconds
11. Fast green, 4% in 1:1:1 clove oil: methyl cellosolve: tertiary butanol, 15-45 seconds
65

12. Absolute ethanol, 15 seconds
13. Differentiate with 2:1:1 clove oil: xylene:tertiary butanol, 15 minutes
14. Xylene, 15 minutes
15. Xylene, 15 minutes
16. Mount coverglasses with 'Namount'

Seed Set Determination (seeds per spike)

In 1974 naturally pollinated spikes were collected from the portions of the selected plants remaining in the original SB$_3$ nursery. All spikes remaining on the plants were collected. The small nursery where cytological material was sampled was not used for seed set determination for there was a lack of an adequate supply of pollen in this nursery. Seed was also collected from the four Oahe types in their original nurseries. In 1975 spikes were sampled at random from pollinator rows in the fifth substitution backcross nursery (SB$_5$) and the number of seeds per spike was determined.

Determination of Male Sterility

Both transplanted and nontransplanted material were checked for male sterility in 1974. A macerated mixture of fresh extruding anthers from the basal florets of central spikelets on at least three separate spikes was stained with 5% acetocarmine, and 400 pollen grains were scored for stainability ("male sterility").

Male sterility was checked in 1975 using 5% acetocarmine and 1% iodine-potassium iodide (IKI) in 70% ethanol. Extruding anthers from
basal florets of central spikelets on six separate spikes were fixed in 3:1 ethanol:acetic acid for later staining. Two separate counts of 400 grains each were made for both acetocarmine and the IKI stained pollen.

Plumpness, coarsely granular cytoplasmic fill, and the presence of three nuclei were the criteria for fertility in the acetocarmine stained pollen. Pollen stained with IKI needed to be plump and well-filled with darkly staining starch granules to be scored fertile.

Photographs of pollen were taken with or without a green filter on 35 mm Panatomic-X film using a Wild automatic photomicroscope.

**Plant Height**

Plant heights for all selected plants in both the transplant nursery and the original SB3 and Oahe nurseries were measured during the summer of 1974. Measurements were made near the time of seed maturity. During 1975 only the transplants were measured (the SB3 nursery had been removed after the previous summer), the heights again being recorded at seed maturity. Measurements were made to the nearest centimeter from the base of the plant to the top of the highest spike representing the majority of the culms. The occasional spike which was twenty or more centimeters above the rest was not taken as representing the majority.
Anther and Spike Measurements

In the 1975 anther lengths, spike lengths, and the number of spikelets per spike were recorded for all the plants at the time of anther extrusion. Six central spikelets from six spikes were fixed in Craf V, and later the lengths of all three anthers in the basal florets were recorded using an eyepiece micrometer in a zoom dissecting scope. Occasionally all the anthers of the basal florets had dehisced or were otherwise absent. In those cases, measurements were taken using the secondary floret or tertiary floret rarely. Only five spikes were sampled from one plant and several plants had one or more anthers deformed or missing from the florets. Therefore, anther lengths were compared using the modification of Duncan's multiple range test suggested by Steele and Torrie (1960) for unequal sample size.

During 1975 ten spikes from each plant were measured from the node of the lowest spikelet to the tip of the highest spikelet. Means were compared using Duncan's new multiple range test (Duncan, 1955). The number of spikelets per spike was also recorded for each of the spikes and the plant means were compared in the same manner.
RESULTS

Description of Plant Types

The twenty-two plants studied varied considerably as to color, growth habit, and height. Colors ranged from bluish to greenish with bluish-green as in the variety 'Oahe' being the most representative color. Some plants were rhizomatous, others were more of a bunch type. Many of the plants had lax leaves, while leaves of others were erect. Lodging was a characteristic common to most plants.

These were subjective observations and no attempt was made to quantify the above characteristics or correlate them with meiotic behavior, sterility or any other factors. They were included merely to indicate that the plants did not represent a phenotypically homogeneous group.

The heights of the plants measured at seed maturity also differed as seen in Table IV. Originally measurements were taken to give some indication if there was a significant transplant effect in 1974, since cytological data were collected from the transplants the same year as transplantation. As can be seen in Table IV, in 1974 all but one of the plants were shorter in the transplant nursery than in the original SB₃ nursery, although not by much in many cases. The heights of the transplants in 1975 were considerably greater than either those in the transplant nursery or those located in the original SB₃ nursery.
Table IV. Plant height at seed maturity of four Oahe, one SB<sub>5</sub>, and seventeen SB<sub>3</sub> intermediate wheatgrass plants

<table>
<thead>
<tr>
<th>Plant Number (Category)</th>
<th>Original Nursery 1974</th>
<th>Transplant Nursery 1974</th>
<th>Transplant Nursery 1975</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Oahe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oahe 1</td>
<td>1.42</td>
<td>1.18</td>
<td>1.44</td>
</tr>
<tr>
<td>Oahe 2</td>
<td>1.20</td>
<td>1.00</td>
<td>1.44</td>
</tr>
<tr>
<td>Oahe 3</td>
<td>1.27</td>
<td>1.08</td>
<td>1.33</td>
</tr>
<tr>
<td>Oahe 4</td>
<td>1.28</td>
<td>1.25</td>
<td>1.51</td>
</tr>
<tr>
<td>(2) SB&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-2-12</td>
<td>.98</td>
<td>.88</td>
<td>1.35</td>
</tr>
<tr>
<td>24-2-7</td>
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<td>.91</td>
<td>1.22</td>
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<td>24-2-55</td>
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<td>.92</td>
<td>1.52</td>
</tr>
<tr>
<td>1-8-89</td>
<td>.61#</td>
<td>.60#</td>
<td>.96</td>
</tr>
<tr>
<td>24-2-76</td>
<td>.84</td>
<td>.87</td>
<td>1.19</td>
</tr>
<tr>
<td>2-17-68</td>
<td>1.44</td>
<td>1.00</td>
<td>1.51</td>
</tr>
<tr>
<td>(3) SB&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-17-83</td>
<td>.65#</td>
<td>.56&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.13</td>
</tr>
<tr>
<td>(4) SB&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-22-68</td>
<td>1.16</td>
<td>.82</td>
<td>1.42</td>
</tr>
<tr>
<td>1-9-79</td>
<td>--</td>
<td>--</td>
<td>1.42</td>
</tr>
<tr>
<td>2-17-20</td>
<td>1.39</td>
<td>1.12</td>
<td>1.41</td>
</tr>
<tr>
<td>2-22-48</td>
<td>.98</td>
<td>.95</td>
<td>1.36</td>
</tr>
<tr>
<td>(5) SB&lt;sub&gt;3&lt;/sub&gt; and SB&lt;sub&gt;5&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-16-2</td>
<td>.82</td>
<td>.80</td>
<td>1.11</td>
</tr>
<tr>
<td>2-16-42</td>
<td>1.33</td>
<td>.84</td>
<td>1.53</td>
</tr>
<tr>
<td>2-16-50</td>
<td>.82</td>
<td>.70</td>
<td>1.22</td>
</tr>
<tr>
<td>2-23-39</td>
<td>1.08</td>
<td>.79</td>
<td>1.15</td>
</tr>
<tr>
<td>29-1-27</td>
<td>.92</td>
<td>.88</td>
<td>1.30</td>
</tr>
<tr>
<td>2-16-88</td>
<td>.62#</td>
<td>.50#</td>
<td>1.06</td>
</tr>
<tr>
<td>1-9-79-31-14</td>
<td>--</td>
<td>--</td>
<td>1.13</td>
</tr>
</tbody>
</table>

# These plants failed to flower in 1974. Measurements recorded are the maximum height of the leaves.

+ SB<sub>3</sub>-2-17-83 produced only two spikes in the transplant nursery in 1974. Both were sampled for cytology. Height recorded is the maximum height of the leaves.
the previous year. So it was difficult to ascribe directly any reduc-
tion of height in 1974 to a transplant effect.

Those plants of category 4, male sterile and meiotically un-
stable were by no means the shortest plants nor the tallest. In 1975
they had heights comparable to those of the four Oahe types (1.41,
1.36, 1.42 and 1.42 meters vs. 1.44, 1.44, 1.33, and 1.51 meters).

Meiotic Characteristics

As can be seen in Table I (p. 51), usually only a small number
of cells could be found in which all the chromosomes could be counted.
Stages where total chromosome counts can be made, such as diakinesis
and anaphase are fairly short, and subsequently only a limited number
of well-squashed cells are counted. Stages such as metaphase I are
longer in duration and there is a greater opportunity for counting a
larger number of cells. Although the bivalents in metaphase I cannot
be counted, the univalents seen lying off the metaphase plate are
easily discernable. Such counts can be used in the absence of the
number of bivalents as a measure of the meiotic stability of the
material.

If one compares the average number of univalents counted in
metaphase I, usually over a larger number of cells, with the number of
univalents in those cells where all chromosomes could be counted
(Table I), it appears that overall the two values are comparable.
Only in a few instances are the two averages widely different (ex. \( \text{SB}_3 \)-1-8-37, 0.3 vs 2.7 and \( \text{SB}_3 \)-1-2-49, 3.8 vs 1.0). These plants were not considered for the cytological study of pollen development.

One of the male sterile plants placed in the meiotically unstable category (category 4) exhibited a very high instability. More than 50% of the cells investigated in \( \text{SB}_3 \)-2-22-48 had greater than 10 univalents. One cell had complete asynapsis, forty univalents and no bivalents. Although the number of univalents and bivalents were the only criteria for classifying plants, some plants exhibited other notable meiotic behaviors. Many of the bivalents of \( \text{SB}_3 \)-2-17-20 were cross bivalents (Schulz-Schaeffer, 1971). In some of the cells all the bivalents exhibited this type of pairing. Two other plants, \( \text{SB}_3 \)-2-17-83 (male sterile) and \( \text{SB}_3 \)-24-2-76 (partially sterile) had a small percentage of quadrivalents.

\( \text{SB}_3 \)-2-16-88 did not flower during 1974 and consequently no meiotic data were collected from this plant. \( \text{SB}_3 \)-1-8-89 also failed to flower but meiotic data had been gathered from a previous year.

**Anther Characteristics (1975)**

The anthers and florets of all twenty-two selected plants were checked visually at the time of anther extrusion and dehiscence for overall appearance, filament elongation, and the presence of a stoma-
Fertile anthers are plump and a deep yellow color when extruded (Fig. 1). All plants except the following had anthers of this appearance: Anthers of male sterile SB\textsubscript{3}-2-17-20, SB\textsubscript{3}-2-22-68, and SB\textsubscript{3}-1-9-79 were pale yellow and lacked the plumpness of anthers containing fertile pollen. Partially male sterile SB\textsubscript{3}-29-1-27 had anthers which were somewhat dried and curled in addition to being pale yellow. Anthers of male sterile SB\textsubscript{3}-2-22-48 were fairly plump and were of a yellow color in between the pale yellow of some of the male steriles and the deep yellow of the fertiles and most of the partially steriles.

All of the anthers of SB\textsubscript{3}-2-16-2 (classified a partially sterile) and most of the anthers from later spikes of SB\textsubscript{3}-2-17-83 (cytological data for the pollen development study had been taken from earlier spikes) were white and "papery". Two anthers of SB\textsubscript{3}-2-17-83 are shown in Figure 2 which are also indicative of all the anthers of SB\textsubscript{3}-2-16-2. The arrow indicates that the base of the anther on the left is modified into a thin papery flap of tissue. The bulges of the microsporangia can be seen above this area. The entire anther on the right has a thin, papery appearance. Anthers of this type which were entirely papery expressed no pollen when macerated and stained with acetocarmine or IKI. While all of the anthers of SB\textsubscript{3}-2-16-2 were papery and no evidence of pollen or PMC's could be found at any stage of development, in SB\textsubscript{3}-2-17-83 these papery anthers were only found in
Figs. 1-2. Fertile and sterile anthers. - Fig. 1. Fertile anthers of the four breeder's genotypes of Oahe intermediate wheatgrass; Oahe 4 (a), Oahe 3 (b), Oahe 2 (c), and Oahe 1 (d). X 8. - Fig. 2. Sterile, malformed anthers of SB-2-17-83. The base of the left anther has been modified into a papery flap of tissue (arrow). The entire anther on the right is papery and contains only a limited microsporangia. X 15.
presumably later spikes. Cytological data for the study of pollen development had been collected from the earliest spikes and no evidence of such deformed anthers was found in these spikes.

In florets with papery anthers often one or more of the three anthers was missing from the floret.

All plants excepting those with papery anthers exhibited filament elongation and anther extrusion. The few florets of SB\textsubscript{3}-2-17-83 with anthers normal in appearance had filament elongation and extruded those anthers.

Male sterile SB\textsubscript{3}-2-22-48 had a small percentage of pistillody when checked at the time of anther dehiscence. Usually all three anthers of an affected floret had been modified into pistillate structures. However, sometimes only one or two were modified and the other anther(s) were normal in appearance.

Each intermediate wheatgrass anther consists of four microsporangia or lobes arranged in two pairs (Fig. 3). Five distinct layers of cells comprise each microsporanum. These are, listed from the outer layer toward the middle: an epidermis, endothecium, middle layer, tapetum, and a layer of pollen mother cells (PMC's). Both the tapetum and middle layer are transitory types of cells. By dehiscence both degenerate, and each microsporangium has only an epidermis and endothecium surrounding the cavity (locule) containing the pollen. Between each pair of microsporangia a longitudinal slit or stomium
Figs. 3-4. Normal morphology of intermediate wheatgrass anthers.

- Fig. 3. Cross section showing four microsporangia arranged in two pairs (light micrograph). Five distinct layers of cells comprise each microsporangium, the epidermis (E), the endothecium (en), the middle layer (m), the tapetum (T), and the pollen mother cells (P), here shown in pachytene-diplotene. The eventual site of the stomium between the members of each pair is indicated by the arrows. X 311.

- Fig. 4. Electron micrograph of the four parietal layers of the anther; vacuolate epidermis with its characteristic cuticle (arrow), endothelial layer (en) containing many chloroplasts, vacuolate middle layer (m) and tapetum (T). X 4635.
nearly the entire length of the anther forms to release the pollen from the anther. The position of the future stomium is shown in Figure 3.

Normal appearing anthers of all plants except male sterile SB₃-2-17-20 demonstrated the presence of a stomium following anther extrusion. The deformed papery anthers of SB₃-2-16-2 and SB₃-2-17-83 were not extruded and lacked a stomium. Three plants, SB₃-2-22-48, SB₃-1-9-79, and SB₃-29-1-27, had anthers which only formed a small stomium. These slits were only one-fourth to one-third the length of the anther compared to a normal stomium three-fourths the length of the anther or greater. Male sterile SB₃-2-17-20 had anthers which failed to form a stomium after extrusion from the florets. A brief comparison of the cytology of the stomium in this plant versus that in a typical fertile plant would be useful at this point. More detail will follow in the discussion. Figure 5 is a drawing of portions of two normal microsporangia near the time of pollen release. The stomium forms between the two microsporangia through the breakage of the specialized connecting epidermal cells (arrow) followed by the separation of the endothecial cells and tapetal membrane of the anther wall in the region of the arrowheads in Figure 5. Note that in the mature anther the tapetal and middle layer cells have degenerated.

However, in SB₃-2-17-20 (Fig. 6) the middle layer and tapetum persist. For a stomium to form, the epidermal cells must sever and
Figs. 5-6. Anthers near the time of dehiscence. Region of the stomium. - Fig. 5. Drawing of a normal anther just prior to dehiscence, showing two adjacent locules (L). The stomium forms through the breakage of the specialized epidermal cells joining the two locules (arrow), followed by rupture of the endothecial cells (e) in the regions indicated by the arrowheads. The tapetal membrane (TM) easily breaks, releasing the pollen grains (P) from the anther. Approximately X 350.

- Fig. 6. Drawing of a nondehiscent anther from SB-2-17-20. Although the epidermal cells joining the two locules may separate (arrow), the tapetal (T) and middle layers (M) persist, possibly impairing further rupture of the anther wall and release of the sterile pollen (P). Approximately X 350.
also three more layers of the anther wall, the endothecium, middle layer and the tapetum must separate in the area indicated by the arrowhead in Figure 6. This latter separation apparently does not occur and the anther walls remain intact preventing release of the sterile pollen.

Anther Length, Spike Length and Number of Spikelets per Spike (Table V)

(a) Anther length. Anther length was analyzed using standard analysis of variance techniques and mean anther lengths were compared using Duncan's multiple range test and grouped according to the categories of Table III. Table V shows the mean anther lengths listed within each category in order of increasing meiotic instability, i.e., increasing four criteria total. Averages over each category are given in parentheses. Although no meiotic data were gathered from SB3-2-16-88, and it had a high percentage male sterility in 1975 (87.3%), it was placed in category 5 with its sibs. It was felt that this plant was probably as variable in this percentage male sterility from year to year as its sibs, and the 87.3% male sterility in 1975 was not indicative of a consistently high percent male sterility which would place it most likely in category 4. As it turned out, all other groups of sibling plants (SB3-2-16-, SB3-24-2-, and SB3-2-22-) were placed in the same category when meiotic behavior was known.
Table V. Mean anther length, spike length, and spikelets per spike of one SB₅, four Oahe, and seventeen SB₃ intermediate wheatgrass plants

<table>
<thead>
<tr>
<th>Plant Number (Category)</th>
<th>Anther Length (mm)**</th>
<th>Spike Length (cm)**</th>
<th>Spikelets** per spike</th>
<th>s—x</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s—x</td>
<td>s—x</td>
<td>s—x</td>
<td></td>
</tr>
<tr>
<td>Oahe 1</td>
<td>7.5 b .05</td>
<td>23.9 cde .53</td>
<td>21.7 def .56</td>
<td></td>
</tr>
<tr>
<td>Oahe 2</td>
<td>(7.2) 7.0 de .03</td>
<td>(24.7) 25.8 bc .52</td>
<td>24.7 bc .61</td>
<td></td>
</tr>
<tr>
<td>Oahe 3</td>
<td>6.7 ef .05</td>
<td>24.0 cde .45</td>
<td>(23.4) 27.0 bc .86</td>
<td></td>
</tr>
<tr>
<td>Oahe 4</td>
<td>7.4 bc .04</td>
<td>25.0 bcd .74</td>
<td>20.3 fg .84</td>
<td></td>
</tr>
<tr>
<td>SB₃-24-2-12</td>
<td>7.1 cde .05</td>
<td>28.0 a .83</td>
<td>23.2 cde .73</td>
<td></td>
</tr>
<tr>
<td>24-2-7</td>
<td>8.2 a .03</td>
<td>21.5 fg .90</td>
<td>21.5 ef .82</td>
<td></td>
</tr>
<tr>
<td>24-2-55</td>
<td>(7.3) 7.5 b .05</td>
<td>(23.6) 25.5 bc .66</td>
<td>26.5 ab .21</td>
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</tr>
<tr>
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<td>15.3 i .54</td>
<td>14.3 j .33</td>
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<td>24.7 bcd .56</td>
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<td>26.5 ab .57</td>
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<tr>
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<td>6.4 fg .20</td>
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<td>17.2 h .51</td>
<td></td>
</tr>
<tr>
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<td>15.5 i .29</td>
<td>15.1 ij .46</td>
<td></td>
</tr>
<tr>
<td>1-9-79</td>
<td>(5.3) 4.9 kl .04</td>
<td>(23.3) 26.2 b .43</td>
<td>18.7 gh .54</td>
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</tr>
<tr>
<td>2-17-20</td>
<td>5.1 jk .12</td>
<td>28.2 a .74</td>
<td>(19.0) 20.8 fg .63</td>
<td></td>
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<td>5.8 hi .16</td>
<td>18.6 h .65</td>
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<td>18.9 gh .67</td>
<td></td>
</tr>
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<td>22.7 ef .79</td>
<td>20.0 fg .76</td>
<td></td>
</tr>
<tr>
<td>2-23-39</td>
<td>4.5 m .07</td>
<td>16.3 i .23</td>
<td>20.7 fg .30</td>
<td></td>
</tr>
<tr>
<td>29-1-27</td>
<td>(5.5) 5.4 ij .06</td>
<td>(19.7) 21.7 fg .39</td>
<td>(19.5) 27.8 a 1.15</td>
<td></td>
</tr>
<tr>
<td>2-16-88</td>
<td>4.6 lm .06</td>
<td>18.3 h .78</td>
<td>17.2 h .25</td>
<td></td>
</tr>
</tbody>
</table>

* Means not followed by the same letter are significantly different at p = 0.05
+ Number in parentheses are averages for each category
On the average the anthers of the plants in category 2 were slightly longer than the anthers of the variety Oahe in category 1 (7.3 mm vs 7.2 mm). Both categories 1 and 2 (meiotically stable) had anthers much longer than those of the plants in meiotically unstable categories 4 and 5. The anthers of those plants meiotically unstable and male sterile (category 4) were as a group the shortest anthers (5.3 mm) although not much shorter than those of plants partially male sterile and meiotically unstable (category 5, 5.5 mm).

Anthers of both meiotically unstable categories were more variable than the anthers of meiotically stable plants as can be seen by comparing the standard errors. $SB_3^-2-16-42$ of category 5 was the only plant of any category which deviated widely in anther length from the others.

The single plant in category 3, $SB_3^-2-17-83$, had an anther length (6.4 mm) midway between the average of the other two meiotically stable categories (7.2 mm) and the two meiotically unstable categories (5.4 mm). $SB_3^-2-17-83$ also had the most variable average anther length ($s_x = 0.20$).

The deformed, papery anthers of $SB_3^-2-16-2$ were not measured.

Within each category not all the mean anther lengths could be declared not significantly different using Duncan's multiple range test; there was a certain amount of variation. However, 50% of the
plants in each of categories 1, 2, and 5 had mean anther lengths not significantly different from other in the same category, while the percentage rose to 75% in category 4.

(b) Spike length. In contrast to anther lengths, average spike lengths as seen in Table V varied somewhat more within each category with the exception of category 1 where all four Oahe types had non-significant differences in average spike length. As a group the averages of categories 1, 2, and 4 were quite similar (24.7, 23.0, and 23.3, respectively), and larger than the average spike lengths of categories 3 and 5 (19.9 and 19.7, respectively). There was no association of spike length with meiotic stability. In fact, except for the very short spike length of $SB_3^-2-22-68$ (15.5 cm), category 4 (male sterile and meiotically unstable) has spike lengths comparable to those of the Oahe types, as would category 2 with the exception of $SB_3^-1-8-89$. $SB_3^-2-16-2$ with deformed, papery anthers had the shortest spike length of all the plants in category 5.

(c) Spikelets per spike. Meiotically stable plants of categories 1 and 2 had average spikelet numbers greater than the plants of meiotically unstable categories 4 and 5 (23.4 and 21.5 vs 19.0 and 19.5, respectively). The single plant in category 3, $SB_3^-2-17-83$ with an average of 17.2 spikelets per spike was lower than any other plant in a meiotically stable category with the exception of $SB_3^-1-8-89$
(14.3). The plant with the shortest spikes also had the least number of spikelets per spike in categories 2, 4, and 5. However, this association did not extend beyond the shortest spike.

Seed Set

The average number of seeds per spike from the SB$_3$ plants was very low in 1974 (Table VI). Only SB$_3$-2-16-42 with an average of 39.9 seeds per spike had an average comparable to the variety Oahe (seed set calculated in 1975). The seed set for the variety Oahe when released as a variety from the South Dakota Agricultural Experiment Station in 1962 was 60.8 seeds per spike. The partially steriles which were meiotically stable had the highest seed set among the SB$_3$'s with the exception of SB$_3$-2-16-42 and SB$_3$-1-9-79 (seed set calculated in a year previous to 1974).

The plants in the SB$_3$ nursery from which seed set was determined in 1974 had much shorter spikes and fewer spikelets per spike than did the plants in the transplant nursery in 1975 (Table V). Only SB$_3$-2-16-42 had values in 1974 comparable to values in 1975.

Unfortunately, in 1974 the harvested spikes were not protected from rodents, and all the spikes from the Oahe types were destroyed and the seed eaten before the spikes could be threshed and measurements taken. Therefore, spike length, the number of spikelets per spike, and seed set could not be determined for these plants.
Table VI.  Seed set, spike length, and spikelets per spike of seventeen SB3 intermediate wheatgrass plants plus seed set of the variety Oahe

<table>
<thead>
<tr>
<th>Plant Number (Category)</th>
<th>Average Seeds per Spike*</th>
<th>Spike Length (cm)*</th>
<th>Avg. Spikelets per Spike*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Oahe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB3-24-2-12</td>
<td>8.6</td>
<td>12.1</td>
<td>13.0</td>
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<tr>
<td>24-2-7</td>
<td>5.1</td>
<td>9.5</td>
<td>12.0</td>
</tr>
<tr>
<td>24-2-55</td>
<td>14.9+</td>
<td>14.4</td>
<td>16.6</td>
</tr>
<tr>
<td>(2) 1-8-89</td>
<td>17.8</td>
<td>FAILED TO FLOWER IN 1974</td>
<td></td>
</tr>
<tr>
<td>24-2-76</td>
<td>16.3</td>
<td>12.3</td>
<td>13.7</td>
</tr>
<tr>
<td>2-17-68</td>
<td>11.5</td>
<td>17.5</td>
<td>14.6</td>
</tr>
<tr>
<td>(3) 2-17-83</td>
<td></td>
<td>FAILED TO FLOWER IN 1974</td>
<td></td>
</tr>
<tr>
<td>2-22-68</td>
<td>4.3</td>
<td>14.5</td>
<td>13.2</td>
</tr>
<tr>
<td>1-9-79</td>
<td>38.1+</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>19.2</td>
<td>16.4</td>
</tr>
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</tr>
<tr>
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<td>12.6</td>
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<td>29-1-27</td>
<td>0.2</td>
<td>7.6</td>
<td>11.8</td>
</tr>
<tr>
<td>2-16-88</td>
<td></td>
<td>FAILED TO FLOWER IN 1974</td>
<td></td>
</tr>
</tbody>
</table>

+ Calculated prior to 1974
* All values from material in the SB3 nursery in 1974. Oahe seed set calculated in 1975 from pollinator rows in the SB5 nursery.
Synchrony of Pollen Development

Paraffin embedded anthers of a basal floret from a spikelet near the middle of a spike and from spikelets near both ends of the same spike were serially sectioned for Oahe 3 (male fertile) and SB⁻２⁻７⁻２０ (male sterile). It was desired to check if pollen development was synchronous within anthers and between anthers of the same floret. This limited study was also done to give an indication if anthers from basal florets of spikelets near the ends of the spike had differing development from those near the middle of the spike.

Late vacuolate microspores (uninucleate microspores with a large central vacuole) were found in all anthers of the spike sampled from Oahe 3. Microspores at that stage of development were the only ones observed in these anthers. All microspores appeared normal as did the degenerated tapetum, and no differences could be detected within anthers, between anthers of the same floret, or between spikelets.

Neither was there any gradient of developmental stages within or between anthers of male sterile SB⁻２⁻１７⁻２０. All anthers had sterile pollen corresponding to the late vacuolate microspore stage in fertile pollen. Doublets (joined pairs) were common and all grains were either uninucleate or anucleate, containing little cytoplasm. A persisting tapetum along the entire length of the anther was common to all anthers.
It was interesting to note that in one anther of each of the three florets sampled, one microsporangium expressed a sterility different from the others. The middle layer of the anther wall had enlarged, and the tapetal and sporogenous cells were degenerated and pressed to a small amorphous mass in the center of the locule.

Pollen Sterility

Male sterility percentages for the twenty-two investigated plants are given in Table VII. The plants are listed according to category and within each category in order to decreasing meiotic stability. The four Oahe types and SB3-2-16-88 have not been investigated meiotically and this order of arrangement does not apply to these plants.

The table is incomplete with respect to some plants for a variety of reasons. The four Oahe types had not been utilized in any research investigation requiring sterility percentages before work began on this thesis in 1974. During 1974 SB3-2-17-83 flowered only in the transplant nursery and then only late in the summer. Only two spikes were produced and these were sampled for the pollen development study. During the summer of 1974 SB3-1-8-89 and SB3-2-16-88 neither flowered in the original SB3 nursery nor in the transplant nursery. SB3-1-9-79 and SB5-1-9-79-31-14 were not included when the original plants were selected for study in 1974. Neither plant had been investigated
Table VII. Percentage male sterility as measured using the stained acetocarmine (AC) and iodine-potassium iodide (IKI)

<table>
<thead>
<tr>
<th>Plant Number (Category)</th>
<th>Original Location*</th>
<th>Transplant Nursery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1973 (AC)</td>
<td>1974 (AC)</td>
</tr>
<tr>
<td>Oahe 1</td>
<td>--</td>
<td>15.0</td>
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<tr>
<td>Oahe 2</td>
<td>--</td>
<td>97.4</td>
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<tr>
<td>Oahe 3</td>
<td>--</td>
<td>13.9</td>
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<tr>
<td>Oahe 4</td>
<td>--</td>
<td>9.4</td>
</tr>
<tr>
<td>SB₃-24-2-12</td>
<td>10.1</td>
<td>14.0</td>
</tr>
<tr>
<td>24-2-7</td>
<td>10.7</td>
<td>14.0</td>
</tr>
<tr>
<td>24-2-55</td>
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<td>14.4</td>
</tr>
<tr>
<td>(2) 1-8-69</td>
<td>99-100</td>
<td>--</td>
</tr>
<tr>
<td>24-2-76</td>
<td>17.5</td>
<td>13.2</td>
</tr>
<tr>
<td>2-17-68</td>
<td>18.2</td>
<td>12.4</td>
</tr>
<tr>
<td>(3) 2-17-83</td>
<td>99.3</td>
<td>--</td>
</tr>
<tr>
<td>2-22-68</td>
<td>99-100</td>
<td>99.0</td>
</tr>
<tr>
<td>1-9-79</td>
<td>98.8</td>
<td>--</td>
</tr>
<tr>
<td>(4) 2-17-20</td>
<td>95.4</td>
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</tr>
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<td>2-22-48</td>
<td>85.7</td>
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<tr>
<td>SB₃-2-16-2</td>
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<td>28.6</td>
</tr>
<tr>
<td>SB₃-1-9-79-31-14</td>
<td>--</td>
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<tr>
<td>SB₃-2-16-42</td>
<td>--</td>
<td>5.9</td>
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<tr>
<td>2-16-50</td>
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</tr>
<tr>
<td>(5) 29-1-27</td>
<td>27.8</td>
<td>78.2</td>
</tr>
<tr>
<td>2-16-88</td>
<td>--</td>
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</tbody>
</table>

* The four Oahe nurseries for each of the Oahe types, the large SB₃ nursery for all SB₃'s except 1-9-79, the SB₃ nursery for SB₃-1-9-79-31-14, and a separate source nursery for SB₃-1-9-79.
meiotically and so neither was eligible for selection. Only after realizing that SB$_3$-1-9-79 had a very high seed set (about 38 seeds per spike) and had maintained its male sterility over four years was it decided to include it in the developmental study. SB$_5$-1-9-79-31-14 was included as a selection of one of SB$_3$-1-9-79's progeny having the highest known seed set as of 1975. Pollen sterility determinations are missing in 1973 from the four plants SB$_3$-2-16-2, -42, -50, and -88 because not all fifteen hundred SB$_3$ plants had been checked for male sterility as of 1973.

In 1974 there was a moderate location difference among some of the fertiles and partially steriles. Where the discrepancy between the two sterility percentages was very large, i.e., SB$_3$-2-16-50 (46.2 vs 12.2) the highest value was always from the plant in the original nursery.

Plants of category 4, male sterile and meiotically unstable, showed fairly good consistency between the two locations with regard to percent male sterility. There was also consistency over all three years for these four plants.

In 1974 Oahe 2 had a very high sterility percentage in both the original and transplant locations, being almost 100% (97.4) in the original Oahe 2 nursery. However, in 1975 sterility was found to be very low, in fact the lowest of the four Oahe types when stained with acetocarmine.
SB\textsubscript{3} -1-8-89 had originally been placed in category 3 using the percentage sterility calculated in 1973. This plant failed to flower during 1974, but sterility when determined in 1975 was found to be low (13.4 and 11.1). SB\textsubscript{3} -1-8-89 on the basis of this sterility was reclassified in category 2. More will be said in the discussion regarding this large variation in sterility between years.

Overall, plants of category 5, metiotically unstable, had the highest male sterility of the partially steriles over all three years, although SB\textsubscript{3} -2-16-42 was low (about .6-12%) while SB\textsubscript{3} -24-2-76 of category 2 was a little high in 1975 (about 25%). Partially male sterile category 2 had percentages only slightly larger than the four Oahe types.

Both of the stains used in 1975, acetocarmine and IKI, gave comparable values over nearly all of the plants. Only with SB\textsubscript{3} -2-17-68 did the larger sterility percentage exceed the smaller by more than a factor of two (3.6 vs 8.0).

Description of Sterility Types in 1975

Micrographs of fertile and sterile pollen are shown in Figures 7-18. The first and third rows of photographs (odd numbers) show pollen stained with IKI. The second and fourth rows (even numbers) are pollen of the corresponding plants stained with acetocarmine (AC).
Figs. 7-18. Fertile and sterile pollen stained with 5% aceto-carmine and 1% IKI. - Fig. 7. Pollen from Oahe 3 stained with IKI. The pollen grains are plump and well-filled with starch granules. A smaller sterile grain lacking starch is seen toward the top of the photograph. X 337. - Fig. 8. Oahe 3 pollen stained with acetocarmine. The two sperm nuclei are indicated by the arrowhead. Occasional sterile grains are seen in the upper left corner. X 337. - Fig. 9. Pollen from SB$_3$-2-17-20 stained with IKI. The pollen grains lack starch and are joined in pairs or quartets by a common wall (arrowhead) as a result of meiotic division failures. X 337. - Fig. 10. SB$_3$-2-17-20 pollen stained with acetocarmine. Note the limited amount of cytoplasm and absence of nuclei. X 337. - Fig. 11. Pollen from SB$_3$-2-22-48 stained with IKI. Only an occasional grain appears normal and well filled with starch. X 337. - Fig. 12. Pollen from SB$_3$-2-22-48 stained with acetocarmine. Most of the pollen has either one (1) or two (2) nuclei. Trinucleate grains with well stained cytoplasm are seldom observed. X 337. - Fig. 13. Pollen from SB$_3$-2-22-68 stained with IKI. An absence of starch and a collapsed appearance is characteristic of all grains. X 337. - Fig. 14. SB$_3$-2-22-68 pollen stained with acetocarmine. Collapsed pollen grains lacking nuclei and cytoplasm were nearly always observed. X 356. - Fig. 15. Pollen from SB$_3$-1-9-79 stained with IKI. A grain with a very slight starch content is seen on the left. Most pollen grains show a complete absence of starch. X 337. - Fig. 16. SB$_3$-1-9-79 pollen stained with acetocarmine. Usually only uninucleate grains with large vacuoles (arrows) characteristic of late vacuolate microspores were seen. X 356. - Fig. 17. Pollen from SB$_3$-2-17-83 stained with IKI. Very seldom was a fertile appearing grain observed. Most showed only a slight starch fill or none at all. X 356. - Fig. 18. SB$_3$-2-17-83 pollen stained with acetocarmine. In the center is a pollen grain with a fertile-like staining reaction, although the grain is small and nuclei are not evident. Most grains have limited cytoplasm, are partially collapsed, and contain either one or no nuclei. X 356.
Pollen typical of that scored fertile is shown in Figures 7 and 8. The fertile pollen, here from Oahe 3, is plump and ovoid to spherical whether stained with IKI (Fig. 7) or AC (Fig. 8). The grains are completely filled, or nearly so, with darkly staining starch granules when stained with IKI. Acetocarmine reveals the cytoplasm of fertile pollen as coarse and granular, completely filling the grains. Also with AC three nuclei can usually be discerned in fertile pollen if the staining of the cytoplasm is not too intense. Empty, smaller grains scored as sterile can be seen in both Figures 7 and 8.

SB3−2-17-20 had very distinctive pollen. The majority of the sterile pollen were single empty grains, but in sufficient number were grains joined by common walls in pairs (doublets) or groups of four (Figs. 9-10). The associations of four occasionally had two large members and two smaller members (Fig. 9), but more often all members were of equal size, resembling the quartets from which they probably arise. Some of the groups of four could be seen to be composed of two joined pairs (doublets) in close association. Starch was absent from all pollen of SB3−2-17-20 and the cytoplasm in the grains was either very limited or absent. Absence of nuclei from the sterile grains was also the usual case. When present, however, they appeared in varying numbers. Single grains could have one or two nuclei. Trinucleate grains were never observed. Single nuclei could be present in one or both members of a doublet. Occasionally one member of a doublet would have
two nuclei while the other member had none. Groups of four could have
one, two, three, or four nuclei in all combinations. However, no more
than one nucleus was observed in a single member of a quartet. The
sterile pollen was often collapsed and many grains were seen with
broken exines and wall deformities.

Empty, partially collapsed pollen was typical for SB\textsubscript{3}-2-22-48
(Figs. 11-12). Sometimes a grain completely or partially filled with
starch (Fig. 11) or staining intensely with acetocarmine would be seen.
Such grains were usually small, only slightly larger than the empty
grains. They were scored fertile, however, on the basis of the stain­
ing reaction and in the case of acetocarmine, the presence of three
nuclei even if the size was not exactly comparable to pollen in fertile
plants. The small sterile grains could contain zero, one, or two
nuclei (Fig. 12). An occasional group of four (quartet) as in SB\textsubscript{3}-2-
17-20 was also observed.

Completely collapsed pollen devoid of starch, cytoplasm, and
nuclei was nearly always seen in SB\textsubscript{3}-2-22-68 (Figs. 13-14). A few
grains with a single peripheral nucleus and a large central vacuole
(late vacuolate stage) were observed. One extremely large grain, per­
haps a common exine around all four microspores of a quartet which
failed to separate, was also counted.

At dehiscence the majority of the pollen from SB\textsubscript{3}-1-9-79 had
an appearance characteristic of late vacuolate microspores when
stained with acetocarmine (Fig. 16). A single nucleus peripheral to a large central vacuole was observed in most of the grains. A complete absence of starch was the usual case in SB\textsubscript{3}-1-9-79 pollen stained with IKI. Some grains, however, had a slight starch reaction when stained with IKI (Fig. 15), although they were not scored as fertile. There was a small percentage (about 2-4%) of normal appearing fertile pollen which was scored as such.

SB\textsubscript{3}-2-17-83 pollen (Figs. 17-18) was usually small, partially collapsed and lacking starch. Only a few grains could be considered fertile (Fig. 17). The sterile pollen contained either one or no nuclei and had a limited amount of cytoplasm (Fig. 18).

Generally, pollen from the partially steriles was either completely normal in appearance or empty and collapsed (totally or partially). Two of the partially steriles of category 5, SB\textsubscript{3}-2-23-39 and SB\textsubscript{3}-29-1-27, had some pollen which, although fairly plump and well stained with acetocarmine, lacked distinct nuclei or contained only one nucleus. Some grains gave a very small starch reaction when stained with IKI.

**Anther Development in Intermediate Wheatgrass**

(a) **General characteristics and terminology.** A brief outline of the differentiated intermediate wheatgrass anther has been given previously. To reiterate, the anther is tetrasporangiate or consists
of four elongated microsporangia. The microsporangia are arranged in two pairs, the partition between the members of each pair breaking down at anthesis to form a stomium or slit which releases the pollen grains from the anther. A vascular area connects the two pairs of microsporangia above the attachment of the filament. Each microsporangium has five layers of cells, four parietal and one sporogenous. They are an outer epidermis, endothecium, ephemeral middle layer, uniserate tapetum and a layer of pollen mother cells (Fig. 3, p. 77). As the anther enlarges, a locule or cavity forms in the center of each microsporangium. Often the term locule is used interchangeably with the term microsporangium but surprisingly little confusion results from this technically incorrect usage.

Ultrastructurally, the four parietal layers appear as in Figure 4, p. 77. The vacuolate epidermis is bounded on the outer tangential surface by a cuticle which becomes thicker as the anther matures. The cells of the endothecium are moderately vacuolate and are characterized by the presence of chloroplasts which many transitorily contain starch granules. The middle layer cells are vacuolate and ephemeral with only their appressed cell walls present at anther maturity. Tapetal cells are typically binucleate and are extremely metabolically active during certain stages of development, the cytoplasm being densely packed with organelles.
(b) Light microscopy of anther development in fertile intermediate wheatgrass with emphasis on sporogenous and tapetal cell development (Figs. 19-41). Figure 19 shows one microsporangium at an early stage of differentiation. An epidermis surrounds the actively dividing cells of the archesporium. These archesporial cells differentiate to produce three more parietal layers to the outside and a sporogenous layer to the inside. The epidermal cells continue to divide to keep pace with the enlarging anther.

After the four layers of the anther wall have been delineated and the center of each microsporangium is filled with sporogenous cells, callose appears between the sporogenous cells in the center of the microsporangia (Fig. 20). This substance is gradually secreted around the whole of each PMC.

Although the layer of callose around each PMC is very thin at this stage and is hard to distinguish, evidence of the initial central deposition of callose is indicated by the callose "tails" which persist on the locular side of the PMC's (Fig. 23).

As meiosis proceeds, the PMC's become rounder, more so if there are fewer cells in the anther, and they are not closely appressed to one another (Figs. 24, 25). Callose tails are absent from the PMC's of Figs. 24 and 25 in pachytene-diakinesis. However, they commonly persist through the quartet stage. The microsporangia have rapidly increased in diameter as indicated by the large locular cavity. At
Figs. 19-29. Light micrographs of early stages in the development of normal anthers as revealed by cross sections in median plane. — Fig. 19. Archesporial stage. Hypodermal cells (arrowhead) actively divide in an early microsporangium to produce the tapetum, middle layer, endothecium and sporogenous cells. The epidermis (Ep) has already differentiated. X 762. — Fig. 20. Premelosis. The layers of the anther have differentiated and callose (arrowhead) appears initially toward the center of the sporogenous mass. X 475. — Fig. 21. Leptotene-zygotene. The tapetal nuclei divide (arrowhead) to produce binucleate tapetal cells. X 381. — Fig. 22. Leptotene-zygotene. The PMC's are wedge-shaped with one face appressed to the tapetal wall. The binucleate tapetal cells can be clearly seen (arrowhead). X 337. — Fig. 23. Zygotene-pachytene. Callose has been secreted around each PMC and prominent callose tails (arrow) persist as remnants of the large initial central deposition of callose. X 337. — Fig. 24. Pachytene. The PMC's become rounded, especially those which are not closely appressed to one another. X 344 — Fig. 25. Diplostene-diakinésis. Callose tails are absent from the PMC's; however, they may persist through the quartet stage. X 337. — Fig. 26. Metaphase-anaphase I. The PMC's flatten; the plane of division is perpendicular to the long axis of the anther. X 350. — Fig. 27. Anaphase-telophase II. Two PMC's (double-headed arrow) are seen after the second division. The plane of the second meiotic division is perpendicular to the first and parallel to the long axis of the anther. The callose wall of one PMC is clearly seen in this photograph. X 316. — Fig. 28. Quartet. The plane of sectioning is such that usually only two members of the quartet are evident. Occasionally, three or even all four microspores can be seen. X 337. — Fig. 29. Quartet stage near the time of callose dissolution. Vacuoles appear in the cytoplasm of the microspores. The tapetal cytoplasm stains intensely obscuring the nuclei. The cytoplasm often will continue to stain deeply until the tapetal cells degenerate. The intense staining and vacuolated appearance of both the tapetum and PMC's in Fig. 27 is unusual for a stage preceding late quartet. X 337.
metaphase-anaphase I (Fig. 26) the PMC's are radially flattened, the plane of division being perpendicular to the long axis of the anther. After the first meiotic division, only one member of each dyad is visible when the anther is seen in cross section.

The plane of the second meiotic division is perpendicular to the first and parallel to the long axis of the anther (Fig. 27). After the second division usually only two microspores of the quartet are visible when the anther is cut cross-section. An oblique section reveals three or perhaps all four microspores (Fig. 28). The divisions are such as to keep one face of each of the microspores pressed to the tapetal wall. The callose wall reaches its maximum thickness at the quartet stage and is clearly evident in Figure 27. From the beginning of meiosis through the early quartet stage, the size and staining of the tapetal cells have remained fairly constant. Only at late quartet stage (Fig. 29) does the tapetum begin to stain darkly obscuring the nuclei. Also at this stage, small vacuoles appear in the cytoplasm of the microspores. The vacuolated appearance of the microspores in Figure 27 as well as the enlarged vacuolated tapetum is unusual for an anther at the second meiotic division. This may represent precocious normal behavior of both the microspores and the tapetum or may be a fixation artifact. This anther was from SB_3-2-16-42, particularly sterile and meiotically abnormal, but was not typical of other anthers from this plant.
At callose dissolution and release of the microspores from the quartets (Fig. 30), small vacuoles are still present in the microspores and the tapetum continues to stain darkly. The time the microspores are released from the quartets to the time several large vacuoles appear in the cytoplasm is termed hereafter the early vacuolate microspore stage of development.

If the microspores are not mechanically disturbed, they will remain peripheral in the locule and close to the tapetum throughout microsporogenesis. As the exine of the pollen wall begins to form, the microspores are oriented with their rudimentary pores facing the tapetum (Fig. 31). This orientation also persists through the rest of microsporogenesis if the microspores are not disturbed.

The small vacuoles in the microspores coalesce to form several large vacuoles and begin the mid-vacuolate microspore stage of development (Fig. 32). The pollen wall exine is thicker and the tapetal cells continue to stain rather darkly. The tapetal cells are still well-organized and retain their individual integrity although there may be slight variations in the contour and radial width of the tapetum.

Sometime during the mid/late-vacuolate microspore stage the tapetal cells begin to senesce visibly, the first indication being a large reduction in width accompanied by a very irregular inner tangential surface (Fig. 33). The microspores have large vacuoles and a
Figs. 30-41. Later stages in the development of normal anthers (light microscopy). - Fig. 30. Microspores at early callose dissol­
uition. Small vacuoles are evident in the cytoplasm. X 337. - Fig. 31. Early vacuolate stage in the young microspore. Pores (open arrowheads) in the developing exine are seen facing the tapetum. X 337. - Fig. 32. Mid vacuolate microspore stage. Vacuoles in the uninucleate micro­
spores increase in size as does the thickness of the exine. The tape­
tal cells are still well-organized. X 341. - Fig. 33. Mid/late-vacu­
olate microspore stage. The microspores are tightly appressed to one another around the periphery of the locule, many taking on a violin-shaped appearance (double-headed arrow). The tapetal cells are reduced in thickness and irregular in shape although retaining their individu­
aleness. X 337. - Fig. 34. Mid/late-vacuolate microspore stage. Tape­
tal radial walls have broken down and membranes are prominent; the characteristic appearance of senescing tapetal cells. X 337. -
Fig. 35. Late vacuolate microspore stage. The center of the micro­
spore is occupied by a large vacuole (V). The tripartite structure of the exine is clearly evident. The tapetum shows further degeneration. X 337. - Fig. 36. Late-vacuolate microspore stage; early binucleate pollen. Just prior to engorging the pollen grains exhibit a collapsed appearance. X 350. - Fig. 37. Early engorging binucleate pollen stage. Generative nucleus (g) is in a position opposite the pore; the vegetative nucleus (v) with a prominent nucleolus is next to the pore. X 762. - Fig. 39. Engorging pollen. The generative nucleus is shown dividing. The tapetal cytoplasm has completely disappeared. X 900. - Fig. 40. Engorging pollen. A large vegetative nucleus (v) with dif­
fuse chromatin and two sperm nuclei are evident. X 1104. - Fig. 41. Engorged pollen. The cytoplasm is packed with starch granules. X 675.
thicker exine. The vacuolate microspores are often violin-shaped probably a result of being closely appressed to one another.

The radial walls of the tapetal cells soon break down and the (tapetal) contents become confluent (Fig. 34). The staining is less intense with membrane systems visibly prominent. By late-vacuolate microspore stage a large single vacuole occupies the center of the microspore with the cytoplasm pushed to the periphery (Fig. 35). The tripartite structure of the pollen wall exine is quite clear in Figure 35. The tapetal cytoplasm has degenerated further and is disappearing.

The microspore nucleus divides to produce a binucleate pollen grain with a vegetative cell and a generative cell. Just prior to the time when the binucleate pollen begins to engorge with starch, collapsed grains are observed (Fig. 36). This appearance is quite consistent for this stage of development and may be a fixation artifact.

As the binucleate pollen begins to engorge with starch, the nuclei become oriented with the generative nucleus and cell in a position opposite the pore, and the vegetative nucleus next to the pore. The pollen pores continue to face or be appressed to the tapetal cells. The vegetative nucleus can be distinguished from the generative nucleus by its diffuse chromatin and one or more very large nucleoli (Figs. 37, 38). The cytoplasm of the tapetal cells is greatly reduced;
it is completely absent in some places (Fig. 38). Figures 37 and 38 show that the middle layer has been reduced to a thin line by this stage. Also the cuticle of the epidermis has become crenulate as the anther matures.

At pollen maturity, the tapetal and middle layer cells disappear and the epidermis and endothecium will be somewhat reduced in thickness. Secondary walls or thickenings form between the radial walls of the endothelial cells during a time from late vacuolate microspore stage to engorging pollen stage, but are hard to distinguish in these photographs. Clearer examples of typical secondary thickenings can be seen in Figures 139 and 140, p. 158.

The generative nucleus divides to produce two sperm nuclei as the pollen grain (vegetative cytoplasm) continues to engorge with starch (Fig. 39). The pollen grain at maturity is then actually three separate cells, each with one nucleus; a large vegetative cell containing most of the pollen cytoplasm and two smaller sperm cells (Fig. 40). Figure 41 shows mature pollen grains densely packed with white starch granules. The cytoplasm is stained very darkly obscuring the nuclei.

(c) Electron microscopy of normal pollen wall development (Figs. 42-52). A discussion of normal pollen wall development is
Figs. 42-49. Electron micrographs of stages in normal exine development. - Fig. 42. Quartet stage. A fibrillar primexine (p) appears between the microspore plasmalemma and the callose wall (c). X 26,550. - Fig. 43. Quartet stage. Densely staining bacula (b) appear in the continuous primexine while the microspores are still encased in callose (c). X 24,300. - Fig. 44. Early vacuolate microspore stage. Within the primexine the bacula increase in size and staining intensity. X 18,250. - Fig. 45. Early vacuolate microspore stage. A darkly staining granular material, possibly sporopollenin, can be seen condensing on the bacula (arrow). The primexine is beginning to stain darker on the edges than in the middle. X 32,850. - Fig. 46. Early-vacuolate microspore stage. The heads of the bacula broaden to give an arrow-shaped appearance. The foot layer or nexine has started to form (arrow). X 41,400. - Fig. 47. Early-vacuolate microspore. The heads of the arrow-shaped bacula begin to fuse (arrow) forming the tectum (roof) of the exine. X 15,500. - Fig. 48. Early/mid-vacuolate microspore. The tripartite structure of the exine has been delineated with channels (arrowheads) appearing in the tectum and nexine, but absent from the bacular shafts (columellae). Primexine remnants can be seen in the cavea (cavity) between the tectum and nexine. X 23,000. - Fig. 49. Mid vacuolate microspore stage. A granular endexine has formed beneath the nexine (arrow). The tips of the arrow-shaped bacula form the spinules of the exine (s). Channels are generally absent from the area of the bacular "arrowheads". X 15,500.
important in that many of the male sterile plants produced pollen with wall malformations.

The first indication of a pollen wall occurs while the microspores are enclosed within the quartet. A fibrillar primexine appears between the callose wall and the plasmalemma (Fig. 42).

The primexine is rather intermittent at first but becomes an even, homogenous layer surrounding each microspore. Densely staining radial rods or bacula which appear in the primexine (Fig. 43) are the next visible event in exine formation. Again, this occurs before the microspores are released from the quartet.

The bacula increase in size in the free microspores after callose dissolution (early vacuolate microspore stage) (Fig. 44). The plasmalemma is seen to be slightly retracted from the elements of the exine throughout pollen wall formation. A granular material (arrow, Fig. 45) can be seen condensed on the bacula as they increase in size at this time. In Figure 45 the primexine is denser (more darkly staining) on the tangential surfaces than in the middle. The heads of the bacula expand to give an arrow-shaped appearance (Fig. 46), and the nexine or foot layer of the exine (arrow, Fig. 46) begins to form on the inner tangential side of the primexine. Also, a line of electron dense material can be seen between the bacular arrowheads on the outer tangential side of the primexine.
Eventually the heads of the arrow-shaped bacula and the material between them fuse to form the tectum or roof of the exine (Fig. 47). Channels (Figs. 48, 49) appear in both the nexine and tectum of the exine but are absent from the bacular shafts (now termed columellae) and immediate region of the bacular arrowhead (Fig. 49). The tips of the bacula form the spinules of the exine. Another element of the pollen wall, the granular endexine, is deposited between the exine and the plasmalemma during the mid-vacuolate microspore stage (Fig. 49).

Figure 50 shows a complete tripartite exine with a tectum, columellae and nexine. Channels about 30nm in diameter open to the surface and to the cavea or space between the tectum and the nexine. The plasmalemma is convoluted and a fibrillar material other than the endexine can be seen in the pockets. This is the first indication of intine formation, the intine being the last element of the pollen wall to appear. There are numerous ribosomes in the cytoplasm and rough endoplasmic reticulum is associated with the developing intine. In Figure 50 it is seen running parallel to the plasmalemma. The intine is further developed in Figure 51. Numerous cytoplasmic channels intertwine in the intine and again endoplasmic reticulum is closely associated with the plasmalemma, although direct continuity was never observed. A mature intine is shown in Figure 52. Channels are still present although less numerous. An exine channel (arrow) can be seen
Figs. 50-52. Electron micrographs of later stages in normal pollen wall formation. - Fig. 50. Early engorging pollen stage. Mature exine with tectum (T), nexine (N), and columella (arrow). Channels open to the surface and to the cavea. The plasmalemma is convoluted and material can be seen in the pockets as the first indication of intine formation (arrowhead). A strip of rough endoplasmic reticulum (ER) runs parallel to the exine. X 15,250. - Fig. 51. Engorging pollen stage. The developing intine (IN) contains many cytoplasmic channels. A fragment of rough endoplasmic reticulum (ER) is associated with the intine although continuity with the plasmalemma or intine channels cannot be seen. X 13,500. - Fig. 52. Engorged pollen stage with mature intine (IN). Intine channels can still be seen although they are not as prominent. Exine channels can be seen opening into the endexine (arrow). X 22,950.

Figs. 53-57. Ultrastructure of normal tapetal orbicular wall formation. - Fig. 53. Quartet stage. The tapetal primary wall (pw) adjacent to the callose wall (c) of the microspore contains many small darkly staining granules. Spherical pro-orbicles (pro-Ubisch bodies) have been extruded from the tapetal cells (T) and lie between the plasmalemma and the primary wall. X 14,750. - Fig. 54. Quartet stage. Many pro-orbicles lie on the plasmalemma beneath the primary wall (pw), and small granules are beginning to appear on their surfaces. Two adjacent pro-orbicles lie in the tapetal cytoplasm along with many poorly preserved mitochondria. X 14,000. - Fig. 55. Quartet stage. A pro-orbicle is being extruded and is seen in a section cut as indicated by the dotted line. X 35,100. - Fig. 56. Quartet stage. The primary wall (pw) is disappearing and pro-orbicles are being extruded through the plasmalemma (arrowhead). X 50,400. - Fig. 57. Early-vacuolate microspore stage. The tapetal cell wall has disappeared and spinules (s) of sporopollenin are forming on the surface of the orbicles (Ubisch bodies). A granular condensation of the spinules similar to that on the bacula in Fig. 45 is indicated by the arrowhead. X 22,000.
opening into the endexine and intine. Many exine channels are obscured when the pollen is mature (compare Fig. 52 with Fig. 50).

(d) Electron microscopy of normal tapetal orbicular wall formation (Figs. 53-64). During late quartet stage small osmophilic granules are seen in the primary wall of the tapetal cells and spherical translucent bodies appear between the tapetal plasmalemma and primary wall (Fig. 53). These spherical bodies are termed pro-orbicles or pro-Ubisch bodies and first appear within the cytoplasm of the tapetal cells during early quartet stage (Figs. 54-56). They have been found by some authors to be membrane bound and associated with ribosomes. However, this was not observed in intermediate wheatgrass. As soon as the pro-orbicles are extruded through the plasmalemma (Figs. 55-56), they become coated with small osmophilic granules, similar to those in the tapetal cell wall (Fig. 54). The cell wall disappears during or shortly after extrusion of the pro-orbicles (Fig. 56). The coated pro-orbicles (pro-Ubisch bodies) are now termed orbicles or Ubisch bodies.

The coating increases in thickness in a manner very similar to the development of the exine. A granular material condenses on the early coating elements, and pointed spinules are formed similar to the arrow-shaped bacula of the exine (Fig. 57). As the Ubisch bodies increase in size, channels appear in the Ubisch body walls (Figs. 58-61).
Figs. 58-64. Ultrastructure of later stages in normal orbicular wall formation. - Fig. 58. Mid vacuolate microspore stage. The sporopollenin coat on the Ubisch bodies is thicker and channels are evident. The pro-orbicular center (arrow) is still as electron translucent as were the original pro-orbicles. X 21,750. - Fig. 59. Mid-vacuolate microspore stage. The sporopollenin coat is thicker still and the channels are seen radiating from a pro-orbicular center which has become more electron dense. The initials of an interconnecting reticulum are indicated by the arrow. X 12,500. - Fig. 60. Mid/late-vacuolate microspore stage. A reticulum of sporopollenin (arrow) now interconnects the Ubisch bodies. X 22,000. - Fig. 61. Late-vacuolate microspore stage. The original material of the pro-orbicular center has disappeared (arrowhead) and the channeled spinulate Ubisch bodies are fully formed as is the tapetal reticulum. A thin line of material, possibly primexine, persists in the cavea of the exine (curved arrow). X 16,000. - Fig. 62. Engorging-engorged pollen stage. The tapetal cytoplasm has disappeared as has most of the middle layer (M). An endothecial cell (EN) containing a chloroplast is seen on the right. X 12,500. - Fig. 63. Engorging pollen stage. Some tapetal cytoplasm (T) is present and a reticulum has formed on all sides of the tapetal cell although Ubisch bodies are present only on the inner tangential surface. The reticulum is somewhat disorganized and a fibrillar component of the "tapetal membrane" is indicated by the arrowheads. The cytoplasm of the middle layer (M) has degenerated to a dark mass. An endothecial cell (EN) is on the left. X 14,500. - Fig. 64. Engorged pollen stage. The mature tapetal membrane lies on the appressed primary walls (pw's) of the middle layer (inner and outer tangential walls) and endothecium (inner tangential wall). The sporopollenin reticulum formed on the outer surface of the tapetum is indicated by the arrow. Ubisch body channels are obscured. X 17,500.

Fig. 65. Normal development of the tapetum (electron microscopy). Pachytene. During meiosis osmophilic granules (arrowheads) appear between the PMC callose wall and the primary wall of the tapetum (T). The tapetal cytoplasm is very dense, containing a large number of ribosomes. X 14,750.
of a diameter similar to that of the exine channels. At first the pro-orbicular center is translucent as were the pro-orbicles, but gradually it becomes darker and finally disappears by the late vacuolate microspore stage.

At the mid-vacuolate microspore stage a reticulum forms between and beneath the Ubisch bodies interconnecting them in a network covering the locular surface of the anther (Figs. 59-60). This network, along with a fibrillar component (Fig. 63) has been termed the "tapetal membrane" and is the only remnant of the tapetal cells present at pollen maturity. The term tapetal membrane is somewhat misleading for there is actually no membrane associated with it. Tapetal membrane is synonymous with orbicular wall.

Figure 61 shows the similarity between the Ubisch bodies and the exine. Composed of a similar material, both have spinules and channels which open to the surface and to an internal space, the cavea or the pro-orbicular center. The formation of both the exine and orbicular wall has also proceeded at the same rate.

The tapetal cytoplasm has completely degenerated and disappeared in Figure 62. Only the tapetal membrane of interconnected Ubisch bodies is present, along with a middle layer with a small amount of tapetal cytoplasm. Figure 63 shows a limited amount of tapetal cytoplasm remaining with a reticulum present on both sides of the tapetum although Ubisch bodies occur only on the locular surface and
to some extent the radial walls. The coating of sporopollenin usually does not completely surround each tapetal cell but extends only partially down the radial walls to allow confluence of the cytoplasms during senescence. The fibrillar component of the tapetal membrane is quite clear in this photograph. The reticulum is somewhat intermittent and disorganized, a feature quite commonly observed. The degenerated middle layer is collapsed and appressed to a thin area.

Figure 64 shows the typical appearance of the tapetal membrane at anther maturity. All cellular residue of the tapetum has disappeared, only the tapetal membrane being present and closely appressed to the cell walls of the middle and endothecial layers. Channels are obscured in the Ubisch bodies. This is a common occurrence and similar to that observed in the exine of the pollen wall at maturity.

(e) Electron microscopy of the normal development and senescence of tapetal cells (Figs. 65-71). From the start of meiosis the cytoplasm of the tapetal cells is very dense. The large number of ribosomes accounts for much of the density and often obscures many of the other features of the cell. Figure 65 shows a tapetal cell and part of a PMC at pachytene. Ribosomes and a few short strips of endoplasmic reticulum are the only organelles quite readily visible in the mildly vacuolate cytoplasm of this photograph. Osmophilic granules are present between the tapetal primary cell wall and the callose
wall of the PMC from the beginning of meiosis to the quartet stage. Figure 66 shows portions of two tapetal cells early in meiosis. Undifferentiated plastids as well as ribosomes and rough endoplasmic reticulum are present. Mitochondria are very numerous throughout the cytoplasm and a few dictyosomes are seen.

The number and amount of ribosomes, plastids and endoplasmic reticulum as well as their uniform distribution in the cell remains fairly constant throughout meiosis. During early stages in meiosis dictyosomes are fewer in number and are often found in areas of the cell away from the locular surface. The number of dictyosomes reaches a maximum at the quartet stage (Fig. 68) and with their associated vesicles are distributed throughout the cell.

At the quartet stage, small osmophilic granules appear in the primary wall of the tapetal cells, being more numerous than the granules which were present during meiosis (Fig. 67) between the tapetal cell wall and the callose wall.

During the early vacuolate microspore stage, small densely staining bodies are often, but not always, seen in the tapetal cytoplasm (Fig. 69). These unidentified bodies occasionally can be observed in earlier or later stages but are most often present during the late quartet-early vacuolate microspore stage. Osmophilic droplets contained within plastids (Figs. 69, 71) can be seen throughout microsporogenesis, but usually the number of droplets and the number
Figs. 66-71. Normal development of the tapetum (electron microscopy). - Fig. 66. Pachytene. The cytoplasm is populated with ribosomes, rough endoplasmic reticulum, mitochondria (m), plastids (p), and dictyosomes (d). X 11,000. - Fig. 67. Quartet stage. Granules in the tapetal cell wall (pw) are quite evident. The cytoplasm is increasingly packed with mitochondria (m), plastids (P), ribosomes and endoplasmic reticulum. A microspore bounded by a callose wall (C) is on the left. X 14,750. - Fig. 68. Quartet stage. The number of dictyosomes (d) and their associated vesicles reaches a peak in the tapetal cytoplasm. X 14,850. - Fig. 69. Early-vacuolate microspore stage. Ubisch bodies extend partially down the radial walls. The cytoplasm is less dense and rough endoplasmic reticulum is more obvious. Plastids (P) sometimes contain osmophilic droplets. Unidentified densely staining bodies (arrow) often appear at this stage. X 12,250. - Fig. 70. Mid vacuolate microspore stage. Cytoplasmic connections form between tapetal cells (arrow). X 12,250. - Fig. 71. Mid/late-vacuolate microspore stage. Membrane systems are quite prominent and tapetal radial walls have started to break down (2). Plastids are filled with osmophilic droplets (1). X 7,410.
of plastids containing such droplets increases toward the later stages of microsporogenesis.

Figure 69 shows more clearly the endoplasmic reticulum present in the tapetum although nuclei are not shown in this photograph. A large amount of endoplasmic reticulum is often present in concentric swirls around the nuclei. An indication of this arrangement is shown in the lower left corner of Figure 69. Also shown in this figure are early Ubisch bodies extending partially down the radial walls.

During meiosis there was only one instance in which connections of any kind were observed between tapetal cells. Plasmodesmata were seen once between two cells during early meiosis. However, at the mid-vacuolate microspore stage cytoplasmic connections appear between the tapetal cells (Fig. 70). This may be the first indication of tapetal radial wall breakdown.

Sometime during the mid/late-vacuolate microspore stage cytoplasmic changes occur indicative of the process of senescence and degeneration in tapetal cells (Fig. 71). The cytoplasm becomes less dense revealing a lattice work of membranes. Ribosomes disappear or are greatly reduced in number. Plastids are often filled with osmophilic droplets. Organelles often become coated with an osmophilic substance. Large membranous vesicular bodies may appear. The cells are reduced radially and are misshaped. Eventually the radial walls break down and the cytoplasmic contents of the tapetum become
confluent. Gradually all organelles and cytoplasmic contents disappear leaving only the interconnected reticulum of Ubisch bodies.

(f) **Ultrastructure of pollen development in fertile intermediate wheatgrass** (Figs. 72-85). In the actively dividing cells of the archesporium (Fig. 72) are found small vacuoles, amyloplasts with starch granules, a few mitochondria and a limited number of ribosomes. The archesporial cells are connected with each other and with the epidermal cells by numerous plasmodesmata.

After the sporogenous cells have differentiated and just before meiosis, callose is deposited between the plasmalemma and primary cell wall of each PMC. The cell wall disappears as the deposition of callose proceeds. The callose is deposited asymmetrically with the greatest portion first deposited on the locular side of the PMC's (Fig. 73). The results in the formation of callose tails (see Fig. 23, p. 100) which may be prominent through the quartet stage. The callose appears to be deposited in layers as evidenced in the quartet of Figure 75.

At the beginning of meiosis, large cytoplasmic channels 300-700nm in diameter interconnect the PMC's through the callose wall (Fig. 74). These connections typically persist until the first meiotic division.
Figs. 72-77. Stages in normal pollen development (electron microscopy). - Fig. 72. Archesporial stage. Actively dividing archesporial cells contain a few small vacuoles (V), starch granules (S) and mitochondria (m). The cells are connected by numerous plasmodesmata (arrowhead). X 10,000. - Fig. 73. Premieiosis; early callose deposition. Callose tails (c) form on the locular side of the PMC's. X 5,890. - Fig. 74. Zygotene-pachytene. Large cytoplasmic channels (arrows) connect the PMC's through the callose walls (C). X 5,795. - Fig. 75. Quartet stage. The callose wall (C) has a layered appearance (arrows). X 4,655. - Fig. 76. Late quartet stage; early bacula formation. Small vacuoles (V) appear in the cytoplasm. X 6,650. - Fig. 77. Early vacuolate microspore stage. The vacuoles in the early free microspore are larger and more numerous. X 4,750.
Throughout meiosis and microspore development until the pollen begins to engorge with starch, the cytoplasm is not very dense and relatively devoid of commonly observed organelles. With the exception of fragments of endoplasmic reticulum and a few ribosomes, most of the inclusions present are unidentifiable following the apparent cytoplasmic reorganization during meiosis. At the quartet stage and through the first mitotic division, a few mitochondria are again recognizable, but many of the inclusions remain undifferentiated and unidentifiable (Fig. 75). Lipid bodies are often seen in the developing microspores following release from the quartets.

After early bacula have formed in the primexine of the microspores, but while they are still enclosed with the quartet, small vacuoles appear in the cytoplasm (Fig. 76). These are not to be confused with the much smaller spherical vacuoles commonly present in the PMC's throughout meiosis (Figs. 73-75). Upon release from the quartet, the vacuoles in the microspores increase in number (Fig. 77) and then in size (Fig. 78). The vacuoles presumably fuse and at the late vacuolate (uninucleate) microspore stage there is only one large vacuole present, nearly filling the entire cell with the cytoplasm pushed to the periphery (refer to Figs. 32, 33, and 35).

Although pore ontogeny will not be discussed in this thesis for lack of an adequate number of representative stages, it appears from the limited data gathered regarding pore structure, to develop in the
Figs 78-84. Electron micrographs of later stages in normal pollen development - Fig. 78. Mid-vacuolate microspore stage. The exine is more developed and the vacuoles are increasingly larger than those of Fig. 77. X 3,895. - Fig. 79. Mid-vacuolate microspore stage. Normal pore structure. The single pore consists of an annulus (a) with an inner operculum (o). A granular Zwischenkörper is present below the operculum (arrow). X 3,420. - Fig. 80. Early engorging pollen stage with early intine (IN) formation. Starch granules (S) are just beginning to form in the amyloplasts (a). Many dictyosomes and mitochondria (m) are present. X 15,500. - Fig. 81. Engorging pollen stage. Starch granules (S) are more prominent. Mitochondria remain numerous and the cytoplasm is packed with ribosomes and associated rough endoplasmic reticulum. X 15,500. - Fig. 82. Mature engorged pollen. The intine (IN) is fully developed and still contains cytoplasmic channels (arrow). Starch granules (S) are large and numerous. The density of mitochondria remains high. A few dictyosomes (d) can still be seen. The exine is evident although many are obscured. X 15,500. - Fig. 83. Engorged pollen, a second variation. Starch granules and mitochondria (m) are present; however, unidentified coated bodies (?) are also numerous. The vegetative nucleus (VN) with its diffuse chromatin is on the right. X 14,750. - Fig. 84. Engorged pollen, a third variation. In addition to the usual population of organelles and starch granules (S), the cytoplasm contains a large number of unidentified vesicles (??V). X 14,000.
manner described for other grasses (Christensen and Horner, 1974).
Brief mention will be made at this point regarding its mature structure (Fig. 79). The single pore or aperture through which the pollen grain germinates consists of a circular annulus which is raised as a result of a thickened lamellated modification of the nexine. Within the annulus is a plug or operculum covering the opening although not continuous with the annulus. Below the operculum is a thick granular area, the Zwischenkörper, which is continuous with the endexine.

After the first pollen mitotic division there occurs a dense repopulation of the cytoplasm with organelles (Fig. 80). Mitochondria and dictyosomes are numerous and an enormous number of ribosomes appear (Fig. 81). Starch granules first appear faintly in amyloplasts (Fig. 80) then increase in size reaching a maximum at pollen maturity (compare Fig. 81 and Fig. 82, the same magnification). In the mature vegetative cytoplasm, numerous lipid bodies are found (Fig. 85) often associated with ribosomes.

Three distinct cytoplasmic types were found for mature pollen in the fertiles and partially steriles. Figure 82 shows the most common type. Starch granules are present in a cytoplasm packed with mitochondria. Pollen from SB_3-1-8-89, -2-16-2, -2-16-42, -2-17-68, -24-2-12, -29-1-27, Oahe 1 and Oahe had this appearance. Three plants, SB_3-2-16-50, SB_2-24-2-76 and Oahe 3, had the characteristics of the first type plus numerous unidentified coated bodies (Fig. 83). These
bodies are smaller (about .3 μm in diameter) than mitochondria and have a homogenous finely granular center area bounded by one and possibly two membranes which are covered by a thin coating of an electron dense substance. The third variation of mature pollen had in addition to the usual organelles (mitochondria, starch granules, dictyosomes, ribosomes, and rough endoplasmic reticulum), a large number of unidentified vacuoles (Fig. 84). These vacuoles are .4-.5 μm in diameter and appear to be bounded by a membrane. The pollen of three plants (Oahe 4, SB3-24-2-55, and SB5-1-9-79-31-14) had this appearance at maturity.

The typical appearance of the two sperm cells is shown in Figure 85. Each lobed cell is bounded by a membrane and contains a multilobed nucleus, ribosomes, endoplasmic reticulum, mitochondria and microtubules. Some inclusions were termed plastids on the basis of their homogenous staining, good preservation and dissimilarity to the smaller more vesiculated mitochondria in the sperm cells. Mitochondria are generally poorly preserved throughout microsporogenesis in this material. However, these "plastids" are of a size comparable to the mitochondria in the vegetative cytoplasm and their shape is by no means definitive of plastids: they may actually be mitochondria. Sperm cells were sampled in only four plants, SB3-29-1-27, -2-17-68, -2-16-42, and -24-2-7, and such "plastids" were seen in sperm from only 1 of these (SB3-29-1-27). A generative cell from Oahe 4 was
observed to contain plastids. Therefore, the sperm cells could be thought to contain them also.

(g) Pollen and tapetum development in partially male sterile Oahe 2 in 1974 (Figs. 86-91). Oahe 2 had a high percentage male sterility (52.4) in 1974. For those sterile anthers to be discussed, the end result of the aberrant development was an empty pollen grain with a malformed, often broken exine (Figs. 86, 88, 91). Both pollen and tapetum development proceeded in all anthers as in fertile intermediate wheatgrass through the quartet stage.

Early development of the bacula within the primexine appeared normal. The next developmental stage observed was that with a rather thick exine so it was not possible to follow intermediate exine development in this plant. The mature exine in the sterile grains was always malformed. Such exines had a channeled tripartite structure but it was very uneven and the columellae were irregularly placed (Fig. 86). Channels were present in the columellae, a feature not seen in normally structured exines. After the cytoplasm had presumably degenerated, only the exine and endexine persisted. Fragments of wall material, probably sporopollenin, were seen dispersed inside the empty sterile grains (Fig. 91).

The exact time of abortion was unknown, but it was probably before the first pollen mitosis. No grains having malformed exines were
Fig. 85. Electron micrograph of engorged normal pollen. The two multilobed sperm cells contain equally lobed nuclei (Nu), plastids (P), mitochondria (m), ribosomes and endoplasmic reticulum. Lipid bodies (L) are seen in the vegetative cytoplasm. X 11,750.

Figs. 86-91. Pollen and tapetum development is partially sterile Oahe 2 during 1974 (e.m. = electron micrograph, all others are light micrographs). - Fig. 86. Mid/late-vacuolate microspore stage. The exine is moderately malformed with channels present in ill-defined columella (e.m.). X 22,050. - Fig. 87. Late-vacuolate microspore stage. Malformed pores are appressed to the tapetal wall (arrow). The degenerating tapetal cells have formed a moderate plasmodium, not unusual in fertile anthers. X 337. - Fig. 88. Mid/late-vacuolate microspore stage. Microspores are devoid of cytoplasm and have irregular and broken exines. X 337. - Fig. 89. Early-vacuolate microspore stage (e.m.). Early degeneration of the tapetum. Very early Ubisch bodies (arrow) are seen on a membranous tapetum. The membranes appear as a lattice surrounding many of the organelles. In addition, the plasmalemma is very irregular in outline. X 12,250. - Fig. 90. Late vacuolate microspore stage (e.m.). The degenerating, membranous cytoplasm contains vesicular plastids filled with osmophilic droplets. Ubisch bodies are few and malformed (arrows). Most of the orbicular wall is reticulum. X 8,250. - Fig. 91. Mid/late-vacuolate microspore stage. Electron micrograph of the sterile microspores in Fig. 88. The endexine (arrow) persists beneath the malformed exine. X 4,655.
observed with normal cytoplasmic structure. If abortion occurred after binucleate pollen was formed, on chance alone one should sample a recognizable stage prior to that and see at least one example of a grain with an abnormal exine but having a normal cytoplasmic fill. Also the amount of tapetal cytoplasm still present after abortion suggest a time before the first mitosis. There was no indication of a delayed tapetal degeneration in this material.

Abnormal pore development was also seen in the sterile grains (Fig. 87). The pore had no resemblance to the normal structure, but appeared as a sharp outward buckle of the exine.

Tapetal development was also found to be aberrant in Oahe 2 during 1974. In some anthers the tapetum was seen to begin degeneration early although this did not result in an early disappearance of the tapetum. Figure 89 shows a tapetal cell which has begun senescence at the early vacuolate microspore stage. Very early Ubisch bodies are seen on the surface. The cell is very irregular in contour and reduced in thickness. A lattice-work of membranes is prominent and surrounds many of the organelles. Normally such degenerative changes do not occur until the mid/late-vacuolate microspore stage.

The anthers either produced fertile pollen or sterile pollen. There did not appear to be both types within an anther. But abnormal Ubisch body formation was always observed, both in anthers with sterile pollen and anthers producing fertile pollen. The Ubisch bodies
were very small and always irregularly shaped (Fig. 90). Some were
canneled but many were so small that neither channels nor a pro-
orbicular center was observed. Also in many anthers few Ubisch bodies
were produced. The tapetal membrane usually consisted of a very heavy
reticulum with widely spaced Ubisch bodies (Fig. 90). The fertile
pollen produced was engorged with starch and had a well-developed inti-
tine. All other cytoplasmic inclusions appeared normal also.

(h) Pollen and tapetum development in male sterile SB₃-2-17-20
(Figs. 92-113). Pollen development in SB₃-2-17-20 was abnormal from
the start of meiosis. Figure 92 shows PMC's at different stages of
development during meiosis I at the same level within an anther. Such
asynchrony was not seen in fertile plants. This asynchronous develop-
ment is again seen following release of the microspores from the quar-
tet. In Figure 93 one cell is seen which has a nuclear structure rem-
iniscent of diakinesis. The size of the cell indicates that it may
indeed be a spore, but the presence of chromosomes is unusual at this
stage.

Division disturbances were commonly observed at SB₃-2-17-20.
From the size of the cells, it is probable that most are second mei-
otic division failures. Figure 94 shows two early microspores with a
common wall between them. In Figure 95 two cells are lagging behind
two other cells with prominent pores and thicker exines. One of the
Figs. 92–99. Pollen and tapetum development in male sterile SB 2-17-20 (light microscopy). - Fig. 92. Meiosis I. Asynchrony of development within the same locule. PMC's are in pachytene, diplotene-diakinesis, and metaphase I. X 337 - Fig. 93. Early-vacuolate microspore stage. A spore with a diakinesis-like nuclear structure is indicated by the arrow. X 337. - Fig. 94. Early-vacuolate microspore stage. A division failure has produced a pair of joined microspores (arrow). X 337. - Fig. 95. Early/mid-vacuolate microspore stage. Two spores have reduced exine formation; one has two micronuclei (arrow), the other consists of two cells within a common exine (arrowheads). The other two microspores with thicker exines and prominent pores appear normal. X 337. - Fig. 96. Mid/late-vacuolate microspore stage. Malformed exines are prominent. Again some microspores consist of two cells within a common exine (arrows). Tapetal cells are intact, although their locular surfaces are highly sculptured. X 337. - Fig. 97. Mid/late-vacuolate microspore stage. Collapsed aborted microspores have a thin malformed exine. Degenerating tapetal cells are reduced in width but their radial walls remain intact. X 337. - Fig. 98. Anther maturity. The tapetal cytoplasm is gone but the tapetal membrane remains highly sculptured. The middle layer (M) persists in many places. Fragments of sterile microspores are observed. Two microspores are joined by a common wall (arrow). X 450. - Fig. 99. Anther maturity. The tapetum persists as does the middle layer. The sterile microspores have thick, uneven walls. The microspore cytoplasm has degenerated and disappeared. X 469.
cells in an earlier stage of development has two prominent micronuclei. The other abnormal microspore is actually two cells within a common exine. Sometimes a very small micrograin was seen attached through a common wall to a larger microspore.

In addition to division failures, pollen wall malformations were often seen in this plant. In fact, the presence of a normal exine was the atypical case. Such wall disturbances, as well as additional examples of division failure producing either "joined" microspores or two microspores within a single exine are given in Figures 96-99. Fragments of exines and microspores are also seen in the locules of Figure 99.

Microspore abortion and degeneration and disappearance of the cytoplasm occurs at varying times but before the first pollen mitosis (Fig. 97).

The tapetum and middle layer of the anthers in SB3-2-17-20 persists often until anthesis. In the mature anther of Figure 98, the middle layer is present in most places, and although the tapetal cytoplasm has degenerated the tapetal membrane system is quite extensive and does not lie flat against the anther wall as is the case in fertile anthers. Figure 99 shows a more obvious persistence of the middle layer and tapetum in the mature anther. Secondary walls or thickenings in the endothecial cells are seen as dark radial bands in Figure 98. The presence of all four original parietal layers of the
anther at maturity as well as secondary thickenings in the endothelial cells becomes important in the discussion of the lack of stomium formation in SB\textsubscript{3}-2-17-20. Some thoughts have already been related previously with regard to Figures 5 and 6.

Another completely different type of sterility was seen in a few anthers of SB\textsubscript{3}-2-17-20. Only one microsporangium in such anthers was affected, however. The middle layer and tapetum enlarged and pushed the sporogenous cells to a small area in the center of the anther (Fig. 100). When this hypertrophy occurred is unknown because only the end result was observed in anthers in which the other three microsporangia were in later microspore stages. Whether the sporogenous tissue degenerated before the tapetum and middle layers enlarged or if it degenerated as a result of mechanical crushing by the hypertrophied is also unknown.

Figure 101 shows an early free microspore of SB\textsubscript{3}-2-17-20 which contains two micronuclei as a result of lagging chromosomes failing to be incorporated into the restitution nucleus following meiosis II. The cytoplasm appears normal, containing lipid bodies which are commonly present in normal microspores. The microspore does not contain many vacuoles, but again this is not an uncommon variation in fertile microspores at this stage.

However, in the early microspores of SB\textsubscript{3}-2-17-20 the bacula are very indistinct in a primexine (Fig. 102) which is often uneven
Fig. 100. Light micrograph of one microsporangium of an anther of male sterile SB-2-17-20. The middle layer (m) and tapetum (T) have hypertrophied. The crushed mass of sporogenous cells lies in the center of the locule. X 450.

Figs. 101-107. Ultrastructure of pollen development in male sterile SB-2-17-20. - Fig. 101. Early free microspore (early vacuolate) stage. Two micronuclei (m) are present. Several lipid bodies are scattered throughout the cytoplasm (arrow). X 5,235. - Fig. 102. Early free microspore. Rather indistinct bacula appear in the primexine (arrow). X 10,000. - Fig. 103. Early free microspore. Higher magnification of Fig. 101. Bacula (b) are few, and the primexine is irregular and intermittent with many gaps (arrows). X 18,000. - Fig. 104. Early/mid-vacuolate microspore stage. The exine is channelled but columella and cavea are absent. The thick granular area (G) may represent a presumptive pore region. X 10,750. - Fig. 105. Mid-vacuolate microspore stage. The exine is mildly irregular with the columella indistinctly placed. The operculum (o) of the pore is fused with the annulus. A granular Zwischenkörper is present below the operculum (arrow). X 5,605. - Fig. 106. Late-vacuolate sterile microspore. A granular endexine (en) persists under the malformed exine. Fragments of sporopollenin are present in the locule outside the microspore. X 5,225. - Fig. 107. Mid/late-vacuolate microspore stage. Three types of abnormal exine are present. A thick solid exine is seen toward the top of the photograph, and a thin solid exine toward the bottom. Both are finely channelled and lack a normal tripartite structure. The components of the exine in the middle (arrow) resemble Ubisch bodies. They are channelled but lack a pro-orbicular center. X 3,230.
and nonhomogeneous. Figure 103 is a higher magnification of Figure 101 and shows indistinct bacula in an irregular intermittent primexine. As the exine increases in thickness, through the deposition of sporopollenin, it often lacks the normal tripartite structure although transversed by channels (Fig. 104). Figure 104 also shows a thickened granular area which is continuous with the endexine beneath a portion of the exine. This may represent the Zwischenkörper and the area may have been a presumptive region for the formation of a pore which subsequently failed to develop.

Another microspore with only a mildly irregular exine has a malformed pore in which the operculum is fused with the annulus (Fig. 105).

Figures 106 and 107 illustrate the abnormal exine types of many of the sterile microspores after they have aborted. The exine may be thick and fractured, retaining the endexine, or it may be rather thin and solid. A fragmented type of exine was also observed, consisting of individual channeled spheroidal components. These resembled Ubisch bodies but lacked a spherical central area.

The ultrastructure of sterile pollen from SB3-2-17-20 exhibiting division errors is shown in Figures 108-109. Figure 108 shows two empty microspores joined by a common wall. Not only is the common wall of atypical structure, but the rest of the exine is abnormal with only a limited cavea. The same type of exine is seen around the two
Figs. 108-113. Further ultrastructural characteristics of pollen and tapetum development in male sterile S. B. 2-17-20. - Fig. 108. Mid/late-vacuolate microspore stage. A common wall has formed between two microspores. X 3,040. - Fig. 109. Mid/late-vacuolate microspore stage. Electron micrograph of Fig. 96. Two cells (1,2) are contained within a common abnormal exine. X 3,040. - Fig. 110. Mid/late-vacuolate microspore stage. Two microspores have formed nearly separate walls (1,2); however, they are joined in several places. X 2,945. - Fig. 111. Mid/late-vacuolate microspore. Degenerating cytoplasm in a microspore with a solid, nontripartite exine. X. 7,750. - Fig. 112. Late-vacuolate microspore stage. The tapetal cytoplasm is degenerating and contains many dense inclusions. The tapetal radial walls (plasmalemma), however, remain intact (curved arrows). A sporopollenin reticulum is present on the radial outer and tangential tapetal wall (straight arrow). The middle layer (M) is not reduced and secondary walls (sw) have formed between the endothecial cells (E). X 2,040. - Fig. 113. Mid-vacuolate microspore stage. The tapetal orbicular wall has an extremely heavy reticulum (arrow), resembling the solid exine of some sterile microspores. X 6,745.
microspores on Figure 109. These two cells have separate plasmalemmas but a common microspore wall. In Figure 110 are two degenerated microspores which have formed essentially separate exines, but the walls can be seen to be joined in several places.

The cytoplasm of a mid/late-vacuolate microspore with a solid exine is observed to be degenerating in Figure 111. A nucleus can not be seen in this microspore, but the thickness of the exine and developmental state of the Ubisch bodies and tapetum suggest that the degeneration is occurring before the binucleate pollen stage.

The persistence of the tapetum and middle layer is illustrated ultrastructurally in Figure 112. The cytoplasm of the tapetum is senescing and contains many unidentified dense bodies and vesiculate inclusions. The plasmalemmas of the radial walls are still intact. Normally, by this stage (late-vacuolate microspore) the tapetal contents would have formed a plasmodium through the breakage of the radial plasmalemmas between the cells.

The middle layer is not reduced as would be the case in anthers of fertile plants. But normal secondary walls are present on the radial and inner tangential surfaces of the endothecial cells.

Abnormalities in the tapetal orbicular wall were also seen in SB3-2-17-20. In Figure 112 a separate reticulum completely surrounds each tapetal cell. In normal anthers there is a gap in the lower portion of the radial walls from which Ubisch bodies or a reticulum is
absent. There is also an unusually high number of Ubisch bodies in the orbicular wall of Figure 112. Figure 113 shows just the opposite. In this sterile anther of SB₃-2-17-20 there are few Ubisch bodies but the tapetal membrane (orbicular wall) is nearly solid with a thick reticulum resembling the exine of some sterile microspores (compare Fig. 111). Part of the fragmented type of exine as in Figure 107 is seen in the upper left portion of the photograph.

(i) Pollen and tapetum development in highly male sterile SB₃-2-22-48 (Figs. 114-133). Tapetum development and senescence as well as Ubisch body and orbicular wall formation were entirely typical of normal behavior (Figs. 114-119, 125).

Pollen wall malformations in the sterile microspores were not always seen but in some instances were observed. Figure 114 shows a small inward buckle of the exine in otherwise normal microspores. In Figure 115 all the microspores were empty and sterile and have irregular, abnormal exines. Two microspores with malformed exines are shown ultrastructurally in Figure 125. One is completely devoid of cytoplasm and the cytoplasm of the other, although containing a few large starch granules, is rather dispersed, lacking the density of normal engorging pollen grains. Another slightly malformed exine is illustrated in Figure 124. The operculum of the pore is abnormally formed in two parts rather than being a solid plug. Also, the cavea
Figs. 114-121. Pollen and tapetum development in highly male sterile SB -2-22-48 (light microscopy). - Fig. 114. Mid/late-vacuolate microspore stage. Microspores appear normal except for a buckle in the exine of one microspore (arrow). Early tapetal degeneration with a highly convoluted locular surface is normal. X 337 - Fig. 115. Late-vacuolate microspore stage. Microspores have an irregular exine and are devoid of cytoplasm. Tapetum is highly degenerated; normal for this stage. X 356. - Fig. 116. Engorged pollen. The pollen cytoplasm is degenerating and has a hypertrophied intine (arrow). The exine appears normal and the middle and tapetal layers have degenerated completely. X 337. - Fig. 117. Engorging pollen. Pollen and tapetum are normal. Only an occasional empty grain is present. X 337. - Fig. 118. Engorged pollen. The pollen appears normal. X 712. - Fig. 119. Anther dehiscence. The stomium is indicated by the arrow. Both fertile appearing pollen and empty, collapsed sterile pollen are seen in the anther locules. X 175. - Fig. 120. Late-vacuolate microspore stage. Degenerated sporogenous and tapetal cells are appressed to a thin dark layer in the center of the locule. X 356. - Fig. 121. Higher magnification of Fig. 120. An epidermis (epi), endothecium (E) with heavy secondary wall thickenings, and an enlarged vacuolated middle layer (M) are present. A possible tapetal cell is indicated by the arrow. X 637.
Figs. 122-128. Pollen development in highly male sterile SB-22-48 (electron microscopy). - Fig. 122. Late quartet stage. A normal appearing primexine (arrow) is deposited while the microspores are still encased in callose (C). A micronucleus (mn) is also observed. X 9,310. - Fig. 123. Early-vacuolate microspore. Normal bacula (arrow) appear in the primexine (arrow). X 18,900. - Fig. 124. Mid/late-vacuolate microspore. Ultrastructure of Fig. 114. Abnormal pore operculum (arrow). Cavea (cavity) of exine is somewhat obscured. Exine channels are very thin and numerous; compare with piece of exine in upper left corner. X 6,750. - Fig. 125. Engorging pollen stage. Two pollen grains with malformed exine; one empty (P1) and the other containing a few starch granules (P2). The tapetal membrane is normal. X 2,220. - Fig. 126. Engorged pollen. Ultrastructure of Fig. 116. Degenerating amorphous cytoplasm contains starch, but is devoid of recognizable organelles. Intine (IN) is abnormally thick. X 4,370. - Fig. 127. Higher magnification of Fig. 126. Granular intine (IN) lacking channels borders an amorphous cytoplasm. The exine appears normal; somewhat obscured exine channels are common in normal engorged pollen. X 11,500. - Fig. 128. Degenerating engorged pollen. A ghost-like nucleus (N) and starch granules (S) lacking a surrounding amyloplast are observed. X 11,500.
between the tectum and the nexine is rather limited and the exine channels are more numerous and smaller in diameter than are the channels of the portion of a normal exine in the upper left corner of the photograph.

Development of the exine was found to be normal through the early stages sampled in this study (Figs. 122-123). Both primexine deposition around the microspores while in the quartet and bacula formation appeared typical for normal development. Later stages of exine deposition between early bacula formation (Fig. 123) and a mature exine were not sampled in this plant. Micronuclei were seen in the microspores of SB$_3$-2-22-48 as they were in most of the SB$_3$ plants.

During 1974 most of the sterility expressed in SB$_3$-2-22-48 was characterized by pollen abortion after starch engorgement. This is shown in Figure 116 and ultrastructurally in Figures 126-128. It was not possible to determine if engorgement had proceeded in a normal manner with respect to the behavior of inclusions in the cytoplasm or if the first and/or second mitotic divisions had occurred, for these stages were not sampled. The degenerating nucleus in Figure 128 is unidentifiable as to whether it is generative, sperm, vegetative, or neither.

The size of the starch granules suggests that at least engorgement had proceeded to a fairly mature state before degeneration began. The intine is unusually thick, but again it is unknown whether this
hypertrophy was present before abortion or was a consequence of the degeneration. The amorphous appearance of the intine and the rest of the cytoplasm can be considered a result of the degenerative processes. Exine formation is normal.

During 1975 plants of SB3-2-22-48 failed to show the degenerative processes following pollen engorgement which were seen the previous summer. In fact, more apparent fertility was observed in 1975 than in 1974 (99.4% vs 87.9%). Figures 117 and 118 show normally engorging and engorged pollen in some anthers sampled in 1975. The starch granules are stained darkly, a feature typical for thick sections stained with toluidine blue in 1975. In 1975 degeneration of the cytoplasm occurred in sterile grains before the pollen engorged with starch (Fig. 117). At dehiscence (Fig. 119) the anthers contained both fertile grains and empty, partially collapsed, sterile grains.

During 1975 several sampled anthers had sterile pollen with abnormal exines. Within such microsporangia, the number of microspores with malformed exines varied from a few to most of those present. Usually the malformations were severe (Fig. 130-131).

The ultrastructure of most of the fertile pollen in SB3-2-22-48 is shown in Figures 132-133. All features are typical of normally engorged pollen with the exception of the unidentified coated bodies described earlier for three other plants (SB3-2-16-20, SB3-24-2-76 and
Figs. 129-133. Further pollen development in highly male sterile SB-2-22-48 (electron microscopy). - Fig. 129. Ultrastructure of the degenerated central mass of Fig. 120-121. Layer of degenerated, crushed sporogenous and tapetal tissue (C) is next to a vacuolate middle layer (M). X 9,250. - Fig. 130. Late-vacuolate microspore stage. Empty microspores are surrounded by a solid malformed exine (MEX). X 9,250. - Fig. 131. Late-vacuolate microspore stage. Another type of malformed exine surrounds an empty sterile microspore. Channels open into a cavea-like space in the exine (arrow). X 9,250. - Fig. 132. Mature engorged pollen. Cytoplasm around the large vegetative nucleus (VN) contains the normal dense population of organelles; mitochondria, dictyосomes, amyloplasts with starch granules (S), ribosomes, and endoplasmic reticulum; plus the same unidentified coated bodies as in Fig. 83, barely resolvable at this magnification (arrows, upper right corner). X 8,740. - Fig. 133. Engorged pollen. Higher magnification of the pollen grain in Fig. 132. The unidentified bodies (?) coated with an electron dense substance may be bounded by a membrane. Mitochondria (m) sometimes contain osmophilic droplets. Dictyosomes (D), Vegetative nucleus (VN). X 23,000.
Oahe 3). These bodies are about 250 nm in diameter and have a layered appearance to the osmophilic coating suggestive of one or two limiting membranes. The internal organization is not suggestive of any other organelle and the bodies were not found to be associated with any specific inclusion. The coated bodies were not seen in engorging pollen nor in the engorged cytoplasm of the few mature grains which had a moderately malformed exine.

Another type of male sterility, similar to that described earlier of a few anthers of SB\textsubscript{3}-2-17-20, was found in one sampled anther of SB\textsubscript{3}-2-22-48 (Figs. 120-121). Two microsporangia of the anther exhibited a hypertrophy of the vacuolate middle layer. The tapetum and sporogenous cells were degenerated and pushed to a small area in the center of the microsporangium. Figure 121 indicates a possible tapetal cell which has not been entirely crushed. The endothecial cells have heavy secondary thickenings, typical for a mature microsporangium. The other two microsporangia in this anther were in the late vacuolate microspore stage of development, but there was no real indication of when the middle layer enlarged or what effect it may have had on the abortion of the sporogenous tissue. The ultrastructure is seen in Figure 129 and it shows the crushed central mass and a cell of the vacuolate middle layer. The think dark line of cytoplasm around the periphery of the middle layer suggests that the middle layer may
be in the process of degeneration also. A thin primary wall surrounds the cells of the middle layer.

(j) Pollen and tapetum development in male sterile SB₃-2-22-68

(Figs. 134-139). Figure 134 shows the end result of the sterility process in most of the anthers of SB₃-2-22-68. The pollen grains are collapsed and devoid of cytoplasm. They are similar in appearance to whole pollen grains stained with acetocarmine or IKI (Figs. 13-14). The tapetal cells preserved poorly in most stages of development in SB₃-2-22-68 leaving a limited number of examples for comparison. However, the available examples gave no indication of a tapetal development and senescence differing from the normal. In Figure 134 a tapetal membrane of typical interconnected Ubisch bodies lies on an endothecial layer with secondary thickenings in the radial walls.

These collapsed grains are seen ultrastructurally in Figure 138. A small amount of degenerated cytoplasm is present. Abortion occurs between the late-vacuolate microspore stage and the time the pollen begins to engorge; before the first pollen mitosis or soon after. Usually the aborted grains have normal exines, but occasionally some wall malformations are seen (Figs. 136, 137). The intermittent but otherwise generally normal exine in Figure 137 is one type of malformed pollen which was not encountered in any other plant. The tapetal debris outside the microspore of Figure 137 is probably due
- **Fig. 134.** Collapsed, sterile pollen \(^3\) (CP) just before dehiscence. A tapetal membrane (TM) is present, along with heavy secondary walls (bandings or thickenings) in the endothecial cells (arrow). X 712. - **Fig. 135.** Abnormal locule (dyad stage in adjacent locules) with hypertrophied, vacuolate middle layer (M) and central mass of crushed tapetal and sporogenous tissue. X 344.
Figs. 136-139. Further developmental anomalies of male sterile SB 3-2-22-68 (e.m. = electron micrograph). - Fig. 136. Early/mid-vacuolate microspore with malformed wall; exine channels are seen in cross section (e.m.). Uneven deposition of sporopollenin is evident. X 4,590. - Fig. 137. Early/mid-vacuolate microspore with a segmented exine (e.m.). Except for a lack of prominent spinules and wall continuity, the exine is fairly well formed. Tapetal debris is shown outside the exine and the spore cytoplasm (SC). X 14,000. - Fig. 138. Collapsed sterile pollen of Fig. 139 just prior to dehiscence (e.m.). The cytoplasm (C) has degenerated, however, exine formation is normal. X 2,580. - Fig. 139. Anther with only three locules; one locule shows a hypertrophied middle layer (M) crushing the tapetal and sporogenous cells. X 194.

Figs. 140-142. Sterility in SB 3-1-9-79. - Fig. 140. Uninucleate, late-vacuolate microspores prior to dehiscence (arrows). Some collapsed microspores are evident. X 350. - Fig. 141. Electron micrograph of early/mid-vacuolate microspore with an uneven nexine (arrow). Some lamella-like structures (L) are seen associated with the nexine. X 6,840. - Fig. 142. Electron micrograph of collapsed late-vacuolate microspores with an uneven nexine (arrow). X 4,560.

Figs. 143-144. Sterility and fertility in SB 3-2-17-83 (light microscopy). - Fig. 143. Collapsed sterile microspores. X 350. - Fig. 144. Engorging pollen from a fertile anther of SB 3-2-17-83. Note the exine is thicker than that of the collapsed microspores of Fig. 149. X 350.
to processing methods which ruptured the tapetal cell walls allowing the cellular contents to flow into the locule. It in all probability does not represent a natural but abnormal rupture of the tapetal cells.

Quite often in SB₃₋₂-22-68 an unusual type of sterility was observed similar to that described earlier for an occasional anther of SB₃₋₂-17-20 and SB₃₋₂-22-48. In the affected microsporangia, one of the parietal layers, usually the middle layer, was hypertrophied and the tissue central to this was degenerated. Figure 135 shows one such microsporangia where principally the middle layer is vacuolated and enlarged. The other microsporangia of this anther are at the quartet stage of development. In all other instances, this type of sterile microsporangium was found in anthers at the late-vacuolate stage of development.

In microsporangia where principally the middle layer was hypertrophied (Figs. 135, 139), a few vacuolate cells can be seen inward to this layer. These may be tapetal cells or perhaps middle layer cells which are out of position. There were cases in which a few of the endothecial cells were enlarged. In other instances endothecial cells and middle layer cells were enlarged in about equal proportion. Except for the epidermis it was difficult to discern a continuous layer of cells in such microsporangia. Usually there were not clear enough delineations to indicate that only the middle layer was
enlarged. Where the middle layer was found to be enlarged at the late-vacuolate, microspore stage, secondary endothecial thickenings were found in only about 50% of the affected microsporangia (Fig. 139).

Anthers were found that had either one or two of this type of microsporangia. In those with two, each "pair" of microsporangia had one normal microsporangium and one with hypertrophied vacuolate cells and a degenerated central mass of tapetal and sporogenous tissue.

Four anthers were found which only had three microsporangia. In three of these anthers, one of the microsporangia was of the above atypical type and the other two were normal (Fig. 139). The other anther with three microsporangia had all three locules filled with sterile collapsed microspores similar to those in Figure 139.

(k) Pollen and tapetum development in male sterile SB$_3$-1-9-79 (Figs. 140-142). Only stages following the release of the microspores from the quartets were sampled for SB$_3$-1-9-79. Tapetal behavior appeared typical for all stages following meiosis. Microspore development also was generally normal as far as it progressed. The appearance at dehiscence was that of normal late-vacuolate microspores (Fig. 140). Only a few microspores were collapsed. Cytoplasmic degenerative changes were not observed. It was as though the microspores had a capacity for further development, but they were interrupted by dehiscence.
There were slight irregularities in wall formation observed for some of the microspores. Figures 141-142 show an uneven deposition of the nexine. This, in a more moderate form, was seen intermittently in fertile anthers, but SB₃-1-9-79 had an unusually large number of microspores with this feature. It was evident in very young microspores as well as in the older spores of Figures 141 and 142. A few lamella-like fragments are seen associated with the nexine in Figure 141. Of all the plants examined this was the only indication of a possible lamellar component of the nexine as reported in other species (Rowley and Southworth, 1967; Sengupta and Rowley, 1974). One abnormal pore was also seen having only a partial operculum.

(1) **Pollen and tapetum development in highly male sterile**

SB₃-2-17-83 (Figs. 143-144). The development of the microspores is quite normal until abortion occurs at a time varying from mid- to late-vacuolate microspore stage. Microspores which aborted toward the early end of this scale are shown in Figure 143. They are collapsed, nearly empty and have a rather thin pollen wall. Compare the thickness of the exine with that of the fertile pollen in Figure 144. A few microspores were seen with unusually small exine channels, but other wall abnormalities were not observed.

The occasional anther which produced fertile pollen in SB₃-2-17-93 (Fig. 144) was entirely normal in its developmental features.
The development and degeneration of the tapetum in both fertile and sterile anthers appeared to be identical and typical for intermediate wheatgrass.

(m) Pollen and tapetum development in partially sterile SB₃ plants (Figs. 145-149). Tapetum development and senescence in the partially steriles showed no difference from that expected for normal development. Usually the occasional sterile grains in these plants were empty and sometimes collapsed. Abortion and disappearance of the cytoplasm occurred near the late-vacuolate microspore stage but before engorgement. In some cases at dehiscence, late-vacuolate microspores and engorging pollen grains were released with the fully mature engorged pollen.

Wall malformations and pore anomalies of the kind already discussed were common among the sterile pollen of SB₃-2-23-39 in 1974. A different type of wall abnormality was seen in some microspores of SB₃-2-17-68. In a locule containing a majority of mid/late-vacuolate microspores were a few early vacuolate spores with an extremely thin nonchanneled exine (Fig. 145). Chromosomes are also evident in this spore, although the cell is evidently not dividing. Otherwise, the cytoplasmic constituents appear normal.

Several anthers of SB₃-29-1-27 contained only sterile pollen. This is in contrast to the other partially sterile plants which had
Fig. 145. Electron micrograph of an abnormal microspore in an otherwise normal locule of SB-2-17-68 at mid-vacuolate stage. The abnormal spore is early-vacuolate and has a thin malformed exine (arrow). The cytoplasm contains many lipid drops (L) and darkly staining bodies, probably chromatin chromosomes (ch). X 5,035.

Figs. 146-149. Electron micrographs of sterile pollen of SB-29-1-27. - Fig. 146. Degenerating pollen. The intine is very thick and amorphous with a lack of cytoplasmic channels. There is an absence of starch in the extremely dark and disorganized cytoplasm. X 10,500.
- Fig. 147. Sterile locule with many degenerating pollen grains. An abnormally thick intine as in Fig. 135 is indicated by the arrow. X 387. - Fig. 148. A colossus (Co), probably part of another malformed exine, has adhered to the exine of a degenerating pollen grain. X 9,000. - Fig. 149. Shown are portions of two collapsed pollen grains with malformed exines and an absence of cytoplasm (1,2). X 4,655.
microsporangia containing both fertile and sterile grains. However, some anthers of SB$_3$-29-1-27 had both sterile and fertile pollen. The pollen degeneration was very similar to that of SB$_3$-2-22-48 in 1974. The pollen in many cases degenerated following engorgement with starch (Figs 146-148). However, the starch granules were much smaller at the time of abortion (not shown). The intine in these grains was fully developed, and sometimes enlarged (Figs. 146, 147). Wall malformations and collapsed microspores were common (Fig. 149). Large pieces of locular debris from malformed pollen walls were seen adhering to the exine of some of the degenerating grains (Fig. 148). Adherence of debris to pollen walls is common in mature pollen grains; large pieces of exine present as debris are rather uncommon in fairly fertile microsporangia.

A summary of the significant events of anther development in male fertile, male sterile, and partially male sterile intermediate wheatgrass is given in Table VIII.
Table VIII. Summary and highlights of anther development

<table>
<thead>
<tr>
<th>Plant Number (category)</th>
<th>Middle Layer</th>
<th>Tapetum</th>
<th>Pollen or Microspore Abortion</th>
<th>Exine</th>
<th>Orbicular Wall - Ubisch Bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oahe, 1, 3, &amp; 4 - 1974 and 1975</td>
<td>Normal-D</td>
<td>Normal- Degenerates during mid/late-vacuolate stage</td>
<td>Usually none - Occasional empty mid/late-vacuolate microspore</td>
<td>Normal-Tripartite structure</td>
<td>Normal-channeled, spinulate Ubisch bodies interconnected by reticulum</td>
</tr>
<tr>
<td>Oahe 2 - 1975 (1) (male fertile)</td>
<td>Normal-Disappears by engorged pollen stage</td>
<td>Normal- Abortion</td>
<td>Mid/late-vacuolate microspore stage</td>
<td>Broken, deformed, abnormal pores</td>
<td>Small malformed Ubisch bodies, few Ubisch bodies, heavy reticulum</td>
</tr>
<tr>
<td>Oahe 2 - 1974 (1) (partially male sterile)</td>
<td>Normal</td>
<td>Often begins degeneration early (at early-vacuolate microspore stage)</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>SB_2-2-22-68 or (4) (male sterile)</td>
<td>Vacuolate and enlarged</td>
<td>Degenerated and crushed, time of abortion unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Persist</td>
<td>Usually persists</td>
<td>Mid/late-vacuolate stage, many division errors; doublets</td>
<td>Deformed</td>
<td>Normal or deformed, often a thick solid reticulum</td>
<td></td>
</tr>
<tr>
<td>SB_3-2-17-20 or (4) (male sterile)</td>
<td>Vacuolate and enlarged</td>
<td>Occasionally enlarged</td>
<td>Degenerated and crushed; time of abortion unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Plant Number (category)</td>
<td>Middle Layer</td>
<td>Tapetum</td>
<td>Pollen or Microspore Abortion</td>
<td>Exine</td>
<td>Orbicular Wall -Ubbisch bodies</td>
</tr>
<tr>
<td>------------------------</td>
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</tr>
<tr>
<td>SB&lt;sub&gt;3&lt;/sub&gt;-1-9-79</td>
<td>Normal</td>
<td>Normal</td>
<td>Some abort following late-vacuolate stage</td>
<td>Normal, but some with mildly irregular nexine</td>
<td></td>
</tr>
<tr>
<td>(4) (male sterile)</td>
<td></td>
<td></td>
<td>Most appear as normal late-vacuolate microspores at dehiscence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB&lt;sub&gt;3&lt;/sub&gt;-2-22-48</td>
<td>Normal</td>
<td>Normal</td>
<td>Mid/late-vacuolate microspore stage or following engorgement</td>
<td>Normal or deformed</td>
<td>Normal or deformed</td>
</tr>
<tr>
<td>(4) (male sterile)</td>
<td>Vacuolate and enlarged</td>
<td>Degenerated and crushed</td>
<td>Degenerated and crushed; time of abortion unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>SB&lt;sub&gt;3&lt;/sub&gt;-2-17-83</td>
<td>Normal</td>
<td>Normal</td>
<td>Mid/late-vacuolate microspore stage</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>(3) (male sterile)</td>
<td></td>
<td></td>
<td></td>
<td>sometimes small exine channels</td>
<td></td>
</tr>
<tr>
<td>SB&lt;sub&gt;3&lt;/sub&gt;-2-23-39</td>
<td>Normal</td>
<td>Normal</td>
<td>Mid/late-vacuolate stage or following engorgement with starch</td>
<td>Often deformed</td>
<td>Normal</td>
</tr>
<tr>
<td>and SB&lt;sub&gt;3&lt;/sub&gt;-29-1-27 (5) (partially male sterile)</td>
<td></td>
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Discussion:

Overview

Male sterility expresses itself in a variety of ways (see Literature Review). Different cytoplasmic male sterile lines have exhibited varying cytological events as have different male sterile genes. If one divides the eighteen substitution backcross plants into eight lines (for example, \( SB_3 - 2-17-20 \), \( SB_3 - 2-17-68 \), and \( SB_3 - 2-17-83 \) would be considered one line, \( SB_3 - 2-22-48 \) and \( SB_3 - 2-22-68 \) another line), then the work on this thesis has also reported varying expressions of sterility between lines. In addition, there were differing expressions of sterility within lines.

The original research intention was to investigate the cytological events of male sterility in intermediate wheatgrass possibly uncovering a cytologically visible clue as to its cause. Coming succinctly to the point, no specific cytological features either within the tapetal or sporogenous cells were found immediately suggestive of causative factors for the male sterility observed in the investigated plants. Except for the irregularities reported in pollen and orbicular wall structure and the isolated cases of tapetal cell persistence (\( SB_3 - 2-17-20 \)) and early degeneration (Oahe 2, 1974) no other significant variations in either internal tapetal or microspore behavior or structure were noted for any of the fertiles, partially steriles, or steriles.
Category Specific Effects

The composition of the categories (Table III, p. 56) was designed to separate any effects from an irregular or unbalanced meiosis from effects due to the cytoplasmic male sterile factors alone. The similarity of some sterility types and tapetal behavior between categories and variations of types within categories, especially category 4, meiotically unstable and male sterile, precluded rigid partitioning of effects resulting from a cytoplasmic male sterility mechanism and those from an abnormal meiosis. However, some similar effects within categories were noted, although not inclusive of all the plants in the categories.

The three plants which exhibited pollen abortion following engorgement with starch were from meiotically unstable categories. One, SB3-2-22-48, was the most meiotically irregular in category 4 (male sterile), and SB3-2-23-39 and SB3-29-1-27 were the most meiotically irregular in category 5 (partially male sterile).

Exine malformations were found among the sterile grains of all categories. However, in the meiotically stable categories only rare instances were noted with the exception of Oahe 2 in 1974. Among many of the plants of categories 4 and 5 (meiotically unstable) pollen wall abnormalities were commonplace, although not in all plants in these categories. Again the most meiotically irregular plants, SB3-2-17-20,
SB₃-2-22-48, and SB₃-29-1-27 had the greatest number of exine malformations.

Three of the four plants of category 4, SB₃-2-22-68, SB₃-2-17-20, and SB₃-2-22-48, had occasional microsporangia in which one or more of the parietal layers (usually the middle layer) became enlarged and very vacuolate pressing the internal layers, including the sporogenous cells, to a thin mass in the center of the locule. This type of sterility was not observed in any other category. The time when the cells began to hypertrophy is unknown, for the earliest stage found with this type of sterility was the quartet stage. In all other instances the unaffected microsporangia in the anther were at the late-vacuolate microspore stage of development. Usually only one, but sometimes two, of the four microsporangia were affected.

Overman and Warmke (1972) reported a similar tapetal hypertrophy in sorghum following release of the microspores from the quartets. This was only one type of tapetal anomaly observed in the several lines they investigated. The formation of partial or total intratapetal syncytia was more common.

A tapetum becoming vacuolate and enlarged during meiosis, finally crushing the microspores while still in the quartets was observed in sweet pepper by Horner and Rogers (1974). In this material as well as in sorghum and intermediate wheatgrass, it was unknown whether the
microspores aborted before being crushed by the enlarged tapetum (or middle layer) or as a result of it.

Horner and Rogers (1974) felt that the other parietal layers were not affected by the abortion since secondary thickenings appeared later in the parietal layers. A similar but not all inclusive observation was made for intermediate wheatgrass. Secondary wall thickenings were observed between the endothecial cells in those microsporangia where the endothecium did not enlarge (Figs. 121, 139).

There appeared to be a definite association of anther length with meiotic instability and with percent sterility (Table V, p. 82). The plants with the shortest anthers belonged exclusively to meiotic unstable categories 4 and 5. In category 5 (partially sterile) the plant with the longest anthers, SB$^3$-2-16-42, had the least sterility. All other plants in this category had shorter anthers accompanying a much higher sterility. All plants male sterile and meiotically unstable (category 4) had short anthers. SB$^3$-2-17-83, although placed in a meiotically stable category was not entirely normal meiotically and had anthers of a length midway between that of the fertiles and the more meiotically unstable steriles and partially steriles.

Partitioning of differences was more difficult when spike lengths were compared. Many of the partially steriles and all but one of the male steriles of category 4 had spike lengths comparable to and in some cases greater than that of the Oahe types. The majority of
the shortest spikes were found among the partially steriles of category 5 (meiotically unstable) but not inclusive of all plants in the category. In general differences in spike length appeared to be quite variable among the backcross plants and consistent in the variety Oahe. Since the spikes were measured from the first node to the tip of the highest spikelet, differences in length could be attributed to differences in rachis length or to differences in spikelet length. From some random measurements not reported, it is probable that spikelet lengths would be considered variable, but not different enough to account for a difference of up to 10 centimeters in spike length between some plants. Actual differences in rachis length were then probably responsible for most of the large differences observed.

Differences in the number of spikelets per spike were also variable even among the Oahe types. The plants with the least numbers of spikelets were distributed in all categories except category 1 (Oahe types). As with spike length the differences in the number of spikelets could not be associated with any particular feature examined in this thesis, other than to say that overall the backcross plants had fewer spikelets than the variety Oahe,

Sterility in Oahe and High Temperatures

High temperatures have been implicated in the production of sterile pollen by Stephens (1937), Peterson (1958), and Meyer (1966).
Meyer (1966) described a positive correlation between the percentage of sterile anthers and the maximum temperature 15 to 16 days before anthesis. A critical period was also noted for relative humidity. The number of anthers which developed was correlated with the relative humidity 22 to 23 days before anthesis. Male sterility has also been reported to be correlated with cool temperatures (Barham and Munger, 1950; Lin and Peterson, 1975). In onions the critical period was found to be from the quartet stage to the first pollen mitosis (Barham and Munger, 1950).

Oahe 2 during 1974 had a high percentage of sterile microspores often with malformed, broken exines. Perhaps the high male sterility observed in 1974 for Oahe 2 was associated with the unusually high temperatures and low rainfall which occurred during June of that year (Table IX). The average high temperature for June of 1974 was 79°F compared to 70°F in June of 1975. The average daily temperature difference between the two years was lower but still significant (62.2°F vs 56.2°F).

Average daily and high temperatures in June of 1974 were also considerably higher than the long term averages over the years 1958-1972. The long term averages were closer to the averages for 1975. A similar situation was observed for precipitation in June. Averages for 1975 (2.72 in.) closely approximated the long term average (2.81), while 1974 was considerably drier than the long term. Taking only
Table IX. Climatic data at the Plant and Soil Science Field Research Station near Bozeman, Montana for three months in 1974 and 1975, and long term averages

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<tr>
<td><strong>1974</strong></td>
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<tr>
<td>June</td>
<td>62.2°F</td>
<td>79.0°F</td>
<td>45.3°F</td>
<td>.43 in.</td>
<td>85.7°F</td>
<td>.09 in.</td>
</tr>
<tr>
<td>July</td>
<td>66.8</td>
<td>84.7</td>
<td>48.9</td>
<td>1.13</td>
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<tr>
<td>August</td>
<td>61.1</td>
<td>75.8</td>
<td>46.3</td>
<td>2.20</td>
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<td><strong>1975</strong></td>
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<tr>
<td>June</td>
<td>56.2</td>
<td>70.0</td>
<td>42.4</td>
<td>2.72</td>
<td>73.1</td>
<td>.97</td>
</tr>
<tr>
<td>July</td>
<td>67.5</td>
<td>82.8</td>
<td>52.2</td>
<td>2.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>60.1</td>
<td>75.0</td>
<td>45.0</td>
<td>1.56</td>
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<tr>
<td><strong>Avg.</strong></td>
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<td><strong>1958-1972</strong></td>
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<td></td>
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<tr>
<td>June</td>
<td>58.1</td>
<td>72.3</td>
<td>43.9</td>
<td>2.81</td>
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<tr>
<td>July</td>
<td>64.6</td>
<td>81.7</td>
<td>47.6</td>
<td>1.33</td>
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<td></td>
</tr>
<tr>
<td>August</td>
<td>64.2</td>
<td>81.2</td>
<td>47.0</td>
<td>1.25</td>
<td></td>
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</tr>
</tbody>
</table>

* From Climatological Data (Montana), U.S. Department of Commerce, Vol. 77-78
+ Caprio, 1972, unpublished data
the last nine days of June during which microsporogenesis up to the late-vacuolate stage principally occurred, the difference between the average high temperatures of 1974 versus 1975 was very large (85.7°F vs 73.1°F). This coupled with the low precipitation during this period (.09 inches vs .97 inches in 1975) might well explain the wall formations and sterility observed for this plant in 1974.

Sterility has been known to be associated with meiotic disturbances (Novak and Betlach, 1970; Jain, 1960) and meiotic disturbances with high temperatures (Jain, 1960; Kaul, 1970). Oahe 2 was not investigated meiotically so further associations could not be made between high temperatures during critical periods, meiotic abnormalities, and subsequent sterility.

**Exine Malformations and Meiotic Disturbances**

The fact that the sterile microspores of Oahe 2 in 1974 more often than not had exine malformations with a high probability of meiotic and spindle disturbances, plus the fact that many of the SB₃ plants especially those meiotically irregular also had wall abnormalities suggests a possible mechanism for exine formation. Could microtubules be involved in the establishment of a pattern for exine formation? Colchicine, widely known as a spindle inactivator, was shown by Heslop-Harrison (1971) and Dover (1972) to affect not only the division of the meiocytes but also exine patterning and the placement
of pores. Meiocytes prevented from dividing sometimes developed a patterned exine but more often exhibited a mispatterning of the exine to a greater or lesser degree. The sensitive period to the drug was early prophase for cleavage errors and diakinesis to metaphase II for exine patterning errors.

Thus, the information for exine formation appears to be transcribed early and may be independent of the spore nucleus. This is further supported by the presence of a patterned exine around tetra-karyotic monads, anucleate spores (Heslop-Harrison, 1971) and cells with reduced chromosome complements (Rogers and Harris, 1969). Rogers and Harris also observed that in micrograins produced from meiotically irregular triploid Linum plants, the thickness of the exine was directly related to the amount of cytoplasm received in the deficient micrograins.

In the intermediate wheatgrass material, exine anomalies were also seen in all grains exhibiting division errors whether monads with two cells or doublets joined by common walls. The degree of exine malformation varied but was inevitably present. Only a few instances of micrograins were observed. These were always seen joined to a larger grain and observed only following degeneration and disappearance of the cytoplasm. Therefore, the presence of nuclei in the micrograins could not be ascertained or discounted, consequently neither could the development of the exine of the micrograin without a
nucleus be ascertained. Pores when present were usually ill-defined similar to that reported by Heslop-Harrison (1971) in which spindle formation was disturbed by colchicine. Multiporate grains were not seen in male sterile intermediate wheatgrass. However, pore number and structure were not intentionally investigated, so the presence of such grains cannot be ruled out.

Bleier (1930) and Bally (1919) cited by Schulz-Schaeffer et al. (1971) reported that in hybrid species the spindles of both parents retain their individuality and activity. In addition to many of the same types of misdivisions as in the intermediate wheatgrass material, Poddubnaya-Arnol' di (1960) observed multiporate grains in similar wheat-Agropyron amphidiploids possibly indicating the activation of both parental spindles. This and the work of Dover (1972) in which the number of pores was thought to depend on the number of spindle pole determinants supports the activation of both parental spindles.

I find it difficult to envision the direct involvement of the spindle apparatus in exine formation. So it is also difficult to view exine malformations as a consequence of conflicting spindle operations from the two parental types.

The involvement of colchicine in pattern abnormalities might suggest the involvement of microtubules other than the spindle in exine formation and patterning. But as yet only Echlin and Godwin (1968b) have reported an association of microtubules with the early
elements of the pollen exine. Groups of microtubules have been found to be associated with the growth of another component of the pollen wall, the intine (Heslop-Harrison, 1968), although the number of tubules seen during rapid growth phase of the intine was relatively few. Microtubules have been thought to have a role in microfibril orientation (Hepler and Newcomb, 1964) and colchicine which inhibits microtubules also has been found to randomize microfibril orientation (Pickett-Heaps, 1966). This, plus the following is nonexperimental argument for a possible role of microtubules in exine development: the primexine does have a cellulosic component (Heslop-Harrison, 1968) suggestive of a microfibrillar organization; the first bacular elements form in the primexine; and in some species the exine elements form around a patterned extracellular plasmalemma glycocalyx.

Microtubules involved in exine formation remains highly speculative and there always remains the possibility that colchicine affects some other pattern determining component as yet unidentified.

Why then would there be an apparent association of meiotic irregularities with exine abnormalities in intermediate wheatgrass? Perhaps it is just that, an association. The two anomalies may be two effects of the same primary causal mechanism. Perhaps microtubules for exine development and for spindle formation originate from the same misprogrammed precursor pool. Remembering that this is hybrid material and still contains a few durum chromosomes, genome imbalance
and conflict of information might be one possible explanation for both effects. Since we feel that cytoplasmic factors are operating in some manner in the sterile material, the effects of the as yet unidentified cytoplasmic sterility factor might also be responsible for the production of one or both effects. Knowing the meiotic behavior of Oahe 2 in 1974 would have helped associate or disassociate spindle disturbances with exine and pore abnormalities.

To compound the problem with further variation, those plants which produced sterile microspores with malformed exines, such as SB₃-2-22-48, did not always do so exclusive of abortive pollen with normal walls. Some anthers of male sterile plants produced pollen which apparently developed normally but aborted following engorgement with starch. Some male steriles (SB₃-1-9-79 and SB₃-2-22-48) also occasionally produced and released perfectly normal appearing pollen.

Whether the fertile-appearing pollen grains of the partially steriles and occasionally present in some of the steriles as ascertained by acetocarmine and IKI are actually viable and capable of fertilization remains unknown. Only test crossing will determine if they are indeed fertile or if they are evidencing a different sterility mechanism which is cytologically invisible.

In general, malformed exines were always associated with sterile microspores. Only in one anther was a mature pollen grain observed with both normal cytoplasmic constituents and a malformed
exine. However, the pollen from this plant, SB$_3$-2-22-48, was also known to abort in some years following engorgement with starch. All other microspores with malformed exines were empty, aborting very early.

Cytoplasmic Male Sterility - Pros and Cons

In the wheat-Agropyron hybrids investigated by Lyubimova (1960) different spikes had varying meiotic configurations as well as differing fertilities. Differing fertilities would also seem to be the case in some intermediate wheatgrass plants. It would indeed be interesting if differing spikes had differing meiotic behaviors which could be correlated with sterility in this material. If so, this would be a case against cytoplasmic male sterility operating in intermediate wheatgrass, and a case for meiotic irregularities as a principal cause of the sterility, but the evidence is against differing meiotic behaviors. The consistency of sterility types found in some of the male sterile plants (SB$_3$-2-17-20, SB$_3$-2-22-68, and SB$_3$-1-9-79) would tend to discount the idea of deficient gametes from an irregular meiosis as responsible for all the sterility observed.

Nor would environment alone acting on the meiosis necessarily be responsible for all the sterility, although it is known that environment does influence meiotic behavior in some hybrids. Lyubimova (1960) demonstrated that wheat-Agropyron hybrids not thought of as
male sterile are more sensitive meiotically to environmental factors, especially temperature and humidity, than the parental forms. In F₁'s from T. vulgare X A. elongatum where the chromosome number is 70 (21 T.v. plus 35 A.e.) 24 to 28 bivalents were found and up to 70% viable pollen grains developed in those anthers going through meiosis at 20°C. At a higher temperature (35 to 37°C) the number of bivalents dropped to 12 to 20 and many more meiotic irregularities in the manner of laggards, fragments, chromatid bridges, and pairings of five or six instead of bivalents produced a higher percentage of sterile pollen. The presence of a somewhat environmentally sensitive cytoplasmic factor or more likely a cytoplasmic-nuclear interaction is seems equally a possibility especially in those plants which have consistent sterility types from year to year. Meiotic determinations were made for only one year in intermediate wheatgrass. Therefore, the years 1974 and 1975 with wide temperature and precipitation differences could not be compared validly for any meiotic differences.

Maintenance of pollen sterility over generations as has been seen in much of the intermediate wheatgrass material is one criterion for cytoplasmic male sterility. However, even some established CMS lines are environmentally sensitive, varying from totally sterile to totally fertile dependent on environmental conditions (Duvick, 1966). One genotype-cytoplasmic combination also may differ from another in environmental sensitivity. That some of the male steriles in
intermediate wheatgrass had a few fertile-appearing grains is certainly not evidence against CMS, but lies in the realm of variation reported for other CMS species.

Lack of a correlation between male sterility and female sterility is also another case for cytoplasmic male sterility. Such is the case for at least one plant. SB\textsubscript{3}-1-9-79 produced about 38 seeds per spike. This value is comparable to the 46.1 seeds per spike produced by the variety Oahe in Bozeman, Montana although it falls short of the 60.8 seeds per spike reported when released as a variety. It is acknowledged, however, that Oahe will not produce 60.8 seeds per spike in all years and in all environments.

Seed set was generally low for all plants including the partially steriles when determined in 1974. Only one plant, SB\textsubscript{3}-2-16-42 had a reasonably high seed set (39.9). The plants from which seed set was determined were located in the SB\textsubscript{3} nursery which was in its fourth year of stand. In addition to the plants being crowded, and June and July being hot and dry, it was noticed that anthesis in the majority of the Oahe pollinator rows preceded that of the SB\textsubscript{3} plants. A combination of all three factors might well be responsible for the overall low seed set especially in view of the fact that the spikes (except for SB\textsubscript{3}-2-16-42) were much shorter than normal in this nursery (Table VI, p. 85). The low seed set probably is not an accurate
representation of the actual potential of the plants under more suitable conditions.

In the entire SB$_3$ generation the numbers of bivalents and univalents agree fairly well with those predicted from the model for the backcross program (Young and Schulz-Schaeffer, 1974). An average of 21 bivalents and 3.5 durum univalents was predicted compared to the 20.2 bivalents and 2.4 univalents actually found. The average number of univalents in the SB$_4$ generation was 0.9, about one-half the predicted average of 1.75 for that generation (Schulz-Schaeffer, 1974). Stabilization of meiosis appears to be progressing at a more rapid rate than expected.

The fact that many lines have maintained nearly 100% male sterility over many backcross generations with increasing meiotic stability, and seed set has steadily increased with each backcross (Schulz-Schaeffer, 1973) is further evidence for cytoplasmic male sterility operating in this material. In the relatively few plants investigated for this thesis, however, specific conclusions about causal mechanisms for the sterility remain elusive. The single plant in category 3, male sterile and relatively meiotically stable, does not offer enough information to differentiate further effects of irregular meioses from nuclear-cytoplasmic interactions alone, other than the apparent association of wall abnormalities with grossly abnormal meioses. SB$_3$-2-17-83 lacked exine abnormalities. This plant
also occasionally produced fertile appearing pollen grains and many anthers were deformed and papery. Such variations within a plant do not facilitate the drawing of conclusions as to the cause of the sterility and in addition make the plant a poor candidate for a breeding program in which consistency from year to year is desired.

A quandary remains as to what sterility mechanisms are operating in intermediate wheatgrass although the discussed patterns suggest cytoplasmic male sterility as defined (see Introduction) as the most likely explanation. Meiotic determinations in further backcross generations will clarify the relationship between certain meiotic states and male and/or female sterility if any correlation does exist. The presence or absence of one or more specific chromosomes even in meiotically stable plants might contribute to the sterility or fertility observed. Along this line, Fukasawa (1953), working with male sterility in *Aegilotricum* found that after one or two backcrosses with *durum* pollen, some plants having one to three extra *ovata* chromosomes as univalents in addition to fourteen *durum* bivalents had 88-92% fertile pollen. Other meiotically normal 28 chromosome sibs were completely male sterile.

**Variation in % Male Sterility - Artificial**

SB₃-1-8-89 on the basis of sterility counts previous to 1974 was initially placed in category 3 (male sterile). However, when male
sterility was determined in 1975, it was found to be only partially sterile (12.2%). This might have not been due to an actual wide variation in sterility from year to year but to an error in counting sterile grains. For the sterility determinations if the anther is sampled too early at the late vacuolate microspore stage, a large vacuole will occupy most of the microspore, and all of the grains will appear small, empty, and sterile when stained with IKI or acetocarmine. With acetocarmine one might often miss the single peripheral nucleus and small amount of cytoplasm which would identify it as a late vacuolate microspore and not necessarily a sterile grain. Such might have been the case with SB$_3^{-1}$-8-89.

**Transplantation Effect**

At the beginning of the cytological study there was concern over a possible transplantation effect, since data was sampled the year of transplantation. For example, SB$_3^{-1}$-8-89, SB$_3^{-2}$-16-88 and for most of the summer SB$_3^{-2}$-17-83 failed to produce inflorescences in the transplant nursery in 1974. SB$_3^{-2}$-17-83 eventually produced only two spikes very late in the season. This was first thought to be a result of the transplantation until it was noted that the same plants failed to produce inflorescences in the original SB$_3$ nursery. The same could be said regarding the male sterility first observed for Oahe 2 in the transplant nursery in 1974. It also had a high sterility in its
originally established location. The observed effects must then be due to some other environmental sensitive factor in the plants affected. In spite of the transplants being generally shorter than the plants in the original locations, probably a transplant effect, transplant shock was not thought to play an important role regarding the differences observed in microsporogenesis.

Lack of Stomium Formation in SB₃-2-17-20

The anthers of only one plant, SB₃-2-17-20, failed to form a stomium at anthesis. The middle layer and the tapetum persisted in this plant, failing to disappear by anther maturity (Fig. 6). In some cases the tapetal cytoplasm disappeared, but the orbicular wall still remained highly crenulated and outlined each previously existing tapetal cell. Normally at anther maturity the tapetal orbicular wall would lie flat against the endothecial inner tangential wall.

The secondary thickenings of the endothecial cells are believed to exert tension on the anther wall as the anther expands approaching maturity. The rapid dehydration of the anther at anthesis plus the hygroscopic nature of the endothecium increases this force until the wall splits in the area of fewest cell layers and least resistance (Figs. 5,6) (Vasil, 1967). Secondary thickenings do form in the endothecial cells of SB₃-2-17-20. However, the highly crenulate orbicular wall at maturity (Figs. 98, 99) suggests that the anther had failed to
expand to the extent normally seen at this stage (pulling out the creases in the orbicular wall, so to speak). This may reduce the tension on the anther wall, and coupled with the persistence of the middle and tapetal layers in the region of the stomium might offer enough resistance to stomium formation even if the anther dehydrates normally at anthesis.

Only in one such anther was there a visible degeneration of vascular tissue. So although vascular disturbances cannot be ruled out as a cause for the lack of stomium formation, they are not visibly evident with any consistency. It can be said at this point that vascular abnormalities were not observed in any other plant, and so also seems unlikely as a reason for any of the other types of sterility observed, where microspores are formed.

**Tapetal Abnormalities**

Surprisingly few tapetal abnormalities were seen in the male sterile and partially sterile plants investigated. The persistence of the tapetal cytoplasm and failure of the radial walls to break down by anther maturity in SB\textsubscript{3}-2-17-20 was atypical and the only instance observed. The unusual presence of an orbicular wall completely surrounding each cell preventing contact between adjacent plasmalemmas might be associated with the failure of the plasmalemmas to rupture.
Although the tapetal cytoplasm often persists, it does so in degenerated form. Senescence appeared to begin and proceed as normally expected. The failure to disappear seems to be cytologically unrelated to the sterility seen in SB₃-2-17-20 for the microspore abnormalities are seen prior to the time after which the tapetum may persist. A persistence of the tapetum in a nondegenerative form might be considered more significant, but this was not seen. A nondegenerative tapetum might be indicative of a generalized failure of tapetal activity possibly preventing the release or transport of nutrients to the developing microspore.

An early degeneration of the tapetum was seen in one anther of Oahe 2 in 1974 but not in any other anthers of this plant. Since this plant produced both fertile and sterile pollen, and the sterility when present was not evident until the mid/late-vacuolate microspore stage, it was impossible to associate an early degeneration of the tapetum in the early vacuolate microspore stage with the anther eventually producing sterile pollen. At the time malformed sterile microspore could be identified, the degeneration of the tapetum appeared normal for that stage. Early tapetal degeneration is certainly significant in that the proper nutrients might then not reach the microspore. Almost all previous publications (Hoefert, 1971; Overman and Warmke, 1972; Christensen and Horner, 1974) have expressed the belief that the tapetum supplies something essential to the normal development of the
microspores. Where early tapetal degeneration has been observed, it was always associated with sterile pollen.

Other than this one instance, the tapetum in all other plants demonstrated no unusual behavior outside the realm of normal variation which might be considered significant relative to the production of sterility. The before mentioned unusually dark staining and vacuolated tapetum of Figure 27 at the second meiotic division was from the partially sterile plant SB\textsubscript{3}-2-16-42. This may well represent a fixation artifact for the plant was very fertile, and ultrastructurally the cells were not unusual and the vacuoles not that prominent. Some vacuolization is quite often seen at various stages in all plants. Fixation and/or processing may just have exaggerated a normal variation.

That tapetal differences need not accompany cytoplasmic male sterility, as was the case for all but one of the CMS intermediate wheatgrass plants investigated, was also shown by DeVries and Ie (1970) for CMS wheat. Using CMS wheat derived from the third backcross generation of CMS Bison (timopheevi cytoplasm) with a mixture of Opal, Gaby, and Orca summer wheats, they found no ultrastructural difference between the tapetal behavior in fertile wheat and that in CMS wheat. Although they did not describe a complete continuum of developmental stages, from their description and photos tapetal behavior also appeared quite similar to that seen in intermediate wheatgrass.
Tapetal Orbicular Wall and Exine Formation

The only other tapetal irregularities observed were orbicular wall malformations. These were seen in only two plants, Oahe 2 and \( \text{SB}_3\)-2-17-20. The Ubisch body malformations due to an irregular deposition of sporopollenin, as well as the relatively few Ubisch bodies present in the tapetal orbicular wall are consistent with an early degeneration and malfunctioning of the tapetum in Oahe 2. This lends support to the belief that the tapetum produces sporopollenin precursors for at least its own wall formation. However, that the exine also is malformed neither supports or discounts the possibility that the tapetum also supplies the sporopollenin precursors for exine formation.

Material resembling sporopollenin in its polymerized form was never seen in the tapetal or spore cytoplasms during the period of exine and Ubisch body formation. The darkly staining bodies present in the tapetum during all stages but especially following the early microspore stage (Fig. 69) remain unidentified; but it is possible that they may represent degenerating organelles. There was no indication that they may be a secretory product eventually released to the surface. They may be the same bodies seen by Risueno et al (1968) associated with the endoplasmic reticulum in Allium, although no such association could be ascertained in intermediate wheatgrass. Certainly they were not released to the surface as seen in Allium.
The small osmophilic granules between the callose wall and the tapetal cell wall during meiosis and the similar sized granules which appeared in the tapetal primary wall at the quartet stage remain unidentified. Numerous granules were not seen in the tapetal wall until the quartet stage in all plants except SB3-29-1-27. In this plant granules were seen consistently in the wall rather than just between the tapetal wall and the callose wall throughout meiosis. Whether these grains represent sporopollenin is also unknown.

A granular material was seen clustered around both the bacula of the just released microspores and the early spinules of the Ubisch bodies (Figs. 45, 57). This might quite possible be sporopollenin precursors polymerizing on the exine and orbicle templates. The idea proposed by Rowley and Skvarla (1975) of a glycocalyx template for exine formation in Canna is extremely significant and is worthy of further investigation in grasses where the exine is less elaborate. The existence of a visible template would lend further support to an exine pattern being established by the meioocyte without direct involvement of the tapetum. It would also support the opinion expressed in this thesis that wall malformations are not the result of a malfunctioning tapetum, and that the template for exine structure is laid down while the microspores are encased in the callose of the quartet. Certainly with the present knowledge it is still conceivable for the
tapetum to secrete and supply to both itself and the microspore the
primary structural material of the exine and orbicular wall, sporo-
pollenin.

The similarity between the abnormal orbicular wall and abnormal
exines in some anthers of male sterile SB₂₃-2-17-20 (Fig. 113) suggest
a latent capacity of both cell types to form the same wall structure.
The tapetal orbicular wall is seen to have a thick exine-like appear­
ance with few Ubisch bodies, and one microspore wall is composed of
individual Ubisch body-like components. Both cell types differentiate
from the same hypodermal tissue in the early microsporangium, so it is
possible that such a capacity for structural similarity might exist in
abnormal material, especially if information responsible for exine
formation is proved to be transcribed early in meiosis. The infor­
mation for not only orbicular wall formation but also exine formation
would then come from diploid tissue.

In intermediate wheatgrass no specific inclusions were found
associated with the developing exine such as the fragments of endo-
plasmic reticulum found perpendicular to the plasmalemma at the base
of the bacula by Skvarla and Larson (1966) in Zea mays. A lamellar
composition to the nexine as noted in Anthurium (Rowley and South­
worth, 1967) was seen neither in Z. mays or sorghum (Christensen et
al, 1972). Neither was there any indication of lamella in the early
exine stages of intermediate wheatgrass. The fragments which appear
to be associated with the nexine in a mid-vacuolate microspore of SB3-1-9-79 (Fig. 141) might be interpreted as indicating a lamellar composition to the nexine. That they might be only fragments of the plasmalemma seems unlikely since they are between the endexine and the nexine. The endexine is always external to the plasmalemma. A complete continuum of stages in nexine formation was not sampled so a lamellar composition of the nexine could not be confirmed or discounted in intermediate wheatgrass. In sorghum (Christensen et al., 1972) in which more intermediate stages were described, three to five layers were associated with the primexine. The basal layer was felt to develop into the nexine with the other layers serving an important but undetermined role in exine patterning. However, lamellar tapes of unit membrane dimension as described by Sengupta and Rowley (1974) were not found to be part of nexine formation. The two peripheral darker layers of the primexine seen in intermediate wheatgrass during bacula development (Fig. 45) appear to form elements of the nexine and tectum. Additional layers as described for sorghum were not observed. However, after the tripartite structure of the exine was delineated, a thin line of material was often seen in the cavea (Fig. 61, p. 114). This often persisted until pollen maturity and may represent remnants of the primexine.
Transfer of Nutrients from Tapetum to Microspore

No indications of a transfer of nutrients from the tapetum to the microspore were evident from the work on intermediate wheatgrass except that as mentioned before whenever tapetal abnormalities were seen, the anther produced sterile pollen. However, the reverse is not true. Anthers producing sterile pollen did not always have a visibly abnormal tapetum.

Christensen and Horner (1974) observed a fibrillar material between the tapetal orbicular wall and the exine of sorghum and a similar substance in the cavea of the exine. The authors thought this might possibly represent material in transit from the tapetum to the pollen. They report the fibrillar material being present only during the vacuolate to engorging pollen stage. However, the photographs also suggest a later stage in which the tapetal cytoplasm has degenerated and disappeared. Therefore, it seems improbable that the fibrillar material would represent nutrient material originating from the tapetum at such a late stage.

An active role of the exine channels and intine channels in the transport of material across the pollen wall was also plausible in the view of Christensen and Horner. A denser material seen in the endexine at the openings of the nexine channels as possible nutrient material passing into the pollen was offered as tentative support for this contention. I too feel that the channels may be a mechanism for
the transport of materials across the pollen wall, especially in the light of the observations on wall tubules by Rowley et al. (1970) and Flynn (1971) (see Literature Review).

Malformed Exines and Sterility

If exine channels are indeed a route for the transfer of nutrient materials to the developing microspore, then the apparent association of gross wall malformations with sterility might be revealed as an actual cause and effect relationship. Abnormal channel systems which fail to form a continuous route through the exine or fail to regulate properly the amount of nutrients entering the microspore would be possible causal factors for the sterility observed, if such speculation regarding exine channels is valid. This would also be compatible with the general lack of tapetal abnormalities seen in male sterile intermediate wheatgrass.

Christensen and Horner speculate that the majority of the tapetal nutrients may enter through the pore for they found the initial accumulation of starch to occur in the pore region which was appressed to the tapetal orbicular wall. An occluded (Fig. 105) or otherwise malformed pore (Fig. 87) might be implicated in pollen sterility for the same argument as above.
Similarities and Differences with Other Grass Species

Microsporogenesis and tapetum development in fertile intermediate wheatgrass appear to be very similar to that described for *Z. mays* (Skvarla and Larson, 1966), wheat (DeVries and Ie, 1970), and sorghum (Christensen et al, 1972; Christensen and Horner, 1974). Some differences were noted, however. The plasmodesmata seen in sorghum connecting the tapetum and PMC's as late as prophase I were not seen in intermediate wheatgrass. No connections were seen between tapetal and sporogenous cells in intermediate wheatgrass after meiosis had begun. Actually no connections were observed past the archesporial stage, but since few premeiotic stages were sampled, the actual time physical connections between the two cell types were severed is unknown.

The vesicles and tubules seen in *Z. mays* between the PMC plasma-lemma and cell wall during callose deposition were not observed in intermediate wheatgrass. The callose, however, appeared to be deposited in layers as was the case for intermediate wheatgrass (Fig. 75). Such a nonhomogenous callose wall was not found in sorghum.

The mature exine of intermediate wheatgrass is nearly identical to that of sorghum and maize. In wheat (DeVries and Ie, 1970) the tectum appears thinner and the cavea larger than that of the other grasses investigated to date.

Judging from the thickness of the exine and presence of early Ubisch bodies on the tapetum, the sterile microspores of CMS wheat
(DeVries and Ie, 1970) appeared to abort during the early/mid-vacuolate microspore stage, although the time of abortion was not mentioned by the authors. The microspores were lobed, partially collapsed, and had a cytoplasm described as degenerated. This is similar to many of the sterile microspores in CMS intermediate wheatgrass. Most aborted after the exine had been well-defined and they were often partially collapsed with little or no cytoplasm. However, exine abnormalities were not observed by DeVries and Ie in CMS wheat. The finding in intermediate wheatgrass also concur in many respects with the review of CMS species by Laser and Lersten (1972). They found that a very high percentage of CMS species have microspores which abort between the quartet stage and the first pollen mitosis, and that the majority of these species were monocots.

Dissimilar Fertile Pollen Types

The majority of the fertile and partially sterile plants had engorged vegetative cytoplasms very similar to that described for wheat (DeVries and Ie, 1970). This appearance is shown in Figure 82. Six other plants, including two of the four Oahe types, had numerous unusual inclusions in the mature vegetative cytoplasm. Three plants, Oahe 3, SB3-24-2-76, and SB3-2-16-50, had spherical inclusions about 0.3 μm in diameter which were bounded apparently by one and perhaps two membranes (Fig. 83). The membranes were coated with an electron
dense substance. These were also found in the fertile-appearing pollen of highly male sterile SB\textsubscript{3}-2-22-48 (Figs. 132-133). The internal structure of the unidentified coated bodies was homogenous and granular. These unidentified inclusions were smaller than mitochondria and were not associated with any particular feature of the cell, but were rather uniformly dispersed throughout the cytoplasm. Membrane bound coated vesicles, up to .06 \textmu m in diameter, apparently arising from dictyosomes, were seen contacting the plasmalemma during intine formation in Lilium (Heslop-Harrison, 1968). These were found in relatively small numbers and were dispersed around the periphery of the cell. An active role in intine formation was theorized for these vesicles. The coated vesicles in intermediate wheatgrass were larger and only observed after the intine had fully formed and the pollen had engorged with starch. Their origin is unknown although dictyosomes are numerous in the cytoplasm.

A larger type of unidentified inclusion was seen in mature pollen of Oahe 4, SB\textsubscript{3}-24-2-55, and SB\textsubscript{3}-19-79-31-14. These appeared as vacuoles containing a more diffuse material than the coated bodies (Fig. 84). It was difficult to determine conclusively that they were membrane bound. However, from fragments of membranes partially surrounding some of the vacuoles, it is probable that they are. As is probably the case with the coated bodies, two membranes may be involved. An electron dense coating of the "membranes" was not
evident around the unidentified vacuoles. The size varied, but they were generally as large or larger than mitochondria. As were the coated bodies the vacuoles were extremely numerous, but again their origin is unknown.

Both unidentified inclusion types were seen in all fertile and partially sterile categories as was the more common type of mature pollen lacking such inclusions.

Although all three types were said to be found in "mature" pollen, the word mature is only relative and was used in reference to pollen following engorgement with starch. There is no assurance that all the pollen was sampled at exactly the same stage and changes following engorgement, possibly having to do with the production of the unidentified inclusions, could have been represented in the pollen sampled. Perhaps both the coated bodies and the vacuoles represent the production of enzymes required for germination with the coated bodies representing just an earlier stage in the development of the larger vacuoles. That they may indeed be mitochondria cannot be discounted. In early germinating seeds, spherical bodies bounded by a double membrane and lacking cristae were seen similar to mitochondria in quiescent cells (Brown, 1972). Mitochondria are known to undergo conformational changes in different metabolic states (Tandler and Hoppel, 1972). In any case it is probable that the inclusions represent real cellular features and are not just fixation artifacts.
Further investigation with closely spaced developmental stages is needed in order to determine the origin and actual structure of the inclusions.

**Sperm Cells, Plastids, and CMS**

Sperm cells from two plants contained organelles which were tentatively identified as plastids. That they are indeed plastids is consistent with a statement given by Larson (1963). "In general, generative cell plastids are difficult to identify because of their smaller dimensions, paucity of internal membranes, and because they do not contain starch . . . mitochondria in generative cells are smaller and have fewer cristae." This description fits the situation observed in intermediate wheatgrass rather well (Fig. 85).

Still, I am hesitant to say conclusively that what were observed were plastids, especially since generative cell plastids are infrequently reported (Larson, 1963; Sanger and Jackson, 1971).

The possible role of plastids in cytoplasmic male sterility can not be discounted. Leaf variation in *Mirabilis jalapa* L. is transmitted only through the egg and the generative cells in this species contain very few or no plastids (Lombardo and Gerola, 1968). In *Pelargonium zonale* Ait. the generative cell contains many plastids and the variegation is transmitted through the pollen.
Even when the "plastids" were observed, they were present in very low numbers. If this generalized lack of plastids could be extended to intermediate wheatgrass as a species, then it is possible that the information for male sterility resides in the plastom of the egg cell originally acquired from T. durum. However, sperm cells from many more plants would need to be observed for the presence or absence of plastids.
SUMMARY AND CONCLUSIONS

Anther and pollen development in male fertile intermediate wheatgrass and cytoplasmic male sterile plants derived from wheat X wheatgrass hybrids (the third substitution backcross to the amphidiploid Tricitum durum X Agropyron intermedium) was examined at the light and ultrastructural levels. The backcross material was divided into four categories on the basis of percent male sterility (partial or total) and meiotic normality and stability. The variety 'Oahe' intermediate wheatgrass was used as control for the investigation.

No specific cytological features were observed in any category which would conclusively indicate the cause of the male sterility in this material. Pollen, tapetum, and anther development in fertile plants was similar in most respects to that described for other grasses.

A variety of sterility types was observed in the male sterile and partially male sterile plants, and many were environmentally sensitive. Two partially sterile plants, SB3-1-8-89 and SB3-2-16-88, and to some extent SB3-2-17-83, failed to produce inforescences during 1974. The fact that late spring and early summer of that year were unusually hot and dry might have been responsible for this behavior. The same factors were probably causative of the high male sterility observed for Oahe 2 in that year. Partially sterile SB3-2-16-2 produced normal anthers in 1974, but all anthers were "papery" and
deformed in 1975 producing no pollen. A substantial proportion of the anthers from presumably later spikes of highly male sterile SB₃-2-17-83 in 1975 were also papery. A limited amount of pistillody was seen in male sterile SB₃-2-22-48 in 1975.

Other plants, especially those classified male sterile and meiotically irregular (category 4), were fairly consistent in percentage and type of sterility from year to year. SB₃-2-22-68 consistently produced empty collapsed microspores, while empty or partially empty microspores joined by common walls or surrounded by common exines were characteristic of SB₃-2-17-20 in both years investigated. SB₃-2-22-48, however, was variable in that in 1974 the pollen aborted following engorgement with starch while the next year abortion occurred before engorgement. Most of the pollen at anthesis in SB₃-1-9-79 appeared as normal late-vacuolate microspores. Whether microspore development was delayed and consequently interrupted by anthesis, or whether the microspores would abort following the late-vacuolate stage in any case is unknown.

SB₃-1-9-79, SB₃-2-17-83, and SB₃-2-22-48 produced a small number of fertile-appearing pollen grains. Anthers of SB₃-2-17-83 producing fertile grains tended to produce all fertile grains while sterile anthers contained only sterile pollen. This was also observed in anthers of partially sterile SB₃-29-1-27. It was unknown whether this all or nothing behavior could be extended to different spikelets or
spikes. Since $SB_3^{-29-1-27}$ is meiotically very irregular, this phenomenon would argue against the possibility that meiotic deficiencies are involved in the production of the sterility.

Three of the male sterile, meiotically irregular plants of category 4, $SB_3^{-2-17-20}$, $SB_3^{-2-22-48}$, and $SB_3^{-2-22-68}$ occasionally had one or more microsporangia in which the middle layer hypertrophied and all inward layers degenerated. Less frequently some of the endothelial and tapetal cells were seen to be also enlarged. A few trilocular anthers were seen in male sterile $SB_3^{-2-22-68}$.

Exine malformations were almost exclusively found in meiotically irregular categories (4 and 5), although not all plants in these categories exhibited such deformities. The most meiotically irregular plants expressed the greatest number of wall deformities. It is unknown whether exine malformations result from nuclear imbalance due to some durum chromosomes being present in addition to those of $A. intermedium$ or as a result of some other factor, perhaps cytoplasmic. However, in view of the work regarding exine and pore disturbances with the application of colchicine and the production of normal exines in chromosome deficient micrograins, it is felt that exine formation is probably under sporophytic control and exine malformations as seen in this study do not result from deficient gametes as a consequence of an irregular meiosis. In view of the consequences of colchicine treatment
the possible role of microtubules in exine formation should be explored.

Oahe 2 was highly sterile and had numerous exine and pore deformities in 1974. That the weather was excessively hot and dry during the time Oahe 2 was undergoing meiosis was believed to be the major factor responsible for the high percentage of sterility observed. If the meiotic behavior of Oahe 2 during 1974 were known, it might illuminate an association of spindle (microtubule) disturbances with exine deformities and sterility.

It is possible that the exine template is laid down while the microspores are still encased in the quartet. The primexine and bacula appear during the quartet stage and sporopollenin apparently is deposited on these early exine elements only after release from the quartets (Fig. 45). Here again, further work is needed regarding the presence of a glycocalyx in the primexine as a template for the control of sporopollenin deposition in grasses.

That deformed exines interfere with the normal transport of nutrients from the tapetum is a possibility in view of the consistent association of deformed exines with sterile microspores. This is also consistent with the fact that tapetal disturbances were generally not observed in the sterile anthers. The inability of the microspore to absorb and utilize nutrients produced by a normal tapetum could be a possibility in this regard. However, there was no visible evidence
gained in this study to support a transfer of material from the tape-
tum to the microspores.

Two plants (male sterile SB$_3^{-2}$-17-20 and partially male sterile
Oahe 2) exhibited tapetal orbicular wall and Ubisch body malformations.
This could not be conclusively associated with any cytoplasmic irreg­
ularity. However, one instance of an early degeneration of the tape­
tal cytoplasm in Oahe 2 was noted, and the cytoplasm in SB$_3^{-2}$-17-20
tended to persist in a degenerated state in some cases. The possibil­
ity that orbicular wall abnormalities are also associated with the
misprogramming of a tapetal plasmalemma-glycocalyx which is used as a
template upon which sporopollenin is polymerized also deserves further
investigation.

The source of the sporopollenin, whether tapetal or sporogenous,
remains unidentified.

Anthers of male sterile plants and meiotically irregular plants
were shorter than fertile anthers and anthers of partially male ster­
ile plants which were meiotically more regular. Spike lengths and
the number of spikelets were more variable and differences could not
be associated with any particular category.

The variety of sterility types observed plus the paucity of
plants in category 3, male sterile and meiotically stable, precluded
any definite statements regarding specific causes of the sterility.
Meiotic irregularities may be involved in some of the sterility, and
exine disturbances appeared to be directly related to sterile microspores. Definite cytologically visible tapetal disturbances were inconsistently observed in only two plants. Probably the consequences of several environmentally sensitive factors were observed in the backcross material.
LITERATURE CITED


Anther and pollen development in male fertile intermediate wheatgrass...