The biology of a male sterile mutant in wheat (Triticum aestivum L.)
by Duane Everett Falk

A thesis submitted in partial fulfillment of the requirements for the degree of .MASTER OF SCIENCE in Agronomy
Montana State University

Abstract:
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The anthers of the male sterile plants were reduced in size and produced no viable pollen. Ovule fertility was normal.

Meiosis was normal in the microspores, but the pollen of male sterile plants degenerated before the mitotic divisions occurred. Aneuploidy was not associated with the male sterility. The cytoplasm did not influence the expression of the male sterility.

Heterozygous plants were completely male fertile and exhibited no detrimental effects from the male sterile alleles.

Four times as much crossed seed was produced per unit of time on male sterile plants as compared to emasculated fertile plants.

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Date October 28, 1977

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by

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

of

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in

Agronomy

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ABSTRACT

A spontaneous male sterile mutant in the F_3 generation of a 'Cheyenne' winter wheat (Triticum aestivum L.) backcross population was controlled by two coupled, duplicate, recessive genes.

The anthers of the male sterile plants were reduced in size and produced no viable pollen. Ovule fertility was normal.

Meiosis was normal in the microspores, but the pollen of male sterile plants degenerated before the mitotic divisions occurred. Aneuploidy was not associated with the male sterility. The cytoplasm did not influence the expression of the male sterility.

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INTRODUCTION

The normal mode of reproduction in hermaphroditic, self-pollinated plant species leads to the evolution of heterogeneous, homozygous populations. The amount of genetic variation within such populations is finite. The 50% decrease in heterozygosity and the rapid approach to homozygosity limit the recombination potential and decrease the chance of superior genotypes emerging.

Genetic diversity is introduced into self-pollinated species by spontaneous mutation and by natural outcrossing. Both of these occurrences are relatively infrequent and do not provide sufficient genetic variation for efficient, continued cultivar improvement.

Artificially induced outcrossing has been used extensively to combine potentially useful genetic variations in such numbers that selection within segregating generations is practical. Artificially created hybrids form the basis for most plant breeding programs in self-pollinated crops.

Hand emasculation, the oldest and most widely utilized procedure of producing crossed seeds, is laborious and time-consuming. Alternative methods such as chilling, chemical gametocides, and cytoplasmic and genetic male sterilities have been employed to facilitate artificial crossing.

Genetic male sterility appears to offer the best combination of efficiency, reliability, and ease of management to facilitate crossing

in wheat. It also has the added benefit of maintaining heterozygosity in segregating populations. Upon removal of the allele for male sterility, populations return to their normal self-pollinating, inbreeding status.

The discovery of a male sterile plant in a winter wheat population led to the initiation of this thesis research. A preliminary investigation indicated that the male sterility was genetic in nature. The objectives of this investigation were to: 1) examine the morphological characteristics; 2) study the cytological nature; and 3) determine the inheritance of the male sterile mutant involved.

LITERATURE REVIEW

Occurrence of Genetic Male Sterility

History

Male sterility as a result of a genetically controlled disruption of microsporogenesis occurs in many plant species (18,23). As early as 1915, Crane (8) described tomatoes (Lycopersicon esculentum L.) which failed to set fruit normally but upon hand pollination produced fruits with an abundance of seed. This male sterility was later found to be due to a single recessive gene (29). Similar phenomena in which plants failed to produce viable pollen but did set seed when cross-pollinated were first observed in corn (Zea mays L.) in 1921 (13), in barley (Hordeum vulgare L.) in 1940 (45), and in hexaploid wheat (Triticum aestivum L.) in 1959 (32).

Gene Frequency and Numbers

Rick (37) found genetic male sterile mutants to occur spontaneously in 0.005% of the 'San Marzano' tomato plants he examined. Hockett and Eslick (22) determined the frequency of naturally occurring genetic male sterility in barley to be 0.0025%.

In separate studies, Rick (36,37) found 5% and 8%, respectively, of the "unfruitful" tomato plants examined to be genetic male steriles. Hockett and Eslick (22) found that 10% of the barley plants identified as "highly sterile" in the field were genetic male steriles. This figure was later corroborated by Foster (16).

While the majority of the male sterile genes described have occurred spontaneously, some have also been induced by mutagens. Fossati and Ingold (14) reported that one of three hundred M_2 rows of the winter wheat variety 'Probus' treated with X-rays segregated for genetic male sterility. Franckowiak et al. (17) induced ten genetic male sterile mutants with ethyl methane-sulfonate (EMS) in a population of 45,000 wheat plants. The $\underline{ms}_3$ gene in barley was induced by acetone treatment (21). Foster (16) found that 17% of the "sterile" plants isolated from the M_3 of a barley population treated with diethylsulfate (DES) were genetic male steriles.

Yamashita (59) isolated more than 250 genetic male sterile mutant barleys from 12,240 M_1 plants treated with gamma rays and the chemical mutagens--EMS, ethyleneimine (EI), and nitrosomethylurea (NMU). Approximately two-thirds of these mutants were completely male sterile, and all except one of them were controlled by a single recessive gene.

Since 1915, eighteen different male sterile genes have been identified in tomatoes (38); forty-four in corn (56); and twenty-six in barley (20). Eight reports of genetic male sterility have been made in wheat (3,15,17,24,27,30,32,43). Two of these mutants are allelic (10).

Rick (37), Hockett and Eslick (22) and Foster (16) maintain that spontaneous genetic male sterile mutants can be obtained in any cultivar of the crops they studied, providing that sufficiently large popu-

lations are examined. Gottschalk and Kaul (18) concluded that male sterile genes are probably present in the genomes of all flowering plants, regardless of the taxa. Genetic male sterility has been described in more than forty-eight plant species in twelve families. Gottschalk and Kaul (18) cite evidence that some of these genes are homologous and represent similar anomolies in different species.

Morphology of Genetic Male Sterile Plants

General Morphology

Genetic male sterile plants are usually indistinguishable from their fertile siblings and normal fertile cultivars until the time of flowering (18,27). Male sterility is easily recognizable at maturity in self-pollinated plants by a reduced seed set (27,32,45) or fruit production (8,28,37) resulting from the failure of the plants to produce viable pollen.

The first indication of male sterility that Crane (8) noticed in tomatoes was that two F_2 plants had failed to set fruit. The anthers of these plants appeared to be "reflexed and somewhat aborted" and devoid of pollen. Lesley and Lesley (28) noted that male sterile tomato plants were often conspicuous by their excessive vegetative vigor and upright habit.

Eyster (13) reported that R. A. Emerson first detected genetic male sterility in corn by a failure of the anthers to extrude. The

sterile spikelets usually remained flattened against the rachis to give a characteristic tassel appearance. Such plants were easily detected at normal pollination time.

Suneson (45) identified male sterile barley plants by widely-opened glumes. This condition persisted for several days after flowering. The florets were observed to close upon pollination. Hockett and Eslick (21) reported that swelling of the unfertilized ovary caused barley flowers to remain open for seven to eight days after normal flowering. The lodicules open the flower briefly at anthesis but close soon after. The flowers close when the unfertilized ovary deteriorates. The spikes of male sterile plants also tended to remain more erect due to a reduction in seed set. Male sterile barley if often infected with ergot (Claviceps purpurea (Fr.) Tul.) under Montana conditions (12). Male sterile plants, in general, usually remain green and continue vegetative development longer than their fertile siblings (18).

Male sterile wheat, like barley, is characterized by opened florets at flowering and a reduced seed set (5,6,15,27,32). Male sterile durum wheat (Triticum durum Desf.) plants tended to head earlier than fertile plants (5). Suneson (49), however, observed the male sterile hexaploid wheat from A. T. Pugsley (32) to be up to two weeks later flowering than the fertile siblings at Davis, California. Some male sterile wheat plants tend to be shorter (27) and less vigorous (27,30, 49) than their fertile siblings. Male sterile 'Saratov 29' wheat

exhibited a greater anthocyanin content than fertile plants (27). Ergot sclerotia have also been observed in the spikes of mature male sterile wheat grown in Montana (14).

Anther and Pollen Morphology

The anthers of some of the male sterile mutants in barley appear to be completely normal and produce stainable pollen while other mutants are characterized by very small, shrunken anthers which fail to dehisce (40). These rudimentary anthers usually contain little or no pollen and the filaments often fail to elongate. Although the various male sterile mutants in barley have very different appearances, the expression of each individual gene is quite stable. More variation in the anther morphology was noted in greenhouse environments than in field-grown populations.

Male sterile wheat plants often possess anthers which are reduced in size (5,45) and may fail to extrude (27). The anthers contain empty pollen grains which are sometimes agglutinated (5,6,27). Some male sterile wheats exhibit a low level of pollen fertility which appears to be affected by unknown environmental factors and modifying genes (3,24,49). One male sterile mutant exhibits varying degrees of anther transformation to nonfunctional ovaries (pistilloidy) (24).

Cytology of Genetic Male Steriles

Chromosome Numbers

Rich (36) reported that much of the unfruitfulness observed in tomatoes was the result of cytogenetic problems arising from aneuploidy. The majority of the sixty-six unfruitful plants isolated from approximately 55,000 plants of three commercial cultivars were aneuploids. Two plants were haploids, two were trisomics, forty-four were triploids, and three were tetraploids. Another fourteen were diploids, of which only four proved to be genetic male steriles. In a later study, Rich (37) found 108 of 150 unfruitful plants to be aneuploids.

Waninge and Zeven (54) and Zeven, Kampmeijer and Enink (60) examined the chromosome numbers of Pugsley's (32) male sterile wheat. They found the 2n chromosome number to vary from 40 to 44 for various plants. Some mixoploids were also observed. Male sterility in this cultivar was not due to aneuploidy.

Microsporogenesis

In an extensive review of genetic male sterility in higher plants, Gottschalk and Kaul (18) concluded that most of the male sterile (ms) genes influence the final stages of microsporogenesis between interphase II and pollen formation. A much smaller group of ms genes affects the very early stages of meiotic prophase and halts the development of microspores at that point. Very few of the known ms

genes function between diakinesis and anaphase II.

In most instances of genetically controlled male sterility, the stage at which the microspore degeneration occurs is highly specific and precise for each ms gene (18,36,37). The behavior of the chromosomes in meiosis is completely normal until the degeneration occurs.

In cytological examinations of meiosis in twelve male sterile corn lines, Beadle (4) found that eight of them went through meiosis normally but did not develop beyond the free microspore stage. Another failed during early prophase the three degenerated over a range of meiotic stages rather than at a specific stage.

Rick (28) found four male steriles in tomato which degenerated in the quartet stage and four more which failed to develop beyond free microspores. In five mutants the microspores collapsed during prophase I while in one other the sporogenous cells aborted before meiosis commenced. In three mutants the breakdown occurred from mid-prophase I to the early microspore stages. Rich (37) also reported an association between the time of breakdown in microsporogenesis and the amount of deformation of the tomato anthers. Genes which cause early microspore abortion are characterized by greatly reduced and degenerated anthers.

Roath and Hockett (42) showed that the degeneration of microspores in the ms mutant in barley occurs during the quartet stage of meiosis. The free microspores of ms_8 failed to undergo mitotic divisions and aborted. A completely normal microsporogenesis and pollen

maturation was observed for $\underline{ms}_6$ mutants. The pollen of this barley mutant lacks a pore and therefore cannot germinate on the stigma (1).

Four cytologically examined genetic male sterile wheat mutants completed meiosis normally but failed to develop beyond the free microspore stage (3,5,6,30).

The stage in which the breakdown occurs does not seem to affect the final result--a failure to produce functional pollen (4,18,36,37, 41).

Megasporogenesis

Most male sterile (ms) genes do not alter the process of meiosis and therefore have little or no influence on megasporogenesis (4,18,21, 36,37). Male sterile plants readily set seed upon being pollinated with viable pollen, indicating normal female fertility (4,5,8,21,27,30, 37). An exception was noted in tomatoes for $\underline{ms}_8$ which causes about 25% of the ovules to abort (37).

Inheritance of Genetic Male Sterility

Gene Action and Number

The majority of the male sterile genes reviewed by Jain (23) and and Gottschalk and Kaul (18) involved single, recessive factors. The heterozygotes were completely male fertile.

All nineteen of the male sterile genes in barley evaluated by

Hockett and Eslick (21) were monofactorial recessives. Rick (38) lists eighteen single, recessive genes for male sterility in tomato and Beadle (3) gives sixteen in corn. Five of the mutants isolated in wheat are single recessive genes (5,6,15,27,30).

There are exceptions to the recessive single gene male steriles, however. Lesley and Lesley (28) reported male sterility in tomatoes controlled by two complementary recessive genes. Beadle (4) mentions two cases in corn which are suggestive of duplicate recessive genes and others that may be more complex than single gene systems. Athwal et al. (3) and Jan (24) postulate that three additive genes control the expression of male sterility in the wheat stocks they investigated. Weaver and Ashley (55) described a dominant gene, $\underline{MS}_7$, in cotton (Gossypium hirsutum L.) which causes male sterility. Another dominant male sterile mutant has been reported in wheat (17).

Gametic Transmission

Most of the male sterile genes act only in the sporophytic (2n) generation and therefore have little or no effect on the gametophytes (23). Lupton and Bingham (30), however, reported a male sterile in which the transmission of the mutant allele is normal through the ovule but is greatly reduced in the pollen of heterozygotes.

Briggle (6) indicated that preferential transmission of the domi-

mant fertile allele may occur in 'Chancellor' wheat. Rick (37) also noted a deficiency of male steriles in segregating F_2 tomato populations.

Cytoplasmic Influences

Gottschalk and Kaul (18) reported that the cytoplasm of certain strains of onion (Allium cepa L.) and carrots (Daucus carota L.) influenced the expression of male sterile genes. When the male sterile genotype was in the fertility restoring cytoplasm, the plant appeared to be completely male fertile. Thus, the male sterile genes were unable to express themselves in certain cytoplasms. Hermsen (19) and Franckowiak et al. (17) have suggested that such a fertile cytoplasm may be found for the male sterile genes in wheat and barley. Krupnov (27) transferred the male sterile gene which he isolated from the wheat cultivar Saratov 29 into the cytoplasm of the cultivar 'Sarrubra,' where it segregated normally to show that the male sterility was expressed in another cytoplasm.

Utilization of Genetic Male Sterility

Crossing Efficiency

Shortly after Suneson (45) found a genetic male sterile mutant in barley, he began using the male sterile plants as female parents to facilitate routine crossing in his breeding program. Riddle and

Suneson (39) found that the stigmas of male sterile plants remained receptive up to ten days after first flowering. Seed set in hand pollinations was increased by clipping the florets back, but seed size was decreased. Open-pollinated seed set ranged from 10.6% with alternate rows of pollen parents and male sterile plants (fertiles were rogued) to 44.9% for male sterile plants in heterozygous backcross and F_2 populations.

Using Pugsley's male sterile wheat, Suneson (49) found that four times as much crossed seed could be produced per unit of time compared to normal varieties which required hand emasculation.

Composite Crosses

Suneson (46) developed Composite Crosses XIV and XV in barley using genetic male steriles as a mechanism which would allow for continued outcrossing and recombination. With such a system, self-pollinated crops can be manipulated like cross-pollinated species with appropriate techniques.

Suneson and Wiebe (50) created barley Composite Cross XXI by crossing 6200 lines from the USDA World Collection of barley onto genetic male sterile plants. This population provides an excellent source of material to be exploited through Suneson's "evolutionary" breeding method (47).

Using Pugsley's male sterile, Suneson et al. (49) combined 235 wheat lines in wheat Composite Cross I. This wheat composite has not

been exploited as widely as the barley composites with male sterile genes in them (48).

Hybrids

In 1960, Wiebe (57) proposed using a recessive male sterile gene (ms) closely linked to a recessive phytocide resistance gene (ddt) for the production of hybrid barley. By spraying a segregating population containing these coupled genes with the phytocide, DDT, all plants not homozygous for the recessive resistance would be eliminated. Because those plants homozygous for the ddt gene would also be homozygous for the coupled ms gene, the entire population remaining would contain only male sterile plants. This male sterile populations would then be pollinated by the desired male parents grown in adjacent blocks to produce hybrid seed.

In 1965, Ramage (34) proposed the balanced tertiary trisomic (BTT) system for producing hybrid barley. The balanced tertiary trisomic plants used by Ramage produced about 70% male sterile progeny because of the reduced transmission of the trisomic chromosome carrying the fertile allele through the pollen. A phytocide resistance gene was proposed to eliminate the 30% of trisomics from the female populations. Another phytocide resistance gene would then be used to eliminate the male steriles from the increase populations.

Wiebe and Ramage (58) added a built-in rogueing mechanism to the

BTT system by using an albino gene coupled to the male sterile gene. Later, Ramage (35) proposed a more complex system using an albino gene, a haplo-viable gene and a megaspore competition gene to streamline the BTT.

Eslick (11) has proposed the use of balanced male sterile and dominant preflowering genes for the selection of an all-female line to be used in hybrid seed production. The dominant genes allow the rogueing of fertile plants before pollination takes place.

In 1972, Driscoll (9) developed his X-Y-Z system for hybrid seed production in wheat. The system employs an added alien chromosome carrying the fertile allele which is not transmitted through the pollen. With the proper manipulations, this system produces an all-male sterile population to be used as the female plot for hybrid seed production.

Taylor et al. (51) and Johnson et al. (25) have proposed the use of a telosomic line in the production of an all-female line for the seed parent of a hybrid. This system, known as the telosomic catalyst, is based upon a reduced transmission of a telocentric chromosome through the pollen. Only alleles, such as ms, on the normal chromosome are passed through the pollen. Crossing the telosomic catalyst onto a male sterile gives all male sterile progeny for hybrid seed production.

The telosomic catalyst, the X-Y-Z system, and the BTT, all take advantage of a discrimination against aneuploidy in the male gametophyte to achieve the preferential transmission of the desired allele on

a normal chromosome.

Hermsen (19) and Franckowiak et al. (17) suggested that a cytoplasm may exist in wheat and barley which may cause a plant homozygous for a ms gene to be phenotypically fertile. This would allow the maintenance of a line homozygous for male sterility. When crossed onto a male sterile plant with a different cytoplasm, all male sterile progeny would be produced. These could then be used in a hybrid program.

Thompson's (52) work with barley in Arizona indicates that hybrid seed production using a genetic male sterile system is economically feasible in self-pollinated cereal crops.

MATERIALS AND METHODS

General Procedures

Greenhouse-grown materials were planted in benches containing pasteurized greenhouse potting soil (1 sand:1 clay:1 peat, by volume), approximately 15 cm in depth. Plants were grown in rows 15 cm apart with 7.5 cm between plants within the rows. A complete nutrient solution was used for watering and supplemental fluorescent lighting of 14-16 hours was provided to simulate long-day conditions.

Spring and fall field plantings were made in 3 m rows 30 cm apart with 10-15 cm between seeds within the row. Planting depth was about 5 cm. The field location was the Plant and Soil Science Field Research Laboratory at Bozeman, Montana. The soil type at this site is the Amsterdam silt loam, a Typic Cryoborall.

Statistical Methods

Comparison of Means

Because of the biological nature of the materials investigated, population sizes and the variances of the populations studied were often unequal. Snedecor and Cochran's (44) weighted t (t') test for independent samples with unequal variances and sample sizes was used to compare the means of the samples being tested. The calculated value of t was found in the usual manner using the following equation:

$$t = (\bar{X}_1 - \bar{X}_2) / \sqrt{(s_1^2/n_1 + s_2^2/n_2)} \qquad \text{(Note: } s_x^2/n = s_{\bar{X}}^2\text{)}$$

the tabulated t value was modified to compensate for unequal variances in populations to give a weighted t (t'). The equation used for calculation of the tabular t' value was

$$t' = [(t_1 \times s_1^2/n_1) +)t_2 \times s_2^2/n_2)]/(s_1^2/n + s_2^2/n).$$

Snedecor and Cochran (44) recommend the assumption that $\sigma_1 = \sigma_2$ be avoided if there is any doubt, thus the modified tabular t (t') was used in preference to the regulat t-test equation. Tests of the equality of variances between populations showed them to be significantly different. A comparison of the calculated t to the tabular t' was used to test the difference of the means at the chosen level of significance. H_o was rejected if the value of the t calculated was greater than t' tabular.

Paired Comparisons

Snedecor and Cochran's (44) equation for paired comparisons was used to determine the probability that differences were significant. The equation used was:

$$t = \frac{\overline{D}}{s_{\overline{D}}}$$

The symbol $\overline{D}$ is used to designate the mean of the difference.

H_o was rejected if the probability was less than .05.

Population Size

Mather's (31) equation to determine the population size required

to obtain a certain class at a given probability level was used to calculate the minimum population necessary in the genetics experiments. The equation is:

$$(\text{1-proportion of the desired type})^N = \frac{1}{\text{(1-probability of desired type)}}$$

where N = the minimum population required at the chosen probability level to obtain one of the desired type.

Source Materials

The source of the male sterility studied in this investigation was a single plant in a 1974 field-grown F_3 head row of 'Yogo' Short Straw 4462/4*'Cheyenne.' This plant contained ergot sclerotia and set only seven seeds on five spikes. Cheyenne (CI 8885) is a widely-adapted hard red winter wheat. Yogo Short Straw 4662 ('Norin' 10/-'Brevor'//3*Yogo,4-6-6-2) is a semi-dwarf, backcross-derived selection utilized as a source of shatter resistance in a backcrossing program with Cheyenne at Montana State University.

The F_3 head row in which the male sterile plant occurred was uniform for height and maturity, but segregated for brown and white chaff. All of the lines derived from the original cross were homozygous for awns and winter growth habit.

The seven seeds on the original male sterile plant were sown in the greenhouse during the winter of 1974-75. Five of the F_1 plants produced were fertile and closely resembled the parental phenotype.

They were designated as Cheyenne male sterile (Cnn ms)-1,-2,-4,-5 and -6 (Figure 1, p. 21).

A sixth plant (Cnn ms-3) was also fertile but exhibited a spring growth habit and was awnletted. Cnn ms-3 probably resulted from an outcross of the original male sterile plant to a nearby awnless spring wheat. An F_2 population from this plant was grown in a preliminary investigation at Bozeman as a field observation plot (Figures 1, p. 21, and 2, p. 22) in 1975. Segregation ratios for awns, stem solidness, growth habit and male sterility were recorded.

The seventh plant (Cnn ms-7) was similar to the parental line in appearance, except that it was male sterile (Figure 1, p. 21).

Derived Materials

Spring Wheats

All of the spring-type wheats studied were descendants of the single F_1 plant Cnn ms-3 (Figures 1, p. 21, and 2, p. 22). Fertile plants in the preliminary F_2 population of 151 plants grown in 1975 provided eighty-two F_3 families in 1976 (Figure 2, p. 22). Crosses were made to the seventeen male sterile plants in the Cnn ms-3 F_2 population using normal cultivars and fertile siblings as male parents. Seed was also produced by open-pollination.

One hundred and forty of the F_1 seeds from the male sterile plants were grown in the greenhouse in the winter of 1975-76. The re-

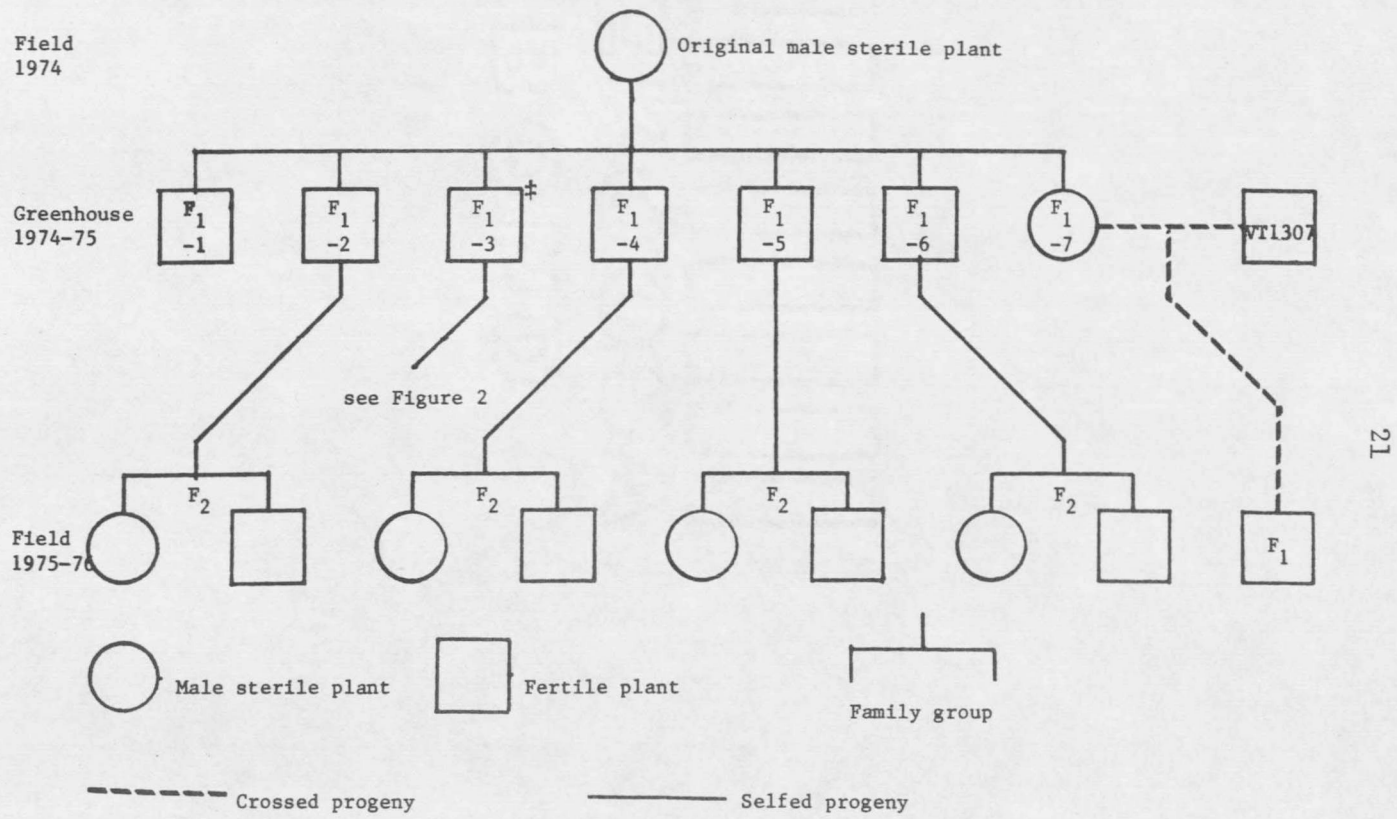

Figure 1. A flow chart of the progeny of the original male sterile plant.

‡This was an awnletted spring-habit plant in the F_1 generation.

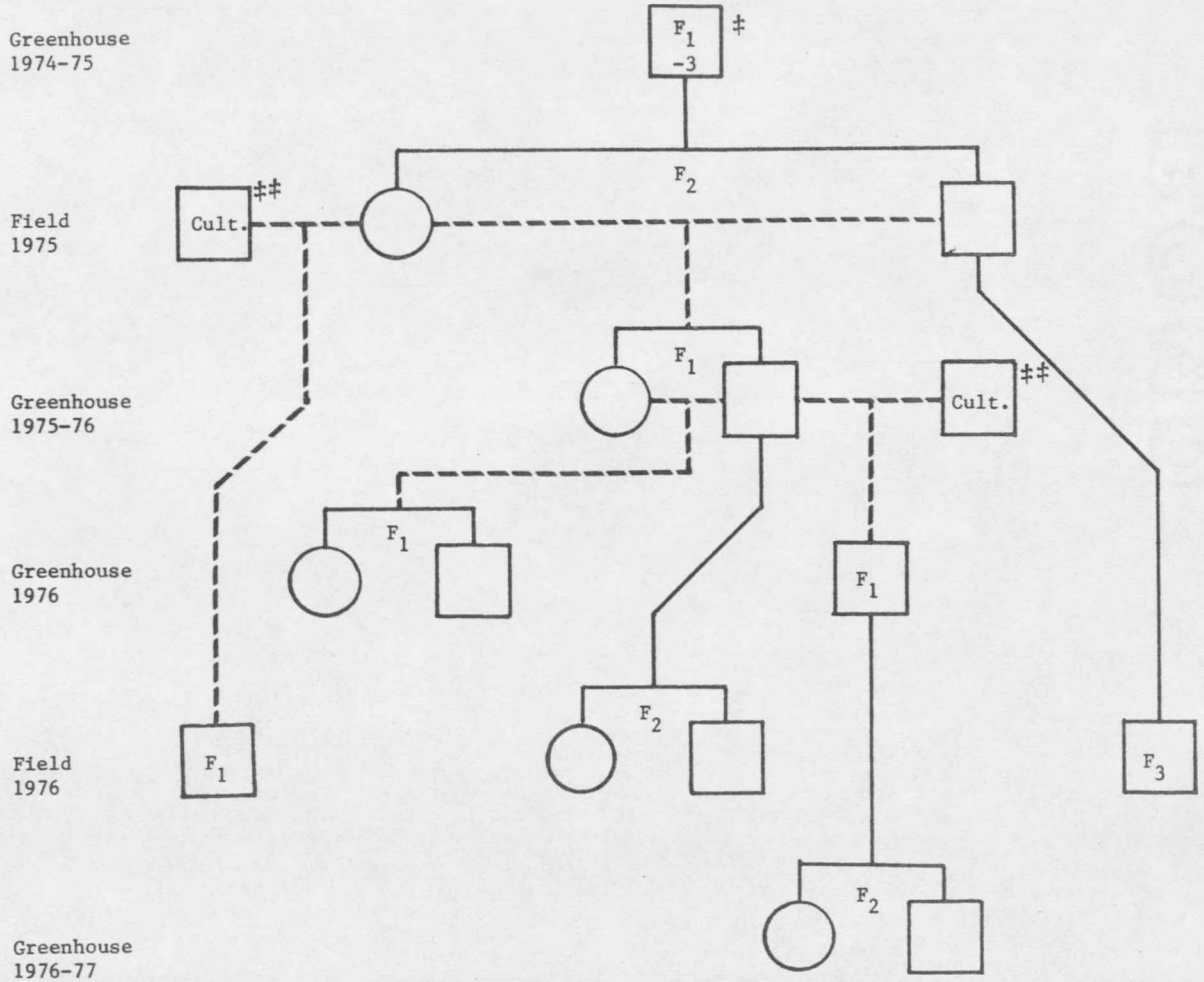

Figure 2. A flow chart of the spring-habit progeny of F_1 #3 from the original male sterile.

‡continued from Figure 1.
‡‡a number of normal, fertile cultivars.

maining eighty-six F_1 seeds from six crosses to cultivars and four open-pollinated spikes were field-grown in 1976.

Five test crosses were made to male sterile plants using fertile heterozygotes in the greenhouse-grown F_1's in 1976 to determine the transmission frequency of the male sterile through the pollen. Reciprocal crosses of three fertile, heterozygous F_1 plants and three normal cultivars were made to determine the transmission of the male sterile factor through the ovule and to study the effect of other cytoplasms on the expression of the male sterility.

Thirty-three F_2 populations of twenty to sixty-four plants each from the fertile F_1 plants in the greenhouse were grown in the field in the summer of 1976. F_1 seed from the testcrosses and reciprocal crosses was grown in the greenhouse during the summer of 1976. Eighteen F_2 populations of thirty plants each from selected lines were grown in the greenhouse in the winter of 1976-77. The plants of the testcross F_1 and reciprocal F_2 generations were classified as male sterile or fertile.

Winter Wheat

The winter wheat F_2 populations grown were the progeny of original F_1 plants Cnn ms-2,-4,-5 and -6 (Figure 1, p. 21). A cross was made to Cnn ms-7 (male sterile) in 1975 to continue this line. A normal fertile winter wheat selection from France, VT 1307, was used as

the male parent.

The three F_1 seeds from this cross, along with ten seeds each from Cnn ms-5 and -6, were started in the greenhouse and then transplanted to the field on May 6, 1976, early enough for vernalization to take place.

About 150-200 seeds from Cnn ms-5 and -6 were field-planted on September 21, 1975. Ninety-six lines of F_5 Yogo Short Straw 4662/4* Cheyenne were fall-planted at Bozeman in 1975 in two replications of four-row plots 3 m long. These populations were observed for male sterility and were used as female parents in crosses for comparative seed set to male sterile plants.

Plant Characteristics

General Morphology

The general appearance of fertile and male sterile plants in spring habit populations derived from Cnn ms-3 grown from May 15, 1975 April 26, 1976 and May 30, 1976 plantings, and winter habit plants sown September 21, 1975 were observed at the field laboratory at Bozeman. Greenhouse materials planted February 1975, January 1976 and November 1976 were also examined visually.

Comparative notes were taken on heading dates, flowering dates, and maturity dates of fertile and male sterile plants in Cnn ms-5 and -6 F_2 populations in 1976. These populations had 134 and 181 plants,

respectively. Individual plants were recorded as headed when 50% of the spikes had completely emerged from the flag leaf sheath. Plants were considered to be flowering when the first spikes on the fertile plants anthesed or when the male sterile plants were first detected by their widely-opened florets. Maturity was determined as the point when the grains in the main tiller spikes were hard and could be easily threshed by hand.

Notes were also taken on these populations for culm diameter, tiller number and development, flower morphology, and general vigor. The mean height of several male sterile and fertile plants was determined as cm from the ground to the tip of the tallest spike, excluding the awns.

Plant heights of fourteen to twenty-four male sterile and fertile plants in each of three F_2 populations of Cnn ms-3/'Crest'(CI 13880)//'Oleson's Dwarf'(CI14497) planted in the greenhouse in November of 1976 were measured in the same manner as above. The mean height of fertile plants was compared to that of the male sterile plants using the weighted t test.

Yield Components

Preliminary notes on Cnn ms-3 F_2 grown in 1975 indicated that the male sterile plants had fewer spikes per plant and fewer spikelets per spike. A more complete examination of these characters was con-

ducted on the winter habit Cnn ms-5 and -6 F_2 populations in 1976. These populations were relatively large (134 and 181 plants, respectively) and somewhat homogeneous.

Fifteen male sterile and four fertile Cnn ms-5 F_2 plants and sixteen male sterile and five fertile Cnn ms-6 F_2 plants were evaluated as to the number of spikes per plant and the number of spikelets per spike. Seeds per spike was not used as a yield component because of the direct effect of male sterility on seed set in wheat.

Sample means were compared using the weighted t test. Samples with similar means (from the t' test) from the two populations were combined for further comparisons of fertile and male sterile plants.

Yield per plant comparisons between F_1 hybrids and the male and female parents were made on a limited number of plants with spring and winter habits in 1976. Seven F_1 hybrid plants of the cross Cnn ms-3/Oleson were compared to five plants of Oleson for height, number of spikes per plant and total yield. They were also compared to Cheyenne for height.

Three winter habit F_1 plants from the cross Cnn ms-7/VT 1307 were compared to the highest yielding plants of Cnn ms-6 F_2 and VT 1307 grown in a similar environment. A single reciprocal cross of VT 1307/Cheyenne which had no male sterility in its ancestry was also included. All plants were grown in greenhouse benches from February 3 to May 15, 1976, then transplanted to the field for vernalization.

The traits measured on each plant were height (to the tip of the tallest spike, excluding awns), spikes per plant, spikelets per spike, seeds per spike, mean kernel weight and total yield.

Because of the limited number of plants examined, the data were compared graphically rather than statistically.

Anther Morphology

The relative size, color and morphology of freshly dissected anthers from the spikes of male sterile and fertile plants were determined by microscope examination. Twenty anthers from randomly-selected primary florets were measured from each spike of twenty fertile and twenty-two male sterile plants from Cnn ms-3/Oleson grown in the greenhouse in 1976. The same number of anthers from twenty-three fertile and twenty male sterile spikes of Cnn ms-6 F_2 field grown in 1976 were also examined. The mean length of twenty anthers from thirteen spikes of field-grown Cheyenne were used as the check.

A weighted t test was used for comparisons of the mean length of the anthers of male sterile and fertile plants in the populations studied.

Cytological Methods

Somatic Chromosomes

Root tip chromosome counts were made to provide an assessment of aneuploidy as a possible cause of the male sterility.

Twenty-five seeds each of Cnn ms-5 and -6 F_2 and forty-two seeds of the five random test crosses of fertile F_1 heterozygotes to male steriles were prepared according to Waninge's (54) modified method of preparing root tip chromosomes utilizing an acetocarmine staining technique. Chromosome numbers were determined and referenced to individual plants which were grown to maturity and scored as male sterile or fertile. The chromosome number of at least five cells per plant was determined.

Microsporogenesis

Pollen mother cells were observed from ten male sterile and ten fertile 1976 field-grown plants from each F_2 population of Cnn ms-5 and -6. Meiosis was also examined in twenty-three greenhouse-grown heterzygous plants from seeds set on male sterile plants in Cnn ms-3 F_2 grown in 1975.

Spikes for this study were harvested in a series of developmental stages from premeiosis through anthesis and preserved in Farmer's solution (3 parts alcohol:1 part glacial acedic acid). Dissected anthers were macerated on a glass microscope slide in a drop of 5% acetocarmine (AC) or 0.5% proprionic-carmine (PC).

At least one hundred pollen mother cells were observed from each plant. An attempt was made to secure a continuous series of stages from premeiosis through mature pollen for all plants. Abnormal divi-

sions were noted as to type and stage of occurrence.

Mature Pollen

Spikes from ten male sterile and ten fertile plants from Cnn ms-5 and -6 F_2 were field harvested at anthesis in 1976. Spikes of fifteen greenhouse-grown heterozygotes from open-pollinated spikes of male sterile plants in Cnn ms-3 F_2 grown in 1975 were also obtained at anthesis for this study. Spikes of field-grown Cheyenne served as checks.

Pollen samples were stained in the laboratory in each of five cytological stains. A 5% solution of aceto-carmine (AC) and a 0.5% solution of proprionic-carmine (PC) were used to detect the chromatin content of the pollen grains. Examinations were made approximately fifteen minutes after staining. A 3% solution of iodine-potassium iodide (IKI) in 70% alcohol and a 3% solution of IKI in glycerine were used to determine the presence of starch in the pollen. Observations were made immediately after staining. A 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in water was used to examine the pollen grains for potential viability. It was not necessary to use a sucrose solution with the TTC for wheat pollen as is needed with barley (40). A two-hour period was sufficient for a complete reduction of the TTC.

Because of the uniformity of the stained materials and the sharp contrast between male sterile pollen and fertile, or normal pollen, data were recorded as "stained" or "unstained" rather than as the

percentage of stained grains for all samples in all stains. At least 95% of all the grains were stained in all samples recorded as "stained." None of the grains were stained in samples recorded as "unstained."

Self-Fertility

Enforced self-pollination by bagging the male sterile heads was used to determine self-fertility. At least one spike of every male sterile plant in Cnn ms-5 and -6 F_2 was enclosed in a glassine pollination bag prior to anthesis in 1976. A total of thirty-two selfed spikes of Cnn ms-5 and thirty-eight selfed spikes of Cnn ms-6 was examined at harvest for seed set.

Spikes on four fertile plants in Cnn ms-5 and five plants in Cnn ms-6 were also bagged to determine the effect of bagging on normal seed set.

Paired comparisons for mean seed number were made between bagged and open-pollinated spikes on the same fertile plants. A t' test (44) was used to compare the mean numbers of seeds per spike for each treatment and each population. Data from populations with similar means were combined for a comparison of treatment effects on male sterile and fertile plants.

Seeds set on selfed spikes of male sterile plants were germinated at 25^{o} C and were to be grown to maturity to determine morphologically if they were selfed seed or accidental cross-pollinations.

This study was terminated when a germinator malfunction killed the seeds.

Ovule Fertility

Ovule fertility of male sterile plants was determined by comparing the mean seed set in bagged, unpollinated spikes with that of bagged spikes on the same plants which had been hand-pollinated. A paired t test was used in this comparison.

The seed set on crossed spikes of male sterile plants was compared to crossed seed set on emasculated fertile plants to determine the relative ovule fertility of male sterile plants. Spikes of several lines of F_5 Yogo Short Straw 4662/4*Cheyenne (selection from the same cross which produced the original male sterile plant) were emasculated in the field in 1976. Crosses were made to forty-two male sterile plants of Cnn ms-5 and -6 F_2 and twenty-three emasculated F_5 plants with thirteen adapted winter wheats as male parents.

The central florets were removed from the spikelets of the fertile plants at the time of emasculation and from the spikelets of the male sterile plants at the time of pollination. The spikes of male sterile plants were bagged at the time of emergence from the flag leaf sheath and those of fertile plants upon the completion of emasculation. The glumes and the lemma and palea were clipped back on all plants to facilitate pollen entry. The "twirling" method of applying pollen to

the stigmas was used in all pollinations with two pollen parent spikes used in each cross to insure fertilization.

The average seed set per crossed spike of the male sterile plants was compared to that of the emasculated fertile plants using a weighted t test (44). Mean kernel weight of the seed set on crossed and open-pollinated male sterile and fertile plants was also compared with the t' test.

Inheritance

Gametic Frequencies

It was decided that the male and female transmission of the male sterile factor should be determined before F_2 and F_3 segregation ratios could be adequately analyzed.

Pollen transmission of the male sterile factor was determined in five testcrosses of a heterozygote to a male sterile made in the greenhouse in 1975. Both male sterile and fertile plants were from seeds set on male sterile plants in Cnn ms-3 F_2 in 1975. A total of forty-two testcross F_1 progenies was grown in the greenhouse during the summer of 1976. The number of male sterile and fertile plants in each cross was recorded. The data from all five crosses were summed together because the number of plants in each family (5-12 plants) was considered to be too small to test accurately individually. The assumption was made that the transmission of the male sterile through the

male gametes was the same for all five heterozygotes. The ovules fertilized by male gametes carrying the male sterile factor should produce male sterile F_1 plants.

Ovule transmission of the male sterile factor was determined by crossing a normal fertile cultivar to an emasculated heterozygote. Oleson's Dwarf, an early, dwarf, hard red spring wheat was used as the male parent. The F_1's were grown in the greenhouse during the summer of 1976. The F_2 populations from these crosses were scored as "segregating" or non-segregating" for male sterility in the greenhouse in the winter of 1976-77. Those F_2's from female gametes carrying the male sterile factor should have produced segregating F_2 populations.

The transmission of the male sterile through the male was statistically compared to that of the female using a goodness of fit chi-square test.

Gene Action and Number

The gene action involved in conditioning the expression of the male sterility was determined by observing the phenotypes of F_1 plants for which the genotype was known, and the proportions of the different phenotypes in the F_2 and F_3 populations.

A Punnett square was constructed using male and female transmission ratios to predict the F_2 segregation for male sterility. The predicted F_2 ratio was compared to actual data with a goodness of fit

chi-square test.

Cytoplasmic Influence

Fertile heterozygotes from crosses made to male sterile plants in Cnn ms-3 F_2 in 1975 were crossed to three emasculated normal cultivars. The cultivars Oleson's Dwarf; 'Rescue' (CI 12435), a solid-stemmed hard red spring wheat; and 'Nugaines' (CI 13968), a soft white winter wheat were chosen to represent an array of cytoplasm backgrounds. The F_1's were grown in the greenhouse during the summer of 1976. The F_2 populations from these crosses were scored as "segregating" or "non-segregating" in the greenhouse in the winter of 1976-77 as in the Ovule Transmission study. Notes were taken on the general appearance of male sterile and fertile plants in these populations.

The degree of expression of the male sterility in these diverse cytoplasms provided an indication of the degree to which the cytoplasm influences the studied source of male sterility.

Lethality

The unusual 8:1 segregation for male sterility in the preliminary population prompted an investigation designed to detect lethality in the populations which may have eliminated some genotypes. Twenty-five seeds of Cnn ms-5 and fifty seeds of Cnn ms-6 were germinated in a growth chamber and the resulting seedlings grown to maturity in the greenhouse. The percentage of germinated seeds and the number of

plants reaching maturity were recorded.

Linkages

When possible, crosses to male sterile plants were made with pollen parents carrying contrasting alleles for discernible morphological characters. In the course of this study linkage was tested between the male sterility and awns; chaff color; stem solidness; and growth habit. Contingency chi-square tests were used to determine the magnitude of the interaction (linkage) of the characters being tested. The linkage of male sterility to growth habit was determined by comparing the segregations of male sterility in populations homozygous for the genes governing growth habit (based on homogeneity) to populations segregating for growth habit. Both winter and spring growth habit populations were used for comparisons to the populations of plants heterozygous for growth habit. A contingency chi-square test was used to determine if the segregations were homogeneous in all populations.

RESULTS AND DISCUSSION

Preliminary Investigation

In order to establish that the male sterility was genetic, the F_2 progeny of a fertile F_1 plant (Cnn ms-3) was field planted in 1975. This plant was chosen because of its spring growth habit and as an outcross the progeny should segregate for several morphological characters.

The F_2 population segregated for awns (91 awnless:45 awned), stem solidness (35 solid:63 semi-solid:38 hollow), growth habit (136 spring:15 winter) and male sterility (119 fertile:17 male sterile) (Table 1, p. 37). The F_2 population from a fertile F_1 plant segregated for male sterility indicating that the male sterility is heritable and recessive in nature.

The segregations for awns, stem solidness and growth habit were not significantly different from the expected ratios (33). The segregation of the male sterility (119:17), however, did not fit the expected ratios for an F_2 segregation of one, two or three loci.

The male sterile plants flowered and ripened later than the fertile segregates in the population. They were also generally shorter and had fewer mature spikes at harvest than fertile plants. Both crossed and open-pollinated spikes set seed, indicating that the ovules were fertile.

The male sterility which has been consistently observed in progeny from male sterile plants of the original line has not been detected

Table 1. Classification of segregates of Cnn ms-3 F_2 grown in 1975.

Character				Number of Plots
Spring habit,	Awnletted,	Solid Stem,	Fertile	19
			Male Sterile	3
		Semi-solid,	Fertile	43
			Male Sterile	2
		Hollow Stem,	Fertile	19
			Male Sterile	5
	Awned,	Solid Stem,	Fertile	11
			Male Sterile	2
		Semi-solid,	Fertile	15
			Male Sterile	3
		Hollow Stem,	Fertile	12
			Male Sterile	2
Winter habit‡				15
			Total	151

‡Did not head from this spring planting.

in any of the sister selections from the source population (Yogo Short Straw 4662/4*Cheyenne), even though a considerable number of lines have been agronomically evaluated. The male sterility probably is a spontaneous mutation rather than a recombination of genes from the parental lines. Such mutations have occurred in tomatoes (37), barley (16,21, 45) and wheat (3,5,6,16,24,27,30,32,43).

The lines developed by crossing siblings to the male sterile plants should be nearly identical to the original line and nearly isogenic for male sterility. Since the original male sterile plant occurred in a Cheyenne back population selected for shatter-resistance (96% Cheyenne genes), it should be adapted to Montana growing conditions.

Although the initial F_2 population demonstrated that the male sterility was probably recessive and controlled by a genetic mechanism, it did not reveal the number of loci involved. The results of a comprehensive investigation of the biology of the male sterile mutant follow.

Plant Characteristics

General Morphology

The male sterile plants in the populations studied were indistinguishable from their fertile siblings until the time of flowering. At anthesis the male sterile plants were easily recognized by their

persistent, widely-opened lemma and palea. This phenomenon was observed two to five days after anthesis started in the fertile plants in the population. The florets of the male sterile plants remained open for up to two weeks. This gave the spikes a translucent appearance when backlighted, making the male sterile plants easily discernible from the fertile plants, as in barley (21).

Awned male sterile spikes had a "bushy" appearance because of the widely-spread awns. This feature was not so readily detected in awnless plants.

The male sterile plants were observed to flower later than the fertile plants, as noted by Suneson (45). This may have been due to not detecting them at a stage comparable to anthesis in fertile plants. Lodicule action was not observed to open the florets of the male sterile plants as it does in fertile plants at the time of anthesis. In barley the florets of the male sterile plants do not open until two or three days after anthesis when the ovary begins to swell (21). The male sterile plants would not be detected until this ovule swelling occurs and would appear to be "flowering" later than the fertiles in which flowering is detected by anther extrusion and floret opening.

The florets of the male sterile wheat spikes closed about ten days after first opening, similar to barley (21). This was probably due to the deterioration of the unfertilized ovary. The florets of receptive spikelets closed soon after pollination.

As the seeds in fertile spikes matured and filled, the florets began to open. At maturity, the fertile spikes were enlarged and the awns widely divergent because of the seeds within. At the same time, the unfertilized ovaries on male sterile spikes had deteriorated and collapsed, causing the florets to close. The awns on male sterile spikes were appressed to the rachis at maturity, giving the spike a narrow, slender appearance. An occasional seed in an open-pollinated floret caused a single awn on that floret to be sharply divergent.

The anthers of the male sterile plants did not extrude like those of fertile plants. The filaments failed to elongate and the stigmas were often observed protruding from the opened flowers. Male sterile plants could be identified after the florets had closed by the absence of the empty, extruded anthers hanging outside the flowers. In the field no anther extrusion was observed in male sterile plants. In the greenhouse, an occasional anther protruded from the open lemma and palea, similar to male sterile barleys (38).

The spikes of the male sterile plants remained upright because of the reduced seed set, while those of fertile plants bent under the weight of seeds. Male sterile spikes usually contained less than ten seeds while fertile spikes often had more than fifty seeds. These results are similar to male sterile and fertile barley observed at Bozeman (21).

Male sterile plants remained green and did not mature like nor-

mal fertile plants. The male steriles continued to produce secondary tillers with poorly-developed spikes which seldom set seed. Most of the tillers on fertile plants mature to a uniform height, while the tillers of male sterile plants were of greatly differing heights which gives a "clumpy" appearance reminiscent of many perennial grasses. The peduncles on male sterile plants were generally smaller than on fertile plants.

In both Cnn ms-5 and -6 F_2 populations, a random sample of five male sterile and five fertile plants averaged eighty-four cm and ninety-six cm in height, respectively. The consistent difference in heights of plants in Cnn ms-5 and -6 F_2 populations suggests a pleiotrophic effect; however, insufficient data preclude such a determination.

The mean heights of male sterile and fertile plants in three greenhouse-grown F_2 populations of Cnn ms-3/Crest//Oleson were not significantly different (Table 2, p. 42). A great deal of variation for height (37-93 cm) was observed in these three populations.

In general, the male sterile plants were less vigorous than their fertile siblings at maturity, but until anthesis there was no discernible difference. The apparent difference in later developmental stages may be due to the difference in seed set and the subsequent effects this has on the hormone balance within the plant.

Table 2. Comparisons of the mean height of fertile and male sterile plants in three F_2 populations of Cnn ms-3/Crest//Oleson. (Data from Appendix Table 1, p. 94.)

	F_2 #1		F_2 #1		F_2 #1	
	Fertile	Male Sterile	Fertile	Male Sterile	Fertile	Male Sterile
$\bar{X}$	63.22	67.7	70.72	55	67.9	69
N	18	6	11	3	14	2
$s^2_{\bar{X}}$	9.4	7.91	5.6	13	16.6	1
$t_{.05}$	2.110	2.571	2.228	4.303	2.160	12.706
t' tabulated‡	2.321		3.678		2.759	
t' calculated	1.077		3.645		.262	
	Accept H_o		Accept H_o		Accept H_o	

‡Equations for t' from Snedecor and Cochran (43); see also Statistical Methods.

Yield Components

Data from the preliminary study (see Preliminary Investigation, p. 36) indicated that the male sterile plants had fewer tillers per plant and fewer spikelets per spike than fertile siblings in Cnn ms-3 F_2 grown in 1973. Cnn ms-3 F_2 was a small heterogeneous population, therefore, these data were not considered to be conclusive.

The male sterile and fertile plants in Cnn ms-5 F_2 averaged 15.6 and 19.0 spikes per plant, respectively (Appendix Table 2, p. 95). The male sterile and fertile plants in this population had respective means of 15.6 and 17.5 spikelets per spike.

The male sterile plants in Cnn ms-6 F_2 averaged 12.2 spikes and the fertile plants 22.8 spikes (Appendix Table 2, p. 95). The male sterile and fertile plants in Cnn ms-6 F_2 had means of 15.2 and 19.7 spikelets per spike, respectively.

Comparisons of the fertile and male sterile plants in Cnn ms-5 F_2 with their respective counterparts in Cnn ms-6 F_2 were not significantly different for either spikes per plant or spikelets per spike (Appendix Table 3, p. 97).

The yield component data from male sterile and fertile plants in Cnn ms-5 F_2 were combined with similar data from Cnn ms-6 F_2 (Appendix Table 4, p. 98).

The resulting combined averages of 15.2 and 18.7 spikelets per spike for male sterile and fertile plants differed significantly (Table

3, p. 45). The fertile plants in both Cnn ms-5 and -6 F_2 had a significantly greater number of spikelets per spike than the male sterile plants.

The number of spikes per male sterile plant (13.7) did not differ significantly from spikes per fertile plant (21.1) in the combined populations. The variance of the mean of the combined data ($s^2_{\bar{X}}$) was more than ten times greater for the fertile plants (13.5) than it was for the male sterile plants (1.2). This variability did not permit a statistical separation of the means. Although the difference in the number of spikes was not statistically significant, the fertile plants averaged 50% more spikes than the male sterile plants in the combined populations.

Only well-developed spikes on each plant were counted in this study. Most of the spikes on fertile plants developed completely while the majority of the tillers on male sterile plants failed to develop normally and so were not counted. The reduction in the number of well-developed spikes, like the continued tiller initiation mentioned earlier, was possibly a result of imbalanced hormones in the plant due to reduced seed set.

The yield components of F_1's were measured to examine the effect of the male sterile genes in the heterozygous condition.

The F_1's from the cross of Cnn ms-3 F_2 male sterile by Oleson were intermediate in height between Oleson and Cheyenne (Figure 3, p.

Table 3. Comparisons of yield components of male sterile plants to fertile plants in the pooled populations, Cnn ms-5 and Cnn ms-6 F_2. (Data from Appendix Table 4.)

	Spikes/Plant		Spikelets/Spike	
	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants
$\bar{X}$	21.11	13.73	18.71	15.2
N	9	33	9	31
$S^2_{\bar{X}}$	13.47	1.23	.39	.24
$t_{.05}$	2.306	2.030	2.306	2.042
t' tabulated	2.283		2.205	
t' calculated	1.925		4.422	
	Accept H_o		Reject H_o	

47). They had more tillers and a greater total yield of grain than Oleson, the only parent tested for these characters. The F_1 heterozygotes and Oleson's Dwarf were grown as spring wheats which precluded a legitimate comparison to the winter wheat Cheyenne for yield because of the greatly different environments. The heterozygous plants appeared to be morphologically normal.

F_1 plants from the cross of Cnn ms-7 (male sterile) by the French cultivar VT 1307 (Figure 1, p. 21) were compared to the F_1 of a cross of VT 1307 by Cheyenne and to fertile plants from Cnn ms-6 F_2 and to VT 1307 grown in the same environment. Individual plants from these four populations were evaluated for plant height, spikes per plant, spikelets per spike, seeds per spike, kernel weight and total grain yield. These data are illustrated as Figure 4, p. 48).

The reciprocal heterozygotes were similar for all components except the number of spikes per plant and the number of seeds per spike. The heterozygotes approached the mid-parent values for spikelets per spike and kernel weight. The F_1 plants were slightly taller than either parent and approximately equal to the higher parent in total yield on a per plant basis. The heterozygous plants were very similar in morphology and maturity and appeared to be completely normal.

No abnormal morphological features were observed in the heterozygous plants studied. Measurements of several yield components revealed no gross detrimental effects from carrying the male sterile

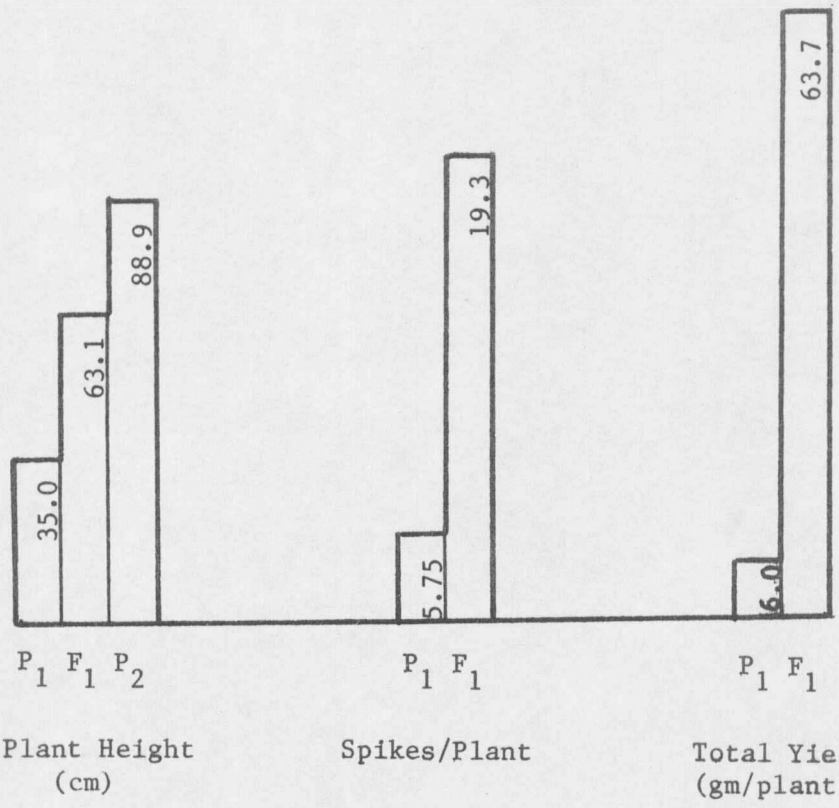

Figure 3. Histograms of several characteristics in the parents and F_1's of the cross Cnn ms-3/Oleson.

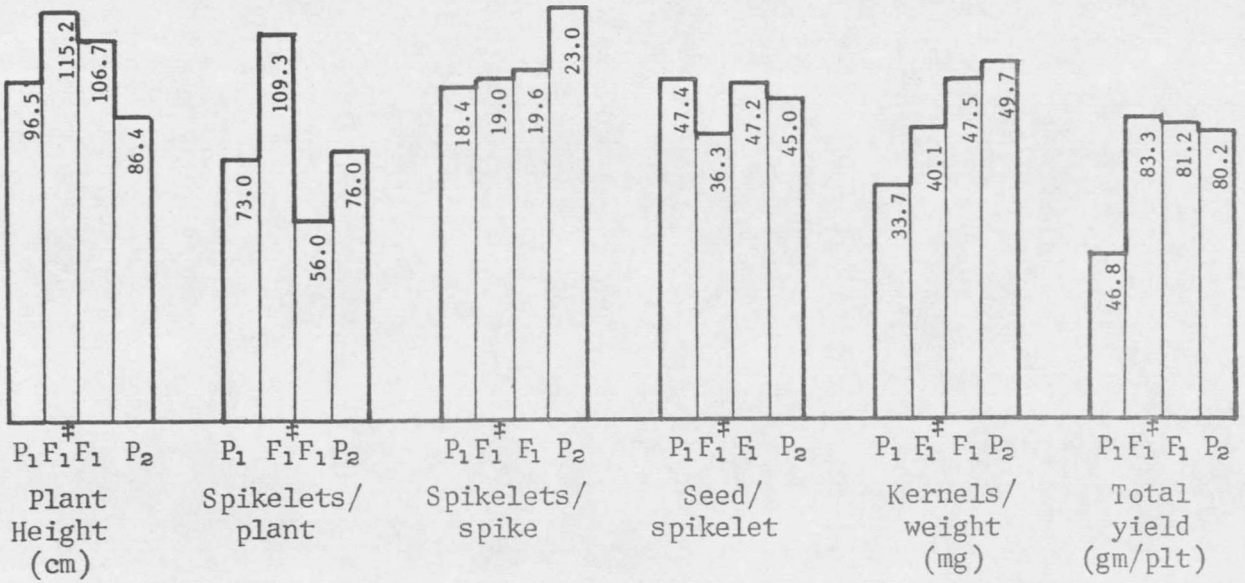

Figure 4. Histograms of several yield components of F_1's and parents in the crosses Cnn ms-7/VT 1307 and VT 1307/Cheyenne.

‡Female parent was a male sterile plant.

genes in the heterozygous condition in the populations studied.

Anther Morphology

The anther lengths of the male sterile plants in Cnn ms-3 F_2 (1.87 mm) and Cnn ms-6 F_2 (1.96 mm) were significantly shorter than the anthers of their fertile siblings (2.35 and 2.55 mm, respectively) (Table 4, p. 50). Cheyenne anthers (2.16 mm) were significantly smaller than those of fertile plants in Cnn ms-6 F_2 (2.55 mm), but were larger than male sterile anthers (1.96 mm) in the same population (Table 5, p. 51). Variability for anther size was noted in the materials studied (1.87-2.55 mm); however, the male sterile anthers were smaller than those of related fertile plants in both segregating populations (Figure 5, p. 52).

The anthers of male sterile plants were shrunken and shriveled at anthesis while those of fertile plants were enlarged and filled with pollen. The anthers of all field-grown and most greenhouse male sterile plants were four-lobed at maturity and contained no stromium. Male sterile anthers were a much paler yellow than fertile anthers and reduced filament elongation prevented anther extrusion from the florets at anthesis. When identified by floret opening, the ovaries of male sterile plants were noticably swollen but normal in other respects (Figure 5, p. 52).

Greenhouse-grown male sterile plants sometimes exhibited anther

Table 4. Comparisons of the mean anther length of male sterile plants to fertile siblings in Cnn ms-3 F_2 and Cnn ms-6 F_2 populations. (Data from Appendix Table 5.)

	Cnn ms-3		Cnn ms-6	
	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants
$\overline{X}$	2.35	1.87	2.55	1.96
N	20	22	23	20
$s^2_{\overline{X}}$	.001	.0005	.001	.002
$t_{.05}$	2.093	2.080	2.074	2.093
t' tabulated	2.089		2.087	
t' calculated	12.393		10.772	
	Reject H_0		Reject H_0	

Table 5. Comparisons of the mean length of the anthers of Cheyenne to male sterile and male fertile plants of Cnn ms-6 F_2.

	Cheyenne Cnn ms-6		Cheyenne Cnn ms-6	
	Normal	Fertile Plants	Normal	Male Sterile Plants
$\overline{X}$	2.16	2.55	2.16	1.96
N	13	23	13	20
$s^2_{\overline{X}}$	.001	.001	.001	.002
$t_{.05}$	2.16	2.74	2.16	2.093
t' tabulated	2.451		2.673	
t' calculated	8.720		3.65	
	Reject H_o		Reject H_o	

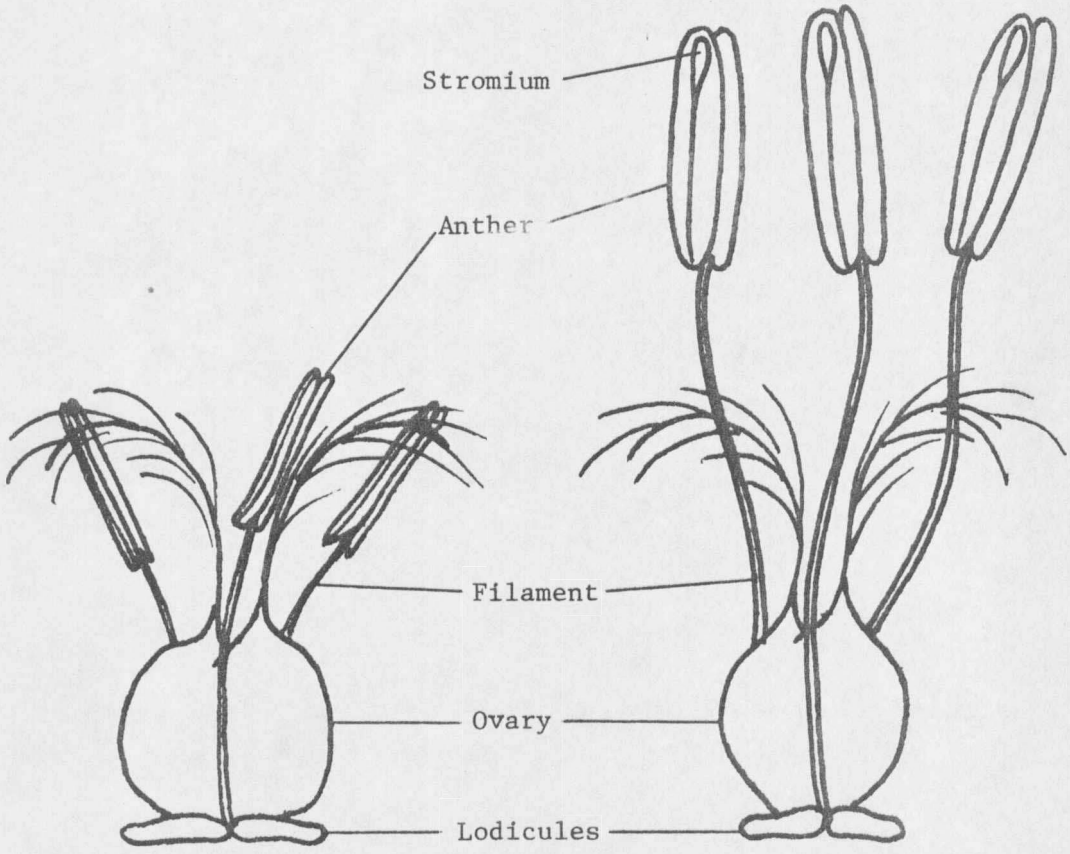

Figure 5. A comparison of the staminate and pistilate characteristics of male sterile (left) and normal, fertile wheat plants.

extrusion and some of the extruded anthers had a stromium. Pollen from these anthers was non-staining with aceto-carmine and IKI, and, therefore, sterile. No selfed seed was set in these greenhouse materials. The variability in the expression of male sterility in the greenhouse environment was consistent with observations reported in barley (38).

The anthers of male sterile plants often turned yellow before the florets opened while fertile anthers remained green until anthesis. Male sterile plants were identified prior to anthesis by this characteristic. With practice, this technique proved to be fairly reliable in locating male sterile plants before the florets opened to be contaminated by wind-borne pollen. Male sterile plants were identified up to five days before the florets opened by this procedure. Some size difference was also discernible at this time.

Cytology

Somatic Chromosomes

An examination of the somatic chromosomes of plants from F_2 populations of Cnn ms-5 and -6, known to segregate for male sterility, determined the role of aneuploidy in the male sterility.

Forty-nine of the fifty seedlings observed from the two populations contained the euploid compliment of forty-two chromosomes. A single plant of Cnn ms-6 F_2 was trisomic ($2n + 1 = 43$). This plant was fertile, had a normal seed set, and was morphologically indistinguish-

able from other fertile plants.

The forty-two testcross F_1 plants examined were all euploid with a chromosome number of 2n = 42.

Aneuploidy, then, was not a probable cause of the male sterility. The aneuploidy present was not associated with the male sterility.

Microsporogenesis

A complete, overlapping series of meiotic stages from premeiosis through mature pollen was observed for most of the male sterile and fertile plants in the sampled F_2 populations of Cnn ms-5 and -6. Both male sterile and fertile plants exhibited a predominantly normal meiosis. No evidence of chromosomal abnormalities was detected.

Some cells (less than 1%) showed a precocious division of chromosomes or a late division of chromosomes during anaphase I. These rare abnormal divisions occurred in both male sterile and fertile plants. A low frequency of such abnormal divisions is not unusual in normal wheat cultivars (24), and it is not likely that these misdivisions caused the complete male sterility in an allohexaploid such as wheat.

The meiotic divisions in the twenty-three random heterozygous plants studied were normal. Very few misdivisions (less than 0.5%) were observed and pairing at diakinesis was normal.

The male sterility studied does not appear to be the result of a

chromosomal abnormality.

Pollen development from the quartet stage to anthesis was also observed in the same materials. Pollen development of the heterozygotes was indistinguishable from that of normal fertile plants. The free microspores underwent two mitotic divisions to give rise to a single vegetative nuclei and two generative nuclei (7).

In the male sterile plants the quartets separated to form free microspores, but pollen development was arrested before the mitotic divisions occurred in the pollen. The nucleus and the cytoplasm disintegrated to an amorphous mass which disappeared completely. The empty exine was all that remained of each pollen grain (Figure 6, p. 56).

Mature Pollen

Mature pollen grains of male sterile and fertile plants from Cnn ms-5 and -6 F_2 and heterozygous plants from crosses made to male steriles in Cnn ms-3 were stained to detect chromatin and starch in the grains and a vital stain was used to determine potential viability. Cheyenne was used as the normal fertile check variety.

Pollen from Cheyenne and the fertile F_2 segregates and from the heterozygotes stained positively in the aceto-carmine (AC) and proprionic-carmine (PC) indicating the presence of chromatin. It also stained with the two IKI stains to show that starch had been stored in the

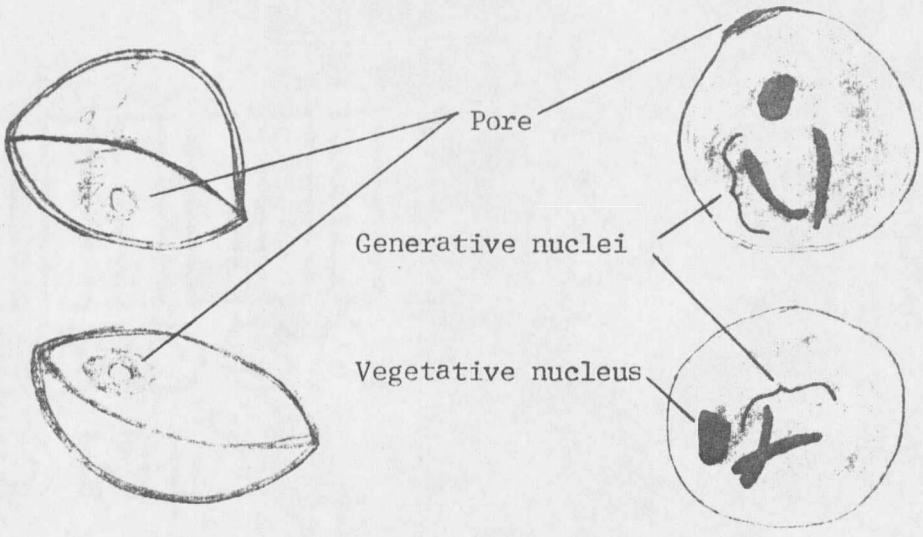

Figure 6. A comparison of the pollen grains of a male sterile plant (left) to those of a normal, fertile plant. Both samples were stained with proprionic carmine.

pollen grains. The fertile segregates from both F_2 populations and the heterozygotes gave similar reactions to the normal Cheyenne pollen used as a check in that all samples were classified as "stained."

The pollen from the male sterile plants was unstained with AC, PC or IKI. This pollen lacked chromatin and starch in the mature grains. It was therefore assumed to be incapable of germinating or effecting fertilization.

The fertile segregates, the heterozygotes and the normal Cheyenne, all gave a positive reaction in the TTC. The pollen from heterozygous plants stained uniformly, indicating no detrimental effect on the sporophytes containing the male sterile alleles.

Roath (40) stated that a positive test for chromatin and starch is not a guarantee that pollen is capable of fertilization, but a negative test is definite evidence that pollen is sterile.

The pollen of male sterile plants was completely devoid of cytoplasmic and nuclear contents at maturity. The grains were usually collapsed and shriveled, but they did contain a normal-sized pore(Figure 6, p. 56). The male sterility appeared to be due to a physiological disruption in the maturing stages of pollen development similar to that reported by Joppa et al. (26) in cytoplasmic male sterile wheat.

Self-fertility

In order to determine the degree and stability of the male ste-

rility within populations, spikes of male sterile plants from Cnn ms-5 and -6 F_2's were enclosed in glassine pollinating bags to prevent outcrossing. Several spikes of fertile plants in the same populations were also bagged to serve as treatment checks. The two populations did not differ for the mean seed set on selfed (34.5 and 41.2 seeds/spike) and open-pollinated fertiles (45.2 and 59.7 seeds/spike) and selfed male steriles (0.17 and 0.18 seeds/spike) (Table 6, p. 59). These data were combined for comparisons between fertile and male sterile plants in both populations.

Cnn ms-5 F_2 open-pollinated male sterile plants set significantly less seed (5.8 seeds) than Cnn ms-6 F_2 male steriles (13.3 seeds) Table 6, p. 56). The two field-grown populations were adjacent and flowered at about the same time. The pollen load should have been similar for both populations. The number of spikes per plant and spikelets per spike were not statistically different for the male sterile plants in both populations (Appendix Table 3, p. 97).

The means of the combined data from Cnn ms-5 and -6 F_2 populations for seed set on bagged fertile plants were compared to that of unbagged fertile plants in the same populations to determine the effect of bagging on seet set. Seed set on bagged spikes of fertile plants was compared to that of bagged male sterile plants in the same combined populations.

Unbagged fertile spikes had significantly higher amounts of seed

Table 6. Comparisons of the number of selfed and open-pollinated seeds set on fertile and male sterile plants in Cnn ms-5 F_2 with those in Cnn ms-6 F_2.

	Fertile Plants				Male Sterile Plants			
	Selfed		Open-Pollinated		Selfed		Open-Pollinated	
	Cnn ms-5	Cnn ms-6	Cnn ms-5	Cnn ms-6	Cnn ms-5	Cnn ms-6	Cnn ms-5	Cnn ms-6
$\bar{X}$	34.5	41.2	45.2	59.7	0.17	0.18	5.78	13.3
N	4	5	4	5	15	18	15	18
$s^2_{\bar{X}}$	2.08	22.94	13.72	24.44	.003	.006	.26	2.36
$t_{.05}$	3.182	2.571	3.182	2.571	2.145	2.110	2.145	2.110
t' tabulated	2.622		2.791		3.122		2.113	
t' calculated	1.339		2.347		.105		4.646	
	Accept H_o		Accept H_o		Accept H_o		Reject H_o	

for seed set on bagged fertile plants were compared to that of unbagged fertile plants in the same populations to determine the effect of bagging on seed set. Seed set on bagged spikes of fertile plants was compared to that of bagged male sterile plants in the same combined populations.

Unbagged fertile spikes had significantly higher amounts of seed than the bagged fertile spikes (53.3 and 38.2 seeds/spike, respectively) (Table 7, p. 61). The bagged male sterile spikes in the same populations averaged only 0.17 seeds per spike.

Bagging the spikes of fertile plants reduced the seed set by 25%. This reduction was probably due to an increase in humidity within the glassine bag which reduced pollen viability and/or an increase in temperature which may have caused ovule or embryo abortion.

A comparison of fertile spikes enclosed in glassine crossing bags with bagged male sterile spikes demonstrated the male sterility effect alone. Only twelve seeds were set on a total of seventy male sterile spikes, while the bagged fertile plants in the same populations averaged 38.2 seeds per spike. The twelve seeds produced were probably the result of contamination before the spikes were bagged, or defective bags which allowed pollen entry. All the male sterile plants which had seeds in bagged spikes had other bagged spikes with no seed in them.

A study examining the origin of the twelve seeds from the selfed male steriles was terminated when a germination chamber overheated

Table 7. Comparisons of the seed set on bagged fertile spikes with that of open pollinated fertile spikes and bagged male sterile spikes from Cnn ms-5 and -6 F_2. (Data from Appendix Table 7.)

Paired t Test			
Fertile Plants			
Open-Pollinated	Selfed		Difference
54.4	34.0		20.4
38.4	31.0		7.43
40.1	35.0		5.14
48.0	38.0		10.0
43.5	33.0		10.5
70.0	42.0		28.0
56.0	28.0		28.0
70.0	50.0		20.0
59.0	53.0		6.0
$\bar{X}$ 53.27	38.22	Σ	135.47
		N	9
		$\bar{X}$	15.05
$t = \frac{\bar{D}}{s_{\bar{D}}} = \frac{15.05}{3.06} = 4.918$		R	5.14-28
		ΣX^2	2712.03
		CF	2039.12
.005>P>.001		s_X^2	84.11
		s_X	9.17
Reject H_o		$s_{\bar{X}}^2$	9.35
		$s_{\bar{X}}$	3.06
		CV	.609

t' Test		
Selfed Spikes		
	Fertile	Male Sterile
$\bar{X}$	38.22	0.177
N	9	33
$s_{\bar{X}}^2$	8.10	.0026
$t_{.05}$	2.306	2.030
t' tabulated	2.306	
t' calculated	13.365	
Reject H_o		

and destroyed the germinating seeds.

It was concluded from pollen examinations (see Mature Pollen, p. 55) and self-fertility studies that the male sterile plants are completely male sterile as a result of producing only inviable pollen.

Ovule Fertility

The lack of seed set on the male sterile spikes could have been caused by reduced female fertility as well as male sterility.

A test of the ovule fertility was made by pollinating bagged spikes of male sterile plants in Cnn ms-5 and -6 F_2 populations with pollen from normal, fertile cultivars.

The mean of 13.4 seeds per crossed male sterile spike was significantly greater than the mean of 0.18 seeds per unpollinated spike. Paired comparisons involved spikes from the same plants (Table 8, p. 63).

Ovule fertility was further examined by comparing the average seed set on crossed spikes of male sterile plants to that of crosses made to closely related emasculated fertile plants.

Male sterile plants in Cnn ms-5 and -6 F_2 had similar numbers of seeds per crossed spike (14.1 and 15.8, respectively; Appendix Table 9, p.105). Data from crossed male sterile plants in the two populations were combined to compare the female receptivity of male sterile plants and emasculated fertile plants.

The number of seeds per crossed male sterile spike (15.3) was

Table 8. Paired comparisons of the mean number of selfed and crossed seed set per spike on male sterile plants.

Plant Number	Number of Selfed Seeds/Spike	Number of Crossed Seeds/Spike	Difference
Cnn ms-5 -2	0	20	20
-3	0.5	17	16.5
-4	0.25	14.3	14.05
-5	0.3	2	1.7
-7	0.5	17	16.5
-9	0	19	19
-10	0	10	10
-14	0.5	14	13.5
-15	0	2	2
Cnn ms-6 -1	0	7.3	7.3
-2	1	11	10
-3	0	1	1
-5	0	19	19
-6	0	24.5	24.5
-7	0	20	20
-8	0	20	20
-9	0	19	19
-13	0	13	13
-14	0	8.5	8.5
-15	0	17	17
-17	0	16	16
-18	0.5	15.3	14.8
-19	0.5	1	.5
$\bar{X}$	0.176	13.39	

$t = \frac{\bar{D}}{s_{\bar{D}}} = \frac{13.21}{1.45} = 9.110$

$P < .001$

Reject H_o

Σ	303.85
N	23
$\bar{X}$	13.21
R	.5 - 24.5
Σx^2	5074.12
CF	4014.12
s_x^2	48.18
s_x	6.94
$s_{\bar{X}}^2$	2.09
$s_{\bar{X}}$	1.45
CV	.525

significantly greater than seeds per crossed, emasculated spike (12.9) (Table 9, p. 65).

These data indicated no reduction in ovule fertility of male sterile plants compared to emasculated fertile plants. The 20% greater seed set on the male sterile plants suggested that some damage to the ovaries occurred during the emasculation of fertile plants.

The kernel weight of open-pollinated fertile plants was significantly higher than open-pollinated male sterile plants in Cnn ms-5 F_2 (Table 10, p. 66). In Cnn ms-6 F_2, however, the open-pollinated male sterile plants had significantly larger seeds. The kernel weights of crossed seed on male sterile and fertile plants were similar (17.7 and 15.0 mg, respectively) indicating no detrimental effects of the male sterile condition on seed development.

The lower seed weight on the male sterile plants in Cnn ms-5 F_2 cannot be explained from the data and may be due to a genetic difference in the two populations. The larger seeds on male sterile plants in Cnn ms-6 F_2 are probably due to the fact that there are fewer seeds in the male sterile spikes (Table 7, p. 61) which results in larger seed through component compensation.

Inheritance

Gametic Frequencies

In five random testcrosses of fertile heterozygotes onto male

Table 9. Seed set upon crossing male sterile spikes to normal cultivars compared to that of emasculated fertile spikes in similar crosses. (Data from Appendix Tables 8 and 10.)

	Fertile Plants	Male Sterile Plants
$\bar{X}$	12.87	15.31
N	23	42
$s^2_{\bar{X}}$	.37	1.54
$t_{.10}$‡	1.717	1.684
t' tabulated	1.690	
t' calculated	1.765	
	Reject H_o	

‡Note that t is being tested at P = .10.

Table 10. Comparisons between the kernel weight of open-pollinated fertile and male sterile plants in Cnn ms-5 and -6 F_2's and between crossed seed on male sterile plants and emasculated fertiles in Cnn ms-6 F_2. (Data from Appendix Table 11.)

	Cnn ms-5		Cnn ms-6			
	Open-pollinated		Open-pollinated		Crossed	
	Fertile	Male Sterile	Fertile	Male Sterile	Fertile	Male Sterile
$\overline{X}$	32	29.6	26.6	35.0	15	17.68
N	2	14	3	24	2	5
$s^2_{\overline{X}}$	0.04	.72	.69	2.74	1.96	4.25
$t_{.05}$	12.706	2.160	4.303	2.069	12.706	2.776
t' tabulated	2.720		2.518		5.910	
t' calculated	2.753		4.536		1.075	
	Reject H_o		Reject H_o		Accept H_o	

sterile plants, the transmission of the male sterile through the pollen was 1.7 fertile gametes to each gamete with the male sterile factor (Table 11, p. 68). The female transmission was 2.0 gametes with the fertile genotype to each gamete with the male sterile (Table 12, p.69). These two ratios were not statistically different from the ratio of 2:1 (Table 13, p. 70). It was concluded that the transmission of the male sterile was equal in both male and female gemetes in heterozygous plants.

The 2:1 ratio of fertile to male sterile gametes in both pollen and ovule did not fit the 1:1 or 3:1 ratios expected for single recessive genes or two complimentary recessive genes (Table 14, p. 71). Because the same ratio occurred in both male and female gametes, the 2:1 ratio was thought to be the result of a meiotic occurrence.

Gene Action and Number

The genes responsible for the male sterile phenotype appeared to act in the homozygous, recessive condition. All F_1 plants were completely fertile and all F_2's segregated for male sterility in a ratio approximating 8:1 (Table 15, p. 72). One or both of these phenomena would not have occurred with dominant or epistatic genes causing the male sterility.

The number of genes governing the male sterility is not immediately obvious from the testcross (Table 11, p. 68) or F_2 data (Table 15, p. 72). The total of all the F_2 populations gave a ratio of 7.8

Table 11. Transmission of the male sterile through the male gametes (pollen).

Male Sterile/F_1 (Pedigree)	Number of Fertile Plants	Number of Male Sterile Plants
Cnn ms-3-13-8/Cnn ms-3-10-12	5	3
Cnn ms-3-22-1/Cnn ms-3-4-5	11	1
Cnn ms-3-8-3/Cnn ms-3-12-3	3	2
Cnn ms-3-8-3/Cnn ms-3-19-1	1	4
Cnn ms-3-4-4/Cnn ms-3-12-2	4	4
Total	24	14
Ratio	1.71:1	

Table 12. Transmission of the male sterile through the female gametes (ovules).

F_1/Normal Cultivar Pedigree (F_2 ppm No)	Number of Fertile Plants	Number of Male Sterile Plants	Ratio (F:S)
Cnn ms-3-5-3/Oleson(1)‡	18	6	3:1
(3)	13	0	
(6)‡	11	3	3.7:1
(7)‡	14	2	7:1
(8)	19	0	
(9)	13	0	
(10)	24	0	
(23)	18	0	
(26)	15	0	
(30)	29	0	
Cnn ms-3-5-5/Oleson(1)	9	0	
(2)	17	0	
(4)‡	7	5	1.4:1
(5)	11	0	
(7)‡	15	4	3.7:1
(9)	21	0	
(10)	23	0	
(11)‡	14	2	7:1

	Nonsegregating:Segregating
	12:6
Ratio	2:1

‡Segregating for male sterility.

Table 13. Comparisons of the ratio of transmission of the male sterile through the male gametes to that of the female gametes (2:1).

Class	Number Observed	Number Expected	X^2	Expected Ratio
Fertile	24	25.33	.48	2
Male Sterile	14	12.67	.10	1
		Total	.58	
	Degrees of freedom		1	
			.5>P>.25	
			Accept H_o	

Table 14. Comparison of gametic transmission frequencies to expected monogenic and digenic complimentary ratios. (Data from Tables 11 and 12.)

	Observed Ratio	Expected One-Gene Ratio (1:1)	X^2	Expected Two-Gene Ratio (3:1)	X^2
Fertile	36	28	2.29	42	.86
Male Sterile	20	28	2.29	14	2.57
		Total	4.58		3.43
	Degrees of freedom		1		1
			.05>P>.025		.1>P>.05
			Reject H_o		Reject H_o

Table 15. Segregations for male sterility in F_2 populations.

Pedigree of F_2 Population	Number of Fertile Plants	Number of Male Sterile Plants	Ratios (F:S)		
			3:1	8:1	15:1
Cnn ms-3/Oleson-8	33	5		.75-.50	
-10	53	6		.90-.75	
Cnn ms-3/Oleson-2	48	3			>.90
-4‡	36	5		.90-.75	
-5	37	5		.90-.75	
-8	37	7		.50-.25	
-9	54	10		.50-.25	
-10	25	3		>.90	
Cnn ms-3/SIB-1	58	4			>.90
-2	50	10		.25-.10	
-3	34	4		>.90	
-8	36	8	.50-.25		
/Crest-3	43	3			>.90
/VT1307-3‡	23	4		.75-.50	
/OP-7-3	28	1			.75-.50
-11-1	35	6		.50-.25	
-12-1	29	3		.90-.75	
-2	23	4		.75-.50	
-3	53	6		.90-.75	
-13-2	51	7		.90-.75	
-14-2	25	6	.50-.25		
-16-1	49	5		.75-.50	
-3	51	11	.25-.10		
-19-6	37	5		.90-.75	
-8‡	35	5		.90-.75	
-20-1	33	3		.75-.50	
-21-1	52	4			.90-.75
Cnn ms-3 F_2‡	119	17		.75-.50	

(Continued)

Table 15. (Continued.)

Pedigree of F_2 Population	Number Fertile Plants	Number of Male Sterile Plants	Ratios (F:S)		
			3:1	8:1	15:1
Cnn ms-5 F_2 ‡‡	119	15		>.90	
-6 F_2 ‡‡	155	26		.25-.10	
Cnn ms-3/SIB-3-2	13	8	.25-.10		
/OP-15-1	39	15	.75-.50		
-19-5‡	25	8	>.90		
/Crest-5-5	51	2			.50-.25
/OP-10-1	59	3			.75-.50
-19-3‡	51	3			.90-.75
TOTAL	1699	240		.10-.05	

‡Segregating for winter habit.

‡‡Homozygous for winter habit.

fertile plants to each male sterile plant. Using 1/9 as the expected frequency, Mather's (31) equation for calculating the population size required to give a 90% probability of at least one male sterile in a population required that twenty or more individual plants be examined.

$$\text{i.e., } (8/9)^N = 1/10$$

$$N\log 8/9 - \log 1/10$$

$$N(0.051152) = -1$$

$$N = 19.5$$

Thirty-six of the F_2 populations grown had the necessary number of plants (Table 15, p. 72). Chi-square tests for the ratios of 3:1, 8:1, and 15:1 gave twenty-two F_2 populations which most closely fit an 8:1; eight fit a 15:1; and six fit a 3:1. The total of the thirty-six populations best fit the 8:1 ratio, although with a low probability ($.10>P>.05$). All of those populations which gave acceptable 3:1 ratios were a result of sibling crosses or open-pollinations. Eight of the F_2 populations produced from hand cross pollinations gave 8:1 segregations and three gave 15:1 segregations. These segregations provide evidence that two loci may be involved in the expression of male sterility (i.e., 15:1's), but that some factor gives a consistently greater proportion of male sterile plants than would be expected from two independent loci (i.e., 8:1's). A single locus controlled the male sterile condition in certain instances (i.e., 3:1's).

A Punnett square constructed from the gametic transmission data

(Table 13, p. 70) predicted a 8:1 segregation ratio, assuming dominance of the fertile alleles.

♀ \ ♂	2 Fertile (F)	1 Male Sterile (MS)
2 Fertile (F)	4 F F	2 F MS
1 Male Sterile (MS)	2 F MS	1 MS MS

Genotype	Phenotype
4 F F	8 Fertile
4 F MS	
1 MS MS	1 Male Sterile

F_3 families from the above F_2 fertile plants should occur in a ratio of one segregating family for each nonsegregating family. Among thirty F_3 families from fertile plants in Cnn ms-3 F_2 grown in 1975, twelve segregated for male sterility and eighteen did not (Appendix Table 12, p.109). These data were not significantly different from the expected 1:1 based on the Punnett Square (Table 16, p. 76).

The gametic transmission data, the F_2 data and the F_3 data were consistent enough to propose a model which best explains the observed segregations for male sterility. The predominance of the observed 8:1 ratio in the F_2 populations and the prediction of this ratio from gametic transmission data suggest that it is the actual ratio. Addi-

Table 16. Test of the goodness of fit of the F_3 segregations to the expected 1:1 ratio. (Data from Appendix Table 12.)

Class	Number Observed	Number Expected	X^2	Ratio
Segregating	12	15	.6	1
Non-segregating	18	15	.6	1
Total	30	30	1.2	2
		Degrees of freedom	1	
		.50>P>.25		
		Accept H_o		

tionally, the occurrence of both 3:1 and 15:1 ratios offer evidence that two distinct, duplicate genes were involved in producing the male sterility.

Two duplicate genes can give more than the expected number of homozygous recessives with coupled linkage. The combined data from thirty homogeneous F_2 populations (Appendix Table 13, p.110) were put into Allard's (2) maximum likelihood formula for a 15:1 ratio to calculate linkage intensity.

i.e., $(a + b + c)\left(\frac{-2P}{4-P^2}\right) + d\left(\frac{2}{P}\right) = 0$ (Repulsion)

$(a + b + c)\left(\frac{2(1-P)\ddagger}{3 + 2P - P^2}\right) + d\left(\frac{2}{P - 1}\right) = 0$ (Coupling)

at $P = .30$

$1461(.3988) + 201(-2.857) = \text{Score}$

$582.6 - 574.2 = \text{Score}$

$+8.3898 = \text{Score}$

Information per plant (ip) = 1.140

Information for the population (Ip) = 1.140 x 1662

$Ip = 1894.68$

$\text{Correction} = \frac{\text{Score}}{Ip}$

$\text{Correction} = \frac{8.3898}{1894.68}$

$P = +.0044$

‡For coupling, $P = (P - 1) \times -1$.

A Punnett square was constructed using the crossover value of .304 to predict F_2 genotypes and F_3 segregation ratios (Table 17, p. 78). The predicted F_3 segregations were then compared to actual data (Table 18, p. 79). For this comparison, segregation ratios less than 8:1 were classified as 3:1's and the expected 44:1 class was combined with the nonsegregating class because of the improbability of identifying this population with the numbers of plants grown. All other segregations were classified as 8:1's due to population size and heterogeneity of the segregations. The predicted numbers were not significantly different from the actual numbers and so the hypothesis that two coupled, complimentary genes cause the male sterility being studied was accepted. All of the data supported this theory, although some of the probabilities were low (i.e., .25-.10).

Cytoplasmic Influence

The cytoplasms of some species are known to influence the expression of male sterility (18). Crosses of heterozygous plants to emasculated plants with unrelated cytoplasms were made to determine if the male sterility would be expressed or altered in any way.

Several of the F_2 populations from crosses to the three different cytoplasms segregated for male sterility (Table 19, p. 80). No partial sterility was observed in any of the populations. Some very stunted plants with abnormal spikes appeared to be aneuploids, but

Table 17. Punnett square used to predict F_2 genotypes and F_3 segregations with a crossover value of P = .304.

♀ \ ♂	AB‡ (.348)	Ab (.152)	aB (.152)	ab (.348)
AB (.348)	.121	.053	.053	.121
Ab (.152)	.053	.023	.023	.053
aB (.152)	.053	.023	.023	.053
ab (.348)	.121	.053	.053	.121

F_2 Genotypes ($Gamete_1$/$Gamete_2$)	% of Population	F_3 Segregations
AB/AB	12.1	—
AB/Ab	10.6	—
AB/aB	10.6	—
AB/ab	24.2	8:1
Ab/Ab	2.3	—
Ab/aB	4.6	44:1‡‡
Ab/ab	10.6	3:1
aB/aB	2.3	—
aB/ab	10.6	3:1
ab/ab	12.1	male sterile in F_2

‡Symbols A and B used to designate the two genes involved, aabb is the male sterile.

‡‡The only male steriles in this F_3 would be the result of one-fourth of the double crossovers which would occur 2.3% of the time with this linkage intensity (see above table).

Table 18. Test of the goodness of fit of the F_3 segregations predicted from the linkage model to actual data. (Data from Appendix Table 12.)

Class	Observed Numbers of Families	Expected Numbers of Families	X^2	Expected Ratio
Non-segregating‡	18	14.5	.845	48.3
8:1	9	8.25	.068	27.5
3:1	3	7.25	2.491	24.2
Total	30	30	3.404	
		Degrees of freedom	2	
			.25>P>.10	
			Accept H_o	

‡Includes the 44:1 class.

Table 19. Test of the influence of other cytoplasms on the expression of male sterility.

Pedigree (F_2 No)	Number of Fertiles	Number of Male Steriles	Expected Ratio
Rescue/Cnn ms-3-10-1 (21)	13	0	--
(4)	16	0	--
(6)‡	15	2	7.5:1
(14)	13	0	--
(17)	8	0	--
Nugaines/Cnn ms-3-4-8 (2)‡	7	1	7:1
(3)	21	0	--
Oleson/Cann ms-3-10-1 (4)	21	0	--
(5)	14	0	--
Oleson/Cnn ms-3-5-3 (4)‡	14	1	14:1
(5)	12	0	--
Oleson/Cnn ms-3-4-8 (1)	17 + 4‡‡	0	--
(2)	12 + 6‡‡	0	--

‡Families segregating for male sterility.

‡‡Plants are fertile but appear to be aneuploids.

produced viable pollen and set seed.

The cytoplasmic background did not appear to affect the male sterility. The 7:1 and 14:1 ratios of fertile to male sterile plants were consistent with those observed in many of the F_2 populations (Table 15, p. 72).

Lethality

A seed or seedling lethal character associated with the male sterility may have been responsible for the 8:1 segregations of F_2's and the 2:1 segregations in testcrosses by eliminating certain genotypes from the populations.

Laboratory germination tests of seed lots from Cnn ms-5 and -6 F_2's gave 96% and 100% germination, respectively. All germinated seeds had normal-appearing coleoptites and primary roots. All the seedlings grew to maturity and a segregation for male sterility was observed in both populations. No lethality or abnormalities of any kind were detected in these tests.

Linkages

A linked marker gene would be helpful in locating male sterile plants in a population since male sterile plants cannot be detected until flowering.

Based on F_2 and/or F_3 data, the male sterility segregated independently of awns (Appendix Table 15, p.112), chaff color (Appendix

Table 16, p.113), stem solidness (Appendix Table 17, p.114), and growth habit (Appendix Table 18, p.115). The only genes for which linkage was detected are the male sterile genes themselves (see Gene Action and Number, p. 67).

Potential Use

The general morphological and cytological aspects of the male sterile mutant stock evaluated in this study are similar to many of the known genetic male sterile mutants in barley and wheat. Genetically, it differs in that all of the known ms genes in barley act as monogenic recessives while the mutant evaluated was controlled by two recessive, duplicate genes. Although the investigated male sterility did not segregate in the desired ratio of three fertiles to one male sterile, the observed ratio produces reasonable numbers of male sterile plants in manageable populations.

The mutant male sterile wheat studied had some characteristics which make it suited for use in a male sterile facilitated recurrent selection program. It was completely male sterile in the homozygous recessive condition, insuring 100% outcrossing on male sterile plants. The heterozygotes showed no detrimental effects of carrying the allele and produced pollen normally. Some lines (Cnn ms-6 F_2) had a high degree of cross pollination under normal conditions in Montana.

The male sterile plants were easily distinguished from the

fertile plants at flowering and at maturity; two stages in which identification may be necessary in a breeding program.

The male sterile plants also would be useful in a conventional crossing program. Twenty percent more crossed seed was set on male sterile plants when compared to crossed seed set on emasculated plants of the same background (15.3 vs. 12.9 seeds). The crossed seed was slightly larger on the male sterile plants (17.7 vs. 15.0 mg). One line (Cnn ms-6 F_2) set seed on open-pollinated spikes almost as well as it did on hand-crossed spikes (13.3 vs. 15.8 seeds). The seed on the open-pollinated spikes was considerably larger than crossed seed set on emasculated spikes (17.7 vs. 35 mg) (Appendix Table 19, p.116).

It takes approximately fifteen minutes for an experienced technician to emasculate one spike of a normal wheat cultivar, and another five minutes for the actual crossing. About .65 seeds are produced per minute of labor input (average seed set 12.9 seeds/cross). With male sterility, the fifteen minute emasculation is completely eliminated, and the only time-consuming operation is the pollination itself. About three crossed seeds are produced on these plants per minute of labor (mean seed set 15.3 seed/cross). Using male sterile plants for females in crossing is approximately $4\frac{1}{2}$ times as efficient as using emasculated materials. Such crosses to male steriles are useful in a backcrossing program because the F_2 will again segregate for male sterility.

The male sterile studied does not lend itself directly to hybrid

development because of its digenic nature (8,25,51). However, it could make a very useful addition to a breeding program producing hybrid cultivars as a means of evaluating a wide range of materials for heterosis. Because the male sterility is a result of a recessive condition, all heterozygous plants are completely fertile and may be evaluated for yield.

Genetic male sterility, such as the mutant studied, might also be incorporated into the existing aneuploid stocks being used for genetic studies in wheat. Lines of aneuploids segregating for male sterility would provide a much greater opportunity to increase the size of the F_1 populations being studied. The aneuploid stocks with the male sterile genes could be easily maintained by selecting several fertile plants in each population segregating for the male sterility, but homozygous for the aneuploidy. Because meiosis is normal in the male sterile plants, aneuploid plants in a segregating population could be identified cytologically, if necessary. Marker genes on the critical chromosome and a preflowering marker gene linked to the male sterile gene should make such a system quite efficient.

The male sterile mutant studied is potentially useful in a conventional variety development program as a female parent in crosses. It could be used in a male sterile facilitated recurrent selection population as an outcrossing mechanism. The male sterility may also be incorporated into existing aneuploid stocks to increase their use-

fulness by eliminating the necessity of emasculation. It could be a helpful tool in evaluating heterotic combinations in conjunction with a hybrid-development program, or may be used directly in a hybrid program as a female line if the proper mechanisms are evolved.

SUMMARY

The male sterile plant discovered in the Cheyenne winter wheat backcross populations in 1974 apparently was the result of a spontaneous mutation. The six F_1 plants grown from the seed on the original male sterile plant were fertile and segregated for male sterility in the F_2 generation, indicating that it was controlled by a recessive genetic mechanism. Based on gametic transmission ratios and F_2 and F_3 segregations, two coupled, complimentary, recessive genes are responsible for the expression of male sterility and the 8:1 segregation observed.

Male sterile plants were morphologically similar to fertile plants until anthesis. At flowering, male sterile plants were identified by widely-opened florets and the lack of extruded anthers. The anthers were about 25% smaller than normal, fertile anthers and did not shed pollen. Almost no seed was set on enforced selfed spikes of male sterile plants.

Mature male sterile plants had numerous poorly-developed tillers, and greatly reduced seed set in the well-developed main spikes. Ergot sclerotia were sometimes present in the florets of the male sterile plants.

The male sterility was very similar to known genetic male sterilities in wheat and other crops in many respects. Meiosis was normal up to the point at which microspore degeneration occurred. The abnor-

mal microspore development was stable in several environments. Male sterility was complete with no viable pollen being produced on the male sterile plants. Female fertility was unimpaired, with a normal seed set resulting from artificial cross-pollinations. Aneuploidy was not associated with the pollen sterility. Diverse cytoplasms did not affect the expression or segregation of the male sterility.

Heterozygous plants were completely fertile and phenotypically normal. No detrimental effects of the male sterile alleles in the heterozygous condition were detected.

This source of male sterility has the potential for eliminating emasculation in producing artificially crossed seeds. The number of crossed seeds set on male sterile spikes was greater than that on emasculated heads. About 25% as much time is involved in creating a crossed seed with the male sterile plants compared to normal cultivars which require emasculation.

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APPENDIX

Appendix Table 1. Heights of male sterile and fertile plants in Cnn ms-3/Crest//Oleson.

	F_2 #1		F_2 #6		F_2 #7	
	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants
	80, 68	72	66,78	50	55,37	68
	80, 56	70	77,75	53	74,52	70
	86, 82	56	72	62	93, 63	
	73, 55	76	75		52, 74	
	52, 53	65	74		82, 79	
	52, 59	67	53		79	
	72, 64		60		76	
	62, 53		73		76	
	43, 48		75		58	
Σ	1138	406	778	165	950	138
N	18	6	11	3	14	2
$\overline{X}$	63.22	67.7	70.72	55	67.9	69
R	43-86	56-76	53-78	50-62	37-93	68-70
Σ_x^2	74818	27710	55642	9153	67494	9524
CF	71946.9	27472.67	55025.8	9075	64464.3	9522
s_x^2	168.9	47.47	61.62	39	233	2
s_x	13	6.89	7.85	6.24	15.3	1.4
$s_{\overline{X}}^2$	9.4	7.91	5.6	13	16.6	1
$s_{\overline{X}}$	3.06	2.81	2.37	3.6	4.1	1
CV	.206	.102	.111	.113	.225	.020

Appendix Table 2. Yield components for each plant sampled in Cnn ms-5 and -6 F_2 populations in 1976.

	Cnn ms-5				Cnn ms-6			
	Spikes/Plant		Spikelets/Spike		Spikes/Plant		Spikelets/Spike	
	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants
	22	14	18	14.5	21	20	18.5	15.7
	16	27	17.25	15.3	20	13	19	15.5
	23	15	18	15.7	9	16	17.5	17.5
	15	18	16.6	15.1	36	19	22	17.7
		20		14.8	28	12	21.5	16.4
		10		14.5		7		13
		13		15.2		31		15.8
		23		15.7		5		14.3
		15		16.2		11		13.3
		19		15.6		1		16
		12		17		9		14.7
		16		15.8		7		12.5
		3		16.3		9		16
		16		16.4		12		14.5
		13		15.6		16		15
						9		15.7
						11		
						11		
Σ_x	76	234	69.85	233.7	114	219	98.5	237.6
N	4	15	4	15	5	18	5	16
$\bar{X}$	19	15.6	17.5	15.58	22.8	12.17	19.7	15.22

(Continued)

Appendix Table 2. (Continued.)

	Cnn ms-5				Cnn ms-6			
	Spikes/Plant		Spikelets/Spike		Spikes/Plant		Spikelets/Spike	
	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants
R	15-23	3-27	16.6-18	14.5-17	9-36	1-31	17.5-22	12.5-17.7
Σ_x^2	1979	4092	1221.12	3648.11	3002	3421	1955.75	3741.34
CF	1444	3650.4	1219.75	3641.01	2599.2	2664.5	1940.45	3708.81
s_x^2	178.3	31.54	.46	.5	100.75	44.5	3.82	2.17
s_x	13.35	5.62	.68	.71	10.04	6.67	1.96	1.47
$s_{\bar{X}}^2$	44.58	2.10	.11	.03	20.15	2.47	.76	.14
$s_{\bar{X}}$	6.77	1.45	.34	.18	4.49	1.57	.87	.37
CV	.703	.360	.039	.046	.440	.458	.099	.097

Appendix Table 3. Comparisons of the yield components of male sterile and fertile plants in Cnn ms-5 F_2 with those of Cnn ms-6 F_2. (Data from Appendix Table 2.)

	Spikes/Plant				Spikelets/Spike			
	Fertile Plants		Male Sterile Plants		Fertile Plants		Male Sterile Plants	
	Cnn ms-5	Cnn ms-6	Cnn ms-5	Cnn ms-6	Cnn ms-5	Cnn ms-6	Cnn ms-5	Cnn ms-6
$\bar{X}$	19	22.8	15.6	12.17	17.5	19.7	15.58	15.22
N	4	5	15	18	4	5	15	16
$s^2_{\bar{X}}$	44.58	20.15	2.10	2.47	.11	.76	.03	.14
$t_{.05}$	3.182	2.776	2.145	2.110	3.182	2.776	2.145	2.131
t' tabulated	33.197		2.283		2.827		2.133	
t' calculated	1.567		1.401		2.359		.873	
	Accept H_o		Accept H_o		Accept H_o		Accept H_o	

Appendix Table 4. Pooled data on yield components for male sterile and fertile plants in Cnn ms-5 and -6 F_2 populations. (Data from Appendix Table 3.)

	Spikes/Plant		Spikelets/Spike	
	Fertile Plants	Male Sterile Plants	Fertile Plants	Male Sterile Plants
Σ_5	76	234	69.85	233.7
Σ_6	114	219	98.50	237.6
Σpooled	190	453	168.35	471.3
N_5	4	15	4	15
N_6	5	18	5	16
Npooled	9	33	9	31
Σ^2_{x5}	1979	4092	1221.12	3648.11
Σ^2_{x6}	3002	3421	1955.75	3741.34
Σ^2_xpooled	4981	7513	3176.87	7389.45
$\bar{X}p$	21.11	13.73	18.71	15.2
CFp	4011.11	6218.45	3149.08	7165.28
$s^2_x p$	121.24	40.45	3.47	7.47
$s_x p$	11.01	6.36	1.86	2.73
$s^2_{\bar{X}} p$	13.47	1.23	.39	.24
$s_{\bar{X}} p$	3.67	1.11	.62	.49
CVp	.522	.464	.099	.180

Appendix Table 5. The mean length (in mm.) of twenty anthers per head in field-grown wheat F_2 populations in 1976.

	Cheyenne	Cnn ms-3 (Spring)		Cnn ms-6 (Winter)	
	Normal Anthers	Fertile Anthers	Male Sterile Anthers	Fertile Anthers	Male Sterile Anthers
				2.4	
	2.2, 2.1	2.2, 2.2	1.9, 1.9	2.3, 2.9	2.1, 2.1
	2.2, 2.2	2.7, 2.2	1.9, 1.7	2.4, 2.6	1.7, 1.9
	2.1	2.6, 2.3	1.7, 2.0	2.3, 2.5	2.2, 1.8
	2.2	2.5, 2.2	1.8, 2.0	2.3, 2.5	2.1, 2.3
	2.5	2.4, 2.4	1.9, 2.0	2.5, 2.7	1.7, 1.9
	2.1	2.5, 2.2	1.8, 1.8	2.5, 2.6	1.9, 1.8
	2.0	2.3, 2.1	2.0, 1.8	2.6, 2.8	2.2, 1.7
	2.1	2.4, 2.2	2.0, 2.0	2.7, 2.5	2.1, 2.2
	2.2	2.2, 2.3	1.9, 1.9	2.8, 2.3	1.7, 2.0
	2.2	2.5	1.7, 1.7	2.6, 2.7	1.8
	2.0	2.6	1.9, 1.8	2.6, 2.6	2.1
Σ	28.1	47.0	41.1	58.7	39.3
N	13	20	22	23	20
$\overline{X}$	2.16	2.35	1.87	2.55	1.96
R	2.0-2.5	2.1-2.7	1.7-2.0	2.3-2.9	1.7-2.3
Σ_x^2	60.93	111.00	77.03	150.45	77.97
CF	60.74	110.45	76.78	149.81	77.22
s_x^2	.016	.029	.012	.029	.039
s_x	.126	.170	.109	.170	.199
$s_{\overline{X}}^2$	.001	.001	.00005	.001	.002
$s_{\overline{X}}$	.035	.038	.023	.036	.044
CV	.058	.072	.058	.067	.102

Appendix Table 6. Mean number of seeds per spike of selfed and open-pollinated spikes of fertile and male sterile plants in Cnn ms-5 and -6 F_2's in 1976.

Cnn ms-5				Cnn ms-6			
Fertile Plants		Male Sterile Plants		Fertile Plants		Male Sterile Plants	
Selfed	Open-Pollinated	Selfed	Open-Pollinated	Selfed	Open-Pollinated	Selfed	Open-Pollinated
34	54.4	0	3.3	33	43.5	0	4.3
31	38.4	0	4.2	42	70	1	26
35	40.1	.5	6.4	28	56	0	9.3
38	48	.25	6.3	50	70	1	3.4
		.3	6.8	53	59	0	17
		0	3.7			0	20
		.5	5.7			0	15.5
		.5	4.6			0	14.4
		0	3.6			0	17
		0	7			.3	7.8
		0	9			0	6.3
		0	4.7			0	23
		0	9			0	9.2
		.5	8.4			0	19
		0	4.0			0	5.9
						0	16
						.5	13.5
						.5	11.9
Σ 138	180.9	2.55	86.7	206	298.5	3.3	239.5
N 4	4	15	15	5	5	18	18
$\bar{X}$ 34.5	45.2	0.17	5.78	41.2	59.7	.18	13.3

(Continued)

Appendix Table 6. (Continued.)

	Cnn ms-5				Cnn ms-6			
	Fertile Plants		Male Sterile Plants		Fertile Plants		Male Sterile Plants	
	Selfed	Open-Pollinated	Selfed	Open-Pollinated	Selfed	Open-Pollinated	Selfed	Open-Pollinated
R	31-38	38.4-54.4	0-.5	3.3-9	28-53	43.5-70	0-1	3.4-26
Σ_x^2	4786	8345.93	1.152	555.37	8946	18309.25	2.59	3907.99
CF	4761	8181.2	.433	501.13	8487.2	17820.45	.605	3186.68
s_x^2	8.33	54.91	.051	3.87	114.70	122.2	.117	42.43
s_x	2.89	7.41	.227	1.97	10.71	11.05	.342	6.51
$s_{\bar{X}}^2$	2.08	13.72	.003	.26	22.94	24.44	.006	2.36
$s_{\bar{X}}$	1.44	3.70	.058	.51	4.79	4.94	.080	1.53
CV	.084	.164	1.335	.341	.260	.185	1.900	.489

Appendix Table 7. Pooled data for seed set on bagged and open-pollinated spikes of fertile plants and bagged spikes of male sterile plants of Cnn ms-5 and -6 F_2. (Data from Appendix Table 6.)

	Fertile Plants		Male Sterile Plants
	Selfed	Open-pollinated	Selfed
Σ_5	138	180.9	2.55
Σ_6	206	298.5	3.3
Σpooled	344	479.4	5.85
N_5	4	4	15
N_6	5	5	18
Npooled	9	9	33
Σ^2_{x5}	4786	8345.93	1.152
Σ^2_{x6}	8946	18309.25	2.59
Σ^2_xpooled	13732	26655.18	3.742
$\overline{X}p$	38.22	53.27	0.177
CFp	13148.44	25536.04	1.037
$s^2_x p$	72.94	139.89	.084
$s_x p$	8.54	11.83	.29
$s^2_{\overline{X}} p$	8.10	15.54	.0026
$s_{\overline{X}} p$	2.85	3.94	.051
CVp	.223	.222	1.638

Appendix Table 8. Crossed seed set per spike of emasculated YGSS 4662/4* Cnn, and Cnn ms-5 and Cnn ms-6 male steriles in 1976.

Yogo Short Straw 4662/4*Cnn		Cnn ms-5		Cnn ms-6	
Female/Male		Female/Male		Female/Male	
Cross	Seeds/ Spike	Cross	Seeds/ Spike	Cross	Seeds/ Spike
F_5 #157/Cnn	10	Cnn ms-5/CRT	20	Cnn ms-6/CRT	20
/	8	/	17	/	22
/	12	/SND	17	/	18
/	15	/	19	/SND	29
159/	11	/	15	/	25
161/	9	/CNN	17	/	17
/	12	/	5	/CNN	1
/	15	/	2	/	8
/	14	/WLT-8	17	/	20
173/	16	/SIB	24	/	1
/	14	/	2	/	3
/	8	/	14	/VT1307/Cnn	21
/	10			/	26
175/	12			/Cnn ms-7/VT 1307	18
/	16			/VT1307	20
183/89-170	12			/MSC/FRD	20
186/Cnn	19			/	23
/	15			/MAR	11
264/	16			/YGSS 2469	16
282/	15			/Gaines	21
/	10			/SIB	17
284/	12			/	1
	15			/	19

(Continued)

Appendix Table 8. (Continued.)

Yogo Short Straw 4662/4*Cnn		Cnn ms-5		Cnn ms-6	
Female/Male		Female/Male		Female/Male	
Cross	Seeds/ Spike	Cross	Seeds/ Spike	Cross	Seeds/ Spike
				Cnn ms-6/SIB	22
				/	6
				/	16
				/	1
				/	24
				/	22
				/	6
Σ_x	96		169		474
N	23		12		30
$\bar{X}$	12.87		14.1		15.8
R	8-19		2-24		1-29
Σ_x^2	3996		2947		9550
CF	3809.39		2380.1		7489.2
s_x^2	8.48		51.54		71.06
s_x	2.91		7.18		8.43
$s_{\bar{X}}^2$	.37		4.29		2.37
$s_{\bar{X}}$	.61		2.07		1.54
CV	.226		.509		.533

Appendix Table 9. Comparison of the crossed seed set on the male sterile plants in Cnn ms-5 and -6 F_2's. (Data from Appendix Table 8.)

	Cnn ms-5	Cnn ms-6
$\overline{X}$	14.1	15.8
N	12	30
$s^2_{\overline{X}}$	4.29	2.37
$t_{.05}$	2.201	2.045
t' tabulated	2.145	
t' calculated	0.659	
	Accept H_o	

Appendix Table 10. Pooled data for crossed seed set per spike on male sterile plants in Cnn ms-5 and -6 F_2's. (Data from Appendix Table 8.)

Σ_5	169
Σ_6	474
Σpooled	643
N_5	12
N_6	30
Npooled	42
Σ^2_{x5}	2947
Σ^2_{x6}	9550
Σ^2_xpooled	12497
$\bar{X}p$	15.31
CFp	9844.02
$s^2_x p$	64.71
$s_x p$	8.04
$s^2_{\bar{X}} p$	1.54
$s_{\bar{X}} p$	1.24
CVp	.525

Appendix Table 11. Kernel weight (in mg.) of open-pollinated and crossed seeds from fertile and male sterile plants in Cnn ms-5 and -6 F_2 in 1976.

	Cnn ms-5		Cnn ms-6			
	Fertile	Male Sterile	Fertile		Male Sterile	
	Open-Pollinated	Open-Pollinated	Open-Pollinated	Crossed	Open-Pollinated	Crossed
	31.8	25.9	27.0	13.6	35.3	17.2
	32.2	26.9	27.8	16.4	30.3	15.0
		26.9	25.0		34.7	18.5
		31.0			23.6	20.9
		29.1			32.3	16.8
		28.3			28.6	
		28.9			38.8	
		25.2			37.8	
		31.4			44.2	
		35.5			36.3	
		30.5			34.7	
		35.5			38.8	
		31.2			33.9	
		26.9			27.4	
					36.9	
					30.3	
					32.7	
					41.0	
					36.5	
					32.2	
					27.9	
					65.4	
					26.2	
					33.8	
Σ	64	414.2	79.8	30	839.6	88.4
N	2	14	3	2	24	5
$\bar{X}$	32	29.6	26.6	15	35.0	17.68
R	31.8-32.2	25.2-35.5	25-27.8	13.6-16.4	23.6-65.4	15-20.9
Σ_x^2	2048.08	12385.54	2126.84	453.92	30885.12	1647.93

(Continued)

Appendix Table 11. (Continued.)

	Cnn ms-5		Cnn ms-6			
	Fertile	Male Sterile	Fertile		Male Sterile	
	Open-Pollinated	Open-Pollinated	Open-Pollinated	Crossed	Open-Pollinated	Crossed
CF	4028.0	12254.40	2122.68	450	29372.0	1526.91
s^2_x	.08	10.09	2.08	3.92	65.79	21.25
s_x	.28	3.18	1.44	1.98	8.11	4.61
$s^2_{\bar{X}}$	.04	.72	.69	1.96	2.74	4.25
$s_{\bar{X}}$	.2	.85	.83	1.4	1.66	2.06
CV	.009	.107	.054	.132	.232	.261

Appendix Table 12. Segregations for male sterility in F_3 families from fertile F_2 plants of Cnn ms-3.

Family Number	Number of Fertile Plants	Number of Male Sterile Plants	Ratio
154	24	1	24:1
155	34	4	8.5:1
157	54	3	18:1
158	24	1	24:1
161	24	4	6:1
189	20	2	10:1
198	21	3	7:1
199	31	4	7.75:1
205	43	3	14.3:1
209	34	3	11.3:1
220	24	2	12:1
222	22	2	11:1
152	22	0	--
156	32	0	--
159	39	0	--
163	50	0	--
178	30	0	--
185	36	0	--
186	55	0	--
195	40	0	--
202	35	0	--
208	23	0	--
210	36	0	--
221	26	0	--
223	53	0	--
225	41	0	--
227	25	0	--
228	27	0	--
229	24	0	--
230	41	0	--

Appendix Table 13. Homogeneous grouping of thirty of the F_2 populations tested for male sterile segregations. (Data from Table 15, p. 72.)

Pedigree	Number of Fertile Plants	Number of Male Sterile Plants	X^2
Cnn ms-3/Oleson-8	33	5	.04
-10	53	6	.21
Cnn ms-3/Oleson-2	48	3	1.85
-4‡	36	5	.00
-5	37	5	.00
-8	37	7	.60
-9	54	10	.75
-10	25	3	.05
Cnn ms-3/SIB-1	58	4	1.86
-2	50	10	1.18
-3	34	4	.09
-8	36	8	1.53
/Crest-3	43	3	1.34
/VT 1307-3‡	23	4	.19
/OP-7-3	28	1	2.04
-11-1	35	6	.25
-12-1	29	3	.22
-2	23	4	.19
-3	53	6	.21
-13-2	51	7	.00
-14-2	25	6	1.54
-16-1	49	5	.41
-3	51	11	1.86
-19-6	37	5	.00
-8‡	35	5	.01
-20-1	33	3	.48
-21-1	52	4	1.29
Cnn ms-3 F_2‡	119	17	.02
-5 F_2‡‡	119	15	.10
-6 F_2‡‡	155	26	.88
TOTAL	1461	201	19.18

Degrees of Freedom 29

.95>P>.90

Ratio 7.27:1

Accept H_o

Appendix Table 14. Table of the phenotypes of the F_2 populations segregating for male sterility and awns.

Pedigree	Number of Awnless, Fertile Plants	Number of Awnless, Male Sterile Plants	Number of Awned, Fertile Plants	Number of Awned, Male Sterile Plants
Cnn ms-3/Oleson-5	30	3	7	2
-8	29	6	8	1
-10	21	2	4	1
Cnn ms-3/Crest-1	10	1	3	1
Cnn ms-3/SIB-2	10	5	3	3
Cnn ms-3/SIB-2	39	9	11	1
-8	24	7	12	1
Cnn ms-3/OP-11-1	25	2	10	3
-15-1	31	12	8	3
-16-7	14	2	2	1
-19-5	16	6	9	2
Total	249	55	77	19

Appendix Table 15. Contingency chi-square test to determine linkage between awns and the male sterility. (Data from Appendix Table 14.)

Class	Observed Number of Plants	Expected Number of Plants	X^2	Expected Ratio	Degrees of Freedom
Awnless, Fertile	249	225	2.56	9	
Awnless, Male Sterile	55	75	5.33	3	
Awned, Fertile	77	75	.05	3	
Awned, Male Sterile	19	25	1.44	1	
Total	400	400	9.38	16	3
Awnless	304	300	.05	3	
Awned	96	100	.16	1	
Total	400	400	.21	4	1
Fertile	326	300	2.25	3	
Male Sterile	74	100	6.76	1	
Total	400	400	9.01	4	1

Interaction X^2 9.38 - (9.01 + .21) = .33

Degrees of freedom = 1

.75>P>.50

Accept H_o

Appendix Table 16. Contingency chi-square test to determine linkage between chaff color and the male sterility. (Data from F_2 Cnn ms-6, 1976.)

Class	Observed Number of Plants	Expected Number of Plants	X^2	Expected Ratio	Degrees of Freedom
Brown, Fertile	80	84.4	.23	9	
Brown, Male Sterile	14	28.1	7.07	3	
White, Fertile	48	28.1	14.09	3	
White, Male Sterile	8	9.4	.21	1	
Total	150	150	21.60	16	3
Brown	94	112.5	3.04	3	
White	56	37.5	9.13	1	
Total	150	150	12.17	4	1
Fertile	128	112.5	2.14	3	
Male Sterile	22	37.5	6.41	1	
Total	150	150	8.55	4	1

Interaction X^2 21.60 - (12.17 + 8.55) = .88

Degrees of freedom (3-2) = 1

.50>P>.25

Accept H_o

Appendix Table 17. Contingency chi-square test to determine linkage between stem solidness and the male sterility. (Data on stem solidness from F_2 Cnn ms-3, 1975, and data on male sterility from F_3 families of these same plants in 1976. See also Table 1 and Appendix Table 12.)

Class	Observed Number of Plants	Expected Number of Plants	X^2	Expected Ratio	Degrees of Freedom
Solid, Fertile	6	2.5	4.90	1	
Solid, Segregating	2	5	1.8	2	
Semi-solid, Fertile	9	5	3.2	2	
Semi-solid, Segregating	6	10	1.6	4	
Hollow, Fertile	3	2.5	.10	1	
Hollow, Segregating	4	5	.20	2	
Total	30	30	11.8	12	5
Solid	8	7.5	.03	1	
Semi-solid	15	15	0	2	
Hollow	7	7.5	.03	1	
Total	30	30	.06	4	2
Fertile	18	10	6.4	1	
Segregating	12	20	3.2	2	
Total	30	30	9.8	3	1

Interaction 11.8 - (.06 + 9.8) = 1.94

Degrees of freedom (5-3) = 2

.50>P>.25

Accept H_o

Appendix Table 18. Contingency chi-square test to determine linkage between growth habit and the male sterility. (Data from Table 15.)

Class	Observed Number of Plants	Expected Number of Plants	X^2	Expected Ratio‡	Degrees of Freedom
Spring, Fertile	1136	1158.2	.43	59.7	
Spring, Male Sterile	167	144.8	.43	7.5	
Segregating, Fertile	289	294.2	.09	15.2	
Segregating, Male Sterile	42	36.8	.73	1.9	
Winter, Fertile	274	271.1	.03	14.0	
Winter, Male Sterile	31	33.9	.25	1.7	
Total	1939	1339.0	1.96		5
Spring	1303	1299.1	.01	67	
Segregating	331	329.6	.01	17	
Winter	305	310.2	.09	16	
Total	1939	1938.9	.11		2
Fertile	1699	1723.6	.31	8	
Male Sterile	240	215.4	.31	1	
Total	1939	1939.0	.62		1

Interaction $1.96 - (.11 + .62) = 1.13$

Degrees of freedom = 2

$.75>P>.50$

Accept H_o

‡The expected ratio used for fertiles to male steriles was 8:1.

Appendix Table 19. Comparisons of number and kernel weight (in mg.) of crossed and open-pollinated seeds on male sterile plants in Cnn ms-6 F_2. (Data from Table 10, Appendix Table 6 and Appendix Table 8.)

	Seed Number/Spike		Kernel Weight	
	Crossed	Open-Pollinated	Crossed	Open-Pollinated
$\bar{X}$	15.8	13.3	17.68	35.0
N	30	18	5	24
$s^2_{\bar{X}}$	2.37	2.36	4.25	2.74
$t_{.05}$	2.045	2.110	2.776	2.069
t' tabulated	2.077		2.499	
t' calculated	1.149		6.551	
	Accept H_o		Reject H_o	

Falk, Duane E

The biology of a male sterile mutant in wheat