STUDIES ON POLLINATIONS AND MALE-STERILITY IN RECIPROCALS OF A DOUBLE-CROSS CORN HYBRID

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1947

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE COLLEGE

OF AGRICULTURE AND APPLIED SCIENCE

1948

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INTRODUCTION

In 1946, field observations revealed low yields in the double cross hybrid K2275 (33-16 x Ky27) X (K55 x K64). These low yields were the result of poor pollinations. Instead of the ears being fully filled, as is the case with complete fertilization, there were only a few kernels scattered at random over the cob. These poor pollinations were found only in field scale plantings planted with seed of the pedigree (33-16 x Ky27) X (K55 x K64). A similar situation in farmers' fields of other white hybrids in Kentucky, Tennessee, and Indiana had previously been described in correspondence. The hybrids exhibiting the characteristic in that area came to be known as "scattergrain" hybrids.

The occurrence of such a "scattergrain" condition in a commercial hybrid would render the hybrid practically worthless from the standpoint of field scale production. Farm plantings of commercial hybrids that gave these poor pollinations resulted in losses to the farmers of sufficient magnitude to cause instigation of law suits against the seed companies.

The high yielding performance of K2275 (33-16 x Ky27) X (K55 x K64) in test plots, where other hybrids produced abundant pollen, gave strong indications this hybrid was fully female fertile and produced normal and receptive silks. Poor seed sets did not occur under such circumstances but when K2275 was grown alone in a field poor pollinations appeared. Consideration of these two factors suggested that in isolated plantings there was an insufficient supply of viable pollen being produced by the plants of (33-16 x Ky27) X (K55 x K64) to effect complete fertilization.

Comparison of the pedigrees of the hybrids that exhibited the "scattergrain" characteristic, Josephson and Jenkins (1948), showed that in each case, the seed had been made up using 33-16 as the female parent of the single cross that was used as the female parent of the double cross. This was also true for K2275. Observations made in the field by Josephson and Jenkins (1948) lead them to the conclusion that male sterility was responsible for the poor seed set. The presence of male sterility in only one of the reciprocal crosses indicated to these investigators that the sterility factor was inherited cytoplasmically. The continuity of 33-16 cytoplasm, provided by its use as the seed parent of the single-cross seed-parents, gave further evidence to substantiate the theory of cytoplasmic inheritance. This hypothesis advanced by Josephson and Jenkins (1948) would evidently apply to K2275.

Although it is known that the three permutations of a double cross maize hybrid may differ in yield, it is generally accepted that the single cross parents of a double cross, or the inbred parents of a single cross, may be used interchangeably as the male or female parent without variation in yield or other characteristics. Some exceptions to the general rule that reciprocal crosses are alike were already known: Chlorophyll variegations which were present in one maize cross and completely absent in the reciprocal cross have been reported by Rhoades (1943), Anderson (1923) and Demerec (1927). A case of cytoplasmic inheritance of male sterility in corn was reported by Rhoades (1933). Another such exception to the general rule was indicated by the observations on fields of the "scattergrain" hybrids.

Investigations were started in the spring of 1947 with the general field observations made by Josephson and Jenkins (1948) and those observations made in fields of K2275 as a background. The object of the investigations reported in this paper was to determine the cause of the small number of seeds produced on the cobs of K2275 and to determine whether or not there was a practical and reliable manner of avoiding the sterility in producing the hybrid. The reason for not abandoning this hybrid for another that did not exhibit the male sterility is that several years are required to test a hybrid before it can be reliably released to farmers for commercial production. K2275 had been tested and proven to have outstanding yielding ability as well as other agronomically desirable characteristics.

REVIEW OF LITERATURE

Male sterility has long been a subject of investigation in both plants and animals. The characteristic has several types of expression, varying from completely sterile to partially sterile. Male sterility has been found to be inherited by simple Mendelian inheritance, complementary genic factorial inheritance, cytoplasmic inheritance, and varying combinations of cytoplasmic and genic inheritance.

At least 18 different Mendelian factors for male sterility in corn were reported by Beadle (1932). Each of these factors, most of which are simple recessives, have been given letter designations from m_{s1} through m_{s16} and variable-sterile (v_{a2}) and warty anthers (w_a). No evidence of female sterility was found in any plants carrying any of these factors. Burnham (1941) reported a simple recessive gene (pa) which caused a partial sterility of maize.

Eyster (1921) reported a simple recessive factor for male sterility, and designated the factor ms. This gene causes the plants to differ from normal plants only in the tassels and anthers. The anther sacs remain undeveloped and no pollen forms. The tassels produced by plants homozygous for ms closely resemble immature normal plants in that the glumes do not open, however, they can be distinguished by close examination as young normal tassels are more plump than the tassels of sterile plants. No intermediates were found by Eyster (1921) and the heterozygotes were normal in appearance.

A study of semi-sterility by Brink (1929) gave evidence that two of the linkage groups of corn (B-lg and P-br) were affected. It was found that semi-sterility was the result of a translocation.

A characteristic called polymitotic, causing male sterility in maize, was described by Beadle (1931). Polymitotic closely resembles male sterility phenotypically. It is a simple Mendelian recessive giving an F_2 phenotypic ratio of 3 normal to 1 sterile. The characteristic is the result of the occurrence of multiple mitoses-like divisions following a regular meiotic division of the microsporocytes. Normal development of pollen is characterized by two mitoses following meiosis, however, in this case there are at least four mitotic divisions followed by degeneration of the resulting cells.

A review of the literature on cytoplasmic inheritance may be introduced by referring to an article by Sirks (1938). Examples of cytoplasmic inheritance in animals cited by this author are sinistrality in snails reported in 1930 by Boycott and his co-workers, and the findings of Toyama, published in 1931 and those by Tanaka, reported in 1924, of their investigations on silkworms. The article by Sirks (1938) has a bibliography of 68 articles on plasmatic inheritance. A further indication of the importance and frequent occurrence of cytoplasmic inheritance is given in the statement of Owen, Correns and Sirks, that it has been found that male sterility in cross pollinated plants is more commonly explained by cytoplasmic inheritance than by genic or Mendelian inheritance, Owen (1945). According to Rhoades (1931), "When the transmission of a character has been conclusively proved to be independent of nuclear factors, it must be inherited through the cytoplasm."

Plasmatic inheritance was divided into three classes by Sirks (1938), they are: 1. An apparently wholly independent action of the plasm in the production of characters without any influence on the part of the genotype; 2. Different reactions caused by different cytoplasms on the same genotype; and 3. Elimination of zygotes or gametes of definite genotypical constitution and other nuclear irregularities under the influence of the plasm. Examples of each type of cytoplasmatic inheritance were described by the author.

A chlorophyll variegation in maize was found by Anderson (1923) which resulted in a pale green color instead of the normal dark green. A single plant was found that had conspicuous pale green stripes. An S_1 progeny was obtained from the original plant. Pollen from a plant in this S_1 progeny was used on an unrelated plant exhibiting the character Japonica and on several normal green plants. Only normal green plants were obtained in the F_1 progenies from these crosses. The S_1 progeny from the original pale green striped plant segregated into the following groups: three normal green, four pale green striped and twenty-one entirely pale green plants. None of the plants that were entirely pale green survived.

Examination of the ears by Anderson (1923) from which the seed was taken, disclosed that the seed carrying the various factors of chlorophyll variegations were not distributed at random over the cob, but were found in large well defined areas; all seed from each area giving the same type progeny. Only seed from a plant that exhibited the pale green striping would give rise to new plants that had the variegation. When pollen from a variegated plant was used on normal green plants, all normal green offspring were produced. This breeding behavior was conclusive evidence to the author that the chlorophyll variegation was inherited through the cytoplasm of the maternal parent.

Demerec (1927) described another chlorophyll variegation found in maize, in which there were pale green, variegated and normal green seedlings. Pollen of a variegated plant was mixed with pollen from an unrelated plant which possessed a factor that would produce a xenia effect and the pollen mixture used to pollinate an ear on the variegated plant. By separating the seeds that exhibited xenia from the others, it could be determined which were selfed seeds and which were crossed seeds. Upon examination of the progeny, it was found that variegated plants occurred with equal frequency in the offspring of both the selfed lines and in the crossed family. When the pollen mixture was used on a normal green plant, all offspring were normal green and bred true. This led to the conclusion of the author that the variegation was inherited only through the female gametes.

A case of male sterility in _Zea mays_ was investigated by Rhoades (1931) and determined to be inherited through the cytoplasm. A cross of the original male-sterile plant by an unrelated normal plant gave an F_1 population of 45 plants, all of which were male sterile. Later generations gave some families that bred true for male sterility, others having both normal and male-sterile plants, and rarely families that were completely normal. Rhoades found: 1. Replacement of the original chromosomes in the male-sterile line with chromosomes from normal lines had no effect on sterility. Nine of the ten linkage groups were free from sterility; 2. Pollen from partially sterile plants carry no transmissible factors, either genic or cytoplasmic, for male sterility; 3. The genetic contribution of the male parent to a male-sterile plant has no demonstrable effect on the degree of sterility in the progeny; and 4. Cytologic investigation shows meiotic division in microsporogenesis to be normal. The pollen degenerates after the first vegetative division.

A case of genicaly induced chlorophyll variegation in maize was investigated by Rhoades (1943) and found to be transmitted to succeeding generations through the cytoplasmic contribution of the maternal parent to the egg cell and so to the young plant. The character known as Iojap was found to be brought about by an irreversible mutation of the plastid primordia in the young zygote when the factor <u>ij</u> was present in a homozygous condition. Iojap is a white striping of green plants and is transmitted to the F_1 population when plants exhibiting the character are used as the seed parent and crossed to a normal plant. The reciprocal of this cross was completely free of the striping. Cytoplasmic inheritance of this type is known to be through the plastids, which are physical entities. In other cases of plasmatic inheritance, the nature of transmitting entities in the cytoplasm can only be conjectured.

A statement made by East (1934) indicated the obstacle confronting investigators attempting to interpret the results of cytoplasmic inheritance: "This is the type (of scientific problem) where two (or more) virtually inseparable and difficulty controllable variables coexist, and where only one reacts neutrally to our measuring devices, yielding data interpretable quantitatively when neglecting the other."

The close knit relationship of the genome and the cytoplasm in the processes of inheritance are demonstrated by the manner of inheritance of male sterility in flax reported by Chittenden (1927), male sterility in sugar beets described by Owen (1942), the transmission of mutated plastid primordia found in corn by Rhoades (1943) and the male sterility that is used for "wholesale emasculation" for commercial production of hybrid onion seed that was studied by Jones and Clarke (1943).

A male-sterile condition that appeared in a cross of Procumbent flax by common Tall flax (Linum usitatissimum) but which was entirely absent in the reciprocal cross was investigated by Chittenden (1927). The Procumbent flax, which is a new type that bred true, appeared in a plot of Linum grandiflorum. These two species normally cross and produce normal fertile hermaphroditic offspring. The theory advanced by Chittenden (1927) for the inheritance of this male sterility, and borne out by experimentation, was that there was an interaction of a negative gene 'T' contributed by the Tall flax, with the cytoplasm contributed by Procumbent flax, which would give only sterile plants when the gene 'T' was in a homozygous state. A ratio of 3 fertile to 1 sterile was obtained in the F_2 generation and two of the fertile plants segregated in the F_3 generation for 3 normal to 1 sterile. When the cross was made in the reciprocal manner using Tall flax as the seed parent with pollen from Procumbent flax, no sterility was found in any succeeding generation. The same problem was studied by Bateson and Gairdner (1921). These latter authors believed that the presence of male sterility in only one of the reciprocal crosses was due to somatic segregation which occurred in the female parent. When common Tall flax was used as the female parent the negative gene for sterility was relegated to the male-side. When Procumbent flax was the seed parent this segregation did not occur. Gairdner (1929) made further studies on the problem and arrived at conclusions that were in agreement with those of Chittenden.

The inheritance of male sterility in onions was described by Jones and Clarke (1943). The male-sterile plants produce no viable pollen, but they set seed readily when hybridized with pollen from normal male-fertile plants. When male-sterile plants were crossed to normal male-fertile plants, the F_1 generation segregated into three families having different breeding behavior. Some progenies were male-fertile, others were completely male-sterile, whereas, still others produced both male-sterile and male-fertile plants in a 1:1 ratio.

The authors explained this breeding behavior as being due to the presence of recessive nuclear factor ms contributed by a normal plant interacting with 'S' cytoplasm of the male-sterile plant. When the recessive factor ms was present in a homozygous condition in 'S' cytoplasm, completely male-sterile plants resulted. When the cross was made reciprocally, the 'S' cytoplasm was not introduced by the pollen and only the dominant allelomorph Ms for male fertility was transmitted to the F_1 progeny which bred true for the male fertility.

A report of complimentary effects of cytoplasm and Mendelian factors in the transmission of male-sterility to new generations in sugar beets was made by Owen, (1942). The nature of the inheritance of the male-sterile condition indicated the presence of two types of cytoplasm. The two types of cytoplasm were named Normal and Sterile by the investigator and given the letter designations of N and S. When plants with N cytoplasm were used as the female parent in a cross with plants having S cytoplasm only normal hermaphroditic plants that produced normal and abundant pollen resulted. In the reciprocal of this cross, varying amounts of viable pollen were produced by the F_1 generation. Most of the breeding behavior of the cross was explained by assuming there were two Mendelian factors present which have complementary effects to influence the degree of sterility in connection with the S cytoplasm.

Plants having "sterile" cytoplasm were assumed to carry the dominant Mendelian factors XX and ZZ for normal pollen production. Plants having normal cytoplasm were assumed to be of the constitution N for normal cytoplasm, and xx and zz, the Mendelian factors for sterility. When both X and Z are present in the homozygous condition, in either normal or sterile cytoplasm, only the normal hermaphroditic condition results. In the F_1 generation of the cross S XX ZZ x N xx zz, there is more or less normal pollen produced, depending on favorable or unfavorable environmental conditions. The segregation in the F_2 generation is as follows:

S xx zz - Male-sterility with white empty anthers

S Xx zz -
S xx Zz - Semi-male sterile with yellow anthers
S XX zz - but little or no viable pollen
S xx ZZ -

S Xx Zz - More or less normal pollen depending upon
S XX Zz - favorable or unfavorable environmental
S Xx ZZ - conditions, but the anthers sometimes fail to open.

S XX ZZ -

Crosses between male sterile female plants (S xx zz) and normal plants (N xx zz) produced only completely male-sterile offspring. When semi-sterile plants (S Xx zz) were crossed with normal pollen plants (N xx zz) half of the F_1 population were semi-sterile and half were completely sterile. Reciprocal crosses using normal plants (N xx zz) as the seed parent with pollen produced by semi-sterile plants (S Xx zz) gave only normal hermaphroditic offspring. The unique nature of the inheritance of this male-sterility in sugar beets has facilitated the wholesale "emasculation" of plants for the production of hybrid seed.

Artschwager (1947) made cytological investigations of male sterility in sugar beets, of the type described by Owen (1942), that revealed the cause of the sterility to be due to disintegration of the microspores. Normal pollen mother cells and normal microspores were produced but the microspores failed to develop. Degeneration of the microspores was associated with the development of either a periplasmodium or cellular tapetum. Instead of the tapetal cells slowly breaking down to furnish nutrients for the development of the young spores, as is the theory of normal pollen production, the tapetal layer in the male-sterile sugar beets makes a 'run away' growth, forming a cenocytic mass. This tapetal plasmodium either crowds the microspores too closely for normal growth or a toxic substance may be given off that causes the disintegration to take place.

An interesting case of male sterility in potatoes was reported by Salaman and Lesley (1922). A type of partial sterility was obtained in a cross of the varieties Edgecote Purple x Edzell Blue and was seemingly absent in the reciprocal cross. The degree of sterility was quite variable; pollen was produced normally by some plants while only a few pollen grains were produced by other plants. Although cytoplasmic inheritance was suggested as a possible explanation for the occurrence of the sterile plants, the authors believed the sterility to be carried by the egg nuclei. The reason for this belief was that there is no evidence that the cytoplasm of the egg cells of one plant may vary in constitution. It was the contention of Salaman and Lesley (1922) that such a cytoplasmic variation would be necessary to produce the different degrees of sterility that were found in the progeny of a single plant if the sterility was inherited cytoplasmically.

MATERIALS AND METHODS

General Materials and Methods

Kansas 2275 is made up of the four inbreds Indiana 33-16 and Kentucky 27 crossed to make one parent and Kansas 55 and Kansas 64 crossed for the other single cross parent. The seed for those fields in which the poor seed sets were found was made on a commercial scale using Indiana 33-16 x Ky27 as the seed parent and K55 x K64 as the pollen parent of the double cross. The studies reported here were made on seed combined in the manner of this original cross (33-16 x Ky27) X (K55 x K64), and the reciprocal of this cross (K55 x K64) X (33-16 x Ky27). The practice of writing the female parent first in the pedigrees of both single crosses and double crosses has been followed throughout this paper.

Detailed studies were made on nine different field plantings of both crosses during the spring, summer and fall of 1947. Data were taken on plant heights, the number of sterile tassels and the number of top fired plants in each of these plantings. The effect of date of planting, soil fertility and soil moisture on the number of sterile tassels also was studied in the plantings. Seed set studies, by means of hand pollinations, were conducted in two of the plantings to determine the effectiveness of pollen produced by the reciprocal crosses. A comparison of grain yields was made on one planting in this group. A study of the amounts of pollen shed by individual plants of the two reciprocal crosses was made on two of the plantings. Records of yield were taken on three other plantings, all of which were grown under different environments. A small planting of (33-16 x Ky27) X (K55 x K64) was grown in the greenhouse during the winter of 1947-1948 to provide pollen for microscopic examination and cytologic studies.

A number of plantings under widely varying conditions of growth and varying dates of planting were made because earlier observations pointed toward a considerable influence of environment and planting date on the expression of the male sterility. The sterility was believed to be more prevalent in mid-late plantings. Several plantings were made to afford a better opportunity of finding the sterility and to determine what environmental factors induced it.

Two privately controlled commercial hybrids which had previously exhibited sterility were included in two of the plantings made for detailed study to compare their response with that of K2275. The pedigrees and names of the commercial hybrids used in this investigation are withheld as confidential information of economic importance, as the seed was furnished solely for the purpose of experimentation.

The difference between the reciprocal crosses of K2275 with respect to the male sterility suggested the possibility of the existance of other differences between them. The large number of plants to be studied limited the search for differences to characteristics on which data could be easily and accurately collected. Because of the relative ease and the rapidity of taking notes in the field, plant heights and top firing were selected as the characteristics to be given detailed study, thus furnishing data on one morphological and one physiological trait in addition to the study of the tassels.

Measurements of plant height were made in seven of the plantings that were given detailed study. The measurements were taken in inches by measuring from the top of the soil at the base of the plant to the tip of the tallest leaf, i.e., the leaves were raised and the one extending the highest was used as the maximum height of the plant. Measurements were taken in the same manner for all plantings and all plants measured were selected at random within each plot.

The tassel of each plant in all nine of the plantings studied in detail was given a classification of either normal or sterile. Only those plants which failed to shed a visible amount of pollen were classed as sterile; if pollen was present, even though in greatly reduced quantity, the tassel was classified as normal. Attempts were made in early observations to estimate the amount of pollen being shed by abnormal tassels in comparison to normal tassels thus making a quantitative classification of those plants producing pollen but in limited amounts. This practice was abandoned as the examinations often lead to erroneous conclusions as to the amount of pollen being produced. The practice of classifying the tassels as either normal or sterile without regard to degree of sterility was followed throughout the entire experiment.

The plants in this experiment were observed to exhibit three different expressions of male sterility. In two of the types of sterility, abnormal anthers were exserted from the glumes, but in the third type no anthers were exserted. Each type of sterility was recorded separately as the examinations were made.

Notes on the top firing of each plant were taken at the time the tassel classifications were made in seven of the plantings made for detailed study. Top firing is used in this paper to mean the wilting, curling, and killing of tissue of the upper leaves and/or sun blasting of the tassel of the plant. Analyses of the data were made by the use of Chi square as a test for significant differences.

Pollen yields were determined on two of the plantings included in the experiment for intensive observations. The pollen yield was determined volumetrically in millileters by measuring the pollen collected in kraft paper tassel bags over a period of approximately 24 hours. The measurements were taken about the third day after the tassel had begun to shed pollen, as this was observed to be the period of greatest pollen shedding.

These measurements of pollen were made by placing the tassel bag over the tassel in the morning and removing it the following morning. The contents of the bag were then sifted through a common wire household strainer to remove the anthers and other foreign matter. A small funnel was used to direct the pollen into a glass cylinder graduated in o.5 millileters to make the measurements as nearly accurate as possible. All pollen measurements were taken in the field immediately after removing the bag from the plant.

Microscopic studies of pollen were attempted in the field, but were found unsatisfactory because of the conditions under which the work had to be carried out. Although some valuable information was gained from microscopic study, the use of a 10x hand lense was found to be much more convenient and gave indications of the particular abnormalities that could be more closely scrutinized in the laboratory.

All statistical analyses made on the data obtained in the fields were carried out in the manners described by Snedecor (1946) and Paterson (1939).

The following plantings were made for detailed study:

(1) The two north border rows of the yield test field on the Agronomy Farm.[1]

(2&3) Two planting dates in the observation plot on the Agronomy Farm.

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[1]The Agronomy Farm of the Kansas Agricultural Experiment Station, Manhattan, Kansas.

(4) One block of heat treated material on the east end of the yield test field on the Agronomy Farm.

(5) Two blocks in the south-east corner of the yield test field on the Agronomy Farm.

(6&7) Two planting dates in the top cross plot on the Agronomy Farm.

(8) The five south border rows of the yield test field on the Agronomy Farm.

(9) Three rows through the hand pollination nursery at the regional nursery of the Soil Conservation Service, Manhattan, Kansas.

The following plantings were made for yield comparisons:

(1) One range of alternate plots of the reciprocal crosses of K2275 in the yield test field at Silver Lake, Kansas.

(2) One range of alternate blocks 20 rows wide of each of the reciprocal crosses of K2275 in the yield test field at Horton, Kansas.

Descriptions of Individual Plantings

Planting Number One. The first planting of the two reciprocal crosses was made in the two north border rows of the yield test field April 30, 1947. A randomized block field design of seven replications was used. Each replication was two rows wide and 32 hills long. The hills were approximately 42 inches apart. Sixty-six days after planting, plant heights were taken on eight plants in each plot. Tassel observations were made daily after the plants began to shed pollen. As each tassel developed and had ample time to shed pollen, it was given a classification of normal or sterile. At about this stage of development ear shoots were bagged with glassine shoot protector bags in preparation for making hand pollinations. Pollen yield studies were carried out when the plants were in the correct stage of development. Hand pollinations were made to determine the relative effectiveness of the pollen of the two reciprocal crosses. These hand pollinations were made on plants of like pedigree, plants of the reciprocal cross, and some self pollinations were made. A seed set study was made on the ears resulting from these hand pollinations. The individual ears were given a grade based on an arbitrary scale of from 0 to 10; a grade of 0 being used to designate a complete absence of grain and the grade 10 was used to designate what was considered to be 100 percent seed set. The scale used in making these ear classifications is represented by the ten ears shown in Plate VI.

Plantings Number Two and Three. The second and third plantings of the reciprocal crosses of K2275 were made in the observation nursery of the Agronomy Farm. The planting was made in four blocks of approximately the same size. One block of each of the reciprocal crosses was planted May 12 and the other two blocks were planted May 21, 1947. July 7, plant heights were taken on 100 plants in each block. The tassel classifications and top firing counts were made August 4 and 5. These two characteristics were taken on only one-half of the rows chosen at random in each block.

Planting Number Four. The fourth set of plantings were of seedlings of both reciprocal crosses that had previously been given a heat treatment. Approximately 300 seeds of each of the reciprocal crosses of K2275 were germinated in blotting paper at 30° centigrade and were kept in the germinator until the shoots were from one-half to one inch long. The young seedlings were then placed in an electric oven and kept at 50° centigrade for one hour. They were then put in pots and kept in the greenhouse until they were from four to six inches in height. At this time, May 23, the plants were transplanted to the field. A complete set of control plants were handled in the same manner except for the heat treatment. The field plan was a randomized block of six replications. The heights of all plants in the block were measured 65 days after they were transplanted. Tassel classifications were made on the entire planting August 2. A reliable statistical analysis could not be made on top firing because of the small number of plants fired at the time the observations were made, August 2, 1947.

Planting Number Five. The two blocks of the reciprocal crosses of K2275 in the southeast corner of the yield test field on the Agronomy Farm were planted May 9, 1947. Tassel classifications were made on approximately 200 plants in each of these blocks August 4; the number of plants top fired also was recorded at this time.

Planting Number Six and Number Seven. The two planting dates represented in the top cross planting were May 30, and June 7. This planting was primarily for producing top-cross seed but the pollen rows were observed for these investigations. Plant heights were taken on 100 plants in each of the 16 rows on July 8. The tassel classifications and top firing notes were taken on August 6 for the earlier planted rows and August 18 for those planted June 7.

Planting Number Eight. Another planting of this study was the five border rows of the south side of the yield test field at the Agronomy Farm. Three commercial hybrids were included in this planting in addition to the two reciprocal crosses of K2275. All three of the commercial hybrids were used in the planting, but because of a shortage of seed, only two of them could be included in any one replication. The reciprocal crosses of K2275 and the commercial hybrids for each replication were planted in a randomized block field design of five replications. The plots were 40 hills long and the hills were 42 inches apart, arranged to conform to the check-row plan of the yield test field. These rows were planted May 30, 1947. The height of ten randomly selected plants in each plot was measured 36 days after planting. The number of sterile tassels present was determined August 13, and the number of top fired plants was recorded at the same time.

Planting Number Nine. The ninth planting of the series for detailed study was made June 4, at the regional nursery of the Soil Conservation Service, southwest of Manhattan. These three rows were made into a double split-plot field design. Half the planting was irrigated and the other half not irrigated. The whole plots, which were 47 feet long, were divided on fertilizer. The fertilizer used was ammonium nitrate, applied at the rate of 100 pounds of nitrogen per acre. The plant height measurements were taken 41 days after planting. Pollen yield measurements were taken on those plants that were in the correct stage of development August 9, but were confined to the irrigated section of the planting. A series of hand pollinations were attempted with pollen of various sources but the data obtained from them were considered unreliable as all pollen, regardless of source, gave very poor seed sets. This was believed to have been caused by the severe climatic conditions that prevailed during the time the pollinations were made. Tassel classifications were made on all plants in the planting. The number of top fired plants was recorded on the irrigated section August 8 and on the unirrigated section August 18.

EXPERIMENTAL RESULTS

Plant Heights

The data obtained from measuring 1,544 plants of seven different ages in seven plantings are combined in Table 1. Table 1a gives the results of analyses of variance for plantings numbered 1, 8 and 9.

The data were analyzed by analysis of variance and tests for significant differences were made by use of the F test.

The data in Table 1 show there is no consistent difference in plant heights between the reciprocal crosses of K2275, regardless of age of the plants or the date of planting.

Table 1. Summary of plant heights.[1]

Planting number	Pedigree of ear parent	Date of planting	Age when observed	Total no. measured	Avg. height (inches)
1	K55 x K64	April 30	66	56	58.08
	33-16xKy27	" "	66	56	57.55
2	K55 x K64	May 12	56	100	53.04*
	33-16xKy27	" "	56	100	50.72
3	K55 x K64	May 21	47	100	47.82
	33-16xKy27	" "	47	100	49.29*
4	K55 x K64	May 23	55	72	67.69
	33-16xKy27	" "	55.	72	69.15
6	K55 x K64	May 30	39	100	46.59
	33-16xKy27	" "	39	100	45.88
7	K55 x K64	June 7	31	100	33.74*
	33-16xKy27	" "	31	100	32.53
8	K55 x K64	May 30	36	100	35.69
	33-16xKy27	" "	36	100	36.38
9	K55 x K64	June 4	41	216	58.13
	33-16xKy27	" "	41	216	57.80

1 All tests for significance made by the F test described by Snedecor (1943).
* Indicates that the value of F exceeds that required for significance at the 5% level.

Although differences, significant at the five percent level, were found in plantings number 2, 3 and 7 they should not be considered as conclusive evidence that the reciprocal crosses of K2275 differ in plant height. This is readily evidenced by the fact that (K55 x K64) X (33-16 x Ky27) was significantly taller in planting 2, and plants of the reciprocal cross (33-16 x Ky27) X (K55 x K64) were significantly taller in planting number 3.

The field in which plantings 2 and 3 were made has a pronounced slope and terraces have been erected to prevent rapid runoff. The heavy soil type prevented adequate drainage between the terraces during the heavy rains in the spring of 1947. This resulted in a highly varied growing environment for the plants, some were nearly "drown", while other plants on the terrace ridges were well drained and grew well. These factors further point toward a possibility of obtaining unusual results from such a planting.

The appearance of a significant difference between the two reciprocals in planting 7 is believed to be due to soil variability, probably to soil moisture availability. The rows in which the measurements were made were unintentionally located in the field so as to favor the cross (K55 x K64) X (33-16 x Ky27).

It is shown in Table 1a that irrigation produced a pronounced effect on plant height, the response being approximately equal in both the reciprocal crosses. The analysis of data from planting number 9, made at the soil conservation nursery, furnished evidence there is no consistent and measureable effect on plant height at 41 days after planting by addition of 100 pounds of nitrogen per acre. Both reciprocal crosses again show a comparable response to a given environmental change.

Table 1a. Summary of analyses of variance for plant heights.

Sources of variation	D.f.	Mean square
Planting #1, North border rows		
Between reciprocals	1	8.04
Between replications	6	127.90
Reciprocals x replications	6	126.98
Error	98	297.05
Planting #4, Heat treated material		
Between varieties	2	25.77
Between replications	5	46.22
Varieties x replications	10	44.85
Error	126	32.93
Planting #8, South border rows		
Between reciprocals	1	23.80
Between replications	4	23.62
Reciprocals x replications	4	19.05
Error	190	13.75
Planting #9, Soil Conservation Nursery ##		
Whole plots:		
Irrigation	1	786.9586**
Reciprocals	1	0.6305
Replications in irrigation	4	128.8516
Irrigation x reciprocals	1	0.1026
Remainder (a)	4	27.6974
Sub-total_1	11 S.S.= 1,413.8769	
Sub-plots:		
Fertilizer	1	13.9996
Fertilizer x irrigation	1	42.1614
Fertilizer x reciprocals	1	0.5921
Fertil. x irrig. x recip.	1	14.0003
Remainder (b)	8	17.2431
Sub-total_2	12 S.S.= 208.6979	

Analysis of variance of double split-plot field design.
** Indicates that the value of F exceeds that required for significance at the 1% level.

Top Firing

Top-firing observations were made on 6,867 plants of the reciprocal crosses of K2275. Statistical analyses were made on the data to determine whether or not the reciprocal crosses were equally susceptible to top firing. The data are summarized in Table 2. All analyses were made by Chi square.

In every planting, the cross (K55 x K64) X (33-16 x Ky27) was found to have a higher proportion of plants top fired than the reciprocal cross (33-16 x Ky27) X (K55 x K64). The Chi square test proved the differences between the proportions top fired to be highly significant in three of the five plantings on which comparative analyses were made. This is strongly indicative of a consistent difference in the ability of the plants of the reciprocal crosses of K2275 to withstand unfavorable climatic and soil moisture conditions.

A significant difference in the percentages of plants top fired was found in one instance in planting number 9, when the soil moisture content was approximately the same for both of the reciprocal crosses of K2275. Although this difference could be due to chance variation, it is not considered likely.

When neither fertilizer nor irrigation water was applied, there was a significant difference between the reciprocal crosses; however, when fertilizer was applied and irrigation water was not, the difference was not significant, but as before the cross (K55 x K64) X (33-16 x Ky27) had a higher proportion of

Table 2. Summary of top firing.

Planting number	Date observed	Number of plants observed	Average % Plants Top Fired 33-16xKy27 Female	Average % Plants Top Fired K55 x K64 Female	Calculated value of chi-square	Probability
2	Aug. 4	549	10.63	14.34	2.8353	.09
3	" 5	486	Nonhomogeneity within either cross			
5	" 4	451	3.52	5.80	1.319	.25
6	" 6	1,330	11.71	22.83	32.13**	.01
7	" 18	2,111	Nonhomogeneity within either cross			
8	" 13	920	35.57	45.09	8.652**	.01
9#	" 18	261	5.88	15.20	6.151**	.01

\# Data from that portion of planting number nine that received neither irrigation water nor fertilizer.

** Indicates that the chi-square exceeds the value at the 1% level for 1 degree of freedom.

plants top fired than the reciprocal cross. This further indicates that the offending cross, (K55 x K64) X (33-16 x Ky27), is more susceptible to top firing under adverse soil moisture conditions and low levels of soil fertility than the reciprocal cross.

Although three of the five tests made gave significant differences, the results should not be considered as conclusive evidence that an actual difference exists in susceptibility to top firing between the two reciprocal crosses.

The nonhomogeneity within crosses, referred to in Table 2, indicates that different rows of the same cross planted the same date did not exhibit comparable proportions of top-fired plants. This situation made a statistical comparison of the two reciprocal crosses invalid. Nonhomogeneity within the crosses in two of the plantings indicates further tests should be made to obtain more critical information.

It was found in the irrigated section of planting 9 and in plantings 2 and 5 that when small percentages of the plants in both reciprocal crosses top fired, the difference between the crosses was not statistically significant. This intimates that if the reciprocal crosses do differ, the difference exists only through a range of adverse conditions which cause a high percentage of top firing.

Types of Male Sterility

The three types of male sterility exhibited by K2275 are easily distinguished from one another in the field. In the first type, the anthers are smaller than normal, malformed and dry in appearance. These anthers are illustrated in Plate I. The lodicules and filaments of tassels exhibiting this type of sterility apparently function normally and the small dry anthers are usually completely exserted from the glumes. The filaments of such anthers lack turgidity but are tough and seemingly quite strong. It is not uncommon to find a large proportion of the small dry anthers attached to the tassels two to three weeks after they were exserted. Usually only a few anthers are found on normal tassels after this period of time. A lateral branch from a sterile tassel, typical of this type of sterility, along with a branch from a normal tassel, is pictured in Plate II.

Beadle (1932) describes a male sterility that is very similar to this type found in K2275. The sterility found by Beadle (1932) is due to a simple recessive factor m_{s6}.

These small dry anthers produced by K2275 were found to contain some pollen but never dehisced and shed. Microscopic examination of the contents revealed both normal appearing and small shrunken, abnormal pollen grains. It was estimated that 70 percent of the pollen grains in the anthers were abnormal. The abnormal pollen grains are irregular in size and shape. Under the microscope, it was seen that the cytoplasm was drawn into a small localized mass only partially filling the pollen grain. Abnormal pollen grains had a pale yellow color and were nearly transparent in contrast to the darker color and translucent appearance of normal pollen grains.

EXPLANATION OF PLATE I

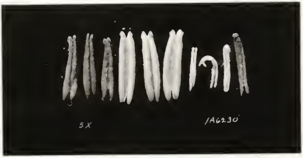
Anthers of K2275 (33-16 x Ky27) X (K55 x K64) grown in the greenhouse during the winter of 1947-1948.

The three anthers on the left were from a plant that usually exserts the anthers but do not dehisce and shed pollen. These anthers are typical of the third type of expression of sterility exhibited by this cross.

The three anthers in the center are normal and were shedding abundant pollen.

The anthers on the right were taken from a plant that was 100 percent male sterile. Anthers of this type were usually exserted but are characterized by this small, dry appearance at the time they come out of the glumes.

PLATE I



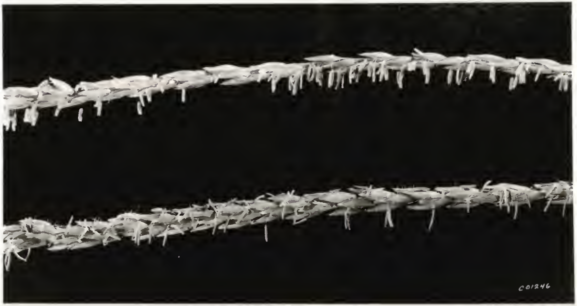
EXPLANATION OF PLATE II

Tassel branches of two plants of K2275 (33-16 x Ky27) X (K55 x K64). The upper branch is from a normal tassel that was shedding pollen freely. Note the plump appearance of the anthers and the even, 'flexible' appearance of the glumes.

The lower branch is from a tassel that was 100 percent sterile. Notice the shriveled anthers that are also shorter in length. The 'stiff' glumes with the veins in the glumes very evident, and the way the entire floret tends to lie flat to the rachis is quite typical.

These branches were in about the same stage of development. These tassel branches were from plants grown in the greenhouse but the sterility is typical of that found in the field.

PLATE II



A second expression of male sterility by plants of K2275 was characterized by fully exserted anthers. The anthers were rather plump and had a turgid filament but did not dehisce and shed pollen. The appearance of these anthers may be compared to normal anthers by referring to Plate I. At the bottom end of the anthers, where splitting usually starts, the anthers were "pinched" and dry. This "pinched" condition apparently prevents the opening of the pore at the bottom end of the anthers, and the reduced amount of pollen in the anthers does not cause sufficient pressure to rupture the anthers at any other point so the pollen may be discharged. Pollen from anthers of this type was studied microscopically. A photomicrograph of pollen grains, after they were removed from an anther of this type, is given in Plate III. Plate IV is a photomicrograph of pollen from a normal anther. An estimated 20 percent of the pollen grains contained in the second type of abnormal anthers were small and malformed. These abnormal pollen grains could not be distinguished from those described in the first type of sterility.

Germination tests of pollen from the abnormal anthers were made. Attempts were made to germinate the pollen by dusting a few pollen grains on short sections of fresh silks on the cover glasses of van Tieghem cells. All such trials were complete failures. Artificial media of agar and dextrose, described by Blair and Loomis (1941), was used with some success.

Germination tests were made to determine whether or not the apparently normal pollen, in that obtained from abnormal anthers, functioned as well as that from normal plants. Although the results from the tests were eratic, it was observed, that in general, a lower proportion of "normal" pollen grains from abnormal anthers formed germ tubes than did pollen from normal anthers. As nearly as could be determined from these tests, the abnormal pollen grains were completely non-functional. Not only did the abnormal pollen grains fail to form germ tubes, but they seemed to be entirely inactive. When placed in distilled water, they merely floated to the surface and did not swell and burst as normal pollen grains did.

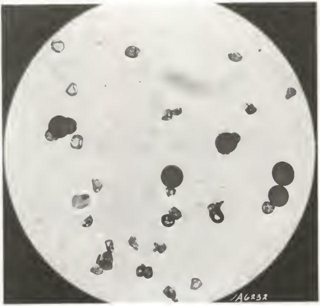
The third type of male sterility is quite different phenotypically from the first two described. This sterile condition is one in which the glumes rarely open at any time and the anthers are very seldom exserted. Tassels of this type closely resemble those of immature plants. This expression of male sterility is characterized by a tough leathery appearance of both the central spike and the lateral branches. The florets tend to be held closely to the rachis which gives the tassel a rigid appearance in contrast to the flexible appearance of normal immature tassels. A typical lateral branch of a sterile tassel along with a branch from a normal tassel is pictured in Plate V.

EXPLANATION OF PLATE III

Pollen from an abnormal anther of a plant of (33-16 x Ky27) X (K55 x K64). The small shrunken pollen grains are typical as seen under lower power (approximately 100x) of the microscope. The abnormal pollen grains are light yellow in color and nearly transparent. The cytoplasm can be seen to be withdrawn into a localized mass.

The large pollen grains are normal in appearance.

PLATE III

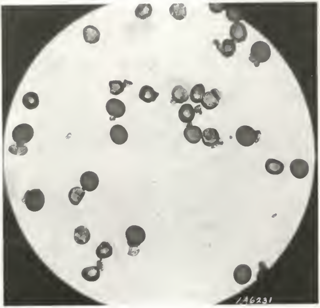


EXPLANATION OF PLATE IV

Pollen from a plant of (33-16 x Ky27) X (K55 x K64) that was shedding normally. Some abnormal pollen grains can be seen.

The pollen tubes that some of the pollen grains appear to be producing, are thought to be artifacts as the pollen grains were on a dry slide with no cover glass.

PLATE IV



This third type of sterility closely resembles a male sterile condition described by Eyster (1921). The male sterility reported by Eyster (1921) is attributed to the recessive Mendelian factor ms.

Anthers removed from the glumes of the sterile tassels were about one-third the size of normal anthers and were found to contain a very small proportion, of normal appearing pollen grains. No attempts were made to determine the viability of pollen from such tassels.

This third type of sterility is probably the most severe in its action as it resulted in tassels which shed no pollen whatever. Although the tassels classified as "sterile" in these investigations were shedding no pollen whatever, it was not uncommon to find anthers of the first two types on tassels that were shedding pollen.

Tassel Classifications

Tassels on 3,589 plants of the pedigree (33-16 x Ky27) X (K55 x K64) and 3,349 plants of the reciprocal double cross were examined for sterility. The numbers of plants of each classification and the ratios of normal to sterile are given for each planting in Table 3.

Statistical analyses were not made on the data of tassel classifications because of the obvious difference existing between the two reciprocal crosses in expression of male sterility.

EXPLANATION OF PLATE V

Tassel branches from two plants of K2275 (33-16 x Ky27) X (K55 x K64). The upper branch showing the general failure of the glumes to open and very few anthers being exserted. The anthers shown on this branch are shriveled and dry as they come out of the glumes and have small amounts of pollen, approximately 70 percent of which is abnormal.

The lower branch is from a normal tassel of a different plant of the same pedigree.

Note the open glumes, long anther filaments and plump anthers.

These two branches are in very nearly the same stage of development. These plants were grown in the greenhouse but the characteristics of male sterility are typical of field grown plants.

PLATE V

It will be noted in Table 3 that as the planting date was delayed there was a tendency for a greater proportion of the plants of the cross (33-16 x Ky27) X (K55 x K64) to produce sterile tassels. The influence of soil moisture on the percentage of male-sterile plants was shown in planting number 9; the proportion of sterile tassels in the non-irrigated section of the planting being nearly twice that found in the irrigated section.

Chi-square analysis of the data shows there were no significant differences between the proportions of plants that produced sterile tassels on the fertilized plots and those on unfertilized plots. This indicates that the nitrogen supply in the soil had little if any effect on the expression of male sterility in (33-16 x Ky27) X (K55 x K64).

From Table 3 it is determined that an average of 62.72 percent of the plants (33-16 x Ky27) X (K55 x K64) produced tassels that were entirely sterile. By using this average percentage for 1000 observations, the 99 percent confidence interval table shows that a minimum of 59 percent of the tassels of this cross can be expected to be sterile.

The data presented in Table 3 were considered as conclusive evidence that all three types of male sterility exhibited by (33-16 x Ky27) X (K55 x K64) were virtually absent in the reciprocal cross (K55 x K64) X (33-16 x Ky27). The two plants found in plots of (K55 x K64) X (33-16 x Ky27) maybe due to accidental mixing of the seed when planting. These two plants may also have arisen naturally as it is not uncommon to find an occasional sterile tassel in any corn planting.

Table 3. Summary of tassel classifications.

Planting number	Pedigree of ear parent	Planting date	Total plants observed	Normal tassels	Total sterile tassels	Reduced ratio (N:S)
1	33-16xKy27 K55 x K64	April 30 " "	388 414	167 414	221 0	1:1.3
2	33-16xKy27 K55 x K64	May 12 " "	235 319	99 319	136 0	1:1.4
3	33-16xKy27 K55 x K64	May 21 " "	283 203	146 203	137 0	1:0.9
4	33-16xKy27 K55 x K64	May 23 " "	86 67	45 67	41 0	1:0.9
5	33-16xKy27 K55 x K64	May 9 " "	226 214	87 214	139 0	1:1.6
6	33-16xKy27 K55 x K64	May 30 " "	610 517	227 516	383 1	1:1.7
7	33-16xKy27 K55 x K64	June 7 " "	886 858	197 858	689 0	1:3.5
8	33-16xKy27 K55 x K64	May 30 " "	432 347	79 347	353 0	1:4.5
9 I	33-16xKy27 K55 x K64	June 4 " "	238 226	87 225	151 1	1:1.7
9 Ni	33-16xKy27 K55 x K64	June 4 " "	205 185	50 185	155 0	1:3.1
9 I & F I & Nf	33-16xKy27 33-16xKy27	June 4 " "	120 115	48 36	72 79	1:1.5 1:2.1
9 Ni & F Ni & Nf	33-16xKy27 33-16xKy27	June 4 " "	79 126	22 28	57 98	1:2.5 1:3.5

I = Irrigated
Ni = Not irrigated
F = Fertilized
Nf = Not fertilized

The reduction in stand of (K55 x K64) X (33-16 x Ky27) indicated in Table 3, is partially due to lower germinating seed and because those plants having sun-blasted tassels, those plants too young for accurate classification and plants infested with insects were not counted as having been examined for male sterility.

The three types of sterility found in (33-16 x Ky27) X (K55 x K64) occurred with the same relative frequencies regardless of planting date in seven of the eight plantings. The low proportion of type three that was observed in the irrigated portion of planting 9 may be an indication that the more favorable the growing conditions, the less severe the sterility factors are in their action. The number of plants exhibiting each of the three types of sterility is given in Table 4.

Table 4. Number of plants exhibiting each type of sterility.

Planting number	Planting date	No. of male sterile plants		
		Type 1	Type 2	Type 3
2	May 12	48	27	61
3	" 21	29	28	80
5	" 9	28	31	80
6	" 30	95	124	164
7	June 7	186	110	393
8	May 30	109	60	184
9 I	June 4	59	46	46
9 Ni	" "	52	24	79

I = Irrigated portion of planting.
Ni = Not irrigated portion of planting.

Pollen Yield Measurements

The quantity of pollen shed in approximately 24 hours by 88 apparently normal individual plants of the pedigree (33-16 x Ky27) X (K55 x K64) was compared by analysis of variance to that shed by 94 plants of the reciprocal double cross. The average pollen yield for each replication is presented in Table 5 and the results of the analyses of variance are given in Table 5a.

A highly significant decrease was found in the amount of pollen shed by the plants of (33-16 x Ky27) X (K55 x K64) when compared to that amount collected from plants of the reciprocal cross (K55 x K64) X (33-16 x Ky27). This shows that there is a smaller number of pollen grains present in a planting of the cross (33-16 x Ky27) X (K55 x K64) than would be indicated by only the proportion of plants that were 100 percent sterile. From Table 5, the average pollen yield of (33-16 x Ky27) X (K55 x K64), in the two plantings, was calculated to be 30.29 percent of the average yield of (K55 x K64) X (33-16 x Ky27). By applying the upper limit of the 99 percent confidence interval, it was found from this experiment that the "normal" tassels of (33-16 x Ky27) X (K55 x K64) could be expected to produce a maximum of 40.0 percent as much pollen as the reciprocal cross (K55 x K64) X (33-16 x Ky27).

Table 5. Volume of pollen shed in 24 hours by reciprocal crosses of K2275.

Replication number	Pedigree of ear parent	No. plants sampled	Average pollen yield (milliliters)
Planting #1			
1	33-16xKy27	4	1.32
	K55 x K64	4	3.95
2	33-16xKy27	4	1.68
	K55 x K64	3	4.87
3	33-16xKy27	4	1.05
	K55 x K64	1	5.40
4	33-16xKy27	4	1.38
	K55 x K64	6	3.73
5	33-16xKy27	6	1.17
	K55 x K64	7	3.23
6	33-16xKy27	5	2.46
	K55 x K64	9	3.36
7	33-16xKy27	6	1.27
	K55 x K64	8	3.02
Planting #9			
1	33-16xKy27	7	0.886
	K55 x K64	7	2.943
2	33-16xKy27	10	0.490
	K55 x K64	10	2.920
3	33-16xKy27	8	0.263
	K55 x K64	8	2.425
4	33-16xKy27	7	0.300
	K55 x K64	7	2.771
5	33-16xKy27	7	0.314
	K55 x K64	7	1.043

Table 5a. Summary of analyses of variance on pollen yield.

Sources of variation	Degrees of freedom	Mean square
Planting # 1		
Between reciprocals	1	81.0509***
Between replications	6	1.3281
Reciprocals x replications	6	1.9733
Error	57	1.2395
Total	70	
Planting # 9		
Between reciprocals	1	77.30925***
Between replications	4	3.23432
Reciprocals x replications	4	1.82229
Error	68	0.99306
Total	77	

*** Denotes the value of F exceeds that necessary for significance at the 0.1% level.

Seed Set Studies

A series of 150 hand pollinations were made in planting 1 to determine the relative fertilizing ability of pollen produced by the reciprocal crosses of K2275. The effectiveness was measured by classifying the seed set of each ear resulting from the hand pollinations. The seed set grades of 1 to 10 are illustrated by the 10 ears shown in Plate VI.

One-hundred thirty-three pollinations were made using pollen from apparently normal plants of (33-16 x Ky27) X (K55 x K64) and pollen from plants of the reciprocal cross. The average seed set obtained in each replication is given in Table 6. The data show that the seed sets obtained from pollen produced by (K55 x K64) X (33-16 x Ky27) were very comparable regardless of whether it was used on ears of the same pedigree or on ears of the reciprocal cross. Comparable seed sets were also obtained from both crosses when (33-16 x Ky27) X (K55 x K64) was used as the source of pollen. This is evidence of equal receptivity of the silks of both the reciprocal crosses to pollen produced by either of the crosses.

Comparison of the average seed sets obtained from the two sources of pollen gives a highly significant difference. This highly significant difference was determined by an analysis of variance, summarized in Table 6a, of the seed set grades given the individual ears. The variation, attributable to parental combinations, was divided into its component parts to give further indications as to the exact source of the variability obtained in the seed sets. This analysis gave statistical support to the evidence that the differences in seed sets are due almost entirely to the source of pollen.

The poor seed sets obtained from pollen of (33-16 x Ky27) X (K55 x K64) are probably due to two main factors. The first of those factors is a deficient quantity of pollen grains to fertilize each ovule. It was shown by the pollen measurements that a significantly lower volume of pollen is produced by plants of this pedigree. The second factor to consider as being responsible for the low percentage of fertilization is the viability of that pollen shed by these plants.

As determined by fiducial limits, the average pollen yield of apparently normal tassels of (33-16 x Ky27) X (K55 x K64) was

EXPLANATION OF PLATE VI

Seed set classification scale used in determining the comparative viability of pollen from the reciprocal crosses of K2275.

The arbitrary classes are of hand pollinated ears grown in the field in the summer of 1947. The range is from zero (0) for no seed as the barren cob on the right, to ten (10), which was considered 100 percent seed set, illustrated by the two ears at the left.

PLATE VI

Table 6. The average grade of seed sets resulting from hand pollinations.

Replication number	Average grade of seed set obtained			
	Ear parent			
	(33-16xKy27) X (K55 x K64)		(K55 x K64) X (33-16xKy27)	
	Pollen parent		Pollen parent	
	(33-16xKy27) (K55 x K64)	(K55 x K64) (33-16xKy27)	(33-16xKy27) (K55 x K64)	(K55 x K64) (33-16xKy27)
1	5.4	6.2	6.4	7.8
2	7.3	8.5	9.5	7.8
3	2.7	8.8	4.4	9.3
4	2.5	8.9	1.3	8.4
5	4.0	6.2	2.8	5.4
6	3.0	6.0	2.5	7.6
7	5.0	7.8	5.5	4.8
Mean	4.27	7.46	4.63	7.30

Table 6a. Summary of analysis of variance of data given in Table 5.

Sources of Variation	D.f.	S.S.	Mean square
SxS vs. FxS#	1	0.446	0.446
SxF vs. FxF#	1	0.121	0.121
SxS & FxS vs. SxF & FxF#	1	60.623	60.623**
Parental combinations	3	61.190	
Replications	6		6.308
Interaction	18		2.949
Total	27	152.13	

\# S Designates (33-16xKy27) X (K55 x K64) - "Sterile".
F Designates (K55 x K64) X (33-16 x Ky27) - "Fertile".
** Indicates the value of F exceeds that required for significance at the 1% level.

40 percent of that produced by the reciprocal cross. It was determined from Table 6 that pollen produced by (33-16 x Ky27) X (K55 x K64) gave seed sets that were 60 percent as good as those produced by pollen from the reciprocal cross. This may indicate that the reduced volume of pollen was wholly responsible for the poor seed sets, however, it should be considered that in making hand pollinations, considerable care is taken to direct the pollen collected in the tassel bag onto the silks and that a great many pollen grains are deposited on each silk. These factors indicate that a large proportion of that pollen produced by (33-16 x Ky27) X (K55 x K64) is not viable.

The remaining 17 hand pollinations were made from tassels that were classified as sterile. The average grade of 0.9 was obtained for the seed sets resulting from these sterile tassels. When it is considered that any ear with one kernel was given a grade of 1, it is readily evidenced that the addition in yield from such tassels would be very small.

Yield Comparisons

A planting of the two reciprocal crosses along with a male-fertile commercial hybrid was made in the yield test field at Silver Lake, Kansas. The three hybrids were randomized in three-plot blocks and replicated eight times across the field. From this planting, the average yield of (33-16 x Ky27) X (K55 x K64) was 17.93 pounds of ear-corn per plot and the average of the reciprocal cross was 17.78 pounds per plot. The individual plot yields of the reciprocal crosses are given in Table 7. These data are typical of the uniform yielding ability of the two crosses when sufficient pollen is available to give good seed sets on (33-16 x Ky27) X (K55 x K64).

The yield data taken from the planting in the yield test field at Horton, Kansas are indicative of the yields that may be obtained from the reciprocal crosses in isolated plantings. This planting was made along the south end of the yield test field next to a "turn-row" for an adjoining wheat field and several of the end plants were injured or entirely destroyed. The seed of (K55 x K64) X (33-16 x Ky27) was of poor quality and gave an uneven stand. The yields from these plots could not be adjusted for missing hills to compensate for differences in stand because the seed was drilled in the rows instead of being planted in check rows. These factors probably account for some of the unusually low yields that deviate significantly from the mean plot yields, but the general trends in yield are evident.

Table 7. Plot yields produced by the reciprocal crosses of K2275 in randomized two row plots at Silver Lake, Kansas.

Plot number*	Pedigree of ear parent	Yield per plot# (pounds)	Plot number*	Pedigree of ear parent	Yield per plot# (pounds)
1	33-16xKy27	19.5	2	K55 x K64	17.3
5	" "	15.8	4	" "	17.4
8	" "	17.2	9	" "	18.1
12	" "	18.6	11	" "	17.5
15	" "	18.1	13	" "	15.7
17	" "	19.4	18	" "	18.6
19	" "	17.2	21	" "	18.1
24	" "	17.6	23	" "	19.5
Mean	" "	17.93	Mean	" "	17.78

* Plot numbers 1 through 24 were assigned in succession. The commercial hybrid in the planting has been omitted from the table as irrelevant data.
\# Yields were adjusted for missing hills.

The data presented in Table 8 show the general uniformity of the individual plot yields of the cross (K55 x K64) X (33-16 x Ky27). The trend toward lower yields in the central plots in both blocks of (33-16 x Ky27) X (K55 x K64) is also seen in Table 8. Poor seed sets which caused the low yields were observed in both blocks of (33-16 x Ky27) X (K55 x K64). For purposes of a 't' test, the first two plots (four rows) on each side of each block were considered as guard rows. This left a buffer of eight rows between the reciprocal crosses, thus making each cross nearly dependent upon its own pollen for fertilization. When the yields of the remaining six plots in each block of (K55 x K64) X (33-16 x Ky27) were compared to those of (33-16 x Ky27) X (K55 x K64) a highly significant value for 't' was obtained.

Table 8. Plot yields produced by the reciprocal crosses of K2275 in alternate 10 plot blocks at Horton, Kansas.

Plot number*	Pedigree of ear parent	Yield per plot (pounds)	Plot number	Pedigree of ear parent	Yield per plot (pounds)
1	K55 x K64	14.7	21	K55 x K64	17.1
2	" "	16.3	22	" "	16.6
3	" "	13.9	23	" "	16.7
4	" "	13.5	24	" "	16.0
5	" "	9.5	25	" "	16.1
6	" "	11.6	26	" "	17.3
7	" "	16.5	27	" "	16.1
8	" "	17.9	28	" "	15.3
9	" "	18.6	29	" "	13.3
10	" "	12.7	30	" "	12.7
Mean	" "	14.52	Mean	" "	15.72
11	33-16xKy27	10.7	31	33-16xKy27	12.7
12	" "	8.0	32	" "	9.8
13	" "	8.3	33	" "	6.8
14	" "	8.1	34	" "	6.2
15	" "	8.3	35	" "	8.4
16	" "	10.1	36	" "	7.7
17	" "	8.4	37	" "	5.1
18	" "	10.6	38	" "	5.0
19	" "	9.5	39	" "	7.0
20	" "	13.1	40	" "	6.6
Mean	" "	9.51	Mean	" "	7.53

* Plots were numbered consecutively from west to east. Each plot was two rows wide and 35 feet long. Rows were 42 inches apart.

The data from the Silver Lake planting have shown that the reciprocal crosses of K2275 have potentially equal yielding ability and the results from the Horton planting show the effect of a deficient pollen supply on the yield of (33-16 x Ky27) X (K55 x K64). Further evidence in agreement with this was found in comparisons of yields of the two reciprocals from rows of equal length grown in the top-cross planting on the Agronomy

Farm at Manhattan, Kansas. On both the north and south sides of the planting, three rows of (K55 x K64) X (33-16 x Ky27) were planted adjacent to three rows of the reciprocal cross. The yield from the center row of (33-16 x Ky27) X (K55 x K64) on the north side of the planting was 57.3 pounds and that of the reciprocal cross was 68.7 pounds. This difference is not considered to be greater than could be expected between two such rows of the same cross. The yields from the rows along the south side of the top-cross planting show the effect of a lack of pollen produced by (33-16 x Ky27) X (K55 x K64). The yield of (33-16 x Ky27) X (K55 x K64) was 11.8 pounds; the yield of the reciprocal cross was 56.5 pounds. In this case, the reciprocal crosses were not separated by guard rows but their locations in the field prevented a great deal of cross pollination from occurring. The male-sterile cross, (33-16 x Ky27) X (K55 x K64), was the third row south of the reciprocal cross (K55 x K64) X (33-16 x Ky27). Although this distance is not great, it is proposed that the prevailing southerly winds carried most of the pollen from the male-fertile rows north-ward. This made the row of (33-16 x Ky27) X (K55 x K64), from which the low yield was obtained, dependent upon pollen produced within that row and the south border row that was of the same pedigree.

GENERAL DISCUSSION

Observations made in fields of the reciprocal crosses of K2275 have revealed the presence of three expressions of male sterility in (33-16 x Ky27) X (K55 x K64) and the absence of all three expressions in the reciprocal cross (K55 x K64) X (33-16 x Ky27). The male sterility in (33-16 x Ky27) X (K55 x K64) was found to be the same as that exhibited by three privately controlled commercial double cross hybrids that were included in this study. These observations are in agreement with the observations Josephson and Jenkins (1948) made on a large number of double-cross hybrids which included the same three commercial double crosses and the reciprocal crosses of K2275.

Inheritance of Male Sterility

A series of single crosses, three-way crosses and double crosses made by Josephson and Jenkins (1948) furnished evidence to these investigators that the male sterility of (33-16 x Ky27) X (K55 x K64) was inherited through the cytoplasm of the inbred line 33-16 and controlled by a minimum of two genes contributed by the male parents. High sterility was found in two of 12 single crosses, when 33-16 was used as the female parent and in 11 cases when 33-16 was the seed parent of the three-way crosses. These results indicated to Josephson and Jenkins (1948) that two of the inbred lines used as pollen parents in the single cross combinations carried both genes that were required for sterility and that each of the inbreds used in the 11 three-way crosses carried one of the genes. High sterility was not found in any case in either the single crosses or the three-way crosses when 33-16 was not used as the seed parent. It was found from the investigations reported in this paper and in the investigations of Josephson and Jenkins (1948) that the proportion of plants exhibiting male sterility of (33-16 x Ky27) X (K55 x K64) was influenced by environmental changes.

When male sterility in corn was found to be due to genic inheritance, as reported by Beadle (1932), Eyster (1921), Brink (1929), Beadle (1931) and Burnham (1941), both of the reciprocal crosses exhibited the sterility with equal frequency.

A case of cytoplasmic inheritance of male sterility in corn was reported by Rhoades (1931). A cross between the original male-sterile plant and a normal plant gave an F_1 generation that was completely sterile. When male-sterile plants in the F_1 progeny were crossed to normal plants, the offspring were all male sterile. This breeding behavior differed from that of male sterility in (33-16 x Ky27) X (K55 x K64), in that all the plants of (33-16 x Ky27) X (K55 x K64) were not male sterile and the male sterility occurred only in certain cases in the F_1 progeny, when 33-16 was used as the female parent in single crosses.

Chittenden (1927) reported a male sterility in flax that occurred in only one of reciprocal crosses. According to Chittenden (1927) this was due to a recessive Mendelian factor that expressed its self only in the environment of certain cytoplasm. A male sterility in onions was studied by Jones and Clarke (1943) and found to be caused by a cytoplasmic contribution of the female parent interacting with a recessive Mendelian factor (ms) from the male parent. Rhoades (1943) found a chlorophyll variegation, called Iojap, that was present in only one of reciprocal crosses and concluded the variegation was caused by the gene ij. When the gene ij was present in a homozygous condition, it caused an irreversible mutatuion of the plastid primordia in the young zygote. In each of these cases, the abnormality arose in the F_2 generation and in each case after the abnormality arose it was exhibited by the succeeding generations, which again is a different breeding behavior than that of male sterility in (33-16 x Ky27) X (K55 x K64).

A case of male sterility in sugar beets, reported by Owen (1942), was quite similar to the male sterility in (33-16 x Ky27) X (K55 x K64). Owen (1942) found the male sterility in sugar beets to be due to a cytoplasmic contribution of the female parent in combination with two recessive factors contributed by the male parent. According to the information presented by Josephson and Jenkins (1948), it appears that the genic factors that interacted with the cytoplasmic contribution of 33-16 to cause male sterility in (33-16 x Ky27) X (K55 x K64) are dominant in their action. Owen (1945) reported that environmental changes from favorable toward unfavorable conditions increased the degree of sterility expressed by sugar beet plants heterozygous for the

Mendelian factors. It was found in the plantings reported in this paper, that as growing conditions became more unfavorable, the percent of male-sterile plants increased. The proportion of plants top fired was used here as an "indicator" of unfavorable environment.

The Effect of Male Sterility on Yield

The high yields of grain produced by (33-16 x Ky27) X (K55 x K64), when it was planted in plots two rows wide and located adjacent to plots of many other hybrids that produced abundant pollen, indicated it was fully female-fertile. Results of hand pollinations made with pollen from both the reciprocal crosses showed that the silks of (33-16 x Ky27) X (K55 x K64) were as receptive as the silks of the reciprocal cross (K55 x K64) X (33-16 x Ky27).

By comparing yields of the reciprocal crosses of K2275 in eight replications in the same planting, it was shown that (33-16 x Ky27) X (K55 x K64) possessed yielding ability equal to that of the reciprocal cross. A comparison of the yields in plantings where the two crosses were dependent on their own pollen, showed that (33-16 x Ky27) X (K55 x K64) gave definitely lower yields than the reciprocal cross (K55 x K64) X (33-16 x Ky27). The effect of an abundant supply of pollen was found by husking-back the ears of the block of (33-16 x Ky27) X (K55 x K64) in planting 5. This block was planted adjacent to a yield-test planting of yellow corn. Those hills of (33-16 x Ky27) X (K55 x K64) nearest the yellow corn produced ears bearing mostly yellow kernels. As the distance from the yellow corn increased, fewer yellow kernels were found and the "scattergrain" condition appeared.

It was found in the observations made in this experiment that an average of 62.72 percent of the plants of (33-16 x Ky27) X (K55 x K64) produced sterile tassels. Josephson and Jenkins (1948) reported finding in their plantings, that an average of 58.3 percent of the plants of (33-16 x Ky27) X (K55 x K64) produced sterile tassels. These investigators also reported that from general observations, appreciably less pollen was produced by hybrids having 33-16 as the seed parent of the seed-parent single cross than was produced by plants in normal hybrids. These observations were confirmed by the pollen yield studies made in this investigation.

Examinations of more than 3,000 tassels of (33-16 x Ky27) X (K55 x K64) in nine different plantings revealed that by the use of fiducial limits, a minimum average of 59 percent of the plants could be expected to be completely male sterile. It was found by volumetric measurements that the remaining 41 percent of the plants could be expected to produce on the average a maximum of 40 percent as much pollen as plants of the reciprocal cross. This means that in the plantings studied in these investigations only 17 percent as much pollen was produced by (33-16 x Ky27) X (K55 x K64) as was produced by (K55 x K64) X (33-16 x Ky27).

The investigation of seed sets obtained from hand pollinations have shown, that when the pollen collected from an apparently normal plant of (33-16 x Ky27) X (K55 x K64) in 24 hours was placed directly on the silks of a plant of either of the reciprocal crosses of K2275, the seed set obtained was 60 percent as good as from pollen from (K55 x K64) X (33-16 x Ky27). This may indicate that a portion of the pollen shed by apparently normal plants of (33-16 x Ky27) X (K55 x K64) was incapable of fertilizing the ovules.

It has been estimated that about 45,000 pollen grains are produced by dent corn for each ovule, Robbins (1931, p. 167). It was shown that (33-16 x Ky27) X (K55 x K64) produced 17 percent as much pollen as was produced by a normal hybrid. This indicated that, under conditions of this experiment, approximately 7,650 pollen grains were produced for each silk present in plantings of (33-16 x Ky27) X (K55 x K64). It was considered that between 60 and 80 percent of the pollen grains shed by this cross were fertile, thus reducing the number of functional pollen grains to less than 6,500 per silk. When the portion of space occupied by the tuft of silks on an ear shoot is compared to the space of a field, it becomes evident that a very great number of pollen grains must be provided for at least one to fall on each silk.

Plant Heights and Top Firing. Although there is no known relationship of either top firing or plant heights to male sterility, the presence of male sterility in only one of the reciprocal crosses of K2275 suggested the possibility that they may also differ in other characters. Severe top firing has been found to cause male sterility in corn by 'blasting' the tassels, however, in this experiment sun-blasted tassels could easily be distinguished from the sterile tassels found in (33-16 x Ky27) X (K55 x K64).

No evidence was found that indicated a consistent difference in plant heights of the two reciprocal crosses.

The data presented indicate there may be a difference between the reciprocal crosses of K2275 in susceptibility to top firing. Under favorable growing conditions, similar proportions of the plants of the two reciprocals exhibited top firing. It was indicated that as the severity of the environmental conditions increased the difference in proportion of plants top fired became greater between the reciprocal crosses. This indicates that a range of conditions exists in which the reciprocal crosses of K2275 exhibit significantly different amounts of top firing. If such a difference does exist, the degree of difference, the range of conditions and an explanation for the difference would need to be determined by more exactly controlled and detailed studies than were conducted in this experiment.

SUMMARY AND CONCLUSIONS

A series of eleven plantings were made of the reciprocal crosses of K2275. These plantings were made to furnish material for determining the extent of male sterility in (33-16 x Ky27) X

(K55 x K64) and the possible effect on seed set and to determine whether or not the sterility was present in the reciprocal cross (K55 x K64) X (33-16 x Ky27).

It was determined from individual tassel examinations that (33-16 x Ky27) X (K55 x K64) exhibits three expressions of male sterility. All three types of expressions of the sterility were virtually absent in the reciprocal cross. A minimum of approximately 59 percent of the plants of (33-16 x Ky27) X (K55 x K64) could be expected to be male sterile under the conditions of these tests. The male sterility found in this cross was present in varying degrees but was of such proportion in each of the plantings to have caused an economically important reduction in the yields. Male sterility was of such rare occurrence in the reciprocal cross that it was of no economical importance.

Records of the yields of both reciprocal crosses indicate that under experimental yield-test conditions (33-16 x Ky27) X (K55 x K64) was equally as productive as the reciprocal cross. Yields produced by the reciprocal crosses, when they were largely dependent upon their own pollen, showed that the yields of (K55 x K64) X (33-16 x Ky27) were significantly greater (at the 1% level) than the yields of (33-16 x Ky27) X (K55 x K64). The low yields of (33-16 x Ky27) X (K55 x K64) were caused by an insufficient supply of viable pollen that resulted in poor seed sets which were absent in (K55 x K64) X (33-16 x Ky27).

The male sterility found in this hybrid is highly subject to environment, especially soil moisture availability. As environmental conditions become more severe, a greater proportion of the plants were found to be male sterile. The sterility exhibited by (33-16 x Ky27) X (K55 x K64) is expressed in the greenhouse in the same manner as in the field.

Treatment of very young seedlings at 50° C. for one hour in an electric oven seemed to have little or no effect on the expression of male sterility in either of the reciprocal crosses. All three expressions of male sterility found in other plantings of (33-16 x Ky27) X (K55 x K64) were present in this planting and none of the plants of the reciprocal cross were observed to be male-sterile. It was also found that heat treatment of the seedlings had no measurable effect on the height of the plants at 55 days after planting.

These conclusions are in direct contrast to the observations made by Jones (1947) on a similar experiment.

A study of pollen yield measurements made on the two crosses indicated that approximately 40 percent as much pollen was shed from "normal" plants of (33-16 x Ky27) X (K55 x K64) as was shed from plants of (K55 x K64) X (33-16 x Ky27). This was the maximum amount of pollen shed by the normal plants of the cross (33-16 x Ky27) X (K55 x K64) under conditions of this experiment, as determined by the confidence interval at the 99 percent level.

Seed set classifications made on ears resulting from hand pollinations revealed that pollen from apparently normal plants of (33-16 x Ky27) X (K55 x K64) gave seed sets approximately 60 percent as good as pollen from the reciprocal cross. Microscopic examination of pollen taken from abnormal anthers that were not shedding pollen revealed an estimated ratio of 1 normal to 4 abnormal pollen grains. It was concluded that a proportion of the pollen shed by apparently normal plants of (33-16 x Ky27) X (K55 x K64) was also abnormal. This abnormality was considered to be at least partially responsible for the reduction in seed set on the hand pollinated ears.

There was no consistent significant difference between the reciprocal crosses in plant height as determined by measurements of the growing plants in the field. Plant heights were found to be significantly effected by soil moisture but the response to added soil moisture was approximately equal in both the reciprocal crosses.

Observations made on the proportions of plants top fired indicated that the reciprocal crosses may differ in their susceptibility to adverse environmental growing conditions. The cross (K55 x K64) X (33-16 x Ky27) had a consistently but not always significantly higher proportion of plants that were top fired than did the reciprocal cross. Climatological data and soil moisture levels that would permit definite conclusions to be drawn on this characteristic were not recorded in this experiment.

ACKNOWLEDGMENTS

The writer wishes to acknowledge his indebtedness to Dr. Loyd A. Tatum, who served as major instructor, for his many helpful suggestions and guidance in conducting the investigations; for his unselfish devotion of time, and for the use of land on which the plantings were made. The writer also wishes to acknowledge the aid of Dr. H. C. Fryer in planning the field designs and making the statistical analysis of the data. Appreciation is expressed to Dr. H. H. Laude and to Mr. A. L. Clapp for their helpful suggestions and to the wife of the writer, Mrs. Florence M. Vandeventer, for her continued inspiration throughout this study and her help with the clerical work.

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