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Cover photo: Fertile and sterile tassels at the time of pollen shedding. This photo and Figures 1, 4 and 5, courtesy of Funk Brothers Seed Co. Figure 2, courtesy of J. W. Hardin, Orval Ehrhardt Farms, Chenoa, Illinois.

POLLEN RESTORING GENES

by

D. F. Jones, H. T. Stinson, Jr., and U. Khoo

Hybrid corn is the product of crossing each year selected inbred strains with superior germplasm adapted to the special conditions prevailing in each region where corn is grown. The first crosses of naturally pollinated corn plants were studied by Charles Darwin in England and reported in his book on "Cross and Self Fertilization in the Vegetable Kingdom" in 1876.

W. J. Beal, starting in 1876 in Michigan, conceived a novel method of cross pollination. He planted the varieties to be crossed in adjacent rows and emasculated the female lines by pulling the tassels. Thus he insured hybridization; thus was born the detasseling technique that was destined to be so important in the hybrid corn industry; and thus he obtained pollen sterility by mechanical means.

Fortunately for Beal, and for the seed corn industry, seed corn growers, and consumers, the corn plant is constructed in such a way as to make the production of hybrid seed feasible. The seed bearing flowers, clustered in the lateral ear, are separated from the pollen producing flowers in the tassel at the top of the stalk. It is a simple matter to pull out these tassels before pollen is shed from all of the plants used as females for seed production. Such plants are effectively emasculated and are sterile as far as pollen functioning is concerned.

Removing the tassels from short inbred plants is relatively easy and inexpensive. Pulling out the tassels from tall first generation hybrid plants in the production of double crossed seed is a much more difficult and expensive undertaking.

About fifteen million bushels of seed corn are needed to plant the eighty million acres of field corn grown each year in the United States. The latest report of the United States Department of Agriculture, Agricultural Marketing Service, in 1955 states that 89.2 per cent of this acreage is planted with hybrid seed. Another twenty million acres are also grown each year in other parts of the world. Pulling out the tassels from 300,000 acres of seed fields in this country has proved to be a major undertaking. Finding sufficient temporary help to do this at the right time is difficult and expensive. Pollen shedding comes on rapidly and is completed in about a week or ten days. If the tassels are not removed completely just before shedding begins the seed cannot be used for planting. If the tassel is removed too soon the plant is injured and seed yield is drastically reduced. Some seedsmen found detasseling such a difficult problem that they were about to give up hybrid seed production before methods of eliminating detasseling were developed.

For many years, beginning in 1917, many ways of producing hybrid seed corn without sterilizing the pollen by detasseling were tried at The Connecticut Experiment Station. Until a method of restoring pollen fertility to cytoplasmic sterile seed parents was discovered none of these was successful. The method now used is described in Connecticut Bulletin 550, "The Production of Hybrid Corn Seed Without Detasseling."

The idea of using a sterile tassel condition to eliminate detasseling began with the discovery of dioecious corn by the senior author in 1927. In the experiments that led to the production of dioecious corn there were progenies in which all of the plants had barren ears. The plants were otherwise entirely normal. These sterile ears resulted from a combination of recessive genes that could be easily controlled to give only seed sterile plants. No practical use has been made of seed sterile corn plants, but if seed sterile plants were possible why not produce pollen sterile plants? Such plants obviously could be used for the production of hybrid seed without emasculation.

A search was therefore instituted for gene controlled pollen sterility. Such a gene was found in 1923 and reported by Singleton and Jones (1930). It occurred in an inbred line of Sanford White flint corn. These gene sterile plants when crossed by any normal pollinator would be restored to complete pollen fertility in the final double cross.

This method was found to be very difficult to use, however, because of manipulative difficulties. This gene was closely linked to the yellow-white endosperm gene. By using this gene linked with either the yellow or the white allele for endosperm color, it was possible to produce seed parents that were tassel-sterile. The trouble, however, is that there was about six per cent of cross-overs plus a small amount of hetero-fertilization. Another difficulty was to transfer these two linked genes to the seed parent inbreds. Furthermore, the seeds had to be separated by color and this was tedious and expensive and only about half of the parent seed could be used for planting. With all of these technical difficulties, this method has never been used commercially but still has possibilities, especially if some method can be discovered whereby the gene sterile plants can be made temporarily fertile by some chemical or other treatment.

Still another possibility was to find pollen sterility that was cytoplasmically inherited, that is, transmitted only by the seed parent. In 1931 Rhoades (1931, 1933) made the important discovery of a sterile tassel condition in corn that has its basis in the cytoplasm. He showed that this could be incorporated and maintained in many different genotypes and some of the progenies would remain all pollen sterile. A similar condition had been found many years earlier in a number of species in other families.

This cytoplasmic sterile condition appeared to be the answer to the problem for a while. It seemed obvious that if the female lines could be made pollen sterile, one would not have to detassel them. The practical trouble showed up very soon, however. Since cytoplasmic sterility passes down through the female line, the progeny which were sold to the farmer would also be pollen sterile and the farmer would find himself with a field full of cobs and no grain.

This method of producing hybrid plants, however, did succeed quite well in the onion and sugar beet crops. In these crops the farmer does not sell the seed, but rather the vegetative part of the plant, and therefore it does not matter whether the flower is sterile or not.

Not having found a practical use, the Rhoades strains of pollen-sterile corn eventually were lost. Additional sources of pollen sterility were found by P. C. Mangelsdorf at the Texas Agricultural Experiment Station and by M. T. Jenkins at the United States Department of Agriculture, and these have been used as the basis for experimental work discussed herein.

A very serious problem that had to be solved in the use of cytoplasmic

sterile pollen was the variability of pollen production in the sterile seed parent plants. Progenies that were completely sterile when grown in the summer were highly fertile in the greenhouse during the winter. This was due possibly to the differences in the length of day. In many cases the sterility was constant in one inbred but when transferred to another line the plants would be highly fertile. Even in the same inbred in successive generations or the same seed grown in different seasons pollen production was highly variable.

These difficulties were not met with in the onion. The first sterile onion seed parents used were propagated as vegetative clones. A clone is genetically uniform and the sterile onion used for seed production showed little variation in pollen abortion under the usual conditions where onion seed is produced. Furthermore, the onion is grown for bulbs and the pollen or seed sterility or fertility of the final crop is of no importance. Hence, the use of cytoplasmic sterility in onion did not offer any promise of usefulness to corn breeders. It is interesting to note that the use of cytoplasmic sterility in seed corn production when known was immediately applied to the grain sorghums.

The same knowledge of basic principles that made hybrid corn possible, also made feasible the production of seed without detasseling. These important principles were the control of heredity in both the seed parent and pollen parent. The pure line concept first formulated by Johannsen in Denmark for self fertilized plants was applied by Shull to the cross fertilized corn plant. The experiments of East and of Hayes finally resulted in the method of selection in self-fertilized lines developed by the senior author (1920) to obtain the superior germplasm now used in the production of hybrid corn. This same control of the heredity in both the seed and pollen parents is necessary for the use of cytoplasmic sterility. Without this genetic control seed propagated inbreds and single crosses would not be sterile under variable conditions of culture and dependable pollen production could not be restored in the final hybrid.

Methods of Pollen Restoration

The first method used restored pollen in the farmer's field by producing two kinds of seed of the same genetic composition—one portion with sterile tassel and one with normal tassel. These two kinds of seed were then mixed in a proportion that produced adequate pollen to give a satisfactory crop. This method of mixing sterile and fertile varieties has long been used by fruit growers. Pollen sterile or imperfect flowered varieties of strawberries are commonly interplanted with perfect flowered varieties to insure adequate pollination. Self sterile varieties of fruit and nut trees are regularly grown in mixed plantings to give the necessary amount of cross-pollination. There seemed to be no reason why this method would not work equally well with corn and it was used as soon as sterile inbreds could be produced.

Promising sterile single crosses were grown in 1947 and 1948 at the Mt. Carmel farm and a crossing field using sterile and fertile seed parents of the same genetic composition with one common pollinator was grown there in 1949 (Jones & Mangelsdorf, 1951). The two lots of seed were mixed and grown in many parts of this country with such satisfactory results that this method was rapidly put to commercial use as fast as the necessary sterile inbreds could be produced. The usual proportions are two-thirds sterile and one-third fertile plants. The two lots may be grown in alternating rows or blocks in the same

Figure 1. A seed production field in which all of the seed parent plants have been detasseled.

field and mixed in the field at time of harvest, or they may be produced in separate fields and mixed in the warehouse after shelling.

In the process of transferring the cytoplasmic condition of pollen abortion to many of the standard inbreds widely used in the production of hybrid seed it was soon noted that some lines were easy to sterilize and some were difficult. A few of the first crosses of cytoplasmic sterile plants by standard inbreds such as Hy, P8, 38-11, W22, and P39 produced many plants with a considerable amount of pollen production. On account of these results it was necessary to devise a method called paired progeny selection in order to produce lines that were completely sterile. In this method an individual plant of the inbred to be sterilized is self pollinated and pollen from this same plant is put on the silks of sterile plants. The two progenies, one fertile, the other sterile or partially sterile, are grown in adjacent rows. If the plants are all sterile in one member of the pair, this progeny and its paired fertile line are used to continue the backcrossing. All fertile or partially fertile backcrosses with their fertile mates are discarded.

In this process it was recognized that there must be genes in the chromosomes capable of restoring normal fertility to these cytoplasmic sterile plants. This interrelation of plasmagenes and chromogenes has been described (Jones, 1950). It was realized at this time that selection by the paired progeny method for fertility restoration should be as effective as selection for sterility. This was done later for two inbreds, W22 and 38-11, and we now have selections of these inbreds with one type of sterile cytoplasm that are completely restored to normal pollen production.

The first crossed plants of sterile by normal inbred lines that appeared to

Figure 2. A foundation seed production field in which all of the seed parent inbred plants have sterile tassels.

be completely normal in pollen production were (Illinois A sterile × A158) (Dioecious 15.17 ♀ × sk). This was a cyto-sterile tassel female crossed by a gene sterile seed male in 1947 and grown in 1948. Out of the 15 plants in one progeny, 5 appeared to be completely normal in pollen production. In this same year other crosses were highly fertile, producing anthers and some pollen on all plants. These crosses were (AS × M14) and (WF9S × P8). Several plants were self-pollinated in each lot and produced a full set of seed. In 1949 several crosses produced plants that were all fully fertile. The most fertile were: AS × B164, C106T × Oh41, C106T × Ky21, and (WF9S × 38-11) Ky21 (the letter S or T in the pedigree gives the source of the sterile cytoplasm).

In the years from 1944 to 1950 more attention was given to the development of completely sterile inbreds that could be used to produce satisfactorily sterile seed parents. This was the most important thing to do first. No method of restoring pollen would be of any value unless it were possible to have good sterile seed parents. A number of inbreds already in use in the production of commercial hybrids have been found to be good restorers. Many of the standard inbreds have been converted to restorers and these can be used to give complete restoration if this is desirable. Most seedsmen are convinced that about 50 percent of the normal amount of pollen production is more desirable than complete restoration. This can usually be obtained by using one good restorer in combination with one non-restoring inbred as the pollinator of the final double cross. The non-restoring inbred can be cytoplasmic sterile. This method avoids all detasseling in both of the foundation single crosses as well as in the final double cross. It also provides a check on the functioning of the restored pollinator before the hybrid is grown for farm production.

Sources of Cytoplasmic Pollen Abortion

The S type of pollen abortion

Cytoplasmic sterile corn plants were first grown at this Station in 1944 from two lots of seed obtained through the kindness of Dr. M. T. Jenkins of the United States Department of Agriculture. These were labeled CI2077 circle 8 Ms × 675 (Ill.A) and CI2077 circle 6 Ms × 657 (I234). About 15 plants of each were grown. The plants appeared to be normal in every respect except that the tassels were completely devoid of extruded anthers. Upon examination the anthers were present within the glumes but were devoid of any normal pollen grains. All plants were completely pollen sterile. Both lots were crossed by several standard inbreds—Illinois A, Hy, Indiana WF9, and P8.

This source of cytoplasmic pollen abortion had been grown at the Arlington Farm in 1937 and was a stock of iojap × teopod originally obtained from Dr. E. W. Lindstrom at the Iowa Agricultural Experiment Station. It had been crossed by a chromosome 7 linkage stock containing *v*$_5$ *fr*$_6$ *ra gl*. The original teopod plants shed little or no pollen. An occasional plant had been selfed. This stock was not related to the cytoplasmic material described earlier by Dr. Rhoades. It is now designated the S type of pollen abortion.

In 1945 the first generation backcross of (CI2077 × 675 Illinois A) by A showed a few anthers on some plants but these anthers shed no visible amount of pollen. In the first cross by WF9 one plant produced anthers and a small amount of pollen. The first cross by P8 produced many anthers and much pollen on nearly all plants. Also the first cross by Hy gave three plants with considerable pollen. Only plants without anthers were selected for further crossing for the paired progeny selection program and in a few generations backcrossed lines were obtained that were completely sterile in the summer growing season in Connecticut.

The T type of pollen abortion

In 1945 an additional source of cytoplasmic pollen abortion was received through the kindness of Dr. J. S. Rogers at the Texas Agricultural Experiment Station. This had the notation of (G.J.39-25-1 × 203). Four different lots of seed were sent, representing crosses of this sterile stock by the inbreds K4, Kys, Tx173D and Tx203. This type of sterility had been found earlier by Dr. P. C. Mangelsdorf at the Texas Station in crosses of Golden June field corn and a sweet corn. Later sterile plants were found in the original white seeded Mexican June variety from which Golden June had been derived.

In Connecticut the cross with Tx203 failed to germinate but the other three lots produced progenies that were almost completely sterile tassel. In the K4 cross there was one plant with a few anthers and some pollen. In the Tx173D there were two plants with a small amount of pollen. The cross with Kys showed no anthers on any plants and these aborted anthers were all devoid of normal pollen grains. This completely sterile progeny was crossed by many of the later maturing field corn inbreds and also by P39 and C13 sweet corn. This source of cytoplasmic pollen abortion is now designated the T type.

Additional sources of pollen abortion

The S and T types of cytoplasm are genetically different by their behavior

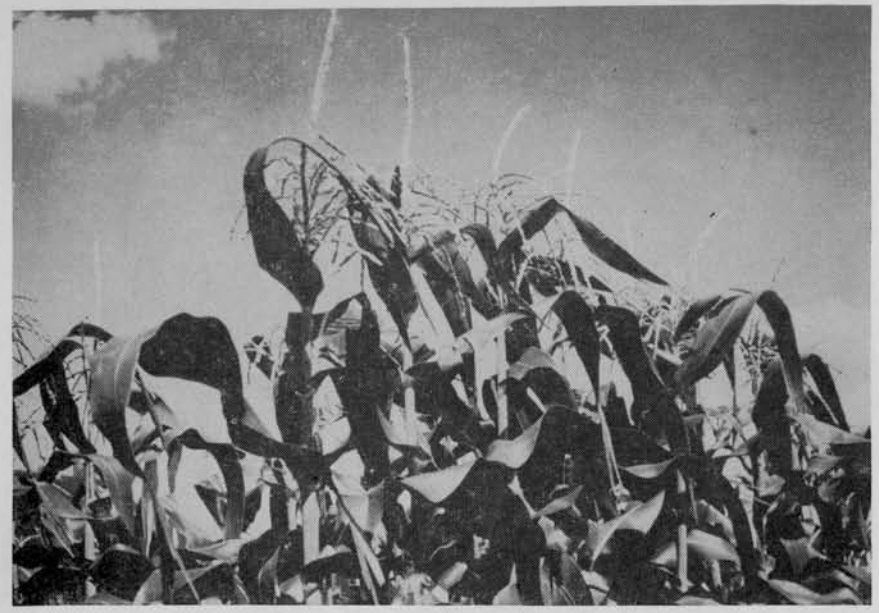

Figure 3. A sterile seed parent single cross resulting from the cross pollination of a sterile inbred and a non-restoring pollinator inbred.

in crosses with restoring inbreds as will be shown later. Many additional sources of cytoplasmic pollen abortion have been found and are now in the process of being converted to common genotypes and tested for similarity or dissimilarity with the S and T types and each other. Eight of these new sources have been designated by letters of the alphabet from A to H.

The sources of these different lots of seed are as follows:

A. A yellow seeded 12-rowed flint from Turkey received from the United States Department of Agriculture, Foreign Plant Introduction, Number 171 892. Sterile plants in this lot were pollinated by A158. Later generations of this cross were again crossed by WF9. The first crosses produced some anthers but shed little or no pollen.

B. A yellow tropical flint received from Dr. F. G. Brieger in Brazil in June, 1948, and first grown in Connecticut in 1949. The plants grew 11 to 12 feet in height and flowered in September. There were anthers and some pollen on nearly all plants. The most sterile plants were pollinated by A158 and K4 and these crosses were in turn pollinated by WF9. Completely sterile lines of backcrossed A158 and WF9 have been established but a four generation backcrossed progeny of W22 produced 10 partially fertile and 8 sterile plants in 1956.

C. Sterile plants in a segregating progeny of *Vg,sy,j,v*$_{16}$ received from Dr. G. F. Sprague at the Iowa Agricultural Experiment Station in 1952. The normal *vg* plants all had sterile tassels. A158C3 plants were all sterile in 1956 and WF9C1 had 2 partially fertile with the remaining plants all completely sterile in the same year.

D. Sterile plants in a B9 *Vg su* stock received from Dr. W. C. Galinat at the Wisconsin Agricultural Experiment Station in 1954. Crossed by WF9 the

Figure 4. A seed production field in Illinois in which normally fertile seed parents with tassels removed are growing in the same field with a sterile tassel version of the same seed parents.

first generation plants were all completely sterile in tassel in 1955 and in 1956 had two partially fertile plants.

E. Sterile plants in a segregating progeny of a sterile stock of Penna. 51-6 × Penndale sweet corn received from Dr. J. E. Wright at the Pennsylvania Agricultural Experiment Station in 1954. Two generations of backcrossing by WF9 have produced some plants with anthers and some pollen in each generation.

F. Sterile plants in a progeny of iojap stock received from Dr. M. M. Rhoades at the Illinois Agricultural Experiment Station in 1954 with the designation 16801Ms × M14. This planting had a few plants showing anthers but apparently no pollen was released. Plants showing no anthers were crossed by WF9. The resulting progeny had 3 partially fertile and the remaining plants sterile in 1956. Other sterile plants crossed by A158 were completely sterile in 1956.

G. Sterile plants in a segregating progeny received from Dr. C. C. Wernham at the Pennsylvania Agricultural Experiment Station in 1954 with the designation (463 × 471) 2077. There were 11 plants with many anthers and much pollen. There were a few sterile plants. Two of these were pollinated by WF9 and one of these two backcrossed progenies in 1956 produced all sterile plants. The other had two partially fertile and the remaining sterile plants.

H. A completely sterile progeny received from Dr. L. M. Josephson at the Kentucky Agricultural Experiment Station in 1956 with the designation R1201. This is a backcrossed WF9 line from Ky27 sterile originally out of Indiana 33-16.

The Manifestation of Cytoplasmic Pollen Abortion in Maize

Horticulturists have long been familiar with fruits whose flowers produce little or no pollen. Varieties of fruit are propagated vegetatively so the inheritance of this pollen abortion is of little practical importance in strawberries and grapes where it is quite common. Varieties of this type are grown in spite of their handicap of producing little or no pollen because they are more productive or more desirable in other respects. They must be interplanted with normal pollen producing varieties that flower at the same time in order to be properly pollinated.

Correns (1908) was the first to study the inheritance of pollen abortion. In two genera in different families, Satureia, a mint of the family Labiatae, and Cirsium, a thistle of the family Compositae, Correns found that pollen abortion in some cases was transmitted by the seed parent relatively uninfluenced by the pollen parent. In this respect this character differed from the usual transmission of dominant and recessive mendelian units of heredity.

Bateson and Gairdner (1921) also studied a maternally inherited condition of pollen abortion in crosses of related species of flax (Linum). This was later shown to be subject to genic as well as cytoplasmic control (Chittenden, 1927). A similar situation has been described by Jones and Clarke (1943) in the onion (Allium). This type of sterility has been used for the production of hybrid seed. Fortunately the onion can be easily propagated vegetatively as well as by seed, and it is therefore not entirely dependent upon pollination. Moreover, the onion is grown for its edible bulb and pollen fertility is not involved in the production of the final crop.

Pollen abortion has also been reported in many other genera, notably Epilobium, Oenothera, Nicotiana, Petunia, Streptocarpus, Sorghum and Zea. Maternal inheritance of a chlorophyll abnormality in maize was described by E. G. Anderson (1923). Cytoplasmic pollen sterility in maize has been reported by several investigators. The first case was described by Rhoades (1931, 1933). Other investigators reporting similar conditions in other forms of maize are Gini (1940), Jones and Everett (1949), Jones (1950, 1951, 1954), Jones and Mangelsdorf (1951), Rogers and Edwardson (1952), and Edwardson (1955).

Josephson and Jenkins (1948) studied a condition known as "scatter grain" in which a number of hybrid varieties of corn grown in Kentucky, Tennessee, and Indiana failed to set seed properly. This was found to be due to insufficient pollen for proper fertilization. It resulted when certain inbreds were used as the seed parent of the single cross used for the production of seed of the final double cross. The inbred chiefly involved in these hybrids with a poor set of seed was Indiana 33-16. This is the source of the H type of cytoplasmic sterility listed on page 10.

The peculiar characteristic of this inbred is the fact that the inbred itself is normally fertile. It was not itself a sterile line, but pollen abortion sufficient to produce a noticeable failure of seed production was brought about in crosses with certain other inbreds. The genic composition of the pollen parent controlled the amount of pollen produced. When used as a seed parent in experimental hybrids the failure to produce adequate pollen was not noted, since in trials of single crosses and double crosses there was always abundant pollen from

fertile plants growing in adjacent rows in the same field. It was only when these partially sterile hybrids were grown separately in farm fields that the pollen deficiency was noted.

When tested in crosses with 12 other lines, two of these pollinator lines were found to give high percentages of pollen abortion in combination with 33-16. These were K63 and Mo.ZRF. Other lines gave a low percentage of sterile plants and a number of lines produced crosses with 33-16 that were completely normal, or almost so.

Many double crosses had high sterility when 33-16 was used as the seed parent of the seed parent single cross, but when Ky21 was used as one of the parents of the pollinator single cross all pollen sterility disappeared. In their publication, Josephson & Jenkins (1948) were interested primarily in preventing the scatter grain condition that had caused considerable loss to corn growers. They made no mention of the possible use of completely sterile seed parents to eliminate detasseling in seed fields. From the evidence previously obtained at the Connecticut Station it seemed probable that Ky21 had the genic constitution necessary to restore normal pollen production to plants that were partially sterile due to some cytoplasmic condition. It was not known that this inbred would restore pollen production to other completely sterile inbreds. Crosses were first made of the inbred Ky21 and the single cross Ky36 × Ky21 on C106T sterile. The hybrid plants were restored completely in the single cross of C106T × Ky21, and half of the plants were restored completely in the three-way cross of C106T × (Ky36 × Ky21). These results indicated clearly that restoration was

Figure 5. The two rows with light colored tassels are pollinators. The six rows between the pollinator rows are seed parents composed of two fertile rows (tassels removed) and four sterile rows (tassels not removed).

brought about by a dominant gene showing the usual mendelian segregation. Additional results reported in 1950 and here confirm this.

The unusual behavior of Indiana 33-16 must be noted. Seed of this line was obtained from the Kentucky Agricultural Experiment Station and grown in Connecticut in 1951. A small progeny of some 15 plants were all normally fertile. Three plants were crossed by C106 and grown in 1952. These F_1 plants were also completely fertile. Two of these plants were self pollinated and the F_2 generation grown in 1953 and again 15 plants were all normally fertile. The fact that this line is fertile when selfed and crossed with many inbreds, but highly sterile in F_1 combinations with certain inbreds, is most unusual. All the inbreds used to restore the S and T types of sterile cytoplasm have dominant genes that are always expressed in the F_1 and later generations. In the case of Ind.33-16 the manifestation of fertility and sterility is apparently quite different.

Expression of Cytoplasmic Pollen Abortion and Restoration

Microsporogenesis in cytoplasmic male sterile plants has been normal in all plants examined. The only indication of meiotic irregularity is the report of Chang (1954) that microsporocytes of A158T and A158S sterile plants were smaller than those of normal A158 when measured in sectioned material at prophase I (for A158S) and metaphase I (for A158T). Chromosome behavior, at any rate, appears to be normal. Ten bivalents are present at pachytene, chiasmata appear at diplotene, and anaphase disjunction occurs in a regular fashion. The second meiotic division takes place normally, giving rise to normal-appear-

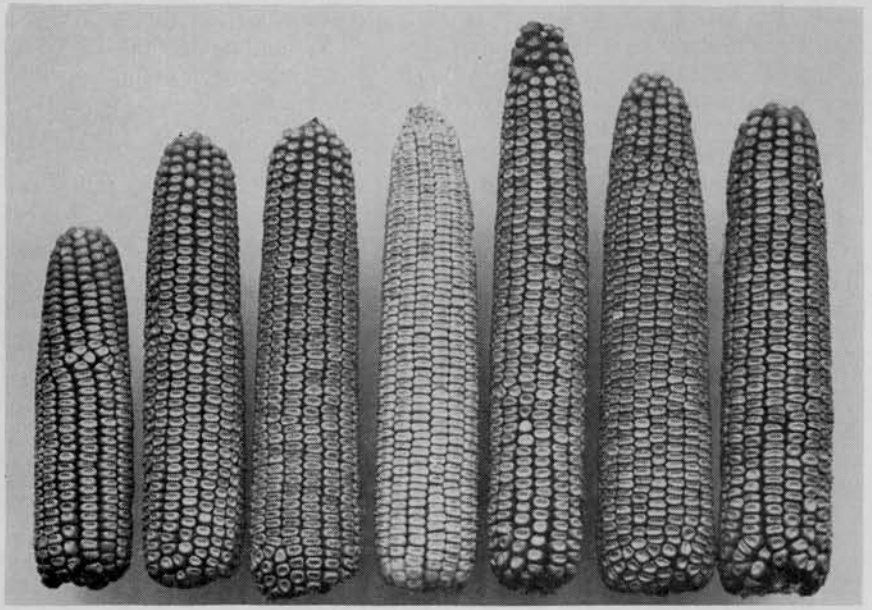

Figure 6. Ears of single cross restoring pollinators. The four ears on the left are crosses of two restorer inbreds. The three ears on the right are crosses of a sterile inbred by a restorer inbred. The first lot will restore 100 per cent and the second lot will restore 50 per cent of the plants in the final hybrid grown in the farmers' fields.

ing spore quartets. The young microspores immediately after release from the spore quartet are also indistinguishable in fertile and sterile plants. The first signs of degeneration become evident during subsequent stages of microspore growth and development. The earliest visible indications of abortion are evidenced by an inhibition in the development of the microspore wall and germ pore. In addition, the cytoplasm of degenerating microspores appears to be more highly vacuolate and to display a more ordered alignment of cytoplasm inclusions than the cytoplasm of normal microspores at comparable stages of development. Size differences are also apparent, microspores from sterile anthers being noticeably smaller at an early stage of microspore growth. The exact stage at which visible differences between the microspores of normal and sterile anthers are manifest seems to vary in different plant material, even where the same type of sterile cytoplasm is involved. In some sterile anthers examined binucleate microspores are rarely seen, whereas in other material binucleate spores are regularly produced, indicating that degeneration may take effect before or after the first post meiotic mitotic division. In any event, at maturity the aborted pollen grains are in most cases small, more or less transparent bodies which are devoid of starch.

An additional criterion by which anthers of normal and male sterile plants with T type cytoplasm may be distinguished has been found in the free amino acid content of anthers as revealed by the technique of paper chromatography. Comparison by this technique of anthers from the sterile F_1 hybrid, C106T8 $\times$ A158, with anthers from the normal fertile hybrid, C106 $\times$ A158, in various stages of anther development shows that anthers in premeiotic stages and in meiotic stages up to quartet formation are identical with respect to free amino acids. The first difference appears at the quartet stage, sterile anthers invariably beginning to show an accumulation of alanine. The build-up of alanine in sterile anthers increases during the stages of microspore development, eventually reaching a two to three-fold increase over the alanine content of normal anthers. Normal anthers also ultimately accumulate free alanine, but only at later stages of pollen development. Sterile anthers, therefore, undergo a precocious accumulation of alanine. It will be noted also that the first differences in amino acid content appear before any visible differences in sporogenous tissue are observed.

The precocious accumulation of alanine beginning in the quartet stage and increasing during the microspore stages appears to be characteristic of T cytoplasm, for it has been seen in all T sterile lines thus far examined (C106, A158, WF9, WF9-4). Anthers from sterile plants with the A, B, or S type of cytoplasm, on the other hand, have failed to show a precocious accumulation of alanine. The amino acid chromatography studies thus offer another line of evidence that the different sources of sterile cytoplasm are not identical.

At later microspore stages a second ninhydrin-positive substance builds up in T sterile anthers in excess of the amount in fertile anthers. This substance, distal to alanine on the paper, has not yet been identified. At late stages of pollen development, other differences have been observed in the relative contents of proline and asparagine, the former being virtually absent in sterile (T) but present in fertile anthers, and the latter present in greater amounts in sterile anthers.

Anthers from restored fertile plants of the F_1 hybrid C106TF5 (Ky) $\times$ A158 are chromatographically identical with anthers from the normal fertile hybrid C106 $\times$ A158. Thus, the effect on free amino acid content associated

Figure 7. A single cross restorer pollinator in a seed production field at the Mt. Carmel farm growing in 1954.

with T cytoplasm is not evident when this type of cytoplasm is combined with restorer genes from Ky21. The effect of the restorer genes which results in a normal free amino acid content does not, however, permanently alter the T cytoplasm, for anthers of segregating sterile plants from restored fertile plants (heterozygous for restorer genes) show the amino acid pattern typical of sterile anthers.

What meaning these findings have for the cause and mechanism of cytoplasmic male sterility is, at present, not clear. They do serve, nevertheless, to characterize further in a descriptive way the differences between plants with normal and T type cytoplasm.

The mechanism which causes pollen abortion in plants with sterile cytoplasm is largely unknown. One question that arises in this connection is whether the abortion process is initiated in sporophytic or gametophytic tissue. The fact that the first abnormalities in development are seen only shortly before (chromatographic evidence) or shortly after (visible degeneration) the haploid microspores are liberated might suggest the sterility-inducing system operates in the gametophyte. On this view, pollen abortion occurs in plants with sterile cytoplasm because the cytoplasm of the microspore (when present with certain genomes) lacks in some way the ability to carry out one or more functions necessary for normal growth and development. The particle hypothesis advanced by Gabelman (1949) assumes a gametophytic effect. According to this hypothesis the factor causing male sterility is a particle which is usually distributed regularly to daughter cells during mitosis, but is randomly distributed at meiosis. The presence of one or more particles in a microspore leads to pollen abortion; microspores which by chance fail to receive a particle at meiosis develop into

Figure 8. The same field as in Figure 7 showing the sterile seed parents and restoring pollinator (one row is a normal fertile that has been detasseled).

functional pollen grains. Although present in sporophytic cells and tissues, the hypothetical particle exerts its visible effect only in the developing gametophyte.

Unfortunate for the particle hypothesis, but certainly not proof against it, has been the failure to find with a variety of techniques and the light microscope any cytoplasmic inclusions peculiar to sterile plants. Perhaps the most suggestive evidence is that given by Rhoades (1933) in describing the first case of cytoplasmic pollen abortion in maize. In material fixed in Benda's fluid and stained in Heidenhain's iron-alum hemotoxylin Rhoades found marked differences in the size, shape, and number of certain cytoplasmic elements in microspores from normal and male sterile plants. These elements, which Rhoades suggests are plastids or plastid primordia, were large, spherical bodies in abortive microspores, whereas the same structures in normal microspores were thin, rod-shaped inclusions which were more numerous and more evenly distributed in the cell. Rhoades was careful to point out that there was no basis for deciding whether the abnormal cytoplasmic inclusions were a cause or an effect of microspore degeneration.

Other investigations, including those of Gabelman (1949), Edwardson (1953), and Chang (1954), as well as work performed in this laboratory, have failed to reveal consistent differences in the appearance of the cytoplasm of microspores from normal and male sterile plants. The techniques employed have mostly been those which would preserve and stain plastids and mitochondria, or which would detect the presence of structures containing ribose or deoxyribose nucleic acids.

In summary, it may be said that if the gene-cytoplasmic interaction responsible for arrested microspore development does take place in the gametophyte, the nature of the system involved remains obscure.

An alternative possibility may be that the events leading to pollen abortion have their inception in sporophytic tissues. In onion and sugar beet where cases of cytoplasmic male sterility which are genetically similar to the one in maize are known, striking alterations in one sporophytic tissue, the tapetum, have been reported. In both cases meiosis is normal, as in maize. In onion anthers at the time of microspore liberation certain tapetal cells, usually centrally located, become greatly hypertrophied and invade the anther sacs containing the microspores, where the hypertrophied cells degenerate in a manner different from normal tapetal cells (Monosmith, 1928). Degeneration of the enlarged tapetal cells is followed shortly, in most cases, by death of the microspores. In cytoplasmic male sterile sugar beets the tapetal cells form a periplasmodium after the completion of meiosis, the plasmodium making pseudopodium-like incursions into the anther cavity (Artschwager, 1947). As a result of the growth of the plasmodium the microspores are crowded together in the anther sac, but the microspores appear normal until the plasmodium disintegrates completely.

Pollen abortion in cytoplasmic male sterile sugar beets is not always associated with these tapetal abnormalities, however, for the tapetum in some flowers remained cellular and degenerated normally. The two types of behavior were observed in flowers of the same cluster, but a given flower showed only one type.

These observations in onion and sugar beet may indicate that the primary event leading to pollen abortion takes place in the tapetum, that is, that the cytoplasmic effect is mediated through sporophytic tissue. That abnormal behavior of the tapetum could bring about microspore degeneration is understandable in view of the generally held role of tapetal cells in the nutrition of sporogenous tissue of the anther (Cooper, 1952; Painter, 1943).

The situation in corn with respect to the role of the tapetum in cyto-sterility is less clear. Chang (1954) reported that tapetal cells in sterile A158 lines were thicker in transverse section than tapetal cells of normal A158 at the three stages of anther development measured. At two of the stages studied, prophase I and early pollen formation, however, the sterile lines differed significantly among themselves in tapetal thickness. In addition, the tapetum of the sterile A158 anthers persisted longer and showed a higher incidence of endomitosis than normal tapetum. The hypertrophy, enhanced endomitotic activity, and longer persistence characteristic of the tapetum in sterile anthers were interpreted by Chang to represent stages in degeneration rather than causes of degeneration. On the basis of his cytological observations of the degeneration process Chang was inclined to attribute microspore deterioration to an insufficient supply of carbohydrates and other essential nutrients, that is, to a starvation process. Chang does not suggest what might be the cause of the inadequate nutrient supply in the microspores nor does he indicate whether the functional alterations responsible for the starvation take place in the microspores themselves or in some sporophytic tissue. If this view of nutritional disturbance is correct, it is possible that the cause may be due to alterations initiated by the sterile cytoplasm in either the gametophyte or sporophyte.

The above account of investigations into the expression and causes of pollen abortion serves to emphasize that the nature of the gene-cytoplasmic effect which leads to male sterility in maize is still in doubt. It is not possible to state with

Figure 9. A close-up view of the restorer pollinator on the left (M14 x Oh41) and a sterile seed parent on the right (W22 x B9) showing the production of hybrid seed corn without detasseling and without mixing.

certainty whether the events responsible for pollen degeneration take place in sporophytic or gametophytic tissues. Those two alternatives are not, in fact, mutually exclusive, since processes taking place in both types of tissue may be adversely affected by the presence of sterile cytoplasm with certain genotypes. It is clear that certain combinations of cytoplasms and nuclear genes bring about a break-down in some function or functions vital for the development of microspores into normal pollen grains. The interaction of nuclear genes and cytoplasm must be a specific one, since other characters in male sterile plants are unaffected. The question of the time, place, and nature of this interaction in maize is no more or no less puzzling than any question of differentiation, where always the problem exists of understanding how cells presumably endowed with identical genetic complements come to assume different physiological and morphological aspects.

The behavior of restored sterile plants may have some bearing on the question of the time and place of expression of the cytoplasmic-genome interaction causing pollen abortion. Important here is the manner in which restoration to normal pollen production is brought about by the restorer genes. Observations made on plants with sterile cytoplasm and heterozygous for a single pair of major restorer genes strongly suggest that some event occurring in sporophytic tissue is responsible for pollen restoration. If the event took place in the haploid gametophyte, such heterozygous plants would not be expected to produce, on the average, more than 50 percent normal pollen, since all the microspores would possess sterile cytoplasm, but only one half would contain the restorer gene. Actually, however, plants heterozygous for a pair of restorer genes have in many cases been restored to normal pollen production (95 to 98 percent

good pollen). Furthermore, if the restorer genes act in the gametophyte, heterozygous plants would transmit only the restorer allele through the pollen, since pollen grains containing the non-restorer allele would be non-functional. If this were the case, progeny from the cross male sterile × heterozygous restorer would consist of fertile plants only. In fact, however, such backcrosses yield sterile and fertile plants. The non-restorer allele is, therefore, transmitted through the pollen. These observations clearly indicate that the restorer genes must express themselves in the sporophyte.

These observations in turn raise the question of the relationship between the action of the restorer genes and the events which cause pollen abortion. A general question is whether restorer genes have the ability to function in sterile cytoplasm in such a way that the normal cellular reactions necessary for pollen development take place, thus removing the block in development produced by sterile cytoplasm and non-restorer genes, or whether restorer genes set up alternative pathways which lead to the production of functional pollen, thereby circumventing the block. On the first alternative, it is possible that the critical step in the action of restorer genes takes place at the same stage when, in sterile plants, pollen abortion is initiated. If this is the case, the critical stage in restored sterile plants would be in the sporophyte, since, as indicated above, pollen restoration appears to be brought about by sporophytic processes. Thus, if restorer genes act in this way, it follows that the event which causes pollen abortion also takes place in the sporophyte.

Nevertheless, even though restorer gene action may remove the block to normal development, this action does not necessarily have to take place at the same stage at which the event leading to pollen degeneration occurs in sterile plants. Restorer genes could, for example, by their action in the sporophyte inhibit or suppress some reaction of sterile cytoplasm in microspores that prevents normal growth, the inhibition persisting in the male gametophyte even in the absence of the restorer gene. This inhibition cannot be permanent in the cytoplasm of the female gametophyte, however, since completely sterile plants can be recovered from restored sterile individuals.

If the second alternative holds, that is, if restorer genes function by forming alternative pathways to pollen production, it is also quite possible that pollen abortion and restoration may be separated in time and place of occurrence.

In attempting to determine the mode of action of restorer genes the chromatographic studies mentioned earlier are of interest. It was pointed out that the pattern of free amino acids in restored sterile plants (with T cytoplasm and Ky21 restorer genes) is indistinguishable from that of normal plants. This means that the Ky21 genes "restore" a normal free amino acid content as well as male fertility to plants with sterile cytoplasm, and although the relationship between the altered amino acid content and the cause of sterility is unknown, it is clear in all material examined that the alterations have invariably been associated with the T cytoplasm effects on pollen development. Thus, insofar as the free amino acid content of anthers reflects the processes that bring about pollen abortion, the restorer genes apparently do not set up alternative pathways, but rather remove or suppress the effects of sterile cytoplasm and allow normal development. The situation in maize is interesting in light of the finding in Neurospora that the gene which gives normal growth in strains with poky cytoplasm does not restore to normal the cytochrome system (Mitchell and Mitchell, 1956).

Measuring Pollen Abortion

The time and pattern of pollen shedding

In the process of converting inbreds to the cytoplasmic pollen sterile condition and the conversion of these sterile inbreds to restorers it is necessary to have some reliable measure of normal pollen production. The examination of anthers from small pieces of tassel under a low power microscope gives a fairly accurate measure of the amount and degree of pollen abortion. The staminate flowers are collected shortly before the time of normal dehiscence and usually preserved in acetic-alcohol until they can be examined. The proportion of normal, well filled pollen grains can be determined approximately but this method is slow and tedious. It also gives no information about dehiscence and ability of the pollen to function.

Pollen examinations over a period of years indicated that there was a close correlation between the amount of normal pollen produced and the time and pattern of pollen shedding. Tassels with anthers well filled with normal pollen grains begin shedding at the time of first silk emergence or before. Anthers that had any appreciable amount of partially filled or completely aborted pollen were usually delayed in appearance until after the first silks appeared, and the anthers did not follow the usual pattern of emergence. The normal pattern of pollen shedding is anthers extruded first below the tip of the central spike. Extrusion then extends evenly to the tip and the base of the central spike, followed or accompanied by the appearance of anthers on the lateral branches near the tips of the upper branches extending evenly to the tips and the bases of all the branches. Any delay in the appearance of anthers beyond the appearance of the first silk on any part of the tassel, or the first appearance of anthers on the lateral branches or at the tip or base of the main spike, or gaps without anthers, is usually an indication of some degree of pollen abortion. In some partially or completely sterile plants the anthers may be filled with normal appearing pollen grains but these anthers are not extruded or if extruded the pollen may not be released.

By using these manifestations plants can be easily and quickly classified in the field at the time of silking. The plants in a segregating population can be

Table 1. Differences in pollen restoration in the same season, two replications grown in 1956

Pedigree	Number of Plants Total	Plants with Anthers Number	Plants with Anthers Percent	Difference Percent
(WF9S × Ky21)-1	95	95	100	
	88	85	97	3
(WF9T × Tx127)-1	97	65	67	
	87	48	55	12
(C106T × Ky21)-1	115	103	90	
	92	76	83	7
(KysT × NC77)-1	119	101	85	
	89	73	82	3
(K4T × NC77)-1	105	86	82	
	97	78	80	2
CI7T(Ky21 × Tx127)	114	114	100	
	89	89	100	0

put in four arbitrary categories: (1) normally fertile, (2) partially fertile with about 50 percent or more of normally released pollen, (3) partially fertile with about 50 percent or less of normally released pollen, (4) completely sterile. In the first category the normal functioning of the pollen has been checked many times by self and cross pollinations made by hand. The last category shows no anthers as long as the silks are receptive, and the anthers are devoid of any normally appearing pollen grains.

For convenience in classification the first three categories are put in one group (and listed in the tables as plants with anthers) to compare the effectiveness of this method of testing segregation. In 1956 six progenies were grown in replicated plantings, about 100 plants in each replication. Five of the lots were F_2 selfed progenies of crosses of S and T sterile inbreds by the pollen restoring inbreds NC77, Tx127, and Ky21. One lot was the F_1 cross of a T sterile inbred by the single cross (Ky21 × Tx127). The differences in the percent of plants with or without anthers appearing ranged from 0 to 12 percent. None of these differences is significant ($P = .14$ for the largest difference).

Seasonal differences in pollen restoration

Using this method of field examination and the same arbitrary classification of plants with and without anthers, the differences shown by the same F_2 segre-

Table 2. Seasonal differences in pollen restoration in the F_2 generation of crosses of restoring inbreds on two types of sterile cytoplasm in different inbreds

Pedigree	Year Grown	Number of Plants Total	Number of Plants With Anthers	Percent With Anthers	Percent Without Anthers
(WF9T × Ky21)-1	1955	94	58	62	38
	1956	77	46	60	40
(38T × Ky21)-1	1955	126	56	44	56
	1956	124	82	66	34
(C106T × Ky21)-1	1955	95	75	79	21
	1956	302	257	85	15
Total	1955	315	189	60	40
Total	1956	503	385	77	23
(WF9T × Tx127)-1	1955	90	43	48	52
	1956	184	113	61	39
(38T × Tx127)-1	1955	122	35	29	71
	1956	132	96	73	27
(C106T × Tx127)-1	1955	101	66	65	35
	1956	116	82	71	29
Total	1955	313	144	46	54
Total	1956	432	291	67	33
(KysT × NC77)-1	1955	91	64	70	30
	1956	208	174	84	16
(K4T × NC77)-1	1955	117	83	71	29
	1956	202	164	81	19
Total	1955	208	147	71	29
	1956	410	338	82	18
(WF9S × Ky21)-1	1955	92	69	75	25
	1956	183	180	98	2

Table 3. Summary of seasonal differences in pollen restoration in the F_2 generation of crosses of several restoring inbreds on T sterile cytoplasm

Inbred used as Pollen Parent	Year Grown	Number of Plants		Percent	
		Total	With Anthers	With Anthers	Without Anthers
Ky21	1955	315	189	60	40
	1956	503	385	77	23
Tx127	1955	313	144	46	54
	1956	432	291	67	33
NC77	1955	208	147	71	29
	1956	410	338	82	18
Totals					
Observed	1955	836	480	57	43
Calculated (9:7)		836	470	56	44
Observed	1956	1345	1014	75	25
Calculated (3:1)		1345	1009	75	25

gating progenies were determined for the two growing seasons of 1955 and 1956. The same lots of seed were planted each year and the results averaged for the three pollen restoring inbreds given above. In 1955 the growing season up to the time of flowering was dry and above normal in temperature. The leaves were wilted and rolled on many days. The 1956 season was quite adequate in moisture before flowering and temperatures were normal. The results combined from the three inbreds used as pollinators in 16 different selfed F_2 progenies with T cytoplasm as shown in Tables 2 and 3 are as follows:

	Number of Plants		Percent	
	With Anthers	*Without Anthers*	*With Anthers*	*Without Anthers*
1955 Observed	480	356	57	43
Calculated 9:7	470	366	56	44
1956 Observed	1014	331	75	25
Calculated 3:1	1009	336	75	25

The agreement in 1955 with a 9:7 calculated ratio and in 1956 with a 3:1 calculated ratio is remarkably close. This indicates that in the relatively unfavorable season of 1955 two restoring genes were needed for the plants to show any anthers. In the more favorable season of 1956 only one restoring gene was necessary. However, while the analysis of variance shows a significant difference between seasons it also shows a significant difference between crosses. For this reason the figures cannot be combined to show the number of genes involved. The separate lots shown in Table 2 are very erratic in their segregation. Undoubtedly different numbers of genes are involved and these probably vary in potency in different seasons. For example, in both seasons Ky21 gave higher percent restoration than Tx127 with the same inbreds as seed parents.

Segregation of pollen restoring genes in inbreds used as pollen parents and seed parents

When the inbreds NC77, Tx127, and Ky21 used as restorers on T cyto-

Table 4. Pollen restoration in the F_1 and F_2 generations of crosses of inbreds used as seed and pollen parents

Pedigree	Year Grown	Number of Plants		Percent	
		Total	With Anthers	With Anthers	Without Anthers
		F_1 Generation			
WF9S × Ky21	1954	5	5	100	0
WF9S × Tx127	1954	8	8	100	0
WF9T × Ky21	1954	8	8	100	0
WF9T × Tx127	1954	12	12	100	0
KysT × NC77	1954	11	11	100	0
K4T × NC77	1954	13	13	100	0
CI7T × (Ky21 × Tx127)	1955	129	129	100	0
CI7T × (Ky21 × Tx127)	1956	203	203	100	0
Inbreds used as pollen parents on T cytoplasm, F_2 generation, grown 1955-56					
Ky21 Observed		818	514	70	30
Calculated 3:1		818	614	75	25
Tx127 Observed		745	435	58	42
Calculated 9:7		745	419	56	44
NC77 Observed		618	485	78	22
Calculated 3:1		618	464	75	25
Inbreds used as seed parents with T cytoplasm, F_2 generation, grown 1955-6					
WF9T Observed		445	260	58	42
Calculated 9:7		445	250	56	44
38T Observed		504	269	53	47
Calculated 9:7		504	284	56	44
C106T Observed		614	480	78	22
Calculated 3:1		614	460	75	25
KysT Observed		299	238	80	20
Calculated 3:1		299	224	75	25
K4T Observed		319	247	77	23
Calculated 3:1		319	239	75	25

plasm are studied separately it is found that they segregate differently in F_2 selfed progenies. All of these inbreds used alone or in single cross combinations produced all normally fertile plants in the F_1 generation grown in 1954, 1955, and 1956 in combination with WF9T, 38-11T, C106T, KysT, K4T and C17T (not all combinations were grown in each of the three years). These F_1 fertile plants were selfed and the F_2 segregating progenies were grown in 1955 and 1956 and the results combined.

Of the three restoring inbreds used as pollen parents Tx127 segregated in a 9:7 ratio, Ky21 and NC77 segregated in 3:1 ratios. The differences between observed and calculated are not significant. Of the inbreds used as seed parents with these pollinators WF9T and 38-11T segregated in a 9:7 ratio, while C106T, KysT, and K4T segregated 3:1. Again the differences between observed and calculated are not significant. However, in view of the wide differences in the two different years and in different progenies the results are only indicative of differences in the number of restoring genes involved in the crosses of the different inbreds used.

Segregation of fertile and sterile plants in backcrossed progenies

That different numbers of genes are involved in the restoration of different

sterile inbreds is also borne out by the behavior of backcrossed lines in the process of conversion to complete restoration. Many of the standard corn belt inbreds widely used in the northeastern and north central corn growing regions are in process of conversion by taking the S or T cytoplasmic sterile versions of these inbreds, crossing them as females by several different restoring inbreds, followed by backcrossing the restored fertile plants repeatedly on the sterile inbreds. These inbreds have been backcrossed from two to six generations and then self pollinated for one or two additional generations. The segregation of fertile and sterile plants is quite different in many inbreds. A few illustrations are given here.

A158 is completely sterile in both S and T types of cytoplasm and in five additional sources (A, B, C, E, and F). No anthers shedding pollen appeared on any plants in 10 backcrossed generations in the S cytoplasm and 5 generations in the T cytoplasm. Both the S and T steriles are completely restored by Ky21. Anthers appear and pollen is shed in normal amount about 5 days before the

Table 5. Segregation of fertile and sterile plants in backcrossed and selfed A158 inbreds with seven different sources of sterile cytoplasm

Pedigree	Pollen Production Fertile	Sterile	Date* of First Tassel	Silk
A158	all†		31	4
A158S10		all	—	3
A158SF5-1A (Ky)	all		2	7
A158SF4-1A (Ky)	all		3	6
A158SF4-2A (Ky)	all		31	4
A158T5		all	—	31
A158TF4-1A (Ky)	10	4	31	7
A158TF4-2A (Ky)	16	4	2	6
A158A4		all	—	2
A158B6		all	—	3
A158C3		all	—	2
A158E2		all	—	1
A158F1		all	—	1

* Date of first tassel and silk are for July and August. Numbers above 25 are in July. Numbers below 25 are in August.
† From 15 to 20 was the usual number of plants in each progeny, in some cases the actual number was not recorded.

first silks appear in the original, fertile inbred, and this same pattern is shown by the restored fertiles. The backcrossed S steriles in 5 generations of backcrossing and 1 generation selfed usually produced no sterile plants. Small progenies of 15 to 20 plants were grown each generation but several progenies were grown each year. The fact that few sterile plants appeared indicates that there are a number of genes, any one of which alone can restore pollen production to the S type of sterile cytoplasm.

The backcrosses on the T type of sterile cytoplasm have segregated approximately 1:1 sterile and fertile in each backcrossed generation, and 3:1 in each selfed generation although the total numbers are small.

The inbreds C103 and Kr (187-2) also give clear cut segregation, 1:1 in backcrossed, and 3:1 in selfed progenies having T sterile cytoplasm. They have not been tested with the S type. A fairly large number of progenies have been grown. The Kr inbred has been selfed twice after backcrossing 4 and 5 generations and a number of progenies in F_3 give all fertile plants as expected. Again,

the time of shedding of pollen in relation to silk appearance is about the same for the restored as for the original fertile inbreds except for the one backcross of Kr with Oh29. Although segregation is 1:1 the time of pollen shedding is delayed three days beyond the first appearance of silks. This agrees with other results that the restorers in Oh29 are not as effective as those from Ky21 and other good restorers.

Table 6. Segregation of fertile and sterile plants in backcrossed and selfed C103 inbreds

Pedigree		Pollen Production Fertile	Sterile	Date of First Tassel	Silk
C103		all		13	17
C103T8			all	—	17
C103TF3(Ky)		5	8	13	20
C103TF3(Ky)		7	5	14	14
C103TF3(Ky)		6	1	11	14
C103TF3(Ky)		3	4	13	20
C103TF3(Ky)		7	11	10	14
	Total	28	29		
C103TF2-1A(Ky)		11	3	14	18
C103TF2-2A(Ky)		11	4	13	17
C103TF2-1A(Ky)		11	4	13	17
C103TF2-2A(Ky)		11	4	14	16
C103TF2-1A(Ky)		17	3	13	16
C103TF2-2A(Ky)		11	7	14	17
C103TF2-1A(Ky)		11	2	13	—
	Total	83	27		

The behavior of WF9 and Hy inbreds is quite different. WF9 is completely sterilized by both S and T cytoplasms, also by four other sources. Five additional sources have given a few partially fertile plants in the first or second generations of backcrossing.

WF9 T and S sterile plants are completely restored by Tx127, Ky21, and WF9T by I153 and many other lines. These restored T steriles have been backcrossed on WF9T sterile for 1 to 3 generations and have all segregated into fully fertile or completely sterile plants. In 22 progenies grown in 1956 there are 382 fertile and 933 sterile plants. This is a significant departure from a 1:1 ratio, being a 1:2.4 ratio. This indicates that WF9T sterile requires more than one restorer gene to produce pollen, and these genes must all be present to be effective. They are complementary in their action.

Selfed progenies of (WF9T × Ky21) grown in 1955 and 1956 (Table 2) gave 104 fertile and 67 sterile plants which is fairly close to a 9:7 ratio, and one backcrossed progeny (Table 8) gave 39 fertile and 120 sterile plants, a very close 1:3 gametic ratio, again indicating two dominant complementary genes for fertility. The F_2 generation of crosses with Tx127 and Oh41 gave fewer fertile plants, indicating more than two genes involved or less potency in the dry year of 1955.

Table 7. Segregation of fertile and sterile plants in the backcrossed and selfed Kr inbreds

Pedigree	Pollen Production Fertile	Pollen Production Sterile	Date of First Tassel	Date of First Silk
Kr	all		13	14
KrT6		all	—	14
KrT7		all	—	13
KrTF1(Oh29)	7	7	10	7
KrTF6(Ky)	7	6	12	14
KrTF6(Ky)	11	6	13	14
KrTF6(Ky)	4	10	14	16
KrTF6(Ky)	10	4	14	14
KrTF6(Ky)	9	6	14	17
KrTF6(Ky)	12	6	15	16
Backcross Total	60	45		
KrTF5-1A(Ky)	11	4	13	14
KrTF5-1A(Ky)	15	2	13	18
KrTF5-1A(Ky)	9	4	13	14
KrTF5-1A(Ky)	11	4	14	16
KrTF5-1A(Ky)	9	9	13	17
KrTF5-1A(Ky)	14	3	14	16
KrTF5-1A(Ky)	8	4	14	16
KrTF5-1A(Ky)	10	3	16	16
KrTF5-1A(Ky)	9	3	13	15
KrTF5-1A(Ky)	14	3	14	19
KrTF5-1A(Ky)	15	3	15	17
KrTF5-1A(Ky)	13	3	13	17
F_2 Total	138	45		
KrTF4-1B(Ky)	all		11	13
KrTF4-1B(Ky)	13	4	13	16
KrTF4-1B(Ky)	all		13	16
KrTF4-1B(Ky)	10	4	13	16
KrTF4-1B(Ky)	all		11	13
KrTF4-1B(Ky)	all		13	15
KrTF4-1B(Ky)	all		14	16
Segregating F_3 Total	23	8		
F_2 and F_3 Grand Total	161	53		

In contrast to the results with WF9 is the behavior of Hy. Five slightly different lines have been sterilized by T cytoplasm and restored to full fertility. Hy has been a difficult line to sterilize and to restore completely. After 5 generations of backcrossing both S and T sterile lines produce some partially fertile plants. When restored by Ky21, C236, and Tx127 the backcrossed lines give 98 fertile to 39 sterile plants, which approximates a 3:1 gametic ratio. These totals include three progenies with a total of 35 plants in which all the plants are fertile. They do not include the four progenies that are all or nearly all sterile. These sterile progenies probably result from partially fertile plants that were used for backcrossing. These same fully fertile backcrossed lines self-fertilized give 109 fertile and 7 sterile plants, which is close to a 15:1 F_2 ratio. Both results indicate two genes of which either one alone or both together can

Table 8. Segregation of fertile and sterile plants in backcrossed WF9 inbreds with 9 different sources of restoring genes on T cytoplasm

Pedigree	Pollen Production Fertile	Pollen Production Sterile	Date of First Tassel	Date of First Silk
WF9	all		10	11
WF9T8		all	—	15
WF9TF1(AFR)	9	13	6	6
WF9TF3(Ky)	5	12	10	9
WF9TF3(Ky)	5	9	10	9
WF9TF2(A344)	9	11	7	5
WF9TF2(W153R)	4	13	8	8
WF9TF2(I205D)	4	14	9	8
WF9TF1(I205DH)	3	12	5	6
WF9TF2(I205DH)	6	9	7	8
WF9TF2(M14FCD)	5	11	10	7
WF9TF2(Tx127)	3	14	13	10
WF9TF2(Oh41)	3	9	5	5
Total	56	127		
(WF9T3 × Ky21)WF9	39	120	13	13
(WF9T × W153R)WF9	29	76	3	4
(WF9T × W153R)WF9	45	74	5	6
(WF9T × A344)WF9	31	86	5	5
WF9T6(WF9T × A344)	21	77	5	3
(WF9T × I205D)WF9	29	83	6	7
WF9T7(WF9T × I205DH)	37	70	7	8
WF9T6(WF9T × I205DH)	47	80	10	9
(WF9T6 × Oh41)WF9	24	71	14	7
(WF9T3 × Tx127) WF9	24	69	10	7
Total	326	806		

Table 9. Segregation of fertile and sterile plants in backcrossed and selfed Hy inbreds

Pedigree	Pollen Production Fertile	Pollen Production Partial	Pollen Production Sterile	Date of First Tassel	Date of First Silk
HyI	all			9	10
HyIT5			all	—	8
HyITF3(Ky)	12	1	9	9	10
HyITF4(C236)	8		7	9	10
HyITF4(C236)	8		8	7	8
HyITF6(Ky)	9		9	7	9
Hy	all			13	17
HyT7			all	—	16
HyTF3(Ky)	16		1	11	14
HyTF3(Tx127)			all	—	14
HyTF3(Tx127)		2	14	16	14
HyTF3(Ky)	10		4	13	16
HyTF3(Ky)		1	17	—	16
HyT7			all	—	17
HyTF4(C236)	14			13	16
HyTF4(C236)	15			10	13
HyTF6(Ky)	6			10	13
Backcross Total	98		39		
HyTF5-1A(Ky)	12			10	13
HyITF5-1A(Ky)	15		3	8	9
HyITF5-2A(Ky)	15		4	10	10
HyTF2-1A(Ky)	12			13	16
HyTF3-1A(C236)	14			13	16
HyTF3-2A(C236)	14			13	15
HyTF3-1A(C236)	14			9	14
HyTF3-2A(C236)	13			6	6
F_2 Total	109		7		

restore fertility. WF9 therefore seems to be recessive for at least two complementary genes and Hy recessive for at least two duplicate genes for pollen restoration, and the dominant alleles of all these genes are present in the restorers used.

Duvick (1956) has studied the segregation of pollen restoring genes in numerous crosses on WF9T sterile. He finds that there are at least two dominant complementary genes plus one or more dominant, duplicate modifying genes necessary for full pollen fertility under the conditions which prevailed in Iowa during this test. He also finds considerable variation in different seasons and in different locations. The results obtained in Connecticut with WF9 agree with his findings in Iowa except that more of the modifying genes are necessary in the midwest than in the cooler and more humid conditions in Connecticut.

The Separation of S and T Restorers

It has been shown previously that Ky21 will restore both the S and T types of cytoplasmic sterility. The S sterile inbred A158 restored by Ky21 and backcrossed on A158S sterile for four generations was tested on a number of unrelated S and T sterile lines, as shown in Table 10. In six crosses on S sterile it restored all of the plants except in one progeny where there was one sterile plant. The remaining (re) plants were all fertile. Although the backcrossed restorer was heterozygous for the restoring genes in all six backcrosses, all of the plants were fertile except one. When these same restored lines were crossed on the same inbreds with T sterile cytoplasm, the resulting F_1 plants were all sterile in eight progenies and were segregating sterile and fertile in two progenies. Therefore, in the process of backcrossing on S sterile cytoplasm the T restoring genes were lost in 8 out of 10 progenies. In the backcrosses on S sterile

Table 10. Pollen restoration in crosses of sterile inbreds restored by backcrossing, on T and S cytoplasm

Pedigree	Pollen Production Fertile	Sterile	Date of First Tassel	Silk
A73T3 × A158SF4(Ky)		all	—	24
A73S4 × A158SF4(Ky)	all		21	24
A73S4 × A158SF4(Ky)	re	1	21	25
A374S4 × A158SF4(Ky)	all		24	25
A374S4 × A158SF4(Ky)	all		25	26
A374T3 × A158SF4(Ky)		all	—	25
A374T3 × A158SF4(Ky)		all	—	25
A375T4 × A158SF4(Ky)		all	—	24
A375T4 × A158SF4(Ky)	11	8	24	24
W9T3 × A158SF4(Ky)		all	—	8
W9T3 × A158SF4(Ky)		all	—	20
M14S7 × A158SF4(Ky)	all		24	25
M14S7 × A158SF4(Ky)	all		25	26
WF9T3 × M14SF4(Ky)		all	—	25
WF9T3 × M14SF4(Ky)		all	—	25
WF9T7 × M14SF4(Ky)	4	14	1	28

they have been retained in all six. This is further proof that the pollen restoration is due to genes in the chromosomes and that there are separate genes capable of restoring S and T cytoplasm independently. The backcrossed restored A158T lines have also been tested on S and T cytoplasm. Crossed on S sterile they give all sterile progenies or segregating sterile and fertile progenies. Crossed on T sterile they give all fertile progenies or segregating sterile and fertile progenies. Again there is a separation of S and T restoring genes.

The Performance of Restored Sterile Lines in Crosses with Inbreds and Single Crosses

Since it has been shown that restored sterile inbreds perform differently in crosses with different inbreds, it is important to know how they will restore the sterile seed parent single crosses widely used in the production of double cross hybrids. Table 11 gives the results of several restored sterile lines after 0 to 5 generations of backcrossing. Five different inbreds restored by Ky21 and backcrossed 0 to 4 generations and crossed on WF9T, CI7T, W24T, and C102T produced 80 completely fertile and 57 entirely sterile plants. This deviates significantly from a 1:1 ratio ($P < .05$).

Several different KrTF5 restorers were also crossed on (WF9T × W22) and (WF9T × R2) single crosses. The first produced 69 fertile and 45 sterile and the second 48 fertile and 47 sterile. This segregation of completely fertile

Table 11. Pollen production in crosses of sterile inbreds restored by Ky21 on sterile inbreds, single crosses and restored sterile inbreds

Pedigree	Year Grown	Pollen Production Fertile	Sterile	Date of First Tassel	Silk
WF9T × I205TF1(Ky)	1955	8	6	28	27
WF9T × KrTF4(Ky)	1955	10	6	26	26
WF9T × KrTF4(Ky)	1955	7	10	1	5
WF9T × Os420TF1(Ky)	1955	10	7	1	1
WF9T × C103TF1(Ky)	1955	15	4	29	1
CI7T × LkTF(Ky)	1955	9	10	1	2
W24T × KrTF5(Ky)	1956	11	8	8	8
C102T × KrTF4(Ky)	1956	10	6	6	6
Total		80	57		
(WF9T × W22)KrTF5(Ky)	1956	16	6	8	7
(WF9T × W22)KrTF5(Ky)	1956	8	6	7	31
(WF9T × W22)KrTF5(Ky)	1956	14	9	10	8
(WF9T × W22)KrTF5(Ky)	1956	6	7	7	7
(WF9T × W22)KrTF5(Ky)	1956	11	7	10	8
(WF9T × W22)KrTF5(Ky)	1956	14	10	7	7
Total		69	45		
(WF9T × R2)KrTF5(Ky)	1956	8	8	10	9
(WF9T × R2)KrTF5(Ky)	1956	10	8	8	10
(WF9T × R2)KrTF5(Ky)	1956	9	11	8	14
(WF9T × R2)KrTF5(Ky)	1956	11	7	6	9
(WF9T × R2)KrTF5(Ky)	1956	10	13	6	9
Total		48	47		
Oh51A-TF5 × KrTF5	1956	7	8	2	3
Oh51A-TF5 × KrTF5	1956	15	3	2	2
Oh51A-TF5 × KrTF4	1956	13	4	4	5
Total		35	15		

and sterile plants was quite clear-cut. The agreement with the expected 1:1 ratio is close in one case, but not in the other. The crosses on (WF9T × W22) give an excess of fertile plants. The deviation from a 1:1 ratio is significant ($P < .05$).

Three of these KrTF backcrossed lines were crossed on Oh51ATF5 (I153), a sterile line restored by I153 and backcrossed five times. Both lines were heterozygous for restoring genes from two different sources. The three lots gave 35 fertile and 15 sterile which is close ($P > .50$) to the 3:1 ratio expected if there is only one gene involved. This is evidence that I153 and Ky21 have one gene in common capable of complete restoration under the conditions which prevailed in the favorable season of 1956.

Naturally Restoring Inbreds

A number of inbreds developed by various experiment stations and the United States Department of Agriculture have been found to be capable of partial or complete restoration in combination with many S and T sterile lines. Many of these inbreds have been tested by Edwardson (1955) and by Duvick (1956). The following tables give the results obtained in Connecticut in 1955 and 1956.

The inbreds NY16, I205R, TER, and Mo940 restored various T sterile inbreds completely as shown in Table 12. In all of these crosses of T sterile inbred by restorer the F_1 plants shed pollen from one to five days before the first silk appeared. CI64 tested on four different inbreds gave variable results. Crossed on W24T and CI7T it restored all of the F_1 plants completely but there was a delay of two or three days in pollen shedding beyond the appearance of the first silk. This is an indication that this inbred is not a good restorer. In a cross with CI21E-T it restored all plants partially and with KrT it restored only two plants while the remaining plants were all completely sterile. Since KrT is completely restored by several good restorers as shown in the previous tables, CI64 cannot be depended upon to do a good job. The inbred A206 restored all plants in a cross with A158S sterile and pollen was shed three days before any silks appeared.

Table 12. Pollen restoration in crosses of miscellaneous inbreds on T and S cytoplasm

Pedigree	Pollen Production			Date of First	
	Fertile	Partial	Sterile	Tassel	Silk
A34T × NY16	all			28	29
WF9T × I205R	all			5	7
KrT × CI64		2	re	—	8
CI21ET × CI64		all		13	10
W24T × CI64	all			11	9
CI7T × CI64	all			14	13
KrfT × TER	all			14	19
KrfT × Mo940	all			14	16
A158S × A206	all			2	5

The widely used, early maturing inbred I153 is a good T restorer. This has been reported in many tests in different locations. In Connecticut a number of related lines, such as W153R, A344, and A293, are also good restorers as shown

in Tables 13 and 14, and in previous tabulations. In all crosses on T sterile lines and single crosses restoration is complete and pollen shedding begins at the same time or before silk emergence.

In crosses on M14S and (WF9S × M14), as shown in Table 13, I153 and related lines either give all sterile plants or restore only partially all or some of the plants. They are therefore good lines to differentiate between S and T cytoplasm. W22 in contrast with I153 restores S but not T.

Table 13. Pollen restoration in crosses of I153 and related lines on T and S cytoplasm

Pedigree	Year Grown	Pollen Production			Date of First	
		Fertile	Partial	Sterile	Tassel	Silk
WF9T × I153	1955	all			24	25
Oh51AT × W153R	1955	all			24	24
A340T × W153R	1955	all			20	20
W22T × W153R	1955	all			25	25
AT × W153R	1955	all			25	25
B8T × W153R	1956	all			31	31
W24T × W153R	1956	all			2	2
Oh43T × W153R	1956	all			30	30
KrT × W153R	1956	all			31	31
CI21ET × W153R	1956	all			4	5
C102T × W153R	1956	all			30	1
M14S × W153R	1955		all		25	24
M14S × W153R	1955		all		26	25
Oh51AT × A344	1955	all			25	25
A340T × A344	1955	all			18	20
A374T × A344	1955	all			25	25
WM13T × A344	1955	all			18	18
W22T × A344	1955	all			25	25
A311T × A293	1956	all			3	5
(A375T × J557)A293	1956	all			3	6
(WF9T × W22)A293	1956	all			3	6
(WF9S × M14)I153	1955		2	14	27	27
(WF9S × M14)I153	1955		7	8	23	27
(WF9S × M14)I153	1955			15	—	25

In crosses with several T sterile single crosses I153, W153R, and I153TF (fertile with sterile cytoplasm) produced only fertile F_1 plants with no delay in pollen shedding as shown in Table 14. The fertile I153 with T cytoplasm used as a pollinator gave the same result as the original line with normal cytoplasm. Furthermore, several restored sterile single crosses, also with T cytoplasm, were used as pollinators on several sterile single cross seed parents. These double crosses produced 39 fertile and 28 sterile plants. This is not a significant departure from a 1:1 ratio ($P > .20$), although there is an excess of fertile plants.

It should be noted that the results given in Table 8 indicate more than one gene necessary to restore WF9T sterile. However, when WF9 restored sterile is used as a pollinator on B9T, Oh43T, and W22T single crosses only one gene is needed to restore. Probably some of the fertility restoring genes that are comple-

Table 14. Pollen restoration in crosses of I153 and related lines on T sterile single crosses

Pedigree	Pollen Production Fertile	Sterile	Date of First Tassel	Silk
(WF9T × W22)I153	all		25	26
(WF9T × Hy)I153	all		26	26
(WF9T × Hy)W153R	all		26	27
(WF9T × R61)I153	all		27	27
(WF9T × B14)I153	all		27	29
(WF9T × B14)I153	all		23	25
(WF9T × B14)W153R	all		23	25
(Os420T × WF9)W153R	all		25	27
(Os420T × WF9)W153R	all		23	26
(Os420T × WF9)I153	all		25	27
(Os420T × WF9)I153TF	all		25	27
(Os420T × WF9)I153TF	all		25	27
(B9T × W22)(WF9T × W153R)	7	5	27	27
(Oh43T × M14SF)(WF9T × W153R)	4	9	26	26
(W22T × B9)(WF9T × W153R)	15	1	24	25
(B9T × W22)(WF9T × A344)	7	6	23	24
(W22T × B9)(WF9T × A344)	6	7	23	24
Total	39	28		

mentary in their action on WF9 are present in these other inbreds and are not needed in the pollinator. The evidence is not adequate to prove this.

Some of the restored double crosses were grown in several places in the mid-west in 1955, 1956, and 1957 and were reported to have about half of the plants shedding pollen normally. They were also productive and promising as early maturing varieties.

The I205 line which has been widely used in the corn belt is easily sterilized by the T cytoplasm. However, several related lines now being grown by seedsmen have the ability to restore pollen completely to all sterile lines that they have been tested on. The origin of the line designated R205 or I205R is not known. It is thought to be a recovered line produced by Dr. E. W. Lindstrom at the Iowa Agricultural Experiment Station in his studies on convergent improvement. This line is quite similar in plant and ear characters to I205 but is more disease resistant. Whatever its origin it proves to be not only an improved line in many respects but is also a good T restorer. Crossed on (WF9T × Hy), a widely used seed parent, it produced all fertile plants in the F_1. The first plants of I205R grown in Connecticut proved to be outcrossed. Individual plants were numbered and self-pollinated. Pollen from several of these same plants was also crossed on sterile seed parents to test their pollen restoring ability. The results are shown in Table 15. Plants numbered 4 and 14 show segregation in some combinations. Crosses of plant number 13 on three different sterile seed parents gave only fertile plants.

Sterile I205 with T cytoplasm restored by Ky21 and backcrossed two generations restored half of the plants completely in three crosses on sterile seed parents. However, there was a delay of two days in pollen shedding in one cross.

The segregation of 34 fertile to 21 sterile indicates more than a single restoring gene difference.

Table 15. Pollen restoration in crosses of I205 non-restoring and outcrossed I205 restoring inbreds on T sterile single crosses

Pedigree	Pollen Production Fertile	Sterile	Date of First Tassel	Silk
(WF9T × B9)I205		16	—	6
(WF9T × Hy)I205R	22		6	11
(WF9T × Hy)I205R-4	16	6	7	7
(WF9T × B7)I205R-4	15	1	9	9
(WF9T × 38-11)I205R-13	22		5	8
(WF9T × Oh7A)I205R-13	20		7	6
(R2T × CI21E)I205R-13	26		6	6
(WF9T × B8)I205R-14	14		8	11
(WF9T × B9)I205R-14	19		6	10
(WF9T × B6)I205R-14	9		8	9
(WF9T × B14)I205R-14	18	2	8	8
(WF9T × Os420)I205R-14	22		9	10
(WF9T × C103)I205R-14	19	1	5	10
(WF9T × B9)I205TF2(Ky)	12	6	5	5
(WF9T × B9)I205TF2(Ky)	6	10	9	7
(WF9T × B9)I205TF2(Ky)	16	5	5	5

Partial Restorers

There are a number of inbreds now being used as pollinators in commercial hybrids that have the ability to restore pollen production fully or partially in some combinations but are completely sterile in other combinations. Inbreds of this type are very erratic in their performance and are subject to wide environmental and seasonal variation. Pollen shedding is usually delayed until after the silks appear.

Table 16. Pollen restoration in crosses of M14 on T cytoplasm

Pedigree	Year Grown	Pollen Production Fertile	Partial	Sterile	Date of First Tassel	Silk
W23T × M14-1	1955			all	—	29
WF9T × M14-1	1955			all	—	28
WF9T × M14-1	1955			all	—	1
(WF9T × Oh51A)M14-1	1956			all	—	6
(WF9T × Oh51A)M14-2	1956	18		1	6	4
(WF9T × Oh51A)M14-3	1956	12		5	8	6
(WF9T × Oh51A)M14-5	1956	14		2	8	6
(WF9T × Oh51A)M14-6	1956	10		12	8	5
(WF9T × Oh51A)M14-7	1956		6	11	8	6
(WF9T × Oh51A)M14-10	1956	11		10	8	6
(WF9T × Oh51A)M14-11	1956			all	—	6
(WF9T × Oh51A)M14-13	1956	2	3	15	—	6
(WF9T × Oh51A)M14-14	1956			all	—	4

Inbreds of this type are M14, Oh29, Oh41, and A71. The pollen restoring ability of the first three of these inbreds is shown in Tables 16 and 17. The M14 line used in the crosses given in Table 16 is a composite of four lines widely

Table 17. Pollen restoration in crosses of Ohio 29 and Ohio 41 on T cytoplasm

Pedigree	Pollen Production			Date of First	
	Fertile	Partial	Sterile	Tassel	Silk
1955					
Oh26T × Oh29	all			30	31
W23T × Oh29	15		2	29	28
WF9T × Oh29	all			2	4
R2T × Oh29	3		3	10	3
Oh33T × Oh29		13	7	—	3
I205T × Oh29	12		6	—	4
LkT × Oh29	all			4	4
Oh7T × Oh29		6	re	—	4
CI7T × Oh29	all			6	8
1956					
CI7T × Oh29	all			13	13
W24T × Oh29	all			10	10
Oh43T × Oh29		all		10	7
KrT × Oh29		re	1	10	8
CI21ET × Oh29		re	1	15	13
C102 × Oh29	all			9	9
1955					
W22T × Oh41	1	2	re	2	2
W23T × Oh41		re	2	1	29
WF9T × Oh41		11	10	3	11
WF9T × Oh41			all	—	1
R2T × Oh41	all			4	1
Oh33T × Oh41			all	—	1
Oh7T × Oh41		3	10	—	4

used in the corn belt. The numbered M14 lines are progeny selections from this composite after intercrossing and selfing. Lines M14-1, 11, and 14 produced only sterile plants in crosses with T sterile inbreds and sterile single crosses. None of the other selections restored completely but several gave a high proportion of fertile plants. However, in every case there was a delay of two to three days in time of pollen shedding which is an indication that M14 is not a good T restorer.

Ohio 29 and 41 are both very erratic in their pollen restoration behavior. In many crosses (as shown in Table 17) Oh29 restored pollen completely with no delay in time of shedding. Ohio 41 restored in only one combination in 1955 and in this there was a delay of three days in pollen shedding.

When these partial restorers are used together as single cross pollinators (Table 18) they do a reasonably good job in many combinations with standard sterile seed parent single crosses. They give completely fertile, partially fertile of all gradations, and entirely sterile plants in varying proportions. The total amount of pollen produced ranges from roughly 25 to 75 percent of normal

Table 18. Pollen restoration in double crosses of the partial restoring inbreds Oh29, Oh41, and M14 as single cross pollinators

Pedigree	Pollen Production			Date of First	
	Fertile	Partial	Sterile	Tassel	Silk
1955					
(WF9T × W22) (Oh29 × Oh41)		9	7	3	5
(WF9T × B14) (Oh29 × Oh41)	3	2	11	5	5
(WF9T × C103) (Oh29 × Oh41)	6	1	10	3	5
(WF9T × C103) (Oh29 × Oh41)	4	2	9	1	3
(WF9T × B14) (Oh29 × Oh41)	9		8	3	3
(WF9T × B14) (Oh29 × Oh41)	8	3	3	1	1
(38T × WF9) (Oh29 × Oh41)	10		5	30	5
(38T × WF9) (Oh29 × Oh41)		10	9	1	1
(WF9T × 38-11) (Oh29 × Oh41)	12		8	1	5
(WF9T × 38-11) (Oh29 × Oh41)		11	6	2	5
(WF9T × 38-11) (Oh29 × Oh41)		11	5	1	1
(WF9T × Hy2) (Oh29 × Oh41)	6	3	6	2	—
	58	52	87		
1956					
(WF9T × B17) (Oh29 × Oh41)	17	1	1	10	9
(R2T × Oh7) (Oh29 × Oh41)	16	5	3	11	11
(R2T × Oh7A) (Oh29 × Oh41)	12	4	5	13	10
(R2T × CI21E) (Oh29 × Oh41)	15	3	2	10	10
	60	13	11		
1956					
(WF9T × Hy) (Oh41 × Oh29)	16		4	10	10
(WF9T × Hy) (Oh41 × Oh29)	17		5	10	8
(WF9T × Hy) (Oh41 × Oh29)	15		3	7	8
(WF9T × Hy) (Oh41 × Oh29)	14	6	2	11	10
(WF9T × CI21E) (Oh41 × Oh29)	9	5	6	12	12
(WF9T × 38-11) (Oh41 × Oh29)	13	3	3	10	8
(WF9T × Oh7A) (Oh41 × Oh29)	5	3	12	12	12
(C103T × B14) (Oh41 × Oh29)	4		16	16	10
	93	17	51		
1955					
(W22T × B9) (M14 × Oh41)	14		2	1	1
(WF9T × Oh26) (M14 × Oh41)	12		3	29	31
(WF9T × Oh51A) (M14 × Oh41)		1	13	3	1
(WF9T × B9) (M14 × Oh41)	15			1	31
(WF9T × I205D) (M14 × Oh41)	12		1	1	1
(WF9T × Hy) (M14 × Oh41)	10		7	2	5
(WF9T × CI21E) (M14 × Oh41)		15	3	5	12
(WF9T × 38-11) (M14 × Oh41)	2	3	7	3	5
(WF9T × C103) (M14 × Oh41)	2	2	8	1	1
(Os420T × WF9) (M14 × Oh41)	6	3	4	1	3
(N37T × Os420) (M14 × Oh41)	9		3	3	1
(P8T × MoG) (M14 × Oh41)	8		2	1	5
	90	24	53		

with a delay of one to six days in time of shedding which could be quite insufficient for good grain production.

Several of these double crosses were grown in many different locations throughout the north central corn growing region and showed about the same amount of pollen production in all locations in both 1955 and 1956. Some of them were outstanding in stalk characters and grain production. They will need careful testing before they can be relied upon for adequate pollination when grown alone.

When these partial restorers are compared with complete restorers as shown in Tables 4 and 19 the difference is readily apparent. Ky21, Tx127, and NC77 in all combinations as single cross pollinators restored all sterile seed parents completely in all locations where they were grown in 1955, 1956, and 1957. One combination showed four steriles in a progeny of 14 plants. These could be outcrosses. There was no delay in pollen shedding in any combination. In some cases pollen was shed 12 days before any silks appeared. This is too wide a difference for good pollination. These plants were grown in 1955 when the plants suffered severely from lack of moisture before and at the time of flowering. With this premature pollen shedding normally fertile plants would not be pollinated properly.

Table 19. Pollen restoration in double crosses of two complete restorers compared with one complete with one partial restorer in single cross pollinators

Pedigree	Pollen Production		Date of First	
	Fertile	Sterile	Tassel	Silk
(WF9T × CI21E) (Ky21 × Tx127)	16		2	—
(WF9T × 38-11) (Ky21 × Tx127)	14		3	13
(WF9T × 38-11) (Ky21 × Tx127)	10		3	14
	40			
(WF9T × CI21E) (NC77 × Ky21)	16		7	8
(WF9T × 38-11) (NC77 × Ky21)	16		2	13
(WF9T × 38-11) (NC77 × Ky21)	16		3	16
	48			
(WF9T × CI21E) (NC77 × Tx127)	10	4	5	8
(WF9T × CI21E) (NC77 × Tx127)	17		8	8
(WF9T × 38-11) (NC77 × Tx127)	14		13	14
(WF9T × 38-11) (NC77 × Tx127)	14		3	15
	55	4		
(WF9T × Hy) (Oh41 × Ky21)	9	5	29	2
(WF9T × C103) (Oh41 × Ky21)	5	8	3	5
(WF9T × Hy) (Oh41 × Tx127)	9	6	3	—
(WF9T × C103) (Oh41 × Tx127)	9	6	2	5
	32	25		
(WF9T × Hy) (Ky21 × A71)	1	4	1	4
(WF9T × C103) (Ky21 × A71)	8	8	1	8
(WF9T × C103) (Ky21 × A71)	9	8	1	5
(WF9T × B14) (Ky21 × A71)	7	5	1	3
(WF9T × B14) (Tx127 × A71)	7	10	30	29
	32	35		

Since all combinations of these three good restorers produced all fertile plants when crossed on sterile seed parents (leaving out of consideration the four possible outcrosses) they must have the same dominant genes for pollen restoration, otherwise sterile plants would appear in the first generation. Duvick (1956) obtained the same results in crosses of Ky21 with other complete restorers.

When partial restorers, such as Oh41 and A71, are used in combination with a complete restorer, such as Ky21, as single cross pollinators (Table 19) they seem to add nothing to the restoring ability of these complete restorers. Oh41 × Ky21 gave 32 fertile and 25 sterile plants in four double cross combinations. Ky21 × A71 produced 32 fertile and 35 sterile plants. These are the results that are expected when a good restorer is used with a non-restoring or sterile inbred as shown in Table 14.

Pollen Production in Commercial Hybrids

One question remains that has not received a satisfactory answer: How much pollen is necessary or desirable to produce a normal crop of corn under all conditions? The method of restoring fertility by mixing seed of sterile and fertile plants is widely used and has given satisfactory results. Varying proportions of the two kinds of seed have been mixed, ranging from 25 percent to 50 percent or more of fertile plants. The usual proportion is one-third fertile and two-thirds sterile.

Usually this seed is mixed in the field at the time of harvest. In the process of grading the proportion of sterile and fertile seeds may vary widely in the different grades due to the fact that the two lots of seed have grown differently. They may differ in size or shape of kernels and more of one kind and less of the other will be represented in the different grades. The two different kinds of seed may germinate differently and flower at different times.

A sampling of mixed lots of seeds actually planted and an examination of mixed fields at time of pollination shows that the proportion of fertile and sterile plants may fluctuate widely. Counts have shown the fertile plants to be as low as 15 to 20 percent. Under normal conditions even this low proportion of plants producing pollen has given a normal crop of grain. This indicates that corn normally produces a vast excess of pollen that is not needed under most conditions.

Wind pollinated plants usually produce vast quantities of pollen. This is a factor of safety necessary for survival under widely varying environmental conditions where individual plants may be widely spaced. Under the favorable conditions of close planting in fields that are well adapted to corn culture this excess of pollen probably is not needed and may be undesirable. If the nutrients needed to produce this excess of pollen can be diverted to seed production there may well be an increase in grain production. This possibility needs further investigation.

For the present actual experience in field production indicates that 50 percent of normal pollen production is entirely adequate for average conditions and that this amount still leaves a margin of safety for extremely unfavorable conditions. Under conditions of extreme heat and low moisture other factors limit

grain production in addition to lack of proper pollination. High temperatures and low humidity can make silks non-receptive and there may be a failure of grain production where there is an abundance of normal pollen.

The Productiveness of Fertile, Sterile, and Restored Sterile Hybrids

Seedsmen report higher seed yields from sterile seed parents and many trials indicate higher yields of the double crossed hybrids produced in commercial crossing fields on these higher yielding sterile seed parents. Several factors are involved in these comparisons. One is the injury that results from tassel pulling and removal of one or more leaves with the tassel at a critical stage in the development of the plant. This injury is often followed by infection by the smut fungus and other organisms also reducing yields. Another factor is the self-pollination resulting from the failure to remove all of the tassels before pollen is released. This is a more serious loss than most seed producers are willing to admit. It is quite apparent in hybrid sweet corn which is usually produced as single crosses. Inbred plants in single crossed sweet corn are usually unmarketable. In double crosses inbred plants are reduced about 25 percent in yield. This reduction is not so noticeable but is nevertheless a real loss.

Table 20. Grain production of hybrids with sterile and fertile tassel

Pedigree	Percent Moisture	Yield Bu/Acre	Percent Sterile	Moisture Fertile	Bu/Acre Sterile	Bu/Acre Fertile
(WF9 × 38-11) (C103 × Oh43)	27	114	26	28	119	109
(WF9 × 38-11) (C103 × Oh43)	29	113	29	28	115	111
(WF9 × 38-11) (C103 × Oh43)	24	109	25	24	104	114
(WF9 × 38-11) (C103 × B14)	23	109	23	22	109	108
(WF9 × 38-11) (CI21E × C103)	28	106	28	27	109	103
(WF9 × 38-11) (Hy12 × C103)	26	105	26	26	103	107
(WF9 × 38-11) (Oh45 × C103)	26	105	26	26	107	103
(WF9 × 38-11) (C103 × B14)	27	103	27	26	102	105
(WF9 × 38-11) (C103 × B14)	25	103	24	26	101	104
(WF9 × 38-11) (Hy × C103)	27	103	28	26	95	111
(WF9 × 38-11) (Oh45 × C103)	28	103	28	27	99	106
(WF9 × 38-11) (C103 × B14)	27	102	28	25	103	100
(WF9 × 38-11) (CI21E × C103)	28	101	29	28	108	94
(WF9 × 38-11) (Hy12 × C103)	27	100	28	26	93	106
(WF9 × 38-11) (CI21E × C103)	31	100	31	31	106	93
(WF9 × 38-11) (CI21E × C103)	29	99	27	30	96	102
(WF9 × 38-11) (C103 × Oh43)	26	99	26	25	105	92
(WF9 × 38-11) (Hy12 × C103)	25	98	25	26	95	100
(WF9 × 38-11) (Oh45 × C103)	27	97	26	29	102	93
(WF9 × 38-11) (Hy × C103)	27	97	27	27	101	94
(WF9 × 38-11) (Hy × C103)	26	96	27	26	103	89
(WF9 × 38-11) (Oh45 × C103)	30	96	30	30	98	94
(Os420 × WF9) (Oh43 × C103)	27	96	26	27	93	98
(WF9 × 38-11) (Hy × C103)	27	95	26	27	97	93
(WF9 × 38-11) (Hy12 × C103)	28	91	29	27	90	91
(WF9 × Oh26) (A158 × Oh43)	24	90	26	22	95	84
Average	27	101	27	27	102	100
L.S.D. for varieties 14						
L.S.D. for sterile versus fertile 3.3						
Average of 4 entries of each pedigree						
(WF9 × 38-11) (C103 × Oh43)	27	109	26	26	111	107
(WF9 × 38-11) (C103 × B14)	26	104	26	25	104	104
(WF9 × 38-11) (CI21E × C103)	29	102	29	29	105	98
(WF9 × 38-11) (Oh45 × C103)	28	100	28	28	102	99
(WF9 × 38-11) (Hy12 × C103)	27	99	27	26	95	101
(WF9 × 38-11) (Hy × C103)	27	98	27	27	99	97

When seed is produced by hand pollination all of these factors are eliminated. Where hand pollinated seed is used a direct comparison of the productiveness and maturity of the sterile and fertile versions of the same hybrids can be made. There is the possibility that the sterile and fertile combinations may not be exactly the same in genotype but these differences are small if the inbred used as the seed parent of the seed parent single cross is closely similar in type to the fertile inbred. Greater differences are likely to occur if one or more restoring inbreds are used in the pollinator.

In the comparison given here in Table 20 only completely sterile and normally fertile versions of the same hybrid are compared. Twenty-six different combinations were grown in a replicated trial in 1954. Maturity as shown by the percent of moisture in the grain at harvest and the yield of grain in bushels per acre are given. There is no difference in moisture for the average of the 26 entries and only two bushels difference in yield. Six of the varieties were made up in four different lots using different seed and pollen parent single crosses. In four of these six hybrids the sterile version yielded more than the fertile. In one the yield was the same and in only one of the six lots did the fertile combination yield more. The greater yield of the sterile version was significant in three cases.

Restored Sterile Hybrids

Restored sterile hybrids produced on standard sterile seed parent single crosses with pollinator single crosses made up of various combinations of one or two natural restoring inbreds, restored sterile inbreds, or combinations of the two have been grown in preliminary trials in Connecticut and in a number of places in the north central corn growing regions. Some of these restored sterile hybrids have been made with a pollinator single cross composed of a sterile inbred crossed by a restorer inbred as mentioned previously. None of these experimental hybrids has been tested adequately as yet for pollen production in isolated fields. Many of them have been tested for yield and other agronomic characters in replicated trials and appear to be quite promising. They produced

from 25 to 75 percent of the normal amount of pollen. Some of them are listed here:

(W22T × B9) (WF9T × A344)
(B9T × W22) (WF9T × W153R)
(WF9T × 38-11) (Oh29 × Oh41)
(WF9T × 38-11) (Oh29 × M14)
(WF9T × B14) (Oh29 × Oh41)
(WF9T × B14) (Oh29 × M14)
(WF9T × Hy) (Oh29 × M14)
(WF9T × Hy) (Oh29 × M14)
(WF9T × C103) (Oh29 × Oh41)
(WF9T × C103) (Oh29 × M14)
(WF9T × W22) (Oh29 × Oh41)
(WF9T × Oh43) (W24 × I205R)
(WF9T × C103) (Oh29 × M14)
(WF9T × Os420) (I205R × Oh41)
(WF9T × Hy) (I205R × Oh41)
(WF9T × Hy) (I205R × N15)
(WF9T × B14) (I205R × N15)
(WF9T × Oh51A) (I205D × M14)
(WF9T × Hy) (I205D × M14)
(WF9T × W22) (I205D × M14)
(WF9T × M14) (KrTF × Os420)
(WF9T × Oh43) (KrTF × I205R)

Pollen Restoring Inbreds

Inbreds that are natural restorers or have been produced by backcrossing in Connecticut and elsewhere are listed in Table 21. These include only yellow seeded lines suitable for commercial production in the northern part of the corn growing areas. Many of the converted lines have not been backcrossed long enough to be restored to the original genotype and have not been self-fertilized sufficiently to be made homozygous for the restoring genes. They will all need further selfing, selecting, and testing for pollen restoring ability in the specific combinations they are to be used, and for local adaptation. Seed of most of the restorers listed is available for experimental purposes only with the understanding that it will be tested both for pollen restoration and combining ability in the areas that the seed is to be grown and in the combinations of inbreds with which it is to be employed before it is used for commercial production.

All of these inbreds can be used as sources of restoring genes to convert other inbreds to pollen restoration. A good way to do this is to select one or more restoring inbreds of about the same maturity and the same combining ability and general performance in hybrids as the inbred to be converted. If a sterile version of this inbred is available it is a simple procedure to cross on to this sterile line as the seed parent. Select fully fertile plants in the resulting progeny and backcross on to the sterile inbred. Continue the selection and backcrossing until the inbred is converted to type and gives equal performance, in the combination it is to be used with, as the original fertile line. As long as it is backcrossed it will give only about 50 percent of fertile plants in each backcrossed generation and in crosses on sterile seed parents. By selfing, these heterozygous plants can be made homozygous and should then give complete pollen restoration. However, they must be tested in all combinations in which they are to be used and these test crosses must be grown under the same latitude, soil, and seasonal conditions that the final hybrid is to be grown.

If sterile versions of the inbred to be converted are not available crosses should be made on restored sterile restorers. Any of the inbreds labeled TF or SF in the accompanying list are suitable for this purpose if they have been tested and are known to be good restorers under the conditions where the hybrids are

Table 21. Restoring inbreds—natural and restored sterile

T restorers	
NY16	Oh29
A158TF(Ky)	Oh29TF(KrT)
A293	Oh29TF(I205T)
A344	Oh41
I153	Oh41TF(C106T)
W153R	Oh43TF(Ky)
I153TF(C106T)	Oh51A-TF(I153)
M14F	B14TF(Mo940)
M14TF(C106T)	I205TF(Ky)
HyTF(Ky)	I205R
HyTF(C236)	Os420TF(Ky)
WF9TF(Ky)	KrTF(Ky)
WF9TF(I153)	C103TF(Ky)
Oh7TF(Ky)	C106TF(Ky)
	LkTF(Ky)
S Restorers	
A158SF(Ky)	Ill.K
A206	Ill.L
M14SF(Ky)	Oh41

The letters and numbers in parentheses give the original source of the restoring genes or the sterile cytoplasm.

to be grown. Fully fertile plants from these crosses can be used as seed parents for backcrossing by the inbred to be converted as pollinators. Having sterile cytoplasm the plants should continue to segregate into fertile and sterile plants in each backcrossed generation. Only fully fertile plants should be selected as seed parents for further backcrossing. This is most important.

In these backcrossed progenies the most fertile plants are the first to flower. Usually they have finished shedding pollen before the inbred plants of the recurrent parent are available for pollination either as pollen parents or seed parents. For this reason the two lots used in backcrossing must be planted at different times in order to bring them into flowering at the same time. These repeated plantings can be avoided by selecting restorers of earlier or later maturity, depending upon which way the backcross is to be made.

While converting an inbred to pollen restoration it is possible to improve the line in other characters. By using sources of restoring genes, that have as well other desirable qualities that may correct some of the weaknesses of the line to be converted, considerable improvement is possible. In actual practice it is much easier to lose desirable qualities than it is to add to them.

By selecting in the first cross or early generations of backcrossing it is possible to create new inbreds that may be quite different in maturity and in their ability to transmit to hybrids other desirable qualities, as well as have the ability to restore pollen to sterile seed parents. Good restoring inbreds are potentially quite valuable. Successful efforts to produce such inbreds should be as well rewarded in the future as the production of good inbred strains of corn has been in the past.

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