THE INHERITANCE OF RESTORER FACTORS IN TWO LOTS OF NEBRASKA 542437 WINTER WHEAT

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CHAPTER I

GENERAL INTRODUCTION

Interest in the possibility of hybrid wheat being grown on a commercial scale has continued to increase since information was released in late 1962 that the necessary male sterile and fertility restorer mechanisms were available.

Cytoplasmic male sterility in wheat was discovered by Kihara (29) and Fukasawa (14, 15) and developed in bread wheats by Wilson and Ross (46) who substituted the nucleus of Triticum aestivum into Aegilops caudata L., Aegilops ovata L., and T. timopheevi Zhuk. cytoplasm. It soon became apparent that the T. timopheevi male sterile was the most promising of the three sources of cytoplasmic male sterility. The discovery of genotypes that would restore fertility in the presence of sterile cytoplasm was made in 1962 by Schmidt, Johnson, and Maan (39) and by J. A. Wilson as cited by Livers (31). The Nebraska wheat population, N542437, gave fertile hybrids when used as a pollinator on its sister pollen sterile females. This population has a winter growth habit and is adapted to Oklahoma conditions. Additional reports of restorer material are beginning to appear from various sources throughout the world.

In a cytoplasmic-genetic sterile system, of crop plants in general, fertility restoration occurs by the action of one or more dominant genes. However, there is considerable variation both among and within species

as to the number of genes controlling fertility restoration. In corn, different gene systems control fertility restoration in different cytoplasms. Complementary dominant genes Rf_1 and Rf_2 are required for restoration in T (Texas male sterile) cytoplasm (10, 12) and a dominant gene, Rf_3, is required for restoration in S (Connecticut sterility-inducing) cytoplasm (7, 11). A number of genes which express partial fertility are also noted (4, 5, 24, 38). In sorghum there appears to be one dominant restorer gene of major effect but partial restorer genes of varying number and degree of expression are required in some environments (21, 33, 34). In pearl millet, evidence indicates that restoration of fertility in the 'Tift 28A' (Tifton, Georgia) cytoplasmic male sterile female is accomplished by one major dominant gene, Ms, although modifiers are known to have some effect on restoration (8).

Preliminary genetic investigations indicate that fertility restoration of wheat is simply inherited and most investigators (2, 11, 18, 22, 31, 48) suggest that at least two complementary genes are necessary.

This study was designed to assess the inheritance of restorer factors and their expression in different environments. The subject matter is divided into two systems of classification, and the correlations pertaining to them. These are as follows: genetic ratios based on seed-set classification, genetic ratios based on pollen classification, relationship between pollen and seed-set classification, and correlation of restoration in different environments. Each chapter is reported, with some modifications, in the style and form required by the scientific journals in the author's field. This style allows individual consideration of each division of subject matter in a somewhat concise

manner. The general discussion and the summary and conclusions are included as separate chapters.

CHAPTER II

MATERIALS AND METHODS

Materials

The restorer material involved in this study consisted of 13 restorer lines; six lines from lot 1 and seven from lot 2 of the Nebraska restorer population 542437. The pedigree of Nebraska 542437 is as follows: T. timopheevi x (Hussar-Hard Federation)^2 x (Comet-Hussar-Hard Federation) x Nebred.

The two lots of Nebraska restorer material originated from seed composited from fully fertile F_2 plants derived from the cross sterile 542437 x fertile 542437. Both lots had a common male-sterile female parent, N542437, but the pollen parent of lot 1 presumably differed from lot 2 in degree of restoration as shown by the segregating classes in Table I. Reports from Nebraska (23) indicated that the F_2 population from cross 627 (lot 1) had two major genes for restoration, whereas in cross 628 (lot 2) a single major gene with possible minor genes was indicated. The cytoplasm was sterile in both restorer lots.

The Oklahoma Agricultural Experiment Station received these two lots of seed from Dr. J. C. Craddock[1/] in July, 1963. They carried the designation N542437^A x N542437^R F_3. The lot 1 (two gene) restorer population, grown as plot 5892 in 1964 at Stillwater was assigned the

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1/ Dr. J. C. Craddock, USDA,ARS,CRD, Beltsville, Maryland.

TABLE I

SEED SETTING FERTILITY OBSERVED IN THE F_2 FROM TWO CROSSES OF MALE STERILE X MALE FERTILITY-RESTORER COMMON WHEAT TYPES IN NEBRASKA 1/

Population	Total No. Plants	0%	1-5%	25%	50%	75%	90%	100%	Total %
F_2 from Cross 627 (Lot 1-2 major genes)	277	2.9	9.0	5.4	11.2	18.1	9.4	44.0	100
F_2 from Cross 628 (Lot 2-1 major gene)	207	11.1	14.5	10.1	18.4	14.0	12.1	19.8	100

1/ V. A. Johnson, J. W. Schmidt, and P. J. Mattern. Hybrid Wheat in the United States. Paper No. 1954, Journal Series, Nebraska Agric. Expt. Sta.

selection number Stw 645892. The adjacent plot 5893 was the lot 2 (one gene) restorer population and the assigned selection was Stw 645893. The individual plant selections from each lot used in this study were identified by plot and plant number. For example, plant selection number two from Stw 645892 is designated as 5892-2.

A total of 26 lot 1 and 28 lot 2 plants were grown as F_3s in the field in 1964. At least two heads of each plant were bagged in order to provide selfed seed for subsequent studies on the restorer parents. Testcrosses were made with a cytoplasmic male sterile 'Bison' female using pollen from individual plants of both lots.

Ample testcrossed seeds were available for six lot 1 and seven lot 2 plants; therefore, the 13 lines derived from these plants were used in the study. Since the 13 lines were selected only on the basis of adequate testcross seed, they can be considered as representative or random samples of the two restorer populations. Two check varieties, 'Triumph' and Bison, were included in each location. The agronomic and quality data for each of these restorer lines and check varieties are shown in Table II.

The three types of populations tested in the present study were as follows:

1) Restorer parents - the F_4 restorer lines were evaluated in 1965 and F_5 lines in 1966. These lines trace to an individual F_3 plant. The seed for each restorer parent came from selfed heads.

2) Testcrosses - the A x R crosses were made in both 1964 and 1965 and the F_1 hybrids were grown in the respective 1965 and 1966 seasons in order to study the genetic constitution of the different restorer lines. Testcross as defined for this study is a cross of a restorer

TABLE II

AGRONOMIC AND QUALITY DATA FOR 13 RESTORER LINES
GROWN AT STILLWATER, OKLAHOMA, IN 1966

Restorer Line	Date Headed	Weight Per Bushel (lbs.)	Yield (Bu/A)	Mixing 1/ Time	Specific Sedimentation	Wheat Protein (Percent)
Lot 1 (2 Gene)						
5892-2	5-05	57.8	32.7	3.4	3.20	16.4
5892-5	5-09	59.0	31.5	4.6	4.80	14.9
5892-7	5-08	59.5	36.2	3.6	4.79	15.3
5892-9	5-11	59.7	38.3	3.0	3.21	15.3
5892-10	5-10	57.0	32.5	3.3	2.93	15.4
5892-25	5-07	56.5	30.0	5.7	4.98	15.3
Lot 2 (1 Gene)						
5893-2	4-30	60.3	46.4	3.0	3.83	13.6
5893-3	4-29	61.0	39.8	2.6	3.95	14.3
5893-13	4-30	59.8	30.3	3.7	4.35	14.3
5893-15	4-29	60.0	39.0	5.0	4.05	15.0
5893-18	4-28	60.3	46.1	3.4	4.04	14.7
5893-20	4-29	60.8	42.2	6.0	4.14	14.5
5892-22	4-29	60.2	39.9	2.5	2.46	14.3
Checks						
Triumph	4-27	60.0	35.0	2.5	2.81	14.6
Bison	5-06	60.0	31.3	4.0	3.21	15.2

1/ Mixograph mixing time readings in minutes.

line, whether homozygous or heterozygous, to a double recessive (male sterile) type female. This definition of testcross does not conform to that given by Allard (1) and others but is, none-the-less, a cross to test genotype of a restorer line.

3) F_2s - the F_1 hybrids from the A x R testcrosses were grown as F_2s in 1966 also, in an attempt to determine the genetic constitution of the restorer lines.

Procedures

The three locations selected for this study were the glass greenhouse, plastic greenhouse, and field on the Stillwater Agronomy Farm. The locations in which each population was grown and the number of plants representing each restorer line during 1965 and 1966 are given in Table III. The methods of handling the material are given by years as follows:

Procedures for 1965

The restorer parents and testcross seedlings for all locations were first established under greenhouse conditions in plant bands during October, 1964.

The seedlings were transplanted in the field on October 28, 1964. The field plot size was a single row five feet in length for each parent line and four feet for each testcross. The row spacings were one foot and the plant spacings within the rows were six inches.

The plant populations grown in the greenhouse were placed outside for eight weeks to effect vernalization, which was completed in December, 1964. The parent lines grown in the glass greenhouse were transplanted

TABLE III

SUMMARY TABLE OF LOCATIONS FOR EACH POPULATION GROWN AT THE STILLWATER STATION DURING THE YEARS 1965-1966

Year	Population					
	Parent		Testcross		F2	
1965	Glass Greenhouse	(8) 1/				
1965	Plastic Greenhouse	(8)	Plastic Greenhouse	(8)		
1965	Field	(10)	Field	(8)		
1966	Glass Greenhouse	(8)	Glass Greenhouse	(2)		
1966	Plastic Greenhouse	(8)	Plastic Greenhouse	(8)	Plastic Greenhouse	(12)
1966	Field	(8)	Field	(20)	Field	(90)

1/ Number in parenthesis = number of plants per restorer line.

to clay pots and placed on greenhouse tables. The parent and testcrossed lines grown in the plastic greenhouse were transplanted into a soil bed in early December, 1964, using 12-inch spacings between plants.

Procedures for 1966

The F_2 populations involving each restorer line as well as testcrosses and the restorer parents themselves were grown in the field and plastic greenhouse. The restorer parents and testcrosses were also grown in the glass greenhouse. However, because of limited space, the F_2 populations could not be grown in this location. The seed for all populations in the glass and plastic greenhouse was again planted in plant bands in the greenhouse on November 1, 1966. All potted plants for the glass greenhouse were moved inside during the month of December, but transplanting into the plastic greenhouse soil bed was delayed until January 15, 1966, to avoid the excess growth that occurred during the 1965 season. Six-inch intervals were used between plants in the plastic greenhouse.

Plot size of the field plantings was one 10-foot row for the parent and testcross nurseries and three 10-foot rows for the F_2 populations. The restorer parents, testcrosses, and F_2s involving each restorer line were represented by 8, 20, and 90 plants respectively, and spacings between plants were 12, 6, and 4 inches respectively.

Adequate fertilizer was applied at each location for optimum plant growth. Water was added to the pots and soil bed of both greenhouse locations as needed but no supplemental moisture was applied in the field, other than a minimum amount in 1966 to establish the transplants.

Observations

The following observations were made for individual plants of the parent lines, testcrosses, and F_2s in all locations for both years:

Date headed - the date when the first head was completely emerged from the boot.

Pollen readings - three anthers were taken from the base, middle, and tip of the primary spike just prior to dehiscence. The pollen grains were stained with an IKI staining solution and subsequently examined with a light microscope at X150 magnification. Readings were based on 200^{+} pollen grains per anther. Anthers were grouped into the following pollen classes: 1) dark - completely dark staining grains, 2) intermediate - range from light black to nearly clear, and 3) sterile - completely non-staining grains. Percent fertile pollen of both individual plants and the three areas of the spike were based upon the combined percentage of dark and intermediate grains per spike as follows:

$$\text{Percent fertile pollen} = \frac{\text{No. of dark grains + no. of intermediate grains}}{\text{Total no. grains in all three classes}}$$

The inclusion of the intermediate pollen class with the dark pollen class may cause a slight bias in the fertile pollen reading, but the majority of intermediate grains were a light shade of black and were presumed to be fertile; although possibly immature.

Seed-set counts - the primary spike was analyzed separately followed by an analysis of five additional heads, thus providing a reliable estimate of plant fertility. The spikelets of the primary spike were numbered from the base to the tip and then divided into three equal parts: base, middle, and tip. The number of lateral (first and second)

florets and seeds for each area were determined. Seed-set values for the five additional heads were established by counting the total number of lateral florets and seeds. All central florets were counted separately and the basal and tip spikelets were disregarded. Percentage seed set was determined as follows:

$$\text{Percent lateral seed set} = \frac{\text{Number of lateral seeds}}{\text{Number of lateral florets}}$$

In order to reduce the possibility of cross pollination, the first six heads of each plant were wrapped with tissue paper which was secured with a paper clip. This method was used for all locations in 1965 but it was adopted primarily because there is no other effective way known of insuring self pollination of wheat in the field without injury. The main disadvantage of this technique is possible reduction in seed set caused from wrapping the head too tight. Dialyses tubing was used instead of tissue paper in both greenhouse locations during 1966; however, because of the strong winds and rapid pollen shed, no enforced selfing was possible in the field.

Nomenclature

Since hybrid wheat is in the developmental stage and terminology has not as yet been standardized, some classification on nomenclature is in order.

The ABR system of nomenclature as used by sorghum workers is generally used in reports dealing with hybrid wheat and is used in this study to designate the various types of lines. The genotypic symbols used here with reference to restoration, however, correspond to those used for corn. The combined classification system then is as follows:

"A" designates the cytoplasmic male sterile line. According to Livers (31), who first used the Rf designation in wheat, the A-line genetically, assuming two genes for restoration, is $rf_1rf_1rf_2rf_2$ in male sterility-inducing cytoplasm. In seed production fields, the A-line is the seed parent.

"B" designates the sterility maintainer line. It is the pollen fertile, non-restorer counterpart of the male sterile line. In a two-gene restorer system, the B-line genotype is $rf_1rf_1rf_2rf_2$ in normal (fertile-inducing cytoplasm). It does not restore fertility.

"R" designates the fertility restorer line that induces normal pollen fertility in the F_1 of a cross with a cytoplasmic male sterile line. The R-line can have either normal or sterile-inducing cytoplasm. Genetically, the R-line, following the corn terminology, has Rf,rf genes that restore pollen fertility. These are given subscripts to denote different loci. A theoretical example of two dominant genes controlling fertility restoration with the expected genotypes and degree of fertility for each is shown below.

Genotype	Degree of Fertility
Rf_1---Rf_2---	complete fertility
Rf_1---rf_2rf_2	partial fertility
$rf_1rf_1Rf_2$---	partial fertility
$rf_1rf_1rf_2rf_2$	complete sterility

In cases where the restorer line is segregating with respect to restorer genes, individual plants from this line which are involved in testcross or F_2 studies may be heterozygous for one or more restorer genes. Since this could be the situation in the present study, the testcross and F_2 populations were tested for goodness of fit to the

theoretical genetic ratios shown in Table IV. These calculated ratios are based on three fertility classes and include two-gene ratios with one or both genes segregating.

TABLE IV

CALCULATED GENETIC RATIOS FOR ONE AND TWO GENE INHERITANCE OF FERTILITY RESTORATION FROM TESTCROSS AND F_2 GENERATIONS

	Testcross			F_2 Population		
Predicted Genotype 1/	Fertile	Partial Fertile	Sterile	Fertile	Partial Fertile	Sterile
2 gene homo. dom. $Rf_1Rf_1Rf_2Rf_2$	1	0	0	9	6	1
2 gene hetero. $Rf_1rf_1Rf_2rf_2$	1	2	1	9	30	9
2 gene--1 homo. dom., 1 hetero. $Rf_1Rf_1Rf_2rf_2$ or $Rf_1rf_1Rf_2Rf_2$	1	1	0	9	18	5
1 gene homo. dom. Rf_1Rf_1 (incomplete dominance)	0	1	0	1	2	1

1/ homo. dom. = homozygous dominant; hetero. = heterozygous

Statistical Treatment

Chi-square probability levels were used to test the goodness of fit to the various genetic ratios regarding restoration in each of the 13 restorer lines. The probability values were obtained from the chi-square distribution tables by Fisher and Yates (13).

Evaluation of the degree of fertility for each population in the present study was based on the percent fertile pollen or percent seed set. The association between percent fertile pollen and seed set was determined by calculating linear correlation coefficients (r). Comparisons of seed-set percentage in different locations and years were

studied also by linear correlations. Test of significance of these correlations was based on n-2 degrees of freedom and the probability values were taken from the table by Fisher and Yates (13).

CHAPTER III

GENETIC RATIOS BASED ON SEED-SET CLASSIFICATION

Although some form of seed-set analysis has been used from time to time in determining the degree of fertility in the wheat spike, recent work on the development of hybrid wheat has resulted in a renewed interest in this technique as a measure of fertility restoration. Seed-set analysis is important as it relates to restoration since the development of seed in the spikes of A x R F_1 hybrid plants will be dependent on the presence of the restorer genes required to counteract the effect of cytoplasmic sterility.

Information regarding the use of seed-set analysis in determining fertility restoration in wheat has been rather limited since the discovery of the restorer genes in Triticum aestivum in 1962. One of the few published sources of data for restoration based on seed-set percentage was the classification of the Nebraska restorer F_2 populations (Table I) reported by Johnson et al. (23). On the basis of the estimated seed set, these workers grouped plants into seven fertility classes. The extreme classes of 0 and 100% were designated sterile and fertile, respectively. The plants expressing intermediate degrees of seed set were placed in one of the following seed-setting fertility classes: 1-5, 25, 50, 75, and 90% fertile. They reported that seed set in this material followed a definite pattern, starting at the base of the spike and progressing toward the tip (e.g. 75% fertile indicates that the lower

three-fourths of the spike contains seed but the upper one-fourth is sterile). These workers used this seed-setting pattern as the criterion to form the several fertility classes. Tsunewaki (43) estimated the degree of fertility restoration in Triticum aestivum ssp. compactum from the seed-setting percentages of bagged heads. Percent seed set was calculated from the number of normally developed first and second florets and the number of seeds set in them. He used eight fertility classes to describe the variation in seed set in the parental and segregating populations. Kihara (29) and Fukasawa (14) used percent seed set in the normally developed florets to estimate fertility of plants with various chromosome numbers during successive backcrossing programs. Fukasawa (17) used seed-set percentage calculated from the number of florets pollinated and number of seeds set to express the effect of cytoplasm on the degree of crossability between different genera and species. Rajki et al. (36) used the number of seeds per spike to express fertility differences in studies of fertility restoration in a series of F_1 hybrids.

In using seed-set analysis to determine the degree of restoration in A x R wheat hybrids, two problems may be encountered which might bias the results of the analysis. One of these involves the fertility of the central florets. The lateral florets of a wheat spikelet in standard varieties are developed first and normally contain seed. However, the degree of development in the flowers of the central florets appears to be more highly influenced by environmental conditions. The author has noted seed set of the central florets ranging from 50-100% for the heads of the Bison variety when grown in the glass greenhouse environment, but less than 5% of the central florets set seed during the same

year under limited moisture conditions in the field. Since one objective of this study was to measure the degree of fertility restoration based on genotype, only the seed set in the lateral florets will be considered in as much as this appears to be the most reliable method.

The second problem in determining fertility is the influence of environment on the expression of the restorer genes. Although prior to the initiation of this study the extent of environmental effect on the expression of sterility and fertility in the Nebraska stock was not known, it was suggested by the Nebraska workers (22) that some combinations of light intensity, photoperiod, and temperature may be possible factors contributing to variation in seed set involving restorer material. This situation will be treated in more detail in Chapter VI.

Experimental Results

It is of upmost importance for the female parent used for test-crosses to remain completely sterile in order to evaluate the expression of the fertility genes of different restorer lines. The male sterile line, $N542437^{A}$ x $Bison^{2}$, serving as the female parent in this study remained very stable, having no seed set when isolated from pollen sources in the three environments tested (Appendix Tables XVI and XVII).

The range and average seed set for each of the R-lines, A x R F_1 hybrids, and check varieties were quite variable within and among locations for both years of the study (Appendix Tables XVI and XVII). Although the range of seed set within each restorer parent line and check variety was large, it was even larger within the F_1 hybrids.

Restorer Parents

Seed-set determinations were made on the first fully emerged (selfed) head of each plant in the restorer lines followed by an analysis of five additional heads. Fertility of the plant was expressed on the basis of both the first head and five additional heads as shown in Table V. The average readings for the first selfed head were higher than those of the five additional heads from the field and glass greenhouse locations. The readings on plants grown in the plastic greenhouse were variable and did not fit this pattern. A comparison of average seed-set readings indicated that the first head to emerge was a good measure of the actual fertility in the various varieties and lines; however, the average of five heads is probably the most reliable, therefore was used in studying the genetic ratios.

Comparisons were made of the restorer lots and check varieties based on the combined averages for the three locations (Table V). There was essentially no difference in the combined average seed-set readings for the check varieties and lot 2 restorer lines; however, the lot 1 restorer line had consistently lower readings. The grand averages for the checks, lot 2, and lot 1 restorers were 79.8, 79.3, and 74.9%, respectively. As shown in Table V, the rankings of seed-set percentages were consistently higher in the field than in the glass greenhouse. The only exception was a higher percentage for the selfed heads of lot 1 in the glass greenhouse in 1965. Seed sets in the glass greenhouse were higher than those in the plastic greenhouse with the exception of the checks in 1966. The average seed-set readings of all restorer lines and checks for both years are presented in Table VI.

TABLE V

COMPARISON OF SEED SET AMONG LOCATIONS BASED ON THE READINGS OF SELFED RESTORER LINES FOR 1965 AND 1966

	Average Percent Seed Set Between Locations (8 plants/restorer line and check)						
	1st Selfed Head/Plant			5 Additional Heads/Plant			
Location	1965	1966	Avg.	1965	1966	Avg.	Grand Average
Lot 1 (6 lines)							
Field	83.5	92.2	87.8	80.1	89.7	84.9	86.4
Glass Greenhouse	89.8	84.9	87.4	74.0	83.7	78.8	83.1
Plastic Greenhouse	47.9	56.3	52.1	49.2	68.0	58.6	55.4
Average	73.7	77.8	75.8	67.8	80.5	74.1	74.9
Lot 2 (7 lines)							
Field	92.2	89.8	91.0	85.3	84.9	85.1	88.0
Glass Greenhouse	88.6	84.3	86.4	81.1	79.8	80.4	83.4
Plastic Greenhouse	58.7	73.4	66.0	56.3	76.9	66.6	66.3
Average	79.8	82.5	81.2	74.2	80.5	77.4	79.3
Checks (Tmp and Bsn)							
Field	92.2	83.6	87.9	90.8	78.2	84.5	86.2
Glass Greenhouse	90.6	75.8	83.2	78.1	64.1	71.1	77.2
Plastic Greenhouse	69.8	81.8	75.8	64.8	87.4	76.1	76.0
Average	84.2	80.4	82.3	77.9	76.6	77.2	79.8
Grand Average	79.2	80.2	79.7	73.3	79.2	76.2	78.0

TABLE VI

AVERAGE PERCENT SEED SET BY LOCATION FOR ALL RESTORER LINES AND CHECKS GROWN IN 1965 AND 1966

Rank	Location	Average Percent Seed Set
1	Field	86.9
2	Glass Greenhouse	81.2
3	Plastic Greenhouse	65.9

The difference in seed-set percentages of 5.7% between the field and glass greenhouse indicated that the glass greenhouse was a slightly more critical environment for fertility expression than the field. The comparisons of field vs. plastic greenhouse and glass greenhouse vs. plastic greenhouse express differences of 21.0 and 15.3%, respectively; which, of course, indicated that the plastic greenhouse was the most critical environment for determining fertility of the restorer lines. A more critical (less fertile) environment will give a more realistic expression of the degree of fertility present in a restorer line than a highly fertile environment.

Testcross and F_2 Populations

The seven fertility classes described by Schmidt and Johnson (40) constituted the initial classification system used in the present study for the parent, testcross, and F_2 populations (Appendix Figures 1 and 2). When comparing the restorer parent plants for percent seed set, five restorer lines equaled or exceeded the best check variety in both years. These lines involved one lot 1--two-gene restorer (5892-25), and four lot 2--one-gene restorers (5893-3, -13, -15, -22).

The lack of plants falling into the 0 and 1-5% fertility classes for the testcross populations caused the frequency distribution curves to be skewed to the right toward higher fertility; also, the percentage of plants in the 90 and 100% classes was very low. The shortage of fertile plants resulted in a higher percentage of plants within the intermediate or partial fertility range and the curves are similar to a normal distribution. The relatively small number of plants for the testcrosses of each restorer line in 1965 made the curves appear somewhat flat in comparison with the 1966 distributions involving larger numbers.

All F_2 frequency distribution curves tended to resemble a normal distribution. One noted difference was that the peaks for all lot 1 (two-gene) restorers, except 5892-2 and 5892-5, were near the 75% fertility class, and all lot 2 (one-gene) restorers, except 5893-2, had a high peak at the 50% fertility class. This reflects the higher degree of fertility in the lot 1 (two-gene) restorer. This classification showed no marked differences among the various generations of each restorer line other than variation in the peaks.

Since this system of classification consisting of seven fertility classes failed to distinguish between restorer lines, a second system of classification was adopted. The classes and reasons for their use are as follows:

a) Sterile class (0-25% seed set) - the range is based on the degree of natural cross pollination from a series of male sterile rows included in this study which averaged 23% seed set. It was impossible to insure controlled selfing on the F_2 populations grown in the field during 1966 because of strong winds; therefore, it is likely that some

cross pollination occurred in sterile or partially fertile heads.

b) Partially fertile class (26-74%) - the intermediate classes of the previous system were not obviously different so were grouped together.

c) Fertile class (75-100%) - a peak of 75% was common for the seed-set distribution of check varieties so the fertile class included any plants which exceeded this amount.

The percentage of plants in each of the seed-set classes for test-cross and F_2 populations of this second classification system are given in Table VII. The F_2 populations grown in the field in 1966 appeared to fall into two logical groups. These groups are not strictly lot 1 and lot 2 as might be expected. Considering only the percentage of plants in the fertile class for this F_2 population, all of the lot 1 lines (except 5892-2) fall within the range of 31-51% fertile plants. Line 5893-2 also fits this range for the first group. The remaining six lines of lot 2 and line 5892-2 of lot 1 have fertile plant percentages ranging from 7-23% to form the second group. These groupings correspond to those explained for the frequency distributions in the first classification system.

Johnson et al. (23) stated that restorer genes function as incomplete dominants and their research indicates that complete fertility restoration in the F_1 hybrid requires at least two genes. If this is true, then the A x R testcross F_1 of a one-gene homozygous restorer would be only 50% fertile and a two-gene A x R testcross would give a fully fertile F_1 hybrid. In applying the incomplete dominant theory for one gene to the testcross populations in Table VII, none of the restorer lines had all plants in the partially fertile class as expected

TABLE VII

SEED SET CLASSIFICATION OF FERTILITY RESTORATION FOR THE RESTORER LINES AS EVALUATED BY TESTCROSS AND F_2 POPULATIONS IN 1965 AND 1966

	1965 Testcross - Field				1966 F_2 Population - Field				1966 F_2 Population - Plastic Greenhouse			
			Partial				Partial				Partial	
Restorer Line	Total No. Plants	Fertile 75-100%	Fertile 26-74%	Sterile 0-25%	Total No. Plants	Fertile 75-100%	Fertile 26-74%	Sterile 0-25%	Total No. Plants	Fertile 75-100%	Fertile 26-74%	Sterile 0-25%
Lot 1 (2 Gene)					(Percentage of plants falling in each class)							
5892-2	8	62.5	37.5	0.0	88	22.7	72.7	4.5	12	41.7	33.3	25.0
5892-5	8	62.5	37.5	0.0	83	33.7	61.4	4.8	12	41.7	33.3	25.0
5892-7	8	75.0	25.0	0.0	88	31.8	61.4	6.8	11	36.4	54.5	9.1
5892-9	8	87.5	12.5	0.0	88	47.7	50.0	2.3	11	36.4	63.6	0.0
5892-10	8	87.5	12.5	0.0	90	51.1	44.4	4.4	10	30.0	50.0	20.0
5892-25	8	50.0	50.0	0.0	90	44.4	54.4	1.1	11	63.6	0.0	36.4
Lot 2 (1 Gene)												
5893-2	8	75.0	25.0	0.0	89	40.4	56.2	3.4	12	33.3	41.7	25.0
5893-3	8	37.5	50.0	12.5	88	11.4	83.0	5.7	12	33.3	66.7	0.0
5893-13	8	62.5	25.0	12.5	88	11.4	81.8	6.8	12	0.0	58.3	41.7
5893-15	8	50.0	50.0	0.0	88	17.0	78.4	4.5	12	33.3	58.3	8.3
5893-18	8	12.5	37.5	50.0	85	7.1	91.8	1.2	12	8.3	33.3	58.3
5893-20	8	87.5	12.5	0.0	87	14.9	77.0	8.0	12	16.7	75.0	8.3
5893-22	8	50.0	50.0	0.0	89	18.0	78.6	3.4	12	16.7	58.3	25.0
Triumph (Check)	10	100.0	0.0	0.0	20	80.0	20.0	0.0	11	100.0	0.0	0.0
Bison (Check)	10	100.0	0.0	0.0	20	89.7	10.3	0.0	11	63.6	36.4	0.0

if one homozygous dominant gene controls fertility restoration.

Chi-square probability levels were used to test genetic ratios regarding restoration in this material. The testcross and F_2 populations for each restorer line shown in Table VIII were analyzed for all possible genotypes of a two-factor hypothesis and a one-factor, homozygous dominant hypothesis. Greater emphasis was placed on the F_2 populations from the field in 1966 since they consisted of a much larger number of plants (approximately 90) than the other testcross and F_2 populations. The testcross populations grown in the field in 1965 showed enough difference among genetic ratios to give support to results of the field F_2s, but the F_2 populations grown in the plastic greenhouse did not fit any of the genetic ratios, probably because of small population size. The F_2 field data in 1966 for five restorer lines (5892-5, -7, -9, -10, and 5893-20) showed a good fit to at least one ratio of two-gene inheritance. The range of actual chi-square probability values calculated for the best fit ratios of these restorer populations was .37-.018 which exceeds the accepted .01 value. The populations of lines 5892-5 and -7 fit the two-gene (one homozygous dominant, one heterozygous) ratio for both testcross and F_2 generations. Restorer lines 5892-9 and -10 were the only populations that fit two-gene homozygous dominant ratios for testcross and F_2 generations in both the field and plastic greenhouse locations. The eight remaining restorer populations had highly significant probability values for all two-gene ratios for the F_2 generations being grown in the field.

Preliminary reports from Nebraska (23) indicated that fertility restoration of the lot 1 populations may be controlled by two genes and the lot 2 population by one gene. Since the actual genotypes of the

TABLE VIII

CHI-SQUARE PROBABILITY VALUES FOR DIFFERENT GENETIC RATIOS OF TESTCROSS AND F_2 POPULATIONS BASED ON SEED SET CLASSIFICATION

	1965 Testcross - Field				1966 F_2 Population - Field				1966 F_2 Population - Plastic Greenhouse			
Restorer Line	2 Gene Homo. Dom. 1:0:0	2 Gene Hetero. 1:2:1	1 Gene Homo. 1 Gene Hetero. 1:1:0	1 Gene Homo. Dom. 0:1:0	2 Gene Homo. Dom. 9:6:1	2 Gene Hetero. 9:30:9	1 Gene Homo. 1 Gene Hetero. 9:18:5	1 Gene Homo. Dom. 1:2:1	2 Gene Homo. Dom. 9:6:1	2 Gene Hetero. 9:30:9	1 Gene Homo. 1 Gene Hetero. 9:18:5	1 Gene Homo. Dom. 1:2:1
Lot 1-2 Gene												
5892-2	<.005	.25-.10	.90-.75	<.005	<.005	<.005	<.005	<.005	.05-.02	.10-.05	.50-.25	.50-.25
5892-5	<.005	.25-.10	.90-.75	<.005	<.005	<.005	.02-.01	<.005	.05-.02	.10-.05	.50-.25	.50-.25
5892-7	.25-.10	<.005	.50-.25	<.005	<.005	<.005	.10-.05	<.005	.50-.25	.50-.25	.90-.75	.50-.25
5892-9	.75-.50	<.005	.25-.10	<.005	.05-.02	<.005	<.005	<.005	.25-.10	.25-.10	.50-.25	.25-.10
5892-10	.75-.50	<.005	.25-.10	<.005	.50-.25	<.005	<.005	<.005	.10-.05	.75-.50	.95-.90	.95-.90
5892-25	<.005	.25-.10	1.00	<.005	<.005	<.005	<.005	<.005	<.005	<.005	<.005	<.005
Lot 2-1 Gene												
5893-2	.25-.10	<.005	.50-.25	<.005	<.005	<.005	<.005	<.005	.02-.01	.50-.25	.75-.50	.90-.75
5893-3	<.005	.75-.50	.75-.50	<.005	<.005	<.005	<.005	<.005	.25-.10	.25-.10	.50-.25	.25-.10
5893-13	.05-.02	.05-.02	.50-.25	<.005	<.005	<.005	<.005	<.005	<.005	.10-.05	.02-.01	.25-.10
5893-15	<.005	.25-.10	1.00	<.005	<.005	<.005	<.005	<.005	.50-.25	.50-.25	.90-.75	.50-.25
5893-18	<.005	.50-.25	<.005	<.005	<.005	<.005	<.005	<.005	<.005	<.005	<.005	.05-.02
5893-20	.75-.50	<.005	.25-.10	<.005	<.005	.02-.01	<.005	<.005	.02-.01	.75-.50	.50-.25	.25-.10
5893-22	<.005	.25-.10	1.00	<.005	<.005	<.005	<.005	<.005	<.005	.90-.75	.75-.50	.90-.75
Triumph (Ck)	1.00				<.005				1.00			
Bison (Ck)	1.00				<.005				<.005			

Chi-square probability <.01 = highly significant evidence against the hypothesis.

two lots are not known, all one- and two-gene genetic ratios were applied to each of the restorer lines from both lot 1 and lot 2. The probability values of all 13 restorer populations in Table VIII showed highly significant deviations from the theoretical one-gene homozygous dominant ratio for both the testcross generation in 1965 and F_2 generation grown in the field in 1966. The lot 1 restorer lines did not fit a one-gene ratio but neither did the lot 2 lines fit as was expected.

Discussion

The genotypes of the different restorer lines must be accurately estimated in order to develop a fertility restorer that will remain fully stable in varying environments. Difficulties will arise if the conditions under which the segregating generations are grown fail to differentiate between the presence of one and two or more restorer genes. Observations at the Nebraska Station indicate that this can and will happen (22).

The first classification system in the present study using the seven seed-setting fertility classes failed to show differences among the restorer lines from the lot 1 and lot 2 populations other than variation in the peaks of the F_2 frequency distribution curves. The second classification system involving only three classes (fertile, partially fertile, and sterile) resulted in more pronounced differences in seed-set fertility among the individual restorer lines. The two classification systems did agree upon the division of the 13 restorer lines into two groups on the basis of plant numbers in the fertile and partially fertile classes. The division was not strictly lot 1 and lot 2 as had been expected. Four lot 1 and one lot 2 lines were of

the same magnitude and expressed a higher degree of seed-set fertility than the remaining two lot 1 and six lot 2 lines.

The high percentage of fertile plants for three restorer lines (5892-9, 5892-10, and 5893-20) coupled with the lack of sterile plants for the testcross populations of the second classification system (Table VII) indicates that two genes may be involved in restoration of fertility and are present in all these lines. Chi-square probability values based on the F_2 populations from the field in 1966 indicate that these three restorer lines and two additional lot 1 lines were a good fit to one of the two-gene ratios. The restorer lines showing good fit for the two-gene genetic ratios were not in complete agreement with respect to the two groups earlier formed on the basis of plant numbers in the fertility classes. With the exception of the five restorer lines fitting two-gene ratios, the remaining eight restorer lines showed highly significant deviations from all of the calculated one- and two-gene ratios. Line 5893-18 definitely did not fit either a one- or two-gene ratio and the actual chi-square values of 31.86 and 43.87 were extremely high in comparison to the other lines. This line must be deficient for at least one essential, strong major gene as indicated in the high percentage of sterile plants (Table VII).

When considering all plants of the check varieties in Table VIII to be fully fertile, low probability values ($<.005$) were observed for Bison in both the plastic greenhouse and field in 1966, but Triumph was low only in the field. These variations can only be attributed to environment because the check varieties are known to be very fertile.

CHAPTER IV

GENETIC RATIOS BASED ON POLLEN CLASSIFICATION

If pollen readings could be used as reliable determinants of fertility, the time and effort involved in converting conventional lines and varieties to restorers could be substantially reduced. Theoretically, by collecting anthers from the primary head and observing pollen, the fertility of the F_1 could be determined before pollen shed, and additional heads of the plant could be utilized for crossing in a restorer conversion program. The degree of restoration of the F_1 used for backcrossing could be verified by selfing the primary head and making seed-set counts.

Some of the early work involving pollen classification as a measure of fertility in cytoplasmic male sterile wheats was reported by two Japanese workers. Kihara (29) used pollen classification to observe the changes in plant fertility while substituting the *T. vulgare* nucleus into the *Ae. caudata* cytoplasm in one trial, and restoring the *T. vulgare* nucleus to its own cytoplasm by successive backcrosses following an initial cross of *T. vulgare* x *Ae. caudata* in another trial. Fukasawa (14, 15, 16, 17) made extensive studies on substitution and restoration of the nucleus (genome) in *Aegilotricum* using the same backcrossing technique reported by Kihara. Pollen fertility and, in most cases, seed set were used to assess the fertility of the progeny in subsequent backcrosses. Fukasawa (18) also classified backcross and F_2

plants of ovata cytoplasmic male sterile x T. dicoccoides var. Kotschyanum restorer using pollen fertility readings, and concluded that restoration derived from dicoccoides is not controlled by a single factor, but that two or more genes are involved.

Just prior to the initiation of the present study, Livers (31) reported on the inheritance of fertility restoration in a T. timopheevi x Marquis[3] F_3 population (Kansas restorer). The testcross and F_2 generations were classified according to the microscopic appearance of the pollen. The three pollen classes reported were normal, partially fertile, and sterile. His data agreed with a two-factor hypothesis based on chi-square tests and he designated these factors as Rf_1 and Rf_2. Livers (31) indicated that the heterozygote, $Rf_1rf_1Rf_2rf_2$, produced by crossing male sterile Bison with the new restorer was fully fertile. He suggested that the partially fertile segregates contain either the dominant gene Rf_1 or Rf_2 but not both and that the sterile class consisted of the recessive genotype $rf_1rf_1rf_2rf_2$ combined with T. timopheevi cytoplasm.

The three fertility classes (fertile or normal, partially fertile, sterile) used by Livers (31) were adopted for pollen classification in this study.

Experimental Results

Restorer Parents

The range and average percent fertile pollen for the restorer lines and testcrosses grown in three locations during 1965 and 1966 are given in Appendix Tables XVI and XVII. The A-line, $N542437^A$ x Bison[2], plants serving as a standard of comparison for pollen classification had

completely nonfunctional pollen in all locations for both years. The conventional varieties, Triumph and Bison, showed very wide ranges of pollen fertility within individual locations. These checks are pure line varieties and should be completely fertile, but pollen readings for Bison during 1966 were as low as 32% and 35% fertile in the plastic greenhouse and glass greenhouse respectively. This variation in the check varieties made it difficult to assess the actual pollen fertility of the individual restorer lines.

Each F_4 restorer line grown in 1965 traces to the seed of an individual F_3 plant grown the previous year. If the F_3 plant was homozygous for restorer genes, as some were expected to be, then there should be no segregation observed in the selfed F_4 progeny in 1965 or F_5 progeny in 1966. In considering the glass greenhouse location for 1965 (Appendix Table XVI), plants of all but two of the restorer lines showed high average percent fertile pollen with very little fluctuation. Data from this location would suggest that a number of restorer lines were homozygous dominant for restorer gene(s). However, the restorer plants grown in the other two locations in 1965 and the two locations from which pollen readings were obtained in 1966 (Appendix Table XVII) had variable pollen readings. This variability is probably due to the effect of environment.

Testcross and F_2 Populations

The data presented in Table IX shows pollen fertility for the 13 restorer lines, with plants grouped in three pollen fertility classes. A high percentage of fertile plants for both testcross and F_2 populations involving restorer line 5892-9 is evident. This suggests that the

TABLE IX

FERTILE POLLEN CLASSIFICATION OF FERTILITY RESTORATION FOR THE RESTORER LINES AS EVALUATED BY TESTCROSS AND F_2 POPULATIONS IN 1965 AND 1966

	1965 Testcross - Field				1966 Testcross - Plastic Greenhouse 1/				1966 F_2 Population - Plastic Greenhouse			
	Total	Partial			Total	Partial			Total	Partial		
Restorer Line	No. Plants	Fertile 75-100%	Fertile 26-74%	Sterile 0-25%	No. Plants	Fertile 75-100%	Fertile 26-74%	Sterile 0-25%	No. Plants	Fertile 75-100%	Fertile 26-74%	Sterile 0-25%
Lot 1 (2 Gene)				(Percentage of plants in each class)								
5892-2	8	37.5	50.0	12.5	8	50.0	50.0	0.0	12	50.0	33.3	16.7
5892-5	8	62.5	12.5	25.0	7	71.4	28.6	0.0	12	58.3	33.3	8.3
5892-7	8	75.0	25.0	0.0	7	42.8	57.1	0.0	10	50.0	50.0	0.0
5892-9	8	100.0	0.0	0.0	8	87.5	12.5	0.0	11	81.8	18.2	0.0
5892-10	8	100.0	0.0	0.0	8	50.0	50.0	0.0	10	30.0	60.0	10.0
5892-25	8	62.5	37.5	0.0	8	87.5	12.5	0.0	11	54.5	18.2	27.3
Lot 2 (1 Gene)												
5893-2	8	87.5	12.5	0.0	8	75.0	25.0	0.0	12	41.7	50.0	8.3
5893-3	8	50.0	12.5	37.5	8	50.0	50.0	0.0	12	50.0	41.7	8.3
5893-13	8	50.0	25.0	25.0	8	37.5	62.5	0.0	12	8.3	66.7	25.0
5893-15	8	37.5	62.5	0.0	8	100.0	0.0	0.0	12	58.3	41.7	0.0
5893-18	8	0.0	25.0	75.0	8	62.5	25.0	12.5	12	16.7	50.0	33.3
5893-20	8	62.5	37.5	0.0	8	87.5	12.5	0.0	12	58.3	33.3	8.3
5893-22	8	25.0	37.5	37.5	8	62.5	37.5	0.0	12	58.3	33.3	8.3
Triumph (Check)					8	100.0	0.0	0.0	11	100.0	0.0	0.0
Bison (Check)	10	80.0	20.0	0.0	8	62.5	37.5	0.0	11	63.6	36.4	0.0

1/ No pollen readings could be made for the field material in 1966 due to weather conditions during the heading and pollination period.

line was homozygous for one or more restorer genes. Additional fertile populations were restricted to an individual restorer line in a particular location. The absence of sterile plants in a population is a good sign of fertility. There were a number of populations having no sterile plants in either of the testcross locations but populations involving only three restorer lines were void of sterile plants for all generations and locations. Of these three populations, two showed a high percentage of partially fertile plants while the third line, (5892-9) discussed previously, was highly fertile.

In order to further study the inheritance of restoration in these lines, chi-square values were calculated, based on fertile pollen readings, for three possible two-gene and one one-gene genetic ratios, identical to those used for seed-set data in the previous chapter. The probability values for the different genetic ratios are given in Table X. Results were strikingly different for the one-gene homozygous dominant ratio compared with the two-gene ratios for the testcross populations of both the field in 1965 and the plastic greenhouse in 1966. All probability values were smaller than the .01 level, indicating there was little evidence to suggest a one-gene control of restoration in any of these lines.

Two lot 1 and six lot 2 testcross populations grown in the field in 1965 had probability values $<.01$ and consequently did not fit the two-gene homozygous dominant ratio. The probability values for testcross and F_2 populations involving line 5892-9 showed a good fit to a two-gene homozygous dominant ratio for both years. Two lot 1 restorer lines (5892-5 and -7) were in agreement with the two-gene (one homozygous dominant, one heterozygous) ratio in all testcross and F_2 generations.

TABLE X

CHI-SQUARE PROBABILITY VALUES FOR DIFFERENT GENETIC RATIOS OF TESTCROSS AND F_2 POPULATIONS BASED ON FERTILE POLLEN CLASSIFICATION

	1965 Testcross - Field				1966 Testcross - Plastic Greenhouse				1966 F_2 Population - Plastic Greenhouse			
Restorer Line	2 Gene Homo. Dom. 1:0:0	2 Gene Hetero. 1:2:1	1 Gene Homo. 1 Gene Hetero. 1:1:0	1 Gene Homo. Dom. 0:1:0	2 Gene Homo. Dom. 1:0:0	2 Gene Hetero. 1:2:1	1 Gene Homo. 1 Gene Hetero. 1:1:0	1 Gene Homo. Dom. 0:1:0	2 Gene Homo. Dom. 9:6:1	2 Gene Hetero. 9:30:9	1 Gene Homo. 1 Gene Hetero. 9:18:5	1 Gene Homo. Dom. 1:2:1
Lot 1-2 Gene												
5892-2	<.005	.75-.50	.75-.50	<.005	<.005	.25-.10	1.00	<.005	.50-.25	.02-.01	.25-.10	.25-.10
5892-5	.05-.02	.05-.02	.05-.02	<.005	.25-.10	.05-.02	.75-.50	<.005	.95-.90	<.005	.10-.05	.05-.02
5892-7	.25-.10	<.005	.50-.25	<.005	<.005	.50-.25	.95-.90	.01-.005	.75-.50	.05-.02	.25-.10	.10-.05
5892-9	1.00	<.005	.02-.01	<.005	.75-.50	<.005	.25-.10	<.005	.25-.10	<.005	<.005	<.005
5892-10	1.00	<.005	.02-.01	<.005	<.005	.25-.10	1.00	<.005	.25-.10	.75-.50	.90-.75	.75-.50
5892-25	.01-.005	.05-.02	.90-.75	<.005	.75-.50	<.005	.25-.10	<.005	.02-.01	<.005	.05-.02	.05-.02
Lot 2-1 Gene												
5893-2	.75-.50	<.005	.25-.10	<.005	.25-.10	<.005	.50-.25	<.005	.75-.50	.25-.10	.75-.50	.50-.25
5893-3	<.005	.10-.05	<.005	<.005	<.005	.25-.10	1.00	<.005	.90-.75	.02-.01	.25-.10	.25-.10
5893-13	.01-.005	.25-.10	.10-.05	<.005	<.005	.50-.25	.90-.75	.01-.005	<.005	.75-.50	.50-.25	.50-.25
5893-15	<.005	.50-.25	.90-.75	.01-.005	1.00	<.005	.02-.01	<.005	.75-.50	<.005	.50-.25	.02-.01
5893-18	<.005	<.005	<.005	<.005	.05-.02	.05-.02	.50-.25	<.005	<.005	.50-.25	.25-.10	.75-.50
5893-20	.01-.005	.05-.02	.90-.75	<.005	.75-.50	<.005	.25-.10	<.005	.95-.90	<.005	.10-.05	.05-.02
5893-22	<.005	.75-.50	.01-.005	<.005	.01-.005	.05-.02	.90-.75	<.005	.95-.90	<.005	.10-.05	.05-.02
Triumph (Ck)					1.00				1.00			
Bison (Ck)	.25-.10				.01-.005				<.005			

Chi-square probability <.01 = highly significant evidence against the hypothesis.

Testcross and F_2 data for only one lot 2 restorer line (5893-2) expressed agreement, having two different two-gene ratios showing a good fit.

Discussion

The number of classes that can be used effectively in describing percent fertile pollen of a population are dependent upon the size of the population, and the ability to distinguish between degrees of cellular staining. In the present study, plants with completely fertile or sterile grains were not difficult to distinguish, other than ghosts which result from breakage of the cell wall of a fertile grain allowing the cellular content to escape. Other plants, however, had pollen grains with varying degrees of stainability. The intensity of staining appeared to depend upon the stage of maturity of the anther. Based on these observations, the three pollen classes (fertile, partially fertile, sterile) were found to be most suitable for the limited number of plants representing each restorer population in this study.

Since the original lot 1 and lot 2 Nebraska restorer populations were derived by bulking the most fertile plants from F_2 segregating populations, some of the plants in these populations were assumed to be heterozygous and some homozygous for restorer genes. In order to assess the genetic control of restoration in this material, four calculated one- and two-gene genetic ratios were applied simultaneously to the testcross and F_2 generations of each restorer line in both lot 1 and lot 2 populations. It was first noted that the deviations from the calculated one-gene homozygous dominant ratio were highly significant for all 13 restorer populations. This was true for all testcrosses

grown in the field in 1965 and plastic greenhouse in 1966. This complete agreement for all restorer lines in two locations and different years indicates that full fertility restoration is not controlled exclusively by one homozygous dominant gene for these restorer lines.

The two-gene homozygous dominant ratio for the testcrosses grown in the field in 1965 (Table X) divided the 13 restorer populations into a group of five showing a good fit and eight showing highly significant deviations from the calculated two-gene ratio. None of the latter group were consistent with the remaining two-gene ratios. Only the testcross and F_2 populations of restorer line 5892-9 showed a good fit for a two-gene homozygous dominant ratio in both years. This line expressed a high degree of restoration and appeared to be the most stable restorer in all testcross and F_2 populations based on the pollen data.

Comparison of the probability values for the testcrosses in the field in 1965 with those in the plastic greenhouse in 1966 showed very little agreement for some restorer lines. Greater confidence was placed in the results of the testcross data from 1965 since the crosses for each restorer line were made from a known individual F_3 plant. The testcrosses for a given restorer in 1966 were made from a group of eight F_4 parent plants. These F_4 plants could have been segregating for restorer genes, therefore, would not be comparable with the 1965 crosses.

The inability to show differences between ratios in the F_2 populations grown in the plastic greenhouse in 1966 were mainly attributed to the small population size; only 12 plants represented each restorer line. With a small population, the difference due to misclassification or environmental effect on one plant could result in considerable bias.

The observed pollen readings of the check varieties, Triumph and Bison, were compared with an expected 100% fertile pollen class. The resulting probability values (Table X) for Triumph in the two generations of the plastic greenhouse were a perfect fit. The plants of Bison were in agreement with the expected 100% fertile pollen class for the testcross nursery in the field during 1965 but showed no agreement for the other two generations in 1966. The lack of fertile pollen in the anthers of Bison appeared to be due to an environmental effect resulting in sterility in the heads.

CHAPTER V

RELATIONSHIP BETWEEN SEED-SET AND POLLEN CLASSIFICATION

In the final analysis, the restoring capacity of an individual plant or restorer line is determined by the amount of seed set in the F_1 hybrid resulting from an A x R cross. The question of whether pollen classification is reliable in determining this restoring capacity is of considerable importance since pollen classification offers several advantages over seed-set classification.

Although both pollen and seed-set classification have been used in studies involving restoration, there is very little information in the literature regarding the relationship between percent fertile pollen and seed set.

Fukasawa (14) reported on the fertility of wheat plants with varying chromosome configurations using percent fertile pollen and percent seed set. In this study it was reported that pollen readings averaged from 5 to 45% higher than seed-set readings. Kihara (29) used fertile pollen and seed-set percentages to measure the fertility of each backcross generation in substituting and restoring the nucleus of T. vulgare. He reported a wide variation in both pollen and seed fertility in the early backcross generations but the readings were reasonably close in the later generations.

Maunder as cited by Miller and Pickett (34) related seed set to pollen fertility in sorghum. He reported that seed set was a good indicator of pollen fertility when fertile pollen fell within the 20 to 65%

range. However, seed-set estimates of pollen fertility were often low when fertile pollen was less than 15% and high when fertile pollen exceeded 65%. In making a large number of A x R crosses, Miller and Pickett (34) used estimates of stainable pollen instead of seed-set counts to measure fertility of the R-lines used as pollen parents. However, the F_1, F_2, backcross, and testcross progeny were rated for percent stainable pollen and seed set and they confirmed the earlier report of Maunder that seed-set and pollen readings are not identical when fertile pollen exceeds 65% or is less than 20%.

Determination of the actual fertility of an individual plant could depend on the area of the spike examined and the technique (either seed-set or pollen classification) employed in measuring fertility. Johnson et al. (23) reported that when fertility was incomplete, seed set without exception, proceeded from the base to the tip of the spike. Wilson (47) also observed that sterile florets occurred primarily in the upper portion of the spike in partially sterile hybrid wheat plants. The inheritance of restoration of the Kansas restorer (31) was determined from the percentage of fertile pollen in the anthers of the spikelets near the tip of the spike.

Fertility differences of the restorer material in the present study were determined by dividing the spike into three equal parts (base, middle, and tip) and classifying each for percent fertile pollen and seed set. The association of fertile pollen and seed set in determining fertility was evaluated by linear correlation. The relationship between genetic ratios based on the two fertility classification methods was also determined by comparing appropriate chi-square probability values.

Experimental Results

Fertile pollen and seed-set percentages for the testcross nursery grown in the field in 1965 were selected as representative of readings for the base, middle, and tip areas of the spike. Percentages of fertile (dark) pollen and seed set for individual plants from testcross populations and two check varieties are given in Appendix Tables XVIII and XIX. In conventional varieties, seed set normally ranges from 85 to 90%. The eight Triumph check plants in this study averaged 84.4% seed set in the tip area of the spike; therefore, any restorer plant equal to or exceeding this value in seed set was regarded as fertile. The testcross plants of restorer line 5892-7 had a high percentage tip fertility and was reasonably consistent with six of the eight plants having higher readings than Triumph for both fertile pollen and seed-set percentages. Association between fertile pollen and seed set for tip fertility was also generally good for the Bison check with all but one of the pollen and seed-set readings indicating fertile plants. Association between fertile pollen and seed set for the tip area of the testcrosses of restorer line 5893-18 was good although none of the plants in this line were classified as fertile. In contrast, several lines had a high percentage of plants showing fertile pollen in the tip but the same plants had low seed-set percentages in this area. The general trend between fertile pollen and seed set in the tip of the spike was that when percent fertile pollen was low, seed set was reduced.

The average percent fertile pollen and seed set for testcrosses of each restorer line are summarized in Table XI. Considering the averages for the tip region of the spike, testcrosses of two restorer lines, 5892-7 and 5892-10, show a very close relationship between fertile pollen

TABLE XI

SUMMARY OF AVERAGE PERCENT FERTILE POLLEN AND SEED SET READINGS, BY SPIKE AREA, FOR TESTCROSSES INVOLVING 13 RESTORER LINES GROWN IN THE FIELD LOCATION IN 1965

Restorer Line Involved in Testcross	Average readings of 8 plants/testcross								
	% Dark Pollen				% Seed Set				
	Base	Middle	Tip	Average	Base	Middle	Tip	Average	D 1/
5892-2	75.08	68.85	55.91	66.61	97.05	91.87	70.43	86.60	+19.99
5892-5	57.68	72.25	66.02	65.32	86.45	82.10	73.26	80.60	+15.28
5892-7	80.21	92.76	77.48	83.48	94.37	92.71	77.98	88.35	+ 4.87
5892-9	83.38	85.15	88.96	85.83	86.26	93.13	78.30	85.90	+ 0.07
5892-10	88.95	93.25	87.00	89.73	94.58	87.30	87.98	89.95	+ 0.22
5892-25	67.42	81.55	82.87	77.71	70.02	78.85	61.55	70.14	- 7.57
5893-2	91.20	86.50	83.47	87.05	91.15	91.15	69.51	83.98	- 3.07
5893-3	50.13	57.10	54.38	53.91	73.12	65.73	45.65	61.50	+ 7.59
5893-13	63.26	53.71	64.25	60.41	77.08	81.05	55.85	71.32	+10.91
5893-15	81.58	59.46	43.70	61.58	84.58	87.70	75.82	82.70	+21.12
5893-18	26.01	20.26	14.56	20.28	37.37	41.46	34.65	37.83	+17.55
5893-20	70.55	82.85	78.61	77.33	93.33	96.46	82.23	90.67	+13.34
5893-22	49.20	49.30	42.86	47.12	82.51	84.16	55.10	73.92	+26.80
Triumph (Check)					89.60	96.67	84.45	90.24	
Bison (Check)	86.98	84.46	91.60	87.68	93.33	98.43	98.75	96.84	+ 9.16
Average	69.40	70.53	60.00	66.64	83.39	84.58	70.10	79.36	+12.72

1/ D = Difference in percent fertility between seed set (+) and dark pollen (-).

and seed set. The percent fertile pollen and seed set for these lines were 77.48 vs. 77.98% and 87.00 vs. 87.98% respectively.

When three regions of the spike (base, middle, and tip) are considered separately, seed set was lower for 13 entries (12 R-lines and Triumph) in the tip area than the other two regions of the spike. No appreciable difference in seed set with regard to spike region was noted for two entries (5892-10 and Bison check). Pollen readings were more variable than seed set in the tip area. In seven testcrosses the low percent pollen fertility was observed in the tip area of the spike but three testcrosses and the Bison check were fertile in all three areas of the spike. In comparing the averages of all entries for each area of the spike, it was noted that percent dark pollen is essentially the same for the base (69.40) and middle (70.53) regions of the spike. Also the percent seed set for these two regions of the spike was very similar (83.39:84.58). Both percent dark pollen and seed set in the tip area were considerably less than the other areas of the spike. The average seed-set readings for the tip areas of all lines were approximately 10% higher than fertile pollen readings.

The average percent seed set of the testcross populations was higher than the average percent fertile pollen for all but two testcrosses as indicated in Table XI. An average across the three regions of the spike results in a difference of +12.72% between pollen and seed-set readings. Considering average seed-set readings for the individual testcross populations, testcrosses of five restorer lines were classified as fully fertile (85%$^{+}$) and three other lines approached full fertility (80% or better). Four testcrosses had a marked reduction in both pollen and seed-set percentages. When readings were consistently low,

especially as observed in testcrosses of line 5893-18, it was assumed that either a major gene or possibly several minor genes for fertility restoration were missing from the genotype.

The linear correlation coefficients between percent lateral seed set and dark staining pollen of F_4 and F_5 restorer parents evaluated in 1965 and 1966 are given in Table XII. In contrast to the good agreement between fertile pollen and seed-set readings in the testcross data, the restorer parent lines showed very little association in most environments. The highest associations between percent fertile pollen and percent seed set occurred in the 1966 plastic greenhouse environment; six restorer lines and the Bison check were either significant or highly significant and four other populations approached significance.

The comparison of pollen and seed-set percentages for the average of all A-, B-, R-lines and A x R F_1 hybrids in Appendix Tables XVI and XVII showed that the average fertile pollen readings were consistently higher than average seed set for the glass greenhouse and plastic greenhouse for both years. The differences in averages of fertile pollen and seed set for all entries ranged from 4.5 to 12.4%. The field results in 1965, however, had a higher average seed set for all entries (7.1%) than that for average fertile pollen.

The two-gene homozygous dominant ratio based on seed-set classification (Table VIII) showed that the testcross and F_2 populations involving restorer lines 5892-9 and 5892-10 exceeded the .01 probability level and therefore were considered to fit this ratio. The same ratio for the populations of these two lines based on pollen classification (Table X) showed good fit for line 5892-9 in all locations but only one location for line 5892-10 fit the ratio. The testcross and F_2 populations of

TABLE XII

CORRELATION COEFFICIENTS (r) BETWEEN LATERAL SEED SET AND DARK STAINING POLLEN FOR 13 RESTORER LINES GROWN IN THREE ENVIRONMENTS IN 1965 AND 1966

	1965			1966	
Restorer Line	Glass Greenhouse	Plastic Greenhouse	Field	Glass Greenhouse	Plastic Greenhouse
Lot 1 (2 Gene)					
5892-2	0.08	0.82*	0.55	0.06	0.82*
5892-5	0.89**	0.07	0.78**	0.34	0.99*
5892-7	-0.46	0.40	0.22	0.22	0.88**
5892-9	0.02	0.45	0.86**	0.57	0.55
5892-10	-0.51	0.90**	0.80**	0.11	0.06
5892-25	-0.16	-0.17	-0.15	-0.20	0.74*
Combined Lines 1/	0.98**	0.50	0.56	0.63	0.64
Lot 2 (1 Gene)					
5893-2	0.31	0.08	0.70	0.75*	0.64
5893-3	0.19	0.58	0.11	0.35	0.70*
5893-13	0.34	0.002	0.13	0.17	0.11
5893-15	-0.47	0.93**	-0.25	-0.13	0.34
5893-18	-0.17	0.51	0.08	0.04	0.97**
5893-20	0.54	0.74*	0.90**	-0.16	0.67
5893-22	0.68	0.33	0.04	0.72*	0.59
Combined Lines 1/	0.95**	0.74	0.52	0.89**	0.99**
Triumph (Check)	0.06	-0.11		0.22	-0.31
Bison (Check)	0.20	0.65	-0.03	0.87**	0.80*

1/ Combined lines = means of the restorer lines are used.
* Significant correlation value, P = .05.
** Highly significant correlation value, P = .01.

restorer lines 5892-5 and 5892-7 showed good fit to the two-gene (one homozygous dominant, one heterozygous) ratio for seed-set classification and fertile pollen classification. Two populations of lot 2 restorer lines showed fit to two-gene ratios; the F_2 population of restorer line 5893-20 fit a two-gene heterozygous ratio based on seed-set classification and all testcross and F_2 populations of restorer line 5893-2 fit a two-gene homozygous dominant ratio based on fertile pollen classification. The remaining seven restorer lines failed to fit the one- and two-gene ratios for fertility restoration using either fertile pollen or seed-set classification.

Discussion

Conventional varieties such as Triumph and Bison used as checks in this study have normal cytoplasm and usually have a high seed-set percentage; however, they commonly have an occasional vacant floret. These sterile florets do not appear to be confined to a specific region but occur at random throughout the spike. The main exceptions to this are drought or freeze injury which may be confined to a particular area. From earlier reports (22, 39), it appeared that in partially restored A x R hybrids, sterile florets did not occur at random throughout the spike but occurred in a definite pattern; with sterility progressing from the tip downward. For this reason, in the present study the spike was divided into three areas (base, middle, and tip) permitting an evaluation of pollen and seed-setting fertility of each area. This pattern of reduced fertility of the tip region was evident in the material under investigation in this study and generally occurred in both pollen and seed-set classification. On the basis of the sterility

pattern discussed above, it was assumed that the middle region of the spike would have a slightly lower average fertility than the base region. However, the fertility of the middle region was found to be essentially the same as the base whether measured by pollen fertility or seed set.

By using the testcross nursery for comparing seed-set and fertile pollen readings (Table XI), it was also possible to evaluate the degree of fertility restoration in the restorer lines. Testcross populations involving eight of the restorer lines exceeded 80% seed set. The field location in 1965 appeared to be a very fertile environment giving a full expression of the restorer genes. Seed-set percentages were consistently higher than fertile pollen percentages for this location. This implies that, as long as enough fertile pollen was available to cause dehiscence of the anther, environmental conditions in the field allowed fertilization and seed development. In the glass greenhouse and plastic greenhouse locations (Appendix Tables XVI and XVII), consistently higher readings were noted for average fertile pollen than for average seed set. The results of Fukasawa (14) that pollen readings averaged from 5 to 45% higher than seed set are generally consistent with the observations noted in the present study.

Except for the plastic greenhouse location in 1966, the association between seed set and dark staining pollen of the individual selfed restorer populations were highly variable (Table XII). In the plastic greenhouse, either percent lateral seed set or percent dark staining pollen could be used to determine the degree of fertility restoration.

The lack of association between the two methods of classification within most locations makes their degree of relationship questionable. However, the agreement between genetic ratios based on fertile pollen

and seed set must be considered. Restoration in the original Nebraska F_2 population from cross 628, designated as lot 2, was reported to be under the control of a single major gene although there was some possibility that minor genes were also present (23). The expression of minor genes could not be demonstrated with the present data, however, if there was only one major dominant gene for restoration, then either a large number of minor genes with small effect or a few minor genes with larger effects would be required for complete restoration. There were four lot 2 lines in the comparisons of the average fertile pollen and seed-set data (Table XI) which appeared to lack these necessary genes. These lines expressed reduced pollen and seed-set percentages for all three areas of the spike. Five of the seven lines that failed to fit a one- or two-gene ratio were from the lot 2 population. This was unexpected when the testcross and F_2 populations of two restorer lines from lot 2 showed fit to one of the two-gene ratios. Each line showed agreement for only one method of classification, either fertile pollen or seed set.

The lot 1 Nebraska material, cross 627, was reported to have two major genes for fertility restoration (23). Of the six lines in this study which originated from the lot 1 material, populations of four lines were shown to fit the hypothesis that fertility restoration is controlled by two major genes based on goodness of fit from each classification. Two lines showed good fit for two homozygous dominant genes and two lines indicated two genes (one homozygous dominant, one heterozygous) for fertility restoration. None of the restorer lines showed complete fertility restoration across all environments tested, but the four lines which fit a two-gene ratio had higher readings for fertile

pollen and seed set and higher correlation coefficients than the remaining lines. Restorer line 5892-9 appeared to be the most fertile since it fit the genetic ratios for two homozygous dominant genes in all generations for both pollen and seed-set classification.

CHAPTER VI

CORRELATION OF RESTORATION IN DIFFERENT ENVIRONMENTS

Fertility restoration of the F_1 hybrid is an essential factor in the successful development and utilization of hybrid wheat. Fertility restoring lines (pollen parents) are being developed by selection from lot 1 and lot 2 of the Nebraska restorer material and by transferring the restorer genes into a series of standard varieties and experimental lines. To be assured that the restorer genes are being maintained, an environment is needed that will allow a reliable expression of the restorer genes and one that can be duplicated in subsequent generations.

Wilson (47) indicated that the degree of pollen fertility expressed in the F_1 hybrids of wheat was determined by the number of fertility factors carried and by the effect of environment. He also reported that as far as the A x R systems in spring wheat are concerned, fertile environments were found in some areas of the southern latitudes with high temperature conditions. In these particular areas wheat hybrids which were only partially fertile in other environments were completely fertile. Schmidt et al. (40) reported marked effect of environment on seed-setting ability of wheat plants grown in both the greenhouse and field. Fukasawa (18) suggested that the pollen fertility of semi-fertile plants of male sterile dicoccum x T. dicoccoides appeared to be influenced considerably by environmental conditions. Kihara (30) reported that the fertility of F_1 hybrids depends to a large extent

upon the external conditions, namely late sowing, humidity, and low temperature. Schmidt et al. (40) indicated that restoration in related material was lower when it headed during a prolonged cloudy period as compared with material heading during bright sunshine. It is possible that light intensity as well as temperature affects the stability of sterility and restoration.

Jones et al.(25) reported that two restorer genes were needed for fertility in the unfavorable season of 1955 in the T (Texas) cytoplasm of corn, while in the 1956 season only one restorer gene was necessary. It was also indicated that a number of inbred pollinators in corn may restore pollen production fully or partially in some combinations but may be completely sterile in others due to wide environmental and seasonal variation (25). Duvick (10) reported that the number of genes required for complete restoration in corn varies considerably in different seasons and locations; for example, corn grown in Iowa required more modifying genes than that grown in the cooler, more humid conditions in Connecticut.

Kidd (28) reported the effect of temperature on the restorer genes of sorghum. His data suggested that at temperatures above 95°F which are desirable for sorghum, three dominant modifying genes will give complete pollen fertility without the dominant major gene.

Burton and Athwal (8) suggested that the variation in the mean seed set observed in the F_1 hybrids of pearl millet, between the same A-line and different R-lines, would be expected because of differences in genotypes and their interactions with environment.

In the present study, the effects of environment on seed set and pollen fertility were determined by calculating correlation coefficients.

Associations were observed between locations in the same year, and the same location in different years.

Experimental Results

Correlations of seed set and dark staining pollen for each restorer line in Table XII were made by locations for both years. In general, the r values were highly variable in magnitude. The lack of consistent correlations between locations would likely be attributed to the variation in response of pollen formation and seed set to different macro-environments. The individual restorer lines were also quite variable to different micro-environments as indicated in the previous chapter. Restorer 5892-2 was the only line with significant positive correlations in the same location for two years. Significant positive correlations were obtained for line 5892-5 for three of the five locations.

The lot 1 and lot 2 populations, represented by the combined lines, resulted in more consistent correlations among the test sites than did the individual restorer lines. The combined values were computed from the means of seed set and dark staining pollen for all plants of each restorer line, thus, it appears that part of the effects of micro-environment were averaged out.

One of the planned procedures for studying environmental differences was to compare different locations for each of the restorer lots and checks using percent lateral seed set. The linear correlation coefficients of these comparisons are given in Table XIII. Only restorer lines from the lot 2 population showed significant positive correlations for the plastic greenhouse vs. field locations in 1965. None of the remaining restorer populations were significantly correlated for either year.

TABLE XIII

CORRELATION COEFFICIENTS (r) BETWEEN LOCATIONS BASED ON SEED SET PERCENTAGE FOR LOT 1, LOT 2 RESTORER LINES AND CHECK VARIETIES GROWN DURING 1965 AND 1966

	1965				1966			
Comparisons	Lot 1	Lot 2	Checks	Combined Value 1/	Lot 1	Lot 2	Checks	Combined Value 1/
Glass Greenhouse vs. Plastic Greenhouse	0.30	0.55	0.54*	0.53*	0.30	0.52	0.83**	0.17
Glass Greenhouse vs. Field	0.14	0.65	0.92**	0.42	-0.59	0.13	0.64**	-0.05
Plastic Greenhouse vs. Field	0.52	0.86*	0.36	0.73**	0.34	0.34	0.29	-0.41

1/ Combined value = the average percent lateral seed set for each restorer line in lots 1 and 2 and check varieties.

* Significant correlation value, P = .05.

** Highly significant correlation value, P = .01.

On the other hand, the check varieties had signficant positive correlations for the glass greenhouse vs. plastic greenhouse and glass greenhouse vs. field in both years. Failure of the restorer lines to respond the same in the locations being compared indicates significant R-line x environment interaction. Part of this effect could be due to differences in cytoplasm. The check varieties had normal cytoplasm but all R-lines had sterile cytoplasm.

Linear correlation coefficients for years, using percent lateral seed set for comparison, are presented in Table XIV. There was a wide range in degree of seed set among the R-lines and check varieties reported earlier (Appendix Tables XVI and XVII), but comparisons of the combined average seed set of each restorer population and the check varieties in Table XIV for two different years showed significant positive correlations. The lot 2 lines in the glass greenhouse had a probability value $>.10$; therefore, were approaching significance. Correlation coefficients for the field location were low and nonsignificant for both restorer lots and the combined values for all entries. It appears that the field was the most variable environment in the two years during which this study was conducted.

Discussion

It is apparent from data reported in Tables XII and XIII that fertility of the material under study, based on fertile pollen and seed set, is influenced greatly by environmental conditions. The linear correlation coefficients for each set of locations compared indicated high R-line x environment interaction based on the lot 1 and lot 2 restorer lines. The check varieties, however, showed significant

correlations between the glass greenhouse vs. plastic greenhouse and glass greenhouse vs. field for two years. Adaptation was first considered as an explanation for these differences, however, the data (Table II) indicated that the restorer lines and checks responded similarly in the same environment as far as heading date, yield, and other agronomic and quality characters were concerned. The major difference between the restorer lines and check varieties is the presence of restorer genes in a sterile cytoplasm for the former, and normal cytoplasm and the absence of restorer genes for the checks. The lack of correlation in the restorer material is assumed to be an environmental affect upon the fertility restorer genes and/or sterile cytoplasm system.

TABLE XIV

CORRELATION COEFFICIENTS (r) BETWEEN YEARS BASED ON SEED SET PERCENTAGE FOR LOT 1, LOT 2 RESTORER LINES AND CHECK VARIETIES GROWN DURING 1965 AND 1966

	1965 vs. 1966			
Location	Lot 1	Lot 2	Checks	Combined 1/ Value
Glass Greenhouse	0.92**	0.67	0.58*	0.61*
Plastic Greenhouse	0.81*	0.76*	0.86**	0.80**
Field	0.33	0.35	0.88**	0.12

1/ Combined value = the average percent lateral seed set for each restorer line in lots 1 and 2 and check varieties.

* Significant correlation value, P = .05.

** Highly significant correlation value, P = .01.

The sterile cytoplasm (A-line) was stable with regard to fertility in all environments encountered in this study. There is the possibility, however, that the plasmon of the A-line, in combination with the genome of the R-line may cause change in metabolic activity of the restorer lines and thus increase sensitivity to environment. The affect of

sterile cytoplasm upon the restorer lines could be evaluated by comparing the same genes in both normal and sterile cytoplasms. Such comparisons were not possible with this material since the Nebraska restorer lines were developed only in sterile cytoplasm.

There was a much higher association of seed-set fertility in the restorer populations between years than among locations in the same year. From these data, it appears that the chances of duplicating an environment from year to year for a dependable expression of the restorer genes would be most likely in the plastic greenhouse. Earlier findings showed that the plastic greenhouse was the most critical location for the expression of the fertility restorer genes and also resulted in a higher correlation of dark staining pollen vs. seed set than the other environments. It is apparent from these results that the plastic greenhouse would make a good location for the development of suitable restorer lines.

Fluctuations in temperature and humidity, both within and among locations, are known to affect the floral organs of the wheat plant. The high temperatures often encountered during the early spring also make heading date an important factor in Oklahoma. Table XV shows the range and average heading date for each location for the two years of this study. It is worth noting that there is approximately a month's difference in average heading date among locations for both years. The wide range in heading date within locations, coupled with fluctuating temperatures may have had a marked affect upon the expression of the restorer genes. Brooks and Brooks (6) reported differences in pollen sterility in sorghum plants of different maturity dates and suggested that the cause of sterility in sorghum may be a temperature-sensitive

mechanism.

TABLE XV

RANGE AND AVERAGE HEADING DATES OF VARIOUS RESTORER POPULATIONS IN THREE ENVIRONMENTS FOR 1965 AND 1966

Year	Location	Heading Date From First Head	to Last Head	Average Date Headed
1965	Glass Greenhouse	Feb. 14	Mar. 28	Feb. 24
1965	Plastic Greenhouse	Mar. 21	May 7	Apr. 15
1965	Field	Apr. 30	May 18	May 6
1966	Glass Greenhouse	Feb. 27	Mar. 31	Mar. 9
1966	Plastic Greenhouse	Mar. 1	May 11	Apr. 16
1966	Field	Apr. 27	May 18	May 3

After observing the wide differences in response of the restorer material to environments, the author suggests that additional studies are needed, possibly using fewer lines and larger populations in the different environments. This should provide further information regarding the degree of environmental effect upon the restorer genes, and might help to identify the optimum levels of temperature, light, and humidity for maximum expression of the restorer genes.

CHAPTER VII

GENERAL DISCUSSION

The two main objectives of this investigation were to study the inheritance of the Nebraska restorer populations and to assess the effect of environment upon the expression of the restorer genes. Two major limiting factors in the study were 1) the small quantity of testcross seed available, and 2) limited space for growing genetic populations in the two greenhouse environments. The original plan was to combine the testcross data for all locations and the F_2 data for all locations to provide a large number of plants for determining genetic ratios. When the locations were combined, a high percentage of partially fertile plants occurred in most of the restorer lines and differences noted in individual populations were usually obscured. The frequency distribution curves (Appendix Figures 1 and 2) resembled a normal distribution and none of the populations showed fit to the one- and two-gene genetic ratios. Thus, it was necessary to study inheritance of the fertility genes on the basis of individual locations.

For a successful restorer conversion program, a method must be used by which the restoring potential of the R-line being developed can be evaluated. In the early stages of hybrid wheat research the degree of self fertility of the restorer line containing sterile cytoplasm appeared to be a good indication of hybrid fertility; however, a number of wheat workers now suggest that the only way to accurately assess the

restoring capacity of an R-line is by a testcross evaluation program.

The major genes for restoration in Nebraska lot 1 and lot 2 restorer material are derived from Triticum timopheevi. This material also may have factors for fertility coming from the 'Turkey' wheat derivative, 'Nebred,' as well. 'Cheyenne,' also a Turkey derivative, has been reported by Nebraska workers (23) to result in fairly high fertility in F_1 hybrids when crossed with male sterile lines having T. timopheevi cytoplasm. This suggests that Cheyenne contains a gene or genes for restoration. From similar crosses with Cheyenne, Livers (32) obtained sterile F_1 hybrids and a segregation of 1:1 (fertility:sterility) in backcrosses to Cheyenne. Since the fertility of the F_1 hybrid was not restored, he considers the genes for fertility in Cheyenne as recessive fertility genes.

It may be necessary to utilize genes for partial restoration in common wheat if fully stable and effective restorer lines are to be developed. Plant selections from a number of varieties are reported to have some degree of fertility restoration when crossed with male steriles having T. timopheevi cytoplasm. According to Livers (32) 'Blackhawk,' 'Relief,' and 'Itana' all have some restoration ability. Fertility genes, similar to those of Cheyenne, or weak restoration genes are also suspected in plants of the varieties 'Crockett,' 'Gage,' 'Lancer,' 'Scout,' 'Selkirk,' and 'Chinese Spring'; however, other plants that can be effectively sterilized can be selected from these same varieties. This indicates that 'pure-line' varieties may be heterogeneous for a character such as fertility restoration. With one possible exception, all of the varieties mentioned above have Turkey or Turkey derivatives in their pedigree. The only possible exception to this is the variety,

Chinese Spring, whose parentage is unknown. Partial restoration of fertility in these varieties has been observed primarily when growing the F_1 progeny or backcross generation from an A-line x variety cross during a male sterile conversion program. From the limited amount of information available, it appears that these genes would be called modifier or minor genes and several could be involved in restoration. From monosomic analysis by Robertson and Curtis (37), chromosomes 1B, 2B, 3D, 6A, and 6B of Chinese Spring appear to carry modifying genes for restoration. Johnson et al. (22) suggested that minor genes, as well as major genes, may be involved in the Nebraska lot 1 and lot 2 material. The results obtained in the present study suggest that a number of minor genes may be involved.

Another possible explanation for the failure of certain lines to fit a one- or two-gene ratio is that at least three major genes are required for complete restoration. Since Triticum aestivum is a hexaploid, each of the three genomes may furnish at least one major restorer gene. According to Wagenaar (44) T. timopheevi has the same two genomes, A and B, as found in T. aestivum. The T. timopheevi species may be furnishing genes for genomes A and B in the present restorer material, but the lack of a gene or genes for genome D may be causing instability in restoration. Porter et al. (35) recently found fertility restorer genes from the world wheat collection. They suggested that some of these genes may come from the D genome, and they may not be included in the crosses of T. timopheevi x T. aestivum, which involve relatively few hexaploid wheats.

The inheritance patterns of the restorer lines used in this study were tested using genetic ratios based on both seed-set and fertile

pollen classification. The agreement between these methods for the testcross and F_2 populations was generally good. However, the association between seed set and fertile pollen for the restorer parents themselves among the different environments in which they were grown was very low. The lack of agreement between the percentages of dark staining pollen and seed set must be attributed to either 1) collection of anthers at improper stage of maturity or 2) differential effect of environment on the anther from meiosis to dehiscence and the early stages of seed formation. Regarding the first point, anther maturity in a wheat spike begins first in the central portion of the spike and progresses toward the base and tip. Anthers in the tip region are the last to dehisce. In the preliminary pollen counts from anthers in different stages of maturity, the intensity of staining and consistency of readings were found to be dependent upon the maturity stage of the anther studied. To avoid the differences in development along the spike, anthers were collected from the three areas of the spike at varied intervals to get pollen at the same stage of maturity. Development of the anther proceeds from an immature green through a gradual change of light yellow to a golden yellow color at dehiscence. Even though collections for pollen counts were made in the medium yellow stage just prior to anther dehiscence there may have been differences in maturity of the pollen grains regardless of attempts to prevent such differences.

Considering the second possibility for lack of correlation between fertile pollen and seed set, the anther must contain a certain quantity of fertile pollen in order for dehiscence to occur. According to Rajki and Rajki (36) this threshold is at least 50% fertile pollen grains. Another factor affecting this threshold is prevailing meterological

conditions during flowering. A high percentage of sterile flowers occurred in the Stillwater greenhouse crosses when temperatures exceeded 90°F. Medium temperatures and high humidity appear to be the most desirable conditions for optimum pollen shedding and seed set expression of greenhouse material. The data reported by Rajki and Rajki (36) support these observations in that, at low atmospheric humidity (30 to 35%), 60 to 65% fertile pollen grains are necessary for anther dehiscence, but in warm weather, at high atmospheric and soil humidity, 45 to 55% fertile pollen grains are sufficient. The period that pollen remains viable from anther dehiscence until fertilization is also influenced by temperature and humidity (45). This should not have a marked effect upon self pollination within an enclosed wheat flower unless extremes of weather conditions occur.

Sterility of a partially fertile wheat plant is observed first in the upper portion of the spike and is referred to as tip sterility. As fertility decreases, sterility progresses down the spike toward the basal spikelets. The exact causes of this sterility pattern of the spike are not known but it has been suggested that this has a physiological and biochemical basis. According to Wilson (47), the florets at the tips of the heads appear to flower last; and consequently, their anthers develop late and miss the maximum pollen shedding period. Johnson et al. (23) indicated that male sterility may be due to a nutritional or physiological block that occurs below the spike. Such a block would be expected to affect the most distil florets first. The prominent wrinkling and shriveling of seed produced on male sterile plants suggests that proper nutrition is lacking during the period of endosperm development.

The central portions of the anthers of A-line plants were reported by Joppa et al. (26) to have poorly differentiated vascular bundles when compared with the male fertile line. The inadequate development of the vascular tissues could reduce solute transport into the stamens of male sterile plants. The lack of solute movement into the stamens could explain the pollen degeneration in male sterile individuals which was reported by Fukasawa (14, 15) to occur shortly after tetrad formation. Fukasawa (15) also indicated that large amounts of nutrition substances such as nucleic acid and waxy materials obtained from the tapetum cells were essential for the maturing of microspores. Extensive studies of anther development and microsporogenesis by Chauhan et al. (9) indicated that male sterility resulted from improper nourishment of the developing microspore due to abnormal behavior of the tapetum. Similar work involving the metabolic activity and reduction of the tapetal layer in barley lead Kaul et al. (27) to conclude that lack of food material from the tapetum caused pollen degeneration. Rapid disappearance of starch granules from the degenerating tapetal cells of male fertile lines, following the first mitotic division was noted by Joppa et al. (26) in wheat and Singh and Hadley (41) in sorghum; however, degeneration in the male sterile lines was slow, with cells persisting up to flowering in both wheat and sorghum.

Preliminary comparisons of pollen grains and anther development of male sterile and normal male fertile varieties were made in the present study. In the early boot-stage, the pollen grains of the male sterile plants were wrinkled while the grains of the normal varieties remained plump. As the male sterile head developed and the anthers should have been expanding, they began to contract and were extremely shriveled at

the time of pollination for the male fertile plants. These observations are consistent with the findings in corn by Rogers and Edwardson (38) and in sorghum by Maunder and Pickett (33). The latter suggest some essential substance for maintenance or development of pollen grains is apparently lacking.

The substance may be of a biochemical nature. Fukasawa (19) reported that the male sterile ovata strain had accumulated the amino acid asparagine while another free amino acid, proline, was lacking. Jones et al. (25) observed similar results in T (Texas) sterile cytoplasm of corn and while anthers from fertile plants contained an adequate quantity of proline, they had limited asparagine content. Another free amino acid, alanine, accumulating in the sterile anthers during the stages of microspore development, eventually reached a two- to three-fold increase over the alanine content of normal anthers. The presence of restorer genes in a sterile cytoplasm are reported to result in normal free amino acid content, but do not, however, permanently alter the T (Texas) cytoplasm, since sterile plants from segregating heterozygous restorers show amino acid patterns typical of sterile anthers. Gableman (20), assuming a gametophytic effect, advanced the hypothesis that the presence of one or more particles in a microspore would lead to pollen abortion. The particle has not been identified and the only suggestion was that it could be a virus. It was suggested that a particle was distributed regularly to daughter cells during mitosis, but was randomly distributed at meiosis. If sterility was under gametophytic control, 50% of the F_1 hybrids would be sterile. Brooks and Brooks (6) concluded from studies of cytoplasmic male sterility in sorghum that pollen abortion was controlled by a sporophytic rather than a gametophytic mechanism.

Together the nucleus and the cytoplasm form an integrated system -- the cell --, although each has its own genetic units, i.e. nuclear genes and plasmagenes or cytogenes (3, 42). Kihara (29) concludes that the plasmon and genome independently maintain their individuality though they interact with each other in metabolic activities. It is assumed that cytoplasmic male sterility involves an interaction of nucleus with cytoplasm since restorer genes are known which insure pollen fertility even though present in sterile cytoplasm.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

A total of 13 restorer lines were evaluated for fertility restoration potential using seed-set and fertile pollen classification systems for restorer parents, testcrosses, and F_2 populations. Tests were conducted for the F_2s in 1966 and the parents and testcrosses in both 1965 and 1966. The populations were grown in three environments. These were glass greenhouse, plastic greenhouse, and field. Chi-square probabilities were used to test the different genetic ratios of the restorer lines. Correlation coefficients were used to measure the association between 1) dark staining pollen and lateral seed set, 2) locations in the same year, and 3) the same location in different years for the lot 1, lot 2 restorer lines, and check varieties.

All restorer lines showed highly significant deviations from the theoretical one-gene homozygous dominant ratio. These results suggest that either two major genes or one major gene and additional minor genes are required for restoration in this material.

Definite differences in degree of restoration were noted among the various restorer lines but the division was not strictly between lot 1 and lot 2. There were four lot 1 and two lot 2 restorer lines that fit a two-gene ratio based on fertile pollen and/or seed-set readings. The remaining seven restorer lines failed to agree with any of the one- and two-gene genetic ratios.

Based on the results of this study, the inheritance of fertility restoration in lot 1 and lot 2 of the Nebraska material appears to be more complex than a simple one- or two-gene control as previously suggested (23). The restorer lines fitting a two-gene ratio were variable in expression of restoration in different environments.

The inability to differentiate among genetic ratios in some populations was attributed to the limited number of plants, coupled with the effect of environment within individual locations.

The lowest percentages of both dark staining pollen and seed set were found in the apical region of the spike. The central and basal regions had essentially the same pollen and seed-set fertility.

In summarizing tip fertility results based on the testcrosses in 1965, three restorer lines (5892-7, -9, -10) showed a close association between pollen and seed-set readings. These same restorer lines showed a good fit to a two-gene genetic ratio; therefore, they must contain a higher and more stable degree of fertility restoration than the remaining restorer lines.

Correlations indicated that the influence of environment was an important factor in the expression of pollen and seed-set fertility.

There was little association of percent seed set among locations for the restorer populations. However, there was generally good agreement for the normal check varieties. These differences were attributed to a significant restorer and/or sterile cytoplasm x environment interaction.

Significant positive correlations for seed set were noted between years (1965 and 1966) for the glass and plastic greenhouses; however, little association was observed between years for the restorer lines

grown in the field location.

Of the three locations, the field was the most fertile environment followed by the glass greenhouse and the plastic greenhouse. This indicates that the plastic greenhouse location was the most critical environment for the expression of restorer genes. This location also had the highest association for dark staining pollen vs. seed set. Thus, the plastic greenhouse appeared to be the most dependable environment for the conversion and testing of restorer lines.

Environmental conditions appeared to be the primary determinant of the degree of association between fertile pollen and seed-set readings as indicated by lack of significant linear correlation for the restorer parents in most locations. However, pollen readings were in general agreement with seed-set readings for the genetic ratios based on the testcross and F_2 populations, indicating that they could serve as a reliable classification method for determining inheritance of the restorer material.

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APPENDIX

APPENDIX TABLE XVI

PERCENT FERTILE POLLEN AND SEED SET IN 13 RESTORER LINES AND THEIR TESTCROSSES GROWN IN THREE LOCATIONS IN 1965

Line or Hybrid	Glass Greenhouse					Plastic Greenhouse					Field				
	No. of Plants	% Fertile Pollen Range	% Fertile Pollen Avg.[1]	% Seed Set Range	% Seed Set Avg.[1]	No. of Plants	% Fertile Pollen Range	% Fertile Pollen Avg.[1]	% Seed Set Range	% Seed Set Avg.[1]	No. of Plants	% Fertile Pollen Range	% Fertile Pollen Avg.[1]	% Seed Set Range	% Seed Set Avg.[1]
A Nebr 542437[A] x Bsn[2]	8	0	0.0	0	0.0	8	0	0.0	0	0.0	10	0	0.0	0	0.0
B Bison CI 12518	8	92-98	95.7	83-100	93.6	8	39-92	76.1	30-87	68.2	10	58-96	87.5	81-100	92.8
B Triumph CI 12132	8	88-97	93.1	68-96	87.6	8	64-97	85.4	60-80	71.4	10	88-97	93.1	82-100	91.7
R 5892-2	8	94-98	95.6	89-100	93.5	8	34-77	60.9	4-54	32.7	10	33-96	80.7	21-100	76.5
R 5892-5	8	3-98	85.0	4-100	73.2	8	37-81	61.1	4-79	38.5	10	64-96	86.9	32-100	79.6
R 5892-7	8	90-97	94.8	92-100	95.0	8	8-92	66.0	29-69	52.6	10	64-97	88.3	29-94	73.8
R 5892-9	8	91-98	94.8	79-100	91.0	7	39-72	57.7	38-72	59.1	10	57-97	86.1	61-100	88.4
R 5892-10	8	95-99	96.5	89-97	93.5	8	47-93	68.8	12-64	40.1	10	63-97	90.8	74-97	89.9
R 5892-25	8	92-96	94.6	85-100	92.6	8	64-92	82.9	35-82	64.5	10	64-97	89.9	77-100	92.8
R 5893-2	6	68-98	86.6	23-90	63.6	6	24-89	69.7	17-86	48.7	6	41-92	64.5	79-100	88.5
R 5893-3	8	90-98	94.9	78-100	93.9	7	49-96	70.3	61-82	73.2	10	58-90	73.7	88-100	95.1
R 5893-13	8	93-99	96.3	82-100	93.8	8	34-94	71.1	50-85	64.7	10	76-95	87.3	92-100	95.9
R 5893-15	8	93-97	95.2	86-100	94.2	8	56-95	84.8	42-84	74.7	10	43-97	85.6	89-100	96.1
R 5893-18	8	93-96	93.9	90-100	95.2	8	2-97	41.9	4-83	49.6	10	36-98	79.4	81-97	91.8
R 5893-20	8	80-99	93.3	67-93	81.6	8	27-93	52.6	14-72	40.7	10	38-96	77.9	52-100	82.0
R 5893-22	8	93-98	96.0	95-100	97.8	8	38-98	73.7	4-89	59.4	9	90-98	94.2	90-100	95.9
msBsn[2] x 5892-2 F_1						8	20-70	53.3	28-65	47.9	8	7-93	61.4	71-97	87.2
5892-5 F_1						8	26-77	54.7	11-72	46.3	8	13-90	66.1	36-97	80.4
5892-7 F_1						8	55-86	64.5	40-81	56.4	8	63-96	83.6	53-97	88.0
5892-9 F_1						8	21-82	58.9	31-94	57.8	8	82-90	86.2	68-100	86.4
5892-10F_1						8	8-86	46.7	44-83	68.0	8	82-94	89.8	66-100	89.5
5892-25F_1						8	57-85	68.8	33-85	59.7	8	59-93	78.4	31-94	70.1
5893-2 F_1						7	63-89	75.7	0-98	70.0	8	61-96	87.1	72-94	83.8
5893-3 F_1						7	17-94	59.8	0-75	50.7	8	8-92	54.3	21-86	61.2
5893-13F_1						8	55-88	75.5	11-80	52.2	8	15-96	60.6	6-97	71.6
5893-15F_1						8	28-92	70.4	32-97	71.0	8	34-95	60.1	62-97	82.8
5893-18F_1						8	0-92	52.7	0-77	37.1	8	4-67	20.0	10-88	39.0
5893-20F_1						8	46-92	72.0	6-75	54.3	8	43-95	76.2	78-100	90.4
5893-22F_1						8	11-93	52.7	45-88	66.6	8	2-95	47.2	34-91	73.4
Average			93.8		89.3			65.3		56.3			76.3		83.4

[1] Average of the first spike for the designated number of plants.

APPENDIX TABLE XVII

PERCENT FERTILE POLLEN AND SEED SET IN 13 RESTORER LINES AND THEIR TESTCROSSES GROWN IN THREE LOCATIONS IN 1966

Line or Hybrid	Glass Greenhouse					Plastic Greenhouse					Field				
	No. of Plants	% Fertile Pollen Range	% Fertile Pollen Avg.[1]	% Seed Set Range	% Seed Set Avg.[1]	No. of Plants	% Fertile Pollen Range	% Fertile Pollen Avg.[1]	% Seed Set Range	% Seed Set Avg.[1]	No. of Plants	% Fertile Pollen[2] Range	% Fertile Pollen[2] Avg.[1]	% Seed Set Range	% Seed Set Avg.[1]
A Nebr 542437[A] x Bsn[2]	8	0	0.0	0	0.0	8	0	0.0	0	0.0	8			0	0.0
B Bison CI 12518	8	35-99	79.7	30-100	68.4	8	32-96	72.2	15-100	70.6	8			50-100	89.2
B Triumph CI 12132	8	63-96	87.5	58-96	83.2	8	78-98	83.8	82-100	92.9	8			14-100	78.0
R 5892-2	8	86-95	90.7	62-90	80.9	8	10-93	83.0	4-72	48.3	8			64-100	88.4
R 5892-5	8	60-93	82.9	40-95	70.3	8	31-84	65.8	4-82	45.7	8			85-100	95.4
R 5892-7	8	63-97	88.7	74-95	85.8	8	38-94	73.3	9-82	56.3	8			80-100	91.3
R 5892-9	8	67-93	86.4	70-96	88.1	8	61-94	77.2	40-80	57.8	8			73-95	86.9
R 5892-10	8	83-97	91.0	54-100	87.6	7	51-89	69.8	21-91	59.1	8			79-100	94.0
R 5892-25	8	83-96	92.4	69-92	84.7	8	89-96	93.0	50-97	70.4	8			89-100	96.9
R 5893-2	8	58-96	81.8	52-94	74.2	8	64-92	84.1	21-94	74.6	8			76-97	90.4
R 5893-3	8	86-99	93.4	91-100	97.0	8	45-97	84.1	31-89	77.8	7			91-100	95.0
R 5893-13	8	88-97	92.6	87-100	93.0	8	92-98	94.4	34-93	77.9	8			82-97	90.3
R 5893-15	8	93-97	95.3	71-100	87.5	8	59-98	88.8	63-91	79.2	8			78-100	88.8
R 5893-18	8	70-93	85.8	86-96	90.4	8	32-95	69.8	22-89	61.8	8			64-100	85.9
R 5893-20	8	57-94	72.6	26-84	62.0	8	20-94	65.8	20-87	60.9	8			74-100	90.2
R 5893-22	8	86-98	92.4	57-96	86.1	8	58-98	84.1	43-100	81.9	8			75-97	88.4
msBsn[2] x 5892-2 F_1	2	23-28	25.0	48-64	55.3	8	45-82	72.8	35-85	55.9	20			4-92	57.1
x 5892-5 F_1	2	18-74	48.1	19-62	47.9	8	31-92	72.8	0-97	68.9	20			38-94	75.0
x 5892-7 F_1	2	74-84	79.2	79-89	84.3	7	52-91	74.9	4-81	42.7	20			16-100	68.8
x 5892-9 F_1	2	50-76	64.0	61-62	61.1	8	65-96	85.1	59-97	83.0	19			25-84	67.2
x 5892-10F_1	2	69-82	75.6	68-70	68.8	8	57-85	73.8	0-94	64.3	20			2-84	65.4
x 5892-25F_1	2	72-81	76.4	88-94	91.4	8	44-94	81.8	71-89	79.0	20			30-92	81.0
x 5893-2 F_1	2	65-80	72.9	52-59	55.6	8	49-96	81.0	35-95	79.6	19			55-98	74.4
x 5893-3 F_1	2	31-63	47.2	23-35	28.8	8	34-95	70.2	0-92	56.8	20			26-68	46.0
x 5893-13F_1	2	87-88	88.1	62-90	75.9	8	55-93	72.4	0-82	61.3	19			21-91	66.0
x 5893-15F_1	2	49-94	67.9	4-82	41.8	8	90-100	94.5	50-93	72.2	20			37-86	65.1
x 5893-18F_1	2	58-88	73.1	8-71	42.3	8	3-93	69.8	0-85	61.1	20			4-75	48.5
x 5893-20F_1	2	3-7	4.7	0	0.0	8	30-95	83.2	0-88	57.8	20			15-67	45.2
x 5893-22F_1	2	36-62	48.9	0-45	23.8	8	40-88	72.4	0-91	50.8	18			1-81	51.4
Average			80.8		68.4			78.4		66.0					77.2

[1] Average of the first spike for the designated number of plants.

[2] No pollen readings could be made for the field material due to weather conditions during the heading and pollination period.

APPENDIX TABLE XVIII

PERCENT DARK POLLEN AND SEED SET FOR THREE SPIKE AREAS OF TESTCROSSES INVOLVING SIX LOT 1 RESTORER LINES GROWN IN THE FIELD LOCATION IN 1965

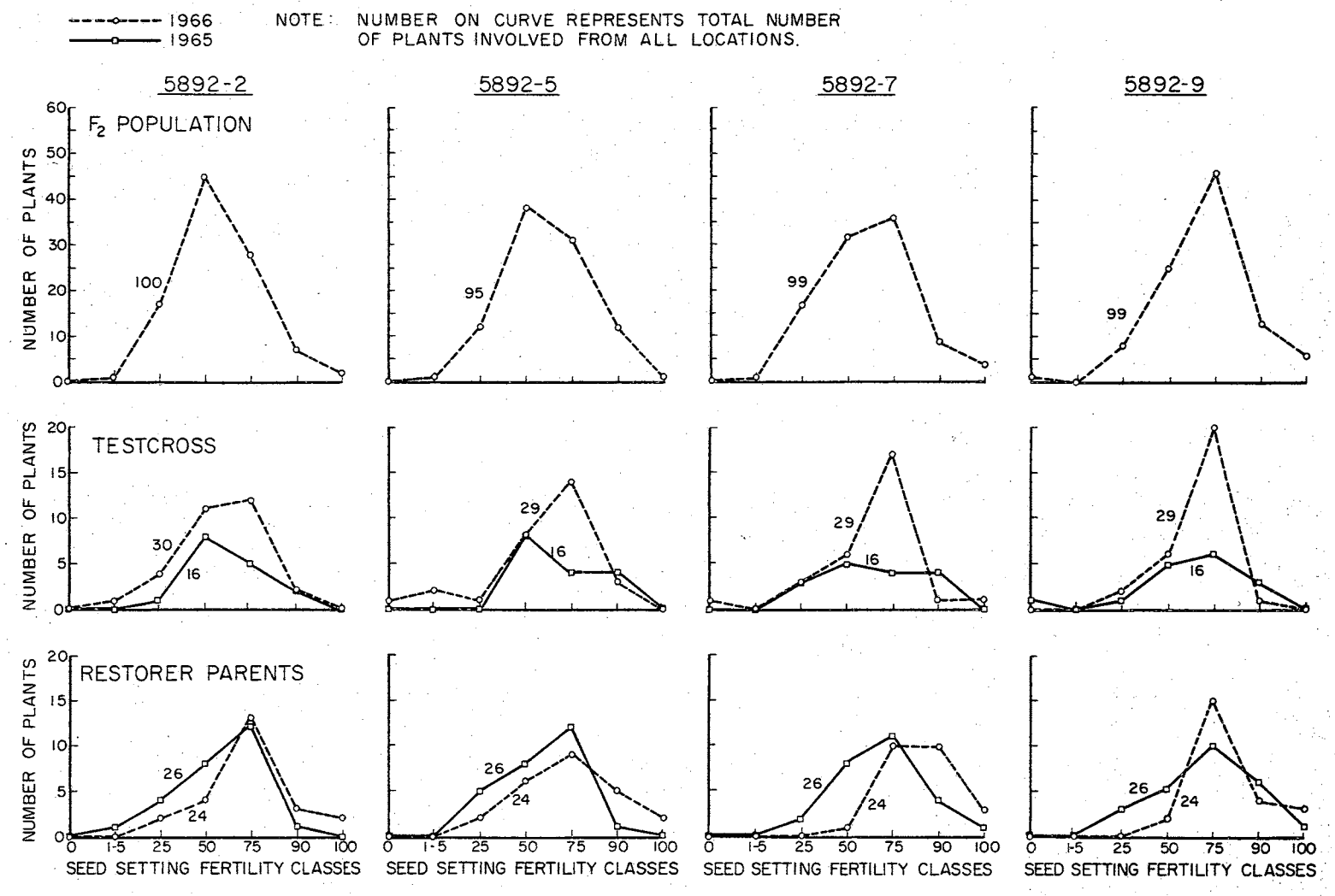
Testcross		ms x 5892-2 F_1		ms x 5892-5 F_1		ms x 5892-7 F_1		ms x 5892-9 F1		ms x 5892-10 F1		ms x 5892-25 F1		Triumph (Ck)		Bison (Ck)	
Plant No.	Area of Spike	% Dark Pollen	% Seed Set	% Dark Pollen	% Seed Set	% Dark Pollen	% Seed Set	% Dark Pollen	% Seed Set	% Dark Pollen	% Seed Set	% Dark Pollen	% Seed Set	% Dark Pollen[1/]	% Seed Set	% Dark Pollen	% Seed Set
1	Base	96.2	100.0	5.7	33.3	92.6	100.0	69.6	91.7	88.6	66.7	78.4	28.6	80.0	91.3	91.7	
	Middle	50.5	100.0	20.0	41.7	90.9	70.0	87.7	91.7	86.2	58.3	62.5	35.7	100.0	14.2	100.0	
	Tip	93.0	58.3	13.0	33.3	0.0	0.0	87.7	10.0	68.4	71.4	57.5	33.3	100.0	65.2	100.0	
2	Base	90.1	100.0	88.1	100.0	66.7	100.0	86.6	100.0	90.1	100.0	0.0	50.0	91.7	30.4	100.0	
	Middle	87.7	83.3	84.4	91.7	85.1	90.0	84.4	100.0	95.2	91.7	87.0	70.0	100.0	99.0	87.5	
	Tip	90.5	60.0	76.3	57.1	50.0	84.6	88.1	100.0	97.1	80.0	83.3	50.0	90.9	96.6	100.0	
3	Base	96.2	100.0	87.3	100.0	50.0	90.0	90.9	91.7	72.2	100.0	66.7	83.3	90.0	93.4	100.0	
	Middle	90.9	100.0	87.7	100.0	96.2	100.0	81.6	91.7	97.6	100.0	67.3	91.7	90.0	88.1	100.0	
	Tip	92.2	83.3	85.1	83.3	93.4	90.9	93.4	83.3	88.1	78.5	85.5	100.0	77.8	97.1	90.0	
4	Base	90.9	90.0	83.3	100.0	98.0	91.7	88.5	80.0	91.7	90.0	91.9	83.3	91.7	94.8	90.0	
	Middle	92.6	90.0	95.2	90.0	94.3	91.7	91.7	100.0	97.1	90.0	88.1	91.7	91.7	98.0	100.0	
	Tip	1.5	25.0	91.3	84.6	97.1	83.3	90.5	91.7	92.6	92.3	90.0	85.7	90.9	95.7	100.0	
5	Base	62.4	90.0	84.4	100.0	50.0	83.3	92.2	66.7	94.8	100.0		100.0	100.0	98.0	87.5	
	Middle	50.0	70.0	92.6	100.0	94.3	100.0	72.7	91.7	93.4	91.7	89.7	100.0	100.0	91.9	100.0	
	Tip	72.0	100.0	84.4	90.0	94.8	90.0	95.2	100.0	85.1	90.0	79.4	81.8	72.7	96.6	100.0	
6	Base	50.0	100.0	91.3	91.7	93.4	90.0	66.7	100.0	91.7	100.0	95.2	91.7	80.0	98.5	90.0	
	Middle	17.4	100.0	89.3	91.7	92.6	100.0	93.0	100.0	91.3	100.0	92.2	100.0	100.0	97.1	100.0	
	Tip	86.6	90.0	87.7	83.3	90.9	91.7	90.5	75.0	92.6	100.0	93.0	83.3	69.2	89.7	100.0	
7	Base	81.6	100.0	20.9	83.3	94.8	100.0	83.3	80.0	87.7	100.0	89.3	83.3	91.7	94.3	87.5	
	Middle	78.7	100.0	15.8	41.7	95.2	100.0	80.0	80.0	94.3	100.0	79.4	91.7	100.0	92.6	100.0	
	Tip	2.9	76.9	1.5	54.5	98.0	91.7	81.6	84.6	87.7	100.0	87.3	50.0	92.3	98.5	100.0	
8	Base	33.3	100.0	0.5	83.3	96.2	100.0	89.3	80.0	94.8	100.0	50.5	40.0	91.7	95.2	100.0	
	Middle	83.0	91.7	93.0	100.0	93.5	90.0	90.1	90.0	90.9	66.7	86.2	50.0	91.7	94.8	100.0	
	Tip	8.6	70.0	88.9	100.0	95.7	91.7	84.7	81.8	84.4	91.7	87.0	8.3	81.8	93.4	100.0	

1/ Pollen readings were missed.

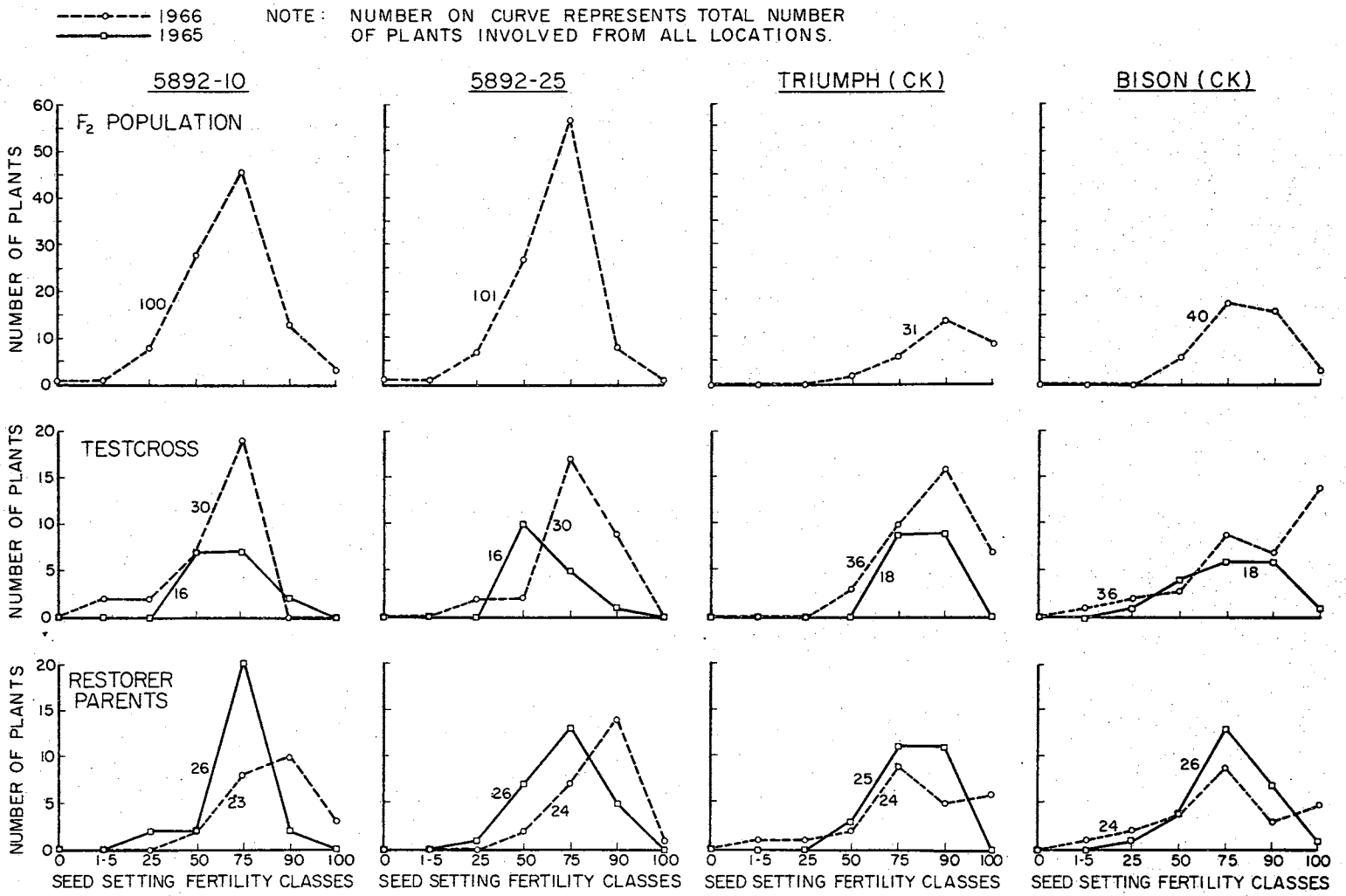
APPENDIX TABLE XIX

PERCENT DARK POLLEN AND SEED SET FOR THREE SPIKE AREAS OF TESTCROSSES INVOLVING SEVEN LOT 2 RESTORER LINES GROWN IN THE FIELD LOCATION IN 1965

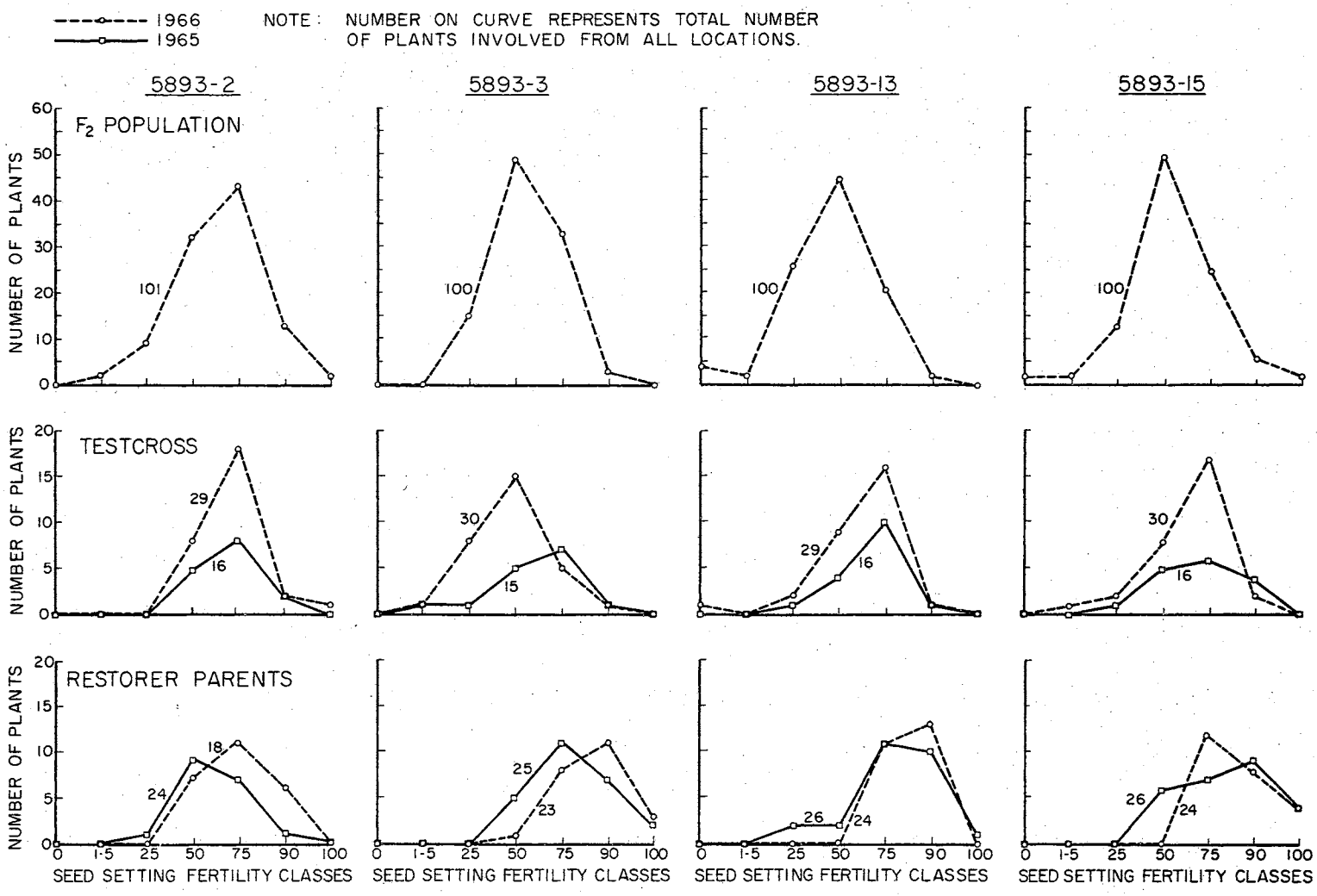
Testcross Plant No.	Area of Spike	ms x 5893-2 F_1 % Dark Pollen	ms x 5893-2 F_1 % Seed Set	ms x 5893-3 F_1 % Dark Pollen	ms x 5893-3 F_1 % Seed Set	ms x 5893-13 F_1 % Dark Pollen	ms x 5893-13 F_1 % Seed Set	ms x 5893-15 F_1 % Dark Pollen	ms x 5893-15 F_1 % Seed Set	ms x 5893-18 F_1 % Dark Pollen	ms x 5893-18 F_1 % Seed Set	ms x 5893-20 F_1 % Dark Pollen	ms x 5893-20 F_1 % Seed Set	ms x 5893-22 F_1 % Dark Pollen	ms x 5893-22 F_1 % Seed Set
1	Base	91.7	80.0	3.9	25.0	51.2	0.0	66.7	91.7	4.8	57.1	96.6	91.7	93.0	91.7
	Middle	87.7	100.0	6.1	16.7	1.0	16.7	76.3	83.3	1.5	50.0	96.2	100.0	63.2	83.3
	Tip	93.0	72.7	12.6	20.0	2.0	0.0	23.1	66.7	6.1	8.3	92.6	78.6	87.0	50.0
2	Base	88.9	80.0	10.3	41.7	6.1	41.7	81.0	75.0	11.9	28.6	87.0	90.0	2.0	90.0
	Middle	50.0	80.0	20.0	50.0	13.4	41.7	16.7	58.3	18.7	50.0	92.6	80.0	33.3	100.0
	Tip	40.6	58.3	42.9	30.0	23.1	70.0	14.5	90.0	22.2	27.3	84.8	66.7	8.3	41.7
3	Base	85.1	87.5	10.3	83.3	94.8	100.0	50.0	70.0	14.9	20.0	17.0	90.0	1.0	20.0
	Middle	94.8	87.5	14.6	41.7	86.2	100.0	50.0	90.0	9.1	10.0	90.9	100.0	3.3	50.0
	Tip	74.6	55.5	10.3	25.0	74.1	66.7	1.5	33.3	13.0	55.6	26.7	75.0	2.4	33.3
4	Base	88.5	90.0	50.0	90.0	96.2	100.0	76.9	80.0	25.0	83.3	90.9	83.3	83.3	100.0
	Middle	86.2	80.0	90.5	80.0	93.9	100.0	13.0	70.0	33.3	91.7	88.9	100.0	14.2	90.0
	Tip	91.7	81.8	86.6	55.6	97.1	69.2	20.0	66.7	21.6	30.8	88.5	78.6	66.7	58.3
5	Base	89.3	100.0	50.0	100.0	40.0	100.0	95.2	90.0	33.3	10.0	92.2	100.0	61.6	100.0
	Middle	93.4	100.0	89.6	91.7	20.6	100.0	91.9	100.0	16.0	10.0	95.7	91.7	91.4	100.0
	Tip	97.1	83.3	59.1	69.2	90.1	50.0	98.0	92.3	4.8	33.3	92.2	100.0	33.3	75.0
6	Base	94.3	100.0	89.7	75.0	89.7	100 0	92.2	100.0	9.0	0.0	83.3	91.7	45.5	100.0
	Middle	88.5	90.0	94.3	62.5	25.0	100.0	92.6	100.0	11.1	20.0	23.9	100.0	76.9	75.0
	Tip	83.0	50.0	91.1	55.6	84.3	90.9	94.8	91.7	2.4	25.0	71.5	92.3	31.8	54.5
7	Base	95.2	100.0	98.0	100.0	94.8	100.0	99.0	100.0	96.2	100.0	17.4	100.0	93.4	91.7
	Middle	97.1	100.0	52.4	83.3	94.8	90.0	95.2	100.0	66.7	90.0	88.4	100.0	97.6	91.7
	Tip	95.2	81.8	83.4	63.6	52.4	54.5	84.7	90.9	33.4	76.9	76.9	66.7	96.7	81.8
8	Base	96.6	91.7	88.9	70.0	33.3	75.0	91.7	70.0	13.0	0.0	80.0	100.0	13.8	66.7
	Middle	94.3	91.7	89.3	100.0	94.8	100.0	40.0	100.0	5.7	10.0	86.2	100.0	14.5	83.3
	Tip	92.6	72.7	50.0	46.2	90.9	45.5	13.0	75.0	13.0	20.0	95.7	100.0	16.7	46.2



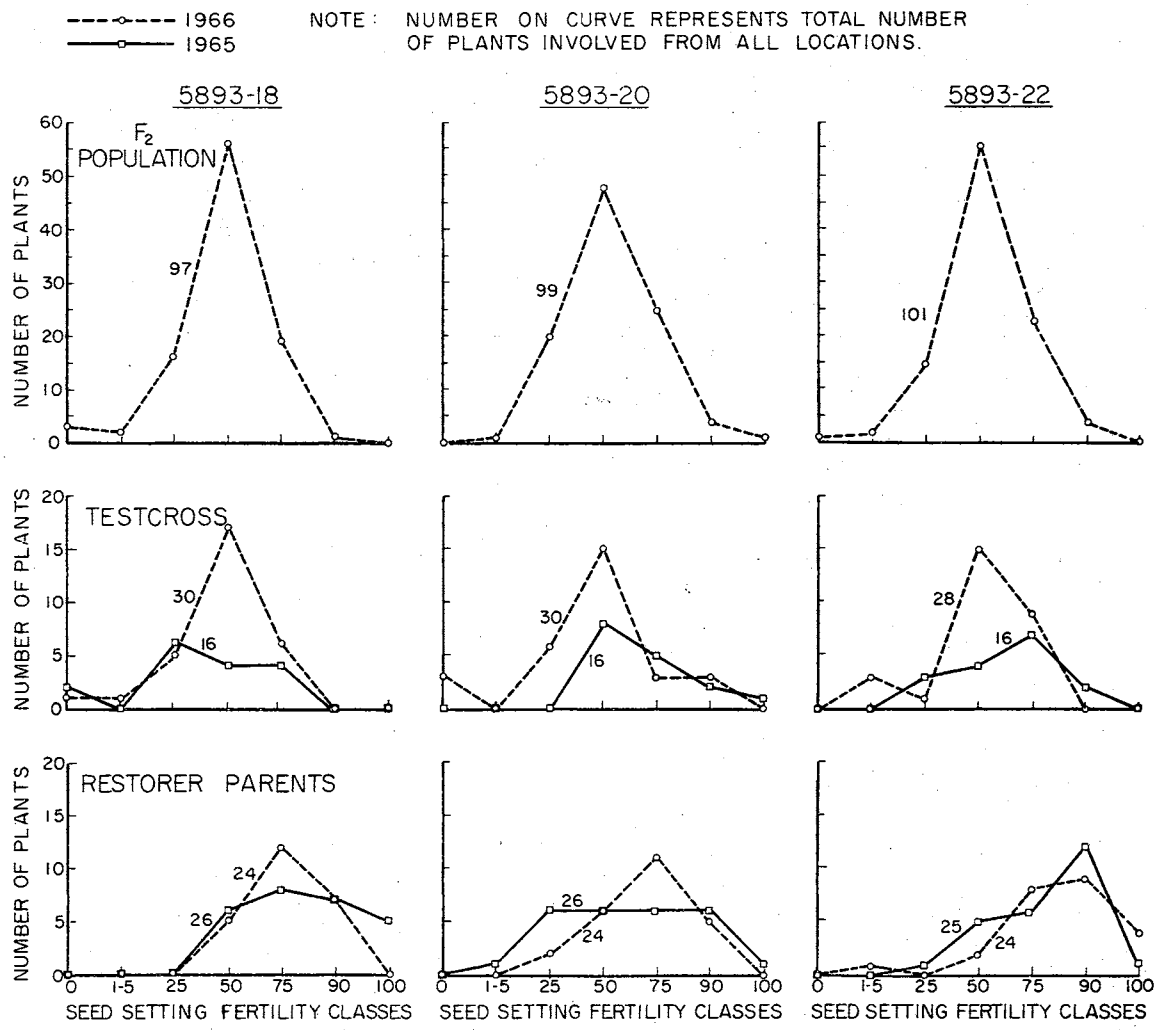
Appendix Figure 1. Frequency distributions for the restorer parents, testcrosses, and F_2s of six lot 1 restorer lines grown in 1965 and 1966.



Appendix Figure 1 (Cont.) Frequency distributions for the restorer parents, testcrosses, and F_2s of six lot 1 restorer lines grown in 1965 and 1966.



Appendix Figure 2. Frequency distributions for the restorer parents, testcrosses, and F_2s of seven lot 2 restorer lines grown in 1965 and 1966.



Appendix Figure 2 (Cont.) Frequency distributions for the restorer parents, testcrosses, and F2s of seven lot 2 restorer lines grown in 1965 and 1966.

VITA

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