HETEROSIS IN RELATION TO SUNFLOWER BREEDING

J. Unrau

[Received for publication June 14, 1947]

Reprinted from Scientific Agriculture, 27 : 9. September, 1947

HETEROSIS IN RELATION TO SUNFLOWER BREEDING[1]

J. UNRAU[2]

[Received for publication June 14, 1947]

Investigations carried on with sunflowers at the Dominion Forage Crops Laboratory, Saskatoon, since 1936 clearly indicate that further improvement in yield and other important agronomic characteristics will be contingent upon the development of techniques and methods of utilizing heterosis. Although basically similar to the utilization of heterosis in hybrid corn, in sunflowers, because of differences in floral structure and mode of pollination, the techniques of inbreeding and crossing on a large scale will be different. To make a program of hybrid sunflower seed production on a commercial scale successful, satisfactory methods of producing inbred and crossed seed and of selecting and testing for combining ability in the breeding program must be worked out. This paper presents the results of tests of diallel crosses produced by hand pollination and in natural crossing plots and a comparison of methods of testing for combining ability.

LITERATURE REVIEW

The effects of inbreeding and crossing on vigor and yield in sunflowers were noted by Morozov (6) and Jagodkin (2) in Russia. These investigators suggested the production of hybrid sunflowers on a commercial scale. Unrau and White (13) reported a 60.8% increase in seed yield and a much greater uniformity in a hybrid compared to unimproved Mennonite. They observed a positive relationship between high percentage crossing and yield, and attributed a low proportion of crossed seed obtained in natural crossing plots to a lack of simultaneous flowering of the two inbred lines, high self-fertility of the female line or a combination of both factors.

The combining ability of large numbers of inbred lines in corn is usually determined by the use of the top cross using varieties or inbred lines as the testers. This method of testing was developed by Jenkins and Brunson (5). Jenkins in 1935 (4) showed that inbred lines displayed their individuality and desirability as parents of crosses when tested early in the inbreeding program. Sprague and Tatum (9), believe that for testing a large number of inbred lines of corn the top cross should be used for discarding lines having poor general combining ability and that specific combining ability must be determined by diallel crossing of the remaining lines. Sprague (10), found that where a corn variety was used as a tester in a top cross, at least ten plants must be used to give an adequate representation. Tysdal *et al.* (12), have suggested the polycross test in alfalfa to test the combining ability of lines.

MATERIALS AND METHODS

Crossing Studies

In 1943 plants of four inbred Mennonite lines, two introduced Russian lines and the variety Sunrise were reciprocally crossed by hand pollination without emasculation. Two plants of each line were crossed with two plants from every other line. The reciprocal transfer of pollen was

[1] Contribution from the Dominion Forage Crops Laboratory, Saskatoon, Sask., being a condensation of part of a thesis submitted to the Graduate School of the University of Saskatchewan in partial fulfilment of the requirements for the Degree of Master of Science.

[2] Formerly Agricultural Assistant, Dominion Forage Crops Laboratory, Saskatoon, Sask., at present Research Assistant in Agronomy, Washington State College, Pullman, Washington.

performed by shaking the pollen of one plant into the paper bag isolator and applying the pollen to the newly opened stigmas of the other plant with a cotton swab. This reciprocal transfer of pollen was performed every second day until flowering was completed. Flowering usually extended over a period of from 7-10 days.

Except for one Mennonite line all possible combinations of the lines listed above were attempted in space isolated natural crossing plots. Where the two lines of a paired combination were known to differ markedly in flowering date, the earlier flowering line was seeded at one or more later dates to obtain simultaneous flowering. The plots were space isolated by distances of half a mile or more. All plots were visited every second day until flowering was completed in order to make the necessary hand crosses and to record flowering dates of each line. Due to uncontrollable factors some plots were failures.

In 1944 seed from the successful reciprocal single-cross combinations produced by hand pollination was used to sow a yield test. In the case of seed from the natural crossing plots, since in 1943 a record was made of the date of flowering of each of the two lines at each date of seeding in each plot and seed from each date of seeding was harvested separately, it was possible to select for the yield test seed from those dates of seeding which had resulted in simultaneous flowering of the two lines in each natural crossing plot. A modified Latin square design with 6 replications and of single row plots was used. The Kemp V-Belt seeder was used in seeding this and test to be described below. Planting was done at a rate to give an approximate spacing of 6 inches between plants in the rows. In both this and the test described below the rows were 36 inches apart. Plots were 20 feet long with the centre 16 feet being harvested for yield determinations.

In 1943 the six lines used in the natural and hand crossing program were also planted in an isolated polycross plot. Each line in the polycross plot was harvested separately. Two plants in each line were hand pollinated with composite pollen from all the other lines. In addition open pollinated seed from the three Mennonite inbred lines used in the polycross plot was obtained from the breeding nursery. This material was tested in 1944 using a simple lattice design in duplicate providing four replications. Size of plots and method of planting were the same as in the previously described test.

In both tests data were obtained on seed yield, plant height, head diameter and flowering date. Seed size was determined by weighing three random 100 seed samples from the seed of each plot and expressing the average on the basis of 1000 seeds. Weight per bushel was obtained by one determination from the seed of each plot. Percentage kernel was determined by the use of a mechanical huller on a 10 gram sample from the seed of each plot. Percentage oil based on the dry weight of the whole seed was determined from a composite sample of seed from all the replicates. The oil analyses were conducted by the Oil Seeds Laboratory, Saskatoon, Sask. Wherever hybrid plants and hybrid seed could be visually distinguished the percentage of hybrid plants and seed from hybrid plants was obtained.

Descript‹‹ n of Lines and Varieties

Me›ı onite is unimproved material, variable for height (usually ranging from 3.5-4.5 f et) for degree of branching, date of flowering and maturity, for seed size and shape and pattern of striping. Seed size varies from 50-75 grams per 1000 kernels with an oil content of between 20 and 28 per cent.

Line A—Strain Number S-37-25-2-1-1-1 is the most vigorous inbred line obtained from Mennonite and has large seed of low oil content.

Line B—S-37-49-2-3-3-1-2 is another inbred line from Mennonite which has shown great uniformity from the third inbred generation onward. It has deep purple pigmentation in leaves, stems and stigmas. The seed when selfed is brown striped but seed from F_1 plants involving this line are solid purple irrespective of the direction of the cross. It is shorter stemmed than Mennonite but has shown some undesirable basal stem weakness. The seed weighs 40-50 grams per 1000 kernels and has an oil content of 30-35 per cent.

Line C—S-37-388-1-1-5-4 is very similar to line B, but lacks the purple pigmentation, is shorter and stronger stemmed and is less vigorous. It has small striped seed of high oil content.

Line D—S-37-402-1-1-3-1-2 is a white seeded inbred Mennonite line taller than the other inbreds and has non-branching strong stems. Oil content is low. Yield of seed is low.

Line E—An extremely early line introduced from Russia under the name "Early Saratov". Extremely low in yield and lacking in uniformity, but of value in a breeding program because of its earliness.

Line F—Released in 1942 under the name Sunrise. This line is the result of four generations of inbreeding from a Russian introduction received in 1937. Sunrise is somewhat shorter and stronger stemmed than Mennonite, has small plump seed with an oil content of usually 30 per cent or over. This line possesses immunity to the sunflower moth, *Homeosoma ellectellum*. According to histological studies by Putt (8), this immunity is due to the presence of an amorphous pigment band between the second and inner seed coat layer. Preliminary studies (7) have indicated this band to be inherited as a simple dominant. To avoid confusion it will be referred to as line F throughout the paper.

Line G—Is another Russian introduction made in 1937 and inbred since then. In all characteristics very similar to line F except that it usually grows 3-4 inches taller and is a few days earlier. The seed, also, is immune to the sunflower moth.

RESULTS AND DISCUSSION

Test of Single Crosses

Yield, Plant Height, and Percentage Crossing

For the sake of brevity only a portion of the data obtained from the 1944 test of hand and naturally pollinated single crosses is presented in Table 1.

As shown in Table 1 highly significant yield differences were obtained among the various single cross combinations. In general the yields obtained appeared to be due to the interaction of percentage crossing and combining ability. On the basis of these two factors, the single crosses can be dealt with in the following four groups:

Group 1. Combinations producing high yield due to the high percentage crossing obtained and to the parents having good combining ability.

Group 2. Combinations producing lower yields due to the intermediate crossing obtained but in which the high proportion of total seed produced by the hybrid plants indicates that the parents possessed good combining ability.

Group 3. Combinations which in spite of a high percentage of crossing produced relatively low yields due to the poor combining ability of the parental lines.

Group 4. Combinations resulting in poor yields due to the low percentage crossing obtained and the low combining ability of the parents.

TABLE 1.—THE YIELD AND OTHER CHARACTERISTICS OF SINGLE CROSSES PRODUCED BY HAND POLLINATIONS AND IN NATURAL CROSSING PLOTS IN 1943 AND TESTED IN 1944

Cross or variety	Yield, lbs. per acre		Plant height (inches)				Per cent hybrid plants in progeny		Per cent of total seed produced by hybrid plants	
	Hand cross	Natural cross	Hand cross		Natural cross		Hand cross	Natural cross	Hand cross	Natural cross
			Hybrid	Self	Hybrid	Self				
B × A	1967	—	42.8	35.1	—	—	65.7	—	87.2	—
A × B	1447	—	41.0	35.5	—	—	28.5	—	64.1	—
F × A	2045	—	47.4	38.3	—	—	64.8	—	94.7	—
A × F	1773	—	45.7	40.2	—	—	79.8	—	95.5	—
C × A	1852	—	46.6	38.5	—	—	69.2	—	—	—
C × G	2005	—	46.9	—	—	—	99.3	—	—	—
G × C	1278	—	44.0	38.1	—	—	29.3	—	—	—
C × B	1935	1705	45.6	41.5	45.8	36.4	59.5	67.2	63.8	—
B × C	1662	1188	42.0	38.4	39.0	—	25.0	—	57.3	—
C × F	1985	1915	44.8	—	47.3	—	98.3	95.8	—	—
F × C	1640	1685	37.3	37.3	45.4	39.8	56.2	46.3	—	—
D × E	1325	1700	39.2	34.3	45.2	40.6	—	72.7	—	85.8
E × D	752	995	37.3	—	40.8	—	—	—	—	—
D × B	—	1757	—	—	47.2	40.0	—	77.7	—	—
B × D	—	1213	—	—	40.3	34.7	—	21.7	—	—
D × F	1743	1798	47.5	41.8	45.0	38.3	74.0	84.0	—	—
C × E	778	1795	39.1	30.5	42.3	38.0	19.2	84.3	—	—
E × C	—	1015	—	—	39.2	33.5	—	—	—	—
A × G	1512	—	47.5	36.2	—	—	40.7	—	74.9	—
G × A	1452	—	45.8	39.2	—	—	42.3	—	83.3	—
C × D	1657	1388	41.2	40.2	41.8	38.8	83.7	92.0	98.2	94.6
D × C	1262	1418	39.8	33.5	44.8	37.4	—	—	—	—
B × E	1098	1610	39.8	30.4	39.6	37.4	31.3	—	60.0	—
E × B	913	1165	38.5	30.0	44.3	33.9	45.8	—	75.7	—
F × E	—	1227	—	—	41.9	37.3	—	—	—	—
E × F	—	768	—	—	39.1	32.0	—	—	—	—
B × G	—	1276	—	—	41.3	34.9	—	60.8	—	—
G × B	—	637	—	—	43.9	41.3	—	20.7	—	—
F × G	1048	825	40.3	—	39.9	—	—	—	—	—
G × F	937	613	41.7	—	40.8	—	—	—	—	—
D × G	—	1500	—	—	45.4	—	—	—	—	—
G × D	—	840	—	—	43.6	41.3	—	—	—	—
Mennonite	1740		47.8							
Line E	773		35.1							
L.S.D.	309		9.0							

On the above basis of grouping, the hand pollinated single crosses B × A, F × A, C × A, C × G, C × B, C × F, D × F and the natural crosses C × B, C × F, D × E, D × B, D × F and C × E would come in the first category where high yields were obtained from a good degree of crossing and good combining ability of the parental lines.

The performance of line C is noteworthy. It was the female parent in seven of the 13 above mentioned combinations. This fact is evidence that it has high general combining ability. In addition in most cases a high degree of crossing was obtained when line C was the female parent. It is apparent, therefore, that line C is a good female parent for use in commercial production of single cross seed. This fact has been utilized in the hybrid variety Advance which was named and licensed in 1945. The variety is a single cross hybrid of C × F.

FIGURE 1. Single Cross G × C. Heterosis is evident indicating good combining ability. Percentage crossing 29.3.

The second group would include crosses such as A × B, B × C, A × G and G × A. These crosses are characterized by intermediate yields, a low percentage of hybrid plants but a high proportion of the total seed being derived from the hybrid plants. This latter fact indicates that either or both parents of these crosses had high combining ability.

In the last two groups low combining ability with or without low crossing was responsible for low yields. The combinations C × D and F × G undoubtedly belong in the third and fourth grouping, respectively. In C × D crossing of 92.0 per cent was obtained, yet the yield was significantly below that of the Mennonite check, indicating that the specific combining ability of these two lines was probably low. Lines F and G are both Russian introductions showing a great deal of morphological similarity, while C and D both have been developed by selective inbreeding from Mennonite. It may well be that in these combinations poor specific combining ability was caused by the presence of similar genes or gene complexes influencing vigor. Results at Minnesota (14), (1) and (3) on corn have indicated that inbreds of diverse genetic origin gave higher yields in crosses than did related ones. Other crosses where poor specific combining ability appeared to be responsible for low yields were E × B and B × G. In the single cross E × B with 45.8 per cent crossing the yield was 913 pounds per acre, only 145 pounds above the yield of inbred line E. The yield of B × G from the natural crossing plot with 60.8 per cent crossing was only 1276 pounds per acre, being significantly lower than that of the Mennonite check. However, lines B and G when used as parents in combination with other lines have given high yielding single crosses. Thus C × G yielded 2005 pounds per acre, C × B 1935 pounds per acre, B × A 1976 pounds per acre and A × B with 40.7 per cent crossing yielded 1512 pounds per acre. These results clearly indicate that from the standpoint of yield specific combining ability is very important.

In the single cross combinations, where good combining ability existed, marked heterosis was displayed by greater height and plant development of the hybrid as compared to the selfed or sibbed plants. Figure 1 clearly illustrates such a condition. In the single cross F × A yielding 2045 pounds per acre, hybrid plants attained a height of 47.4 inches, while the selfs or sibs averaged only 38.5 inches in height. C × D and G × B are examples where poor combining ability resulted in little heterosis as expressed by yield and height. Thus in the combination C × D the difference between hybrid and selfed or sibbed plants was only 1.0 inches in the hand pollinated cross, and 3.0 inches in the natural cross.

It is significant that, even in the highest yielding single crosses, the height of plants was below that of Mennonite. In addition these single crosses were uniform for this character, making them much more desirable from the standpoint of ease of harvesting. Figures 2 and 3 illustrate the uniformity of the single cross C × F (Advance) as contrasted to Mennonite, for height, stem type and strength, date of flowering and other characters.

With the exception of the cross C × E, there was close agreement between the hand and naturally pollinated combinations in respect to

FIGURE 2.—Single Cross C × F (Advance) from seed from natural crossing plot. Note uniformity for height, flowering date, etc.

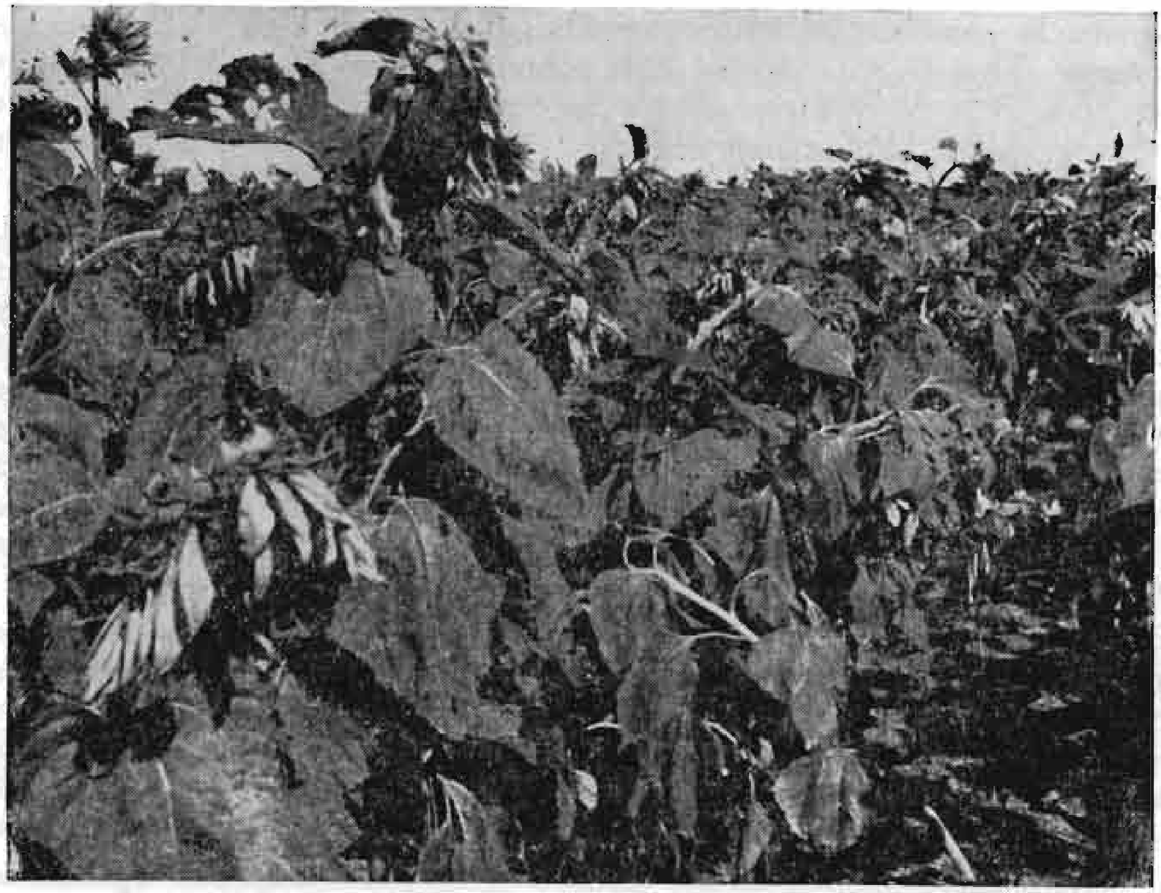

FIGURE 3. Mennonite. Lack of uniformity for height, stem strength and date of maturity as displayed is undesirable from standpoint of harvesting with a combine.

yield and percentage crossing. This fact is important from the standpoint of testing new inbreds for percentage crossing and combining ability. An illustration of this is found in the results of the hand and natural cross combinations C × F. When produced by hand pollination the cross C × F had 98.3 per cent crossing and yielded 1985 pounds per acre. This same cross produced by natural crossing had 95.8 per cent crossing and yielded 1915 pounds per acre. In the reciprocal the hand pollinations resulted in 56.2 per cent crossing with a yield of seed of 1640 pounds per acre while the same cross produced in a natural crossing plot had 46.3 per cent crossing and yielded 1685 pounds of seed per acre. Hence for this combination similar results were obtained whether hand crossed seed from two plants or naturally crossed seed from many plants was used. The reason for disagreement in the results of the cross C × E when produced by hand as compared to natural crossing was undoubtedly due to lack of homozygosity of line E. Thus the two plants of line E used in the hand pollinations did not give a true picture of the line either as to combining ability or its value as a pollen parent. The results on all the other lines inbred for 3-4 generations indicate that the value of inbred lines as parents in single crosses can be quite adequately determined by reciprocal hand pollinations of two plants of each line.

Providing that flowering of the lines in a natural cross combination occurred simultaneously, percentage crossed seed produced by any line was undoubtedly affected primarily by its degree of self-fertility or the amount of good pollen produced by the other line. Thus the difference obtained between the reciprocals of the combinations of C with F and C with G can be interpreted as being due to the lower self-fertility of line C, the high self-fertility of lines F and G and the fact that they were good pollen producers.

The single cross C × F produced in natural crossing plots for three successive years has consistently resulted in a high percentage crossing and a high yielding F_1. However, in the reciprocal cross the percentage crossing, and consequently the yield, has been lower. In 1945 the single cross C × F was licensed for commercial production under the name Advance. Obviously only seed from line C of a crossing plot should be distributed unless, after testing, it is found that in certain crossing plots line F produced a sufficiently high percentage of crossed seed to warrant distribution of such seed as Advance.

Among the single crosses listed above another noteworthy feature is that in crosses involving parents having good combining ability in every case percentage crossing of 59.5% or higher resulted in high yields of seed which came mostly from hybrid plants. As pointed out in a previous paper (13), this was undoubtedly due to the greater vigor of the hybrid plants which were able to depress the low yields of the weaker selfs or sibs. In the case of combinations having high combining ability it appears plausible that the effect of lower crossing percentages might be counteracted by increasing the seeding rate so as to have more hybrid plants per unit length of row, and the greater vigor of the hybrids would crowd out or nullify the effect of the selfed or sibbed plants. Tysdal and Kiesselbach (11) present data showing that this phenomenon occurs in alfalfa. However, controlled experiments would be necessary before such a practice could be recommended in commercial hybrid sunflower production.

The natural crossing plots used in this study have had equal numbers of rows of the two parents. In the case of a combination such as C × F, where F is highly self-fertile and a good pollen producer and seed from it cannot be used commercially as it has been grown in the past, it might be possible to use a fairly low proportion of inbred F in a crossing plot and still obtain high percentage crossing unto line C. In that event the total amount of crossed seed produced from a crossing plot would be greatly increased. There is the further possibility that under such conditions the percentage crossing in line F would be higher than if equal numbers of rows of both lines were grown. In addition the proportion of F seed to the total seed produced in such a crossing plot would be low so that the composite might have a high enough percentage crossing to enable distributing all the seed as hybrid seed. Until this is ascertained, it will be necessary to test the seed from line F to determine percentage crossing before its sale or distribution.

Determination of percentage crossing would be facilitated if the line used as the female parent carried a marker gene expressing itself in the seedling stage. Such a character is the purple pigmentation carried by line B. It should be relatively simple to transfer this character to a line such as C by the backcross method using line C as the recurrent parent for 4-5 generations.

Date of Flowering

The flowering dates are summarized in Table 2. Since no differences existed for this character in reciprocals of a combination, only the flowering dates of a cross in one direction are presented.

TABLE 2.—DAYS FROM SEEDING TO FLOWERING OF INBRED LINES AND THEIR SINGLE CROSSES

Line or variety	Number of days from seeding to flowering when						
	Inbred	Crossed with					
		A	B	C	D	E	F
A	85						
B	82	77					
C	84	81	81				
D	85	—	80	76			
E	66	—	71	74	72		
F	87	78	80	82	79	77	
G	85	77	81	79	78	—	83
Mennonite	78						

Since hybrid and selfed or sibbed plants of a single cross were growing in the same rows, a direct comparison between them for this character was possible. With the exception of hybrids involving line E, all single crosses were earlier than either parent. In the cross C × F the hybrids flowered in 82 days, five days earlier than line F and two days ahead of line C. In C × G the hybrids flowering in 79 days were five and six days earlier than line C and G, respectively. It is noteworthy that these two combinations were also in the group having high yield because of high percentage crossing and good combining ability when line C was used as the female parent. The hybrids involving line E had a flowering date intermediate between those of the two parent lines, but in every case closer to that of line E.

It should be mentioned that while in Mennonite the flowering date was earlier than in some of the hybrids, the period over which this process extended was much longer since there is a great lack of uniformity in Mennonite for this character. In the single crosses, on the other hand, the hybrid plants flowered and ripened at the same time and the period in which this process takes place is much shorter so that harvesting would actually not be delayed materially even in crosses flowering later than Mennonite.

Seed Characters

In Table 3 the summarized data on seed characters of some of the single crosses and Mennonite are presented.

Plump seed uniform for shape and of small size was one of the objectives when the project was originally started. The data clearly show that some of the single crosses had seed with very desirable characteristics. To a large degree the original objective had been reached in the single crosses C × G and C × F. Besides producing significantly higher seed yields, they had

TABLE 3.—SEED CHARACTERS OF SOME SINGLE CROSSES AND MENNONITE FROM TEST IN 1944

Cross or variety	Wt. per 1000 seeds (grams)		Wt. per bushel (pounds)		% Kernel		% Oil	
	Hand cross	Natural cross	Hand cross	Natural cross	Hand cross	Natural cross	Hand cross	Natural cross
A × F	61.2	—	29.2	—	51.5	—	30.3	—
F × A	60.5	—	29.0	—	53.4	—	31.7	—
C × G	52.6	—	29.7	—	56.2	—	33.4	—
G × C	52.9	—	29.3	—	56.9	—	33.4	—
C × F	48.1	50.2	29.7	30.3	55.7	56.8	33.4	33.3
F × C	51.4	55.6	29.3	28.8	55.9	55.9	33.4	33.3
D × F	—	52.6	—	26.7	—	50.6	—	30.0
D × E	55.0	—	26.2	—	52.8	—	28.6	—
B × D	—	41.4	—	28.2	—	58.8	—	27.6
Mennonite	63.5		24.9		48.8		27.3	
L.S.D.	5.1		1.8		2.7		—	

an oil content of 6 per cent above that of Mennonite. In addition, since F and G both carry the dominant factor for resistance to sunflower moth damage the seed produced by the hybrid plants of the single crosses C × F and C × G is also resistant. From the standpoint of all characters considered these single crosses, therefore, represent a very material improvement over Mennonite. In Figure 4, seed of lines C and F, the F_1 of C × F and Mennonite are shown.

The desirable seed characteristics of lines F and G were also present in other single crosses involving these lines. While line A was not tested in 1944, in previous years its seed has had an oil content of around 20.0 per cent. The single cross F × A had an oil content of 31.4 per cent, with very desirable seed size and shape. The single crosses D × F D × E and B × D are also interesting from the standpoint of the relationship of oil content of the inbred and the single crosses. Line D, which has low oil content, was present as one of the parents in the three crosses just mentioned. When F was the other parent the oil content was 30.0 per cent, while in the other two the oil content was little above that of Mennonite.

Reciprocal crosses were similar for seed characters as they were for yield, if the percentage crossing was similar. Also, the hand pollinated single crosses produced seed similar to that produced from natural crossing plots provided the lines had been inbred for a sufficient period of time to attain a high degree of homozygosity.

PRELIMINARY STUDIES ON METHODS OF TESTING FOR COMBINING ABILITY

The results of a test on polycross seed of six lines grown in an isolated polycross plot, of seed produced on plants of each of the six lines by hand pollination with composite pollen from the other lines, and of open pollinated seed of the three Mennonite inbred lines from the 1943 breeding nursery, are summarized in Table 4.

TABLE 4.—RESULTS OF THE TEST ON METHODS OF STUDYING COMBINING ABILITY—1944

Line	Method of crossing	Yield (lbs. per acre)	Height (inches)	Head diam. (inches)	Bus. wt. (lbs.)	Wt./1000 seeds, grams	% Kernel	% Oil	Days to flower
C	Poly-cross	1393	43.4	5.2	26.9	47.8	52.0	29.2	75.2
C	Hand cross	1196	40.0	5.0	26.9	43.8	51.0	29.0	79.3
C	Open pol.	1377	38.8	5.0	25.0	54.2	52.0	29.9	76.5
B	Poly-cross	883	38.1	4.9	25.8	42.6	52.0	28.6	79.0
B	Hand cross	862	31.4	5.2	24.9	39.9	58.0	29.8	81.0
B	Open pol.	1270	35.3	5.4	26.5	53.3	49.0	28.7	75.5
D	Poly-cross	1251	41.4	5.3	25.1	49.8	48.0	28.1	77.5
D	Hand cross	1207	41.0	5.4	25.0	49.0	51.0	27.4	80.2
D	Open pol.	1307	39.7	4.9	24.1	56.0	48.0	27.5	77.7
E	Poly-cross	961	38.1	5.6	27.8	56.8	55.0	27.9	66.0
E	Hand cross	1011	38.4	5.0	27.1	49.4	55.0	29.8	74.0
F	Poly-cross	1077	39.6	5.3	26.1	48.4	52.0	27.8	81.2
F	Hand cross	1130	38.9	4.8	26.9	51.0	54.0	28.7	75.8
G	Poly-cross	931	40.0	5.3	27.2	46.8	52.0	27.9	79.5
G	Hand cross	558	38.9	4.9	27.4	37.0	55.0	27.9	80.5
Mennonite check		1014	39.9	4.5	26.4	55.4	49.0	26 0	76.8
L.S.D.		222	5.9	N. Sig.	1.8	6.7	—	—	—

In these studies line C behaved very much as would be expected from the results of single crosses where it gave high yielding single crosses when used as the female with all lines but D. The high yields of line C in this test undoubtedly were due to high percentage crossed seed, regardless of the method used, and to good general combining ability with the pollen parents.

The poor performance of line B is hard to explain. It may be due, in part, to the presence of a fairly large number of plants that had lines E or G as the male parent. The poor specific combining ability with these two lines resulted in low yields in the test of single crosses. It may also be due to the fact that a lower proportion of seed was crossed than in line C in the hand pollinations and the polycross plot. In any case, these studies were inadequate to test the value of this line as a potential parent of single crosses.

From the test of single crosses (Table 1) it was concluded that line D had poor specific combining ability with line C. Furthermore, it was not a parent in any single crosses that gave as high seed yields as when line C was used. On the basis of these studies on methods of testing combining ability lines C and D would be about equal, and the percentage hybrid plants must have been high to have given yields significantly above the Mennonite check.

The results with seed from line E are interesting. Very likely little crossed seed was produced as the result of open pollination in the polycross plot, since it had almost completed flowering when the other lines began. If most plants produced were selfs or sibs, the low yield and extreme

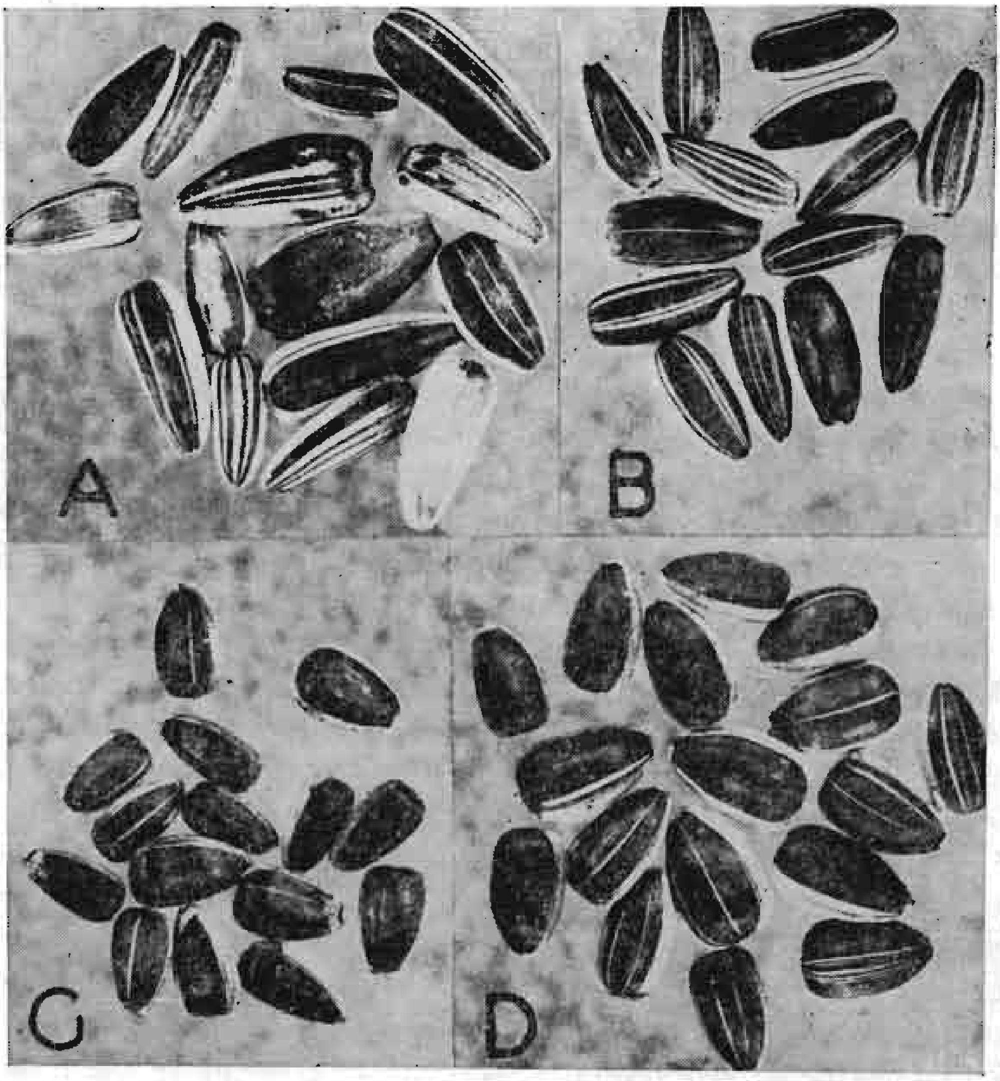

FIGURE 4. A—Seed of Mennonite.
B—Seed of line C.
C—Seed of line F. (Sunrise).
D—Seed of the F_1 of C × F. Note plumpness and uniformity for shape and size. (About 1¼ actual size).

earliness would be accounted for. The hand pollinated plants were the later flowering plants in the line. More crossed seed was probably produced and delay in flowering date and higher yield likely were due to the presence of hybrid plants in this treatment. Also, since this line is not uniform the plants used for hand pollinations may have carried genes for lateness.

The results of lines F and G in these studies can be discussed together. Both of these lines are highly self-fertile as was indicated by the low percentage crossing of the various combinations when these lines were used as the female parents. The low yields from these studies, without a knowledge of the self-fertility of these lines, would indicate that they had low general combining ability. However, the test of single crosses showed that the highest yielding single crosses had either F or G as the male parent. Low percentages of crossing in the seed whether produced in the polycross plot or by hand pollination was, undoubtedly, the cause of the depressed yields.

From the single cross test and these latter inadequate studies, it appears that the best method to test inbred lines efficiently for combining ability would be to use two tester strains in a modified top cross. Examples of two such lines could be C and F. Inbreds having as low self-fertility as line C could be tested as female parents with line G. Those as highly self-fertile and as pollen productive as line F could be tested as male parents with line C. The possibility of using the hand pollination technique in material that has been inbred for 3-4 generations would make such a system quite rapid and efficient provided the testers used have a wide enough range of good general combining ability.

SUMMARY

1. Single crosses significantly outyielding the Mennonite check, and highly uniform for desirable morphological characteristics have been produced by hand pollinations and in natural crossing plots.

2. Single crosses with high percentage of crossed seed and good specific combining ability gave the highest yields. Low yields were caused by poor specific combining ability, low percentages of crossed seed or both of these factors.

3. Good combining ability resulted in marked heterosis of the hybrid plants as manifested by greater height and general plant development.

4. Percentage crossing of 60 or over was adequate to give high yields in crosses the parents of which possessed good combining ability.

5. Testing the possibility of using heavier seeding rates of combinations having good specific combining ability, but crossing percentage below 60 per cent, has been suggested.

6. The minimum amount of line F in a crossing plot of F and C to still give high crossing on to line C should be ascertained.

7. To enable determination of percentage crossing in the F seed from a crossing plot of F and C (Advance) in the seedling stage, it has been suggested to transfer the purple pigment character from line B to C by the backcross method of breeding using line C as the recurrent parent.

8. The data on seed characters further showed the improvement in oil content, plumpness, size and uniformity of shape of the hybrids having lines F or G as one of the parents. These hybrids, in addition, had seed immune to sunflower moth damage.

9. With the exception of hybrids involving line E, all were earlier than either parent entering the cross. When E was one of the parents, the flowering dates were closer to that of line E than to the other parent.

10. The results on yield and seed characters clearly indicated that where lines had been inbred for 3-4 generations, reciprocal hand pollinations using two plants of a line gave reliable information regarding the yield and other characters of a combination.

11. The preliminary studies on methods of testing for combining ability indicated that the use of the polycross would, at best, be limited to the testing of fairly self-sterile lines, and that in these it would be impossible to distinguish those where general combining ability was fairly similar.

12. A method of using two tester lines of good general combining ability is suggested. Fairly self-sterile lines should be tested as female parents with a good pollen producing tester line. Lines as self-fertile as F should be tested as male parents with the self-sterile tester line.

ACKNOWLEDGMENTS

The writer wishes to express his thanks and appreciation to Dr. W. J. White, Officer-in-Charge, Dominion Forage Crops Laboratory, Saskatoon, for the generous guidance, the many helpful suggestions throughout the study and for reading and criticizing the manuscript. The technique of hand pollination used throughout the study was developed by Mr. E. D. Putt. The writer wishes to express his thanks and appreciation to members of the staff who have at various times helped with the field work and note-taking.

Thanks are also due to the Superintendent of the Forest Nursery Station, Sutherland, Sask., and to the farmers who co-operated in providing space for the various isolated crossing plots.

REFERENCES

1. Hayes, H. K. and I. J. Johnson. The breeding of improved selfed lines of corn. Jour. Am. Soc. Agron., 31 : 710-724. 1939.
2. Jagodkin, I. G. Application of method of inbreeding and diallel crossing in sunflower growing. Selektsija i Semenovodstvo. No. 1 : 21-27, 1937. Plt. Brdg. Abst., 8 : 65. 1937-1938. (Original not seen).
3. Johnson, I. J., and H. K. Hayes. The value in hybrid combinations of inbred lines of corn selected from single crosses by the pedigree method of breeding. Jour. Amer. Soc. Agron., 32 : 479-485. 1940.
4. Jenkins, M. T. The effect of inbreeding and selection within inbred lines of maize upon the hybrids made after successive generations of selfing. Iowa State College Jour. Sci., 9 : 429-450. 1935.
5. Jenkins, M. T., and A. M. Brunson. Methods of testing inbred lines of maize in crossbred combinations. Jour. Am. Soc. Agron. 24 : 523-530. 1932.
6. Morozov, V. K. Practical utilization of the phenomenon of heterosis in the sunflower. Semenovodstvo 4 : 69-72, 1934. Plt. Brdg. Abst., 5 : 143. 1934-1935. (Original not seen).
7. Putt, E. D. Observations on morphological characters and flowering processes in the sunflower (*Helianthus annuus* L.) Sci. Agric., 21 : 167-179. 1940.
8. Putt, E. D. Histological observations on the location of pigments in the akene wall of the sunflower (*Helianthus annuus* L.) Sci. Agric., 25 : 185-188. 1944.
9. Sprague, G. F. and L. A. Tatum. General vs. specific combining ability in single crosses of corn. Jour. Amer. Soc. Agron., 34 : 923-932. 1942.
10. ——————, ———. An estimation of the number of top-crossed plants required for adequate representation of a corn variety. Jour. Amer. Soc. Agron., 31 : 11-16. 1939.
11. Tysdal, H. M. and T. A. Kiesselbach. Hybrid alfalfa. Jour. Amer. Soc. Agron., 36 : 649-667. 1944.
12. ——————, ——————, and H. L. Westover. Alfalfa breeding. Coll. of Agric., University of Nebraska. Res. Bull 124. 1942.
13. Unrau, J. and W. J. White. The yield and other characters of inbred lines and single crosses of sunflowers. Sci. Agric., 24 : 516-528. 1944.
14. Wu, Shao-Kwei. The relationship between the origin of selfed lines of corn and their value in hybrid combinations. Jour. Amer. Soc. Agron. 31 : 131-140. 1939.