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Interest in the principles of alfalfa breeding has increased greatly with the growing "forage consciousness" of the country at large and the more specific interest developed through cooperation in the Alfalfa Improvement Conference. As is true of many other forage crops, information on the breeding behavior of alfalfa is meager. During the past few years, however, the genetics and principles of reproduction of this crop have come to be better understood and it is the purpose of this paper to bring together the available information pertaining to the improvement of alfalfa and to add certain suggestions with respect to future possibilities.

The new data herein reported were obtained through a cooperative alfalfa breeding program conducted jointly by the Nebraska Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Information will be presented on the amount and manner of natural crossing, the effects of inbreeding and hybridization, and the possible commercial utilization of hybrid vigor in alfalfa.

Some of the more important publications concerning alfalfa improvement previous to 1937 have been reviewed by Tysdal and Westover *(39)*.[3] Several papers have since appeared and these will be considered together with an examination of various methods of improvement such as mass selection and strain building, which can now be considered in the light of recent results.

OBJECTIVES OF BREEDING

A first requisite in any breeding program is to establish objectives. This was thoroughly done at the initial meeting of the Alfalfa Improvement Conference held at Lincoln, Nebraska, in 1934. This organization, composed of workers in the United States and Canada interested in the production or testing of new, improved alfalfas, has in recent years at the various conferences become a clearing house for information and materials connected with the alfalfa improvement program under way throughout the country. At these conferences it has been recognized that regional needs and conditions determine the type of alfalfa best suited to various parts of the country but that this did not preclude the desirability of developing an alfalfa adapted to a very wide region. It has also been emphasized that the number of improved alfalfas released should be held to the smallest number consistent with regional requirements, in order to minimize the difficulties of seed increase under isolation, and its distribution from producing to consuming areas.

That forage and seed yield, forage quality, and stand longevity are vitally affected by susceptibility to diseases, insects, winterkilling, and drouth was stressed at the first conference. Overcoming these weaknesses and at the same

[1] These studies have been conducted cooperatively by the Nebraska Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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[3] Numbers in parentheses are references to complete citations on pages 44 to 46.

time maintaining favorable genetic factors for yield and desired growth habit, are the chief aims of the alfalfa breeder. Interest is also manifest in the development of special-purpose alfalfas suitable for grazing, soil erosion control, and other special soil conditions. The degree of improvement attained by breeding will depend upon the success in removing heritable weaknesses and upon the superior genetic constitution with respect to favorable growth genes which lead to superior hybrid vigor. The diseases and insects of greatest concern are bacterial wilt (*Phytomonas insidiosa* McC.; Bergey *et al.*), leaf spot (*Pseudopeziza medicaginis* Lib.; Sacc.), leaf blotch (*Pyrenopeziza medicaginis* Fckl.), other leaf spots and stem blackening caused by *Ascocyta* spp., *Phoma* spp., and *Pseudomonas medicaginis* Sackett, pea aphids (*Macrosiphum pisi* Kltb.), and potato leafhoppers (*Empoasca fabae* Harris).

Aside from possible special-purpose alfalfas, such as for pasture, there appears to be definite need for at least three major types which may be designated regionally as northern, central, and southern. It is hoped that alfalfas may be developed that are suitable throughout each of these latitude belts. Throughout the northern region resistance to cold and wilt is very desirable. Because of the greater prevalence of leaf spot, leaf blotch, and leafhoppers east of Nebraska and the Dakotas, resistance to these diseases and pests is apparently of greater importance than west of these states. For this reason it may be advisable in the early stages of breeding to produce both a northwestern and a northeastern alfalfa. Stand longevity under the conditions where the alfalfa is grown is of prime importance in all of these regions. Since much of the east, because of generally unfavorable conditions for seed production, depends largely upon the central west and west for seed supplies, it would be very desirable eventually to grow in the central west and west an alfalfa eminently adapted to eastern conditions.

BASIC CONSIDERATIONS OF INTEREST IN BREEDING

Relation of Characters

Aside from forage yield at least four other economic characters are of paramount importance in an alfalfa. These are quality, longevity, seed productivity, and resistance to diseases and insects. Disease resistance, particularly to such a disease as bacterial wilt, is obviously correlated with stand longevity. Cold resistance is also related to stand longevity in northern latitudes. Numerous tests with existing varieties have shown that there is a slight negative correlation between cold resistance and yield. Thus, under the most ideal conditions, northern varieties do not yield as much as southern, nondormant, and relatively cold-susceptible varieties. Therefore the plant breeder must accept certain limitations, because fall dormancy constitutes a type of hardening in preparation for low temperatures. No evidence other than this general physiological response is at hand to show that disease resistance, stand longevity, and high yield cannot be combined in a single variety.

More research has been reported concerning the relation of quality and seed productivity to forage yield than concerning the relations between disease resistance and yield. Freeman (*14*), Hackbarth and Ufer (*17*), Fleischmann (*13*), Helmbold (*18*), and others found a slight negative correlation between forage yield and quality in alfalfa. High-yielding plants were taller, more upright, more sparsely leaved, and had thicker and more woody stems than low-yielding plants. These workers agree, however, that these characters do not show complete linkage and that among a sufficiently large number of plants it should be possible to find individuals with the desired combination of high quality and yield.

This opinion is further substantiated by observations in various current breeding programs. To obtain such individuals, rigid selection must be carried out in large populations. Lower crude fiber and higher protein content are among the more important characters involved in the quality of alfalfa. The protein content of alfalfa hay has been demonstrated to be closely correlated with the percentage of leaves. In recent investigations carried out at the Nebraska Station, it was determined that plants differ remarkably in leafiness even though in the same stage of growth. In one test in which the leaves were removed from the stems, dried, and weighed, the leafiness varied from 40 to above 70 per cent in different plants. In another comparative test conducted in 1940, samples were taken from different strains and varieties, and the leaf content was found to vary from 47.8 to 62.1 per cent.

In still another test during the summer of 1940, replicate samples were taken from field plots in each of three cuttings and the percentages of leaves determined during the following winter. On a dry-weight basis, the percentages of leaves averaged 40.6 for the first cutting, 53.2 for the second, and 46.0 for the third. Nineteen varieties and strains were represented in the comparison and these varied from 43.3 to 49.3 per cent leaves as an average of the three cuttings. Some of the alfalfas showed a uniformly high percentage of leaves for all cuttings, while others were uniformly low. This great variation in percentage of leaves between different plants and between different varieties and strains suggests the possibility of selection for increased leafiness and emphasizes the importance of quality as related to retention of leaves through selection for resistance to leaf diseases as well as increasing the percentage of leaves.

It is well known that fresh alfalfa hay is a valuable source of many vitamins. Vitamins A, B, E, G, and K are all found in this crop. Though no direct data are available on the variations in vitamin content of different varieties and strains, it may be assumed that differences exist and that future studies may include selection for higher vitamin content.

A recent paper by Smirnova (*31*) has indicated that the color of foliage in alfalfa may be an indication of the protein content, being higher in dark green specimens of *Medicago sativa* and *Trifolium pratense* than in light green specimens. The content of crude protein in the leaves of *M. sativa* was 21.23 and 29.43 per cent, respectively, in light green and dark green forms; corresponding figures for the stems were 7.75 and 9.13 per cent. The stability of the color character provided a criterion for easy selection of valuable forms. If this proves to be the case under all conditions, it might form a simple guide for selecting higher protein strains.

Hackbarth and Ufer (*17*), Fleischmann (*13*), and others have shown a low positive correlation between seed production and forage production, though it is often suggested that selection for high forage production may result in lowering the seed yields. In the Nebraska breeding nursery, consisting of several thousand spaced plants, each plant is harvested separately and yield of forage and seed determined. Inasmuch as many plants are retained for several years, it has been possible to correlate seed and forage yields of these plants within the same year, and between years. Results of such correlations are reported in Table 1. Fifteen separate correlations were calculated, seven between seed and forage productivity, four between seed productivity of the same plants in different years, and four between forage productivity of the same plants in different years. All correlation coefficients were positive and were significant as determined by the $P=0.05$ value. In general, the correla-

TABLE 1.—*Coefficients of correlation between seed, forage and seed, and forage yields of individual alfalfa plants over a period of three years, Lincoln, Nebraska.*

Correlating	n	r	Age of plants	Planting space
			Yrs.	*In.*
1938 seed with 1938 forage	894	+.4406	2	30 x 30
1938 seed with 1938 forage	109	+.2051[1]	1	30 x 30
1939 seed with 1939 forage	910	+.4948	2–3	30 x 30
1939 seed with 1939 forage	594	+.4827	1	15 x 30
1940 seed with 1940 forage	1244	+.6310	1	15 x 30
1940 seed with 1940 forage	761	+.4739	2	30 x 30
1940 seed with 1940 forage	169	+.4057	2	15 x 30
1938 seed with 1939 seed	639	+.5201	2–3	30 x 30
1938 seed with 1940 seed	537	+.3387	3	30 x 30
1939 seed with 1940 seed	655	+.4187	2–3	30 x 30
1939 seed with 1940 seed	301	+.2152	2	15 x 30
1938 forage with 1939 forage	637	+.6867	2	30 x 30
1938 forage with 1940 forage	537	+.5865	3	30 x 30
1939 forage with 1940 forage	679	+.7878	2	30 x 30
1939 forage with 1940 forage	280	+.6923	2	15 x 30

[1] $r = +.1886$ for P value of 0.05 when $n = 109$.
$r = +.2470$ for P value of 0.01 when $n = 109$.

tions of the forage production of the same plants from one year to the next were higher than those between seed and forage production of the same plants in the same year, which in turn were higher than those between seed production of the same plants from one year to the next. From these correlations it may be concluded that the plants producing greater forage are not necessarily deficient in seed production.

From the results of the correlations between seed production one year with that of the same plants the following year and also two years later (1938 with 1940), it seems clear that there is a tendency for the highest producing plants one year to be the highest in following years, but in some cases the correlation is rather low. The correlations between the forage productivity one year with the same plants the following year average consistently high, indicating more consistency in this character than in seed productivity. This also indicates that the determination of forage productivity would ordinarily be more reliable than the determination of inherent seed productivity.

The Genetics and Cytology of Alfalfa

It is highly desirable that the breeder understand the mode of inheritance of specific characters, the number of factors involved, and whether they are dominant or recessive. While such information would aid in the selection and recombination of characters, it is still comparatively meager and investigations along these lines should be pursued.

The inheritance of flower color, leaf shape, and stem structure have been studied by Korohoda (*28*), and of black and white seed coat by MacVicar (*29*).

Peltier and Tysdal (*30*) have suggested that three major genetic factor pairs for resistance to the wilt disease may explain their results with inbred lines, though they did not consider this conclusive. It was also concluded that the inheritance of cold resistance is due to a number of factors as no distinct factorial segregation was found. Brink, Jones, and Albrecht (*3*) conclude that "resistance to bacterial wilt in alfalfa behaves in inheritance as an intergrading character and probably rests upon a complex genetic basis. A factorial interpretation is at present impossible." Armstrong and Gibson (*1*) and Burton (*6*) have reported concerning the inheritance of a number of morphological characters.

Many workers, including Dann (*12*), Tysdal (*35*), Carlson (*8*), and others have found that self-fertilization results in much less seed production per flower compared to cross-fertilization of comparable flowers. Kirk (*27*) has found a high degree of natural self-tripping and self-fertility in certain autogamous plant selections and their derived selfed lines and F_1 hybrids. These plants produce relatively high yields of seed in the absence of insects or artificial tripping.

Recent contributions on the cytology and embryology of alfalfa have been made by Cooper (*9*), Cooper, Brink, and Albrecht (*10*), Cooper and Brink (*11*), and Brink and Cooper (*4*). They have pointed out the differential pollen-tube growth of self- and foreign-pollen, the partial incompatibility of self-pollen, and the far greater abortion of embryos following self-fertilization as compared with embryos developed from cross-fertilization. Armstrong and White (*2*) have studied the pollination mechanism of alfalfa. It was their conclusion that "in the act of tripping, the stigmatic surface is ruptured and the released stigmatic content initiates pollen germination. Rupturing of the stigmatic surface is essential to penetration by the pollen tubes. Pollen germination occurred in 84 per cent of the tripped flowers and in less than one per cent of the untripped flowers examined."

Brink and Cooper (*4*) agree with the theory of the stigmatic membrane, but suggest that under certain environmental conditions the view that the membrane constitutes an absolute block to effective pollination cannot be accepted. Results in Wisconsin in 1936 showed that 12 per cent of the flowers were tripped, whereas 34 per cent of the flowers set pods, indicating, contrary to Armstrong and White, that there is considerable seed setting without tripping. Carlson (*8*) has also reported that alfalfa may set seed rather freely without tripping of flowers. On the other hand, the results of an extensive cooperative study (*35*) suggest that seed setting without tripping is the exception rather than the rule and that for profitable seed production under usual conditions, tripping by insects is necessary. Regardless of the divergence in results indicated above, all workers agree that cross-pollination (including tripping) invariably results in an increase in seed production over comparable untripped flowers, thus substantiating the conclusion that tripping is beneficial for seed production.

Fryer (*15*) has reported the chromosome numbers for 17 of the more common species of alfalfa. The somatic number for eight of these is 32, while 16 and 14 are typical of six and three species, respectively. The chromosome numbers of additional *Medicago* species are given in the appendix of the article by Tysdal and Westover (*39*). All of the standard varieties grown in this country have a somatic number of 32 and are readily intercrossed. The cooperative breeding at the Nebraska Station involves the five species: *M. sativa, M. falcata, M. media, M. gaetula,* and *M. glutinosa,* all with 32 chromosomes. Since many *Medicago* species have 16 chromosomes it appears not improbable that those with 32 are tetraploid. Moreover, a review of the inheritance studies indicates that, in many cases at least, the genetic ratios obtained fit the expected segregation in an autotetraploid better than the complicated disomic ratios reported, and tetrasomic ratios often explain those cases for which no satisfactory disomic ratios were found. To illustrate, in a study of inheritance of leaf shape in alfalfa, Korohoda (*28*) reported an F_2 segregation from a cross between *Medicago sativa* and *M. falcata,* in which the shape typical of *M. sativa* was observed in 28 plants, typical of *M. falcata* in 196 plants, and intermediate in 424 plants. He found it impossible to explain the results on the commonly accepted diploid inheritance assuming two, three, or four genetic factors. When, however, these results are analyzed on the assumption of a single-factor difference in an autotetraploid as illustrated below, a very close fit is obtained between the observed and the calculated ratios. In this explanation it is assumed that there is random chromatid assortment and that the triplex (*AAAa*) gives the same phenotype as the quadruplex (*AAAA*), and the duplex (*AAaa*) and simplex (*Aaaa*) give the intermediate type. The calculations are illustrated as follows:

Inheritance of Leaf Shape (Observed data from Korohoda)

Parents	*AAAA* x *aaaa*		
F_1	*AAaa*		
F_2	1 *AAAA*, 5.3 *AAaa*	9.1 *AAaa*, 5.3 *Aaaa*	1 *aaaa*
	6.3	14.4	1
Observed	196	424	28
Calculated	188.3	429.9	29.8

$X^2 = 0.554$

P value between 0.70 and 0.80.

From this evidence it seems highly probable that common alfalfa is a tetraploid. Some evidence indicates it may be an autotetraploid, though it has many allotetraploid characteristics and therefore may be intermediate between the two types. It is hoped to establish its cytogenetic constitution by appropriate genetic and cytological tests now under way.

The Amount of Natural Crossing in Alfalfa

Correct breeding procedure requires a knowledge of the mode of pollination, whether self- or cross-fertilized, and, if both, the extent of each, as well as the behavior of the crop when inbred or crossbred.

Waldron (40) reported at an early date that in North Dakota there was 42.7 per cent crossing between the purple- and yellow-flowered alfalfa, the yellow flowered being the female parent; 7.5 per cent crossing was found in the reciprocal, the purple being the female parent. It seems apparent, however, that crossing within the purple or yellow flowered strains could not be detected, which might explain, in part at least, the low percentage of crossing found.

The amount of natural crossing in alfalfa was found by Burkart (*5*) to be from 67 to 98 per cent in Argentina with an average of 84.5 per cent crossing.

For a number of years information has been sought regarding the amount of natural crossing under Nebraska conditions. In a controlled test to determine the prepotency of foreign pollen, yellow- (*M. falcata*) and purple- (*M. sativa*) flowered alfalfas, growing in the greenhouse, were subjected to self- and foreign-pollen simultaneously. In this test, pollen from the yellow-flowered plant was gathered on the end of a toothpick upon which were snapped the stigmas of a purple-flowered plant. Since the stigmas were surrounded by their own pollen, there would be applied in this manner both self- and foreign-pollen at the same time, and since the plants had different flower colors, the hybrids could be distinguished from the selfed progeny. As an average of five different progenies, 97 per cent of the seed proved to be hybrid. Reciprocal crosses gave similar amounts of cross-pollination. These results would seem to indicate a differential growth of pollen tubes and fertilization in favor of the foreign pollen. This conclusion is in agreement with the histological and embryological findings of Cooper, Brink, and Albrecht (*10*).

Several methods of studying natural crossing in the field have been used. In 1936 a yellow-flowered *M. falcata* plant was exposed to crossing with *M. sativa* under natural field conditions in western Nebraska. Of a total of 479 progeny plants, 96.7 per cent were hybrid.

The same year six inbred lines were exposed to cross-pollination with other lines under natural field conditions. A progeny of 303 plants from open-pollinated seed was grown in a nursery from 1937 to 1940 in comparison with controlled hybrids, self-fertilized progenies of these six lines, and standard varieties. Throughout this period there has been a clear-cut distinction in size and yield between the inbred and hybrid plants. When the open-pollinated group were conservatively classified, it was found that 83 per cent of the plants were hybrid.

In 1939 a white-flowered, white-seeded plant of *M. media* was grown in a nursery and allowed to develop seed under natural conditions. It had previously been determined that this plant was average or above average in self-fertility and that upon selfing the plant produced only white-flowered progeny. The 154 plants resulting from seed produced on the open-pollinated white-flowered plant showed 87.6 per cent cross-pollination. A similar white-flowered plant was grown 100 feet distant from plants with purple blossoms. Of a progeny of 30 from this plant, 28 (93 per cent) have shown cross-pollination.

As an average of the three tests of seed produced under conditions of open-pollination, 89.1 per cent natural crossing was found.

While these percentages are somewhat higher than previously reported for natural crossing in alfalfa, they are probably valid estimates. This conclusion seems even more certain upon consideration of the evidence from: (1) the decline in vigor after self-fertilization, (2) the mode of pollination, and (3) the necessity for tripping *(2, 41)*. It should be remembered, however, that the self-fertility of the plants and the environmental conditions would undoubtedly have a bearing on the amount of natural crossing at any one time. The effects of inbreeding will be shown to be those ordinarily associated with a highly cross-fertilized crop. The mode of pollination has been studied by a number of workers, and though they are not all agreed on the importance of tripping, recent studies (*35*) indicate that tripping of the flowers by insects is a major factor in the production of commercial seed (Figs. 1 and 2). Insect visitation, chiefly by species of *Megachile, Paranomia, Bombus, Andrena, Apis,* and possibly others such as *Chauliognathus,* is very effective in bringing foreign pollen to the stigma, and, as has been shown, the foreign pollen is usually the effective agent in fertilization. Thus the high percentage of crossing may be accounted for largely by insect carriers of pollen.

Performance of Selfed Lines

Several workers have published on the behavior of alfalfa when self-fertilized, although most of the data are on seed productivity rather than on forage productivity. Kirk (*26*) has given the forage yields of a number of

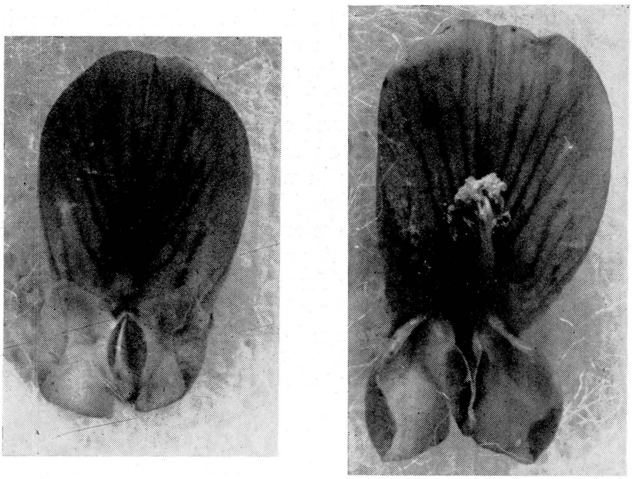

FIG. 1.—LEFT: a single alfalfa flower in full bloom. RIGHT: a "tripped" alfalfa flower showing the male and female parts (stigmatic column) liberated from the keel and pressed against the standard petal. The flowers are borne in racemes as shown in Figure 2.

FIG. 2.—Insects cause most of the tripping of alfalfa flowers, which is necessary for good seed setting. Three types of bees are illustrated showing their manner of visiting alfalfa flowers to gather nectar and pollen. At the top is the bumble bee (*Bombus* sp.) which because of its weight and manner of visitation trips from 30 to 80 per cent of the flowers visited.

In the center is a wild bee (*Melissodes* sp.), which is similar to the common *Megachile* sp., both being very effective, tripping 80 to 90 per cent of the flowers visited. Note that this bee has already tripped a flower at the left, and that it enters the flower in front of the keel, thereby insuring cross-pollination by contact of the stigma with parts of the bee laden with foreign pollen.

At the bottom is a honey bee whose proboscis is inserted at the side of the keel to gather the nectar. In this way very little tripping results, about one per cent of the

flowers visited. If there are a large number of honey bees present, however, they may do considerable tripping by repeated visits.

lines selfed one, two, three, and four generations in comparison with the yield of Grimm. Taking the forage yield of Grimm as 100, the yields of the S_1, S_2, S_3, and S_4 lines were 84, 66, 60, and 54 per cent, respectively. Considering the seed yield of Grimm as 100, the inbred lines averaged 62, 30, and 22 per cent for one, two, and four generations of selfing, respectively.

Torssell (*34*) found much lower seed yields in self-fertilized lines, the relative yields being as follows: open-pollinated, 100 per cent; S_1, 35 per cent; S_2, 27 per cent; and S_3, 12 per cent. Williams (*43*) reports a seed yield of only 12 per cent of the original open-pollinated parent plants after one generation of self-fertilization. Carlson (*7*) found that S_1 lines produced 50 per cent as much seed as the open-pollinated parent, and the S_2 lines 52 per cent of the open-pollinated parent.

Selfing by artificial manipulation of flowers has been included for a number of years as a part of the cooperative alfalfa breeding program in Nebraska. While most of the lines have been selfed for only one or two generations, a

FIG. 3.—Alfalfa nursery at the Nebraska Agricultural Experiment Station at Lincoln. In the foreground are the solid-planted rows of the Cooperative Uniform Alfalfa Nursery test. Nurseries similar to this, containing many new and improved strains, are established in over 40 states. In the background is the space-planted test of inbreds, hybrids, and open-pollinated varieties.

few have been carried into the seventh and eighth generations of inbreeding. A number were tested in comparison with other alfalfa in space-planted nurseries, the rows being 27 inches apart and the plants separated by 18 inches in the row (Fig. 3). The tests included (1) self-fertilized lines, (2) hybrids between these lines, (3) natural outcrosses of these lines, (4) the standard varieties, Grimm, Ladak, and Hardistan, the latter belonging to the Turkistan group. These varieties included those from which practically all of the inbred lines originated. A few of the lines were of Cossack origin. The majority, however, originated from Turkistan and Hardistan. In no case were entries with less than ten plants considered in the results, and in most instances, 30 to 60 plants formed the yield basis. Nurseries were planted in 1936, 1937, and 1938. In all cases two years' yields were obtained by cutting individual plants and weighing them immediately afterwards, at which time the percentage of moisture was fairly uniform for all plants.

The results shown in Table 2 were obtained by averaging the forage yields of the various inbreds and calculating their individual yield in terms of the yield of the three standard varieties, Grimm, Ladak, and Hardistan, grown in the same tests. Similarly the yields of open-pollinated seed from the inbreds were obtained for one year in the same nurseries and these are also reported in this table.

It is clear that both forage and seed productivity are reduced by self-fertilization. As an average of 54 S_1 lines, the yield of forage was 68 per cent of that of the original open-pollinated varieties, while the yield of seed was 62 per cent of that of the original varieties, the original varieties being considered 100 per cent in each case. The forage yield of succeeding generations decreased until it reached a minimum of 26 per cent in the seventh generation of inbreeding, at which point it seemed to level off somewhat, as in the eighth generation

TABLE 2.—*Yields of self-fertilized lines of alfalfa in per cent of the parental open-pollinated varieties, Grimm, Hardistan, and Ladak; average of two years, 1938–39, Lincoln, Nebraska.*

No. of selfed generations	No. of lines tested	Actual yield in % of original parents		Theoretical yield	
		Forage	Seed	Forage	Seed
		%	%	%	%
1	54	68	62	68	62
2	17	48	39	52	43
3	9	59	38	44	33.5
4	13	51	36	40	28.8
5	1	41	29	38	26.4
6	. .	. .	. .	37	25.2
7	1	26	15	36.5	24.6
8	4	28	8	36.3	24.3

there was no further reduction in the yield of the lines. The seed productivity of selfed lines decreased even more drastically than the forage productivity, reaching a yield of 15 per cent of the open-pollinated varieties in the seventh generation and only 8 per cent in the eighth generation of inbreeding.

The theoretical forage yields, which are also included in Table 2, were derived by (1) regarding the average yields of the original open-pollinated varieties, Grimm, Hardistan, and Ladak, as 100 per cent; (2) determining that the yields of 54 first-year selfed lines averaged 68 per cent as great, giving a reduction of 32 per cent; (3) assuming that the yield reductions would lessen by one-half in each succeeding generation. For example, subtracting one-half of 32 or 16 from 68 gives an expected yield of 52 per cent for lines that have been selfed for two generations. The theoretical seed yields were calculated in a similar manner.

These actual and theoretical yields for successive generations are presented in graphic form in Figure 4. The actual forage yields deviate somewhat from the theoretical curve, the earlier generations showing greater productivity, while the later generations show less productivity than the theoretical. The

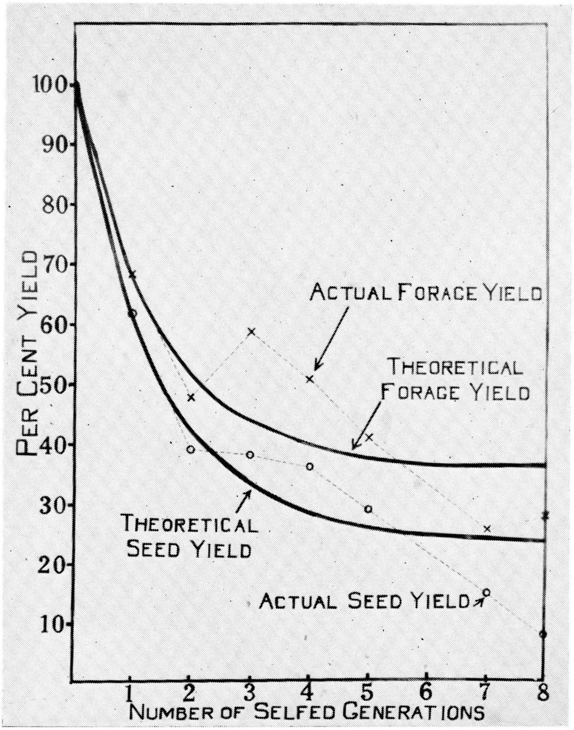

FIG. 4.—Actual and theoretical forage and seed yields per acre from alfalfa that has been subject to self-fertilization for one to eight generations. Data taken from Table 2.

curve, however, fits the data about as well as any that could be constructed, three of the yields of the advanced generations being above and three below the curve. The seed yields fit the theoretical curve somewhat better than the forage yields, with the exception of the eighth generation. In both forage and seed yields of the seventh and eighth generation, the actual yields are lower than the theoretical. This might be because the few lines that were tested were unusually poor. The yield trend of continued selfing in alfalfa is, however, unmistakable.

To give an exact curve of reduction in yield caused by inbreeding in alfalfa would be difficult, as this would necessitate avoidance of any kind of selection. Obviously, selection is practiced among the inbred lines in a breeding and improvement program, and the results herein presented are subject therefore to whatever bias may have resulted from such selection. In some cases, lines were carried for the purpose of simply determining the reduction in vigor rather than for selective purposes. Other lines were discontinued because of decline in seed yield. To indicate the wide range in forage yield of selfed lines, it may be pointed out that the forage yields of S_1 lines varied from 26 to 105 per cent of the original varieties. It should also be pointed out that the polyploid condition in common alfalfa would probably influence the rate of increase in homozygosity upon selfing.

Seed yield is even more variable in selfed lines than forage productivity. Some lines in fairly advanced generations showed increased productivity over the original parent, while others decreased very rapidly. This divergence might be attributed, at least to some extent, to the variability of seed setting in alfalfa in general, including partial or even complete incompatibility, and also, perhaps, to the conditions under which the test is made. Those lines selected for high self-fertility, as autogamous lines, for example, might produce unusually well under conditions of limited cross-pollinating insect activity. The varietal origin of the selfed lines no doubt also plays an important part. Selfed lines from Turkistan origin do not seem to decrease as rapidly as those from Grimm or Ladak origin. It appears that in general the Turkistan group is more homozygous than alfalfas of hybrid origin such as Ladak and Grimm, and it may be that the diversity of origin would produce a greater range and possibly a different type of curve in yields of inbred progenies. Further, when a given plant is chosen for selfing from a mass population, there is no way of knowing whether it resulted from cross- or self-fertilization. The general unanimity, however, in the results of various workers with alfalfa tends to substantiate the principle of a progressive lowering of yield in response to continued inbreeding, as shown in Figure 4, until a state of homozygosity is reached.

Performance of Hybrids

During the progress of the alfalfa breeding program, a number of single crosses between inbred lines have been made by controlled emasculation[4] and subsequent pollination (Fig 5). These hybrids were tested in the above-described space-planted nursery in comparison with their inbred parents, the open-pollinated progeny of the inbred parents, and the standard varieties, Hardistan, Ladak, and Grimm.

The yields of 28 hybrids in grams of green forage per plant, together with the average seed production per plant, are given in Table 3. A large number of hybrids were not included in this table because of an insufficient number of plants upon which to base the yield. All hybrids listed in the table had more than 10 plants, the majority having from 30 to 60. The forage weights are an average for the two years, 1938 and 1939. Seed yields were obtained for the one year, 1939. The technique followed in this unreplicated, space-planted nursery does not permit a very refined interpretation of individual

[4] Emasculation by the suction method has been found to give inadequate control in highly self-fertile material. Sterilization of the pollen by immersing untripped flowers, in the full bloom stage, in 57 per cent ethyl alcohol for 10 seconds has given complete control (*36*) without serious injury to the female organs. The alcohol trips the flowers, which may be pollinated almost immediately after treatment. The strength of the alcohol and period of treatment may need to be varied slightly under some conditions.

yields. The degree of variability is suggested by the green-forage plant yields of the five individual plots of the Grimm check distributed throughout the nursery, which were as follows: 1,263, 1,225, 1,148, 1,168, and 1,056 grams.

Table 3 shows a wide range in yields of the hybrids. Considering the forage productivity, the average of all the hybrids was remarkably close to the average of the checks, being 1,235 grams per plant, compared with 1,289

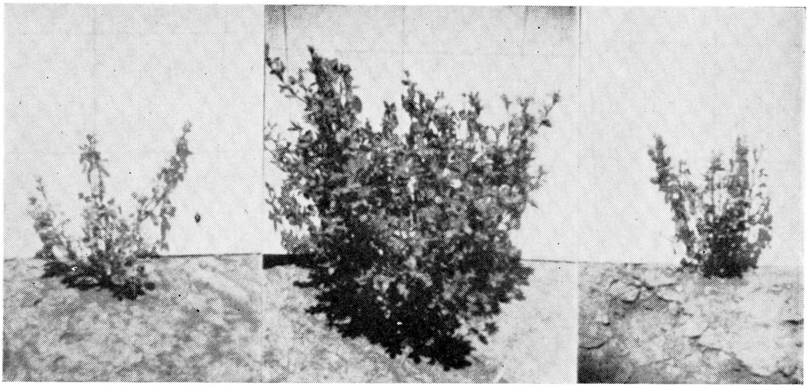

FIG. 5.—Representative plants of two inbred lines and their F_1 single cross. LEFT TO RIGHT: 69137 P-1 female parent, hybrid (No. X28), and 3–702, male parent. Their respective row yields over a two-year period averaged 628, 1797, and 337 grams of green forage per plant. Their seed yields were 0.15, 6.32, and 1.55 grams per plant, respectively. The camera was placed the same distance from each plant.

grams for the checks. On the other hand, the yield of the highest-producing hybrid exceeded the average yield of the checks by 39 per cent, and was higher than any single plot of a check variety. The average of the top ten hybrids was 15 per cent above the yield of the checks.

In a comparison of the hybrids in the field it was clear that certain ones showed a decided amount of hybrid vigor, while others were lacking in this respect, indicating that the combining ability of some of the lines was very good, while relatively poor for others (Fig. 6). It was also observed that a cross between two lines, A and B, for example, produced a high yielding hybrid, while a cross between A and C was mediocre, even though C appeared to be a better line than B.

The average of the ten hybrids producing the greatest amount of seed exceeds the average of the checks by 69 per cent, but the forage production of the same ten hybrids was only 82 per cent of the standard varieties. The seed yield was relatively low for a number of the hybrids in which a selected plant of *M. falcata* was one of the parents. Table 4 presents both the forage and seed yields for one F_1 hybrid and its inbred parents during the two years, 1938 and 1939, in this same nursery. These data suggest that forage and seed yields of an F_1 hybrid may exceed those of standard, open-pollinated varieties.

Since yields were obtained for both the inbred lines and their open-pollinated (chiefly outcrossed) progenies, as well as for hybrids between these lines, it is possible to compare their behavior. In addition to yields of seed and

TABLE 3.—*Forage and seed yields in grams per plant of F_1 single-cross hybrids compared with Hardistan, Ladak, and Grimm checks. Forage yields are averages of two years 1938–39 and seed yields for one year, 1939, Lincoln, Nebraska.*

Hybrid No.	Yield per plant		Relative to the average of the checks as 100	
	Green forage	Seed	Green forage	Seed
	gm	*gm*	%	%
X28	1797	6.32	139	62
X78	1652	6.38	128	62
X32	1571	7.72	122	75
X34	1517	7.69	118	75
X70	1431	2.77	111	27
X82	1400	10.76	109	105
X69	1367	1.87	106	18
X68	1364	4.01	106	39
X65	1355	3.08	105	30
X67	1348	2.36	105	23
X83	1325	8.99	103	88
X58	1307	16.95	101	165
X71	1280	1.26	99	12
X72	1258	5.67	98	55
X24	1249	19.33	97	189
X66	1230	3.28	95	32
X16	1217	26.34	94	257
X77	1151	4.39	89	43
X81	1145	12.52	89	122
X80	1102	9.87	85	96
X62	1071	21.89	83	214
X61	1021	14.53	79	142
X13	977	19.27	76	188
X73	974	9.96	76	97
X60	932	12.93	72	126
X75	907	18.09	70	176
X76	855	9.16	66	89
X74	778	11.09	60	108
Hardistan	1268	9.74	98	95
Ladak	1436	5.43	111	53
Grimm	1164	15.58	90	152
Averages of:				
All hybrids	1235	9.94	96	97
Checks	1289	10.25	100	100
Top ten hybrids in forage	1480	5.30	115	52
Top ten hybrids in seed production	1060	17.29	82	169
Inbred parental lines	646	5.03	50	49
Naturally outcrossed lines	1230	10.95	95	107

forage, detailed observations were made on two diseases, leaf spot *(Pseudopeziza medicaginis)* and black stem *(Ascochyta imperfecta)*, and also habit of growth, *i.e.*, whether prostrate, spreading, or upright growth. Observations on the diseases and habit of growth were recorded on a scale from 1 to 10 as recommended by the Alfalfa Improvement Conference.

Correlations were calculated between the performance of the inbreds and the hybrids, between the hybrids and the outcrossed progeny of the inbreds, and also between the inbreds and the outcrossed progeny of the inbreds. In the correlations between the inbreds and hybrids, the average performance of the two inbred parents was compared with that of the single-cross hybrids.

FIG. 6.—Heterosis shown in the nursery, planted in 1941. Both rows in the center are hybrids. Of these, the one on the left (No. 87–294) resulted from crossing two high combining lines while the one on the right (No. 97–553) shows only mediocre hybrid vigor. The latter yielded almost exactly the same as the average of the commercial varieties Grimm, Hardistan, and Ladak, included in the same test, while the former yielded over 40 per cent more.

Such correlations were calculated for five characters: forage productivity, seed productivity, leaf spot, black stem, and habit of growth. The correlations are given in Table 5. The correlation coefficient between the forage yield of the hybrids and their inbreds is negative but not significant. When it is recalled that many of these inbreds were still fairly heterozygous, the negative correlation may be partially explained because some of the higher-yielding heterozygous lines, when crossed, may not produce as good hybrids as low-yielding homozygous lines. The significant positive correlation obtained between the hybrids and the outcrossed progeny of the inbreds is of interest. This correlation of +.3476 is suggestive of a possible use of the outcrossed seed produced in a selection nursery for testing the combining ability of large numbers of

TABLE 4.—*Yields of seed and forage of a single cross, F_1 compared with that of the inbred parents, open-pollinated inbred parents, and standard varieties. Average of two years 1938 and 1939, Lincoln, Nebraska.*

Kind of alfalfa	Actual yields per plant[1]		Relative yields per plant	
	Green forage	Seed	Green forage	Seed
	gm	*gm*	%	%
Varieties:				
Ladak	1436	5.43	..	..
Hardistan	1268	9.74	..	..
Grimm	1164	15.58	..	..
Average	1289	10.25	100	100
Inbred lines from:				
(a) Old Nebraska field[2]	643	3.47	..	..
(b) Falcata x Turkistan cross[3]	857	2.33	..	..
Average	750	2.90	58	28
F_1 hybrid (a) x (b)	1400	10.76	109	105
Open-pollinated lines:				
(a)	1114	15.53	..	..
(b)	1483	15.97	..	..
Average	1299	15.75	101	153

[1] Yields of 1,000 grams green weight per plant are the equivalent of 3.35 tons of hay (15% moisture) per acre, while ten grams of seed per plant equals 3.17 bushels per acre.
[2] One year selfed.
[3] Two years selfed.

inbreds. Also of considerable significance are the high positive correlations of seed productivity in all comparisons. This indicates that selection for seed productivity in the inbred lines might also result in higher seed producing hybrids.

The problem of inheritance of reaction to foliar diseases is of interest to alfalfa breeders. The observations among inbred lines indicate distinct differ-

TABLE 5.—*Coefficients of correlation between hybrids, their inbred parents, and progeny of the open-pollinated (out-crossed) inbreds for five characters. Forage yields were the averages of two years, 1938 and 1939. Seed yields were obtained in 1939 and the disease notes and habit of growth observations were obtained on individual plants in 1938. All results were taken from the hybrid alfalfa nursery, planted in 1937, Lincoln, Nebraska.*

Character correlated	Number	Correlations between		
		Hybrids and inbreds	Hybrids and progeny of open-pollinated inbreds	Inbreds and progeny of open-pollinated inbreds
Forage	42	—.1643[1]	+.3476	+.2235
Seed	40	+.5581	+.4467	+.5785
Leaf spot	42	+.2084	+.3038	+.3021
Black stem	42	+.2916	+.3305	+.3222
Habit of growth	42	+.4076	+.4679	+.5565
Average		+.2603	+.3793	+.3966

[1] $r = +.3044$ for P value of 0.05 when $n = 42$.

ences with respect to their susceptibility to leaf spot, black stem, and also mildew. The evidence, while not conclusive, supports the theory that selection within inbred lines may result in the production of more resistant hybrids. A significant positive correlation is shown between the behavior of the inbreds and their outcrossed progenies with respect to black stem and leaf spot. This is also true of the relation between hybrids and the outcrosses of the inbreds entering the hybrid combinations. The correlation of the disease infection of the inbreds and their hybrids is positive but not as significant as in the other two groups. The habit of growth is highly correlated in all three groups, indicating that selection for habit of growth in the inbred line will produce a somewhat similar habit of growth in the hybrids.

A study of second-generation hybrids involving three cuttings was made in 1939. It was found that in comparison with the average of the three standard check varieties as 100 per cent, the mean forage yield of four inbred lines was 55 per cent; two F_1 single crosses between them, 96 per cent; and their F_2 progenies, 41 per cent. This test is of special interest in paralleling the experience with corn as to the striking reduction in the F_2. The reduction here indicated is extreme but it may serve to illustrate an important principle.

In a top-cross, *i.e.* a cross between an S_6 and a selected *M. falcata* clone, not inbred, the F_2 generation (seed of which was produced in an isolated block by roguing all pure yellow- and pure purple-flowered plants) produced 3.12 tons of hay per acre as an average of three years' trials in an advanced-nursery replicated test. The open-pollinated progeny of the inbred produced 2.92 tons per acre in the same test, and the progeny of the other parent produced 3.19 tons per acre. Thus the two parents averaged 3.06 tons per acre against the F_2 yield of 3.12. No comparable yield of the S_6 is available but in a space-planted nursery, the selfed line yielded 64 per cent of its original parent. In the F_2 of this cross, therefore, no appreciable reduction in vigor occurred, even though one of the parents was an inbred line. This would probably not be true of all such hybrids.

Variability of Inbred Lines, Hybrids, and Varieties

Since individual plant records were kept on the yield of forage and seed, leaf spot, black stem, and habit of growth of the inbred lines, hybrids, outcrossed progenies of the inbred lines, and the standard varieties grown comparably in these nurseries (Table 3), it was possible to calculate standard deviations and coefficients of variability for these characters. These are given in Table 6 for the five plant characters for each of the four alfalfa groups differing in heterozygosity. As measured by standard deviation, the commercial varieties and line-outcrosses were distinctly the most variable as to forage and seed yield. The inbreds were lowest and the F_1 hybrids were intermediate in standard deviation for both of these characters. Based on the coefficient of variability, however, the value for the inbreds is relatively high because the inbreds had low average yields.

The results for the two foliar diseases are of interest in showing somewhat less variability for the hybrids and inbreds than for the outcrossed progenies and the original varieties. This is particularly true with respect to leaf spot.

The variability of growth habit is least in the hybrids and most in the inbreds. The only explanation that can be offered for the high standard deviation of the inbreds is that probably some of them were still segregating for habit of growth. Other breeding evidence has indicated that inheritance of habit-of-growth character may not be a simple one, resulting in continued segregation in later generations.

TABLE 6.—*The standard deviations and coefficients of variability of inbred lines of alfalfa compared to hybrids, varieties, and outcrossed progenies of the inbred lines of each of the five characters: forage and seed productivity, leaf spot and black stem occurrence, and habit of growth, Lincoln, Nebraska.*

Character	Forage		Seed		Leaf spot		Black stem		Growth habit	
	S.D.	C.V.	S.D.	C.V.	S.D.	C.V.	S.D.	C.V.	S.D.	C.V.
Original varieties	412	37	6.37	71	1.19	83	0.86	95	1.03	17
Outcrosses	411	39	7.34	81	1.23	105	1.02	116	1.26	21
Hybrids	372	31	4.96	71	0.96	67	1.04	97	1.05	17
Inbreds	257	41	4.09	96	1.04	84	0.81	108	1.40	30

Considering the data as a whole, it appears that selfing has reduced the variability with respect to leaf spot reaction and also forage and seed productivity of the inbred lines and hybrids as compared with the outcrossed progenies of the inbreds and the commercial varieties. The decrease in variability of the disease reactions is of particular interest, and shows that selection tends toward greater uniformity in this respect and suggests the possibility of selection within inbred lines for resistance to these diseases.

IMPROVEMENT METHODS

Since the available data show rather clearly that the genetic principles applying to alfalfa are much the same as for corn, it would seem most promising in an alfalfa improvement program to follow the highly successful procedure with corn as closely as the difference in flowering habit will permit. The breeding procedure might be almost identical, but the manipulation of pollination and the mechanics of commercial seed production will necessarily differ. Until male-sterile pistillate-parent lines are available it will be possible to produce pure 100 per cent F_1 hybrid seed only by hand pollination. Because of natural crossing of self-fertile lines there will always be a variable amount of selfed and sibbed seed mixed with the F_1 hybrid seed. Under such production, the commercial seed at least would correspond with hybrid seed corn produced in a poorly detasseled crossing field, permitting 10 to 15 per cent selfing and sibbing. It may also be of interest to the alfalfa breeder to keep informed of progress in the production of hybrid sugar beet seed (*33*), since many of the problems are similar to those found in alfalfa. Speculation is undertaken herein (pp. 34 to 38) as to how well the modern corn breeding practices may be superimposed on alfalfa. Other alfalfa improvement practices are first considered, however, since they have been used longer and have been discussed at greater length in the literature.

Improvement practices logically fall into three main categories: (1) mass selection with its modifications of *strain-building* and *maternal-line* selection,

(2) recombination of selected inbred lines as synthetic varieties and (3) hybridization for use as F_1 hybrids. The resultant improved products may be known as (1) improved selections or strains, (2) synthetic varieties, and (3) F_1 hybrids. There are a number of modified practices by which each goal may be reached. For example, synthetic varieties may be produced either by orthodox hybridization of inbred lines or by the modified "strain-building" procedures.

Mass Selection

This mode of improving cross-fertilized crops recognizes their heterogeneous nature and the continuous segregations and recombinations that occur within them. Many of the individuals present in such a population are heritably superior in one or more respects. To select a single superior individual for seed increase and commercial utilization is hazardous because of the narrow gametic relationship within the resultant crop. To overcome this hazard, many superior plants are selected and their seed is mixed. Plants of the resultant mixture intercross. If all or most of the component plants are homozygous for some favorable character, the new selection is likely to be superior in that respect. In other respects, however, the open-pollinated plants are likely to be inferior to the original selected plants, though not to the original variety, because of the segregation which occurs.

By following some plan of continuous mass selection for a succession of generations, some concentration of favorable factors results, and the character of the crop is definitely and heritably warped in the direction of the selection. No new inheritance is created thereby but the existing factors are merely reassembled. The plan approaches the early varietal improvements in corn by the well known mass selection methods of choosing superior plants and seed ears.

With alfalfa the regional problem with respect to length of growing season is not the same as with corn. However, winterhardiness, dormancy, pest resistance, leafiness, forage and seed productivity, etc., are characters for which selection may be made.

To bring about much improvement in many characters by mass selection is seldom achieved in any open-pollinated crop because of its heterozygous genetic constitution.

Through many years of natural selection and survival of the fittest, a number of important regional strains or varieties of alfalfa have come into existence. During the long periods of time involved, favorable mutations may have arisen and may have been retained by natural selection in part or in all of the population. There are few examples illustrating how man has improved upon the recognized standard varieties by continued mass selection within them. So far as is known, the standard varieties—Grimm, Cossack, Baltic, Ladak, Common, Turkistan, and Hairy Peruvian—are of natural origin.

Hardigan is not far different from its parent variety, Baltic. Viking, a new mass selection of hardy plants surviving the rigors of Canada, has not yet had opportunity to be tested for general superiority, other than cold endurance. In recent correspondence, Sprague speaks of the three New Jersey entries in the Uniform Alfalfa Nursery as being "better adapted strains developed by mass selection." A well organized mode of selection of apparently superior plants accompanied by natural outcrossing with similarly selected plants in the nursery through two to four generations has been used. A65 (New Jersey) is a seed mixture of 29 strains which had passed through four generations of selection subject to outcrossing. A66 (New Jersey) combines eight strains selected in three generations and A67 (New Jersey) includes 22 strains selected for two generations. These three selections have been tested for yield in the uniform alfalfa nurseries *(42)* where they had a very favorable record for forage yield in both 1937 and 1938. In 1938, their respective average forage yields for all stations were 111, 111, and 107 per cent of the average of six standard varieties. Their respective ranks among 85 entries were 2, 2, and 6. The yields of the two highest-producing selections were identical with that of Baltic. In New Jersey these three selections surpassed the average of the six checks by 43, 46, and 40 per cent, respectively, while Baltic surpassed by 23 per cent. With respect to wilt resistance the New Jersey selections averaged 28 per cent as resistant as Hardistan, while Baltic averaged 13 per cent.

The two mass selections from Baltic developed in Michigan, A68 and A69, gave relative forage yields of 109 and 107 per cent, respectively, compared with 111 per cent for Baltic, and averaged 8 per cent as wilt resistant as Hardistan.

On the other hand, certain studies made at the Nebraska Experiment Station are suggestive of the limitations to selection within open-pollinated varieties of alfalfa as an improvement procedure. To determine the extent to which the forage and seed yields of selected individual plants are reflected in the yields of their progenies, 40 plants differing in forage and seed yield were picked for study from among 2,000 uniformly spaced plants of open-pollinated alfalfa growing in an established nursery. Their forage yields varied from 405 to 2,107 grams per plant, while their seed yields ranged from 6.7 to 172.5 grams per plant. Open-pollinated seed of these 40 plants, largely outcrossed, was planted the following year in the same nursery and their respective progenies were correlated with those of the original mother plants as to yield of forage and seed, as well as leaf spot incidence and desirability rating. No significant correlation was shown between the performance of individual plants and that of their immediate progenies as shown in Table 7. This is quite contrary to the high correlation found between inbred lines and their outcrossed progenies as previously reported in Table 5.

If alfalfa history repeats that of corn, it may be fairly safe to assume that these mass selections ultimately will be outdistanced by methods involving the utilization of hybrid vigor. The corn breeders have practically discontinued work with mass selection because they prefer working with definite entities which breed true. With respect to this type of selection the alfalfa breeder has now reached the point where the corn breeder was about 15 to 20 years ago.

Maternal-line selection:. This may be considered as another modification of mass selection, developed and applied by Fryer (*16*) at the University of Alberta especially for improved seed-setting capacity of alfalfa. It consists primarily of establishing about 80 rather large space-planted progenies in adjacent rows in an isolated nursery. These are grown for a four-year period, the more prolific seed-yielding plants in each progeny being carefully studied and noted. Poor progenies and plants are destroyed at the end of the second year. In the third year (after several cycles of improvement), approximately the best

100 plants in 30 or 40 progenies are selected on the basis of the combined seed-yield scores during the two years and also on the basis of vegetative characteristics of the plants. Their seed is harvested and mechanically mixed for

TABLE 7.—*Correlation between the performance of selected open-pollinated parents and their open-pollinated progeny, Scottsbluff, Nebraska.*

Character	Mean Yield, grams per plant			Correlation coefficient parent with progeny		n
	Parent		Progeny			
	1939	1940	1940	1939 with 1940	1940 with 1940	
	gm	*gm*	*gm*	r	r	
Forage	1145	1222	400	—.0574[1]	—.0487	40
Seed	42.68	6.17	8.83	+.0722	—.1380	40
Leaf spot[2]	....	3.34	2.90	....	+.0819	40
Desirability[3]	....	2.31	2.87	....	+.1858	40

[1] $r = +.3126$ for P value of 0.05 when $n = 40$.
[2] Leaf spot, largely *Pseudopeziza medicaginis,* was scored on the basis of 1 = very little infection; 5, medium; and 9, much.
[3] Desirability is a rating given on the general type and forage character of the plant. 1 = very desirable; 5, medium; and 9, very undesirable, such as stemmy, sparsely leaved, etc.

seed increase and distributed as foundation stock for commercial alfalfa seed growers. In the fourth year all plants except about the best 80 in the entire nursery are removed from the field. These 80 plants are permitted to set seed *in situ* under open-pollination. The seed is used the next year to establish 80 new progenies for a new four-year cycle.

Results are reported by Fryer (*16*) from a three-year space-planted yield test (1936–38) of an improved strain compared with three standard varieties. The average yields in tons of cured hay were: Grimm 3.619, Ladak 3.673, Cossack 3.609, and Strain IM 35–1 3.668. In a comparable planting, the IM 35–1, now named Ferax, produced an exceptionally high seed yield. The seed of IM 35–1 planted for these yield tests was developed by maternal-line selection and was prepared by mixing the seed from 31 selected transplanted mother plants that had been grown one year subject to outcrossing in an isolated nursery. Thus the seed which was tested for yield was a mixture from open-pollinated plants.

Strain-building: According to Jenkin (*19, 20*), who is generally credited with originating the term, "Strain-building may be relatively simple or very complicated." It is described as including methods of improvement ranging from the very simplist type of selection such as the formation of a new strain from a single selected plant, through various types of mass selection, to the most complicated selection of a number of plants to form a new synthetic strain. Jenkin describes the most complicated method when he suggests the determination of the gross genetic constitution of each of the basic plants being considered for a new strain by: (1) self-fertilization, (2) diallel-crossing (crossing in all possible combinations), (3) backcrossing F_1 plants from each family to their parents, and (4) intercrossing F_1 plants within each family to study the range of variation in the second generation. Each of the crosses and selfed progenies is tested and only those parent plants having *superior combining* ability and which do not show unfavorable segregation are included in the final established strains. The original plants are retained so that a given result can be repeated at any time.

Jenkin explains that such a program would be too complicated and expensive for general application since it involves a tremendous number of crosses, but it illustrates an extreme, perhaps an ideal procedure to determine the breeding behavior of the plants which make up the composite. As Stevenson (*32*) also has pointed out, modifications of this procedure are usually employed in strain-building. The term strain-building, therefore, covers all types of improvement, with one notable exception—that of the production of F_1 hybrids for commercial use. In all strain-building procedure the ultimate objective is to develop a superior strain characterized by having uniformity with respect to particular characters sought, and with a sufficiently broad gene base so that it will not suffer from close breeding when allowed to produce seed from generation to generation. Thus it differs from the use of F_1 hybrids in which the original cross is remade for each succeeding commercial crop.

Two other features are often mentioned in the original description of strain-building. One of these is the desirability of retaining vigor in the basic, selected plants; this precludes the use of selfed lines in the composite when selfing materially reduces vigor; the other is the opportunity provided by this system for a continuous process of improvement by adding more suitable plants as they become available by further testing and discarding less suitable ones. Whether these two features are made a part of the program depends largely on the type and behavior of the crop which is being improved.

The fact that the term strain-building covers such a multitude of procedures makes it impossible to designate any one procedure as strain-building. It is suggested, therefore, that if the term is used the specific method of improvement be given in detail in each case. The various methods involved in strain-building are not new, although the discussion and illustrations of such methods have undoubtedly added to the knowledge of forage crop improvement and have emphasized the desirability of genetic tests of material entering the composites. In order to avoid confusion it is suggested that, until greater clarification and more restricted usage of the term is established, it be considered a broad term covering practically all forms of strain improvement rather than a specific type of improvement procedure. As a matter of fact, as it is now used the term "strain-building" may be considered synonymous with the term "strain improvement."

Recombination of Selected Inbred Lines as Synthetic Varieties

Expected yields from various synthetic combinations: Data have been presented on the effects of self-fertilization and hybridization in alfalfa. These indicate that the breeding of this crop is governed by essentially the same genetic principles as is corn. In general, with alfalfa, the reduced heterozygosity accompanying inbreeding results in smaller plant size, lower yield, and greater uniformity of heritable characters. First-generation hybrids and outcrosses frequently outyield standard open-pollinated varieties because of superior hybrid vigor and resistance to pests and cold. In the second generation, the productivity of hybrids is lessened and falls midway between that of the F_1 hybrid and the average yield obtained from open-pollinated seed of the two parents. More advanced generations yield the same as the F_2 when the seed is continued by cross-pollination within a representative population. The yield of successive generations will be modified, depending on the amount of selfing and the number of lines. In a single cross between homozygous lines the F_2 generation yields equally whether the seed is produced by selfing or by sibbing, whereas in multiple hybrids the reduction is greater with selfed than with sibbed seed.

From such behavior it is concluded that the most effective breeding practices parallel those with corn rather closely. Jenkins (*21, 22*), Kiesselbach (*23, 24*), and others have outlined many of the principles pertaining to corn, while Kiesselbach (*25*) has discussed the application of corn breeding principles in alfalfa improvement. Undoubtedly maximum performance in alfalfa as in corn may be obtained from first-generation hybrids involving relatively few lines.

When two self-fertile lines or parental hybrids are planted in a natural crossing block, emasculation of one parent, as with corn, is impossible and the resultant seed is a mixture of selfed and crossed seed. This degree of selfing voids the possibility of taking full advantage of hybrid vigor and materially decreases the yield. Such difficulties would be obviated by the use of self-sterile lines or hybrids as parents of the commercial F_1 hybrids. The feasibility of such procedure is discussed later herein.

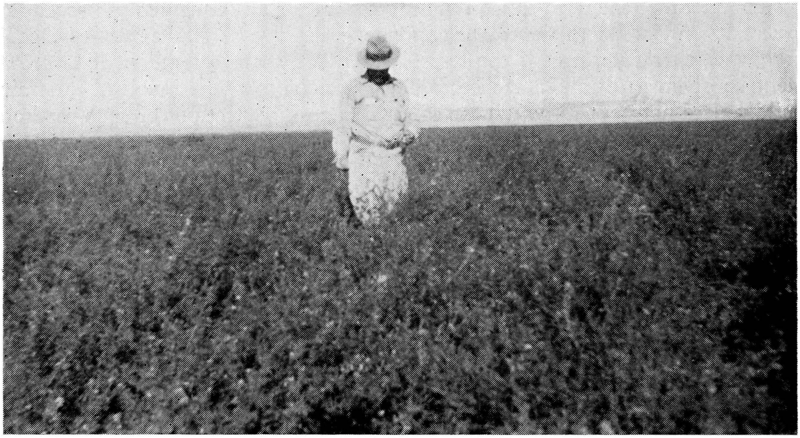

FIG. 7.—A commercial seed-increase field of a productive, bacterial wilt-resistant synthetic variety of alfalfa.

Synthetic varieties (Fig. 7) are regarded as the progenies from the open-pollinated seed of mixtures of inbred lines or of hybrids between them, in a sufficiently advanced generation for reasonable stabilization of their genetic constitution. Since inbred lines and hybrids are variable as to the amount of selfing and yield, actual field performance can be the only reliable criterion of productivity. At this early stage in the breeding program, however, interest attaches to the theoretical yields of synthetic varieties produced under various methods of breeding and degrees of self-fertilization. Such hypothetical yields are presented in Table 8. They are based on the assumptions that either 50, 80, or 90 per cent crossing occurs, that the foundation inbred lines yield either 50 or 75 per cent of the original open-pollinated varieties, and that the F_1 hybrids yield 110 per cent of the open-pollinated varieties. These assumptions are based on the behavior of selfed lines and hybrids reported earlier herein, where it was shown that the majority of lines eventually reduced to 36 per cent in forage yield of the standard variety, but that some of them produced 50 to 75 per cent yield in the second, third, and fourth generations of inbreeding. The results have also shown that desirable hybrids surpass the yields of standard varieties. The ten best F_1 single crosses reported in Table 3 averaged 115 per cent forage yield. The conservative figure of 110 is used in these calculations because it is known to be more difficult to obtain a high-yielding synthetic variety by combining 8 to 16 lines than a high-yielding single cross.

In accordance with the designation adopted by a joint committee of the Alfalfa Improvement Conference and the International Crop Improvement Association, the progeny produced from the first generation of open-pollinated seed would be known as syn-1 (first synthetic generation), the second as syn-2, etc. For convenience this method of designation will be used.

The method used in calculating the yields in Table 8 is diagrammatically illustrated on page 29 for the 4-line synthetic, assuming that sibbing freely occurs, that the selfed lines yield 50 per cent of the standard variety, and the six possible single crosses between them yield an average of 110 per cent of the standard variety. When equal quantities of seed of these four lines are mixed and planted in an isolated increase block, a part of the seed of the immediate progeny will be selfed, part sibbed, and part crossed, depending upon the amount of natural crossing that occurs. Of the resultant seed, that portion which was selfed will continue to yield 50 per cent of the standard variety; that which was sib-pollinated will yield the same as the selfed seed, *i.e.,* 50 per cent. The amount of sibbing will depend upon the proportionate number of sister plants present. With 50 per cent crossing, one-half of the seed will be selfed, one-fourth of one-half, or one-eighth, will be sib-pollinated, and the remaining three-eighths crossed. Five-eighths of the progeny will therefore yield 50 per cent and three-eighths 110 per cent, making an average yield of 72.5 per cent for the syn-1 progeny. This is the yield given in the table for the 4-line hybrid in which 50 per cent crossing of lines yielding 50 per cent is assumed.

The calculations can be somewhat simplified by the fact that the sibbing and crossing together equal the yield of the F_2. In the above diagram the average yield of the crossed progeny (one-eighth sibbed yielding 50 per cent and three-eighths 110 per cent) is equal to 95 per cent (which is the F_2 yield of a double cross yielding 110 per cent whose inbred parental lines yield 50 per cent according to the formula applicable to symmetrical crosses, F_2 yield $= a - [1/n(a\text{-}b)]$ where $a =$ yield of the F_1, $b =$ average yield of all inbred lines, and $n =$ number of inbred lines). The average yield for the syn-1 is therefore 72.5 per cent (one-half 50 plus one-half 95) which is the same as

TABLE 8.—*Theoretical yields in successive generations of synthetic varieties originating from various hybrid combinations and seed mixtures of inbred lines, natural outcrosses, and hybrids.*

Foundation material			Yield of synthetic varieties in percentage of standard variety														
Kind	Basic lines		50 per cent crossing					80 per cent crossing					90 per cent crossing				
	% yield	No.	Before synthesis	Generations of synthesis				Before synthesis	Generations of synthesis				Before synthesis	Generations of synthesis			
				1	2	3	4		1	2	3	4		1	2	3	4
SYNTHETIC VARIETIES FROM MIXTURES (NO SIBBING OCCURRING)																	
Selfed lines	50	2	50.0	80.0	75.0	74.4	74.5	50.0	98.0	79.3	79.0	79.2	50.0	104.0	79.8	79.7	79.8
Selfed lines	50	4	50.0	80.0	81.6	82.1	82.3	50.0	98.0	91.2	91.2	91.3	50.0	104.0	93.3	93.3	93.3
Selfed lines	50	8	50.0	80.0	84.6	85.8	86.1	50.0	98.0	97.0	97.2	97.3	50.0	104.0	100.0	100.1	100.1
Selfed lines	50	16	50.0	80.0	86.1	87.6	88.0	50.0	98.0	99.9	100.3	100.3	50.0	104.0	103.4	103.4	103.5
Selfed lines	75	2	75.0	92.5	89.6	89.2	89.3	75.0	103.0	92.1	91.9	92.0	75.0	106.5	92.4	92.3	92.4
Selfed lines	75	4	75.0	92.5	93.4	93.7	93.9	75.0	103.0	99.0	99.0	99.1	75.0	106.5	100.3	100.2	100.3
Selfed lines	75	8	75.0	92.5	95.2	95.9	96.1	75.0	103.0	102.4	102.5	102.6	75.0	106.5	104.2	104.2	104.2
Selfed lines	75	16	75.0	92.5	96.0	96.9	97.2	75.0	103.0	104.1	104.3	104.4	75.0	106.5	106.1	106.2	106.2
SYNTHETIC VARIETIES FROM MIXTURES (SIBBING OCCURRING)																	
Selfed lines	50	2	50.0	65.0	68.8	69.7	69.9	50.0	74.0	76.4	76.6	76.7	50.0	77.0	78.4	78.4	78.4
Selfed lines	50	4	50.0	72.5	78.1	79.5	79.9	50.0	86.0	89.6	90.0	90.0	50.0	90.5	92.5	92.6	92.6
Selfed lines	50	8	50.0	76.3	82.8	84.5	84.9	50.0	92.0	96.2	96.6	96.7	50.0	97.3	99.6	99.7	99.7
Selfed lines	50	16	50.0	78.1	85.2	86.9	87.4	50.0	95.0	99.5	100.0	100.0	50.0	100.6	103.2	103.3	103.3
Selfed lines	75	2	75.0	83.8	85.9	86.5	86.6	75.0	89.0	90.4	90.5	90.6	75.0	90.8	91.5	91.6	91.6
Selfed lines	75	4	75.0	88.1	91.4	92.2	92.4	75.0	96.0	98.1	98.3	98.3	75.0	98.6	99.8	99.9	99.9
Selfed lines	75	8	75.0	90.3	94.1	95.1	95.3	75.0	99.5	101.9	102.2	102.2	75.0	102.6	103.9	104.0	104.0
Selfed lines	75	16	75.0	91.4	95.5	96.5	96.8	75.0	101.3	103.9	104.1	104.2	75.0	104.5	106.0	106.1	106.1
F_1 single crosses	50	100	110.0	94.7	90.9	89.9	89.7	110.0	103.5	102.9	102.8	102.8	110.0	106.5	106.3	106.3	106.3
F_1 single crosses	75	100	110.0	101.1	98.8	98.3	98.2	110.0	106.2	105.8	105.8	105.8	110.0	107.9	107.8	107.8	107.8
Outcrosses[1]	50	1	80.0	78.8	78.4	78.4	78.3	98.0	88.8	87.9	87.8	87.8	104.0	91.0	90.3	90.3	90.3
Outcrosses[1]	50	8	80.0	85.3	86.6	87.0	87.0	98.0	99.3	99.4	99.4	99.4	104.0	102.8	102.7	102.7	102.7
Outcrosses[1]	75	1	92.5	91.3	90.9	90.9	90.8	103.0	96.8	96.2	96.1	96.1	106.5	98.0	97.5	97.5	97.5
Outcrosses[1]	75	8	92.5	95.5	96.2	96.4	96.4	103.0	103.6	103.6	103.6	103.6	106.5	105.5	105.5	105.5	105.5
SYNTHETIC VARIETIES FROM HYBRIDS (SIBBING OCCURRING)																	
F_1 hybrid	50	4	110.0	87.5	81.9	80.5	80.1	110.0	92.0	90.2	90.0	90.0	110.0	93.5	92.7	92.6	92.6
F_1 hybrid	50	8	110.0	91.3	86.6	85.4	85.1	110.0	98.0	96.8	96.7	96.7	110.0	100.4	99.8	99.7	99.7
F_1 hybrid	50	16	110.0	93.1	88.9	87.9	87.6	110.0	101.0	100.1	100.0	100.0	110.0	103.6	103.3	103.3	103.3
F_1 hybrid	75	8	110.0	99.1	96.3	95.6	95.5	110.0	103.0	102.3	102.2	102.2	110.0	104.3	104.0	104.0	104.0
F_1 hybrid	75	16	110.0	100.2	97.7	97.1	96.9	110.0	104.8	104.2	104.2	104.2	110.0	106.0	106.1	106.1	106.1

[1] Outcrosses produced by open-pollination in a nursery containing a large number of other selected lines and hybrids.

Diagrammatic illustration of the method of calculating the theoretical yield of the first and second generations of synthesis of a composite made by mixing the seed of four inbred lines, assuming the lines each yield 50 per cent of a standard variety, that all of the hybrids from these lines yield an average of 110 per cent of the standard variety, that 50 per cent natural crossing takes place, and that sibbing freely occurs.

Syn-1			Syn-2					
Proportion	Type of material	Yield	Proportion of total population				Type of material	Yield
		%						%
Mixture of seed of 4 lines: $\frac{4}{8}$ selfed, $\frac{1}{8}$ sibbed	$\frac{5}{8}$ inbreds	50	$\frac{1}{2}$ will self			$\frac{5}{16}$ or $\frac{160}{512}$	inbreds	50
			$\frac{1}{2}$ will cross with	$\frac{5}{8}$ inbreds	$\frac{1}{4}$ same	$\frac{25}{512}$	inbreds	50
					$\frac{3}{4}$ not related	$\frac{75}{512}$	F_1	110
				$\frac{3}{8}$ F_1	$\frac{2}{4}$ related	$\frac{30}{512}$	backcross	80
					$\frac{2}{4}$ not related	$\frac{30}{512}$	3-way x	110
$\frac{3}{8}$	F_1	110	$\frac{1}{2}$ will self			$\frac{3}{16}$ or $\frac{96}{512}$	F_2 selfed	80
			$\frac{1}{2}$ will cross with	$\frac{5}{8}$ inbreds	$\frac{2}{4}$ related	$\frac{30}{512}$	backcross	80
					$\frac{2}{4}$ not related	$\frac{30}{512}$	3-way x	110
				$\frac{3}{8}$ F_1	$\left\{\frac{1}{6}\right\}$ $\frac{0.67}{4}$ same	$\frac{6}{512}$	F_2 selfed	80
					$\left\{\frac{4}{6}\right\}$ $\frac{2.66}{4}$ related	$\frac{24}{512}$	F_2 open	95
					$\left\{\frac{1}{6}\right\}$ $\frac{0.67}{4}$ not related	$\frac{6}{512}$	F_1 double x	110
Yield of synthetic		72.5						78.13

obtained in the diagram. The yield of the syn-2 generation can be calculated in a similar manner: five-sixteenths of the population will yield the same as the selfed lines (50 per cent); three-sixteenths, being selfed from the hybrid, will yield 80 per cent; and eight-sixteenths will yield the same as an open-pollinated F_2, *viz.,* 95 per cent. These averaged together give a yield of 78.13 per cent for syn-2.

It is to be recognized that the calculations reported in Table 8 are highly theoretical and may not result in the same response in the field. Among the many things which may alter the field response are the following:

1. *The amount of sibbing:* Table 8 gives the results with and without sibbing within the selfed lines. It is probable that the actual behavior of the crop lies between the two extremes given. For example, if there is 20 per cent selfing it is very likely that there is at least 20 per cent sibbing (depending on the number of lines involved), and perhaps even a higher percentage of sibbing because it is well known that in many self-sterile forage crops it is easier to obtain sibbing than selfing. The amount of sibbing would also depend on the homozygosity of the lines. If they were completely homozygous there would be less sibbing than if they were not homozygous. In these calculations it is assumed that the lines are practically homozygous. In the calculations for all synthetics other than those from selfed lines, sibbing is considered as taking place, because in these cases the plants are not so closely related. While sibbing or lack of sibbing makes a great difference in the yield of syn-1, the differences become relatively insignificant in the syn-2 and more advanced generations of synthesis, particularly with the higher percentages of crossing.

2. *Differential survival of inbreds and hybrids:* It will be shown that inbreds do not survive in the field as well as hybrids.

3. *Total yield not in proportion to relative number of inbred and hybrid plants:* It is probable that a mixture of hybrid and inbred plants will yield nearer to the average of the hybrids than to the numerical ratio between them because of competition, etc.

4. *Differential seed-yielding ability:* This would be especially true between high seed yielding hybrids and relatively low yielding inbreds, resulting in a greater proportion of hybrids.

5. *Compatibilities of crosses and reciprocal crosses may alter the relationships.*

Despite the differential responses which may occur in the field, the results found in Table 8 would seem to merit conclusions on at least three important breeding principles. The first is the low yield which might be expected when a high degree of selfing occurs; the second is the number of generations of synthesis required before essentially reliable yield results may be obtained (Fig. 8), and the third pertains to the yield of the inbred strains. With regard to the use of highly self-fertile lines in the synthetic or to the selection of autogamous plants, the results when 50 per cent selfing occurs show clearly that superior yields cannot be expected. In all cases the yield of the synthesized variety is below 90 per cent. Low yield of highly self-fertile material has been ascertained in local field tests corroborating these theoretical results (unpublished material). This outcome has a very significant bearing on alfalfa breeding, since selection for high self-fertility would appear opposed to maintaining or increasing forage productivity, even though such selection might tend to increase seed yields.

The second consideration, that of the number of generations for synthesis, can be confined to the groups with 80 to 90 per cent crossing because this is most common in alfalfa and because the 50 per cent crossing is included chiefly

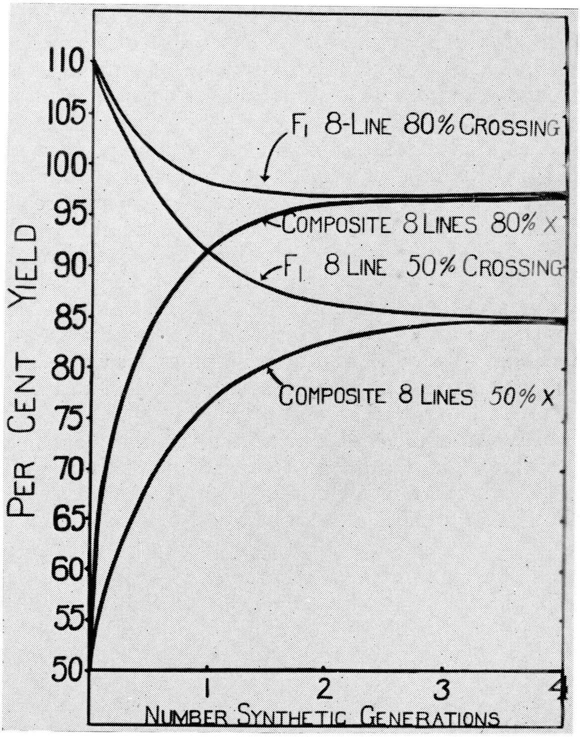

FIG. 8.—Theoretical yields in four successive generations of synthetic varieties originating both as composites and F_1 8-line hybrids, assuming 50 and 80 per cent crossing and sibbing. (Compiled from calculations in Table 8.)

to show the disadvantage of high self-fertility. Considering these two groups, it can be seen that the maximum change in yield between syn-2 and syn-3 is 0.5 per cent, which could easily be within the experimental error of testing. It would appear therefore that testing synthetic strains in the syn-2 generation would give the necessary information on yielding ability. Testing previous to syn-2 may or may not be feasible, depending very largely on the type of material involved.

The third principle has to do with the yield of the inbred lines as reflected in the final synthetic yields. In all the hypothetical cases presented the inbred lines yielding 75 per cent produced synthetics higher in yield than corresponding lines yielding 50 per cent. It is of importance therefore to produce high-yielding inbred lines for use in synthetics or to use little or no inbreeding if equally high combining individuals can be obtained which breed relatively true for the characters desired.

The out-crosses were treated essentially as line-variety crosses in these calculations. The F_1 hybrids were assumed to be symmetrical hybrids made by controlled crossing of the lines to make single crosses, then crossing these to make double crosses, then 8-line, and finally 16-line hybrids.

Actual yields of synthetic varieties: The yields of six synthetic varieties are given in Table 9. These synthetic varieties were produced from selected inbred lines which were allowed to outcross in a nursery containing many such lines. In a few cases the inbred lines were outcrossed once, then reselected and allowed to outcross a second time before entering the synthetic. Seed from 2 to 18 such outcrosses was composited to begin the various synthetic varieties. The composites were tested for yield directly, and at the same time some of the seed of each composite was planted in separate isolated increase blocks, thus producing seed for the first synthetic generation. The yields reported for the original composites were obtained from the 1940 Report of the Uniform Alfalfa Nurseries and the percentages are in terms of the average yield of the standard varieties Grimm, Hardistan, Ladak, Northern Common, Kansas Common, and Baltic. The yields for the syn-1 progenies were obtained in 1939 and 1940 from an advanced-nursery replicated test at the Nebraska Experiment Station, and are reported in terms of the same six standard varieties.

The yields of the synthetic varieties reported in Table 9 are all within 5 per cent of the average of the standard varieties. Some of them yielded more in syn-1 than in the composite, while others yielded less, there apparently being little relationship to the number of outcrosses originally included. It is of interest to note, however, that the average of the syn-1 is 2 per cent higher than the average of the composites. This would correspond closely to the situation shown in Table 8 where the eight outcrosses yielded 98 per cent and the syn-1

TABLE 9.—*Comparative forage yields of six synthetic alfalfa varieties and standard varieties. Yields of composites were obtained from the Cooperative Uniform Nurseries; those for syn-1 from advanced nursery tests at Lincoln, Nebr. The yields are reported as a percentage of the six standard varieties, Grimm, Hardistan, Ladak, Northern Common, Kansas Common, and Baltic, which were in all tests.*

Designation	No. of out-crossed lines composited	Yield of Composites	Yield of Syn-1
		%	%
A92	2	98	97
A106	4	98	103
A110	4	95	102
A112	18	101	105
A113	12	104	97
A114	2	101	105
Av. of synthetics		99.5	101.5
Av. of 6 varieties		100	100

yielded 99.3 per cent with 80 per cent crossing. The differences noted between the various synthetics, aside from errors of testing, may doubtless be accounted for by differences in the amount of crossing in individual lines, or differences in their combining ability. The average, however, closely follows the expected on the basis of the 80 to 90 per cent crossing found under field conditions in this area.

The actual yields also check with the theoretical yields in showing only a mediocre yield despite the rather intensive selection program preceding the final selection of the outcrosses. In no case, however, were the selections previously tested for combining ability and this may be one of the important steps required to produce a superior combination. It should be pointed out, however, that although the synthetic varieties reported in Table 9 are not superior to commercial varieties in yield, they are superior in bacterial wilt resistance, seed-yielding ability, and other agronomic characters as a result of intensive selection practiced during inbreeding, and show that this method of improvement is effective in the establishment of certain desirable characters.

Comparative field survival of plants from inbred lines, hybrids, and standard varieties: In any alfalfa mixture involving the growing of inbred plants in competition with plants of hybrid origin the question logically arises whether the inbred plants, lacking in vigor as they do, would survive as well as the hybrids. The relative survival would undoubtedly depend upon the thickness of planting and intensity of competition. For example, in the first year after planting, a solid-drilled field of alfalfa contained 705,000 plants per acre, whereas three years later it contained only 178,000 plants per acre, without any noticeable reduction in ground coverage *(38)*. It is to be expected that spaced plants would not be subject to such severe elimination; nevertheless even under such conditions considerable elimination does take place.

An opportunity to study the comparative survival of inbred plants, their hybrids, and the original open-pollinated varieties presented itself in the hybrid nursery to which reference has been made. The plants for this nursery were started under comparable conditions in the greenhouse in January, and were transplanted to the field in April, 1937. Rows of the inbred lines were planted between rows of their open-pollinated progeny or their hybrids as the case might be. The rows were spaced 27 inches apart and the plants 18 inches apart in the row. For purposes of this comparison all of the inbreds were averaged together whether they were selfed one or eight generations, though the majority averaged three to four generations of selfing.

The results of the plant survival counts are given in Table 10, together with the percentages based on these counts. The first year includes the transplanting survival, but since all plants were under comparable conditions this should be an indication of their inherent ability to survive. The results show the same trend in survival after the plants were established, thus indicating that survival the first year is a measure of surviving ability. During the second year the plants were subject to a rather heavy grasshopper infestation and to drouth.

From the date of transplanting in April, 1937, to October, 1939, 51.5 per cent of the inbred plants, 60.6 per cent of the open-pollinated plants, and 75.1 per cent of the hybrid plants survived. Putting it another way, if the hybrids had survived 100 per cent the inbreds would have survived only 68.6 per cent.

TABLE 10.—*Summary of plant-survival of selfed lines and their hybrids, and of open-pollinated varieties grown in a space-planted nursery, Lincoln, Nebraska.*

Class	Number of plants			Percentages of plants surviving from		
	Transplanted	Surviving				
	4/37	6/38	10/39	4/37–6/38	4/37–10/39	6/38–10/39
Inbreds	1731	1119	891	64.6	51.5	79.6
Varieties[1]	944	673	572	71.3	60.6	85.0
Hybrids	1640	1393	1231	84.9	75.1	88.4

[1] Included Grimm, Hardistan, and Ladak.

This differential survival would undoubtedly be a factor in the results obtained from mixing seed of hybrids and inbreds as would be done in producing certain types of synthetic varieties. The differential survival would tend toward slightly higher yields of the synthetic, but in most cases the percentage of inbreds is so small that the differences would be slight. Ordinarily the competition would not be extreme in the first few generations of increase because of thin or row planting for seed production.

In the calculations presented in Table 8, differential survival was not taken into consideration.

It is of interest to note that the hybrids were superior in survival to the standard varieties, Grimm, Hardistan, and Ladak.

First Generation Hybrids

The theoretical yields presented in Table 8 indicate that there is an apparent "ceiling" in the yield of synthetic varieties developed by these methods of breeding. This "ceiling" is definitely below that attained by F_1 hybrids free of admixtures with selfed and sibbed seed. The breeding results, both in forage crops and corn, substantiate this theoretical calculation. Not only would F_1 hybrids have a yield advantage, but it would also be easier to "fix" other desirable characters than in a synthetic. For these reasons breeders of alfalfa and other forage crops should thoroughly investigate the possibilities of utilizing F_1 hybrid vigor commercially.

A possible tool for such achievement is the utilization of self-sterility. In the course of the alfalfa breeding program, data have been obtained concerning the self-fertility of a large number of alfalfa plants. In preliminary work approximately 30 plants out of a total of 150 plants showed less than 30 per cent self-fertility, while a number showed less than 10 per cent and one less than 1 per cent self-fertility. With a relatively low percentage of self-fertility the chances are good for a very high percentage of crossing. Even semi-self-sterile individuals under open-pollination are expected to show a very high degree of crossing due to selective fertilization by foreign pollen and differential embryo mortality.

How can these highly self-sterile individual plants be utilized in producing a commercial F_1 hybrid? Many possibilities may be suggested but only one will be discussed. It involves the production of four self-sterile plants which together would combine well to produce two self- and sib-sterile single crosses, which in turn would combine to produce an outstanding double cross. This double cross would need to be disease resistant, at least to bacterial wilt and leaf spots, and should rank high in forage yield and other desirable characteristics. In other words, to make such a program successful the hybrid would need to be outstanding; otherwise synthetic combination would be preferred. The four plants finally selected as the basis for the crosses may be either inbred plants or hybrids, depending upon which type of material is found to have superior combining ability together with satisfactory seed yield as clonal lines.

Assuming that four such plants may be found (and this would not seem impossible), the problem of their propagation arises. For this phase of the program vegetative propagation (Fig. 9) would be the solution. Vegetative propagation of these relatively self-sterile lines would be more feasible than seed propagation, and continuing them in pure form would not then be a problem. The clonally propagated lines would be space-planted in isolated natural crossing blocks to produce the two single crosses.

These two single crosses preferably would be relatively self-sterile (something which does not appear impossible because a number of the self-sterile plants cited above were hybrids, Fig. 10), but high in seed production when allowed to cross. Thus the double cross could be made by mixing the seed of the two single crosses and planting in isolated, commercial seed-increase fields. The double-cross seed would then be used for planting commercial meadows and pastures. Ordinarily seed would not be harvested from these double-cross fields. If occasionally it were more profitable for the grower to raise seed, he could still take advantage of the situation because advanced-generation seed from a highly superior double cross would probably be as productive as standard varieties and at the same time retain the desirable disease resistance. The use of F_2 seed would be subject to the same limitations, especially in regard to reduction in vigor, as with hybrid corn. Another important consideration is the fact that the seed of cold-resistant hybrids could be produced in the favorable, high seed yielding areas of the southwest as well as in the north.

While the use of vegetative cuttings for commercial production may sound impractical, it may not appear so difficult when illustrated by an actual example as follows: Two isolated 25-acre natural-crossing fields each planted with cuttings of two clonal lines would produce annually (at 200 pounds per acre) sufficient F_1 seed of the two single-cross parents to plant 10,000 acres of crossing fields for the production of the double-cross seed, planting at the rate of one pound per acre. Furthermore, the original fields could be under seed production a number of years. Under favorable conditions the acreage would pyramid very rapidly. At the end of five years there could be as much as 50,000 acres of the two single-cross parents established for the production of the double-cross seed. Allowing for failures and perhaps in some cases thicker planting, it would certainly be conservative to estimate that the 50 acres of cuttings would keep at least 10,000 acres of the crossing fields under production. These 10,000 acres should produce sufficient seed (at 4 bu. per acre) to plant 200,000 acres of hybrid alfalfa at 12 pounds per acre for forage production. This acreage established each year would be sufficient to maintain one million acres of hybrid alfalfa under production on a five-year rotation basis (Fig. 11). It is roughly estimated that it would cost \$60.00 to establish one acre of vegetatively propagated plants, though this figure no doubt could be lowered under proper commercial conditions. Cuttings are readily propagated in the open during summer and excessive greenhouse space would not be required.

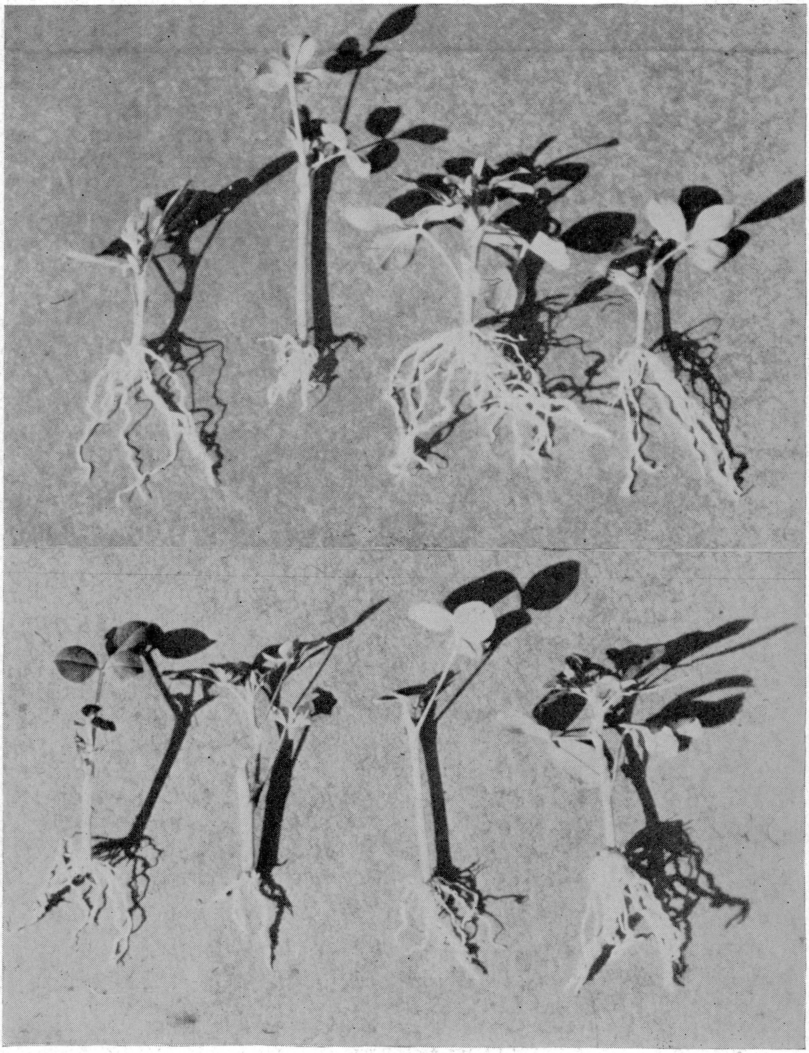

FIG. 9.—Alfalfa is readily propagated by stem cuttings. The cutting should consist of at least one node and portion of an internode. When set in moist sand with the node exposed, the top growth comes from the node and the roots from the cut end of the internode. Treatment with growth substances such as indol butyric acid may slightly stimulate rooting (top four cuttings compared with untreated checks below), but usually the percentage of rooting is so high that such treatment is unnecessary.

These details are given as suggestive of the possibilities of forage crop breeding. Although results cannot be demonstrated for the F_1 double-cross, experimental evidence indicates the desirability of selecting self-sterile, high-combining individuals whether the objective is the production of synthetic strains or F_1 hybrids. The breeding procedure would therefore be identical

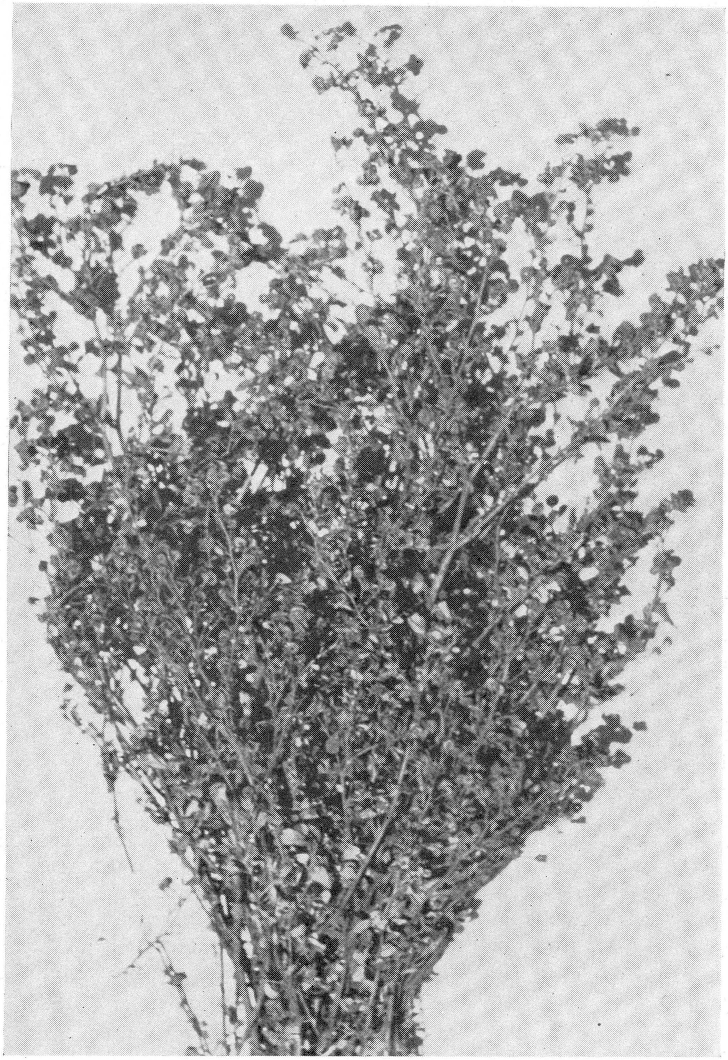

FIG. 10.—A plant of the F_1 hybrid No. 07–758. This hybrid is self-sterile but high in seed production when exposed to cross-pollination by unrelated alfalfa. Two such hybrids with high combining ability would produce the desired commercial F_1 double cross.

up to this point. Since it seems evident that the hybrid would have a yield advantage, the final decision whether to produce synthetic strains or F_1 hybrids will no doubt depend on the economic feasibility of the latter.

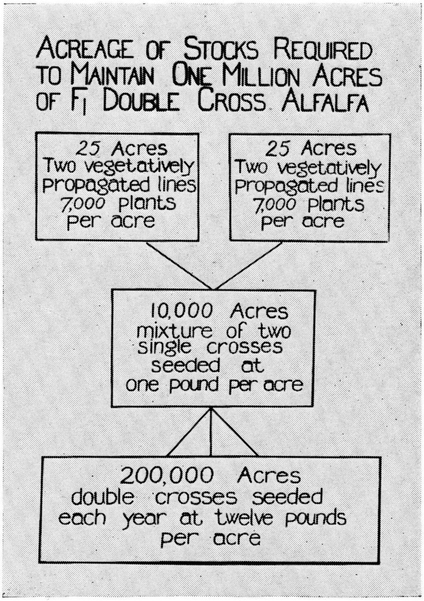

FIG. 11.—Plan for a possible commercial method of producing F_1 seed of a double cross. The two 25-acre foundation fields would be established by transplanting cuttings of four self-sterile plants, two in each block. The seed of the two single crosses from these foundation fields would be mixed and planted in a manner for commercial seed production and from these fields would be obtained the F_1 double-cross seed to be sown in commercial hay fields. With sufficient F_1 double-cross seed produced annually to establish 200,000 acres, these two 25-acre foundation fields could maintain one million acres on a five-year rotation basis. To safeguard against unusually poor seed production or poor establishment of hay fields the acreage of foundation fields could be doubled without being prohibitive.

Developing Lines for Recombination

Inbreeding is not to be regarded as essential in the establishment of foundation material for use in hybridization. It is merely one means to an end. Its objective is to isolate lines that are relatively homozygous for important genetic characters and that possess high-combining ability. One or two years of selfing may be the maximum desired for use in the production of F_1 commercial hybrids. Theoretically, suitable lines may be selected for hybridization without inbreeding, provided they are found not to segregate unduly for undesirable characters. The tetraploid complex in alfalfa undoubtedly makes it more difficult to attain complete homozygosity with respect to any given character, but, on the other hand, such a chromosomal complex results in fewer harmful recessives appearing in the segregating generations. Regardless of the degree of inbreeding, the usual terminology will be employed, as lines,

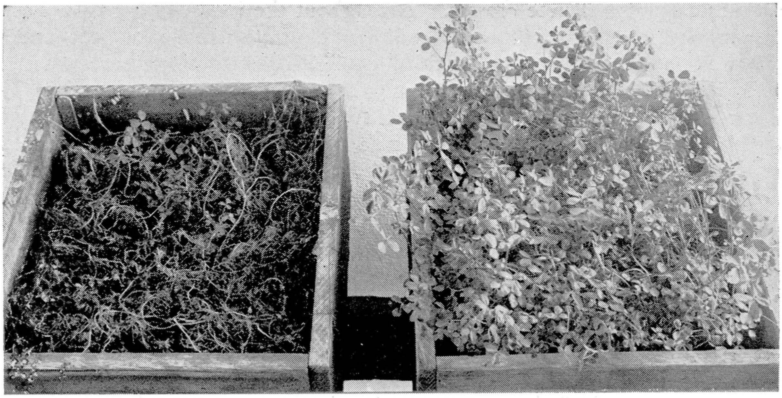

FIG. 12.—Artificial tests for cold resistance. Cold resistance can be determined in the field over a period of years, but often it is desirable to determine cold resistance of strains in shorter periods of time. This is accomplished by using artificially controlled temperature chambers. Results which correlate well with field determinations are obtained in a period of two months. On the left a non-cold-resistant variety, on the right a cold-resistant strain, showing recovery after comparable freezing in a hardened condition.

FIG. 13.—Controlled tests for disease resistance greatly facilitate a breeding program. The above strains have been artificially inoculated with the organism, *Phytomonas insidiosa,* causing the bacterial wilt disease. Inoculation is accomplished by immersing the roots of young plants in a bacterial suspension for 10 to 30 minutes. The strains illustrated above are, left to right: 04–826, a bacterial wilt-resistant selection, three susceptible strains, the center one of which is Grimm, and A144, a resistant selection.

single crosses, and double crosses. Throughout the inbreeding or other selection process, positive selection for the desired characters should be practiced. To facilitate this selection in alfalfa, helpful devices for evaluating the crop with respect to its various agronomic characters such as resistance to cold (Fig. 12), disease (Fig. 13), and insects may need to be provided where opportunity for the expression of the characters is too intermittent to be relied upon under natural field conditions. Thorough field testing, the principles of which have been fairly well established *(37)*, is also desirable. It is necessary to initiate rather large numbers of lines and deal in fairly large populations in order to ferret out the exceptional individuals that possess and are essentially homozygous for the characters sought.

It is not to be expected that individual lines may be developed that are homozygous for all favorable factors. In the production of F_1 hybrid seed the number of factor pairs carrying dominance is increased over that of inbred lines, and evidently they contribute equally whether homozygous or heterozygous.

If the hybrid is to be handled as a synthetic variety, however, both parents should bring in dominance for the specific characters; otherwise they will segregate and the progeny will be variable with respect to the characters in question.

An important element in the success of selecting lines of the desired character is the initial choice of variety or varieties in which to begin the self-fertilization. It is desirable to start with varieties that already incorporate the characters most wanted. To establish basic lines for a top-notch northern hybrid or synthetic variety of alfalfa, one would be justified in selecting within Cossack, Turkistan, Ladak, and Common. Selection within hybrids and also the use of *M. falcata* strains in crosses should be considered. To take advantage of the superior germ plasm found in such bacterial-wilt-susceptible varieties as Baltic, Grimm, and Hardigan, it is suggested that selfing might advantageously follow hybridization of these with some wilt-resistant strains.

Since the use of the line-variety or top-cross method of evaluating the prepotency or combining ability of lines as parents has gained such wide and approved usage in the corn breeding programs of the country, it may be well to inquire whether any comparable practice is available to the alfalfa breeder. The top-cross data as stated by Jenkins *(21)* serve "as a measure of the ability of the lines to impart high average yield to their hybrid progeny." Further, "experience has demonstrated that inbred lines imparting a high average yield to their hybrid progeny may be expected also to produce high yielding individual hybrids . . . Yield prepotency depends upon the number of dominant alleles favorable to yield carried by the different lines and upon their relative importance."

Perhaps the various line-outcrosses developed in a common alfalfa breeding nursery may be regarded as having the equivalent of a common top-cross origin, and that their comparative performance may serve to evaluate the lines. Such usage is considered under the following subject.

The polycross method of testing the combining ability of lines: Because of the characteristics of the crop, it is impossible to produce a large amount of hand-pollinated alfalfa seed. It would also be very difficult to have a sufficient number of isolated blocks (Fig. 14) to plant each line in conjunction with a commercial variety for the purpose of producing a natural top cross, which at best would be only 80 to 90 per cent crossed. It seems appropriate, therefore, to suggest a substitute method which is at once practical and also appears to give the desired results. This method involves the growing of the selected lines together in a single nursery, the open-pollinated seed from each line being tested thereafter for productivity, disease resistance, etc. For the purpose of distinction in discussion, the name *polycross* is suggested for the progeny from seed of a line that was subject to outcrossing with the other selected lines growing in the same nursery. Such polycrossed seed is likely to be 80 to practically 100 per cent hybrid. It is assumed that the various lines are thus exposed to reasonably comparable outcrossing by random samples of pollen. If it is found that variable distance between lines within a large nursery results in nonrepresentative composite pollination of the lines, this could undoubtedly be overcome for practical purposes by randomized rep-

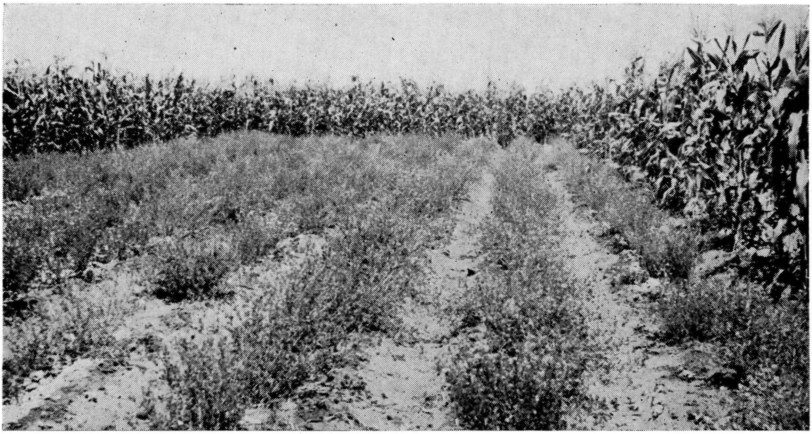

FIG. 14.—Seed of selected strains must be increased in well isolated blocks for preliminary testing. One method is to place such blocks at intervals in a corn field. Usually good seed production is obtained the first year whether the block is started from plants or from seed. The plot shown above yielded at the rate of 150 pounds per acre when planted May 1 and harvested late in September of the same year.

lication of all lines. Seed from the replicate plots would be mixed for use in testing the performance of the polycross. The apparent correlation in the performance of hybrids and the polycrosses of their component lines (Table 5) makes it seem worth while to consider seriously the adoption of this method for detecting those lines with high combining ability. This method has the advantage of producing a considerable amount of naturally outcrossed seed from each line which is necessary for rather thorough progeny testing. In the case of self-sterile lines, these would be included in the polycross nursery as clonal lines of self-sterile plants. Their propagation would be by means of cuttings. Thus a polycross nursery preferably may consist largely of clones of self-sterile plants selected from the breeding nursery the previous year by appropriate self-fertility tests together with observations on seed yielding ability and other desirable characteristics. These plants need not have been previously subjected to inbreeding. Clonal propagation would provide adequate seed from the polycross nursery for thorough testing and would have the additional advantage of affording replication in the polycross nursery to insure random crossing.

While the search for promising F_1 hybrids and a method to make their production commercially feasible is continuing, it may be found desirable for immediate use to select the best combining lines, as determined by the polycross test and composite these to form a synthetic variety. Such a synthetic variety (the term "synthetic" being applied only after two generations of open pollination) should have an advantage over random mixtures because of the superior combining ability of its component lines.

CONCLUSIONS

Although there is evidence that some improvement may be effected by mass selection, a review of the literature and the results reported in this paper would seem to justify the conclusion that the principles of breeding alfalfa, with some modifications, are essentially the same as those which have been established for corn. Under natural field conditions, both crops may be partly self-fertilized but usually are largely cross-fertilized. The difference in principles lies chiefly in the degree of pollen control possible in the commercial production of first-generation hybrid seed. Because of this distinction, it may be practical at the outset to use the open-pollinated synthetic variety in alfalfa rather than the first-generation hybrid as used in corn. Nevertheless, the promise of first-generation hybrids between two, three, or four selected, high-combining, self-sterile lines is so great that this mode of procedure should be fully explored.

A program to develop either a superior synthetic or a hybrid might well include the following steps:

1. *Choice of the original varieties should be given careful consideration:* If varieties susceptible to the bacterial wilt disease, such as Baltic or Common, are to be used the recommended procedure is to cross them with resistant plants, self the resulting hybrids for one generation, inoculate seedlings from the selfed seed with the bacterial wilt organism, allow the survivors to outcross under open-pollinated conditions, and use seed from these outcrosses as indicated under 2. Varieties with considerable wilt resistance and other desirable characters could immediately be entered under procedure 2.

2. *Epidemic nursery:* Inoculate and test a large population of plants from the chosen varieties or outcrosses for bacterial wilt and leaf spot diseases. Where facilities are available, selection also may be made for resistance to cold and insect pests.

3. *Breeding nursery:* Plant the healthy survivors of such tests in the breeding nursery, where they may be observed for seed and forage productivity and for other desirable characters, and tested for self-sterility. Self-sterility is determined by bagging several racemes of buds, tripping the flowers by hand-manipulation when they come to full bloom, replacing the bag and later determining the percentage of flowers forming pods. Less than 20 per cent of the flowers form pods on highly self-sterile plants, while in highly self-fertile

plants often as high as 80 per cent of the flowers form pods. Approximately 10 per cent of an unselected population may be expected to be highly self-sterile. (Unpublished data) It will not be necessary to test all the plants for self-sterility. The simplest way is to select only the superior, high seed-producing plants for this test, thus eliminating considerable labor. The order of procedure throughout the selection program is based on eliminating the undesirable plants by the method involving the least labor first, progressing to those methods wherein more labor and detail are involved.

Not more than 10 per cent of the plants are likely to merit advancement to the polycross nursery based on observations of productivity and other desirable characters. Of this 10 per cent probably not more than one plant in every ten will be sufficiently self-sterile to be included in the polycross nursery. On this basis if 1000 plants survive the disease nurseries, only 10 will be left to enter the polycross nursery. Among the more wilt-resistant varieties only one-third of the plants can be expected to survive inoculation; thus it would be necessary to start with 3000 plants to have 10 for the polycross nursery. The desirable, relatively self-sterile, high seed and forage producing plants are now advanced to the polycross nursery.

4. *Polycross nursery:* To facilitate adequate seed production, randomization, and observation in the polycross nursery, clonal lines are made of the selected plants from the breeding nursery. Further elimination may take place in the polycross nursery on the basis of yield of forage and seed and other characteristics, but the majority of lines will be allowed to produce open-pollinated seed which will be planted in a replicated nursery for performance tests of the various polycrosses. It is important to observe the polycrosses for undesirable segregation since the progeny will afford a relative test on the homozygosity of the various lines. Selfing may be utilized if necessary to secure more information on the segregation of the lines. Those showing undesirable segregation are discarded. It would also be advantageous to appraise the polycrosses by various controlled tests such as cold, disease and insect-pest resistance. The original clones of the best polycrosses could be mixed to form a new synthetic variety.

5. *Production of hybrid alfalfa:* The original clones of the best polycrosses are increased by vegetative propagation so that single crosses, (which are equivalent to double crosses if the original clones were hybrid), in all combinations can be made in separate isolation blocks. Tests should be made of the self-sterility of the single crosses. The ideal situation would be to have self- and sib-sterile single crosses, though even if this is not fully realized it is probable that the double-crossed seed will not contain more than 25 per cent selfed and sibbed seed, (assuming 10 per cent selfing, as found under natural conditions, and 15 per cent sibbing, considering that a large proportion of the sibs have oppositional factors in common). From the tests of the single crosses the performance of all possible double crosses may be predicted. Seed of the most promising predicted hybrids is produced as outlined on page 35, and tested for performance. By corresponding seed production on a larger scale, the best performing double crosses are made available for commercial utilization.

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