GENERAL AND SPECIFIC COMBINING ABILITY OF FIVE ALFALFA CLONES
INCLUDING RECIPROCAL EFFECTS FOR SEEDLING VIGOR AND SEED YIELD

by

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INTRODUCTION

The use of F₁ hybrids for commercial production of such cross-pollinated crops as corn, sorghum, sugar beets, onions, and pearl millet suggests the feasibility of using this technique for alfalfa. Production of F₁ hybrids of commercial value is dependent on the use of breeding material expressing good combining ability. In order to obtain precise estimates of combining ability for quantitative characters in alfalfa, it is necessary to produce all possible single crosses among a number of parents. The single cross seed required is difficult to obtain due to the vegetative reproduction and isolation required; and, subsequently, limited testing of this type has been conducted in alfalfa. Testing breeding material for combining ability based on seed production has been more limited than testing based on forage yield or various other measurements. In this experiment a diallel crossing system was used to test the general and specific combining ability of five alfalfa clones previously selected for good general combining ability.

The report is based on first-year data of a three-year study, and the results are subject to errors which may occur due to variability inherent in the year of establishment. This is especially true for conclusions based on seed production. However, first-year data should be valid for such characteristics as flower color and seedling height. The analysis of seed production and seedling height is designed to measure
the relative amount of general and specific combining ability of the clones involved. Reciprocals of the single crosses were evaluated for flower color, seedling height, and seed yield to check if reciprocal cross progeny give equal performance.
REVIEW OF LITERATURE

General Review

The problem of alfalfa improvement has been of major concern to forage crop breeders for a number of years. Tysdal and Westover (1937) reviewed early improvement work in alfalfa, and a more recent review was presented by White (1949). The importance of using breeding material manifesting high combining ability in the improvement of alfalfa and other crops has stimulated research on this subject. Studies concerned with the use of combining ability have been reviewed by Hayes et al. (1955) for corn and other crops, and by Hanson and Carnahan (1956) for forage grasses. The information on combining ability concerning methods of alfalfa selection available prior to 1948 was reviewed by Bolton (1948) and more recently by Kehr and Graumann (1958).

Problems in Alfalfa Improvement

The development of superior breeding material in forage crops requires the isolation of plants with high combining ability. Breeding material from such plants may be obtained by inbreeding or clonal reproduction. In alfalfa improvement the isolation of clones offers the most practical solution due to the difficulty of obtaining inbred lines. Many workers have reported a reduction in vigor following self-fertilization in alfalfa. Tysdal et al. (1942) reported that forage yield of succeeding inbred generations decreased until it reached a minimum of 26 percent in the seventh generation. The seed productivity of selfed
lines decreased even more drastically than forage yield, reaching a low of 8 percent in the eighth generation of inbreeding. Bolton (1948) reported a drastic reduction in seed yield due to selfing where the average yield of 48 first generation inbred lines was 13 percent of the average yield of the check plots. Brink and Cooper (1936) found that foreign pollen tubes grew faster and were more likely to affect fertilization than self pollen tubes. They also found that the percentage of fertile ovules collapsing when self-pollinated was about five times as great as when cross-pollinated.

Probably the chief need for inbred lines is to duplicate a particular genotype that has superior characteristics and combining ability. However, Tysdal and Kiesselbach (1944) pointed out that in alfalfa (a perennial adapted to vegetative propagation) a genotype can be maintained indefinitely.

In recent diallel combining ability analyses of alfalfa, Pearson (1958) and Carnahan (1960) assumed that reciprocal crosses give progenies with equivalent performance. The assumption is that the self-fertility of the lines involved is equal or that selfed seed of low vigor will be eliminated through competition. Brink and Cooper (1936), Tysdal and Kiesselbach (1944), and Bolton (1948) reported that selfed seed produces seedlings with low vigor and that these seedlings will be eliminated through competition under field conditions. Tysdal and Kiesselbach (1944) and Bolton (1948) tested reciprocal crosses in alfalfa and found that in most cases the reciprocal crosses yield comparable to the single crosses. However, Tysdal and Kiesselbach (1944) found that reciprocal cross 1038 x 1035 yielded less than the cross 1035 x 1038 and that the difference was
due to more selfing in line 1038. He suggested that this situation be checked in planning combinations for possible commercial hybrids. Bolton (1948) reported that most reciprocal cross progenies of plants considered to be relatively self fertile did not differ in either seed or forage yield. The progenies of reciprocal crosses with one line, however, did differ significantly for both seed and forage yield. He concluded that the difference was due to the fact that seed from this plant germinated slowly and resultant stands were later and thinner rather than due to a large proportion of selfed seed. In testing other forage crops for reciprocal effects, Oldemeyer and Hansen (1955) found that although some orchard grass lines tended to be self-fertile, there was no indication that progeny yields were lowered. Knowles (1955) compared reciprocal crosses in brome grass and suggested that where significant differences were observed, they were due to self-fertilization.

Genetic Considerations

Atwood and Grun (1952) presented a comprehensive review of the cytogenetics of alfalfa. They reported that most of the early studies reviewed indicated characters were inherited in a disomic manner, but more recent studies reported notable exceptions to the disomic hypothesis. Tysdal et al. (1942) re-examined a study of leaf shape inheritance that could not be explained on the basis of diploid inheritance. They reported that when the leaf shape study was analyzed assuming tetraploid inheritance, a very close fit was obtained between the observed and the calculated ratios. Tysdal et al. (1942) stated that "from this evidence it seems highly probable that common alfalfa is a tetraploid and that some evidence indicates it may be an autotetraploid, though it has many allotetraploid characteristics." Stanford (1951) studied the inheritance of
flower color in alfalfa and demonstrated conclusively a case of tetraploid inheritance of a dominant factor for purple flower color. In another study of flower color inheritance, Weihing (1951) presented data to show that three independent factors (genes) govern segregations of purple and white in purple and white crosses, and for yellow x white crosses, three independent factors govern segregation of yellow and white progenies. Twamley (1955) reported that purple flower color was due to a sap-soluble anthocyanin pigment, the intensity of which was probably controlled by the number of dominant (purple) alleles present at each of two complimentary loci. In the same experiment Twamley used flower color inheritance in an investigation to determine whether alfalfa was an autotetraploid or an allotetraploid. The results of his experiment are in agreement with the conclusions of Tysdal et al. (1942) and Atwood and Grun (1952) that an unequivocal answer to the problem cannot be given.

**Definition of Combining Ability**

The concept of combining ability has been defined by Hayes et al. (1955) as "the relative ability of a biotype to transmit desirable performance to its offspring," and is becoming increasingly important in testing plant breeding material. This thesis is concerned with the definitions of general and specific combining ability as they were originally defined by Sprague and Tatum (1942). They defined the terms as follows: "The term general combining ability is used to designate the average performance of a line in hybrid combination, ... The term specific combining ability is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved." They made the assumption that general combining ability was dependent on the
additive effect of genes while specific combining ability was dependent on epistatic and dominance effects. Hayman (1957) reporting on the factors associated with heterosis in diploids stated that in the absence of epistasis, general combining ability is composed of both additive and dominance portions and specific combining ability mainly involves dominance. He also stated that when epistasis is present, both general and specific combining ability are expected to contain epistatic portions. Matzinger, Sprague, and Cockerham (1959) assumed that in a diploid crop, general combining ability was primarily a measure of additive gene action and specific combining ability was a deviation from additivity. Jinks (1955) reporting on this same problem concluded that general combining ability is always associated with the presence of epistasis.

Definitions of Combining Ability Tests

Several different tests are used by forage crop breeders to determine combining ability. The main tests used are "polycross," "open-pollinated progeny," "top-cross," and "diallel cross." Since they are generally used in the literature without being defined, a general definition will be given here. Unless otherwise specified, the general reference to the definition of a test is Hayes, Immer, and Smith (1955), Methods of Plant Breeding. The term "polycross" was first proposed by Tysdal, Kieselbach, and Westover (1942) to designate "the progeny from seed of a line that was subject to outcrossing with other selected lines growing in the same nursery." The term "open-pollinated progeny" generally refers to progenies from seed produced on selected plants outcrossed with other plants in any breeding nursery. "Top-crossed progeny" has been suggested to describe progenies grown from outcrossed seed produced from nurseries in which rows of the selected clones or lines are planted alter-
nately with a single commercial variety. Griffing (1956) defined a
diallel crossing system as "one in which a set of P inbred lines is
chosen and crosses among these lines give rise to a maximum of $P^2$ combi-
nations." The "diallel cross" is considered to be the best method to
determine the variance for specific as well as general combining ability
in the same experiment. Sprague and Tatum (1942) first used the system
in testing inbred lines of corn for combining ability. Diallel cross
data can be conveniently set out in a $P \times P$ table and analyzed using
applicable statistics.

Combining Ability Tests and Uses in Alfalfa

Several workers have proposed the use of alfalfa breeding material
of high combining ability to produce a hybrid. Tysdal (1942) tested the
performance of a number of single crosses between inbred lines and reported
that the highest producing hybrid exceeded the average of the checks by 39
percent, and the average yield of the ten top hybrids exceeded the checks
by 15 percent. It was pointed out in this study that not all of the hy-
brids demonstrated a large amount of hybrid vigor, indicating that the
combining ability was not the same for all lines. Tysdal et al. (1942)
and Tysdal and Kiesselbach (1944) proposed the use of alfalfa clones that
are highly self-sterile and possess good combining ability in a double
cross method of hybrid seed production. Bolton (1948) proposed a modifi-
cation of the double cross scheme of Tysdal (1942) based on the assumption
that four non-self-tripping, self-fertile plants might be found whose pro-
genies would have high combining ability. Tysdal et al. (1942) emphasized
the need for male-sterile pistillate lines to produce 100 percent $F_1$ hybrid
seed. Childers (1952, 1960) reported the occurrence of complete male
sterility in alfalfa that did not disturb the normal function of the female
gametophyte and can be maintained and increased under a system of back-crossing.

Tysdal and Crandall (1948) reported the results of comparing polycrosses and top-crosses of seven clones with their crosses when crossed in all combinations. The chief objective of the polycross test was to determine if it would be possible to select clones by means of the yields of their polycross progenies that would give high yielding single crosses. They found that the forage yield of the single crosses between the three clones combining best in the polycross test was 117 percent of the check varieties. This indicates that it would be possible to select high combining clones on the basis of the polycross test. To determine whether it is better to use a common top-cross parent rather than the general polycross progeny to determine combining ability among clones, Tysdal and Crandall compared forage yields of the two tests. This comparison of the two tests indicated that if the top 20 percent of the clones were selected, the same clones would be selected on the basis of either test.

Carnahan et al. (1960) studied the diallel crosses among 14 clones at several locations and reported that seedling vigor and fall growth habit were highly correlated and that each was highly heritable. The variance due to general and specific combining ability of seedling vigor and fall growth habit was investigated, and general combining ability variance was found to be much higher than specific combining ability variance for both characters. Although the variance for specific combining ability was low in magnitude, it was highly significant at each location. The contributions of individual clones to the general combining ability variance (σ_g^2) and specific combining ability variance (σ_s^2) were also reported. Carnahan concluded that the preponderance of additive gene
Effects for seedling vigor and fall growth habit in the study suggest the feasibility of using either polycross or inbred progeny performance to evaluate breeding material and indicate the use of synthetics to capitalize on the additive gene effects. However, he noted that the significant non-additive gene effects present would require the use of first generation hybrids for maximum expression of the genes. A diallel cross system of testing alfalfa was also employed by Pearson (1958) to analyze resistance to common leaf spot, pseudoplea leaf spot, bacterial wilt, winter injury, and stand persistence for general and specific combining ability. The inheritance of all these characters except winter injury and stand persistence is dependent on the additive effect of genes or general combining ability. There was no evidence of specific combining ability indicated for any of these characters except winter injury. Pearson (1958, 1960) suggested that the synthetic performance of characters that are inherited in an additive manner can be accurately predicted from clonal cross data or from general combining ability estimates of parental clones.

Morley et al. (1957) studied general and specific combining ability of 44 F1 alfalfa hybrids derived from ten strains for summer and winter production. The ratio of the estimated components of variation of general combining ability to residual was approximately one for summer yield and seven for winter yield. The ratio of specific combining ability variance to the residual variance was the same for both summer and winter yield. The larger ratio for general combining ability in the winter indicates large differences between strains for winter production. These differences are determined largely by genes with additive effects.

Wilsie and Skory (1948) in studying the forage yield of all possible single crosses obtainable from seven clones noted that some clones were
better combiners than others. A more detailed analysis of the yields of single crosses involving the best and poorest combining clones indicated that crosses involving the best combining clone were significantly higher than those involving the poorest combiner. The relationships between the yield performance of several other types of breeding material were studied by using correlation coefficients. The forage yield of 20 clones correlated with the forage yield of their first generation selfed progenies ($S_1$) gave an $r$ value of $-0.42$, which was almost significant at the 5 percent level. They suggest that this correlation may indicate value in evaluating parent plants by selection in $S_1$ progeny.

Open-pollinated progenies and polycross progenies were found to be significantly correlated with selfed ($S_1$) progenies by Wilsie and Skory (1948), Wilsie (1951), and Davis (1955). This suggests that if a polycross or open-pollination seed could not be produced, then $S_1$ progeny testing could be used to evaluate the general combining ability and/or prepotency of a clone in a breeding program. Wilsie (1951) found that self-fertility was relatively independent of combining ability, since there was no correlation between self-fertility and the yield of polycross progeny. McAllister (1950) also indicated that there was little difference in seed production between high and low combining groups.

Sprague and Tatum (1942), Carnahan et al. (1960), Timothy et al. (1959), Kalton and Leffel (1955), Federer and Sprague (1947), and Roja and Sprague (1952) tested forage crop breeding material for combining ability and found that when the parental breeding material being tested was selected on the basis of good general combining ability, the combining ability variance of the test will be greater for specific than for general combining ability. They have also concluded that when the breeding
material to be tested was not selected on the basis of combining ability
but from a random sample of the population with overall gene frequencies
equivalent to those of the larger population, the combining ability
variance of the test was larger for general than for specific combining
ability.

Studies of Combining Ability in Other Forage Crops

Burton (1951) studied a population of several pearl millet selec-
tions including their F1 and F2 progenies. His studies indicated that
heterosis was manifested in the inheritance of plant yield, stem diameter,
head length, plant height, and internode length. A test for the nature
of gene action in these studies indicated that it was largely arithmetic
(additive) for yield, stem diameter and maturity, but predominantly geo-
metric (over dominant) for plant height. In another study of 818 pearl
millet single crosses involving 296 well-established inbred lines, Burton
(1959) used 18 forage yield trials to estimate the additive and non-
additive genetic variance of the lines. Great variations were observed
between population of inbreds tested: Some exhibited little, others
large amounts of non-additive (over dominant) genetic variance. He
reported that in four of the sets of inbreds studied, the non-additive
genetic variance made up less than 15 percent of the total. If all pearl
millet behaved in this manner, the plant breeder could make close to the
maximum potential advance by using a stabilized synthetic to concentrate
the additive genes within a variety. However, in half of the 818 single
crosses Burton studied, the non-additive genetic variance component made
up over half of the total genetic variance, indicating that rapid yield
advance will be dependent on the use of an F1 hybrid. The value of the
F1 hybrid hypothesis was demonstrated when Starr pearl millet, a stabi-
lized synthetic released in 1950, demonstrated an advance in forage yield of only 33.5 percent of that obtained with Gahia - 1, an F₁ hybrid.

Oldemeyer and Hanson (1955) reporting on combining ability in orchard grass, found that there was a significant correlation coefficient for yield in relating polycrosses and single cross progenies to parents. There was found to be little difference in evaluating combining ability by using a wide polycross of 112 clones or a restricted polycross of 5-7 clones. A significant correlation was also obtained between 1951 and 1952 yield performance. They also reported considerable variation among the single crosses for the respective parents indicating the possible expression of specific combining ability. Correlations of breeding behavior with clonal performance of orchard grass were calculated by Weiss, Taylor, and Johnson (1951). Clones were significantly correlated with their open-pollination progenies in leaf width, panicle number, and winter survival. Low correlations were obtained and virtually no associations were in evidence for forage yield or leafiness. Another set of correlations between single crosses and mean performance of parent clones showed a high degree of associations for winter survival, moderately high correlations for leafiness, leaf width, panicle number, leaf diseases and lodging, and highly variable but low associations for forage yield. Performance of single crosses was correlated with mean performance of open-pollinated progenies of the two parent clones. Hanson et al. (1952) compared the general combining ability of 18 clones of orchard grass and 52 of their inbred lines in broadcast plots. The results showed that the level of inbreeding was not related to combining ability. They suggest that lines within families should be evaluated for combining ability. Kalton and Leffel (1955) evaluated all possible single crosses among 11
clones of orchard grass for general and specific combining ability. It was evident from their study that appreciable differences existed in general combining ability among the parental clones, but that effects attributable to specific combining ability were generally low. Since the clones evaluated were not selected for general combining ability, the large variance for general combining ability was expected. Methods of evaluating brome grass for combining ability have been investigated by several workers. Knowles (1955) reviewed the results from ten tests of brome grass progenies over the period of 1945-1953 to find the usefulness of open-pollinated progenies in assessing the combining ability of selected plants. Open-pollinated progeny tests of selected plants from commercial varieties and strains indicated a moderate range for forage production and a wide variability for seed production. Several first generation synthetics formed on the basis of open-pollination progeny performance showed significantly higher forage yields than commercial check varieties. The combining ability of five brome grass clones was studied by Timothy et al. (1959) using clones, single crosses, and polycrosses progeny. In general they found fair agreement among the rank of the clones and their combining ability. He suggested that, should there continue to be fair agreement of clonal rank and progeny performance, and if additive genetic effects are more important than non-additive effects, then a breeder should consider the possibility of clonal performance alone as a basis of selection for the formation of brome grass synthetics. The five clones were also evaluated in a diallel cross system of testing. General combining ability was more important than specific combining for forage yield, seed yield, leaf spot, bacterial blight, and scald. Specific combining ability was predominant for plant
height, noted to a minor extent for seed yield and general leaf spot, and appeared to be absent for forage yield, bacterial blight, and scald. In summary of the different tests used by Timothy et al. (1959), there was reasonable close agreement in performance of clones, their average single cross performance, and polycross progeny performance of all characters studied except plant height. Murphy (1952) compared the performance of parental clones with that of polycross and selfed progenies of orchard grass, smooth brome, and red fescue. Drilled and Broadcast spaced planting were used and significant positive correlations were obtained between parental and progeny yields regardless of the method of planting. According to these investigations, any of the methods tested could be used in selecting for high yield potential.

In a polycross test the purpose is to judge the parents by their progenies and a practical problem to the plant breeders is to determine what proportion of the total variation in the progenies can be attributed to genetic differences among the female parents. This variation can be given by "H" or Heritability. Heritability of quantitatively inherited characters in forage crops is generally calculated in the broad sense, measuring the additive gene effects by using the following formula presented by Thomas and Kernkamp (1954):

\[
H = \frac{V_G}{V_G + V_e}
\]

where: \( V_G \) = Error mean square

\( V_G = \text{(Clonal or progeny mean square)} - V_e \)

\( r = \text{Number of replications} \)

Heritability ratios and correlation coefficients were used to measure combining ability in smooth brome grass by Thomas and Kernkamp (1954). They illustrated the use of heritability ratios in studying the relation between parent clones and their polycross progenies. It was pointed out that even though there was no correlation between parents and progeny for
protein percentage and seed yield, the heritabilities of the first cutting of brome grass at three different locations of 15, 19, and 25 per cent, would make it appear feasible to select for this character using the data from the three progeny tests. Heritability ratios as used by Thomas and Kernkamp are probably influenced mostly by additive genetic variations among progenies in per cent of total genetic variation. They conclude that heritability ratios are more convenient and useful as an aid to selection than correlation between parent and progeny because only the relationship between the two phenotypes and not the genotypes is measured between parent and progeny.

Heritability in tall fescue was measured by Burton and DeVane (1953) by using heritability ratios as calculated in the broad sense. They in turn used the heritability ratios to calculate the expected gain (S) or advance over the population mean, that a breeder may expect, by using a particular selection system and selecting a certain percentage of the population. S is calculated as follows from the formula presented by Lush (1945):

\[ S = \frac{s \times V_g}{V_p} \]

where: \[ V_p = V_g - \frac{V_e}{r} \]

and \[ s = \text{selection differential} \]

The "S" value for seed yield in Burton and DeVane's (1953) experiment was 161 percent greater than the mean when the top 5 percent of the population was selected (s = 2.06). Other measurements—plant yield rating, yield of green tissue rating, green weight of plants in grams and pounds, greenness rating (used for drought and heat resistance), and disease resistance rating—gave "S" values ranging from 33-72 percent. Weber and Moorthy (1952), in studying soybeans, used heritability ratios in the broad sense referring to the whole genotype as a unit and in contrast
with environmental effects. They found that heritability for yield in the F₁ crosses studied was very erratic and averaged near zero, due to a large environmental variance. The other characters studied, oil content, flowering time, maturity date, period from flowering to maturity, plant height, and seed weight, were affected comparatively less by soil heterogeneity and environmental factors than yield. Consequently, a considerably higher heritability was obtained for them.

Sweet clover was studied by Johnson (1952) with reference to the effectiveness of the recurrent selection technique for increasing general combining ability. The original population was relatively inferior with respect to general combining ability. When first generation selfed lines were selected on the basis of combining ability from the upper ranges of the population of parental plants, nearly 50 percent of the F₁ crosses among the ten selfed lines were significantly higher in yield the first year than the parent varieties. He used a random combination of F₁ crosses to form a synthetic in the second generation and found that individual plants from the synthetic 2 gave a distribution in general combining ability approximately equal to that of the F₁ crosses. Since a large positive gain in combining ability was obtained in a single cycle of recurrent selection, he suggests that this breeding procedure may be an effective method of breeding forage crop plants.
MATERIALS AND METHODS

The clones used in the experiment were selected by Dr. M. W. Pedersen from a spaced planted, open pollinated nursery on the basis of high seed production, resistance to yellow leaf blotch, downy mildew, and other necessary agronomic characteristics. The clones were used by Dr. Pedersen to produce a five clone synthetic named Utah Synthetic C and are identified as Utah numbers 9, 34, 55, 57, and 70. Single cross seed of the clones involved in the experiment was produced under 20 x 20 foot plastic mesh cages. Cross pollination was facilitated by confining a colony of honey bees under each cage. The individual clones making up a single cross were planted in individual, alternating rows within a cage. Seed harvested from alternate rows was kept separate in order to obtain reciprocal cross seed.

A test designed to measure the self fertility of each parent clone was conducted using the five parent clones that were previously established on the Evans Experimental Farm. Five hundred florets on each of the five clones were self-pollinated on July 7 and 8, 1960, using the following technique: The terminal end, and all but ten untripped florets, was removed from ten racemes per plant. The ten florets remaining on each raceme were tripped with a toothpick to accomplish self-pollination. Plants containing self-pollinated flowers were sprayed with Systox, mixed on the basis of six ounces per acre, to control insects. On August 16, 1960, the racemes containing selfed pods were harvested and taken to the legume seed laboratory to be threshed.
The combining ability test nursery was located in "H" field of the Greenville Experimental Farm. The nursery covers a total of 1.7 acres and was divided into six replications with 64 plots 6 x 20 foot within each replication. Only the 20 plots containing the single and reciprocal crosses, out of the 64 plots in the nursery, are used in the combining ability analysis. A roadway is included longitudinally every 24 feet to allow, mainly, insecticide application with a tractor. In preparation for planting, germination tests were conducted on the seed of the crosses used in the experiment. The seed in question was placed in petri dishes containing moistened blotter paper and kept in a constant temperature cabinet. A germination percentage of approximately 90 percent was obtained for all entries except Ranger and the 1953 polycross, both of which germinated at approximately 80 percent. The seed was mixed on the basis of its germination percentage with heat sterilized seed at a ratio of one pound of viable to 2.6 pounds of inviable seed. The mixture of viable and sterile seed was thoroughly blended using a Seedburo mixer and then placed in manila envelopes. The envelopes were each labeled with a randomized plot number for each replication.

A firm seed bed was prepared; and on April 14, 1960, three rows, two feet apart, were planted within each plot. On the basis of 120 square feet per plot, 7.55 grams of viable and 19.69 grams of sterile seed were planted per plot. The nursery was cultivated with a tractor on June 2, 30, July 12 and 20 to remove weeds and provide irrigation furrows. Hand weeding was done at periodic intervals when needed throughout the growing season. The nursery was furrow irrigated on May 2, June 10, 24, and July 14. These dates correspond to periods when the plants in the nursery needed supplementary moisture to maintain growth and development.
After "seed set" was initiated, no more irrigation water was applied so that vegetative growth would be kept at a minimum. Periodic inspection throughout the season indicated that foliage diseases were not a factor in the performance of the varieties and strains of alfalfa.

Insect control was accomplished throughout the growing season by periodically spraying with insecticides, using a tractor equipped with suitable spraying apparatus. The tractor used was driven down the roadways letting the "booms" of the spray unit extend over the plots; consequently, no mechanical injury was inflicted on the plots by the spraying operation. The time to spray and the kinds and amounts of insecticide to apply were determined by the numbers and species of insects estimated to be present in the nursery. An estimate of the insect population was obtained at approximately weekly intervals by sweeping plants with a hand net and counting the number of harmful insects obtained. The lygus bugs, *Lygus elisus* and *L. hesperus*, and green pea aphid, *Macrosiphum pisi*, appeared to be the only harmful insects posing any threat to seed production; and, since lygus bugs were in greatest concentration, plots were sprayed when the number of lygus bugs per sweep was greater than one. Subsequently, the plots were sprayed on June 29 with four ounces of dieldrin per acre, July 12 with eight ounces of dieldrin and six ounces of Systox per acre, and on August 2 and 11 with two and three pounds of Toxaphene per acre respectively.

An estimate of seedling height was obtained on June 14, 1960, by measuring three plants in the center row of each plot. The technique used to obtain the height measurement is as follows: The person measuring took one step down either furrow next to the center row in each plot, placed a measuring stick by his toe, and measured the tallest plant near
his toe. Two more measurements on the center row of each plot were obtained in the same way at two-step intervals. Plant height data were taken on July 11, 1960, using the same technique as outlined for seedling height.

An estimate of the flower color of each entry in the nursery was made on August 4. The color of the flowers in a plot is indicated by a code number chosen by the writer. The code numbers run from 1, which designates predominantly white, to 5, which designates purple.

The seed plots in the nursery were "separated" by hand on August 24 in preparation for harvesting. This was accomplished by walking between the outside rows of each plot and separating the entangled growth between them. The nursery was harvested on September 21, 1960, with a tractor-operated forage mower. The plots were then separated into small piles, staked down, and allowed to dry in the field before threshing. Threshing was carried out in the field on September 26, 27, and 28, using a small combine that had been modified for threshing small alfalfa plots. The forage from each plot was placed on a canvas, weighed, and weight recorded in pounds before threshing. Forage from each plot was threshed individually and, since some unthreshed seed pods were found to be carried over in the chaff, the chaff was collected in a large basket as it came out of the combine and rethreshed. The total chaff from the 64 plots in each replication was also rethreshed and the seed was salvaged for use as a correction for the amount of seed lost per replication. The seed, "in the dirt," from each plot was removed from the machine after each plot was threshed, placed in 25 pound paper bags, and labeled with the field plot number. The amount of seed from each replication was found to be within ±1 standard deviation of the mean; therefore, the mean value
was used as a correction factor for each replication. A clipper-type cleaner was used to separate the seed of each plot from the chaff and other dirt. The cleaned seed was weighed and the weight recorded in grams. Gram weights of clean seed were transposed to pounds per acre before statistical analysis.

**Statistics**

**Analysis of variance for combining ability**

The analysis for general and specific combining ability among the 10 single crosses and reciprocal crosses possible from the 5 parent clones was accomplished by using the diallel technique proposed by Griffing (1956), where one set of single crosses and reciprocals were included but not the parents.

A diallel crossing system is one in which a set of \( P \) clonal lines is chosen and crosses among these lines were made. In this experiment \( P = 5 \) and a maximum of \( P^2 \) or 25 crosses are possible. Since the parental lines are left out of this experiment, we have a total of \( P(P-1) \) or 20 combinations comprised of one set of single crosses \( \frac{1}{2}P(P-1) \) or 10 plus one set of reciprocals \( \frac{1}{2}P(P-1) \) or 10.

Griffing (1956) suggests using the means of the single cross \((i \times j)\) progeny in a diallel table. However, when means are used the sums of squares for combining ability will not add up to the sums of squares for treatments as calculated by the regular analysis of variance. This can be overcome by using means of the single cross progeny in the diallel table of the combining ability analysis and then multiplying the sums of squares by the number of replications in the experiment \((r)\), or by using totals of the single cross progeny in the diallel table and then dividing the sums of squares by the number of replications \((r)\).

In order to clarify the notation used for the diallel analysis, an
example using \( P = 5 \) is given.

\[
\begin{array}{cccccc}
  j_1 & j_2 & j_3 & j_4 & j_5 \\
  i_1 & x_{11} & x_{12} & x_{13} & x_{14} & x_{15} & x_{i1}^- \text{.} \\
  i_2 & x_{21} & x_{22} & x_{23} & x_{24} & x_{25} & x_{i2}^- \text{.} \\
  i_3 & x_{31} & x_{32} & x_{33} & x_{34} & x_{35} & x_{i3}^- \text{.} \\
  i_4 & x_{41} & x_{42} & x_{43} & x_{44} & x_{45} & x_{i4}^- \text{.} \\
  i_5 & x_{51} & x_{52} & x_{53} & x_{54} & x_{55} & x_{i5}^- \text{.} \\
  x_{i1} & x_{i2} & x_{i3} & x_{i4} & x_{i5} & x_{..} \\
\end{array}
\]

Where in this experiment the parents \((x_{i1}j_1', x_{i2}j_2 ... x_{i5}j_5)\) are not included in the main diagonal:

\[
x_{i1}^- = x_{i1}e_{i1} + x_{12} + x_{13} + x_{14} + x_{15},
\]

\[
x_{i1}^- = x_{i1}e_{i1} + x_{21} + x_{31} + x_{41} + x_{51},
\]

Total = \(X_{..} = x_{i1} = x_{12} + x_{13} ... + x_{53} + x_{54}\)

At this point it is necessary to decide whether the experimental material being tested is a chosen or fixed set or a random sample from a population. These assumptions correspond to what is known statistically as a Model I or Model II, respectively. In this experiment the material being tested is a chosen or fixed set and, therefore, is analyzed as a Model I. In Model I, according to Griffing's analysis, the experimental material is to be regarded as the population about which inferences are to be made. The objective is to compare combining abilities of the parents when the parents themselves are used as testers, and to identify the higher yielding combinations. For this testing procedure it is necessary to assume only that the error is normally and independently distributed with mean zero and variance \(\sigma_e^2\).

Other assumptions implicit on the analysis are: (a) The population is random mating and in equilibrium; (b) the genotypes of the parent
clones are considered to be a chosen or fixed set; (c) each parent clone is equally heterozygous i.e. non-inbred; (d) reciprocal crosses do not necessarily give progenies with equivalent performance; (e) each F<sub>1</sub> population studied is representative of the cross i.e. selective or non-random reduction of performance due to weed competition, moisture difference, disease, etc. is not affecting performance; (f) statistical technique developed for diploid organisms may be used on alfalfa, which is believed to be a tetraploid species.

Analysis of variance

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>Expectation of mean squares for Model I</th>
</tr>
</thead>
<tbody>
<tr>
<td>General combining ability</td>
<td>p-1</td>
<td>S&lt;sub&gt;g&lt;/sub&gt;</td>
<td>M&lt;sub&gt;g&lt;/sub&gt;</td>
<td>σ&lt;sup&gt;2&lt;/sup&gt;·2(p-2)·1/p-1·Σri&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Specific combining ability</td>
<td>p(p-3)/2</td>
<td>S&lt;sub&gt;s&lt;/sub&gt;</td>
<td>M&lt;sub&gt;s&lt;/sub&gt;</td>
<td>σ&lt;sup&gt;2&lt;/sup&gt;·2(2/p(p-3)·ΣΣsij&lt;sup&gt;2&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Reciprocal effects</td>
<td>p(p-1)/2</td>
<td>S&lt;sub&gt;r&lt;/sub&gt;</td>
<td>M&lt;sub&gt;r&lt;/sub&gt;</td>
<td>σ&lt;sup&gt;2&lt;/sup&gt;·2(2/p(p-1)·ΣΣrij&lt;sup&gt;2&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Error</td>
<td>m</td>
<td>S&lt;sub&gt;e&lt;/sub&gt;</td>
<td>M&lt;sub&gt;e&lt;/sub&gt;</td>
<td>σ&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Where:

\[
S_g = \frac{1}{2(p-2)} \sum (X_{i.} + X_{.i})^2 - \frac{2}{p(p-2)} X_{..}^2
\]

\[
S_s = \frac{1}{2} \sum \sum (x_{ij} + x_{ij})^2 - \frac{1}{2(p-2)} \sum (X_{i.} + X_{.i})^2 + \frac{1}{(p-1)(p-2)} X_{..}^2
\]

\[
S_r = \frac{1}{2} \sum \sum (x_{ij} - x_{ij})^2
\]

Differences within classes of effects are tested by F ratios, where M<sub>e</sub> = Experimental error for the analysis of variance.

1. To test G.C.A. effects use \( F(df=p-1;m) = \frac{M_g}{M_e} \)
2. To test S.C.A. effects use \( F(df=p-3)/2;m) = \frac{M_s}{M_e} \)

3. To test reciprocal effects use \( F(df=p-1)/2;m) = \frac{M_r}{M_e} \)

The variance of each individual clone for general combining ability may be estimated by:
\[
\sigma^2 g_i = (g_i)^2 - \frac{p-1}{p(p-2)} \sigma^2 \]
where \( \sigma^2 = \frac{M_e}{r} \)

and \( (g_i) = \frac{1}{2p(p-2)} \left[ p(x_{i.} - x_{.1}) - 2 x_{.} \right] \)

The variance of each individual clone for specific combining ability may be estimated by:
\[
\sigma^2 s_{ij} = \frac{1}{p-2} \sum (s_{ij})^2 - \frac{p-3}{p-2} \sigma^2 \]
where \( \sigma^2 = \frac{M_e}{r} \)

and \( (s_{ij}) = \frac{1}{2(p-2)} (x_{i1} + x_{1j} - x_{1.} - x_{.j}) + \frac{1}{(p-1)(p-2)} x_{.} \)

**Levels of significance**

The levels of significance for F-ratios and correlation coefficients in the results of this experiment are indicated by asterisks (*): where (*) = significant at \( P = .05 \) level and (**) = significance at \( P = .01 \) level.

**Heritability**

Heritability ratios (H) were calculated in the broad sense, which measures the mostly additive gene effects, by using the formula presented by Thomas and Kernkamp (1954).

\[
H = \frac{V_G}{V_G + V_e} \quad \text{Where } V_e = \text{Error mean square} \\
\text{and } V_G = (\text{clonal or progeny mean square} - \frac{V_e}{r})
\]

The advance (S) over the population mean, that a breeder may expect by using a particular selection system and selecting a certain percentage of the population, is calculated from the formula presented by Lush (1945).

\[
S = \frac{s V_G}{V_p} \quad \text{Where } V_p = V_G + \frac{V_e}{r} \\
\text{and } s = \text{selection differential}
\]
EXPERIMENTAL RESULTS

Self-fertility of Parent Clones

A test designed to measure the self-fertility of the parent clones was conducted and the results are reported in table 1.

Table 1.— Self-fertility of parent clones

<table>
<thead>
<tr>
<th>Clone number</th>
<th>Flowers tripped per raceme</th>
<th>Number of racemes harvested</th>
<th>Seeds per raceme</th>
<th>Seeds per flower tripped</th>
<th>Number</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>10</td>
<td>38</td>
<td>196</td>
<td>.516</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>10</td>
<td>40</td>
<td>107</td>
<td>.268</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>10</td>
<td>40</td>
<td>208</td>
<td>.520</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>10</td>
<td>37</td>
<td>226</td>
<td>.611</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>10</td>
<td>38</td>
<td>330</td>
<td>.868</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>.557</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The average self-fertility of the clones is .557 seeds per flower tripped. Clone number 34 is lowest in self-fertility, producing only .268 seeds per flower tripped; and clone number 70 was highest, producing .868 seeds. The other clones, 9, 55, and 57, were close to the average value for self-fertility.

Seedling Height

Seedling height of single crosses and reciprocal crosses was analyzed using the analysis of variance and the results are reported in table 2. Variance for the difference between the crosses is significant at the P = .01 level and is partitioned into the variance attributable to
general combining ability, specific combining ability, and reciprocal effects. Highly significant F-ratios were obtained for general combining ability and reciprocal effects, but specific combining ability effects were not significant.

Table 2.—Single cross and reciprocal cross analysis of variance for seedling height

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Mean squares</th>
<th>F-ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>5</td>
<td>44.83</td>
<td>8.96</td>
<td>5.43**</td>
</tr>
<tr>
<td>Crosses</td>
<td>19</td>
<td>77.12</td>
<td>4.06</td>
<td>2.46**</td>
</tr>
<tr>
<td>Gen. C. A.</td>
<td>(4)</td>
<td>(31.31)</td>
<td>(7.82)</td>
<td>5.63**</td>
</tr>
<tr>
<td>Sp. C. A.</td>
<td>(5)</td>
<td>(9.41)</td>
<td>(1.88)</td>
<td>1.35</td>
</tr>
<tr>
<td>Recip. Effects</td>
<td>(10)</td>
<td>(36.40)</td>
<td>(3.64)</td>
<td>2.62**</td>
</tr>
<tr>
<td>Exp. Error</td>
<td>95</td>
<td>125.53</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>257.48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C.V. = 19.0

The contribution of each clone to general combining ability is given in Table 2. General and/or specific combining ability effects can be either positive or negative and indicate whether the combining ability of a clone is greater than (positive) or less than (negative) the average combining ability of the other clones involved in the study. The general combining ability variance of a clone is computed by squaring the combining ability effects and subtracting a constant and, therefore, is of a similar relative magnitude. However, since the combining ability effects are squared, any negative values will be eliminated, and it must be kept in mind that a large positive variance can indicate that a clone performs better or poorer than the average.
Table 3.—General combining ability effects and variances for seedling height

<table>
<thead>
<tr>
<th>Parent clone</th>
<th>General combining ability effects $\bar{G}$</th>
<th>Rank of clones</th>
<th>General combining ability variance $\sigma_2^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.06</td>
<td>3</td>
<td>$-0.0060^{a}$</td>
</tr>
<tr>
<td>34</td>
<td>0.635</td>
<td>1</td>
<td>0.3398</td>
</tr>
<tr>
<td>55</td>
<td>0.199</td>
<td>2</td>
<td>$-0.0239^{a}$</td>
</tr>
<tr>
<td>57</td>
<td>$-0.556$</td>
<td>5</td>
<td>0.2456</td>
</tr>
<tr>
<td>70</td>
<td>$-0.388$</td>
<td>4</td>
<td>0.0507</td>
</tr>
</tbody>
</table>

$^{a}$Negative values for the variance are obtained when the correction term is larger than the sum of squares.

Clones are ranked from 1, which indicates the best general combiner, to 5, which indicates the poorest. On this basis clone number 34 performed best and 57 the poorest. Since the general effects of clone 9 are low and nearer to zero than the effects of the other clones, it performed about average. Negative values were obtained for clones 70 and 57, indicating that they performed lower than would be expected on the basis of the average of all lines involved. Specific combining ability was not significant in the seedling height study and, therefore, the specific combining ability variances and effects are not reported.

Since it is reported in table 2 that reciprocal effects are highly significant, the means and reciprocal effects of the single crosses are given for comparison in table 4.

The reciprocal effect reported in table 4 is the difference between a single cross and its reciprocal and indicates if the reciprocal cross was taller (positive) or shorter (negative) than the single cross. Reciprocal effects are ranked from 1, the reciprocal cross with the greatest reduction in performance, to 10, the reciprocal cross with the greatest
increase in performance. The poorest performing reciprocal crosses which are ranked 1, 2, 3, in order, all contain clone 57 in the maternal position. Reciprocal crosses ranked 4, 5, 6 also performed poorer than the single crosses and all contain clone 70 in the maternal position.

Table 4.—Seedling height, means, and reciprocal effects for single crosses and reciprocals

<table>
<thead>
<tr>
<th>Cross</th>
<th>Seedling height mean (inches)</th>
<th>Reciprocal effect</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>9x34</td>
<td>6.6</td>
<td>+ .8</td>
<td>9</td>
</tr>
<tr>
<td>34x9</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9x55</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55x9</td>
<td>6.6</td>
<td>- .4</td>
<td>7</td>
</tr>
<tr>
<td>9x57</td>
<td>6.6</td>
<td>-1.5</td>
<td>2</td>
</tr>
<tr>
<td>57x9</td>
<td>5.1</td>
<td>+1.1</td>
<td>10</td>
</tr>
<tr>
<td>70x9</td>
<td>5.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9x70</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34x55</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55x34</td>
<td>6.7</td>
<td>+ .1</td>
<td>8</td>
</tr>
<tr>
<td>34x57</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57x34</td>
<td>6.0</td>
<td>-1.4</td>
<td>3</td>
</tr>
<tr>
<td>34x70</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70x34</td>
<td>6.3</td>
<td>- .3</td>
<td>5</td>
</tr>
<tr>
<td>55x57</td>
<td>6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57x55</td>
<td>4.7</td>
<td>-2.0</td>
<td>1</td>
</tr>
<tr>
<td>55x70</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70x55</td>
<td>6.2</td>
<td>- .7</td>
<td>6</td>
</tr>
<tr>
<td>57x70</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70x57</td>
<td>4.7</td>
<td>-1.1</td>
<td>4</td>
</tr>
</tbody>
</table>

A comparison of the means of the clones when crossed in all maternal and paternal combinations gives the relative performance of a clone in relation to other clones and the reciprocal effects. The comparison of these means is given in figure 1, where bar X indicates the average yield of a clone when crossed in the maternal position; bar Y, the yield when crossed in paternal position. The clones are listed in order of
increasing plant height on the abscissa, and seedling height in inches on the ordinate.

![Bar graph showing average seedling height in inches of individual alfalfa clones when used as the maternal parent (X bar) and paternal parent (Y bar) in crosses.]

Figure 1.—Bar graph showing the average seedling height in inches of individual alfalfa clones when used as the maternal parent (X bar) and paternal parent (Y bar) in crosses.

Clone number 34 performed best for seedling height when all possible cross combinations containing 34 were averaged. The clones decrease in performance in order through clones 55, 9, and 70, down to 57, which gave the shortest average seedling height. A comparison of the maternal and paternal crosses for the clones shows that clones 57 and 70 demonstrated the greatest reciprocal cross differences due to a reduced height when used as the maternal parent. Clones 34 and 55 show reciprocal differences due to increased height when used as the paternal parent. No appreciable reciprocal difference was noted for clone 9.

**Plant Height**

Plant height of the crosses in the nursery was nearly uniform at the time the measurement was taken and no significant difference between single crosses was indicated by the F ratio.
Flower Color

An estimate of the flower color of the single crosses and reciprocals was obtained when the nursery was in full bloom. Analysis of variance of these data are reported in table 5. The average flower color of the individual clones when used as paternal and maternal parents in crosses is reported in figure 2.

Table 5.—Single cross and reciprocal cross analysis of variance for flower color

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Mean squares</th>
<th>F-ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>5</td>
<td>2.2</td>
<td>.44</td>
<td>.03</td>
</tr>
<tr>
<td>Crosses</td>
<td>19</td>
<td>107.3</td>
<td>5.65</td>
<td>4.06**</td>
</tr>
<tr>
<td>Reciprocal Effects</td>
<td>(10)</td>
<td>(27.78)</td>
<td>(2.78)</td>
<td>2.00*</td>
</tr>
<tr>
<td>Exp. Error</td>
<td>95</td>
<td>132.4</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>141.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C.V. = 28.5

The analysis of variance for flower color reported in table 5 indicates that crosses are significantly different at the P = .01 level. Reciprocal effects as partitioned out of the variances for single crosses and reciprocals using the method proposed by Griffing (1956) are significant at the P = .05 level. Combining ability variance is not included, for it is a measure of gene effects inherited quantitatively and flower color is a qualitatively inherited character.

In order to show the relative color of each clone and how it performed when crossed in the maternal as well as the paternal position, the average flower color of a clone is given in figure 2, when crossed as the maternal parent (bar X) and paternal (bar Y).
The clones 57, 55, 9, 70, and 34 are listed on the abscissa in order of increasing purple color. The values given on the ordinate represent the relative color of the clones where 1 designates white; 2, more white than purple; 3, even amounts of purple and white; 4, more purple than white; 5, completely purple. Clone 57 contains the most white on the basis of the maternal and paternal average, and the clones increase in purple through numbers 55, 9, and 10 to number 34, which is almost completely purple. Clone 70, which is high in self-fertility, demonstrated a high reciprocal difference; but the highest difference was demonstrated by clone 55, as shown by the height of the bars in figure 2.

Seed Yield

Seed yield of the single and reciprocal crosses was analyzed using the analysis of variance, and the results are reported in table 6. The variance of single and reciprocal cross entries was partitioned into that due to general, specific, and reciprocal effects, using the method proposed by Griffing (1956).
Table 6.—Single cross and reciprocal cross analysis of variance for seed yield

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Mean squares</th>
<th>F-ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>5</td>
<td>337,783</td>
<td>67,557</td>
<td></td>
</tr>
<tr>
<td>Crosses</td>
<td>19</td>
<td>1,089,743</td>
<td>57,355</td>
<td>3.36**</td>
</tr>
<tr>
<td>Gen. C. A.</td>
<td>(4)</td>
<td>(380,438)</td>
<td>(95,110)</td>
<td>5.57**</td>
</tr>
<tr>
<td>Sp. C. A.</td>
<td>(5)</td>
<td>(202,443)</td>
<td>(40,489)</td>
<td>2.37*</td>
</tr>
<tr>
<td>Recip. Effects</td>
<td>(10)</td>
<td>(506,962)</td>
<td>(50,686)</td>
<td>2.97**</td>
</tr>
<tr>
<td>Exp. Error</td>
<td>95</td>
<td>1,623,270</td>
<td>17,091</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>3,050,796</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V. = 18.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The difference between the crosses analyzed in table 6 is highly significant at the $P = .01$ level. The variance attributable to general combining ability and to reciprocal effects is also highly significant. Specific combining ability variance is lower in magnitude but still significant at the $P = .05$ level.

Since the variance for general, specific, and reciprocal combining ability effects are significant in table 7, it is appropriate to calculate the contributions of each clone to these effects. The contribution of each clone to general and specific combining ability is given in table 7. The method proposed by Sprague and Tatum (1942) to compute the variance of individual clones for combining ability is included in table 7 to compare the results obtained from it to the results obtained in the analysis proposed by Griffing (1956). Only the variance for general and specific combining ability is reported when using Sprague and Tatum's analyses. Under Griffing's analysis in table 7 the general combining ability effects are reported in addition to general and specific variance.
The rank of the clones for general and specific combining ability is the same for both analyses. Since Sprague and Tatum's analysis is included only for comparison, the results will be discussed as they appear under Griffing's method. The general combining ability variance is ranked from 1, which indicates the best combiner, down to 5, which indicates the poorest. On this basis clone number 54 was the best combining clone, with number 55 second. Clones 9 and 57 have general effects near zero and, therefore, are about average in general combining ability. The poorest general combining ability was demonstrated by clone number 70.

Specific combining ability variance is also ranked from 1 to 5. Clone 57 had the highest variance and was ranked number 1, which indicates that combinations with 57 performed either better or poorer than could be expected on the basis of the average performance of the lines involved. As the specific combining ability variance decreases it indicates that crosses containing a particular clone are performing more closely to what might be expected on the basis of the average performance of the lines involved. Clone number 55 had the next lowest variance and is followed by clones 9, 54, and 70. Clone 70, having the smallest variance, was, therefore, performing in crosses about as expected on the basis of the average performance of the lines involved.
It was reported in table 6 that specific combining ability effects and reciprocal effects are significant. Therefore, the seed yield means, specific and reciprocal effects for single crosses, and reciprocals are reported for comparison in table 8. The specific combining ability effects are computed by averaging a single cross and its reciprocal and indicate whether a cross performed better (positive) or poorer (negative) than would be expected on the basis of the average of the lines involved. The specific effects are used, mainly, for computing the specific combining ability variance of a clone and no further attempt will be made to discuss them here. The reciprocal effect reported is the difference between a single cross and its reciprocal and indicates if a reciprocal yielded better (positive) or poorer (negative) than its single cross.

Table 8.—Seed yield, means, specific and reciprocal effects for single crosses and reciprocals

<table>
<thead>
<tr>
<th>Cross</th>
<th>Mean seed yield lbs./A.</th>
<th>Specific effect</th>
<th>Reciprocal effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>9x34</td>
<td>707</td>
<td>-32</td>
<td>+58</td>
</tr>
<tr>
<td>34x9</td>
<td>765</td>
<td>-4</td>
<td>+108</td>
</tr>
<tr>
<td>9x55</td>
<td>679</td>
<td>+73</td>
<td>+166</td>
</tr>
<tr>
<td>55x9</td>
<td>787</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9x57</td>
<td>700</td>
<td>+108</td>
<td></td>
</tr>
<tr>
<td>57x9</td>
<td>866</td>
<td>+166</td>
<td></td>
</tr>
<tr>
<td>9x70</td>
<td>606</td>
<td>-36</td>
<td>-28</td>
</tr>
<tr>
<td>70x9</td>
<td>580</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34x35</td>
<td>860</td>
<td>+81</td>
<td></td>
</tr>
<tr>
<td>55x34</td>
<td>809</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34x57</td>
<td>724</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57x34</td>
<td>760</td>
<td>-4</td>
<td></td>
</tr>
<tr>
<td>34x70</td>
<td>706</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70x34</td>
<td>637</td>
<td>-74</td>
<td></td>
</tr>
<tr>
<td>55x57</td>
<td>671</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57x55</td>
<td>634</td>
<td>+27</td>
<td></td>
</tr>
<tr>
<td>55x70</td>
<td>808</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70x55</td>
<td>535</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57x70</td>
<td>735</td>
<td>+15</td>
<td></td>
</tr>
<tr>
<td>70x57</td>
<td>532</td>
<td></td>
<td>-202</td>
</tr>
</tbody>
</table>
Reciprocal effects are ranked from 1, the reciprocal cross with the greatest reduction in performance, to 10, the reciprocal cross with the greatest increase in performance. The poorest performing reciprocal crosses which are ranked 1, 2, and 3, all contain clone 70 in the maternal position. The reciprocal cross ranked number one, 70 x 55, yielded 535 pounds of seed; and its single cross, 55 x 70, yielded 808 pounds, a difference of 272 pounds. The reciprocal cross ranked numbers 2 and 3 yielded 202 and 70 pounds, respectively, less than their single crosses. The total reduction in yield from these three reciprocal crosses containing clone 70 is 544 pounds, which is only 8 pounds below the total reciprocal effects of the seven other reciprocal crosses.

The means of the clones when crossed in all maternal and paternal combinations is given in figure 3, which shows the relative seed yield of the clones and the reciprocal effects. Bar X indicates the average yield of a clone when crossed in the maternal position; and, bar Y, the yield when crossed in the paternal position. The clones are listed in order of increasing seed yield on the abscissa and seed in pounds per acre on the ordinate. Clone 34 yielded the most seed when all possible cross combinations containing 34 were averaged, and the clones decreased in seed yield through 55, 9, and 57 down to 70, which yielded the lowest amount of seed in all combinations. Clone 70 had, by far, the greatest reciprocal differences due to a drastic decrease in yield when used as the maternal parent in crosses. Clone 9 gave a reciprocal difference due to poor performance as the maternal member. However, clones 57, 55, and 34 had reciprocal differences that were due to increased yields when used as the maternal parent.
Heritability

Heritability ratios calculated in the broad sense using the formula by Thomas and Kernkamp (1954) are reported in table 9. Heritability in the broad sense measures the additive gene effects and, therefore, provides an estimate of general combining ability. The advance in performance over the single cross mean, that could be expected by selecting from the top 5 percent of the population, was calculated using the formula presented by Lush (1945) and is also reported in table 9.
Table 9.—Heritability of seedling height, plant height and seed yield

<table>
<thead>
<tr>
<th>Character</th>
<th>Heritability</th>
<th>Advance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling height</td>
<td>23.44%</td>
<td>18.0%</td>
</tr>
<tr>
<td>Plant height</td>
<td>1.49%</td>
<td>.91%</td>
</tr>
<tr>
<td>Seed yield</td>
<td>28.19%</td>
<td>20.0%</td>
</tr>
</tbody>
</table>

Correlations

In order to check if the self-fertility of clones was related to combining ability, correlation coefficients between these two characters were computed and are reported in table 10.

Table 10.—Correlation coefficients between combining ability and self-fertility for seedling height and seed yield

<table>
<thead>
<tr>
<th>Combining ability</th>
<th>Degrees of freedom</th>
<th>Self-fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling height</td>
<td>Gen. C. A.</td>
<td>3</td>
</tr>
<tr>
<td>Seed yield</td>
<td>Gen. C. A.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Sp. C. A.</td>
<td>3</td>
</tr>
</tbody>
</table>

General combining ability for seed yield was correlated significantly with self-fertility. The significant negative r value indicates that for an increase in self-fertility there is a decrease in combining ability. There appears to be a relationship between the general combining ability of seedling height and self-fertility, but the r value of -.80 was not significant at the P = .05 level. The correlation between specific combining ability and seed yield was low and not significant. No correlation was computed for specific combining ability of seedling height because
it was not significant in the combining ability analysis. The general combining ability rank of the clones for seedling height and seed yield had a correlation coefficient of $r = .90$, which was significant with three degrees of freedom at the $P = .05$ level.
DISCUSSION

An evaluation of the literature on alfalfa and other forage crops indicates that improvement is continent on the isolation of breeding material, generally clones with outstanding agronomic characteristics and the ability to transmit these characteristics. The ability of breeding material to transmit desirable characteristics to its progeny is known as combining ability. It is evident that testing breeding material for characteristics that are inherited in an additive manner can be done in a variety of ways. However, the most practical method of testing breeding material for characteristics, inherited additively and/or through dominance, that has evolved thus far is the diallel method. The combining ability analysis of this experiment is, therefore, based on such a method.

It is recognized by the writer that the results of this experiment are based on a relatively small number of clones and on the performance of the plants in the year of establishment.

The range in self-fertility among the five clones used in this study expressed as seeds per flower tripped extended from .27 to .37 with a mean of .56. This range and average is not as great as that reported by some researchers. Wilsie and Skory (1948) tested a number of erect-type alfalfa clones originating as single plant selections from a seven-year old nursery and found the range in seeds per flower tripped to extend from .41 to 2.33 with a mean of 1.23. Wilsie (1951) in another experiment found the self-fertility range of 67 alfalfa clones to
extend from .12 to 1.84 seeds with a mean value of .75 seeds per flower tripped. Pedersen (1953) reported that the range in self-fertility based on the percentage of flowers forming pods of 18 alfalfa clones was 5 to 61 percent with a mean of 35.2 percent. Since the clones used in this experiment were all selected on the basis of high seed production where a large degree of self-fertility would be detrimental, a relatively low range in self-fertility can be expected.

The combining ability results in this experiment are in agreement with the results of other recent combining ability studies in alfalfa, Carnahan et al. (1960) and Pearson (1958), where general combining ability effects were more important than specific effects. The variance for general combining ability was highly significant for both seedling height and seed yield. Specific combining ability variance was low and not significant for seedling height, indicating the performance of individual single crosses was not deviating greatly from the average performance of the clones involved. Specific combining ability variance was also lower than the variance for general combining ability in seed yield but was significant at the P = .05 level.

Several forage crop breeders, Sprague and Tatum (1942), Federer and Sprague (1947), Roja and Sprague (1952), Kalton and Leffel (1955), Timothy et al. (1959), and Carnahan et al. (1960), have concluded that specific combining ability effects will be greater than general combining ability effects when the material tested was previously selected on the basis of good general combining ability. The breeding material tested in this experiment was previously selected on the basis of good general combining and specific combining ability effects are less important than general effects. Pearson (1958) also tested breeding material, assumed
by the writer to have been selected on the basis of good general combining ability, and also found that specific combining ability effects are less important than general effects.

In testing alfalfa breeding material for combining ability, it is generally considered that reciprocal crosses give progenies with equivalent performances. However, in this experiment certain reciprocal crosses did not give progeny with equal performance. Tysdal and Kiesselbach (1944) reported that a reciprocal cross difference noted in their experiment was probably due to a higher degree of selfing in one line which was 18 percent self-fertile as compared to the line it was crossed with which was only 1 percent self-fertile. Bolton (1948) found that reciprocal cross differences in either seed yield or forage yield were not large, even when lines considered relatively self-fertile were used in crosses. He did note a significant reciprocal effect when one particular line was used in crosses, but concluded that differences were due to slow germination of seed from that line and not self-fertility.

Brink and Cooper (1936), Tysdal and Kiesselbach (1944), and Bolton (1948) all conclude that in general, selfed seed will result in seedlings with low vigor and that these seedlings will be eliminated through competition under field conditions.

In the seedling height study, clones 57 and 70 demonstrated the largest reduction in performance when they were used as the maternal parents. Of the two clones, 57 expressed only slightly higher reciprocal effects than clone 70. Clones 9, 34, and 55 all performed better when crossed as the maternal parent in this study. In the seed yield study the largest outstanding reciprocal differences due to a reduction in seed yield when a clone was used as the maternal member of a cross, were expressed by clones 70 and 9. However, in this study clone 70, when
crossed in all possible combinations, expressed the largest reduction in yield, 142 pounds as compared to 67 pounds for 9. Clone 57 expressed the greatest reciprocal effect due to an average increase, when used as the maternal parent, of 92 pounds per acre over that received when used as the paternal member. The ability of clone 57 to combine well in the maternal position probably accounts for its having the highest variance for specific combining ability in seed yield.

The reciprocal cross differences in the flower color study are rather difficult to interpret since clone 55, which was about average in self-fertility, expressed the greatest reciprocal cross effects for flower color. The flower color of clone 55 is predominantly purple but it does contain some white. In the flower color study, the progeny of clone 55 were predominantly purple when it was used as the paternal parent. However, when clone 55 was used as the maternal parent, its progeny contained about equal amounts of purple and white. This suggests that a high degree of selfing occurred when single crosses were made. If this was the case, the vigor of reciprocal cross progenies for seedling height and the seed yield could be expected to be reduced, but reciprocal differences in these two studies were not large enough to account for this.

The contribution of the clones to general combining ability gave a clonal rank that was nearly the same for both seedling height and seed yield, and a correlation of these ranks gave a significant r value of .90. In the seedling height study clone rank for general combining ability, in order of decreasing importance, was: 34, first; 55, second; 9, third; 57, fourth; and 70, fifth. In the seed yield study, the rank was: 34, first; 55, second; 9, third; 57, fourth; and 70, fifth. The top three clones for general combining ability are the same for both
characters; however, 57 was ranked lowest for seedling height and 70 the lowest for seed yield. Specific combining ability was only significant for seed yield, and, therefore, this was the only character for which the contributions of the clones to specific combining ability was calculated. In order of decreasing importance, the clones are ranked 57, first; 55, second; 9, third; 24, fourth; and 70, fifth. A comparison of the clones for the two types of combining ability indicates that 24 is the best general combiner for seedling height and seed yield, and therefore, performs the best on the basis of the average performance of all clones involved. However, clone 24 is ranked fourth for specific combining ability of seed yield and, therefore, yields about as expected on the basis of its average performance. Clones 55 and 9 are ranked second and third respectively for seedling height and seed yield general combining ability and for seed yield specific combining ability. Clone 57, although ranked fifth in seedling height and fourth in seed yield for general combining ability, ranked first in seed yield for specific combining ability. On the basis of these ranks, clone 57 performs poorly in seedling height and seed yield compared to the average of all the clones, but the seed yield of some individual crosses containing 57 is better than would be expected from the average performance of all clones involved. Clone number 70, which is ranked fourth for general combining ability in seedling height and fifth for both general and specific combining ability in seed yield, appears to be the poorest combining clone in all categories.

Wilkie (1951) and McAllister (1950) tested the relationship between self-fertility and general combining ability as based on polycross performance and found that the two characters were not related in their studies. A correlation between self-fertility and general combining
ability of seed yield in this experiment gave a significant negative 
r value of -.95. Even though a small number of clones are used in making 
this correlation, it appears that self-fertility and general combining 
ability of seed yield are related. Heritability ratios, in the broad 
sense which measure the general combining ability of a clone, that were 
calculated in this experiment, substantiate the results of the analysis 
of variance for seedling height, plant height, and seed yield. The 
general combining ability variance was highly significant for seedling 
height and seed yield and relatively high heritabilities of 23.44 percent 
and 28.19 percent respectively were calculated for these characters.

Plant height of the crosses was not significantly different in the 
analysis of variance so no combining ability variance could be partitioned 
in the analysis of variance. A low heritability ratio of 1.49 percent 
also indicates no appreciable difference among crosses for this character.

The advance in production over the mean of the crosses, that a breeder 
may expect by selecting out of the top 5 percent of the population, 
calculated from the heritability ratios indicates that an advance of 18 
and 20 percent could be made in seedling height and seed yield. A 
negligible advance of .91 percent could be made in plant height.
SUMMARY AND CONCLUSIONS

Summary

The general and specific combining ability of five selected alfalfa clones was estimated by analyzing their single and reciprocal crosses for seedling height and seed yield in a diallel crossing system. General combining ability variance was highly significant and larger than specific combining ability variance for both seedling height and seed yield. Contributions of the individual clones for general and specific combining ability of these characters are also reported. Performance of reciprocal crosses varied sufficiently to produce a highly significant variance for reciprocal effects in seedling height and seed yield.

Conclusions

Conclusions made are limited to the five clones used in this study and may be influenced by the fact that all measurements were taken in the year of establishment. Conclusions are:

1. The difference among the single and reciprocal cross entries was highly significant at the P = .01 level for seedling height, seed yield, and flower color, but not significant for plant height.

2. The variance among the single and reciprocal cross entries for seedling height and seed yield when partitioned into that due to general and specific combining ability was found to be greater for general combining ability.

3. General combining ability effects were highly significant at the
P = .01 level for both seedling height and seed yield.

4. Specific combining ability effects were significant at the P = .05 level for seed yield only.

5. The rank of the individual clones for general combining ability of seedling height was: 34, first; 55, second; 9, third; 70, fourth; and 57, fifth. Clone 34 demonstrated the best general combining ability and clone 57 the poorest.

6. The rank of the individual clones for general combining ability of seed yield was: 34, first; 55, second; 9, third; 57, fourth; and 70, fifth. Clone 34 demonstrated the best general combining ability and clone 70 the poorest.

7. The rank of the clones for specific combining ability of seed yield was: 57, first; 55, second; 9, third; 34, fourth; and 70, fifth. Clone 57 demonstrated the best specific combining ability and 70 the poorest.

8. The general combining ability rank of the top three clones for seedling height and seed yield was the same. A correlation of the general combining ability rank of the five clones for seedling height and seed yield gave a significant r value of .90. There appears to be a relationship between the clones in the way they combine for both measurements.

9. Reciprocal cross progenies did not perform equivalent to single cross progenies in this experiment. The difference between single and reciprocal cross progenies was highly significant at the P = .01 level for seedling height, seed yield, and flower color.
LITERATURE CITED


McAllister, D. R. 1950. The combining ability of selected alfalfa clones as related to the self-fertility of the clones, their \( F_1 \) and \( F_2 \) progenies. Ph. D. Dissertation, Iowa State College.


