THE EFFECT OF SELF-AND-CROSS-FERTILIZATION ON SEED SET AND VEGETATIVE VIGOR OF THE PROGENY IN ALFALFA

by

Khairi Sgaier

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I hereby recommend that this dissertation prepared under my direction by Khairi Sgaier

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After inspection of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

M H Schonhorst — 4-19-65
James W. O'Leary — 4-19-65
[illegible] — 4/19/65
[illegible] — 4/20/65

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SIGNED: Khairi Ighaiw

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ABSTRACT

Experiments were conducted to obtain additional information on the effect of self- and cross-fertilization on seed set and vegetative vigor of the progeny in alfalfa. Preliminary investigations were conducted on 40 alfalfa clones under greenhouse conditions during 1963, and self-fertility (SFI) and cross-fertility indices (CFI) were computed. SFI and CFI values ranged from zero to 196.7 and zero to 359.5 respectively. The respective means were 65.5 and 39.2. The populations were slightly skewed toward low self- and cross-fertility.

There was a positive and significant correlation between the total number of pods set and the number of seeds per pod when plants were both self- and cross-fertilized. However, there was no association between self- and cross-fertility.

The ovule number per ovary for eight clones selected for detailed studies ranged from 6 to 15 with an average of 11.4. The number of ovules per ovary differed significantly among clones, plants within a clone and flower position on the raceme. The number of ovules per ovary decreased from the base to the apex of the raceme, but the number was constant among stems of the same plant, and among racemes positioned differently on the same stem. The magnitude of difference in ovule number per ovary was the greatest among clones, followed by

flower position on a raceme, plants within a clone, raceme position on a stem. These differences were 3.7, 0.9, 0.2 and 0.1 respectively. Ovule number did not appear to limit seed production. Moreover, it was believed that there was a "critical number" of ovules per ovary above which there was no increase in the number of seeds per pod.

Self-fertilization of the parent clones resulted in a reduction in the number of flowers per raceme and the viability of pollen of all progenies. A similar reduction in self-fertility index was observed in progenies of six out of the eight clones, whereas, an increase was observed in progenies of two clones. The mean reduction was 51.7, 29.6 and 82.3 per cent respectively.

Length of style for the eight clones ranged from 1.91 to 2.97 mm. The clones did not differ significantly in length of style. The results indicated that this factor did not present a major physical or physiological barrier to self-fertilization.

The term "skip" was suggested to indicate an unfertilized ovule located between the stigma and a fertilized ovule furthest from the stigma. "Skips" were higher in clones with low self-fertility than those with high self-fertility. The pattern of skipping appeared to be at random for a single clone. However, if all clones were averaged, a general decrease in "skips" was observed from the apex to the base of the ovary. The percentage of fertilized ovules was 31.6, 20.4, 8.0 and 3.6 in the apical, sub-apical, supra-basal and basal section of the

ovary respectively.

The diallel analysis indicated that a large part of the total genetic variation observed for cross-fertility was associated with a highly significant general combining ability.

The three treatments (hand pollination without emasculation, and hand pollination of alcohol- and suction-emasculated flowers) did not differ significantly in their effect on seed weight. Reciprocal effects on seed weight were nonsignificant.

Height and yield of dry matter differed significantly for progenies receiving the different emasculation treatments. The progenies of plants receiving suction-emasculation where shorter and yielded less dry matter than those of alcohol-treated plants. Reciprocal effects were highly significant only for the yield of dry matter at ten weeks of age for the three treatments.

The study indicated a positive and significant correlation between the yield of dry matter and seedling height at four and eight weeks of age for all progenies.

INTRODUCTION

Several studies relating to the effect of self- and cross-fertilization on seed set and vegetative vigor in alfalfa have been reported in the literature. Selfing in contrast to crossing was found to reduce the number of flowers, viability of pollen, number of seeds per pod and vegetative vigor of the progeny. However, the problem of inadequate seed set in alfalfa still is not fully understood. More information is needed on the effect of self- and cross-fertilization on seed-set and forage yield of the resulting progeny in alfalfa. Furthermore, a knowledge of the association between self- and cross-fertility and the major plant factors limiting to seed set in alfalfa is important.

Investigators have used various methods of measuring self- and cross-fertility in alfalfa plants. Some have utilized the number of pods set while others have used the number of seeds per pod as indices of fertility. A third group has employed a combination of pod set and number of seeds per pod. Thus, no single criterion has been established for measuring self- and cross-fertility. Also, no work comparing the effect of various methods of emasculation and their influence on seed-set has been found in the literature. In addition, little research has been conducted to determine the possible existence of reciprocal differences in alfalfa crosses.

Recently, combining ability has become an effective tool for determination of the value of alfalfa clones as parents of commercial varieties. A knowledge of the relative importance of general and specific combining ability on agronomic characters will be helpful in the development of future varieties. Information relative to the morphological and genetic factors controlling seed and forage yield may suggest new approaches in handling or contribute to a better understanding of the seed-set and production processes.

The primary objectives of this study were to determine:

1. The effect of self- and cross-fertilization on pod set, average number of seeds per pod, seed weight, and vegetative vigor of the progeny.

2. The effect of viability of pollen, length of style, and variation in ovule number as factors influencing seed production of alfalfa clones.

3. The influence of various methods of emasculation in diallel matings on cross-fertility, general and specific combining ability, reciprocal differences, height and yield of dry matter of the progeny.

REVIEW OF LITERATURE

The literature pertaining to alfalfa in general is voluminous, and the various phases of seed and forage production as influenced by the environmental and plant complexes have received a great deal of attention in the last decade. Self- and cross-fertilization and their subsequent effects play an important role in seed set and vegetative vigor in alfalfa. This area of research is reviewed and an attempt will be made to relate it to recent findings in order to bring into focus the various plant factors controlling seed and forage yield.

Fertility Relationships in Alfalfa

Alfalfa generally is considered a highly cross-fertile species with low self-fertility but under conditions of artificial selfing, fertility of alfalfa plants varies from almost complete self-fertility to almost complete self-sterility (32).

A frequency distribution of self-fertility of 685 open-pollinated plants showed continuous variation that closely approached a normal curve (32). Schonhorst (34) found that frequency distributions from hybrids of high X low viable pollen parents and S_1 progenies of individuals with high pollen viability were skewed toward high viability of pollen. He also found that self-pollinated populations of plants with low pollen

viability had distributions slightly skewed toward low viability; however, about 30 per cent of the self-pollinated plants were intermediate.

Sprague (36) studied the fertility of alfalfa by measuring the amount of seed produced after self-pollination and cross-pollination. All pollinations were made in the greenhouse, and suction emasculation was used before cross-pollination. He found that cross-fertility was greater than self-fertility in every population observed.

According to Rotar and Kehr (32), male sterility is defined as:

> (a) deficiency of male individuals in a dioecious strain. (b) absence or atrophy of male organs in normally bisexual plants, (c) failure to produce normal sporogenous tissues in strains, (d) inhibition at various stages of pollen development yielding incomplete or imperfect pollen, or (e) failure to mature, dehisce, or to function when placed on a compatible stigma.

Childers (14) in his studies on male sterility in Medicago sativa defined male sterility as: (a) lack of pollen, (b) faulty dehiscence of anthers, or (c) tapetal abnormality. He distinguished between two kinds of male sterility--complete and partial. The former was characterized by overgrowth of the tapetum resulting in the degeneration of pollen mother cells. The latter was characterized by a swollen tapetum. Tysdal and Kiesselbach (39) suggested that in using male sterility in production of hybrid alfalfa seed the pollinator may be self-fertilized, and so only part of the seed will be hybrid unless special precautions are taken during planting and harvesting.

Cooper and Brink (17) attributed self-sterility in alfalfa to two phenomena: (a) incompatibility, and (b) early ovule abortion following fertilization. They defined self-incompatibility as the ineffectiveness of male gametes in accomplishing fertilization in the plant from which they arose. Armstrong and White (3) attributed self-sterility in alfalfa to causes other than those advanced by other workers (14, 17, 32). They cited the following causes: (a) meiotic irregularity, (b) high percentage of poor pollen produced by faulty maturation, or (c) self-incompatibility of pollen plants which produce a high percentage of good pollen. For producing hybrid alfalfa seed, sterile plants of types b and c are used.

Factors Affecting Seed Set in Alfalfa

Seed setting in alfalfa as any other process in the plant is controlled by many factors.

Self- and Cross-Compatibility

In classifying plants according to why they may fail to set seed, it is desirable to distinguish between incompatibility and sterility. With incompatibility the pollen and ovules are functional, and unfruitfulness results from physiological hindrance to fertilization usually manifested either through failure of the pollen to germinate on the stigma or through slow growth of the pollen tube down the style (1). Sterility,

on the other hand, is characterized by nonfunctional gametes. It is caused by chromosomal aberrations, gene action, or cytoplasmic influences that upset the development of pollen, embryo sac, embryo, or endosperm (1).

In connection with the problem of incompatibility, Kroh (27) conceived the following picture of the processes which occur on the stigma of self-incompatible Cruciferae after cross- and self-pollination. After cross-pollination, the pollen cutinase system is irreversibly activated by the stigma. Hence, the cuticle of the papillae becomes permeable, and is dissolved at the place where it is in contact with pollen grains. Now moisture, necessary for pollen grain germination, can reach the surface of the stigma, and tubes can enter the stigmatic tissue at this place. After self-pollination, the formation of the activator-enzyme-complex is inhibited by the stigma, and pollen germination and pollen tube penetration in the stigma are blocked.

Linskens (31) associated compatibility in Petunia hybrida with the age of style and concluded that after self-pollination the tubes in unripened styles are less inhibited than in ripe styles. This could account for the well-known experience of getting seeds in self-incompatible flowers if pollination occurs in the bud stage of growth. He also concluded that the physiological barrier which prevents self-fertilization is built up during the growth of the style.

Atwood (4) concluded from a cytological study of self-incompatibility in white clover that the inhibiting action in incompatible pollinations or the stimulating action in compatible pollinations or both must take place very soon after the pollen is placed on the stigma, and that a large part of the blocking effect took place on the stigma before the tubes had reached the style.

According to Lewis (30), self-incompatibility in Oenothera organensis resulted from the specific reaction of an antigen-like substance in the pollen with a preformed antibody-type substance in the style. A disruption of this reaction may lead to a breakdown of incompatibility. An ideal method for the elimination of unwanted antibodies is through their complete absorption by massive doses of antigen. In keeping with the hypothesis of complete absorption, Cohen and Leffel (15) postulated that the large amount of early pollen germination releases excessive amounts of the antigen-like substance which may completely absorb the performed antibody in the style.

Whitehead and Davis (40) reported that self-compatibility of the female parent and cross-compatibility in alfalfa showed significant positive correlations for percentage of pods and for number of seeds per pod ($r = 0.97$ and 0.96). These relationships were significantly different from the corresponding correlations ($r = 0.54$ and 0.47) involving cross- and self-compatibility of the male parent. Moreover, they concluded that the female parent influenced cross-compatibility

and F_1 self-compatibility values more than the male parent, and that differences in self-compatibility cannot be attributed to differences in the percentage of viable pollen.

In the work of Hanson et al. (25) on alfalfa, self-compatibility was determined by the percentage of pods set, the number of seeds per pod, and the number of seeds set per one hundred flowers selfed. The clones differed in percentage of pods setting seed and in seeds set per one hundred flowers selfed, but not in seeds per pod.

Positional Relationship Between the Anthers and Stigma

According to Armstrong and White (3), Coffman divided the progress of the development of alfalfa flowers into four stages: (a) straight bud stage, (b) pointed bud stage, (c) hooded bud stage, and (d) erect standard stage. They found that 80 per cent of the plants shed their pollen in the pointed bud stage in both high and low seed-setting types, but fertilization did not occur in the untripped flowers in spite of the fact that pollination had taken place.

Tripping

Armstrong and White (3) showed that tripping ruptured the stigmatic film which prevented the pollen from making contact with the stigmatic secretion necessary for germination. When the staminal column springs forward, it causes the curved stigma to strike the standard and rupture the membrane.

Automatic tripping takes place frequently, and it is enhanced by high temperature (29). It occurs when the dynamic force present in the staminal column is sufficiently greater than the static force present in the keel.

Mechanical tripping is used for studies on alfalfa breeding, and it can be accomplished by: (a) a lateral pull on one of the wings with a pair of tweezers, or (b) inserting a pointed object such as a pencil point or a toothpick end into the throat of the corolla (3, 11).

Mechanical devices have been designed to trip alfalfa flowers on a field-scale. These machines increased the number of tripped flowers, but in no instance did they result in a significantly greater seed yield (7). The reasons for failure were rather obvious because cross-pollination was not accomplished. Furthermore, several treatments were necessary to cover the extended flowering period of alfalfa, and this repeated manipulation of the plants caused mechanical injuries. Thus, it appears that the construction of a successful mechanical tripping machine will be extremely difficult, if not impossible according to Bolton (7).

For determination of the ease of tripping, Tysdal and Kiesselbach (39) used ethyl alcohol solutions in varying strengths by placing a drop in the throat of the flower and determining which strength was sufficient to cause the flower to trip.

Population Effect of Pollen on Pollen Germination

Brewbaker and Majundar (8), in their studies on eight genera of angiosperms, observed a significant effect of decreasing population size of pollen on pollen germination in vitro. Reduction of percentage pollen germination (Y) occurred linearly in Petunia inflator with a decrease of population size (X) below two hundred grains per 0.01 ml. drop because of the lack of "mutual stimulation." Water extracts of pollen and other plant parts contained a factor or factors which could overcome fully the population effect. The pollen growth factor was dialyzable, insoluble in ether, relatively heat stable, and was not replaceable by kinetin and auxin.

Brewbaker and Majundar (8) proposed that the pollen growth factor (PGF) may be consumed during growth, and that incompatibility inhibits the production, utilization by or transfer to the pollen tube of the PGF. In large populations where optimum germination and growth were obtained, this effect was termed "mutual stimulation." According to these authors, evidence for the existence of a population "grouping" effect in pollen germination was first suggested by Brink.

Pollen Tube Growth and Chemotropism

Cooper et al., (16) reported that Kraus suggested the embryo sacs secreted a substance which attracted pollen tubes. Possibly only a few of the embryo sacs of an individual ovary secrete such a

"chemotactic" substance which may account for the low percentage of fertilization.

In his work on Lilium, Rosen (33) found that the high sucrose requirement for optimal tube growth and the observation that bursting tubes expelled their cytoplasmic contents with considerable force from the tip indicated that the pollen tube has a high turgor pressure. Extension of the pollen tube at its tip would result in the wall yielding to pressure at the point where it was weakest. He postulated that a concentration gradient of an external factor, which could influence the extensibility of the tube wall, might be expected to regulate the direction of the growth of the tube. The interplay of internal turgor pressure and an external agent which controlled wall extensibility could cause the tube to grow in the direction of increasing concentration of the external agent. This external agent controls the extensibility either by inhibiting the hardening of the newly formed wall at the tip or by softening the partially hardened wall in back of the tip.

Another mechanism suggested by Rosen (33) is that the chemotropic factor may inhibit the synthesis of rigid components of the wall. He also stated that negative chemotropism and inhibition of growth may be interpreted by assuming that the factor acts as a wall-hardening inhibitor at low concentration and as a hardening accelerator at high concentration. Alternatively, positive and negative chemotropism may be controlled by two different factors or groups of factors which are

present in different ratios in various tissues and which may diffuse through the medium at different rates.

Atwood (4), in his work on Trifolium repens, reached the conclusion that in white clover there are two interference zones active in preventing the growth of incompatible pollen: (a) one on the stigma, and (b) the other three-fourths of the distance down the style. Once the interference zone in the style has been passed, the tubes appear to have an accelerating growth rate.

Fertility of the Ovules in Relation to Their Position

The ovary of an alfalfa flower usually contains ten to twelve ovules arranged alternately along the ventral suture in serial order (17). The fertility of ovules decreases from the apex of the ovary to the base, and most fertile ovules are in the apical half of the ovary. Cooper and Brink (17) found that pollen tubes grew beyond the mid-region in only a few ovaries.

Barnes and Cleveland (6) studied the effect of ovule position on fertilization. They vacuum-emasculated flowers, and then hand-pollinated the flowers in two crosses. These workers found that the percentage of fertilized ovules ranged from 48 in the first position (nearest to the stigma) to 50 in the twelfth position (furthest from the stigma) in one cross, and from 20 to zero in the first and twelfth position, respectively in another.

Effect of Selfing and Crossing on Reproductive and Vegetative Characters in Alfalfa

Cooper and Brink (17) reported that self-pollination of alfalfa flowers resulted in early wilting of the corolla and that the percentage of collapse of ovules was much higher after selfing than after crossing. The percentage was 34 and 7, respectively. Thus, the net fertility in crossing is approximately five times that in selfing. They also found that the average number of seeds per pod was 1.93 when flowers were hand-selfed compared with 5.44 seeds per pod when they were hand-crossed.

Brink and Cooper (9) stated that the collapse of ovules followed abnormal growth of the somatic tissue adjacent to the embryo sac. The critical factor for survivial seemed to be the manner in which the translocated food was shared between the endosperm on one hand and the inner integument on the other. This investigation further revealed that following self-fertilization the rate of growth of the endosperm was so slow that the balance soon shifted in favor of the integument causing the collapse of the endosperm and eventually terminating the development of the ovule. They also observed a higher survival of fertilized ovules following cross-fertilization as a result of the more active growth of the hybrid endosperm.

Hanson et al., (25) observed a reduction in seed weight in some alfalfa clones when hand-selfed as compared to the weight of seed

resulting from hand-crossing of the same clones. They also found that the seed weight from hand-pollinated crosses was higher than the weight of seed obtained from bee-pollinated crosses. They attributed this difference to the extensive self-pollination of the bee-pollinated crosses.

In comparing seed setting after self- and cross-pollination, Bolton (7) cited the fact that average values for self-fertility are always lower than for cross-fertility. In this respect his results showed an average self-fertility of 1.58 seeds per pod compared with 5.54 seeds per pod for cross-fertility. Wilsie (41) stated that "Most alfalfa plants will show a marked inbreeding depression when selfed, on the average perhaps 20 to 30 per cent in vigor and a great deal more in self-fertility." Whitehead and Davis (40), in determining the self- and cross-fertility of six alfalfa clones under greenhouse conditions, found that 1.7 to 23.1 per cent of the flowers set pods with 1.1 to 1.9 seeds per pod when self-fertilized. Similar values for plants cross-fertilized ranged from 9 to 81 per cent of the flowers setting pods with 2.7 to 6.2 seeds per pod.

The depressing effect of selfing is not limited to the percentage of pods set, seed per pod, or vegetative vigor in alfalfa. Schonhorst (34) found that when plants with high pollen viability were self-pollinated, progeny were produced which were lower than their parents in average amount of viable pollen. The mean amount of aborted pollen for two clones was 3.8 and 7.1 per cent compared with 10.4 and 12.8 per cent for their respective progeny. However, one parent clone had

more aborted pollen than its progeny. The percentage of aborted pollen was 70.1 and 57.3 per cent, respectively. The reduction in viable pollen from parent to progeny in the first two clones was attributed to a general reduction in plant vigor arising from inbreeding depression; whereas, the increase in viable pollen in the third clone probably was due to reaction of genotypes which were influenced by environmental changes.

The report by Tysdal and Kiesselbach (39) on the effect of self-pollination on reduction in forage yield is representative of published accounts. They stated that spaced plants of S_1 lines yielded only 68 per cent as much forage as noninbred plants.

Determination of Pollen Viability

The most widely used techniques for determining the viability of pollen grains are by staining or germination in a sucrose solution (13, 14, 19, 24, 34). Staining is accomplished by acetocarmine or iodine-gelatin. In either case, a newly opened flower is selected and tripped into a drop of stain; the cover-slip is placed, and the pollen grains examined under the microscope. Pollen grains are considered nonviable if they do not absorb the stain.

The sucrose solution used as a medium for germinating pollen grains is an approximate duplication of the nutritive and chemical environment of the stigmatic surface. Ten to twenty per cent sucrose

solutions are widely used. Boric acid may be added in small amounts (100 ppm) to enhance the sucrose translocation in pollen grains as a sucrose-borate complex (33).

Emasculation of Alfalfa Flowers

Ir order to obtain hand-crossing, the alfalfa flower must be emasculated to prevent the self-pollen from affecting fertilization. Many methods have been developed.

Tysdal and Russell (38) established a method which involved clipping the standards, immersing the raceme in 57 per cent ethyl alcohol for ten seconds, rinsing in water for a few seconds, followed by a drying period, and then applying pollen to stigmas. They found it made little difference if pollen were applied immediately after emasculation or as much as an hour after emasculation provided the water was blown off the stigma before the pollen was applied. As a safeguard against injuries to the stigma from immersion for ten seconds in 57 per cent alcohol, Lesins (28) found it possible to reduce this time by half if the stigma was then washed with water.

Another method of emasculation is by suction (1, 7, 36). In this method, a small vacuum nozzle is used to remove anthers and suck adhering pollen from the stigma.

Determination of the Number, Position, and Percentage of Fertilized Ovules

For determining number, position, and percentage of fertilized ovules of alfalfa, Barnes and Cleveland (6) collected flowers 72 hours after pollination, dissected the ovaries and recorded the number and position of fertilized ovules. The fertilized ovules were easily distinguished from the unfertilized by their shape and increased size.

The number of ovules per ovary represents the potential number of seeds per pod. Barnes and Cleveland (5) stated that selection of plants with a high number of ovules per flower should result in maximum seeds per pod. These workers believed there was a relationship between ovule number and seed production and that the latter could be improved by breeding.

Barnes and Cleveland (5) also studied the variability of ovule number per ovary per raceme in both diploid and tetraploid alfalfa plants and the inheritance of ovule number per ovary in diploid alfalfa. Their studies showed that the average number of ovules per floret per raceme was relatively constant among racemes of the same plant. The only obvious difference in ovule number within plants appeared to be associated with the position of florets on the racemes. Significant differences for average number of ovules per floret were found among plants of both tetraploid and diploid alfalfa. Genetic studies indicated that ovule number in diploid alfalfa was controlled by four genes. Three

of the genes showed complete or nearly complete dominance, while the fourth gene expressed a degree of incomplete dominance. Genetic effects of all four loci controlling ovule number were additive. Cooper and Brink (19) examined approximately one hundred alfalfa ovaries and observed a range of eight to fourteen ovules per ovary, and a range in number of fertilized ovules per ovary from one to six with the average being between three and four.

Combining Ability, Diallel Crosses, and Reciprocal Effects

The concept of combining ability is becoming increasingly important in plant and animal breeding. It is especially useful in connection with testing procedures where it is desired to study and compare the performance of lines in a hybrid combination (24).

According to Sprague and Tatum (35), the term "general combining ability" is used to designate the average performance of a line in hybrid combinations. The term "specific combining ability" is used to designate those cases in which certain combinations do relatively better or worse than can be expected on the basis of the average performance of the lines involved. Specific combining ability may result from several causes such as Mendelian segregation and recombination, incorrect genotypic classification, and various types of factor interactions.

Frakes et al., (23) interpreted general combining ability as the relative performance primarily from the additive effects of polygenes, whereas specific combining ability was interpreted as the relative performance primarily from deviations of the additive scheme. They found that natural height and long-stem length were highly significant for the effects of general combining ability, whereas natural width of the plant and stem number were not. Since the degrees of freedom in their analysis of variance were very low, they thought it would appear that the ratio of the mean squares of the general to specific combining ability would be more meaningful. By placing specific combining ability effect equal to one, such a ratio readily showed the relative size of the effects of general and specific combining ability (e.g. 250:1 for natural height).

Carnahan (12) found in combined analyses for locations that the mean square for general combining ability was approximately 50 times that for specific combining ability for both seedling vigor and fall-growth habit. He pointed out that the relationships among mean squares provided estimates of the relative total contributions of the sources of variance. Estimates of the several components of variance permit comparisons on a unit basis and are, therefore, more informative than F ratios in assessing the relative magnitude of additive and nonadditive gene effects.

In determining yield of reciprocals for selfed and hybrid progenies, Hanson et al. (25), using hand- and bee-pollination, reported that reciprocal differences of the hand-pollinated crosses were not statistically significant beyond the seedling stage (four weeks old); whereas, differences among reciprocals of the bee-pollinated crosses were highly significant at all stages of growth. Thus, their results indicated that differences among reciprocals of the bee-pollinated crosses were sizable. This apparently resulted from extensive self-pollination of both parents. Reciprocal differences in seedling vigor were noted also in hand-pollinated crosses, but the differences were negligible beyond the early seedling stage and were attributed to effects associated with differences in seed size. Thus, evidence of maternal effects appeared to be limited to early seedling vigor.

Griffing (24) defined a diallel crossing system as one in which a set of p inbred lines is chosen and crosses among these lines are made. This procedure gives rise to a maximum of p^2 combination. Thus, the p^2 combinations can be divided into three groups: (a) the p parental lines themselves, (b) one set of $\frac{1}{2}p(p-1)$ F_1's, and (c) the set of $\frac{1}{2}p(p-1)$ reciprocal F_1's.

Diallel crossing techniques may vary depending upon whether or not the parental inbreds, the reciprocal F_1's or both are included. With this as a basis for classification, there are four possible experimental methods: (a) parents, one set of F_1's and reciprocal F_1's are

included (all p^2 combinations); (b) parents and one set of F_1's are included but reciprocal F_1's are not ($\frac{1}{2}p(p+1)$ combinations); (c) one set of F_1's and reciprocals are included but not the parents (p (p-1) combinations), and (d) one set of F_1's but neither parents nor reciprocals F_1's are included ($\frac{1}{2}p(p-1)$ combinations). Each method necessitates a different form of analysis. It was pointed out by Griffing (24) that to obtain unbiased estimates diallel crossing method (c) or (d) must be used, i.e., the parents must not be included in the combining ability analysis.

Johnson (26) enumerated two basic advantages with regard to the advantages of a diallel-cross technique when compared to the other methods available. The diallel-cross technique provides a more systematic approach to large scale studies of continuous variation, and a better disciplined analysis of the resulting data. Also, the over-all analysis provides reliable genetic information that would be useful in identifying, in early generations, crosses with the best selection potential.

Evaluation of Vegetative Vigor

Carnahan (12) determined the average height at four weeks and at eight weeks of age and yields of dry weight at nine weeks of age for the evaluation of seedling vegetative vigor under greenhouse conditions. He found that mean height at four weeks of age for 15 crosses was 4.8 per cent higher than the reciprocals of these 15 crosses. This difference was significant at the .05 level of probability. Reciprocals were

highly correlated for both seedling height and forage yield, indicating that these characters were largely controlled by the nucleus. Seed weight was highly correlated with unifoliolate leaf area which, in turn, was significantly correlated with seedling height at four weeks. Reciprocal differences in this study were largely attributable to the relation between seed size and photosynthetic area in seedlings.

Davis and Panton (18) estimated seedling vigor by measuring height of plants at two, three, and four weeks after germination. The plants were grown in a greenhouse under a daylength of approximately $13\frac{1}{2}$ hours. There was a positive and significant correlation between seedling height at four weeks of age and weight of dry matter.

Association Among Characters

Knowledge of the relationships among characters is important to forage breeders, particularly when improvement is desired in a complex character such as yield (25). Frakes et al. (21) found that natural height showed a positive association with dry-matter yield, although not significant for all regrowth periods. They noted a highly significant correlation between plant yield and plant height in alfalfa, with tall plants usually being superior in relative yield capacity. Plants having larger leaves generally produced a greater yield than those with smaller leaves.

Frakes et al. (22), measured dry-matter production per plant, height, natural plant width, and length of the longest stem once in the year of establishment and three times during the year following establishment in another study. This study indicated that the aforementioned characters were measurable and that they could be used to predict the dry-matter production per plant in a spaced-plant nursery. Such variables may then serve as measurable components of yield.

METHODS AND MATERIALS

Preliminary investigations of the effect of self- and cross-fertilization on alfalfa clones were initiated March 20, 1963. Clonal lines of alfalfa used in these studies were obtained from Dr. M. H. Schonhorst and grown in the Agronomy greenhouse on the University of Arizona's Campbell Avenue Farm, Tucson, Arizona. Parent materials were maintained in one-gallon plastic pots in a greenhouse.

Air temperatures and relative humidites were recorded throughout the experiment with a hygrothermograph placed on the same bench as the plants. Aphids and red spider mites were controlled by spraying with malathion and kelthane. Late in the fall of 1963, when day lengths became short, supplemental illumination of an intensity of 20 foot-candles was provided with 300-watt incandescent bulbs to give a 14-hour photoperiod. The longer photoperiod promoted induction, development, and maturation of flowers and seed.

Forty clones were picked at random and used in the preliminary investigation. Indices for self- and cross-fertility were determined and designated as self-fertility index (SFI), and cross-fertility index (CFI). SFI was computed by determining the percentage of pollinated flowers setting pods multiplied by the average number of seeds per pod. CFI was computed by determining the percentage of flowers setting pods,

when emasculated with alcohol and hand crossed, multiplied by the average number of seeds per pod. A clone designated as MAR-48 and high in both self- and cross-fertility was used as a pollen source in all crosses. Clone MAR-5 was used as the pollinator in crossing MAR-48. The two indices were used to evaluate the degree of self- and cross-fertility of the clones.

Eight clones showing various degrees of self- and cross-fertility were selected for further detailed studies from the forty clones used in the preliminary investigation. Four categories combining self- and cross-fertility were established with two clones in each category. Two of the eight clones were both highly cross- and self-fertile, two highly cross-fertile but low in self-fertility, two low in cross-fertility but high in self-fertility, and two were low in both cross-and self-fertility.

Selfing of Alfalfa Flowers

Hand-selfing, was used whereby flowers were tripped with the aid of a pollen-free toothpick. This method involved the application of external force for release of the staminal column and rupture of the stigmatic film by striking against the unclipped standard. Flowers per raceme were counted, and a small paper tag with the number of flowers was attached to the peduncle. At maturity, racemes were harvested, pods per raceme were counted and threshed using a hand thresher to

determine: (a) The percentage of pods set, (b) average number of seeds per pod, and (c) self-fertility index.

Cross-Pollination of Unemasculated and Emasculated Flowers

Each of the eight clones was hand-pollinated without emasculation. Each clone also was used both as female and male parent to produce crosses and their reciprocals. There were twenty-eight crosses and twenty-eight reciprocals.

Flowers per raceme were counted, and if the number of flowers exceeded ten, they were reduced to ten by removing the apical flowers to make the subsequent manipulations easier and more accurate. Standards were clipped with pointed-end scissors, and flowers were tripped with the sharp end of a pollen-free toothpick. Flowers used for a pollen source were tripped with a hard paper boat, and pollen collected on the boat was transferred to the stigma of the female parent. The raceme was tagged as described above.

Hand-pollination of alcohol emasculated flowers was done as previously described (28, 38).

The procedure was as follows:

a. Standards on all flowers were cut off with pointed-end scissors, and if flowers per raceme exceeded ten, they were reduced to ten.

b. Staminal columns were released from the keel using the sharp end of a pollen-free toothpick.

c. The raceme was immersed in 57 per cent ethyl alcohol in three quick movements taking a total of four to six seconds without removing the raceme from the alcohol. This was done to insure better contact between the alcohol and pollen and to completely destroy the pollen.

d. The alcohol-treated raceme was immersed in tap water for approximately 20 seconds to remove the alcohol and any pollen that escaped the alcohol treatment. The water film was believed to serve as a barrier between the stigmatic surface and pollen scattered about from released sexual columns of other racemes.

e. After several racemes had been emasculated and immersed in water, the raceme first treated with alcohol was held by its peduncle and sharp blasts of air were directed at different angles to free the stigma from water and pollen.

f. Flowers from the plant used as a male parent were tripped with a hard paper boat, and the pollen collected was transferred to the stigmas of the emasculated flowers. All racemes were tagged after they were pollinated.

At maturity, racemes were harvested, pods were counted and threshed, and seed count was taken to determine the percentage pod set, average number of seeds per pod, and CFI.

Hand pollination of suction-emasculated flowers was done as previously described except for the method of emasculation where suction instead of alcohol was used.

Prior to suction emasculation, the raceme with tripped flowers was immersed in tap water for approximately 20 seconds to make the removal of pollen easier and to destroy pollen (1, 7, 36). Suction was obtained by running water from a faucet into an aspirator. The stigma and upper part of the style were inserted into the drawn end of a glass tube attached to one end of the aspirator. Each flower was inserted twice into the tip of the suction tube in order to insure complete removal of pollen.

In the two methods of emasculation, alcohol and suction, and where hand pollination without emasculation was used, all standard petals were cut off, and thus the stigmatic film was not ruptured (3). The broad end of a pollen-free tooth pick was passed gently over the stigma to rupture the stigmatic film and to insure contact between the stigmatic surface and pollen from flowers of the clone used as a male parent. Also, to insure the transfer of adequate pollen to the stigma, the number of flowers tripped for pollen was two to three times the number of emasculated flowers. Whenever more than one clone was manipulated and crossed successively, hands were washed with tap water and dried between manipulation of each plant in order to avoid contamination from undesired foreign pollen.

Seed Weight

After seeds were counted for the determination of average number of seeds per pod, the weight in grams of 50 mature seeds taken at random was determined. Seed weight served as a means of evaluating the effect of hand pollination without emasculation, hand pollination of alcohol and suction-emasculated flowers, and reciprocal differences.

Viability of Pollen

Pollen viability was determined by examining a minimum of 200 grains per clone at each sampling. Fresh flowers were detached from the racemes, tripped with a pollen-free needle so that pollen landed in a drop of acetocarmine on a slide. A cover glass was placed over the acetocarmine, and the pollen grains were observed under the microscope at 100X total magnification. Viable pollen was characterized by having a normal round shape and staining red when compared with the abortive nonstaining pollen (19). The number of viable pollen grains was expressed in per cent of the total number of pollen grains (34).

In addition to the examination of pollen from the eight parent clones (S_o), pollen grains of their respective progeny (S_1) arising from hand-selfing of the parents were examined for viability. This procedure determined whether or not there was a reduction in pollen viability from parents to progeny when hand-selfed.

Length of Style and Percentage of Fertilized Ovules

Flowers were detached from racemes 72 hours after hand pollination (6) to determine whether fertilization had taken place (10). The base of the calyx was cut with a dissecting needle, and the pistil was then removed from the staminal column. One dissecting needle was placed at the basal part of the pistil, and a second was used for splitting the pistil longitudinally along the dorsal suture starting at the stigma. The distance from the tip of the stigma to the tip of the first ovule (nearest to stigma) was measured, in mm, for all clones using a dissecting microscope. Data collected were statistically analyzed.

To determine the effect of ovule position on fertilization, flowers were collected 72 hours after they had been self-pollinated by hand and dissected as above. The fertilized ovules were distinguished from the unfertilized ovules by their shape and increased size (6). The number of fertilized ovules in their respective positions from the stigma was determined for the eight parent clones.

Variation in Ovule Number

The variation in ovule number was determined on the earliest flowering racemes of each plant. These racemes were selected to minimize any decrease in ovule number because of time of flowering. Variation in ovule number was determined for flowers of the same raceme by sampling from the base, the middle, and the apical part of

the raceme, from racemes located on different stems of the same plant, and from different plants of the same clone.

The base of the calyx was cut with a dissecting needle and the petals and receptacle were separated from the staminal column. After dissecting, a cover slip was placed over the pistil, and pressure was applied until the ovary split longitudinally. The ovary was observed under a dissecting microscope at 100X total magnification, and the ovules counted. Approximately 6700 ovules were observed and all data were analyzed statistically.

Vegetative Vigor of the Progeny

Seed produced on F_1 plants in the greenhouse in 1963 and 1964 from hand crossing without emasculation, hand crossing of alcohol-emasculated flowers and hand crossing of suction-emasculated flowers, called henceforth treatments, were planted in flats on September 21, 22, 24, 1964.

The flats (21" x 15" x 4") were filled to within 1/2 inch of the top with a mixture made of two parts of soil, one part of sand, one part of peat moss, and one part of perlite. A fungicide containing captan-cadmium, and BHC-iron was spread in a thin layer on top of the soil mixture in each flat, and was then thoroughly incorporated by hand into the mixture. Inoculation with the appropriate nitrogen-fixing bacteria was done at the time of planting the seed. The experiment was designed

as a randomized complete block with three replications. Air temperatures and relative humidities were recorded throughout the experiment with a hygrothermograph placed on the bench.

Seeds from each cross and the reciprocal for each of the three treatments were planted in flats in rows four inches apart. Watering was done every other day for the duration of the experiment. Also, the position of each flat was changed every other day in a clockwise manner to minimize any position effect.

At the third trifoliolate leaf stage of growth, seedlings were uniformly thinned to approximately ten plants per row. Measurement of seedling height was made at four weeks and eight weeks from the date of planting. The plants were cut at the crown level ten weeks after planting, and the roots were counted to ascertain the number of plants per row. The topgrowth was dried to a constant weight in a forced-air oven at 80°C., and weight of topgrowth was expressed on a single plant basis.

Data collected from this study were programmed, processed and analyzed according to Griffing's Method 3 (24). Analyses of variance were determined for the following characters; height, yield of dry matter of progeny produced from seed of the three treatments, reciprocal differences, and the effect of general and specific combining ability on seedling height and yield of dry matter of the progeny.

Description of the Eight Clones Used for Detailed Studies

The eight clones used for the detailed studies were chosen on the basis of the degree of self- and cross-fertility that they had shown in the preliminary investigation. Moreover, it was found from the preliminary investigation that the clones were in an unequal state of heterozygosity. Therefore, a brief summary of information pertaining to the place of origin, history and ancestry of the eight clones used in these studies is presented to help with the interpretation of their performance.

MAR-Clones

Four clones bear the designation MAR which stands for Mesa African root rot resistance. These clones are MAR-30, MAR-35, MAR-40, and MAR-48. The MAR-clones were selected by Dr. M. H. Schonhorst at the University of Arizona's Mesa Experimental Station in an area heavily infested with the fungus causing cotton root rot (Phymatotrichum omnivorum, (Shear) Duggar). Parents of the MAR clones were obtained from the variety African in 1957 for resistance to the spotted alfalfa aphid (Therioaphis maculata, (Buckton). These selections were further screened for two years in an area on the Mesa Branch Experimental Station which was heavily infested with the cotton root rot fungus. African was introduced from Egypt in 1924. The original stock was labeled as U.S.D.A., F.C. 31370 Hegazi alfalfa.

Clone MLR-3

The clone designated as MLR-3 was a selection from the variety Lahontan in 1958 by Dr. M.H. Schonhorst and was screened further through the area containing cotton root rot at the University of Arizona's Mesa Experimental Station. Lahontan was developed by the late O.F. Smith, U.S.D.A., alfalfa breeder, in cooperation with the Nevada Agricultural Experimental Station, Reno, Nevada. It is a synthetic variety with five parent plants selected from Nemastan for resistance to bacterial wilt and the stem nematode.

Clone M-5-20

Clone M-5-20 was selected from plants of the variety African which exhibited resistance to the spotted alfalfa aphid. M-5-20 and M-56-2 were selected in 1957 at the University of Arizona's Mesa Experimental Station by Dr. M.W. Nielson, Entomologist with the Entomology Research Division, ARS, U.S.D.A. Clone M-5-20 was reported by Schonhorst[1] to be low in cross-fertility from data obtained in a polycross nursery. Also, plants of this clone were reported to be unattractive to honeybees, and were characterized by pronounced vegetative vigor and abundant flowers when grown in a spaced-plant nursery at Yuma.

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1. This information was obtained in a private conversation.

Clone M-56-2

Clone M-56-2 was one of 13 selected from an introduction from India called Sirsa Number 9. The introduction number was P.I. 235,736. The selections were based on seedling vigor and resistance to spotted alfalfa aphid.

Tagiura

Tagiura is the only ecotype of alfalfa recognized in Tripolitania, Libya. It was named after Tagiura, an oasis located nine miles to the east of Tripoli, where the ecotype is well adapted and grown on a large scale. It is grown along the coast and in the oases of Tripolitania. The ecotype is known to be a short-day plant with erect stems and very rapid recovery after cutting. Plants show slight winter hardiness and little drought resistance. The seed used for this study was obtained by the author from Sidi Mesri Experimental Station, Tripoli, Libya.

RESULTS AND DISCUSSION

Average minimum and maximum air temperatures and relative humidities of the greenhouse and field during the time of this study are shown in Tables 1 and 2, respectively. The average minimum and maximum temperatures in the greenhouse were 51° and 84°F., respectively. The average minimum and maximum relative humidities were 35 and 97 per cent, respectively (Table 1). Corresponding values in the field were 43° and 76°F., for temperature and 17 and 93 per cent for relative humidity (Table 2). Note that the difference between average minimum and maximum temperatures under greenhouse and field conditions was about the same, 36° and 33°F., respectively; whereas, the difference between the average minimum and maximum relative humidities was less under greenhouse than field conditions (62 and 77 per cent).

Self- and Cross-Fertility

It was believed at the beginning of these investigations that neither the percentage pod set nor the average number of seeds per pod was a good indicator of self- or cross-fertility of alfalfa. Research workers in the past have measured fertility by the number of pods or the number of seeds per pod (36). Where the percentage of flowers

TABLE 1. Average minimum and maximum air temperatures and relative humidities of the greenhouse at the level of the plants for the duration of the study.

Month	Temperature °F.		Relative Humidity per cent	
	Min.	Max.	Min.	Max.
March, 1963	42	85	36	100
April, 1963	45	89	31	98
May, 1963	57	94	17	96
June, 1963	54	98	17	98
July, 1963	53	96	36	97
August, 1963	67	94	60	98
September, 1963	66	93	59	96
October, 1963	60	85	53	95
November, 1963	43	72	34	98
December, 1963	40	73	28	97
January, 1964	36	69	49	99
February, 1964	39	76	42	100
March, 1964	56	93	30	95
April, 1964	41	90	35	97
May, 1964	55	95	19	96
June, 1964	55	97	18	94
Mean:	51	87	35	97

TABLE 2. Average minimum and maximum air temperatures and relative humidities surrounding plants growing in flats outside the greenhouse. Campbell Avenue Farm, Tucson, Arizona.

Month	Temperature °F.		Relative Humidity per cent	
	Min.	Max.	Min.	Max.
October, 1964	51	92	13	99
November, 1964	38	71	15	96
December, 1964	40	65	19	84
Mean:	43	76	16	93

forming pods and the number of seeds per pod were used simultaneously (40), two different values were presented making comparison among clones difficult. Therefore, the two indices self-fertility index (SFI) and cross-fertility index (CFI) were used in this study to evalutate the degree of self- and cross-fertility of 40 alfalfa clones. It was believed that the percentage of flowers setting pods and average number of seeds per pod were two major components of seed production in alfalfa. Furthermore, it was more practical to use one value for comparing various clones instead of two or more.

Self-fertility indices (SFI), the product of the percentage pod set multiplied by the average number of seeds per pod, for the 40 clones are shown in Table 3. The SFI values ranged from zero in three clones to 196.7 in one clone with an overall average of 65.5. Twenty-two clones out of 40 or the equivalent of 60 per cent showed a SFI less than the overall average indicating that the population was slightly skewed toward a low self-fertility (Figure 1). Note in Table 3 that clones MAR-3 and MAR-45 had the same percentage of pod set (25 percent), but they differed in the average number of seeds per pod i.e., 0.7 and 1.2, respectively. It is noteworthy also that clones Caliverde C. and MLR-7 had essentially the same average number of seeds per pod (1.9 and 1.9, respectively), but they differed in pod set (32.1 and 71.9 percent, respectively).

TABLE 3. Self-fertility indices (SFI) and their components of 40 alfalfa clones used for preliminary studies.

Clone Designation	Per cent pod set	Average number of seeds per pod	SFI
M-5-9	0.0	0.0	0.0
N-1412	0.0	0.0	0.0
CAL-377-5	0.0	0.0	0.0
MAR-5	0.1	1.0	0.1
MAR-40	7.1	0.9	6.4
MLR-3	6.0	1.0	6.5
M-5-20	11.6	0.7	8.2
M-56-2	7.2	1.1	8.5
MAR-25	16.9	0.9	16.4
MAR-3	25.0	0.7	17.7
MAR-2	17.4	1.0	18.1
MAR-1	18.7	1.1	21.3
Y-56-1	17.0	1.3	22.5
MAR-43	25.9	1.0	26.9
MAR-45	25.0	1.2	32.2
MAR-4	22.4	1.4	32.7
CAL-E-12	34.6	1.2	42.6
SBPFY	31.9	1.6	51.0
MAR-33	37.8	1.4	53.0
MLR-11	45.1	1.2	56.4
MAR-38	38.3	1.4	57.0
MAR-39	32.7	1.8	58.9
M-56-11	33.9	1.8	61.5
Caliv. C.	32.1	1.9	63.6
MAR-32	29.7	2.2	65.7
MAR-27	50.2	1.6	80.8
MAR-30	54.7	1.7	94.7
MAR-34	51.2	1.9	97.4
RMC-4-8	53.1	1.9	105.7
MAR-9	52.0	2.0	107.9
MLR-15	58.2	1.8	109.4
MLR-26	44.1	2.5	112.0
Tagiura	59.1	2.1	126.5
MAR-48	47.3	2.6	127.2
Y-56-146	60.1	2.1	127.4
MAR-35	26.6	2.0	127.8
MLR-7	71.8	1.9	140.1
MAR-23	63.3	2.5	160.3
RRPMC-2	63.2	2.7	175.2
RRPMC-1	70.2	2.8	196.7

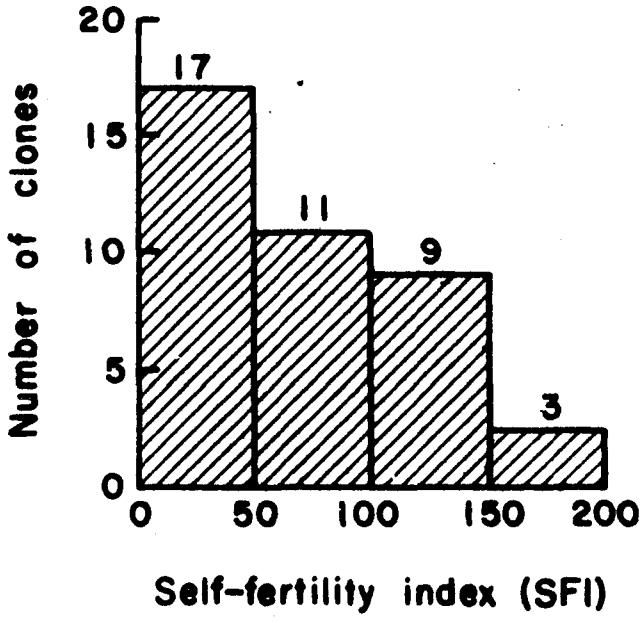

FIGURE 1. Frequency distribution for SFI of 40 alfalfa clones used in the preliminary study.

Cross-fertility indices (CFI), the product of the per cent pod set when flowers were alcohol-emasculated and hand-pollinated multiplied by the average number of seeds per pod for the 40 clones, are shown in Table 4. The values for CFI ranged from zero, in clone Cal. 377-5, to 359.5 in clone MAR-48. The overall average for all crosses tested was 39.2. Eighty-four per cent of the clones tested had a CFI below the average indicating that clones were skewed toward low cross-fertility (Figure 2). It was surprising that the overall average of SFI was much higher than the overall average of CFI (65.5 and 39.2, respectively). It should be pointed out that ethyl alcohol was used for emasculation prior to cross-pollination, and it may have had a detrimental effect on the stigma, style or ovules or all three.

El Tabbakh (20) reported that when alfalfa stem cuttings were dipped into a 50 per cent ethyl alcohol solution for 30 seconds the number of roots was significantly less than when similar cuttings were dipped in distilled water. After the toxic effect of ethyl alcohol had been observed, suction-emasculation was used and cross-fertility was much greater than self-fertility in every clone. These results are in agreement with the findings of Sprague (36) and Whitehead and Davis (40).

Examination of the data showed that two clones, MAR-25 and M-56-2, had approximately the same percentage of pod set, 24.0 and 24.3, respectively; whereas, they differed in the average number of seeds per pod (1.0 and 2.2, respectively). On the other hand, clone MAR-5 and clone

TABLE 4. Cross-fertility indices (CFI) and their components of 40 alfalfa clones used for preliminary studies.

Clone Designation	Per cent pod set	Average number of seeds per pod	SFI
CAL-3775	0.0	0.0	0.0
MAR-5	2.6	1.0	2.6
Y-56-1	6.6	0.7	5.0
MAR-35	6.6	0.8	5.3
MAR-30	4.6	1.3	6.2
MAR-38	5.8	1.2	6.9
MLR-3	5.2	1.3	6.9
MAR-40	14.8	0.5	7.4
MAR-1	3.2	2.5	8.2
M-56-11	6.8	1.5	10.3
MAR-4	12.1	1.0	12.1
M-5-9	12.0	1.1	13.9
MLR-15	12.0	1.3	15.9
MLR-26	9.3	1.7	16.2
MAR-32	20.0	0.8	17.0
MAR-2	6.4	2.6	17.1
M-5-20	25.6	0.7	17.9
MAR-39	14.2	1.3	18.5
CAL E-12	21.4	0.8	19.0
MLR-7	18.3	1.0	19.9
MAR-9	22.0	0.9	20.0
MAR-3	18.4	1.1	20.0
N-1412	15.3	1.5	23.0
MAR-33	15.0	1.6	25.0
MLR-11	12.9	2.0	25.8
MAR-25	24.0	1.0	25.9
MAR-43	25.4	1.0	27.2
MAR-45	22.7	1.2	27.2
MAR-34	19.1	1.4	27.9
RRPMC-2	20.1	1.4	28.5
MAR-23	8.3	3.7	30.9
RRPMC-1	25.0	1.3	32.5
M-56-2	24.3	2.2	53.6
Y-56-146	27.7	2.4	68.0
Caliv. C.	21.1	2.6	85.3
Tagiura	30.0	3.0	90.0
SBPFY	35.0	3.4	120.0
PMC-4-8	35.1	3.4	121.1
MAR-27	36.1	3.9	140.7
MAR-48	76.6	4.6	359.5

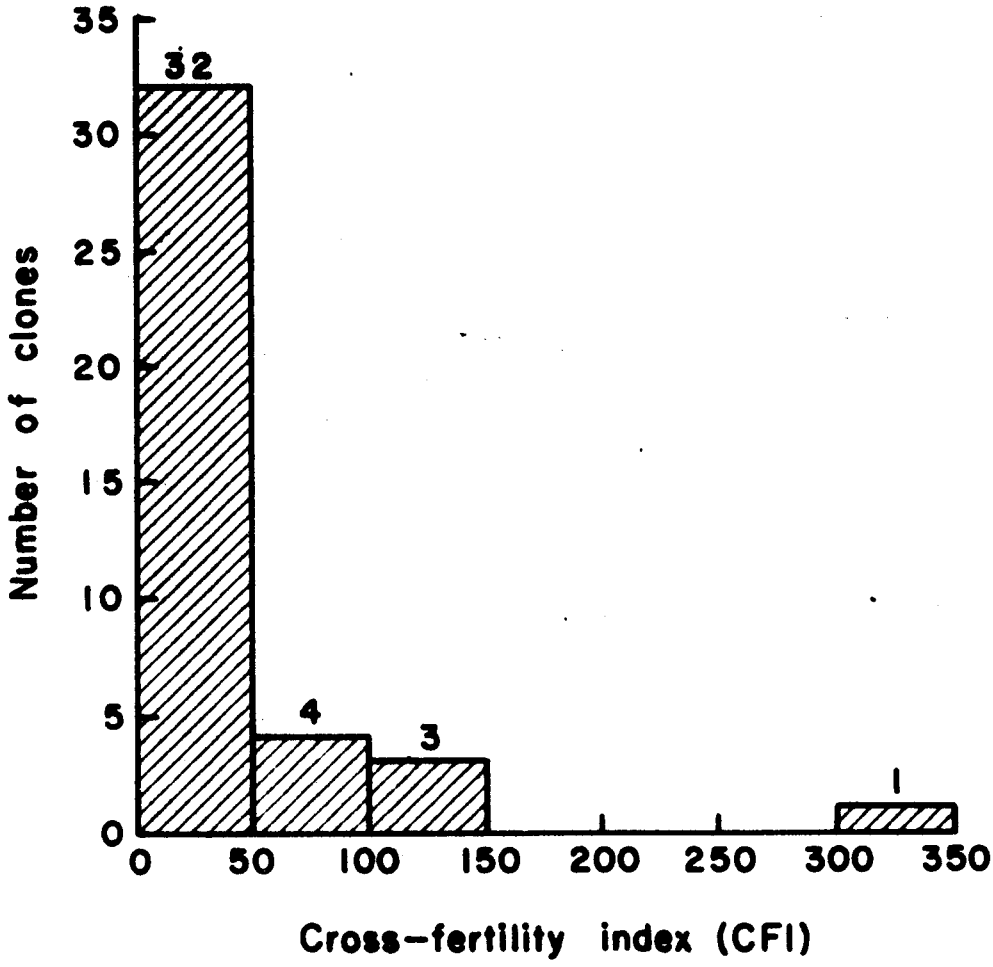

FIGURE 2. Frequency distribution for CFI of 40 alfalfa clones used in the preliminary study.

MAR-4 had the same average number of seeds per pod, 1.0; but, the percentage of pod set in the clones was different (2.6 and 12.1 respectively). It appears that two or more clones may have the same percentage of pod set though they differ appreciably in the average number of seeds per pod. The reverse condition was also found to be true.

Correlation coefficients for the association between the number of pods set and seed set, when flowers were selfed on one hand, and alcohol emasculated and then cross pollinated by hand on the other, were +0.936 and +0.664, respectively. These values would indicate that the number of seeds was affected more by the alcohol than the number of pods. This was further substantiated by the seed to pod ratio obtained when flowers were self-pollinated by hand and compared with flowers that were alcohol emasculated and cross pollinated by hand. The ratios were 1.84 and 1.55, respectively.

Since seeds are mature ovules, the decrease in seed number may be caused by alcohol damage to the ovules. It is interesting to note that the eight clones used for the detailed studies in this report did not show equal response to the alcohol used for emasculation. The ratio of CFI of each clone when pollinated without emasculation to the CFI when flowers were alcohol emasculated and hand pollinated ranged from 7.6 to 1 in clone MAR-35 to 28.8 to 1 in clone MLR-3. This finding was interpreted to mean that the stigma, style, ovary, and ovules wholly or partly in clone MLR-3 were more sensitive to ethyl alcohol than those

of clone MAR-35. Thus, the number of pods set, number of seeds per pod and ultimately the CFI were decreased. These results are in agreement with those of El Tabbakh (20).

Results of the preliminary study indicated that clones showed various degrees of self- and cross-fertility. Some clones were high both in self- and cross-fertility; some were high in self-fertility, and low in cross-fertility; others were low in self-fertility and high in cross-fertility and still others were low in both self-and cross-fertility. Because of the variation in self- and cross-fertility among clones, four categories with two clones in each combination of self- and cross-fertility were selected for detailed study (Table 6). The correlation coefficient ($r = -0.151$, Table 5) for self- and cross-fertility was not significant at the five per cent level indicating no association between self- and cross-fertility. This finding does not agree with those of Whitehead and Davis (40) who reported that self- fertility and cross-fertility of the female parent were positive and significantly correlated for percentage of pods set and seeds per pod. However, these results support the idea of Tysdal and Kiesselbach (39) who advocated the use of cross-fertile, self-sterile alfalfa plants to insure cross-fertilization.

Variation in the Number of Ovules

A summary of the variation in the number of ovules per ovary for the eight clones is shown in Table 7. For different clones, the

TABLE 5. Simple correlation coefficients (r) of certain characteristics of eight alfalfa clones selected for detailed studies.

Characteristics correlated	D.F.	r value
Self-fertility index with:		
Percentage of viable pollen	6	$-0.083^{n.s.}$
Cross-fertility index	6	$-0.151^{n.s.}$
Length of style	6	$+0.161^{n.s.}$
Average number of ovules per ovary	6	$-0.161^{n.s.}$
Cross-fertility index with:		
Length of style	6	$-0.052^{n.s.}$
Average number of ovules per ovary	6	$-0.425^{n.s.}$

n.s. = not significant at the 0.05 level.

TABLE 6. Self- and cross-feritility indices of the eight alfalfa clones used to obtain most of the data in the detailed study.

Clone Designation	SFI	CFI	Designation
MAR-48	127.3	359.6	H X H[1]
Tagiura	126.6	90.0	
MAR-35	127.8	5.3	H X L
MAR-30	94.7	6.2	
M-56-2	8.5	53.7	L X H
M-5-20	8.2	17.9	
MAR-40	1.0	7.4	L X L
MLR-3	6.6	6.9	

(1) H and L represent high and low fertility.

Table 7. A summary of the average number of ovules per ovary for different clones, plants within a clone, stems within a plant, raceme position on a stem, and flower position on a raceme in eight alfalfa clones.

Clone designation	Plants within a clone (1)		Stems within a plant (1)		Raceme position (2)			Flower position (2)			Mean
	1	2	1	2	B	M	A	B	M	A	
MAR-35	10.2	10.0	9.9	10.2	10.0	10.2	9.8	10.5	10.3	9.6	10.0[a]*
MAR-30	10.6	12.2	11.4	11.4	11.5	11.6	11.2	12.1	11.4	10.7	11.4[b]
MAR-48	12.1	12.4	12.5	12.4	12.6	12.6	12.2	13.0	12.6	11.9	12.5[c]
MAR-40	13.0	13.5	13.3	13.2	13.3	13.2	13.3	13.5	13.2	11.9	13.2[c]
MLR-3	9.7	9.4	9.7	9.4	9.3	9.4	10.0	9.5	9.5	9.5	9.5[a]
M-5-20	12.9	13.0	12.8	13.2	13.0	13.0	12.6	13.5	13.2	12.1	12.9[c]
M-56-2	10.4	10.3	10.5	10.3	10.2	10.4	10.5	10.8	10.6	9.8	10.4[ab]
Tagiura	11.9	11.2	11.5	11.5	11.2	11.7	11.6	11.8	11.4	11.3	11.5[bc]
Mean	11.3[a]	11.5[b]	11.4[a]	11.4[a]	11.4[a]	11.5[a]	11.4[a]	11.8[a]	11.5[ab]	10.9[b]	11.4

(1) Each value is a mean of 36 flowers.
(2) Each value is a mean of 24 flowers.
B, M, A represent base, middle, and apex for racemes and flowers.
* Mean values followed by the same letter are not significantly different at the 0.01 level using Duncan's multiple range test.

average number of ovules per ovary ranged from 9.5 in clone M-5-20 to 13.2 in clone MAR-40. The ovule number per ovary for all the clones ranged from 6 to 15. This range compared favorably with the observed range of 8 to 14 ovules per ovary by Cooper and Brink (17). An analysis of variance (Table 8) indicated highly significant differences in the number of ovules per ovary among clones, plants within a clone, and flower position on the raceme. There was no significant difference among stems of the same plant and among racemes of the same stem. The significant difference in ovule number between plants within a clone was not expected. The cause of this variation was not determined.

Flowers at the base of a raceme contained more ovules per ovary than those at the middle which in turn contained more ovules than flowers at the apex. The magnitude of the decrease in ovule number per ovary from flowers at the base to apex of the raceme varied with clones. Clones with a low average number of ovules showed a relatively small decrease from the base to apex, while clones with relatively high average number of ovules showed a greater decrease with a higher position on the raceme. These results are in agreement with those of Barnes and Cleveland (5). In general, the difference in average number of ovules per ovary between basal and middle flowers was less than that between middle and apical flowers. These differences were 0.3 and 0.6, respectively (Table 7).

Table 8. Summary of the analysis of variance for the variation in ovule number per ovary in eight alfalfa clones.

Source of variation		D.F.	M.S.
Total		575	
1	Clones. (2)*	7	135.04**
2	Plants within a clone. (3)	8	8.43**
3	Stem of a plant within a clone. (16)	16	0.87
4	Raceme position. (10)	2	1.02
5	Flower position. (11)	2	35.76**
6	Flower position x racemes. (12)	4	1.70
7	Clones x raceme. (10)	14	1.46
8	Clones x flower position. (11)	14	1.63
9	Clones x flower position x raceme. (12)	28	0.77
10	Raceme x plant x clone. (13)	16	1.77
11	Flower position x plant x raceme. (14)	16	0.88
12	Raceme x flower position x plant within a clone. (15)	32	0.97
13	Raceme x stem of a plant within a clone. (16)	32	1.28**
14	Flower position x stem of a plant within a clone. (16)	32	0.72
15	Flower position x raceme x stem of a plant within a clone. (16)	64	0.57
16	Sampling	228	0.57

* Number in parenthesis refers to mean square used for testing.
** Significant at the 0.01 level.

It was also found that the magnitude of difference in the number of ovules per ovary was the greatest among clones followed by flower position on a raceme, plants within a clone and raceme position on a stem. These differences in the average number of ovules were 3.7, 0.9, 0.2 and 0.1, respectively. The first three differences were highly significant at the one per cent level; whereas, raceme position on the stem was not (Table 8). The only significant interaction was raceme x stem of a plant within a clone, indicating that the variation in the number of ovules among racemes was influenced by stems within a plant within a clone.

Barnes and Cleveland (5) indicated that the number of ovules per ovary represented the potential ability of a flower to produce seed. Thus, it appeared that selection of plants with a high number of ovules per ovary should result in maximum seeds per pod. This study indicated a negative and nonsignificant correlation (r = -0.165 and -0.425) between the average number of ovules per ovary and self-fertility and cross-fertility, respectively (Table 5). This finding may be interpreted to mean that ovule number <u>per se</u> is not the only limiting factor to seed production. Moreover, the possibility may exist that there is a "critical number" of ovules per ovary above which any increase in ovule number results in no increase in seed set. The large, though nonsignificant correlation coefficient (r = -0.425) for the ovule number per ovary and cross-fertility as compared with a smaller r = -0.165 for the

ovule number and self-fertility leads one to postulate that the "critical number" of ovules is lower when alfalfa flowers are crossed than when selfed. This may be because the potential of fertilization is greater, and the demand for food and other factors is heavier in seeds developing after cross-fertilization when produced from self-fertilized ovules.

After observing the gradual decrease in ovule number per ovary from the base of the raceme to the apex, pods from 30 racemes of four clones were classified according to their position on the raceme as basal, middle and apical. Each group of pods was threshed separately and seeds were counted and averaged per pod. The mean number of seeds per pod was 3.4, 2.8 and 2.0 for basal, middle and apical pods, respectively. This decrease in number of seeds per pod from the base to the apex of the raceme was similar to the decrease in number of ovules.

Differences in ovule number associated with flower position on the raceme and a reduced number of seeds per pod from the base of the raceme to the apex can probably be attributed to competition for food among upper and lower flowers. According to Barnes and Cleveland (5), ovules in the lowest flowers have the opportunity to develop because of the direct availability of food, but the competition for food becomes greater as the flowers continue to be formed, and a greater number of ovules fail to develop. They also suggested that more competition for food may occur in plants genotypically provided with a higher number of ovules than in plants with a lower number.

Self-Fertilization and Its Effects on Vegetative and Reproductive Characters

Mean number of flowers per raceme for the eight S_o parent clones and their respective S_1 progenies is presented in Table 9. Means for parent clones ranged from 10.4 to 19.7 flowers per raceme in clones MAR-35 and MLR-3, respectively. Mean number of flowers for S_1 progenies ranged from 5.9 to 14.1 per raceme in S_1 plants of MAR-30 and MLR-3, respectively. These values represented decreases of 48.5 and 71.6 per cent, from their respective parents.

Means for percentage viable pollen of S_o parental clones and their S_1 progenies are shown in Table 9. An analysis of variance of pollen viability showed that clone MAR-48 was significantly lower at the one per cent level than the rest of the clones. The remaining clones did not differ significantly among themselves (Table 10).

This study indicated that when parent alfalfa clones were hand-selfed and the resulting progenies were tested, there was a reduction in the average number of flowers per raceme and the percentage viable pollen in the progeny from all clones. The magnitude of reduction was greater in the average number of flowers per raceme than in the percentage of viable pollen. This reduction was 51.7 per cent and 29.6 per cent, respectively.

The reduction in percentage viable pollen from parent to progeny agrees with the results of Schonhorst (34). He suggested two possibilities

Table 9. The effect of self-fertilization on average number of flowers per raceme, percentage of viable pollen, and self-fertility index (SFI) for eight alfalfa clones (S_O) and their selfed progeny (S_1).

Clone designation	Average number of flowers per raceme		Per cent decrease	Percentage of viable pollen		Per cent decrease	Self fertility index		Per cent increase or decrease
	S_O	S_1		S_O	S_1		S_O	S_1	
MAR-35	10.4	9.2	13.0	88.9	63.5	40.0	128.0	26.0	-80.9
MAR-30	12.2	5.9	106.7	92.2	58.9	56.5	95.0	18.0	-81.0
MAR-48	14.8	8.2	80.5	63.1	47.9	31.7	127.0	6.0	-95.3
MAR-40	12.3	10.0	23.0	92.2	68.4	34.8	1.0	0.0	-100.0
M-5-20	12.6	8.1	55.5	97.2	92.4	5.2	8.0	104.0	+92.3
MLR-3	19.7	14.1	39.7	90.5	65.3	38.6	7.0	4.0	-42.8
M-56-2	11.2	7.6	47.4	93.4	91.7	1.8	8.0	28.0	+71.4
Tagiura	14.5	8.5	70.5	92.7	60.1	54.2	127.0	8.0	-93.7
Mean:	13.5	8.9	51.7	88.8	68.5	29.6	62.6	24.2	

TABLE 10. Summary of the analysis of variance for the viability of pollen in eight parent clones (So) of alfalfa.

Source of Variation	D.F.	S.S.	M.S.	F.
Total	31	3591.00	115.84	
Clones	7	2716.71	388.10	10.64**
Error	24	875.25	36.47	

**Significant at the 0.01 level.

for this reduction, namely: a general reduction in plant vigor arising from inbreeding depression, and the possible occurrence of recombinations of genetic factors producing some S_1 individuals intermediate and low in viable pollen. Thus, the average viability of pollen for the entire group would be lowered. Also, it is believed that the same factors were responsible for the reduction in average number of flowers per raceme.

A decrease in the self-fertility index from parent to progeny was observed in this study in six of the eight clones (Table 9). This finding agrees with those of Schonhorst (34) concerning mean pollen sterility. He attributed the reduction in self-fertility to the general effects of inbreeding depression. The increase in self-fertility in S_1 plants over their parents of clones M-5-20 and M-56-2 of 92.3 per cent and 71.4 per cent, respectively, might have resulted from the segregation and accumulation of a more favorable gene or genes for the development and maturity of seed than for the initiation and development of flowers and viability of pollen. This study showed a maximum inbreeding depression of 106.7 per cent in flowers per raceme, 56.5 per cent in viability of pollen, and 100 per cent in self-fertility (Table 9). These results are in agreement with the prediction of Wilsie (41) who made the following statement: "in such a population of cross-breeding individuals of alfalfa, a certain degree of heterosis is supposed to exist. Most of them will show a marked inbreeding depression when selfed, on the average perhaps 20 to 30 per cent in vigor, and a great deal more in self-fertility."

Since the magnitude of inbreeding depression was not the same in all of the clones studied, it is possible that they were not in an equal state of heterozygosity. Therefore, inbreeding might be used as a useful tool for detecting the degree of heterozygosity in certain alfalfa clones.

Length of Style and Ovary and Percentage of Ovules Fertilized

Data on the length of style and ovary of the eight clones are presented in Table 11. Style length ranged from 1.9 to 2.9 mm. in clones M-56-2 and Tagiura and clone MAR-35, respectively. The average length of style for the eight clones was 2.3 mm. An analysis of variance indicated no significant difference for length of style among the eight clones (Table 12). It is interesting to note that some of the clones with shorter styles were among the lowest in self-fertility; whereas, clones with longer styles were among the highest (Table 11). This may be interpreted to mean that the length of style in alfalfa does not present a major physical or physiological barrier to fertilization when alfalfa flowers are self-pollinated by hand. When the same clones were cross-pollinated by hand after alcohol emasculation, the clones whose style length was below the average for the eight clones had the highest cross-fertility index. Those with a length of style above the average included clones ranging from very low to low cross-fertility (Table 11). These results seem to indicate that foreign pollen is more effective in accomplishing fertilization when the style is short. Thus, it may be

TABLE 11. Length of style and of the ovary, in mm, for eight alfalfa clones used in this study.

Clones	Style length mm	Ovary length mm	SFI	CFI[1]
MLR-3	2.3	2.9	6.6	6.9
MAR-30	2.4	2.5	94.7	6.2
M-56-2	1.9	3.5	8.5	53.7
Tagiura	1.9	2.7	126.6	90.0
MAR-48	2.1	3.1	127.3	359.6
MAR-35	2.9	2.9	127.8	5.3
M-5-20	2.3	4.0	8.2	17.9
MAR-40	2.5	2.1	1.0	7.4
Mean	2.3	2.9	62.5	68.3

[1]Alcohol was used for emasculation prior to crosspollination by hand.

TABLE 12. Summary of the analysis of variance for the distance from stigma to the first ovule (style) in eight alfalfa clones.

Source of variation	D.F.	S.S.	M.S.	F.
Total	31	30.57		
Clones	7	3.28	0.47	$0.41^{n.s.}$
Error	24	27.29	1.14	

n.s. = not significant at the 0.05 level.

desirable to select for "short style" and cross-fertile plants to increase seed yields.

Because of the positive and negative values for self-fertility and cross-fertility with the length of style, it is postulated that the reactions between the self-pollen tube and the tissue of the style and ovary is different from that between the foreign pollen and these tissues.

Ovary length ranged from 2.1 to 4.0 mm. in clones MAR-40 and M-5-20, respectively. The average length of ovary for the eight clones was 2.9 mm. (Table 11). Although these data are of a preliminary nature they seem to indicate no close relationship between self-fertility and the length of ovary.

The percentage of ovules fertilized as a function of their position when flowers were self-pollinated by hand is presented in Table 13. Data in this table showed that clones differed in the number of skips (unfertilized ovules located between the stigma and the fertilized ovule furthest from stigma). Two of the clones highest in self-fertility, Tagiura and MAR-48, had no skips; whereas, two of the clones lowest in self-fertility M-5-20 and MAR-40 had the highest number of skips, three and eight, respectively. The pattern of "skipping" seemed to be at random in some clones, and not necessarily confined to ovules in the base of the ovary. The presence of "skips" in the base of the ovary may be due to the failure of pollen tubes to reach and fertilize the basal ovules; whereas, the "skips" in the apical and sub-apical sections of the

TABLE 13. Percentage of ovules fertilized as a function of their position in the ovary of eight alfalfa clones.

Clone	Ovule position in ovary from stigma												
	1	2	3	4	5	6	7	8	9	10	11	12	13
MLR-3	50[1]	50	50	0	50	50	0	0	0	0	0	0	0
MAR-30	0	31	15	31	23	7	15	7	0	0	0	0	-
M-56-2	36	43	28	14	36	21	14	21	0	7	7	-	-
Tagiura	43	50	27	7	28	21	7	7	0	0	0	-	-
MAR-48	53	37	37	31	42	37	21	16	26	10	10	0	-
MAR-35	33	67	33	0	33	33	33	0	0	0	-	-	-
MAR-40	0	0	25	0	25	25	0	0	0	0	0	25	0
M-5-20	25	0	25	0	50	25	0	25	0	0	-	-	-
Mean	31.6 Apical section			20.4 Sub-apical section			8.0 Supra-basal section			3.6 Basal section			

[1] Average of 10 ovaries per clone
(-) Ovule did not exist in that position.
(0) A skip (unfertilized ovule).

ovary may be attributed, among possibly other things, to the differences in capacity to secrete a "chematactic" substance by embryo sacs which attracts pollen tubes (16).

When the numbers of fertilized ovules was averaged for the eight clones, a general decrease from the apex to the base of the ovary was observed. These results are in agreement with those of Cooper and Brink (19). On a clone basis, the decrease was erratic. If the ovaries were arbitrarily divided into four sections from the stigma to the base for the eight clones combined, the percentage of fertilized ovules was 32 in the apical section, 20 in the sub-apical, 8 in the suprabasal, and 4 in the basal section. These data show a strong tendency toward a reduction in fertilization of the ovules from the apex toward the base of the ovary.

Methods of Emasculation and Their Effects on Agronomic Characters and Genetic Variances

Average cross-fertility indices (CFI) of the crosses and their reciprocals produced from hand pollination without emasculation, hand pollination of alcohol-emasculated flowers, and hand pollination of suction-emasculated flowers are presented in Table 14. A diallel cross analysis for CFI is shown in Table 15. It was apparent that differences among treatments for CFI were highly significant. Comparison of the treatment effect on cross-fertility showed that emasculation with alcohol suppressed the fertility to a level considerably below that of

TABLE 14. Self-fertility indices (SFI) and cross-fertility indices (CFI) for all possible crosses and reciprocals of eight alfalfa clones for the control, alcohol, and suction emasculation.

Clones	Hand-selfing	Control,		Alcohol,		Suction,		MeanCFI
	SFI	CFI (1)		CFI		CFI		
		C	R	C	R	C	R	
MAR-35	128.0	366	342	48	19	423	235	255.5
MAR-30	95.0	323	352	21	27	205	246	195.7
MAR-48	127.0	504	366	50	26	353	135	239.0
MAR-40	1.0	231	337	15	41	98	302	170.7
M-5-20	8.0	18	453	2	70	38	324	150.8
MLR-3	7.0	458	373	22	9	206	158	204.3
M-56-2	8.0	469	355	33	34	268	264	237.2
Tagiura	127.0	574	364	51	19	300	247	259.2
Mean	62.6	367.8	367.8	30.2	30.6	236.4	238.9	

(1) C and R represent crosses and reciprocals, respectively.

TABLE 15. Summary of the analysis of variance for cross-fertility index (CFI) for all possible crosses and reciprocals of eight alfalfa clones for the control, alcohol, and suction emasculation.

Source of variation	D.F.	S.S.	M.S.	F.
Total	167	6189796.20		
Treatment	2	3049120.20	1524560.10	71.65**
General	7	455085.16	65012.16	6.00**
Specific	20	125337.00	6266.85	1.04
Maternal	7	92437.15	13205.31	2.17
Reciprocal	21	127255.44	6059.78	0.28
Error	110	2340561.10	21277.83	

** Significant at the .01 level.

emasculation by suction or the control (Table 16). The drastic reduction from alcohol can be attributed to the detrimental effect of alcohol on the stigma, style, ovary and ovules either singly or collectively at or after the time of emasculation.

The high cross-fertility index in the control where no emasculation was done may be linked to the fact that the stigmas of plants used as female parents had a large amount and more diverse pollen than the stigmas of the female parents following emasculation. In the former, both self and foreign pollen grains were present.

Another possible explanation for the increased cross-fertility in the control was that the increased and diversified pollen may provide optimal germination and growth of pollen tubes under the effect termed "mutual stimulation" by Brubaker and Majundar (8). The reduction in cross-fertility when suction emasculation was compared with the control may be attributed to the lack of "mutual stimulation." In the case of suction emasculation, pollen grains on the stigma were fewer in number and composed of foreign pollen only as compared with the large number of diversified pollen grains in the case of the control.

The diallel analysis for combining ability indicated a large part of the total genetic variation observed for the cross-fertility was associated with a highly significant general combining ability. Effects from specific combining ability were small and not significant (Table 15). The relative magnitude of the ratio of general to specific combining

TABLE 16. Average cross-fertility index (CFI) for all possible crosses and reciprocals of eight alfalfa clones for the control, suction, and alcohol emasculation.

Treatment	Cross-fertility index (CFI)
Control	356.0[a] (1)
Suction emasculated	239.3[b]
Alcohol emasculated	30.5[c]

(1) Values followed by the same letter are not significantly different at the 0.01 level using Duncan's multiple range test.

ability for cross-fertility was high (Table 17). These ratios indicate that general combining ability (additive effects) is more important than specific combining ability (non-additive effects) when breeders seek high cross-fertility in alfalfa. Variation due to reciprocal and maternal effects was not significant (Table 15).

Average weight in grams of 50 seeds for the crosses and their reciprocals produced under the three treatments is shown in Table 18. There was no significant difference among treatments and between crosses and reciprocals. Thus, evidence of maternal effects on seed weight of the clones tested appeared to be negligible. These results agree with those of Hanson et al., (25).

Average seedling height of the progeny at four and eight weeks of age and yield of dry matter at ten weeks resulting from seed produced under the three treatments are shown in Table 19. The diallel analysis in Table 20 indicated that the treatments differed significantly in their effects on height and yield of dry matter of the progeny. Separation of the treatment means by Duncan's multiple range test (37) showed that plant height at four and eight weeks of age and yield of dry matter at ten weeks of age did not differ significantly between the progenies of plants emasculated with alcohol and the control; whereas, the progenies of plants receiving suction emasculation were significantly less than those of the control and alcohol-treated plants (Table 19).

TABLE 17. Mean squares for general (GCA) and specific (SCA) combining ability, and GCA:SCA mean square ratios for some characters in eight alfalfa clones.

Character	Mean squares		Mean square Ratio of GCA: SCA
	GCA	SCA	
Cross fertility index (CFI)	65,012.16**	6,266.85	10.37
Seedling height at 4 weeks	965.65	276.33	3.49
Seedling height at 8 weeks	1,554.13*	1,086.75	5.11
Yield of dry matter at 10 weeks	14,570.35	6,925.19	2.12

* Significant at the 0.05 level.

** Significant at the 0.01 level.

TABLE 18. Average weight (in grams) of 50 seeds, for all possible crosses and reciprocals of eight alfalfa clones for the control, alcohol and suction emasculation.

	Weight of 50 seeds (gms.)		Mean
	C[(1)]	R	
Control	0.125	0.135	0.130[n.s.]
Alcohol emasculated	0.130	0.142	0.136[n.s.]
Suction emasculated	0.141	0.145	0.143[n.s.]
Mean	0.132[n.s.]	0.141[n.s.]	

(1) C and R represent crosses and reciprocals, respectively. n.s. = non significant at the 0.05 level.

TABLE 19. Average seedlings height at four and eight weeks, and yield of dry matter of progeny at ten weeks for all possible crosses and reciprocals of eight alfalfa clones for the control, alcohol, and suction emasculation.

Treatment	Height at 4 weeks (mm)	Height at 8 weeks (mm)	Yield of dry matter at 10 wks. (gms.)
Control	37.47a(1)	71.68a(1)	90.88a(1)
Alcohol emasculated	42.83a	76.98a	121.66a
Suction emasculated	28.13b	50.06b	40.52b

(1) Values followed by the same letter are not significantly different at the 0.01 level using Duncan's multiple range test.

TABLE 20. Summary of analyses of variance for seedling height at four and eight weeks, and yield of dry matter at ten weeks for all possible crosses and reciprocals of eight alfalfa clones, for the control, alcohol, and suction emasculation.

Source of variation	D. F.	Height at 4 weeks	Height at 8 weeks	Yield of dry matter at 10 weeks
Total	503			
Replications	2	30.24	1552.00	9647.35
Treatments	2	9300.06**	34158.80**	281904.00**
General	7	965.65	5554.13*	14570.35
Specific	20	276.33	1086.75	6925.19
Maternal	7	933.46**	2321.67*	7029.39
Reciprocal	21	139.19	659.87	15660.55**
Error	444	164.07	536.47	7689.06

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Since the treatments did not differ significantly in their effect on seed weight (Table 18), there was no explanation for the detected difference between suction-emasculation on one hand and alcohol emasculation and control on the other for height and yield of dry matter of the progeny. When analyzed for the effects of general and specific combining ability, the general combining ability for seedling height at eight weeks of age was significant (Table 20).

The ratios of mean squares of general to specific combining ability suggested by Frakes et al. (23) for seedling height at four and eight weeks of age and yield of dry matter at ten weeks of age are shown in Table 17. The ratios were relatively high especially for cross-fertility index and seedling height at eight weeks of age. Such ratios readily showed the relative size of the effects of general and specific combining ability. These findings are in agreement with those of Frakes et al. (23). Also, this study indicated that the effects of general combining ability on seedling height had appeared rather late (eight weeks of age). Reciprocal differences were highly significant for the yield of dry matter only indicating that these differences persisted for a period longer than that reported by Carnahan (12) and Hanson et al. (25).

The reciprocal effects observed in this study may be attributed to the same sources reported by previous workers (12, 25) namely selfing and effects conditioned by the female parent per se (true maternal

effects). Maternal differences which were part of the reciprocal differences were significant at the one per cent level for seedling height at four weeks of age. Thus, the maternal effects seemed to dissipate with increase in age of the seedling. Also, it can be concluded from this study that significant reciprocal differences can be detected for some but not all characteristics (Table 20).

Association Between Plant Height and Yield of Dry Matter

Correlation coefficients of plant height at four weeks of age with height at eight weeks of age and between these two variables and the yield of dry matter at ten weeks of age are shown in Table 21. Correlation between height at four weeks and height at eight weeks were significant at the one per cent level. Correlation between height at four weeks and yield of dry matter at ten weeks was significant at the one per cent level for progeny from the alcohol and suction emasculation; whereas, the correlation between the variables for the control was significant only at the five per cent level. The association between height at eight weeks and yield of dry matter at ten weeks was significant at the one per cent level for progeny of plants of the control and those receiving alcohol emasculation but was not significant for progeny of those plants receiving suction emasculation. When the three treatments were combined in one analysis, the correlations between height at four weeks and eight weeks of age and between these two and yield of dry

TABLE 21. Single correlation coefficients (r) between height at four and eight weeks, and yield of dry matter at ten weeks for all possible crosses and reciprocals of eight alfalfa clones for the control, alcohol, and suction emasculation.

	Height at 8 weeks	Yield of dry matter at 10 weeks
Height at 4 wks.	C[(1)] .486**	C .43*
	A .459**	A .192**
	S .339**	S .336**
	CAS .554**	CAS .294**
Height at 8 wks.		C .253**
		A .328**
		S .125**
		CAS .373**

(1) C, A, S and CAS represent control, alcohol and suction emasculation and all the three treatments combined.

* Significant at the 0.05 level.
** Significant at the 0.01 level.

matter at ten weeks of age were significant at the one per cent level. These results can be interpreted to mean that there was a positive and significant relationship between height at the two stages of growth on one hand and height and yield of dry matter on the other regardless of the treatment under which seed was produced. The positive and significant relationship between height and yield of dry matter was in agreement with the findings of Davis and Panton (18), Frakes et al. (21, 22) and Whitehead and Davis (40).

SUMMARY

Preliminary investigations were conducted on 40 clones of alfalfa to determine self- and cross-fertility. Eight clones were selected for further detailed studies. The following data were collected: self- and cross-fertility indices, variation in ovule number, self-pollination, length of style, "skips" in fertilization of ovules, and comparison among methods of emasculation. Data were also obtained on height of stem and yield of dry matter of progenies, combining ability, and reciprocal effects. All data were analyzed statistically.

The following results were obtained. Self-fertility indices (SFI) and cross-fertility indices (CFI) were computed for 40 alfalfa clones. The SFI values ranged from zero to 196.7 with an overall average of 65.6. CFI ranged from zero to 359.5. The overall average was 39.2. The populations were slightly skewed toward low self- and cross-fertility. The low CFI may have resulted from the toxic effect of ethyl alcohol on the stigma, style or ovules or all three. Certain clones had the same percentage pod set, but they differed in the average number of seeds per pod. On the other hand, certain clones had the same average number of seeds per pod; whereas, they had a different percentage pod set.

Correlation coefficients for the association between the total number of seeds per pod when flowers were self-pollinated by hand and compared to those alcohol emasculated and then hand pollinated were r = +0.936** and +0.664**, respectively. These values would indicate that the number of seeds per pod was affected more by the alcohol than the number of pods. This was substantiated further by the seed to pod ratios obtained when flowers were hand selfed and compared with flowers that were alcohol emasculated and hand pollinated.

The correlation coefficient value of -0.151 for self and cross-fertility was not significant indicating no association between these factors for the clones studied. Also, the eight clones used for the detailed study did not show equal response to the ethyl alcohol used for emasculation. This was interpreted to mean that the stigma, style, ovary and ovules wholly or partly in certain clones were more sensitive to ethyl alcohol than those of other clones.

The ovule number per ovary for the eight clones ranged from 6 to 15. The overall average for the eight clones was 11.4 ovules per ovary. An analysis of variance indicated highly significant differences in the number of ovules per ovary among clones, plants within a clone, and flower position on the raceme. The significant difference in ovule number between plants within a clone was not expected. Flowers at the base of a raceme contained more ovules per ovary than those at the middle, which in turn contained more than flowers at the apex. The respective

means were 11.8, 11.5 and 10.9. The average number of ovules per ovary appeared to be constant among stems of the same plant and among racemes positioned differently on the same stem. The magnitude of difference in ovule number per ovary was the greatest among clones, followed by flower position on a raceme, plants within a clone, raceme position on a stem. These differences were 3.7, 0.9, 0.2 and 0.1 ovules per ovary respectively.

A decrease in the number of seeds per pod from the base to the apex of the raceme was similar to the decrease in number of ovules. Clones with a low average number of ovules showed a relatively smaller decrease from the base to the apex of the raceme than those with relatively high average number of ovules.

Self-fertilization of the parent clones resulted in a reduction in the number of flowers per raceme and the viability of pollen of all their progenies. A similar reduction in the self-fertility index was observed in progenies of six out of eight clones; whereas, an increase was observed in progenies of two clones. The reduction from parent to progeny was attributed to a general reduction in plant vigor arising from inbreeding depression and possible occurrence of recombinations of genetic factors producing some progenies intermediate and low in number of flowers, viability of pollen and self-fertility. This study showed a maximum inbreeding depression of 106.7 per cent for flowers per raceme, 56.5 per cent for pollen viability and 100 per cent for

self-fertility. The magnitude of inbreeding depression was not the same in all of the clones studied, indicating that they were not in an equal state of heterozygosity.

Length of style for the eight clones ranged from 1.91 to 2.97 mm. No significant differences in style lengths were detected among the eight clones. These data indicated that the length of style did not present a major physical or physiological barrier to fertilization when alfalfa flowers were selfed by hand.

The term "skip" was developed in this study to indicate an unfertilized ovule 72 hours after hand-pollination. Clones differed in the number of "skips" which were higher in clones with low self-fertility than those with high self-fertility. The pattern of "skipping" appeared to be at random on a single clone basis throughout the ovary, but when the number of fertilized ovules was averaged for the eight clones, a general decrease in "skips" from the apex to the base of the ovary was observed. If all clones were averaged and the ovary was arbitrarily divided into four sections from the stigma to the base, the percentage of fertilized ovules was 31.6, 20.4, 8.0 and 3.6 in the apical, sub-apical, suprabasal and basal sections, respectively.

The effect of hand pollination without emasculation, hand-pollination with alcohol- and suction-emasculated flowers (treatments) on cross fertility, seed weight, height at four and eight weeks of age

and yield of dry matter of progenies at ten weeks of age was compared. It was found that alcohol emasculation suppressed the cross-fertility to a level lower than that of pollination without emasculation and suction emasculation. The reduction from alcohol was attributed to the toxic effect of alcohol on the stigma, style, ovary and ovules either singly or collectively at of after emasculation.

Diallel analysis for combining ability indicated that a large part of the total genetic variation observed for cross-fertility was associated with a highly significant general combining ability. Specific combining ability, reciprocal and maternal effects were not significant.

There was no significant difference between the effect of pollination without emasculation and pollination of alcohol-and suction-emasculated flowers and reciprocal effects on seed weight. Thus, evidence of maternal effects on seed weight appeared to be negligible. The emasculation treatments differed significantly in their effects on height and yield of dry matter of the progenies. Reciprocal differences were highly significant only for the yield of dry matter at ten weeks of age indicating that these differences persisted for a period longer than that reported earlier. The reciprocal effects were attributed to selfing and to effects conditioned by the female parent. Maternal effects were highly significant for seedling height at four weeks of age only, indicating that they disappeared with time.

The yield of dry matter of progenies at ten weeks of age positively and significantly correlated with seedling height at four weeks of age. This was true for all progenies resulting from alcohol and suction emasculation. Also, a significant correlation was obtained for yield of dry matter and seedling height at eight weeks of age for progenies resulting from the control and alcohol emasculation. When the three treatments were combined in one analysis, the yield of dry matter correlated significantly with seedling height at four and eight weeks of age for all progenies.

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