Estimates of cross- and self-fertility of cicer milkvetch (Astragalus cicer L.)
by John George Scheetz

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University

Abstract:
The mode of pollination of nicer milkvetch was studied in 1969 and 1970. The floral structure was examined and the common pollinators were identified. Estimates of cross-fertility by open-pollination and self-fertility by artificial selfing by hand and by artificial selfing without hand manipulation were made for 20 cicer milkvetch plants in 1969. Estimates of cross-fertility by open-pollination and selffertility by artificial selfing by hand were made on 30 plants in 1970. Estimates of cross-fertility by open-pollination and estimates of self-fertility by natural selfing in isolation by bees and artificial selfing by hand were made on four clones of cicer milkvetch in 1970. Seeds obtained from the four clones were seeded in flats in the greenhouse to study the effect of inbreeding on cicer seedlings.

The floral structure was found not to eliminate self-pollination. Three species of bumble bees were found to be the common pollinators of cicer milkvetch. Apparently, honey bees are not common pollinators of cicer. Cicer flowers were more fertile during the first few weeks of the flowering period both years. The average artificial self-fertility of all cicer milkvetch plants was 13.10%. The average natural selffertility of four cicer milkvetch clones was 58.86%. The average crossfertility of all cicer milkvetch plants was 62.65%. Selfing had an adverse effect on the growth of cicer seedlings. It was concluded that cicer milkvetch is naturally cross-pollinated with a relatively high degree of self-fertility.

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Date December 9, 1970

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Approved:

Head, Major Department

Chairman, Examining Committee

Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

March, 1971

ACKNOWLEDGEMENT

The author wishes to express his appreciation to the following:

Dr. A. E. Carleton, my major professor, for his guidance and assistance throughout my graduate work.

Mr. J. R. Stroh, Manager of the Bridger Plant Materials Center, for his assistance and suggestions.

The Soil Conservation Service for the use of their staff and facilities. Also for making part of my assistantship available.

Dr. S. R. Chapman for his cooperation in preparing the photographs.

Dr. J. R. Sims for his assistance in preparing this manuscript.

Mr. R. F. Eslick for his assistance in analyzing the data.

Dr. N. L. Anderson for the identification of the insects.

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ABSTRACT

The mode of pollination of cicer milkvetch was studied in 1969 and 1970. The floral structure was examined and the common pollinators were identified. Estimates of cross-fertility by open-pollination and self-fertility by artificial selfing by hand and by artificial selfing without hand manipulation were made for 20 cicer milkvetch plants in 1969. Estimates of cross-fertility by open-pollination and self-fertility by artificial selfing by hand were made on 30 plants in 1970. Estimates of cross-fertility by open-pollination and estimates of self-fertility by natural selfing in isolation by bees and artificial selfing by hand were made on four clones of cicer milkvetch in 1970. Seeds obtained from the four clones were seeded in flats in the greenhouse to study the effect of inbreeding on cicer seedlings.

The floral structure was found not to eliminate self-pollination. Three species of bumble bees were found to be the common pollinators of cicer milkvetch. Apparently, honey bees are not common pollinators of cicer. Cicer flowers were more fertile during the first few weeks of the flowering period both years. The average artificial self-fertility of all cicer milkvetch plants was 13.10%. The average natural self-fertility of four cicer milkvetch clones was 58.86%. The average cross-fertility of all cicer milkvetch plants was 62.65%. Selfing had an adverse effect on the growth of cicer seedlings. It was concluded that cicer milkvetch is naturally cross-pollinated with a relatively high degree of self-fertility.

INTRODUCTION

Cicer milkvetch has been shown to have a high potential as a forage legume. It has the potential of higher hay yields than alfalfa in areas having a short growing season. With no cases of float being reported, cicer milkvetch has a potential of being important as a pasture forage.

The mode of pollination of a species is very important in determining the type of breeding program that is used for improving existing varieties or developing new varieties of that species. There are a number of problems that can occur if a breeding program is developed without the knowledge of the mode of pollination. If the plant breeder believes the crop to be exclusively cross-pollinated and sets up a number of two clone crosses, a high degree of self-fertility can alter his whole study. This is especially true in a crop that has indeterminate flowering as cicer milkvetch does. One clone may have 70% of its flowers open while the other clone may have only 40% of its flowers open at one particular time during the season. If the crop is highly self-fertile, this could result in a high percentage of selfed seeds when the breeder actually believes these seeds to have been developed from cross-pollination. On the other hand, if the breeder knows the crop is predominantly self-pollinated, he does not have to worry about foreign pollen from the neighboring plant. Knowledge of the mode of reproduction of a crop also indicates whether or not pollinators are needed for seed set.

There were no studies on the mode of pollination of cicer milk-vetch (<u>Astragalus</u> <u>cicer</u> L.) reported in the literature. Thus, this study was set up to determine the mode of pollination of this species. The specific objectives were (1) examination of the flower structure, (2) to determine self-fertility by natural selfing in isolation and artificial selfing by hand, (3) to determine cross-fertility by open-pollination, and (4) to determine the effect of inbreeding on cicer seedlings.

LITERATURE REVIEW

Cicer milkvetch (*Astragalus cicer* L.) was introduced into the United States by the Plant Introduction Center from Sweden in 1926 (12). It has been evaluated primarily as a ground cover and secondarily as a forage legume at the western Soil Conservation Service Plant Materials Centers. However, recent studies at the Plant Materials Center, Bridger, Montana, have demonstrated the high potential of cicer milkvetch as a forage legume for the northern Rocky Mountain region and adjacent plains area (6, 10, 12, 26).

The flowers of cicer milkvetch are white to pale yellow in color and are borne in racemes (6, 28). Cicer has good palatability and livestock and big game animals consume it readily (23).

The acceptance of cicer has been limited because of poor stand establishment. A very high percentage of hard seed in cicer milkvetch results in poor germination and thus, poor stand establishment. Recent work at the Bridger Plant Materials Center and Montana State University showed that mechanical scarification increases the germination and field emergence (6). These workers have developed a commercial scarification procedure for cicer milkvetch.

Fertility and Inbreeding

The first step in determining whether a plant is self- or cross-pollinated is an examination of the floral structure. The next step is the isolation of single plants to observe whether or not seeds are produced. The failure of a plant to set seed in isolation is an almost certain indication that the species is cross-pollinated. However, the reverse is not always true because the plant may be cross-pollinated but still be highly self-fruitful. If inbreeding can be carried out without adverse affects, the species is probably self-pollinated (1).

A typical legume flower consists of a standard petal, two wing petals, and two keel petals. The latter are usually partially united and enclose the stamens and stigma. Nine of the ten stamens have their filaments joined to form a ring around the style. The tenth stamen remains free from the others. The five petals are partially united to form a corolla tube (24).

Korean lespedeza, a self-pollinated legume, has two types of flowers on the same plant; chasmogamous and cleistogamous. In the cleistogamous flowers, the pollen germinated before dehiscence. The anthers are close to the stigma, and, therefore, some of the pollen tubes penetrate the anther wall and enter the stigma (24).

In sainfoin, the style protrudes past the anthers. It has been observed that appendages surround the stigma; more work is needed to determine the significance of these appendages. The above structures do not ensure cross-pollination because a bee tends to work all the open flowers on one plant before going to the next plant. This could result in a self-pollinated plant (11).

In red clover, the stigma protrudes slightly above the anthers at the time of flowering. The keel petals form a receptacle, enclosing the staminal tube with a small opening at the tip. The weight of an insect presses the keel down exposing the anthers and stigma. Pollen is dusted onto the insect while foreign pollen carried by the insect is rubbed onto the stigma (24).

Pollen of birdsfoot trefoil is dispersed by a piston apparatus. The keel petals form a conical cavity above the anthers with a small hole at the apex of the cone. The weight of an insect depresses the keel forcing the stamens up into the cone. The pollen is forced out the opening in ribbons covering the underside of the insect with pollen. Further depression of the keel causes the stigma to protrude where it can be covered with foreign pollen carried by the insect (24).

The pollen of alfalfa is dispersed by an explosive action known as tripping. When an insect lands on the keel, his body weight presses the keel down causing the anthers and stigma to snap upward and free of the keel. The staminal column strikes the insect covering his underside with pollen. At the same time, foreign pollen carried by the insect is rubbed on the stigma (24).

In the only reference found on cicer milkvetch fertility, Townsend (32) observed many open-pollinated heads contained 200-300 seeds each. When the isolated flowers were rolled between the thumb and fingers, most plants set some selfed seed, although the amount was generally low. Several plants were fairly self-compatible because 50-62 selfed seeds per head were obtained.

Pharis (21) found that in alfalfa an average of 31.6% of selfed flowers formed pods whereas 74.8% of the flowers that were crossed formed pods. The pods obtained from selfing contained 1.7 seeds per pod and the pods from crossing contained 3 seeds per pod.

In one study, the range of self-fertility in alfalfa was 0.24-4.84 seeds per flower tripped with a mean of 1.72 (17). In another, the mean range in self-fertility extended from 0.12-1.84 with a mean of 0.75 seeds per flower tripped (35). It was noted by Tysdal (34) that self-fertilization results in much less seed production per flower than cross-fertilization. Piper and co-workers (22) found that when alfalfa flowers were tripped they set selfed pods freely, although cross-fertilization resulted in more pods. They also found that cross-fertilization resulted in more seeds per pod than did self-fertilization. An unanticipated high degree of selfing in several two-clone crosses of alfalfa was reported by Hanson and co-workers (14). Additional research must be conducted to determine the extent of natural crossing in alfalfa. In general, alfalfa flowers must be tripped to produce seed, and that ordinarily there is not sufficient automatic tripping to produce satisfactory seed crops (33). Other researchers (2, 7, 15, 20) have shown that untripped alfalfa flowers will form some pods, but the number of pods formed in this way is quite low in comparison to the number of pods formed when the flowers have been tripped. Armstrong and White (2) showed that pollen germination occurred in 84% of the tripped alfalfa flowers and in less than 1% of the untripped flowers. In 1936, Brink and Cooper (5) found that the environment had a great influence on whether or not alfalfa flowers have to be tripped to form pods. In the greenhouse, the flowers must be tripped; whereas, if the environment is right, the flowers set pods freely without tripping.

Temperature and other environmental factors have been shown to greatly influence the magnitude of self-fertility in alfalfa (27, 35). Self-sterility in alfalfa was approximately twice as high in the greenhouse as in the field (35). Self-fertility indices in alfalfa are highest during the first weeks of flowering. In latter weeks, there is considerable variation among plants (19). Kehr and LaBerge (16) reported that the degree of self- and cross-pollination in alfalfa may differ, depending upon materials, planting method, and environment.

In alsike clover, self-compatibility in the greenhouse was significantly correlated with self-compatibility in the field (30). Self-seed set in tetraploid alsike clover is reported not to be limited by the metabolities needed for seed production, but seed set is primarily dependent on the genetic mechanisms controlling self-compatibility (31).

Hanna (13) reported that in sainfoin open-pollinated seed set ranged from 3.7-78.3% with a mean of 35.6%. Self-fertilization ranged from 0-21.4% with a mean of 4.8%. He also stated that this measurement of self-fertility by tripping did not agree with what he observed on plants in isolation. Haaland (11) reported 48% seed set with open-pollination in sainfoin on S_0 plants. When the same plants were crossed by honey bees in two clone combinations, 25% seed set was obtained. When these isolated S_0 clones were selfed with honey bees, 29% seed set was obtained. However, when sainfoin was selfed by hand tripping, only 6% seed set was obtained on the same S_0 plants. He concluded that self- and cross-fertility, as estimated by percent seed set, where flowers were selfed and crossed by honey bees were not significantly different. He also concluded that selfing by bees is more effective than selfing by hand tripping.

In birdsfoot trefoil, artificial pollination data cannot be interpreted in terms of the natural pollination behavior of the plant. A significant difference between self and cross seed set with artificial pollination would suggest that under natural conditions an advantage exists in favor of cross-pollination (4).

In general, there is a lower number of ovules in alfalfa entered by a pollen tube after selfing than after crossing. Cooper and Brink (8) reported that after self-pollination under greenhouse conditions, only 14.6% of the ovules of alfalfa became fertilized; this is in comparison to 66.2% after crossing. Individual plants vary significantly in the proportion of ovules that become fertilized after selfing, and most of these ovules are in the apical half of the ovary. They reported that pollen tube growth was more rapid after crossing than after selfing.

Sayers and Murphy (25) found that pollen tube growth after self-pollination closely paralled pollen tube growth after cross-pollination. These groups of researchers (8, 25) have also shown that a larger percentage of the fertile ovules either abort or collapse after self-pollination then after cross-pollination.

In alfalfa, percent pods set was significantly correlated with both number of seeds per pod (r = +0.88) and number of seeds set per 100 flowers selfed (r = +0.96) (9). The same type of association was found in Montana (18). Pods per floret tripped was significantly correlated to seeds per floret tripped (r = +0.88) and to seeds per pod (r = +0.67). It was concluded that this indicates that all of these estimates of determining self-fertility were equally effective.

In alfalfa, selfing greatly reduces self-fertility and vegetative vigor. Wilsie (36) reported that after one generation of selfing, self-fertility was reduced 80-90% and vegetative vigor was reduced 20-30%. Melton and co-workers (18) concluded that inbreeding does not affect all genotypes of forage crops in the same way or to the same degree.

MATERIALS AND METHODS

Flower Morphology and Pollinators

Mature flowers were collected from cicer milkvetch plants under evaluation at the Montana State University field research center. The flowers were than taken into the lab, dissected, and photographed.

Flowers were collected from a 'Lutana' cicer milkvetch foundation seed field at the Bridger Plant Materials Center. About 50 flowers were taken into the lab, dissected, and all the floral parts measured. An average measurement was then calculated for each floral part.

Pollinating bees were collected from the foundation field of cicer. These bees were identified to species to determine the common pollinators of cicer milkvetch.

Self- and Cross-Fertility of Four Clones

This study was initiated in April 1969. Cuttings were made of four clones of cicer milkvetch that were currently under evaluation at Bozeman. These clones had been designated as 4-14-1-A, 4-14-1-B, 4-14-1-C, and 4-14-6-C and will remain so for this study.

In June of 1969, these clones were taken to the Bridger Plant Materials Center. One plant of each clone was transplanted along Bridger Creek with approximately one mile between plants to insure spacial isolation. Another plant of each clone was transplanted into the 'Lutana' cicer milkvetch breeders block at the Plant Materials

Center. Plants became established during the summer.

Three treatments (1) cross-fertility by open-pollination, (2) artificial selfing by hand, and (3) natural selfing in isolation by bees, were carried out on all clones in the summer of 1970. Estimates of cross-fertility were obtained from open-pollinated plants. Two different estimates of self-fertility were made. One was obtained from artificially tripped flowers and another from the spacially isolated plants. Fertility estimates were made during three periods of the summer. Treatment periods were started on June 29, July 13, and July 20.

The plants in the 'Lutana' breeders block were used to estimate cross-fertility and artificial self-fertility. Both of these treatments were carried out on a single plant of each clone. The decumbent growth habit of cicer milkvetch allowed half of the plant to be covered by a cage with the remaining half to be uncovered. Five racemes, with flowers unopened, were chosen at random, tagged, and left for cross-pollination by native bees on the uncaged half of each plant. Five racemes, with flowers unopened, were chosen at random, tagged, and artificially selfed by hand on the caged half of each plant. Artificial selfing by hand was accomplished by rubbing a toothpick over the anthers and stigma as each flower opened.

The plants along Bridger Creek were used for the natural selfing by bees treatment. Five racemes, with flowers unopened, were chosen at random, tagged, and left for selfing by bees on each clone.

Estimates of fertility were calculated as the number of pods formed per number of available flowers, number of seeds per pods formed, and number of seeds per available flower. All flowers were tripped in the artificial determination of self-fertility. It was assumed that under natural pollination, all treatment flowers of the plants in the breeders block and of the plants spacially isolated were, in fact, tripped.

The data were analyzed using a split plot design. Clones were used as replications, dates as main plots, and modes of pollination as subplots. Duncan's New Multiple Range Test was used for the mean separations between dates and between mode of pollination. Simple correlations between the three different methods of estimating self- and cross-fertility were estimated.

Self- and Cross-Fertility of a Larger Random Sample of Plants

The study was initiated in the summer of 1969. Five plants per date were chosen at random on June 19, July 8, July 21, and July 30 from the 300 plants in the 'Lutana' cicer milkvetch breeders block at the Bridger Plant Materials Center.

Three fertility estimates were made on these 20 plants. These estimates were (1) cross-fertility by open pollination, (2) self-fertility by artificial selfing by hand, and (3) self-fertility by isolation without hand manipulation of the flowers. The estimate of self-fertility by isolation without hand manipulation evaluated the requirement of tripping.

Three racemes, with flowers unopened, were chosen at random and tagged on each of the plants. The number of flowers on each raceme was reduced to 20. One raceme was left for open-pollination by the native bees. The other two racemes were covered with glycine bags. The flowers on one of the racemes, covered with the bag, were artificially selfed by hand. The flowers on the other raceme, covered with a bag, were not tripped. The artificial selfing by hand was accomplished by rubbing a toothpick over the anthers and stigma of each flower. Bags were left on until all flowers had dried up.

A similar study was carried out during the summer of 1970. Ten plants were chosen at random on each of the dates of June 29, July 13, and July 20 from the 300 plants in the 'Lutana' breeders block.

Two estimates of fertility were made on these 30 randomly chosen plants. These estimates were (1) cross-fertility by open-pollination, and (2) self-fertility by artificial selfing by hand.

Ten racemes, with flowers unopened, were chosen at random and tagged on each of the plants being evaluated. Five of these racemes were left for open-pollination by the native bees. The other five racemes were covered with a cage and artificially selfed by hand. Artificial selfing was accomplished in the same manner as in the previous study.

On July 13, 1970, five additional racemes, with flowers unopened, were chosen at random, tagged, and caged on five of the ten plants chosen for that date. This was also done on the four clones described in the previous section. These flowers were left for selfing without hand manipulation.

Estimates of fertility for 1970 were calculated as the number of pods formed per number of available flowers, number of seeds per number of pods formed, and number of seeds per number of available flowers. Estimates of fertility for 1969 and for the additional study on July 13, 1970 were calculated as the number of pods formed per number of available flowers. All flowers were tripped in the artificial determination of self-fertility. It was assumed that in the cross-fertility determination that all flowers were, in fact, tripped. Any flowers that formed pods in the selfing without hand manipulation treatment were assumed to be self-tripped.

The 1969 data were analyzed as a completely randomized design. The 1970 data were analyzed in the same manner with the exception that a sampling error could be estimated since five racemes were used for each treatment on each plant. When fertility was estimated by seeds per pod, one plant for each date was eliminated because no pods were formed for these plants in the artificial selfing by hand method of pollination. Simple correlations between the two different methods of estimating self- and cross-fertility in 1970 were estimated.

Effect of Inbreeding of Seedlings

The seed obtained from the natural selfing in isolation and from open-pollination in the study of four clones was used for this inbreeding study.

The seed from the three dates was combined, keeping each method of pollination for each clone separate. The seed was then hand scarified to increase the germination.

Seed was planted in 14" x 20" flats in the greenhouse on September 21, 1970. A split plot design with four replications was used with the four clones as main plots and the type of progeny as subplots. A single flat was used as a main plot. Eighty seeds were seeded in two rows for the subplot in each flat. The plants were watered with nutrient solution each day. On October 9, 1970, the OP and S_1 seedlings were thinned. An attempt was made to reduce each row to a constant number for both the S_1 and OP in each replication. The seedlings were harvested on November 9, 1970 by cutting them off just above the bottom true leaves. At this time, the number of seedlings for each subplot was recorded. The harvesting was done in this manner so that the plants would not by killed and, therefore, be used for further evaluation. Dry weight in milligrams per seedling was used as the measurement criterion. A split plot analysis of variance was used to analyze the data (Appendix Table 21). Duncan's New Multiple Range Test was used for mean separations.

RESULTS

Self- and Cross-Fertility of Four Clones

The inflorescence of cicer milkvetch is an indeterminate raceme (Plate 1). Individual flower number for a raceme ranged from 13 to 62.

The flower of cicer milkvetch is a typical legume flower (Plate 2). The calyx consists of five sepals united near the top where they separate into sharp teeth 3 mm long. The entire calyx is 8 mm long (Plate 3). The corolla consists of 5 petals: one standard or banner petal 25 mm long and 8 mm wide, the apex emarginate (Plate 4); two wing petals 17 mm long and 3 mm wide, each consisting of a claw and blade (Plate 5); two keel petals which are united and enclose the reproductive parts. When united, the keel petals are 20 mm long and 7 mm wide (Plate 6). The stamens are diadelphous (Plates 9 and 1) and there is one pistil (Plates 7 and 8). The stamens and pistil are equal in length and about 2/3 the length of the standard petal, or 16 mm long (Plate 9). This type of floral structure does not eliminate automatic self-pollination.

Observations on pollinator visitation were made at the Bridger Plant Materials Center. When a bee lands on a flower, the keel petals trip down much in the same manner as alfalfa. When the flower is tripped, there is a snapping noise. After the bee leaves, the keel petals move back up around the stamens and pistils again. The flower will not snap again even though a bee may land on it. It was noted that the bees worked several flowers on one raceme before moving to another

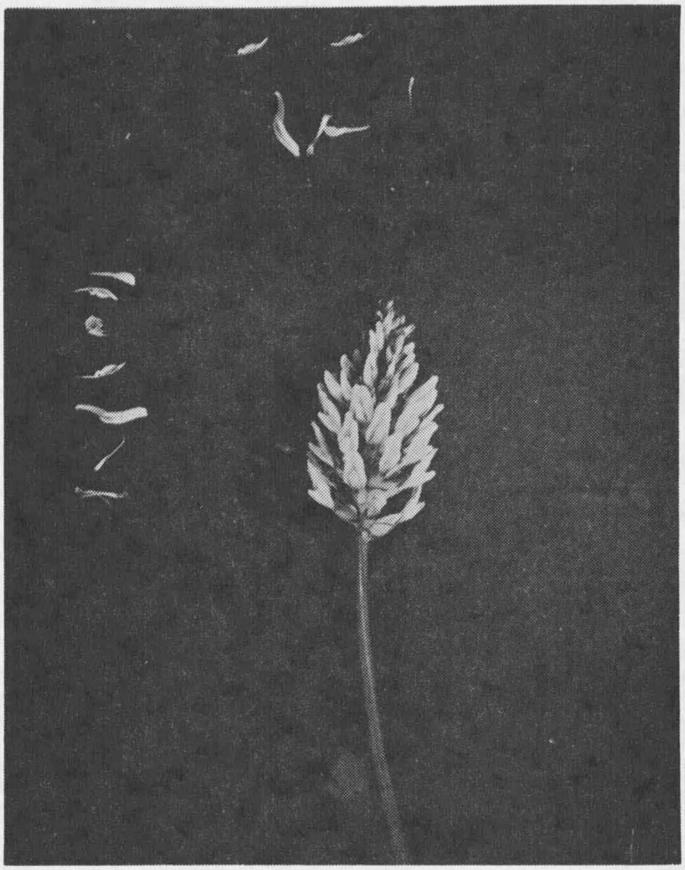

PLATE 1.--The inflorescence of cicer milkvetch

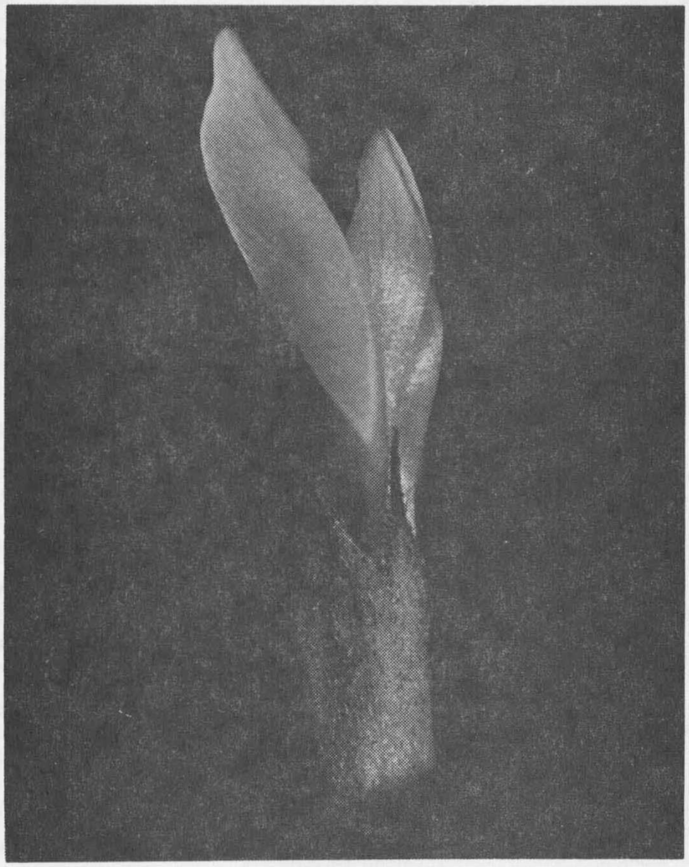

PLATE 2.--The intact flower of cicer milkvetch

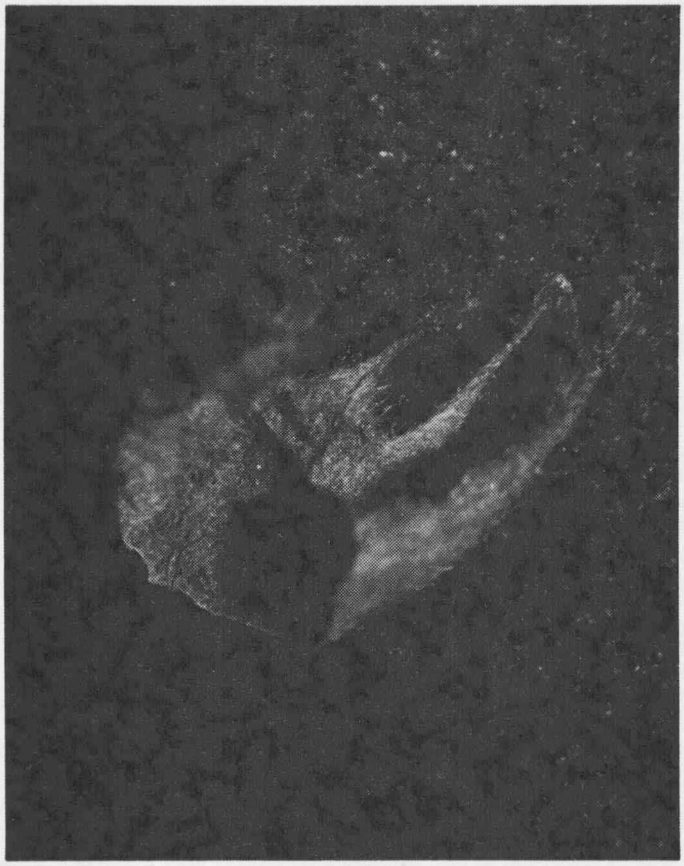

PLATE 3.--The calyx of a cicer milkvetch flower

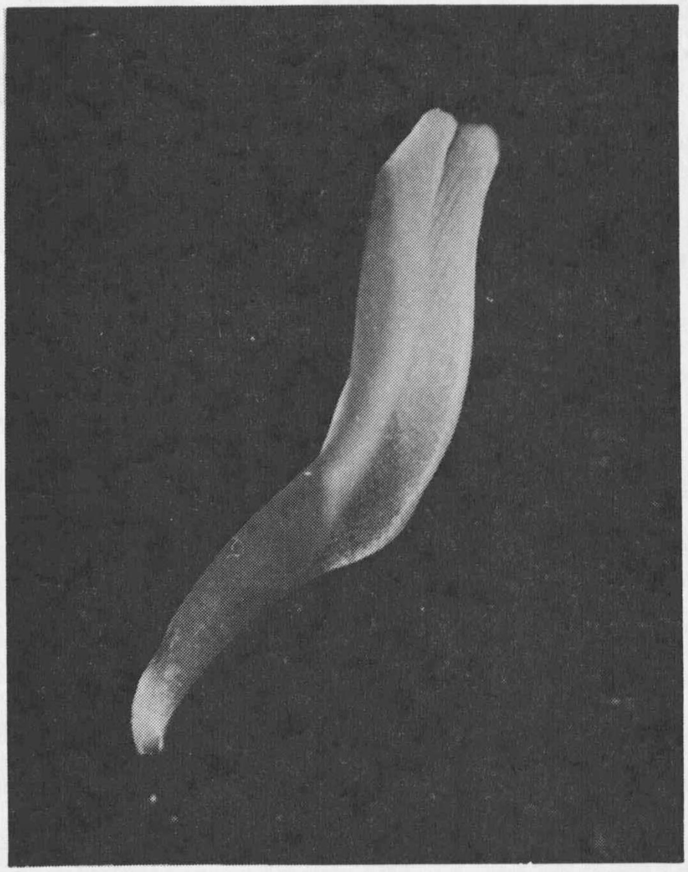

PLATE 4.--The standard petal of a cicer milkvetch flower

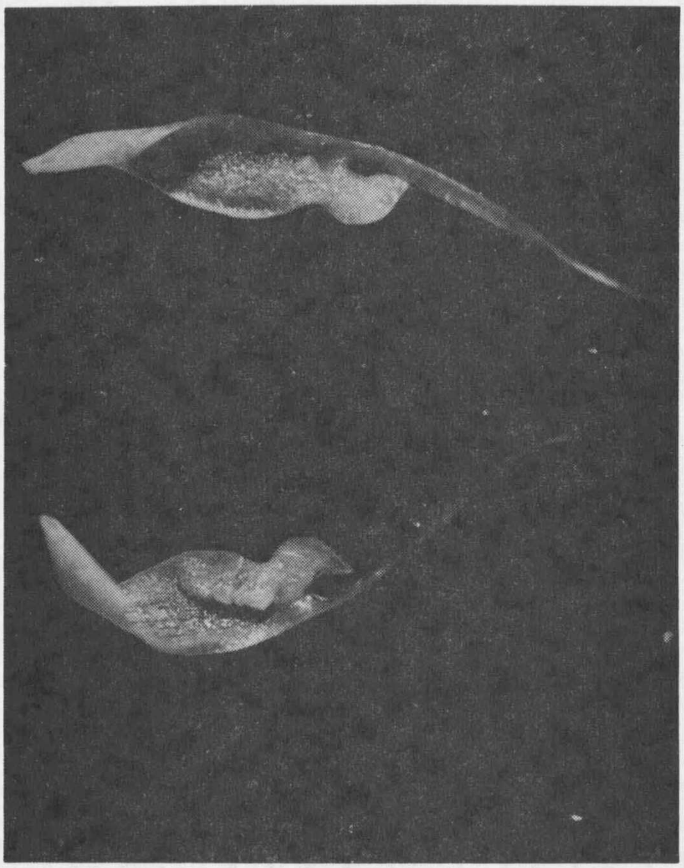

PLATE 5.--The two wing petals of a cicer milkvetch flower

PLATE 6.--The two united keel petals of a cicer milkvetch flower

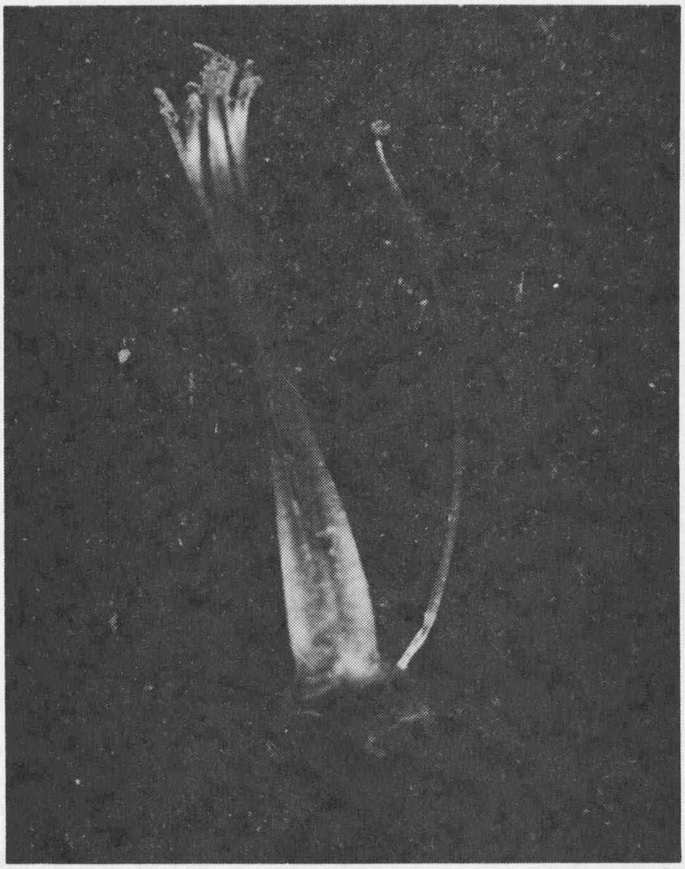

PLATE 7.--The stamens of a cicer milkvetch flower

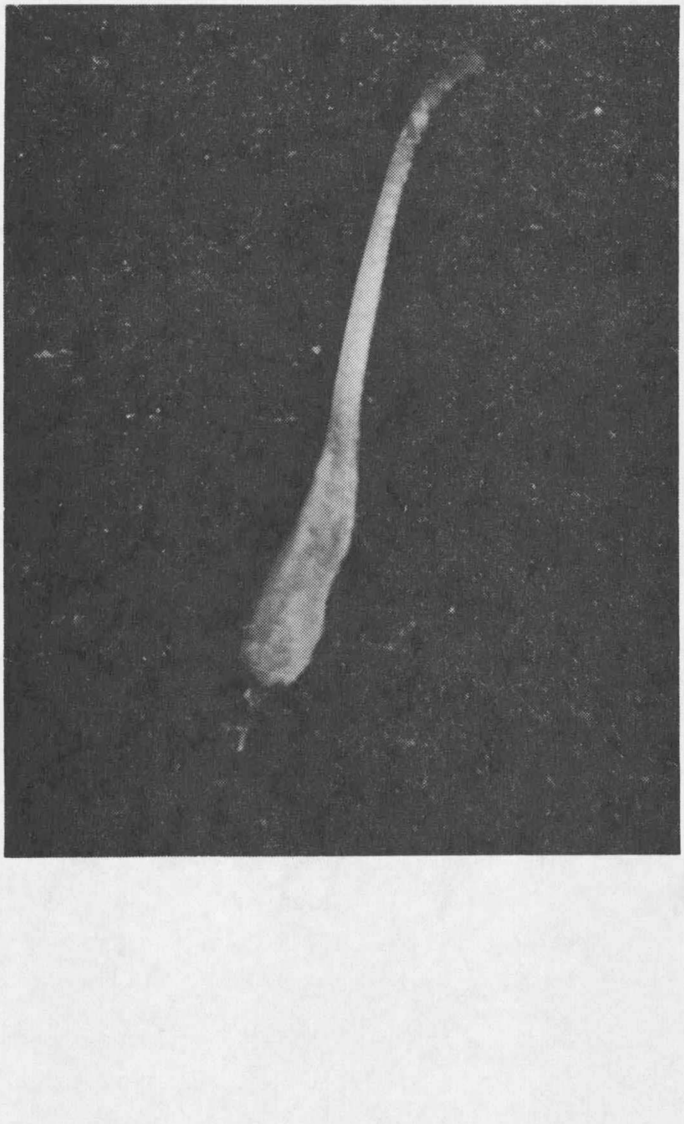

PLATE 8.--The pistil of a cicer milkvetch flower

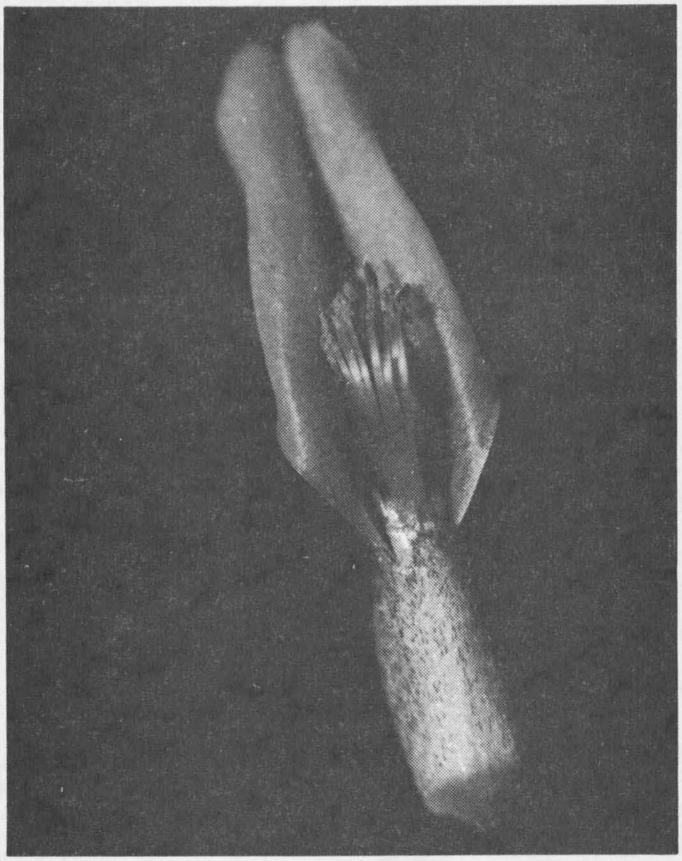

PLATE 9.--A cicer milkvetch flower with wing and keel petals removed

raceme. They worked several racemes on one plant before moving to the next plant. This pattern of visitation could result in many flowers being self-pollinated.

Three species of bumble bees were observed working cicer milkvetch. They were: Bombus appositus (cresson), Bombus centralis (cresson), and Bombus fervidus (Fabricius). Honey bees were not observed working in the foundation field of cicer milkvetch even though there were several hives less than a quarter of a mile away.

Self- and Cross-Fertility of Four Clones

Fertility estimates of the four clones were effected by dates (Table 1). In the mean separation test, fertility estimates of the first date, June 29, were significantly greater than both the second date, July 13, and the third date, July 20. There was no significant

TABLE 1.--Mean fertility for three methods of estimating fertility at three dates

Date	Pods per Available Flowers	Seeds per Pod	Seeds per Flower
June 29, 1970	51.44%a[1/]	3.78a	2.29a
July 20, 1970	42.50%b	3.08b	1.65b
July 13, 1970	35.94%b	3.25b	1.44b

[1/]Means in each column followed by the same letter are not significantly different at P = .05 for Duncan's New Multiple Range Test.

difference between the second and third dates for any method of estimating fertility.

Methods of pollination affected estimates of fertility (Table 2). No difference was found between the cross-fertility by open-pollination (61.76%) and the natural selfing in isolation by bees (58.86%) methods of pollination when the fertility estimates were based on pods per available flowers. However, when the estimates of self- and cross-fertility were based on seeds per pod or seeds per flower, differences were found. Artificial self-fertility estimates were lower than the other two fertility estimates regardless of the method used to determine fertility (Table 2).

TABLE 2.--Mean fertility estimates for three methods of pollination

Method of Pollination	Pods per Available Flowers	Seeds per Pod	Seeds per Flower
Cross-fertility by open-pollination	61.76%a[1/]	5.91a	3.64a
Natural selfing in isolation	58.86%a	2.59b	1.55b
Artificial selfing by hand	9.25%b	1.61c	0.19c

[1/]Means in each column followed by the same letter are not significantly different at P = .05 for Duncan's New Multiple Range Test.

A significant interaction was found for method of pollination and clones when the estimates of fertility were based on seeds per pod. This interaction was not found for the other two methods of estimating fertility. This interaction is shown graphically in Figure 1. All clones appear to respond the same except for a magnitude difference in response.

When estimating fertility by seeds per flower, the date by method of pollination interaction was significant. Figure 2 shows that cross-fertility by open-pollination decreased from date 1 to date 2 and then increased from date 2 to date 3. However, the natural selfing in isolation by bees and artificial selfing by hand remained relatively constant across dates.

The association between the three methods of estimating fertility within a method of pollination are presented as correlation coefficients in Table 3. Pods per available flowers and seeds per flower had moderate to high correlation values for the three methods of pollination. Seeds per pod and seeds per flower had a similar pattern of relationship, whereas the relationship of pods per floret and seeds per pod varied greatly depending upon method of pollination.

The association between natural selfing in isolation by bees and artificial selfing by hand was found for each of the methods of estimating fertility. Pods per available flowers were highly correlated ($r = .63$), whereas seeds per pod were not ($r = .15$). Seeds per flower

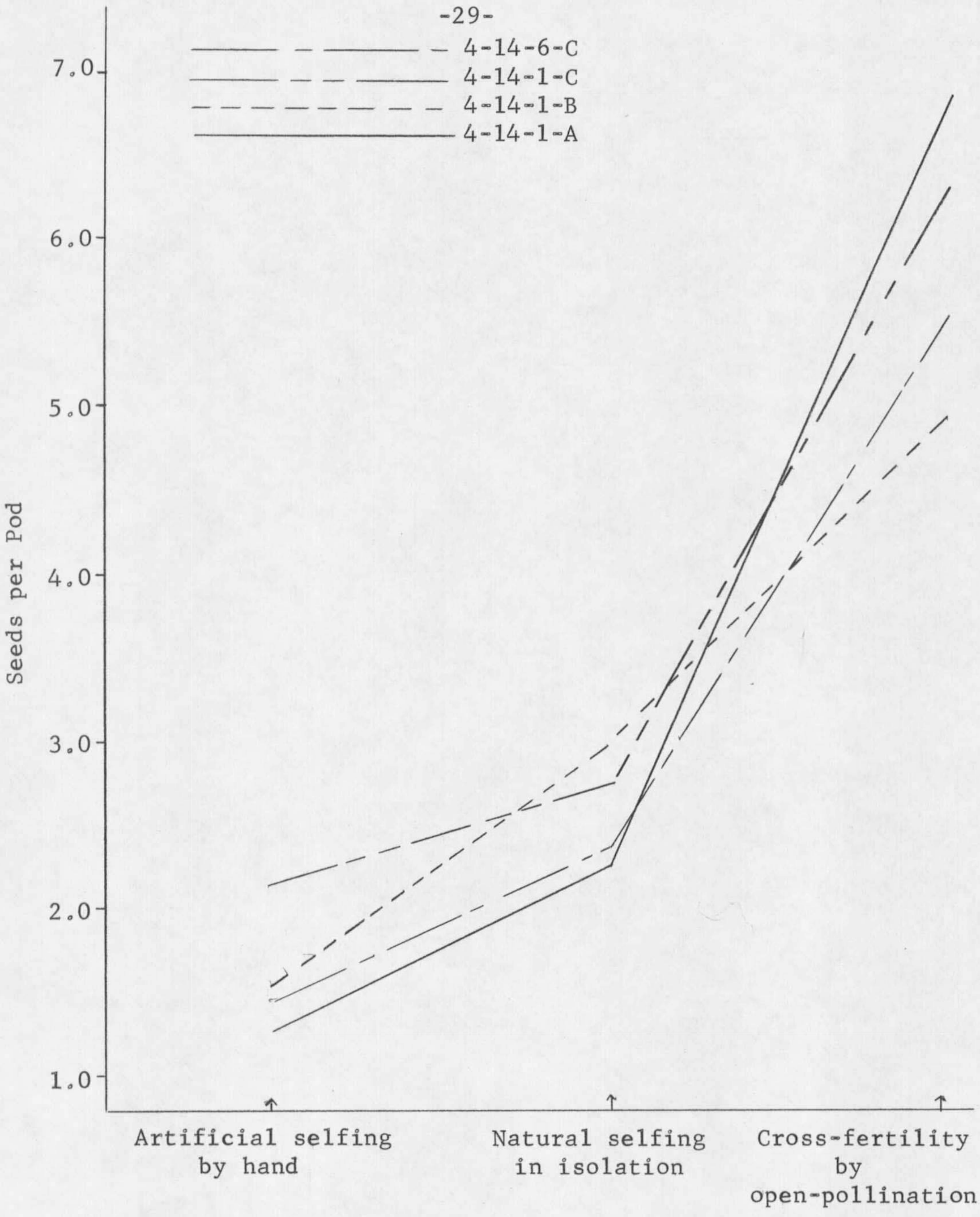

FIGURE 1.--The effect of methods of pollination and clones on fertility estimated by seeds per pod

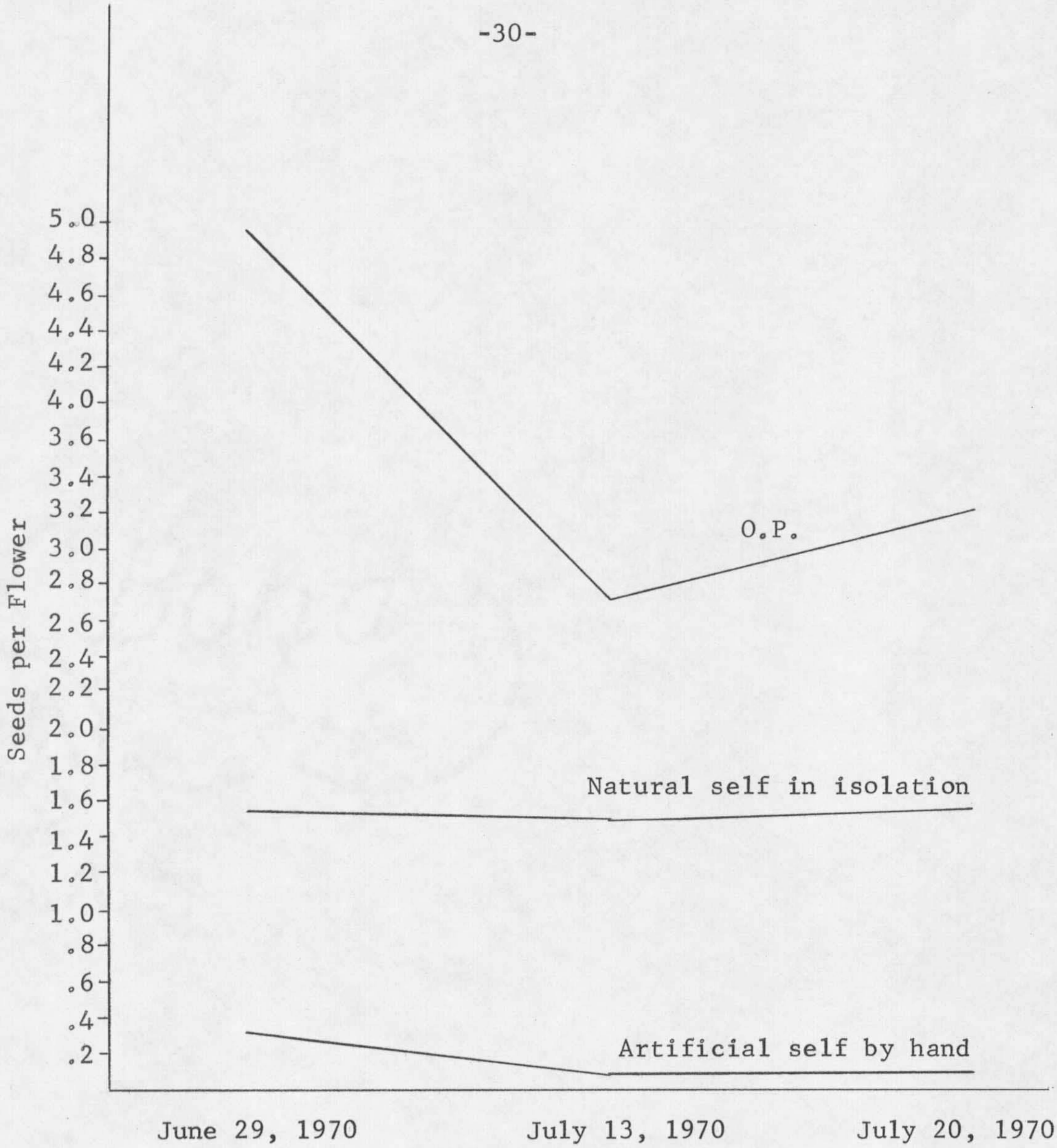

FIGURE 2.--The effect of dates and methods of pollination on fertility when estimated as seeds per flower for four clones of cicer milkvetch

were negatively correlated ($r = -.06$) for the two methods of self-pollination.

TABLE 3.--Correlation coefficients of three methods of estimating fertility for the different methods of pollination

Method of Estimating Fertility	Artificial Selfing	Natural Selfing	Cross-Fertility by Open-Pollination
Pods per available flowers and seeds per flower	+0.41	+0.41	+0.81**
Pods per available flowers and seeds per pod	+0.60*	-0.23	-0.07
Seeds per pod and seeds per flower	+0.72**	+0.49	+0.58*

* Denotes significance at P = .05.

**Denotes significance at P = .01.

Cross- and Self-Fertility of a Larger Sample of Plants

Fertility estimates of pods per available flowers of 20 cicer milkvetch plants in 1969 were affected by dates (Table 4). In the mean separation test, fertility estimates of the first three dates, June 19, July 8, and July 21, were not significantly different. However, the fertility estimates for June 19 and July 21 were significantly greater than the fertility estimate of the fourth date, July 30. There was no significant difference in the fertility estimates of July 8 and July 30.

TABLE 4.--Effect of four dates on a fertility estimate of cicer in 1969

Date	Pods per Available Flowers
July 21, 1969	36.33a[1/]
July 19, 1969	33.00a
July 8, 1969	27.33ab
July 30, 1969	21.00b

[1/]Means followed by the same letter are not significantly different at P = .05 for Duncan's New Multiple Range Test.

Methods of pollination in 1969 affected estimates of fertility (Table 5). A significant difference was found between the cross-fertility by open-pollination (65.25%) and the other two methods of pollination, artificial selfing by hand (19.75%) and artificial selfing without hand manipulation (3.25%). The artificial selfing without hand manipulation estimate of fertility (3.25%) was lower than the artificial selfing by hand estimate (19.75%) (Table 5). The interaction of dates and methods of pollination was nonsignificant.

The cross-fertility by open-pollination estimate of fertility (50.19%) of nine cicer plants was significantly greater than the artificial self-fertility estimates based on either the tripped or not tripped methods of pollination in 1970. There was no difference between artificial selfing by hand (4.20%) and artificial selfing without hand manipulation (0.52%) (Table 6).

TABLE 5.--Effect of three pollination methods on three fertility estimates of cicer in 1969

Method of Pollination	Pods per Available Flowers
Cross-fertility by open-pollination	65.25a[1/]
Artificial selfing by hand	19.75b
Artificial selfing without hand manipulation	3.25c

[1/]Means followed by the same letter are not significantly different at P = .01 for Duncan's New Multiple Range Test.

TABLE 6.--Mean fertility estimates of three methods of pollination on nine cicer milkvetch plants in 1970

Method of Pollination	Pods per Available Flowers
Cross-fertility by open-pollination	50.19a[1/]
Artificial selfing by hand	4.20b
Artificial selfing without hand manipulation	.52b

[1/]Means followed by the same letter are not significantly different at P = .01 for Duncan's New Multiple Range Test.

Dates affected fertility estimates of cicer milkvetch in 1970 (Table 7). When pods per florets were the fertility estimates, June 29 was significantly greater than July 13 and July 20. No difference was found between July 13 and July 20. When seeds per pod were used to estimate fertility, there was no difference between June 29 and July 13 and between July 13 and July 20 but June 29 and July 20 were different. However, when seeds per flower were used to estimate fertility, a difference was found between June 29, July 13, and July 20 (Table 7).

TABLE 7.--Effect of dates on three estimates of fertility of cicer in 1970

Date	Pods per Available Flowers	Seeds per Pod	Seeds per Flower
June 29, 1970	46.19a[1/]	3.25a	19.23a
July 13, 1970	29.77b	3.01ab	12.69b
July 20, 1970	30.93b	2.56b	11.05c

[1/]Means in each column followed by the same letter are not significantly different at P = .05 for Duncan's New Multiple Range Test.

The method of pollination also affected fertility estimates in 1970 (Table 8). Cross-fertility by open-pollination was greater than artificial selfing by hand, regardless of the way fertility was estimated (Table 8).

A significant interaction was found for methods of pollination and dates when the fertility estimates were pods per available flowers and seeds per flower. This interaction was not found for the seeds per pod estimate of fertility. Figures 3 and 4 show that in both fertility estimates, cross-fertility by open-pollination decreased from June 29 to July 13 and from July 13 to July 20. However, artificial selfing by

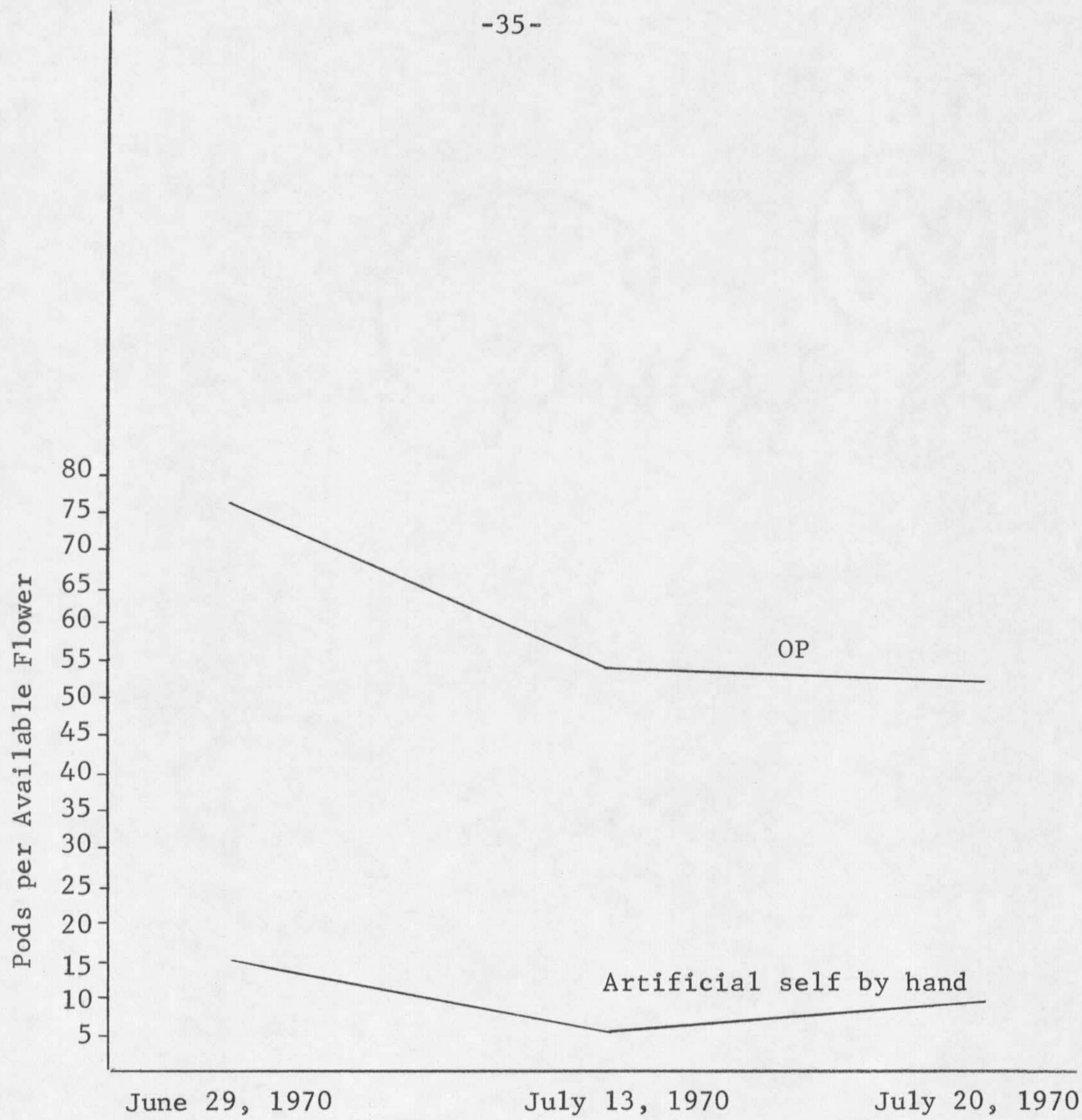

FIGURE 3.--The effect of methods of pollination and dates when fertility was estimated by pods per available flowers for 30 cicer milkvetch plants.

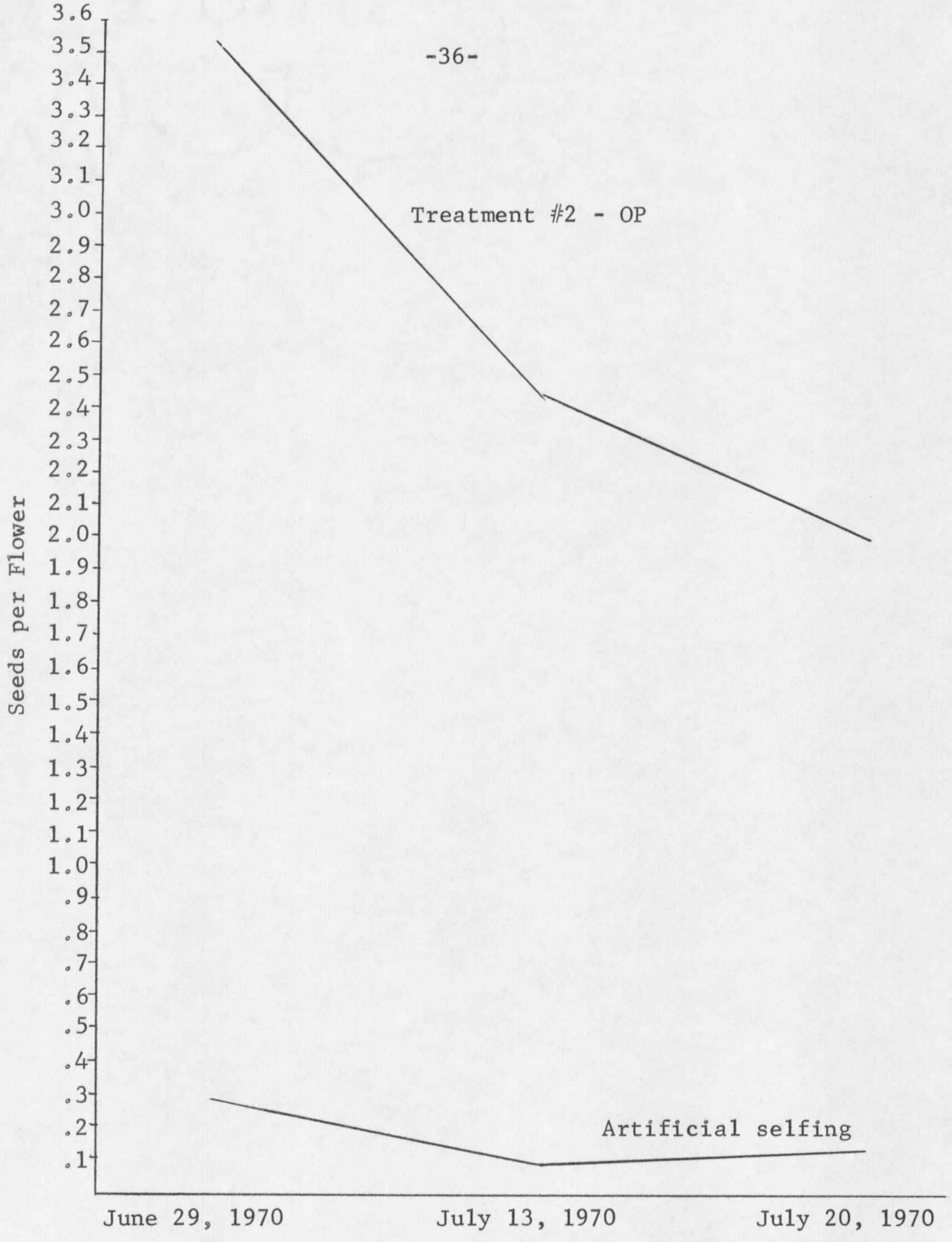

FIGURE 4.--The effect of methods of pollination and dates when fertility was estimated by seeds per flower for 30 cicer milkvetch plants

hand decreased from June 29 to July 13 and then increased from July 13 to July 20.

TABLE 8.--Effect of two pollination methods on three fertility estimates of cicer in 1970

Method of Pollination	Pods per Flower	Seeds per Pod	Seeds per Flower
Cross-fertility by open-pollination	60.95a[1/]	4.33a	2.69a
Artificial selfing by hand	10.30b	1.55b	.18b

[1/]Means in each column followed by the same letter are not significantly different at P = .01 for Duncan's New Multiple Range Test.

The association between the three fertility estimates within a method of pollination are presented as correlation coefficients in Table 9. All estimates of fertility were significantly correlated in the artificial selfing by hand and cross-fertility by open-pollination methods of pollination.

Effect of Inbreeding of Seedlings

Selfing cicer milkvetch for one generation affected the growth of cicer seedlings (Table 10). A highly significant difference was found between seedling weight of the OP (53.28) and the S_1 (36.16) (Table 10). There were differences in inbreeding of the four clones. The interaction of clones and type of progeny was not significant.

TABLE 9.--Correlation coefficients of three estimates of fertility for the different methods of pollination of cicer in 1970

Method of Estimating Fertility	Artificial Selfing	Cross-Fertility by Open-Pollination
Pods per available flowers and seeds per flower	+0.92**	+0.87**
Pods per available flowers and seeds per pod	+0.42*	+0.41*
Seeds per pod and seeds per flower	+0.53*	+0.80**

* Denotes significance at P = .05

**Denotes significance at P = .01

TABLE 10.--Influence of inbreeding on cicer milkvetch seedlings

Type of Progeny	Dry Weight per Seedling in mg.
Open-pollinated	53.28a1/
Selfed	36.16b

1/Means followed by the same letter are not significantly different at P = .01 for Duncan's New Multiple Range Test.

When the reduction in growth per seedling (inbreeding depression) was calculated, the S_1 seedlings yielded 32% less than the OP seedlings. The average dry weight of the OP and S_1 seedlings and the persent inbreeding depression of each clone is presented in Table 11.

TABLE 11.--Mean dry weight per seedling of OP and S_1 seedlings, and the percent inbreeding depression of four clones of cicer

Clone	OP	S_1	Mean Differences	Inbreeding Depression
	mg.	mg.		%
4-14-1-A	46.93	29.71	17.22	37
4-14-1-B	55.61	35.83	19.78	36
4-14-1-C	59.20	37.07	22.13	37
4-14-6-C	51.38	42.04	9.34	18
Average	53.29	36.16		32

DISCUSSION

Flower Morphology and Pollinators

The structure of cicer milkvetch flowers was found to be very similar to the structure of alfalfa flowers as described by Poehlman (24). The tripping of a cicer flower is similar to that of the alfalfa flower with the exception that after tripping the keel petal of cicer will again enclose the reproductive parts, whereas the keel petal of alfalfa will not. It is not known at this time if there is a membrane covering the pistil of cicer as there is in alfalfa. Because of this fact and the fact that the stamens and pistil are equal in length, the possibility of automatic self-pollination is not eliminated.

Three species of bumble bees were found to be the primary pollinators of cicer milkvetch. Even though there were several hives of honey bees within a quarter mile of the foundation field, no honey bees were found working cicer milkvetch flowers. This would indicate that, in the future, the pollination of cicer milkvetch is going to depend on the native bumble bee population. In small acreages of cicer, there has been no shortages of bumble bees noticed. At the Bridger Plant Materials Center, it has been observed that as the number of acres of cicer increases so does the native bumble bee population (29). Therefore, even though the pollination of cicer depends on this population of bees, there is no immediate problem.

Cross- and Self-Fertility of Four Clones of Cicer Milkvetch

There are two ways of studying the mode of pollination of a species. One way is to use a detailed study on a small number of plants. The other way is to study a larger number of plants with less evaluation of each plant. However, data from both types of studies should compliment each other.

Self-fertility of cicer milkvetch was highest during the first few weeks of flowering. Miller and Schonhorst (19) showed the same results for alfalfa.

When fertility estimates were pods per available flowers, the results show that cross-fertility by open-pollination (61.76%) and natural selfing in isolation by bees (58.86%) were not significantly different. These results showed an unanticipated high degree of self-fertility. However, when seeds per pod and seeds per flower were used to estimate fertility, there was a difference between cross-fertility by open-pollination and natural selfing in isolation by bees. Pods may be formed with seed number ranging from only one to nine or ten. This could lead to large discrepancy between the estimates of fertility based on pods per available flowers and estimates based on actual seed numbers. There are several possible explanations for the cross-fertility by open-pollination being greater than natural selfing in isolation by bees in the seeds per pod and seeds per flower estimates of fertility. Natural selfing in isolation was done on plants growing with less moisture than those plants involved in the open-pollinated studies. The lack of moisture may have resulted in fewer ovules developing per ovary. Another explanation is that since the natural selfing was, in fact, self-pollination, fewer pollen grains could have germinated. The pollen grains that did germinate may have had a shorter pollen tube growth than those for cross-fertility by open-pollination and, therefore, did not reach the ovules in the lower part of the ovary. There may have also been a larger number of the ovules fertilized that aborted in the natural selfing than in the cross-fertility by open-pollination. All of these explanations have been found to be reasons why self-pollination results in fewer seeds per pod than open-pollination in alfalfa (8, 25).

Although natural selfing estimates of self-fertility were high in this study, they would not be expected to be as high if other cicer plants were close to the ones being evaluated. The reason being that self pollen is not as effective in the presence of cross pollen. Marker genes must be used now to determine the amount of natural selfing when cross pollen is present.

Natural selfing in isolation by bees was greater than artificial selfing by hand regardless of how fertility was estimated. This indicates that bees do a more effective job of self-pollination than an individual can do by tripping the flower.

The significant interaction for methods of pollination and clones probably resulted because of a difference in magnitude of response of seeds per pod for each clone for the various methods of pollination. This is possible because each clone has a different level of self- and cross-fertility from every other clone.

The interaction for dates and methods of pollination was significant when fertility was estimated by seeds per flower. The artificial selfing by hand and crossing by open-pollination appeared to respond similarly across dates. Each of these treatments had about the same percentage change when fertility was estimated on the different dates. In contrast, the values for natural selfing in isolation did not vary across dates. Since open-pollination and selfing by hand was accomplished on the same plants growing in the breeders field at the Bridger Plant Materials Center, one might expect any environmental conditions which influenced fertility of the plant to influence both these types of pollination equally; natural selfing in isolation was done on clones of the same plants growing in a completely different environment. Thus, an interaction of this type could occur because of differences in environment.

In general, either pods per available flowers, seeds per flower, or seeds per pod have given comparable estimates of fertility in most legume crops. In cicer, these estimates were positively and significantly correlated in some instances and not associated in others. A possible explanation can be drawn from the following linear models of the contributions to different fertility estimates of various factors.