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The objectives of this study were (a) to measure the amount of cross-pollination caused by three bee species at 10, 20 and 40 rods distance from a foreign pollen source, (b) to measure the effects of bee species, planting pattern and season on broad sense heritability estimates for seed yield in alfalfa, (c) to determine the incidence of selfing caused by the bees, (d) to investigate the usefulness of bees as a breeding tool in alfalfa and (e) to determine which of the three planting patterns used in this investigation is the best in effecting the maximum amount of cross-pollination.

Three bee species, the honey bee, *Apis mellifera* L.; the leaf-cutter bee, *Megachile rotundata* F. and the alkali bee, *Nomia melanderi* Ckll. were chosen for this investigation because of their importance as pollinators in alfalfa. Studies under conditions of good isolation for bee species and competitive bloom were necessary

to obtain reliable results. In the center of a three mile square isolated area, seven plots of recessive white-flowered clonally established plants were planted 10, 20, 40, 80, 160, 240 and 320 rods north of a colored-flowered population, and seven plots were planted at the same distance east of the colored-flowered population. Seven planting patterns were used in the east series. Three of these were utilized by the bees described. The number of florets tripped, pods developed and seeds developed in each pod were recorded for each raceme visited by pollen collecting bees. Seed samples from the white-flowered clones were grown in a greenhouse to ascertain whether the seed resulted from cross- or self-pollination.

A record of flower color in 1963 demonstrated that at 10, 20 and 40 rods from a contaminant source honey bees caused 15.7, 11.2 and 5.8 percent cross-pollination; leaf-cutter bees caused 13.1, 4.8 and 8.1 percent cross-pollination while endemic pollinators caused 42.9, 30.9 and 10.0 percent cross-pollination in 1962. An important fact in this regard was that endemic pollinators caused 6.52 percent cross-pollination at a distance of a mile.

Bee species, planting pattern and season caused large fluctuations in broad sense heritability estimates for seed yield in alfalfa. The ratio environmental variance to phenotypic variance gave a good indication which environmental factor caused the most fluctuation in the estimate.

When endemic pollinators were used 94.7, 88.3, 36.3 and 23.1 percent selfed seeds were recorded as occurring in pods with one, two, three and four or more seeds per pod. One seven seeded pod was recorded as possessing three selfed seeds. Data from leaf-cutter bees showed that all of the pod types had over 67 percent selfed seeds. Distance from the contaminant source affected the percentage selfed seeds. At 40 rods only the four or more seeded pods contained any crossed seeds (20 percent)

Leaf-cutter bees trip 27-50 percent more florets per raceme and cause less cross-pollination at 10, 20 and 40 rods than honey bees. Honey bees cause approximately the same amount of cross-pollination at 10 and 20 rods while the value obtained at 20 rods for the leaf-cutter bee was close to one-third of the value at 10 rods. Leaf-cutter bees also showed less preference between colored- and white-flowered alfalfa flowers.

Of several planting patterns considered, maximum cross-pollination was obtained in a plot planted with alternating rows of alfalfa.

SOME FACTORS THAT AFFECT POLLINATION AND SEED FORMATION IN ALFALFA, *MEDICAGO SATIVA* L.

by

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops in the world. Even though this plant species has been cultivated for centuries, many problems regarding its management and culture are yet to be solved. At the present time very little information has been forthcoming comparing pollen dispersal by bees and the formation of seed. Information of this type would be useful to establish the reliability of present isolation standards in alfalfa, to determine the usefulness of different bee species as pollinators and to assess the amount of self-pollination caused by bees. Studies in recent years have established the essential role of wild bees as pollinating agents of alfalfa and have pointed out the value and limitations of honey bees. However, further information is needed in the area of pollinator control, pollen dispersal and related fields if large yields of genetically pure seed are to be produced.

An assessment of the value of three species, the honey bee, *Apis mellifera* L.; the leaf-cutter bee, *Megachile rotundata* F.; and the alkali bee, *Nomia melanderi* Ckll. under good isolated conditions was undertaken. In this investigation an attempt was made to overcome the difficulties that have plagued previous studies. Such problems as how to control pollinator populations or the presence of competing bloom that attracts pollinators from the experimental site have to be resolved before reliable information can be obtained. The information obtained in this study will provide a better understanding of the topic.

The objectives of this study were: (a) to measure the amount of cross-pollination caused by three bee species at 10, 20 and 40 rods distance from a foreign pollen source, (b) to measure the effects of bee species, planting pattern and season on broad-sense heritability estimates for seed yield in alfalfa, (c) to determine the incidence of selfing caused by the bees, (d) to investigate the usefulness of bees as a breeding tool in alfalfa and (e) to determine which of the three planting patterns used in this study is the best in effecting the maximum amount of cross-pollination.

LITERATURE REVIEW

A Basis for Isolation Standards in Alfalfa

The forage legume, alfalfa, requires cross-pollination to insure adequate seed formation. Many agricultural crops which are propagated by seed frequently decline in quality and vigor because of pollen contamination from varieties growing nearby. In spite of the absence of detailed knowledge of isolation for many of our crops, seed growers have established isolation requirements to maintain varietal purity. Isolation requirements for alfalfa have in part been established on the basis of information derived from other crops. Since no relevant information is available for alfalfa a consideration of some of this information in other crops may serve to demonstrate the difficulty in establishing adequate isolation distances between alfalfa varieties.

Because of the uncertainty of pollen movement in cabbage and kale Haskell (18, p. 591) reported that distances of one to two miles between varieties are used in Great Britain to insure adequate isolation. Evidence derived by Bateman (4, p. 257-258) from radish and turnip demonstrated that pollen dispersal is similar over a range of 0 to 160 feet. It was noted that initially there was a rapid reduction in contamination with increasing distance. The amount of contamination in this case decreased from 60 percent contaminated pollen at 20 feet to 13 percent at 80 feet, but at 140 feet it was still six percent. Increasing the isolation distance from 160 feet to 580 feet resulted in no apparent decrease in contamination from a value of one percent. Working with a genetic marker in maize, Bateman (3, p. 245-246) was able to show that contamination declined to one percent at 50 feet north of a contaminant source and 40 feet in a southern direction. Use of glycerined slides to trap pollen grains around a maize plot produced evidence similar to that obtained by the genetic marker method.

In addition to the distance factor Crane and Mather (14, p. 301) were able to demonstrate that the number and pattern of radish plots influenced cross-pollination between two varieties. In an experiment in which two varieties were planted nine inches apart in square blocks with one side in common, contamination was 0.02 percent at 15 feet, while in an experiment in which a single row of plants of one variety was planted at right angles to a square plot of the other variety, contamination was 0.02 percent at 150 feet.

Factors Affecting Seed Formation in Alfalfa

Bees are the primary means by which pollen is distributed from one alfalfa plant to another and the visitation of bees causes the staminal column of the floret to snap forward thus depositing pollen on the bee, and at the same time pollen from the bee is brushed onto the stigma. In this way pollination is effected. However, bee activity is only one of the many factors that affect the amount of seed formed on a plant. Brink and Cooper (8, p. 682) reported that high temperature affected the amount of self-pollination that occurred. They indicated that under these conditions histological observations showed pollen tubes growing down the style before the floret was tripped. This observation is directly opposite to views of other workers who contend that the stigmatic membrane must be ruptured by the force of the stigma striking the standard petal of the floret before pollen can germinate (2, p. 177).

Effective pollination does not mean that seed will form since other events can come about which will prevent the seed from developing. Cooper and Brink (12, p. 453) have noted that male gametophytes are less effective in accomplishing fertilization in the plant from which they arise than in unrelated plants. The same authors reported a difference in survival of ovules containing inbred and hybrid embryos and endosperms; the former class being much more prone to collapse during development than the latter (12, p. 453; 13, p. 213). The time of ovule collapse appears to be a function of genetic interactions resulting from self-pollination. The death of the embryonic seed may then fail at any stage between fertilization and seed maturity. Brink and Cooper (9, p. 545-546) reported that collapsed ovules were apparently brought on by an abnormal growth of the somatic tissue adjacent to the embryo sac. The authors further mentioned that the critical factor for survival seemed to be the manner in which translocated food was shared between the endosperm and the inner integument.

In addition to these internal factors there are a host of external factors that affect seed formation. Armstrong and White (2, p. 175) stated that flowers must be tripped, abundant viable pollen must be shed at the erect standard stage, a proper moisture relationship for pollen germination and pollen tube growth must be maintained to effect fertilization and, after fertilization, water metabolism of the plant must be such as to prevent ovule abortion. The weather conditions are also important and various workers have pointed out that moderate air temperature, low humidity and soil moisture below optimum produced the type of vegetative growth of alfalfa plants that was conducive to storage of high organic reserves resulting in a physiological condition favorable to seed setting (17, p. 131; 21, p. 40-44).

The necessity of tripping bears further discussion since it is so important to good seed yields. Many workers have discussed the necessity of this phenomenon, and it has been shown that tripping by external agents is necessary to obtain large yields (11, p. 511; 22, p. 444-446; 39, p. 572). Tysdal (40, p. 534) emphasized this point when he reported that not more than five percent of the flowers will set seed in the absence of tripping. He also pointed out that such factors as rain, sun or wind were unimportant as tripping agents and that insects are absolutely essential as a tripping agent in alfalfa.

Bees as Pollinators in Alfalfa

The general concensus of opinion is that bees are the primary pollinators of alfalfa. Aicher (1, p. 5) and Sladen (34, p. 125) were among the first investigators to emphasize the importance of wild bees such as species of *Megachile* and *Bombus*. Linsley (25, p. 21), working in California, reported that in addition to these two species, *Melissodes* and *Nomia* species are important. He also noted a scoliid wasp (*Campsomerus plumipes*) accidentally tripping and cross-pollinating alfalfa while it was foraging for nectar. Linsley and McSwain (26, p. 356) did not consider honey bees (*Apis mellifera* L.) as important pollinators due to the fact that wild pollinators fly more rapidly than honey bees, trip more flowers per minute and visit smaller percentages of flowers on any one plant, thus likely accomplishing more cross-pollination. The relative inefficiency of honey bees as compared to wild bees does not infer that honey bees are of no value. The advantage of honey bees is the ease of management and the ease in placing large populations of bees in seed fields which will compensate somewhat for their lack of efficiency. The value of honey bees was mentioned by Vansell and Todd (41, p. 473) when they observed that in Utah only *Apis*, *Nomia* and *Megachile* species of bees were found to be of economic importance in seed production. However, the honey bee was of primary importance in the area because of their predominent numbers and because they foraged alfalfa for pollen due to a lack of any other source of pollen. Menke (29, p. 13-14), working in Washington, also noted the effectiveness of honey bees when there were large populations, but he stated that in areas where alkali bees (*Nomia melanderi* Ckll.) were abundant that honey bees did not work the same bloom. This suggested some form of interspecific competition. He also reported an interesting comparison of the efficiency of alkali and honey bees when he stated that alkali bees trip 95 percent of the florets that they visit while honey bees trip only 0.4 percent.

Biology of Honey, Leaf-Cutter and Alkali Bees

A knowledge of what constitutes an adequate population or which bee species will effect the maximum seed set is important. In order to obtain answers to these problems it is necessary to investigate the biology of the particular bee species.

Honey bees are used throughout the world wherever intensive agriculture prevails, and the species has been extensively studied. Their wide distribution is largely due to the ease of domestication and transportation of colonies from one area to another. The ease of domestication of this bee species is the result of their surprising social organization and ease of maintenance in artifical wooden domiciles. In this way population sizes can be controlled, and whenever the need arises the intact working colony can be transported to the fields of flowering plants.

Very little is known about the wild pollinators, but two of the more important ones, the leaf-cutter bee (Megachile rotundata F.) and the alkali bee (Nomia melanderi Ckll.), will be considered briefly because of their increasing importance in the alfalfa seed producing areas of the Western United States. At the present time alfalfa seed yields have increased in the Western United States because of management practices that have enabled seed producers to build up large populations of alkali bees. Stephen (35, p. 14-15; 37, p. 36-39) has shown that certain areas with a suitable soil texture could be reclaimed for alkali bee beds by maintaining a high water table, cleaning the surface area of weeds and adding sodium chloride to the soil to increase alkalinity. In another paper (36, p. 70) he has shown how to create artificial nesting sites. The method consists of digging a trench 30-36 inches deep, lining it with six mil polyethylene, pouring in two to four inches of gravel, filling the trench with soil of an aggregated clay size particle content of less than seven percent and then adding one pound of sodium chloride per square foot of surface.

Water is added to the gravel layer through pipes installed for this purpose and the bee bed is ready for occupancy.

In addition to the alkali bee there is another bee that is showing considerable promise as a pollinator. Stephen (38, p. 992-993) has been able to devise methods of building up large populations of leaf-cutter bees (Megachile rotundata F.) in a small area. Under normal conditions leaf-cutter bees nest in abandoned burrows of other insects or similar small spaces. Artificial sites such as boxes of milk straws have proved to be well accepted by these bees. If this method of handling alfalfa pollinators is successful, leaf-cutter bees will offer serious competition to the alkali bee.

Bee Foraging Behavior

With some indication of the problems of alfalfa seed production, the next point of interest is how efficient bees are as pollinators. In the first instance the research worker needs a method to associate bee activity with seed yields. Various workers have used dyes and paints of one form or another to obtain an estimate of the quantity of pollen that bees are depositing on the flowers (19, p. 572; 29, p. 173). This method would be useful except bees from another population close by can pick up the marking compound on the flower and distribute it. For this reason direct associations, to date, between seed yield and bees in the field and bee numbers correlated with seed yields. One of the factors involved in a study of this nature is what effect increasing distances from the pollinator domiciles have on seed yield. Very little information is known at the present time on what constitutes the best distance from a field of alfalfa to wild bee domiciles. Some work has been done with honey bees which has proved of value. In the first place there is considerable lack of agreement on the flight range of honey bees; no doubt due to the different areas and populations used for experimentation. Early studies by Eckert (15, p. 284) indicated that honey bees will fly up to eight and one-half miles depending on the availability of flowers. Ribbands (33, p. 226) stated that even though bees are capable of flying long distances, a distance over three-quarters of a mile was detrimental to the strength of the colony. More recently Peer (31, p. 23), using bees which possessed a recessive body color mutant to distinguish them from other honey bees in the area, was able to demonstrate that approximately 27, 56 and 16 percent of the field bee population were foraging at one, two and three miles, respectively, from the domicile site. Walstrom (42, p. 64), working with red clover, concluded that honey bee hives should be placed within 100 to 400 feet of the crop since significant decreases in seed yield were recorded for distances over 400 feet. Also, Walstrom and co-workers (43, p. 52) stated that the decrease in seed yield per acre was approximately six and four-tenths pounds per acre per 100 feet of distance from the colonies. The evidence about foraging distances is not too extensive for wild pollinators, but Bohart (5, p. 15) recorded a flight range of 300 yards for the leaf-cutter bee. Levin and co-workers (23, p. 60) suggested other reasons to account for differences in seed set as a function of distance from the domicile. The reasons cited were (a) whether or not the bees collected pollen or nectar, (b) how much other food was available close to the experimental site and (c) variations occur in the plant population that affect the distribution of bees in the field.

Even though many experiments have been set up to record bee behavior as discussed, no work has been forthcoming about the amount of seed set that definitely can be attributed to bees at varying distances from the bee domicile. The reason for this is that it is difficult to find a suitable bee-free area and a reliable plant marker.

MATERIALS AND METHODS

The data for this study were obtained from a series of 1/20 acre plots established in a semi-arid region of northeastern Oregon.

Plant Material

The scope of the study necessitated the use of a reliable marker which would establish the dissemination nature of pollen by bees. A flower color marker gene was used for this function. Alfalfa plants used in this investigation were from two sources. One was a simply inherited recessive white-flowered clone supplied by R. L. Davis, Purdue University, Lafayette, Indiana. The second source of material was Vernal alfalfa which has colored flowers. In the first instance, in excess of 5,300 vegetative propagules (stem cuttings) of the white-flowered clone were established in 2" x 2" x 3" plant bands in a greenhouse. Shortly thereafter over 2,800 seedlings of Vernal alfalfa were established in plant bands. These two groups of material were then transplanted to the experimental site in April, 1961.

Experimental Site

One of the prerequisites of this investigation was to find an area that was relatively bee free and capable of producing agricultural crops. Such a site was found adjacent to the Columbia River in Morrow County Oregon. The region, a segment of 90, 000 acres recently given to the state by the federal government, is a virgin desert area not previously used for agricultural production. Control of bloom and pollinating fauna was reasonably successful by establishing a nine square mile area for the investigation. The various plots necessary for the experiment were arranged in the center of the nine square mile area in an L-shaped pattern with arms one mile long as seen in Figure 1.

Plot Pattern

In order to acquire maximum information, different planting patterns were used both between arms and within the east arm. The north arm of the L was planted with seven plots each comprised of 504 white-flowered plants. In order to gain information about the reliability of present isolation requirements in the production of certified alfalfa seed the first four plots were planted at distances of 10, 20, 40 and 80 rods. The three additional plots were established at distances of 160, 240 and 320 rods in the event that the first four plots were inadequate for this type of information. Within a given plot, plants were planted on two-foot centers.

In the east arm of the L, the plots were spaced at distances identical to the north series, but each plot had a different planting

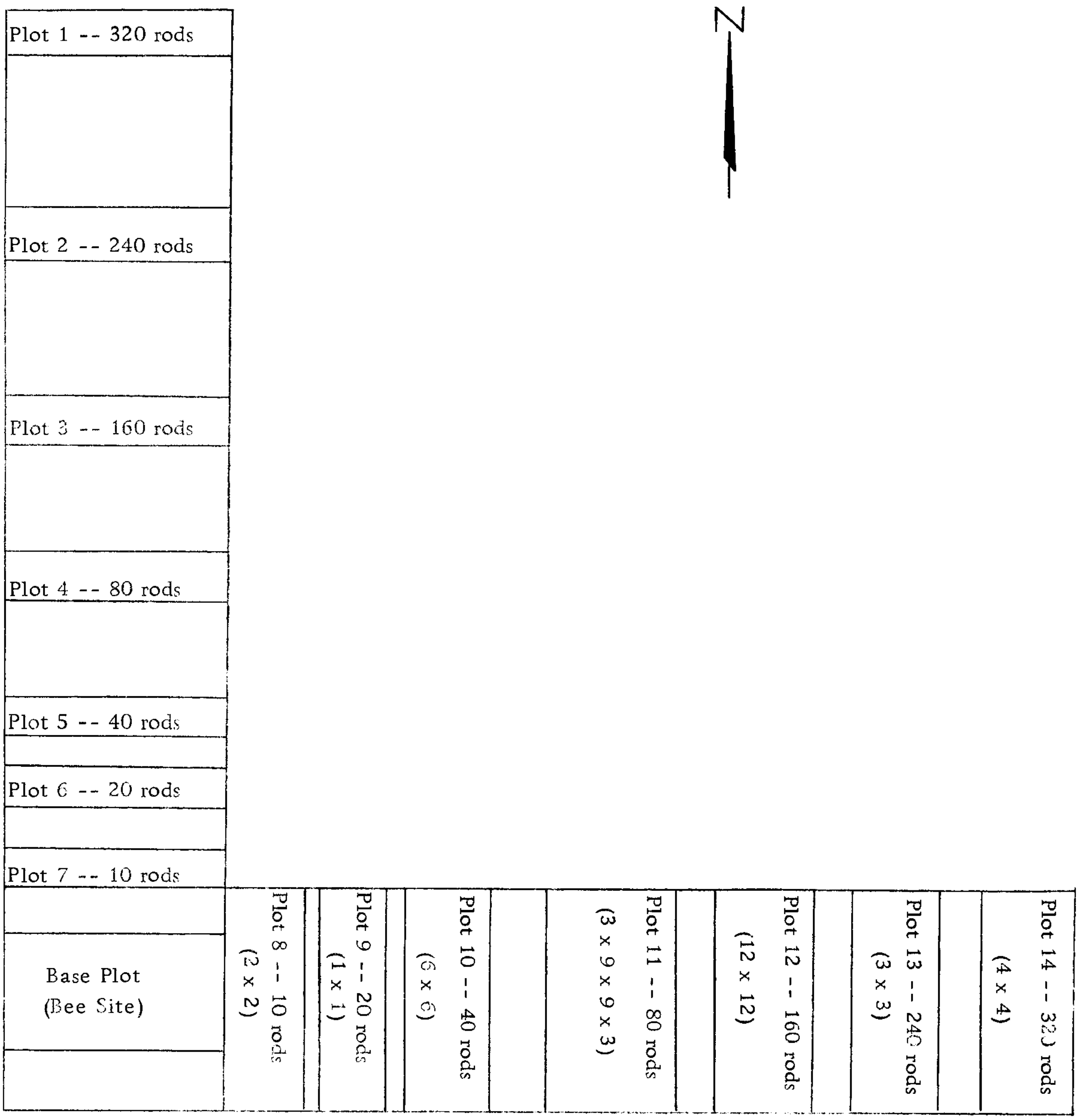

Figure 1. Alfalfa plot plan.

Plot 1-7 -- Recessive white-flowered alfalfa.

Base plot -- Vernal alfalfa (colored-flowers).

Plots 8-14 -- The planting patterns of white- and colored-flowered alfalfa (2x2, 1x1, 6x6, 3x9x9x3, 12x12, 3x3 and 4x4) are described on page 16.

planting pattern of both white- and colored-flowered plants. These different planting patterns were used to demonstrate the affect that planting pattern has on cross-pollination and hence the data derived from this series of plots is independent of the white-flowered series. In addition these plots were used to demonstrate what affects bee species, planting pattern and year have on broad-sense heritability estimates for seed yield. These plots were planted as follows:

Plot 8 -- 10 rods: two rows of white-flowered alfalfa alternating with two rows of colored-flowered alfalfa for 24 rows,

Plot 9 -- 20 rods: alternating rows of white- and colored-flowered alfalfa for 24 rows,

Plot 10 -- 40 rods: six rows of white-flowered alfalfa alternating with six rows of colored-flowered alfalfa for 24 rows,

Plot 11 -- 80 rods: three rows of white-flowered alfalfa, nine rows of colored, nine rows of white and three rows of colored-flowered alfalfa,

Plot 12 -- 160 rods: 12 rows of white-flowered alfalfa adjacent to 12 rows of colored-flowered alfalfa,

Plot 13 -- 240 rods: three rows of white-flowered alfalfa alternating with three rows of colored-flowered alfalfa for 24 rows,

Plot 14 -- 320 rods: four rows of white-flowered alfalfa alternating with four rows of colored-flowered alfalfa for 24 rows.

In order to provide a colored-flower source for the north arm, a plot consisting of 1,008 plants of Vernal alfalfa was established at the intersection of the north and east arms.

Pollinators

Three unrelated species of bees were utilized in this investigation. These were the honey bee, *Apis mellifera* L.; the leaf-cutter bee, *Megachile rotundata* F.; and the alkali bee, *Nomia melanderi* Ckll. Perhaps the most desirable features of these three bee species were the relative ease in controlling population numbers and the ease in establishing introductions.

Management Practices

Each of the 14 plots was divided into three subplots and mowed to maintain one-third of the plot in bloom for each of the three bee species. All plots were weeded regularly and irrigated with sprinklers once a week with two inches of water carried to the plots by a tank truck.

In order to control harmful forage feeding insects two ounces of actual Dieldrin was applied per acre each year. In addition, it was necessary to apply insecticides to control a few species of *Bombus* and *Bembix* during the growing season. Applications of three pints per acre of parathion were used when no introduced pollinators were at the site. Lygus and chalcid control was accomplished by applying three pints of Systox and eight pints of Toxaphene per acre when excessive numbers were observed.

Bee populations were maintained beside the large colored-flowered base plot at the intersection of the north and east arms. In the 1961 season bloom was not available until the end of July. At that time several cores of soil with adult alkali bees were introduced into the experimental site. This introduction failed; as did three successive ones. In late August the alkali bee work was abandoned and a small colony of Caucasian honey bees was introduced. A week later three colonies of Italian honey bees were placed beside the first colony thus increasing the population density beyond five colonies per acre of base plot.

In the first week of September the honey bees were removed and five successive populations of leaf-cutter bees were introduced. A population of 59 bees was unsuccessful, as were populations of 27 bees refrigerated at 45° F for 24 hours, 21 bees refrigerated for 48 hours and two groups of 25 bees retained in two cages for 24 and 48 hours respectively.

In 1962 alkali bee introductions were attempted from June 22 to July 27 and all were unsuccessful. The methods of introduction consisted of introducing larvae in short soda straws, bringing in cores of soil containing adult bees and allowing adult bees to burrow into a four by four foot box of soil when the bees were covered by a canvas. On the first of August a nucleus colony of Italian honey bees composed of one and a half frames of brood and one and a half frames of honey was introduced. Population numbers of honey bees could be adjusted in this way. Two weeks later another nucleus colony composed of Caucasian honey bees was placed beside the first. Finally, a third small colony of Cordovan honey bees (a brown recessive mutant strain) was introduced, increasing the honey bee population to five colonies per acre. After removal of the honey bees in the first week of September, 173 leaf-cutter bees were introduced with no success. A later introduction of 570 bees was successful and a working population of 181 bees was maintained for several days at which time the population was increased to 227 bees to assess the effect of increasing the population size.

In the 1963 season the alkali bee introductions again were a failure although for a few days a population of 145 bees seemed to be established in a 4 x 4 x 3 foot box of soil heated to maintain the temperature at 85° F. However, the population declined very rapidly so that data were not as complete as desired. Once again three populations of honey bees were introduced but only a population size in excess of two colonies per acre was useful to obtain a desirable number of pollen collectors forty rods from the base plot. In the third week of August, 954 leaf-cutter bees were introduced, but the introduction was not a success due to a toxic insecticide residue remaining on the leaves. After several days 497 more bees were introduced and a working population of approximately 150 bees was maintained.

Sampling Methods

In order to gain an accurate estimation of the activity of each bee species, pollen collecting bees were followed in the plots and the tripped florets were recorded. All tripped florets were kept separate from the untripped florets by removing all untripped florets from the raceme. The racemes were labelled with tags and then enclosed in 2-1/2 x 4 inch muslin bags for a minimum of four days to insure that no contaminating pollen was introduced by other bees or insects. In this way the following information was recorded for each bee species: (a) number of plants foraged in each plot, (b) number of racemes bagged on each plant, (c) number of florets tripped on each raceme, (d) number of pods that formed on each raceme, (e) number of seeds that developed in each pod. Where possible 100 racemes were bagged for each bee species or population size in each of the plots that were foraged by the bees. At maturity all racemes were collected, pods counted and then the material taken to a greenhouse at Oregon State University. A 100 seed sample from each of the subplots (three subplots per plot) was then planted in 2 x 2 x 3 inch plant bands so that flower color could be recorded. A temperature of 70° F and additional illumination from fluorescent tubes to increase daylengths to 18 hours were maintained for the growing period. Weekly fertilizer applications of a complete fertilizer were essential to maintain adequate growth because of the small soil area and excessive leaching due to frequent watering.

Information relevant to the occurrence and order of selfed and crossed seeds within a pod were obtained by planting a portion of the seed in the order in which they occurred in the pod. This was facilitated by first soaking the pod to soften it and then carefully removing the seeds in order. It was found that it was advantageous to scarify and soak all seed prior to planting to insure complete germination.

Broad-Sense Heritability Estimates

In this study the plots with alternating rows of white- and colored-flowered alfalfa offered the opportunity to assess the usefulness of deriving broad-sense heritability estimates from clonal material and an open-pollinated variety of alfalfa. All too often these estimates are derived from material where there is no opportunity to observe any variability that may be inherent in the computation. Bee species, planting pattern and year were used in the investigation to determine their effect on the broad-sense heritability estimates for seed yield in alfalfa.

Clonal material and an open pollinated variety of alfalfa can be used to derive components of variance that are used in broad-sense heritability estimates of a given trait. The variance of a non-segregating population such as a vegetatively propagated clone would be environmental since there is no genetic difference between vegetative propagules (10, p. 478; 16, p. 130; 24, p. 769; 20, p. 482; 27, p. 23). To obtain a broad-sense heritability estimate, an estimate of phenotypic variance can be obtained from S_1 populations (27, p. 23; 20, p. 482) or from a genetically mixed population (16, p. 130) grown under the same environmental conditions. Subtraction of the environmental variance from the phenotypic variance leaves the genetic variance with which a broad-sense heritability estimate can then be derived as indicated by the following formula:

$$\text{broad-sense heritability} = \frac{\sigma_G^2}{\sigma_P^2}$$

The magnitude of the change in environmental variance due to bee species, planting pattern and season can be computed from the ratio of

$$\frac{\sigma_E^2}{\sigma_P^2} .$$

The genetic variance is σ_G^2, the environmental variance is σ_E^2 and the total or phenotypic variance is σ_P^2 .

RESULTS

Distance from a Contaminant Pollen Source as a Factor in Cross-Pollination

The data obtained in 1962 (Table 1) indicated that only one and a half percent cross-pollination occurred using *Apis* as the pollinator 10 rods from the contaminant source. No cross-pollination was recorded at 20 and 40 rods. In 1963, 15.7 percent cross-pollination occurred at 10 rods while 11.2 and 5.8 percent cross-pollination occurred at 20 and 40 rods respectively. *Megachile* data in Table 2 demonstrate that in 1963, 13.1, 4.8 and 8.1 percent cross-pollination occurred 10, 20 and 40 rods from the contaminant source. Data from endemic pollinators in Table 3 showed higher values of cross-pollination close to the contaminant source. In 1961, 51.6, 28.4 and 9.3 percent cross-pollination occurred at 10, 20 and 40 rods respectively. In 1962, 42.9, 30.9, 10.0, 6.0, 5.2, 9.3 and 6.5 percent cross-pollination occurred at 10, 20, 40, 80, 160, 240 and 320 rods respectively. Smaller values of 19.6, 9.7, 3.3, 6.5, 4.4, 0.0 and 0.0 were obtained at the same distances in 1963.

Table 1. Frequency of selfed and crossed seeds in alfalfa following pollination by <u>Apis</u> pollinators.

Year	Distance from the colored source*	No. of selfed seeds	No. of crossed seeds	Total seed	Percent cross-pollination
1962	10 rods N	65	1	66	1.5
	20 rods N	51	0	51	0.0
	40 rods N	9	0	9	0.0
1963	10 rods N	88	17	105	15.7
	20 rods N	87	11	98	11.2
	40 rods N	114	7	121	5.8

*See Figure 1, page 15.

Table 2. Frequency of selfed and crossed seeds in alfalfa following pollination by <u>Megachile</u> pollinators.

Year	Distance from the colored source*	No. of selfed seeds	No. of crossed seeds	Total seed	Percent cross-pollination
1963	10 rods N	86	13	99	13.1
	20 rods N	117	7	124	6.0
	40 rods N	124	11	135	8.1

*See Figure 1, page 15.

Table 3. Frequency of selfed and crossed seeds in alfalfa following pollination by endemic pollinators.

Year	Distance from the colored source*	No. of selfed seeds	No. of crossed seeds	Total seed	Percent cross-pollination
1961	10 rods N	60	64	124	51.6
	20 rods N	73	29	102	28.4
	40 rods N	165	17	182	9.3
1962	10 rods N	56	42	98	42.9
	20 rods N	56	25	81	30.9
	40 rods N	90	10	100	10.0
	80 rods N	94	6	100	6.0
	160 rods N	55	3	58	5.2
	240 rods N	68	7	75	9.3
	320 rods N	43	3	46	6.5
1963	10 rods N	78	19	97	19.6
	20 rods N	84	9	93	9.7
	40 rods N	87	3	90	3.3
	80 rods N	86	6	92	6.5
	160 rods N	86	4	90	4.4
	240 rods N	104	0	104	0.0
	320 rods N	53	0	53	0.0

*See Figure 1, page 15.

Broad-Sense Heritability Estimates for Seed Yield

Broad-sense heritability estimates were derived from a segregating and non-segregating population of alfalfa by the formula

$$H._{b.s.} = \frac{\sigma_G^2}{\sigma_P^2} .$$

The effect of bee species, planting pattern and year are considered as factors that can alter a broad-sense heritability estimate for seed yield in alfalfa.

Bee species. From Table 4 it can be observed that quite different heritability estimates are derived when each bee species and the endemic pollinators are considered. For example, in 1962 a range of values of 68.5-95.7 percent was obtained for the estimate in the plot planted to two rows of the white clone and two rows of Vernal alfalfa for a total of 24 rows. An even wider range of 31.7-93.3 percent was observed in the 1 x 1 rowed plot. Similar trends are noted in all plots in each of the two years. Averaging the results of two years demonstrates that in most instances the estimate obtained from *Apis* was much lower than that derived by the other pollinators.

Planting pattern. The influence of the second factor, planting pattern, is noteworthy from the 1962 data in Table 4. In the 2 x 2 rowed plot the heritability estimates ranged from 68.5-95.7 percent. In the next plot, planted to alternating rows of the white-flowered

Table 4. Broad-sense heritability estimates of seed yield in Vernal alfalfa using three bee species (and endemic pollinators), three planting patterns and two seasons.

Pollinator	Year	Distance in rods	Plot pattern ***	σ^2_P	σ^2_G	σ^2_E	$\frac{\sigma^2_E}{\sigma^2_P}$	$H._{b.s.}$
Apis	1962	10	2x2	1.40	1.11	0.29	0.21	79.3
Apis	1963	10	2x2	1.09	0.44	0.65	0.59	40.4
	Average						0.40	59.8
$Megachile_1$*	1962	10	2x2	1.46	1.00	0.46	0.72	68.5
$Megachile_2$**	1962	10	2x2	1.86	1.78	0.08	0.04	95.7
Megachile	1963	10	2x2	0.42	0.35	0.07	0.17	83.3
	Average						0.31	82.5
Nomia	1963	10	2x2	4.08	3.63	0.45	0.11	89.0
Endemic	1962	10	2x2	1.38	1.06	0.32	0.23	76.8
Endemic	1963	10	2x2	1.40	1.19	0.21	0.15	85.0
	Average						0.19	80.9
	Plot average						0.40	77.2
Apis	1962	20	1x1	4.20	2.26	1.94	0.47	53.8
Apis	1963	20	1x1	1.36	0.51	0.85	0.64	37.5
	Average						0.55	45.7
$Megachile_1$	1962	20	1x1	2.72	2.54	0.18	0.07	93.3
$Megachile_2$	1962	20	1x1	1.95	0.62	1.33	0.32	31.7
Megachile	1963	20	1x1	0.94	0.71	0.23	0.25	75.5
	Average						0.22	66.8
Nomia	1963	20	1x1	7.42	6.78	0.64	0.09	91.4
Endemic	1962	20	1x1	2.16	1.93	0.23	0.11	89.4
Endemic	1963	20	1x1	0.63	0.54	0.09	0.14	85.7
	Average						0.13	87.5
	Plot average						0.26	69.8
Apis	1962	40	6x6	1.58	1.14	0.44	0.28	72.2
Apis	1963	40	6x6	1.57	1.50	0.07	0.05	95.5
	Average						0.17	83.9
$Megachile_1$	1962	40	6x6	16.11	15.20	0.91	0.06	94.4
$Megachile_2$	1962	40	6x6	1.00	0.69	0.31	0.31	69.0
Megachile	1963	40	6x6	1.87	1.82	0.05	0.03	97.3
	Average						0.13	86.9
Endemic	1962	40	6x6	0.50	0.39	0.11	0.22	78.0
Endemic	1963	40	6x6	0.48	0.43	0.05	0.10	89.6
	Average						0.16	83.8
	Plot average						0.15	85.1

*population of 181 bees
**population of 227 bees
***see Figure 1, page 15.

clone and Vernal alfalfa for 24 rows, the range was still greater (31.7-93.3 percent) but the range in the third plot, planted to six rows of the white clone and six rows of Vernal alfalfa, was of the same magnitude as that computed in the 2 x 2 rowed plot. Consideration of Table 4 demonstrates that even when an average of two years is computed a wide difference in broad-sense heritability estimates as affected by the three planting patterns is observed. Ranges in heritability values of 59.8-89.0 percent, 45.7-91.4 percent and 83.8-86.9 percent for the 2 x 2, 1 x 1 and 6 x 6 rowed plots were obtained respectively. An interesting feature regarding planting pattern is that the 6 x 6 rowed plot gave the narrowest range in heritability estimates. This fact is especially noticeable in Table 4 since the range is only 83.8-86.9 percent.

Year. The final factor that affects the broad-sense heritability estimate for seed yield is the change in seasons from one year to the next. In Table 4 a marked fluctuation in estimates for most bee species in the two years is noted. The least variation for *Apis* was 37.5-53.8 percent in the 1 x 1 rowed plot and the greatest variation was 40.4-79.3 percent in the 2 x 2 rowed plot. *Megachile* ranged from 68.5 percent in 1962 to 83.3 percent in 1963 for the 2 x 2 rowed plot. The greatest variation did not occur in the 2 x 2 rowed plot as was the case with *Apis* but was in the 1 x 1 rowed plot. Here the 1962 values were 93.3 and 31.7 percent while the 1963 value was 75.5 percent for a single population. It is interesting to note that the estimate derived from endemic pollinators was by far the most consistent from one year to the next. The greatest difference occurred in the 6 x 6 rowed plot with 78.0 percent in 1962 and 89.6 percent in 1963. It is noted that the values for Apis were higher in the 2 x 2 and 1 x 1 rowed plots in 1962 and lower in 1963 whereas in the 6 x 6 rowed plot the value was low in 1962 and high in 1963. However, the small population of Megachile was slightly different, the values were lower in the 2 x 2 and 6 x 6 rowed plots in 1962 and higher in 1963 but higher in the 1 x 1 rowed plot in 1962 and lower in 1963. The values derived in the 1 x 1 and 6 x 6 rowed plots were consistent for both bee species although the trend was from a high value in 1962 to a low value in 1963 for the 1 x 1 rowed plot whereas a low value was obtained in the 6 x 6 rowed plot in 1962 and a high value in 1963. The 2 x 2 rowed plot gave opposite trends for the two bee species. A final comparison of broad-sense heritability estimates is noted if the plot averages are computed. Values of 85.1, 77.2 and 69.8 percent were obtained for the 6 x 6, 2 x 2 and 1 x 1 plots respectively.

Environmental variance. In addition to heritability estimates an indication of the contribution of environmental variance to the total variance can be obtained from the ratio $\frac{\sigma_E^2}{\sigma_P^2}$. Of the three bee species, the largest contribution to environmental variance was obtained with *Apis* as the pollinator in the three plots. Endemic pollinators and *Nomia* gave the smallest ratios. A consideration of the three plot patterns demonstrates that the largest values were obtained in the 2 x 2 rowed plot and the smallest values in the 6 x 6 rowed plot. However, the effect of year was not as clear cut as the two previous factors. In 1963 the ratio of environmental to phenotypic variances for *Apis* increased from 0. 2-0. 6, 0. 5-0. 6 respectively in the 2 x 2 and 1 x 1 rowed plots but the ratio decreased from 0. 3-0. 1 in the 6 x 6 rowed plot. Quite different results were obtained for *Megachile* and the endemic pollinators.

Incidence of Selfing in Alfalfa Pods

Frequency of selfed and crossed seeds. A record of the number of selfed and crossed seeds in different classes of alfalfa pods for endemic pollinators in Table 5 demonstrates that pods with one seed contained 94. 7 percent selfed seed. Pods with two seeds contained 88. 3 percent selfed seed while pods with three and four or more seeds contained 36. 3 and 23. 1 percent selfed seed respectively. Some four seeded pods had all selfed seed and one seven seeded pod had three selfed seeds and four crossed seeds.

Data obtained when *Megachile* was used as the pollinator showed that a very high incidence of selfing occurred. In Table 6

Table 5. Frequency of selfed and crossed seeds per alfalfa pod following pollination by endemic pollinators.

Pod description	No. of pods	No. of selfed seed	No. of crossed seed	Percentage selfed seed
Pods with one seed	38	36	2	94.7
Pods with two seeds:				
two seeds selfed	25	50	0	
one seed selfed	3	3	3	
no seed selfed	2	0	4	
Total	30	53	7	88.3
Pods with three seeds:				
three seeds selfed	10	30	0	
two seeds selfed	3	6	3	
one seed selfed	1	1	2	
no seed selfed	20	0	60	
Total	34	37	65	36.3
Pods with four or more seeds:				
four seeds selfed	3	12	0	
three seeds selfed	1	3	4	
two seeds selfed	4	8	10	
one seed selfed	7	7	25	
no seed selfed	14	0	61	
Total	29	30	100	23.1

Table 6. Frequency of selfed and crossed seeds per alfalfa pod following pollination by <u>Megachile</u> pollinators, 10 rods from a colored-flower population.

Pod description	No. of pods	No. of selfed seed	No. of crossed seed	Percentage selfed seed
Pods with one seed	30	29	1	96.7
Pods with two seeds:				
two seeds selfed	28	56	0	
one seed selfed	1	1	1	
no seed selfed	1	0	2	
Total	30	57	3	95.0
Pods with three seeds:				
three seeds selfed	29	87	0	
two seeds selfed	0	0	0	
one seed selfed	1	1	2	
no seed selfed	0	0	0	
Total	30	88	2	97.8
Pods with four or more seeds:				
four seeds selfed	16	60	1	
three seeds selfed	4	12	5	
two seeds selfed	3	6	6	
one seed selfed	4	4	14	
no seed selfed	3	0	13	
Total	30	82	39	67.8

the percentage selfed seed 10 rods from the contaminating source of pollen was 96.7, 95.0, 97.8 and 67.8 percent for one seeded pods, two seeded pods, three seeded pods and four or more seeded pods respectively. At 20 rods (Table 7) the selfed seed percentage was 96.7 percent for the one seeded pods, 88.3 percent for the two seeded pods, 78.9 percent for the three seeded pods and 79.5 percent for the four or more seeded pods. At 40 rods (Table 8) all seeds were selfed for the one seeded pods, two seeded pods and three seeded pods while the percentage selfed seed for the four or more seeded pods was 77.9 percent. It is noted that at all distances recorded for Megachile a narrow range of 67.8-80.5 percent selfed seeds existed for the four or more seeded pods.

Order of selfed seeds within alfalfa pods. An indication of the variable nature of pollen tube growth within the style is indicated by the data in Table 9 which records the order of selfed and crossed seeds within a pod. Of the 40 pods that contained both crossed and selfed seed, 11 pods contained seeds in the order white to colored seeds (base to apex) while 30 pods contained seeds that were not in this order.

Self-fertility measurements. Any discussion of the incidence of selfing should be considered in light of the fertility of the material used. In Table 10 it is noted that a range of 26.0-45.0 percent fertility for three seasons in the field and one season in the greenhouse.

Table 7. Frequency of selfed and crossed seeds per alfalfa pod following pollination by *Megachile* pollinators 20 rods from a colored-flower population.

Pod description	No. of pods	No. of selfed seed	No. of crossed seed	Percentage selfed seed
Pods with one seed	30	29	1	96.7
Pods with two seeds:				
two seeds selfed	24	48	0	
one seed selfed	5	5	5	
no seed selfed	1	0	2	
Total	30	53	7	88.3
Pods with three seeds:				
three seeds selfed	21	63	0	
two seeds selfed	1	2	1	
one seed selfed	6	6	12	
no seed selfed	2	0	6	
Total	30	71	19	78.9
Pods with four or more seeds:				
four seeds selfed	21	84	1	
three seeds selfed	2	6	2	
two seeds selfed	0	0	0	
one seed selfed	5	5	16	
no seed selfed	2	0	8	
Total	30	95	27	77.9

Table 8. Frequency of selfed and crossed seeds per alfalfa pod following pollination by Megachile pollinators 40 rods from a colored-flower population.

Pod description	No. of pods	No. of selfed seed	No. of crossed seed	Percentage selfed seed
Pods with one seed	30	30	0	100.0
Pods with two seeds:				
two seeds selfed	30	90	0	
one seed selfed	0	0	0	
no seed selfed	0	0	0	
Total	30	60	0	100.0
Pods with three seeds:				
three seeds selfed	30	90	0	
two seeds selfed	0	0	0	
one seed selfed	0	0	0	
no seed selfed	0	0	0	
Total	30	90	0	100.0
Pods with four or more seeds:				
six seeds selfed	1	6	1	
five seeds selfed	3	15	0	
four seeds selfed	19	76	0	
three seeds selfed	0	0	0	
two seeds selfed	3	6	9	
one seed selfed	3	3	11	
no seed selfed	1	0	6	
Total	30	106	27	79.6

Table 9. Order of selfed and crossed seeds per alfalfa pod following pollination by Megachile pollinators.*

No. of pods	Seed no.	Seed origin within pods** Order of selfed or crossed seeds
1	2	S-C
5	2	C-S
1	3	S-S-C
1	3	S-C-S
4	3	S-C-C
2	3	C-S-C
2	4	S-S-S-C
1	4	S-S-C-C
3	4	S-C-S-S
1	4	S-C-S-C
1	4	S-C-C-C
1	4	C-C-S-C
3	4	C-S-C-C
3	4	C-C-S-C
1	4	C-C-C-S
1	5	S-S-S-S-C
1	5	S-C-S-S-C
1	5	S-C-C-C-C
1	5	C-S-S-S-S
1	5	C-C-S-S-C
2	5	C-S-C-C-C
2	5	C-C-C-S-C
1	7	S-S-S-C-S-S-S

*Seed order base to apex of the pod

** C = crossed seed

S = selfed seed

Table 10. Effect of different seasons on the estimate of self-fertility in the white-flowered clone.

Season	No. of tripped florets	Total pods developed	Percentage pod set
1961 (field)	200	90	45.0
1962 (field)	100	26	26.0
1963 (field)	150	41	27.3
1963 (greenhouse)	103	35	34.0
Total average	553	192	34.7

These values were derived from percentage pod set. A comparison of the fertility estimates of several genotypes from Vernal alfalfa using pod set, selfed seed produced and average seeds per pod is presented in Table 11. Rating of the genotypes including the white clone in Table 12 demonstrates that pod set and total seed produced give the same rating. However, rating by average seeds per pod gave a different value. The order of the high and low genotype did not change, but the other three genotype ratings were altered.

Usefulness of Bees as a Breeding Tool

Florets tripped per raceme. Some indication of why one bee species causes more cross-pollination than others can be derived from data presented in Tables 13 and 14. It is noted that in Table 13 *Apis* tripped far more single florets on a raceme than *Megachile*. In addition, the larger population of *Megachile* tripped more florets on a raceme than the smaller population. The difference in tripping on a raceme for each bee species is especially noticeable when the average number of florets tripped per raceme is computed. The larger population of *Megachile*, on the average, tripped almost twice as many florets per raceme as *Apis* in 1962. In 1963 (Table 14) similar observations were noted, but in this year the difference in the average number of florets tripped was not so large. Once again *Megachile* has higher values in the three to six florets per raceme

Table 11. Self-fertility of four genotypes of Vernal alfalfa and the white-flowered clone.

Genotype	No. of tripped florets	Total pods developed	Total selfed seed	Average seed per pod
1	100	49	79	1.6
2	100	27	41	1.5
3	100	53	105	1.9
4	100	13	13	1.0
White clone	100	17	30	1.7

Table 12. Three self-fertility ratings (order of decreasing fertility) of four genotypes of Vernal alfalfa and the white-flowered clone.

Method of rating self-fertility	Rated high to low				
Pods per 100 florets tripped	3	1	2	white clone	4
Seeds per 100 florets tripped	3	1	2	white clone	4
Average seeds per pod	3	white clone	1	2	4

Table 13. Average number of florets tripped per raceme in 1962 in white-flowered alfalfa at several distances from the colored-flowered base plot.

Pollinator	Distance***	No. of trips per raceme 1	2	3	4	5	6	7	8	9	10	Total racemes	No. of florets tripped	Ave. no. of florets tripped
Megachile$_1$*	10 rods	16	13	12	6	0	0	1	0	0	0	48	109	2.27
	20 rods	15	16	13	8	4	1	0	0	0	0	57	144	2.53
Total												105	253	
Average														2.41
Megachile$_2$**	10 rods	10	23	12	5	2	0	0	0	0	0	52	122	2.34
	20 rods	13	14	10	4	2	5	0	1	0	0	49	135	2.76
	40 rods	0	12	17	8	9	3	1	0	0	0	50	177	3.54
	80 rods	1	7	12	10	12	5	0	0	0	0	47	169	3.60
Total												198	603	
Average														3.05
Apis	10 rods	59	26	11	1	2	0	0	1	1	0	101	175	1.73
	20 rods	67	18	12	2	1	0	0	1	0	0	101	160	1.58
	40 rods	48	4	0	0	0	0	0	0	0	0	52	56	1.08
Total												254	391	
Average														1.54

*population of 181 bees.
**population of 227 bees.
***see Figure 1, page 15.

Table 14. Average number of florets tripped per raceme in 1963 in white-flowered alfalfa at several distances from the colored-flowered base plot.

Pollinator	Distance*	No. of trips per raceme 1	2	3	4	5	6	7	8	9	10	Total racemes	No. of florets tripped	Ave. no. of florets tripped
Megachile	10 rods	11	39	32	24	16	10	2	1	0	1	136	453	3.33
	20 rods	27	37	24	16	12	9	1	1	0	1	128	376	2.94
	40 rods	19	38	34	5	5	3	1	0	1	0	106	276	2.60
Total												370	1105	
Average														2.99
Apis	10 rods	49	34	29	10	4	0	1	0	0	0	127	271	2.13
	20 rods	49	43	18	11	4	3	0	0	1	0	129	280	2.17
	40 rods	30	30	23	10	5	0	0	0	0	0	98	224	2.29
Total												354	775	
Average														2.19

*see Figure 1, page 15.

count while Apis had a much higher value for the single tripped raceme count. On the average, Megachile tripped nearly a third more florets per raceme than Apis.

Tripping rate and pod development. Even though one bee species may be recorded as tripping more florets per raceme, this does not necessarily indicate that larger seed yield will result. Data obtained in two years for two pollinators show wide variations in pod set at different distances from a foreign pollen source. At 10 rods from the colored-flowered base plot it was found (Table 15) that when the two trips per raceme count was recorded the percentage pod set was substantially larger than the single tripped raceme count, and even the percentage pod set of the four or five count was larger than the single tripped raceme value. Typical examples in Table 15 are the values obtained for the first population of Megachile. Values of 56.3, 76.9, 55.6 and 58.3 were obtained for one, two, three and four trips per raceme respectively. However, the increase in percentage pod set from 56.3-76.9 did not lead to an increase in average seeds per pod, but rather a decline from 1.9 to 1.8 seeds per pod. Another example is from the Apis data where an increase from 22.0-46.2 percent pod set resulted in no change in the average seeds per pod. Similar examples are found in Tables 16 and 17, but it is noted that in some cases the pod set and average seeds per pod values remain very constant for the three d.stances from the foreign pollen

Table 15. Percentage pod set and average seeds per pod as affected by increased numbers of tripped florets on a raceme in 1962 (10 rods from the base plot).

Pollinator	No. of trips per raceme	No. of racemes	No. of florets	No. of pods	Total seed	Percent pod set	Average seed per pod
Megachile$_1$*	1	16	16	9	17	56.3	1.9
	2	13	26	20	35	76.9	1.8
	3	12	36	20	36	55.6	1.8
	4	6	24	14	25	58.3	1.8
	5	0	0	0	0	0.0	0.0
	6	0	0	0	0	0.0	0.0
	7	1	7	6	12	85.7	2.0
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0
Megachile$_2$**	1	10	10	5	6	50.0	1.2
	2	23	26	19	26	73.1	1.4
	3	12	36	17	27	47.2	1.6
	4	5	20	9	15	45.0	1.7
	5	2	10	7	12	70.0	1.7
	6	0	0	0	0	0.0	0.0
	7	0	0	0	0	0.0	0.0
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0
Apis	1	59	59	13	18	22.0	1.4
	2	26	52	24	33	46.2	1.4
	3	11	33	12	18	36.4	1.5
	4	1	4	3	4	75.0	1.3
	5	3	15	7	10	46.7	1.4
	6	0	0	0	0	0.0	0.0
	7	0	0	0	0	0.0	0.0
	8	1	8	7	13	87.5	1.9
	9	1	9	5	12	55.6	2.4
	10	0	0	0	0	0.0	0.0

*population of 181 bees.
**population of 227 bees.

Table 16. Percentage pod set and average seeds per pod as affected by increased numbers of tripped florets on a raceme in 1962 (20 rods from the base plot).

Pollinator	No. of trips per raceme	No. of racemes	No. of florets	No. of pods	Total seed	Percent pod set	Average seed per pod
Megachile$_1$*	1	15	15	9	18	60.0	2.0
	2	16	32	10	20	31.3	2.0
	3	13	39	17	28	43.6	1.7
	4	8	32	24	42	75.0	1.8
	5	4	20	12	20	60.0	1.7
	6	1	6	5	13	83.3	2.6
	7	0	0	0	0	0.0	0.0
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0
Megachile$_2$**	1	13	13	3	5	23.1	1.7
	2	14	28	5	8	17.9	1.6
	3	10	30	14	15	46.7	1.1
	4	4	16	8	10	50.0	1.3
	5	2	10	4	5	40.0	1.3
	6	5	30	16	25	53.3	1.6
	7	0	0	0	0	0.0	0.0
	8	1	8	7	11	87.5	1.6
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0
Apis	1	67	67	17	23	25.4	1.4
	2	18	36	13	19	36.1	1.5
	3	12	36	15	27	41.7	1.8
	4	2	8	3	3	37.5	1.0
	5	1	5	1	2	20.0	2.0
	6	0	0	0	0	0.0	0.0
	7	0	0	0	0	0.0	0.0
	8	1	8	3	4	37.5	1.3
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0

*population of 181 bees.
**population of 227 bees.

Table 17. Percentage pod set and average seeds per pod as affected by increased numbers of tripped florets on a raceme in 1962 (40 rods from the base plot).

Pollinator	No. of trips per raceme	No. of racemes	No. of florets	No. of pods	Total seed	Percent pod set	Average seed per pod
Megachile$_2$*	1	0	0	0	0	0.0	0.0
	2	12	24	8	10	33.3	1.3
	3	17	51	36	54	70.6	1.5
	4	8	32	18	26	56.3	1.4
	5	9	45	31	52	68.9	1.7
	6	3	18	9	14	50.0	1.6
	7	1	7	6	8	85.7	1.3
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0
Apis	1	48	48	14	19	29.2	1.4
	2	4	8	0	0	0.0	0.0
	3	0	0	0	0	0.0	0.0
	4	0	0	0	0	0.0	0.0
	5	0	0	0	0	0.0	0.0
	6	0	0	0	0	0.0	0.0
	7	0	0	0	0	0.0	0.0
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0

*population of 227 bees.

source. At 10 rods Apis had a value of 22.0 percent pod set and an average of 1.4 seeds per pod. Similarly, at 20 rods the values were 25.4 percent and 1.4 seed while at 40 rods the values were 29.2 percent and 1.4 seeds per pod. However, this situation is a variable one also, as attested by the values obtained in Tables 15, 16 and 17 for the larger population of Megachile. At 10 rods the percentage pod set was 73.1 percent while the average seeds per pod was 1.4. At 20 rods the values were 17.9 percent and 1.6 seeds per pod while at 40 rods the values were 33.3 percent and 1.3 seeds per pod. In one bee species values were obtained 80 rods from the foreign pollen source (Table 18), but as in the other data considerable variation was observed.

Generally, the trends observed in 1963 were similar to those of 1962. But one striking feature is that there are notable differences in average seeds per pod. In Table 19 the Apis data were much higher than the corresponding data in 1962, Table 15. However, the data in Tables 20 and 21 were similar to data in Tables 16 and 17.

Effect of distance on pod set and average seeds per pod. Another factor that is of interest is the effect of bee species and distance of the white-flowered plots from the colored-flowered base plot on pod set and average seeds produced per pod. In Table 22 a decline in 1962 from 40.7 percent at 10 rods to 33.5 and 24.6 percent at 20 and 40 rods respectively is noted. However, the average seeds

Table 18. Percentage pod set and average seeds per pod as affected by increased numbers of tripped florets on a raceme in 1962 (80 rods from the base plot).

Pollinator	No. of trips per raceme	No. of racemes	No. of florets	No. of pods	Total seed	Percent pod set	Average seed per pod
Megachile$_2$*	1	1	1	0	0	0.0	0.0
	2	7	14	6	9	42.9	1.5
	3	12	36	13	20	36.1	1.5
	4	10	40	25	28	62.5	1.1
	5	12	60	22	43	36.7	2.0
	6	5	30	21	26	70.0	1.2
	7	0	0	0	0	0.0	0.0
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0

*population of 227 bees.

Table 19. Percentage pod set and average seeds per pod as affected by increased numbers of tripped florets on a raceme in 1963 (10 rods from the base plot).

Pollinator	No. of trips per raceme	No. of racemes	No. of florets	No. of pods	Total seed	Percent pod set	Average seed per pod
Megachile	1	11	11	5	7	45.5	1.4
	2	39	78	40	62	51.3	1.6
	3	32	96	38	55	39.6	1.5
	4	24	96	32	54	33.3	1.7
	5	16	80	23	32	28.8	1.4
	6	10	60	25	37	41.7	1.5
	7	2	14	3	7	21.4	2.3
	8	1	8	2	3	25.0	1.5
	9	0	0	0	0	0.0	0.0
	10	1	10	3	3	30.0	1.0
Apis	1	49	49	28	57	57.1	2.0
	2	34	68	34	69	50.0	2.0
	3	29	87	49	100	56.3	2.0
	4	10	40	18	32	45.0	1.8
	5	4	20	7	12	35.0	1.7
	6	0	0	0	0	0.0	0.0
	7	1	7	6	9	85.7	1.5
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0

Table 20. Percentage pod set and average seeds per pod as affected by increased numbers of tripped florets on a raceme in 1963 (20 rods from the base plot).

Pollinator	No. of trips per raceme	No. of racemes	No. of florets	No. of pods	Total seed	Percent pod set	Average seed per pod
Megachile	1	27	27	13	17	48.2	1.3
	2	37	74	29	49	39.2	1.7
	3	24	72	27	33	37.5	1.2
	4	16	64	19	35	29.7	1.8
	5	12	60	20	30	33.3	1.5
	6	9	54	17	36	31.5	2.1
	7	1	7	5	15	71.4	3.0
	8	1	8	2	3	25.0	1.5
	9	0	0	0	0	0.0	0.0
	10	1	10	3	3	30.0	1.0
Apis	1	49	49	34	59	69.4	1.7
	2	42	84	56	94	66.7	1.7
	3	18	54	33	60	61.1	1.8
	4	11	44	24	44	54.6	1.8
	5	4	20	7	12	35.0	1.7
	6	3	18	11	17	61.1	1.6
	7	0	0	0	0	0.0	0.0
	8	0	0	0	0	0.0	0.0
	9	1	9	5	10	55.6	2.0
	10	0	0	0	0	0.0	0.0

Table 21. Percentage pod set and average seeds per pod as affected by increased numbers of tripped florets on a raceme in 1963 (40 rods from the base plot).

Pollinator	No. of trips per raceme	No. of racemes	No. of florets	No. of pods	Total seed	Percent pod set	Average seed per pod
Megachile	1	19	19	9	10	47.4	1.1
	2	38	76	24	30	31.6	1.3
	3	34	102	32	44	31.4	1.4
	4	5	20	11	16	55.0	1.5
	5	5	25	11	13	44.0	1.2
	6	3	18	7	8	39.0	1.1
	7	1	7	2	2	28.6	1.0
	8	0	0	0	0	0.0	0.0
	9	1	9	1	1	11.1	1.0
	10	0	0	0	0	0.0	0.0
Apis	1	30	30	19	27	63.3	1.4
	2	29	58	36	67	62.1	1.9
	3	22	66	40	79	60.6	2.0
	4	10	40	18	38	45.0	2.1
	5	5	25	24	49	96.0	2.0
	6	0	0	0	0	0.0	0.0
	7	0	0	0	0	0.0	0.0
	8	0	0	0	0	0.0	0.0
	9	0	0	0	0	0.0	0.0
	10	0	0	0	0	0.0	0.0

Table 22. Percentage pod set and average seeds per pod as affected by bee species and distance of the white-flowered plot from the colored-flowered base plot.

Pollinator	Distance in rods	Year	No. of plots	No. of racemes	No. of florets tripped	No. of pods set	Total seed	Percent pod set	Average seed per pod
Apis	10	1962	37	103	177	72	107	40.7	1.5
Apis	10	1963	31	123	271	142	279	52.4	2.0
Total					448	214	386		
Average								47.8	1.8
Megachile$_1$ *	10	1962	19	50	117	71	126	60.7	1.8
Megachile$_2$ **	10	1962	25	50	121	57	86	47.1	1.5
Megachile	10	1963	29	135	446	172	262	38.6	1.5
Total					684	300	474		
Average								43.9	1.6
Nomia	10	1963	12	34	55	31	43	56.4	1.4
Plot total					1187	545	903		
Plot average								45.9	1.7
Apis	20	1962	38	101	161	54	81	33.5	1.5
Apis	20	1963	33	137	285	170	297	59.7	1.8
Total					446	224	378		
Average								50.2	1.7
Megachile$_1$ *	20	1962	19	59	158	77	142	48.7	1.8
Megachile$_2$ **	20	1962	22	46	126	57	82	45.2	1.4
Megachile	20	1963	23	127	371	135	222	36.4	1.6
Total					655	269	446		
Average								41.1	1.7
Nomia	20	1963	8	19	30	13	16	43.3	1.2
Plot total					1131	506	840		
Plot average								44.7	1.7
Apis	40	1962	26	54	57	14	19	24.6	1.4
Apis	40	1963	22	98	221	133	249	60.2	1.9
Total					278	147	268		
Average								52.9	1.8
Megachile$_2$ **	40	1962	25	50	180	108	164	60.0	1.5
Megachile	40	1963	20	112	292	98	124	33.6	1.3
Total					472	206	288		
Average								43.6	1.4
Nomia	40	1963	7	12	15	10	20	66.7	2.0
Plot total					765	363	576		
Plot average								47.5	1.6

*population of 181 bees.

**population of 227 bees.

***see Figure 1, page 15.

per pod was very close at 10 and 20 rods but declined at 40 rods. Inspection of the *Apis* data for 1963 shows that this is not a consistent trend since pod set values of 52.4, 59.7 and 60.2 percent were obtained while there was a general decline in average seed per pod values at distances of 10, 20 and 40 rods. *Megachile* data were even more irregular because there was no trend in any given year. In 1962 the data from the larger population of *Megachile* showed that pod set was 47.1 percent at 10 rods, 45.2 percent at 20 rods and 60.0 percent at 40 rods. Values of 1.5, 1.4 and 1.5 seeds per pod demonstrate that seeds per pod were equally irregular in this year. However, in 1963 the pod set showed a consistent decline from 38.6 percent at 10 rods at 33.6 percent at 40 rods while the average seeds per pod fluctuated from 1.5 at 10 rods to 1.6 at 20 rods and then decreased to 1.3 seeds per pod at 40 rods. The *Nomia* data were found to be very similar to those obtained from *Megachile*. Consideration of the averages obtained in Table 22 shows that the highest pod set is not necessarily associated with the highest average seed yield per pod as far as *Apis* and *Megachile* are concerned. Values of 47.8 and 50.2 percent pod set were obtained at 10 and 20 rods while the average seed per pod value declined from 1.8-1.7. The association between percentage pod set and average seeds per pod was very close as far as *Nomia* was concerned, but the sample size was small. As the distance increased from 10 to 20 rods the average seed yields and percentage pod sets showed a small decline. However, the values at 40 rods showed the same trend as those at 10 rods for percentage pod set but were quite different for average seeds per pod. The overall averages of bee species in each plot in Table 22 are surprisingly close for both percentage pod set and seeds per pod. The percentage pod set values only ranged from 44.7-47.5 while the average seeds per pod ranged from 1.6-1.7.

Territorial effects. An interesting feature of the pollen collecting habits of two bee species is presented in Table 23. It is noted that out of a total of 37 *Megachile* observed collecting pollen, 36 showed no preference for flower color and they collected from both white and colored flowers. However, *Apis* was quite different, and from a total of 50 bees recorded, seven collected pollen only on the white clone, one was observed collecting from both white- and colored-flowers, while 42 were observed collecting only on the colored-flower plants.

Effect of Planting Pattern on Cross-Pollination

The effect that the type of planting pattern has on percentage pod set and average seeds produced per pod is not a well defined one. A comparison of Tables 24, 25 and 26 shows that there is not a consistent trend for any of the bee species used in each of the plots. The percentage pod set range was 46.0-73.3, 50.0-63.4 and

Table 23. Pollen collection visits of two bee species in plots interplanted with white-flowered and colored-flowered alfalfa, 1963.

Bee species	No. of bees collecting pollen only on white-flowered plants	No. of bees collecting pollen on white- and colored-flowered plants	No. of bees collecting pollen only on colored-flowered plants
Apis	7	1	42
Megachile	0	36	1

Table 24. Pod set and average seed per pod produced in a plot composed to two rows of white-flowered and two rows of colored-flowered alfalfa alternating for 24 rows.

Pollinator	Year	No. of plants	No. of racemes	No. of florets tripped	No. of pods set	Total seed	Percent pod set	Average seed per pod
Apis	1962	20	72	137	63	96	46.0	1.5
Apis	1963	11	47	120	73	128	60.9	1.8
Megachile$_1$*	1962	13	41	65	42	81	64.6	1.9
Megachile$_2$**	1962	15	38	72	39	51	54.2	1.3
Megachile	1963	7	32	117	64	96	54.7	1.5
Nomia	1963	7	17	30	22	50	73.3	2.3

*population of 181 bees.
**population of 227 bees.

Table 25. Pod set and average seed per pod produced in a plot composed of one row of white-flowered and one row of colored-flowered alfalfa alternating for 24 rows.

Pollinator	Year	No. of plants	No. of racemes	No. of florets tripped	No. of pods set	Total seed	Percent pod set	Average seed per pod
Apis	1962	18	54	94	48	96	51.1	2.0
Apis	1963	10	47	85	45	87	52.9	1.9
Megachile$_1$*	1962	15	40	93	59	113	63.4	1.9
Megachile$_2$**	1962	9	18	35	22	45	62.9	2.1
Megachile	1963	6	30	106	53	95	50.0	1.8
Nomia	1963	7	21	46	25	55	54.3	2.2

*population of 181 bees.
**population of 227 bees.

Table 26. Pod set and average seed per pod produced in a plot composed of six rows of white-flowered and six rows of colored-flowered alfalfa alternating for 24 rows.

Pollinator	Year	No. of plants	No. of racemes	No. of florets tripped	No. of pods set	Total seed	Percent pod set	Average seed per pod
Apis	1962	16	52	76	21	31	27.6	1.5
Apis	1963	7	29	43	26	42	60.5	1.6
Megachile$_1$ *	1962	9	18	44	29	40	65.9	1.4
Megachile$_2$ **	1962	10	20	60	31	50	51.7	1.6
Megachile	1963	5	25	42	30	48	71.4	1.6
Nomia	1963	1	3	4	3	6	75.0	2.0

*population of 181 bees.
**population of 227 bees.

27.6-75.0 for the 2 x 2, 1 x 1 and 6 x 6 rowed plots respectively. A clearer picture of the change in pod set is obtained in Table 27. Inspection of this table demonstrates that the highest average percent pod set was obtained in the 2 x 2 rowed plot. However, the values in the other two plots are very similar, differing by only 1.1 percent in the 1 x 1 rowed plot and 4.0 percent in the 6 x 6 rowed plot. Though the largest average was obtained for the 2 x 2 rowed plot, the values of the two bee species Megachile and Nomia indicate that the 6 x 6 rowed plot gave the largest pod set. However, a different situation exists if the average seeds produced per pod is considered. The ranges of the average seeds per pod in Tables 24, 25 and 26 were 1.3-2.3, 1.9-2.2 and 1.4-2.0 seeds per pod for the 2 x 2, 1 x 1 and 6 x 6 rowed plots respectively. Consideration of the averages of the two years in Table 27 shows that the highest value was obtained in the 1 x 1 rowed plot and the lowest in the 6 x 6 rowed plot. It should be

Table 27. 1962 and 1963 averages of pod set and seeds per pod from the white-flowered population.

Pollinator	Plot* pattern	No. of plants	No. of racemes	No. of florets tripped	No. of pods set	Total seed	Percent pod set	Average seeds per pod
Apis	2x2	31	119	257	136	224	52.9	1.7
Megachile	2x2	35	111	254	145	228	57.1	1.6
Nomia	2x2	7	17	30	22	50	73.3	2.3
Total				541	303	502		
Average							56.0	1.7
Apis	1x1	28	101	179	93	183	52.0	2.0
Megachile	1x1	30	88	234	134	253	57.3	1.9
Nomia	1x1	7	21	46	25	55	54.3	2.2
Total				459	252	491		
Average							54.9	2.0
Apis	6x6	23	81	119	47	73	39.5	1.6
Megachile	6x6	24	63	146	90	138	61.6	1.5
Nomia	6x6	1	3	4	3	6	75.0	2.0
Total				269	140	217		
Average							52.0	1.6

*see Figure 1, page 15.

noted that the difference between the 2 x 2 and 6 x 6 rowed plots is not too large. A further significant fact is that both the Apis and Megachile values were highest in the 1 x 1 rowed plot. Nomia had the highest value in the 2 x 2 rowed plot but the value here was only 0.1 seeds per pod higher than that in the 1 x 1 rowed plot.

Endemic pollinators give values of 82.5, 86.5, 70.8, 74.7, 72.8, 73.0 and 64.0 percent cross-pollination 10, 20, 40, 80, 160, 240 and 320 rods from the base plot as listed in Table 28.

Table 28. Frequency of cross-pollination by endemic pollinators for seven planting patterns.

Distance	Plot* pattern	No. of selfed seed	No. of crossed seed	Total seed	Percent cross-pollination
10 rods	2x2	17	80	97	82.5
20 rods	1x1	13	83	96	86.5
40 rods	6x6	26	63	89	70.8
80 rods	3x9x9x3	21	62	83	74.7
160 rods	12x12	22	59	81	72.8
240 rods	3x3	24	65	89	73.0
320 rods	4x4	31	55	86	64.0

*see Figure 1, page 15.

DISCUSSION

The necessity of maintaining adequate isolation between varieties of alfalfa to insure varietal purity is one of the major concerns of the plant breeder and seed certification specialist. It appears from the data recorded that the amount of foreign pollen disseminated by bees is a function of the bee species involved, distance from a foreign pollen source and year. In the case of Apis very little foreign pollen is carried in some years even at a distance of 10 rods. While in another year nine times as much foreign pollen is carried 10 rods. It was observed that the amount of cross-pollination for Apis is similar at distances of 10 and 20 rods and a marked decline is not noted until 40 rods. The values obtained in the one year for Megachile demonstrate a similar amount of cross-pollination as Apis at 10 rods, but there was a marked drop at 20 rods.

The amount of cross-pollination by endemic pollinators was exceedingly high in this study. A pollinator such as a bumble bee carries a very large amount of pollen and forages over wide areas. The values obtained for this information can very likely be primarily attributed to this insect. However, a second insect was thought to play a role, since a wasp (Bembix occidentalis) was observed to accidentally trip florets while it was foraging for nector. This wasp also foraged erratically over a wide area. The amount of pollen carried would be much less for this insect since it has no pollen carrying devices and the pollen would have to adhere to its non-pubescent body. Perhaps the most important fact in regard to endemic pollinators was the distance that foreign pollen was distributed. Six and a half percent cross-pollination is quite high at a distance of a mile but there are three factors to consider. First, the number of plants in this study was quite small and they were spaced at various distances with long distances between with no alfalfa. The percentage cross-pollination might have been much less with a solid planting from the colored-flowered contaminant source. Second, in the summer months the alfalfa bloom constituted the main source of bloom for several miles and hence attracted higher populations of endemic pollinators that would be expected under field conditions. Third, because of the flat, open terrain a concentrated source of flowers attracted insects from extremely long distances and therefore provided an unusually high concentration of insects in a small area.

It is not uncommon to compute broad-sense heritability estimates for characters to ascertain what might be considered the total genetic potential for a particular character. This type of investigation is excellent as long as a reliable estimate can be obtained from the plant material. Even though many broad-sense estimates have been computed from clonal material, certain limitations have been noted. In the first instance, vegetatively propagated clones and plants grown from seed have different root systems which may influence forage growth and seed potential in successive years. The common consensus at the present time is that one to two years of growth are necessary to remove bias caused by this source. The data for the present study were derived in the second and third years after planting and therefore variation from this source should be small. Other factors such as planting pattern, season and the insect pollinator need to be considered. Extreme variations in broad-sense heritability estimates for seed yield in alfalfa were obtained in this study. Of the three variables considered, planting pattern of alfalfa caused the largest extremes in the estimates. The 1 x 1 rowed plot gave the largest values while the 6 x 6 rowed plot gave the least. The difference in the plot estimates is believed to be due to the different foraging behavior and manner by which each bee species carried pollen.

Megachile species were observed to give the largest extremes while the endemic pollinators (*Bombus* and *Bembix* sp.) gave the least extreme values. This might suggest that the foraging behavior of *Megachile* is more subject to environmental influences than the other bee species and endemic pollinators. It is noted that the range in each year was very similar indicating that seasonal effects are not too different.

Perhaps a better indication of what is involved in the broad-sense heritability estimate can be derived from the consideration of the change in environmental variance in relation to total variance. Genetic variance is considered to be constant from year to year assuming no genetic-environmental interaction or no change in this interaction if it does occur. In this way individual factors in the environment can be shown to be the cause of variation in broad-sense heritability estimates. From this source a slightly different picture is indicated. The overall seasonal change is still calculated as being small. Apis pollinators contribute to a much higher environmental variance than the other bee species and the 2 x 2 rowed plot pattern has the highest value not the 1 x 1 rowed plot pattern as might be inferred from consideration of only the broad-sense heritability estimate. It is suggested that consideration of environmental variance is very useful in determining the nature of variation in broad-sense estimates. In addition, the variation induced by the pollinator, season and planting pattern of the material were such as to cast doubt on the use of broad-sense heritability estimates for such a character as seed yield in alfalfa when these values are derived from clonal material.

Prior to this study it was assumed that most of the pods which had three or more seeds were the result of cross-pollination while those pods with two or less seeds were the result of self-pollination (28, p. 13; 32, p. 77). Data in the present study demonstrate that many of the seeds in pods with three or more seeds are the result of self pollination. Even two seeded pods cannot be assumed to be the result of self-pollination as 11.7 percent of the seed from this source was crossed seed. These data were derived at random from sources up to 40 rods from a contaminant source and pollinated by endemic pollinators.

Using Megachile as the pollinator and comparing the effect of three distances of the white clone from a contaminant source an entirely different set of data was obtained. Sixty-seven percent or more seeds of all pod types were selfed in this instance. The pollinator used seems to play an important role in how much foreign pollen is available to effect cross-pollination. Megachile causes much less cross-pollination than endemic pollinators on the basis of this information and this fact is supported by the percentage cross-pollination record in Tables 18 and 19. The endemic pollinators were recorded to cause approximately two to five times as much cross-pollination as Megachile. It is noted also that within a given pod type the amount of self-pollination is higher at 40 rods than at 10 and 20 rods with the exception of the four or more seeded pods. The amount of cross-pollination for this latter class remained quite constant for the three distances. An explanation of higher percentages of self-pollination at increased distances from the contaminant source is advanced on the grounds that as an insect forages, the pollen from the colored source is gradually replaced or covered by the pollen from the white-flowered clone and hence the incidence of selfed seed increases with each successive floret tripped. The possibility also exists that deposition of a single pollen grain from the colored source aids in development of selfed ovules and therefore pods with one or more crossed seeds occur with a variable number of selfed seeds.

With the observation that selfed and crossed seeds occur within the same pod the question arises concerning the order of selfed and crossed seed. Foreign pollen grains might be expected to grow down the style of the flower more rapidly and therefore fertilize all the ovules at the apex of the ovary. The data indicate that this is not the case. It appears that fertilization of ovules in the ovary occurs more or less at random. The more rapidly growing foreign pollen grains do not necessarily fertilize ovules linearly from the apex because selfed and crossed seeds were observed at random in the ovary.

A demonstration of the variable nature of pod set and seed produced as a result of season introduces a difficult problem in the comparison of data in different years. The data demonstrate that a comparison of selfing attributed to different bee species in different seasons would not be a valid one unless it is considered in terms of the season that the information was recorded.

Estimates of the fertility of several genotypes demonstrate that there is good agreement in fertility ratings when pod or seed number is considered. Average seed per pod does not appear to be a good measure of the fertility of a genotype because the number of pods produced is not a good criteria of number of seeds that will be produced. A simple record of total pods produced per unit number of florets tripped is as good a comparative measure as any.

One would expect that the pollinator that tripped fewer florets on a raceme and moved from plant to plant more frequently would produce the most cross-pollinated seed. On the basis of trips per floret *Apis* would be expected to produce more cross-pollinated seed in this study since in both years this species tripped substantially less florets per raceme than *Megachile*. However, to assess a bee's worth purely on the basis of florets tripped per raceme could be misleading. First of all, the manner and means of collecting pollen can be a significant factor because the amount of pollen foreign to a particular genotype is handled by bee species in different ways. *Megachile* carries its pollen on a ventral abdominal brush while *Apis* carries pollen in pollen baskets on the hindleg. It is conceivable then that successive pollen collections by the two species can alter the pollen supply on the insect in different ways and this could influence the amount of cross-pollinated seed produced. Second, the bee that makes fewer pollen collections between different genotypes is likely to deposit more foreign pollen on each genotype and hence produce more cross-pollinated seed.

Efforts to correlate tripping and seed production have usually failed in the past. Pedersen and others (30, p. 178) reported that when one-third of the flowers were tripped 66.4 percent of the cross-pollinated flowers formed pods compared to 44.7 percent when all flowers were tripped. The supposition was that there was not sufficient photosynthetic surface available to support the development of a large number of fruits (6, p. 412). Data obtained in this study generally support the view that increasing tripping rates cause fewer pods and seeds to develop. It appears that the amount of variability obtained both for percentage pod set and average seeds per pod are a primary function of the amount of foreign pollen available to effect pollination and a secondary function of the number of leaves available to produce the necessary photosynthate for the development of pods and seeds. The work in this study points out that one should be cautious about using percentage pod set information since it was demonstrated that this value was not necessarily a good criterion of the magnitude of the more reliable average seeds per pod value.

Assuming that bees pick up partial loads of pollen at the colored-flowered base plot and forage progressively further away into the white-flowered plots it would be expected that more and more pollen from the white-flowered source would accumulate on the bees. If this is true less pods and a smaller average seeds per pod should occur at increasing distances from the colored-flowered base plot.

However at distances up to 40 rods this expected trend was not clearly defined. In some cases there was a decided decline in percentage pod set and average seeds per pod at 40 rods while in other cases the percentage pod set increased and the average seeds per pod values declined. However, the overall averages for percentage pod set and seeds per pod are surprisingly similar and only when considering individual bee species is there some indication of differences at three distances of 10, 20 and 40 rods. Only with Nomia and partially so with Megachile was there a substantial decline in average seed produced per pod. It appears that considerably greater distances from the colored-flowered source are necessary to demonstrate marked declines in average seeds produced per pod.

Of the many environmental factors influencing seed production undoubtedly pollination by the bee is the most important and the least well known. Data by Boren and others (7, p. 188) demonstrated that Apis mellifera has a surprising ability to select certain genotypes in a population and repeatedly return to them. In one instance, one honey bee foraging 45 replicated, randomly arranged alfalfa clones in a greenhouse showed complete specificity to a single clone during a long foraging trip. Similar information was obtained in this study with Megachile and Apis in the white- and colored-flowered plots. However, no attempt was made to establish the identity of individual bees but rather an attempt was made to demonstrate that

Megachile and Apis were collecting pollen from some genotypes to the exclusion of others. Megachile did not seem to show any preference for the white clone or colored bloom, but very definitely Apis did prefer to collect pollen from the purple flowered population of Vernal alfalfa. What constitutes the basis for selection is not known but very likely flower color or volatile flower constituents would be a good possibility.

Computation of data to ascertain the most desirable planting pattern can be derived in three ways. Either from the percentage pod set, from the average seeds produced per pod or from a record of crossed seed produced on the white-flowered clone. Of the methods used, percentage pod set was the most variable since the values obtained by Nomia and Megachile were the highest in the 6 x 6 rowed plot even though the amount of cross-pollination in this plot was less than in the 1 x 1 and 2 x 2 rowed plots. It appears that the most reliable pod estimate should be obtained from the plot which produced the highest quantity of seed for a given number of pods. The data at hand indicate that pod set is not reliable since it has been demonstrated that the largest number of pods does not necessarily yield the largest number of seeds. After all, the largest amount of cross-pollination is expected to be produced in the plot with the most seed but not necessarily to produce the most pods. The fact that the values of all three bee species for all intents and purposes gave the highest estimates in the plot with the highest average seeds per pod is reasonable evidence that the estimate from the average seeds per pod is more reliable than one from percentage pod set. In this investigation the plot planted to alternating rows of white-flowered and colored-flowered alfalfa had the highest average seeds per pod. Information regarding cross-pollination was substantiated by recording flower color for a sample of seeds in each of the seven planting patterns investigated. In this way there was no doubt that more cross-pollination occurred in the 1 x 1 rowed plot.

SUMMARY AND CONCLUSIONS

This investigation was undertaken to gain information about some of the factors that affect seed formation in alfalfa. At the present time very little information has been forthcoming comparing pollen dispersal by bees and production of seed. Information of this type is useful to establish the reliability of the present isolation standards in alfalfa, to determine the usefulness of several bee species as breeding tools to effect cross-pollination in isolated breeding blocks and to ascertain the degree of self-pollination by insects and other related phenomena.

In this study seven plots of a white-flowered clone of alfalfa were placed at various distances from a colored-flowered source and seven plots of different planting patterns were placed at equal distances in another direction. This plot layout thus formed an L-shaped pattern with the color-flowered base plot at the intersection of the two arms of the L. Three bee species, *Apis mellifera* L., *Megachile rotundata* F. and *Nomia melanderi* Ckll., were used to gain an appreciation of their value. Data were also recorded for bees that were endemic to the area. Sampling procedures were such that harvested pods and seed could be definitely attributed to a given bee species.

The conclusions of this study are summarized below.

The frequency of cross-pollination decreases rapidly at 40 rods and 20 rods for *Apis* and *Megachile* respectively. Cross-pollination by endemic pollinators follows a trend similar to *Apis* but exceedingly high values are obtained at 10 and 20 rods. Six and one-half percent cross-pollination was noted in one year one mile from the contaminant population. It is recommended that isolation distances of 10 and 20 rods be eliminated so that Breeders and Foundation seed varieties be spatially isolated 80 rods while Registered and Certified seed varieties be isolated at least 40 rods.

Estimations of broad-sense heritabilities derived from clonal alfalfa and Vernal alfalfa proved to fluctuate markedly in response to bee species, planting pattern and season recorded. It is noted that *Megachile* gave a more consistent estimate in the two years of this study. Consideration of the magnitude of environmental variance in relation to total variance should perhaps be considered with broad-sense heritability estimates since the environmental variance possibly gives a better indication of what comprises the broad-sense heritability estimate.

Use of number of seeds per pod as a criterion to establish whether or not seeds in a pod are crossed or selfed is an uncertain one. Data from endemic pollinators establish that one seeded pods contained 94.7 percent selfed seed, two seeded pods 88.3 percent, three seeded pods 36.3 percent and four or more seeded pods 23.1

percent selfed seed. Data from *Megachile* pollinators demonstrate that seeds per pod fluctuates markedly with bee species and distance of a genetically uniform population from a contaminant source. All pod types from the *Megachile* study produced 67.8 percent selfed seed or more.

The order of crossed and selfed seeds within alfalfa pods is not necessarily selfed seed at the base and crossed seed at the apex as might be expected. The distribution of crossed and selfed seeds seems to be more or less at random, likely depending on the amount of foreign pollen available on the pollinating insect.

Estimates of fertility of several genotypes of Vernal alfalfa and a white-flowered clone demonstrate that fertility measurements vary with the season and that estimates from pod set and selfed seed produced give the same rating while the rating derived from average seeds per pod is quite different.

A comparison of the number of florets that *Megachile* and *Apis* trip per raceme demonstrated that the *Megachile* bee tripped 27-50 percent more florets per raceme than *Apis*.

Generally, an increase in tripping rate caused decreases in pod set and average seeds per pod. Exceptions to this general conclusion were noted, but it is felt that lack of sufficient foreign pollen and adequate photosynthetic area could explain the discrepancies.

One expects less cross-pollination and consequently less pod set and numbers of seeds per pod in a recessive white-flowered population at increasing distances from a dominant flower color source. However, at distances up to 40 rods this observation did not always hold true and therefore greater distances are required to establish consistant declines in seed production.

Of the two bee species compared intensively (*Apis* and *Megachile*) *Apis* appeared to cause more cross-pollination as measured by average seeds produced per pod and caused more cross-pollination at 40 rods from the colored-flowered base plot than *Megachile*. Information derived from the flower color study support the above conclusions regarding the amount of cross-pollination caused by the pollinators.

Megachile were observed to show very little preference between white- and colored-flowered alfalfa while *Apis* definitely preferred to collect pollen from purple colored flowers in the population.

The planting pattern of several genotypes of alfalfa has a marked effect on the amount of cross-pollination that takes place. Of the 1 x 1, 2 x 2 and 6 x 6 rowed planting patterns assessed the 1 x 1 pattern proved to be superior.

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