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A Study of the Clover Seed Chalcid Infestation of Various Alfalfa Varieties in Utah

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A STUDY OF THE CLOVER SEED CHALCID INFESTATION
OF VARIOUS ALFALFA VARIETIES IN UTAH

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Reed C. Bunker

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INTRODUCTION

One of the limiting factors associated with seed production of alfalfa and certain clovers is a small jet-black wasp commonly known as the clover seed chalcid, *Bruchophagus gibbus* (Boheman). In alfalfa seed producing areas this insect is often referred to as the alfalfa seed chalcid or the alfalfa seed chalcis-fly. In local areas of Utah, farmers often refer to this insect as simply "the fly."

Nature of Problem

The clover seed chalcid is a seed destroyer. Each year this insect destroys about 10 to 20 percent of the alfalfa seed crops. At various times infestations as high as 60 percent have been reported in Utah.

The clover seed chalcid is widely distributed. It is found in almost every area of the world where the host plants grow and produce seeds. The exact origin is unknown, but the clover seed chalcid was first described in 1879 in the United States. When first described this insect was thought to be beneficial, but after careful observations the clover seed chalcid was recognized as an important plant pest.

Suitable methods of control have not been developed. Cultural practices are the only method of control being recommended and many of these do not appear practical to seed producers.

Objectives

The primary purpose of this study was to determine if clover seed chalcid infestations were different among alfalfa varieties. The study included 40 alfalfa varieties being grown in varietal trials and commercial varieties in fields.

Several other closely-related projects that developed as the study progressed, included: (a) a study of the chalcid-infested seeds in alfalfa chaff and screenings from threshing machines as sources of adult chalcids; (b) estimation of the numbers of male and female clover seed chalcids and chalcid parasites that emerge from infested seeds collected in pod samples; (c) applying a sucrose solution to blossoms and young seeds to see if adult chalcids might be attracted into an area for possible control; (d) to determine the possible correlation between adult chalcid populations as estimated by sweepings with an insect net and percentages of infested seeds in seed samples; (e) determining the extent of chalcid damage in various areas of plants for more reliable estimates in sampling; and (f) sampling areas within several fields to determine the distribution of chalcids.

REVIEW OF LITERATURE

Classification of Insect

The clover seed chalcid was first described by Howard in 1879 (Comstock 1880). He named this insect *Eurytoma funebris* Howard and placed it in the subfamily Eurytominae, family Chalcidae, and order Hymenoptera. Titus (1904) indicated that Ashmead placed this species in a new genus, *Bruchophagus*. Later, the chalcid was placed in the superfamily Chalcidoidea, family Eurytomidae, and the name was changed to *Bruchophagus gibbus* (Boheman), the present classification.

Most of the Eurytomids are considered beneficial, being parasitic upon harmful insects. When the clover seed chalcid was first described it was thought to be a parasite of the clover seed midge. However, after careful observations by Hopkins in 1896, it was found to be a highly destructive pest of clover seeds (Urbahns 1920).

Kolobova (1950) in Russia reported that 2 races of chalcids and maybe 3 were involved in infestations of clovers, alfalfa, and birdsfoot trefoil. She stated that differences were observed in the time spent in the pupal and adult stages, in biometrical measurements, and that insects collected on either alfalfa or clover would not infest the seeds of the other in cages. Biometrical measurements over a 3-year period showed constant differences in the ratio of abdomen to thorax lengths, length of ovipositor, and shape of eggs. She suggested the subspecies name *medicaginis* for the race of chalcids in alfalfa. Kolobova also indicated that a third race may infest *Lotus corniculatus* (birdsfoot trefoil) since the shape of chalcid eggs produced was different from that of the other 2 races.

Extent of Damage

In the alfalfa, and bur, red, and crimson clover seed producing areas of the world clover seed chalcids are of great importance. However, where these crops are grown for forage rather than for seed these insects are of little significance. Lieberman and Knowlton (1955) reported that the losses from chalcid-damaged seeds can render alfalfa seed production unprofitable.

All immature stages of the clover seed chalcid are completed within infested seeds. Adult females deposit their eggs directly into young, soft, green seeds with one seed being destroyed by each developing larva. The insects pupate within infested seeds and each newly formed adult emerges by gnawing a hole through the seed coat and crawling out. These emergence holes in seeds are indicators of clover seed chalcid damage.

The total amount of damage caused by clover seed chalcids is difficult to determine. Urbahns (1914) discussed the possibility of seeds becoming infested before they have developed enough to support growing larvae. Under these conditions the seeds and larvae are both destroyed. Oviposition may occur after seeds have passed their optimum point for infestation (Sorenson 1930). Seeds appear almost normal and are often difficult to separate from uninfested seeds when infested late in development.

Estimations of chalcid damage are determined by collecting seed samples and counting the numbers of infested seeds present. Sorenson (1930) described the characteristics of infested seeds. He described them as usually being ". . . discolored, misshapen, and more or less dwarfed." Mature infested seeds are usually soft and easily broken. Sometimes they appear almost normal in size, shape, and color; for this reason the samples are usually examined under a binocular microscope.

Sorenson and Knowlton (1951) reported that clover seed chalcids destroy between 0.3 and 63.0 percent of the alfalfa seed crops each year in Utah. Sorenson (1930) reported the average annual infestation of chalcids was 15.84 percent over a 4-year period. This amounted to an average loss of approximately $6.10 per acre each year. Wildermuth (1931) observed that the range of chalcid infestations was from 2 to 85 percent on any one crop and amounted to losses of from less than 30 pounds to about 300 pounds per acre. The extent of chalcid damage is not uniform from time to time within the same area. Freeman (1914) cited an example where about 6 percent of the alfalfa seed was infested at Yuma, Arizona, in June 1910, whereas one year later the infestation was about 50 percent. Urbahns (1914) indicated that seed samples from early crops were about 10 to 30 percent infested with chalcids and those from late were 20 to 70 percent infested. According to Wildermuth (1931), early volunteer alfalfa growing in waste areas is often highly infested by chalcids that emerge early in the spring.

Host Plants

Plants which have been reported as host plants for clover seed chalcids are:

Medicago arabica (Urbahns 1920)
Medicago falcata - alfalfa (Urbahns 1920)
Medicago hispida denticulata - bur clover (Urbahns 1920)
Medicago hispida nigra - bur clover (Urbahns 1920)
Medicago hispida terebellum - bur clover (Urbahns 1920)
Medicago ruthemia (Urbahns 1920)

Medicago sativa - alfalfa (Urbahns 1914 and 1920, Sorenson 1930, and others)
Medicago tuberculata (Urbahns 1920)
Medicago tunetana (Urbahns 1920)
Lotus corniculatus - birdsfoot trefoil (MacDonald 1946)
Trifolium incarnatum - crimson clover (Sorenson 1930)
Trifolium pratense - red clover (Urbahns 1920)
Astragalus douglasi - Douglas or milk vetch (Bridwell 1923)
Oxytropis lamberti - crazy weed (Bridwell 1923)

Distribution of Insect

Clover seed chalcids have a wide distribution. They are found in almost every area of the world where the host plants grow and produce seeds. Urbahns (1914) reported that cultivated alfalfa seed imported from Germany, Turkestan, and Chile; and both cultivated and uncultivated varieties imported from Turkey and Siberia contained chalcid-injured seeds. In the United States, according to Wildermuth (1931), the clover seed chalcid seems to reach the highest numbers in the irrigated sections of the west and southwest.

Description of Insect

Howard gave the first description of adult clover seed chalcids according to Comstock (1880), and Folsom (1909) described the various stages of development.

Adults

Sorenson (1930) described the adult insect as being about one-twelfth inch long and having two pair of nearly colorless wings, which span about one-ninth inch. The adults are ". . . jet black in color, with exception of certain parts of the legs which are yellowish-brown."

Sorenson (1930) discussed several differences between adult male and female clover seed chalcids. Generally, females are slightly larger than males. Male antennae are more distinctly separated than females and have 11 segments; all but the first three segments are coarsely pubescent. Female antennae have 10 segments and bear fine, short hairs. The female abdomen is more closely joined to the thorax, is larger, and somewhat more pointed at the posterior.

Eggs

The eggs of the clover seed chalcid are too small to be seen with the naked eye. Sorenson (1930) described the eggs as being about one-one hundred and twenty-fifth (1/125) inch long, water-colored, and elliptical in shape. One end is pointed and the other is drawn out into a long tube-like structure about two or three times as long as the main part of the egg.

Larvae

Newly hatched larvae are small, about the same size as eggs. After starting to feed they develop a greenish color; by the time the larvae are mature they are white except for a pair of brown mandibles. The full grown larvae are without legs or feet and are grub-like in appearance; they vary in length from about 1.5 to 2.0 mm. (Sorenson 1930).

Pupae

Changes in pupal development were observed by Sorenson (1930). He reported that the pupa is white when first formed and through development the eyes become pink, bright red, brown, and then dark brown. Meanwhile, the abdomen becomes transversely banded with black and appears gray. The head and thorax become pigmented and the entire body becomes jet black except for the eyes. Wings, legs, and antennae are folded next to the body and the insect is encased in a thin, transparent, pupal skin. Fully developed pupae average about one-thirteenth inch in length.

Life Cycle

Length of the life cycle seems to depend upon temperature and moisture content of the seeds. Sorenson (1930) indicated that decreases in the temperature extended the time necessary for insect development and caused adults to become sluggish or inactive. The life cycle may be completed in 30 to 40 days under favorable conditions (Peairs and Davidson 1956) or it may extend over an indefinite period if the conditions are not favorable when the larval stage is completed (Wildermuth 1931). Sorenson (1930) observed that the average length of time required for development from egg to adult was 23 days for summer broods. Vinogrado (1941) in Russia reported that in both field and laboratory observations adults emerged when the mean temperature reached 18 to 20 degrees centigrade and during the winter chalcids emerged in 2 to 3 days when kept at this temperature if the seeds had at least 15 percent moisture.

Spring emergence

According to Lieberman and Knowlton (1955), the hibernating larvae from the previous season's infestation, pupate in the spring with the rise in temperature ". . . and emerge as adults . . . about the time the first flowers appear on alfalfa." In the warm irrigated valleys of the western and southwestern United States emergence may be as early as March, but in the colder northern areas adults may not appear until early June (Wildermuth 1931). In the Uinta Basin of Utah Sorenson (1930) observed clover seed chalcids in alfalfa seed fields about the first 2 weeks of May.

Adults

In the Uinta Basin the first adults that emerged in the spring were males and the population remained predominantly males throughout the season, according to Sorenson (1930). Females appeared several days later and mating occurred. The females then flew about searching for suitable host plants for oviposition. Sorenson also observed that females did not migrate extensively when in fields favorable for oviposition. However, Wildermuth (1931) indicated that these insects are strong fliers and may ascend high into the air where they are carried by winds to neighboring fields. This might be the case when suitable host plants are not available for oviposition.

The extent of activity and length of adult stages, as reported by Urbahns (1914), depends upon the weather conditions. Activity is greatest during hot weather, but the chalcids seek shade in the heat of the day. Apparently, the adults visit alfalfa blossoms to secure food and may live to be several weeks old when weather conditions are moderate. Sorenson (1930) reported that most of the adults confined in breeding cages lived only 2 or 3 days and all were dead within 2 weeks. However, Sorenson also indicated that adults may live longer in the fields under natural conditions.

Sorenson (1930), from field studies conducted in the Uinta Basin, indicated that during average climatic conditions adult chalcid populations gradually increased to a maximum number near cutting time with exception of a slight drop after the first brood emerged. Population numbers dropped when the nights turned cold and disappeared with the first frost in the fall.

Oviposition

After mating, females seek suitable host plants for oviposition. Sorenson (1930) observed that females seek newly formed seeds in a semi-fluid or jelly-like condition and will not oviposit in seeds after they have reached the dough stage or when the seed materials have started to harden. In dissecting thousands of green seeds, Sorenson found that less than 1 percent contained more than one larva or were infested too late for the insects to complete development before the seeds hardened.

The act of oviposition was described by Sorenson (1930). He reported that ". . . the female bends her abdomen ventrally and forward, extrudes her stinger-like ovipositor and thrusts it through the pod and seed coat into the soft substance of the kernel where the egg is deposited." Urbahns (1920) reported that the time involved in oviposition was about 1 minute. Sorenson (1930) observed one female oviposit in six seeds on the same raceme over a 15-minute period.

In order to determine the number of eggs an adult female might oviposit in her life time, Sorenson (1930) dissected 50 fertilized females which had been fed in captivity for 48 hours and examined them under a binocular microscope. "In various individuals dissected the number of eggs was found to range from 24 to 66, with an average of 42.24." These eggs were approximately the same size and shape as eggs oviposited in a normal manner.

Urbahns (1920) reported that ". . . the parthenogenetic habit is well established in the females of this species." He observed that adult females reared from pupae under isolated conditions, oviposited freely when placed in cages containing uninfested green seeds suitable for oviposition. The progeny consisted of all males and the largest number of offspring observed from an individual female was 20.

Egg stage

The egg stage is the shortest of all stages in the life cycle. According to Wildermuth (1931), "The eggs hatch in from 3 to 12 days, depending upon the temperature at the time development is taking place." The average egg stage is about 4 days (Lieberman and Knowlton 1955).

Larval stage

Sorenson (1930) reported that clover seed chalcid larvae may be found in the fields approximately a week after the pods have begun to curl. About 1 day after the larvae are hatched, they start to feed and by the time the pods have ripened most of the larvae have completed their growth (Wildermuth 1931). Only one larva can develop within each seed. Under favorable conditions the larval stage lasts from 10 to 15 days, but Wildermuth indicated that where conditions are too dry at the end of feeding, the larvae may aestivate--go into a resting state. In this condition they may remain within the seeds for periods lasting 1 or even 2 years.

Pupal stage

Urbahns (1914) reported that if sufficient moisture is remaining in the seeds after feeding is completed the larvae at once transform to the pupal stage and remain for a period of 10 to 40 days. Wildermuth (1931) suggested that the pupal stage lasts from 5 to 40 days and Sorenson (1930) reported that the average length of the pupation period is about 12 days. At the end of the pupation period adults emerge and the life cycle is continued.

Generations per year

The length of the growing season apparently determines the number of generations which can develop each year. Wildermuth (1931) reported that as many as six generations may occur in those areas of the western and southwestern United States, where the growing season begins about March and extends until November. In Utah there are two and sometimes three generations each year (Sorenson 1930). Differences in numbers of generations per year have a marked effect on the extent of damage sustained by the clover seed chalcids (Wildermuth 1931).

Overwintering stage

Clover seed chalcids overwinter as mature larvae within seed coats of infested seeds. Lieberman and Knowlton (1955) suggested that the four major places where infested seeds containing overwintering larvae may be found are: (1) on the ground in seed fields from shattering of ripe seeds from pods and from threshing with a combine; (2) in and around chaff stacks where the light infested seeds have fallen and are blown during threshing; (3) on and around volunteer and unharvested host plants; and (4) in uncleaned seeds and the screenings from cleaned seeds. Sorenson (1930) found that about 75 percent of the infested seeds from first crop contained overwintering larvae and about 84 percent of the second. Vinogrado (1941) in Russia reported that 5 out of 6 infested seeds in the threshed portion contained living larvae and 1 out of 13 in the chaff.

Control

Control of clover seed chalcids is one of the major problems confronting alfalfa seed producers.

Chemical control

Very little has been reported on the use of chemicals for clover seed chalcid control. In the Biennial Report of the Utah Agricultural Experiment Station (1932) it was reported that an unsuccessful attempt was made to control the clover seed chalcid in field tests using home-made nicotine dust, dusting sulphur, and Cyanogas at three levels. Sorenson and Knowlton (1951) and Lieberman and Knowlton (1955) reported that satisfactory methods of chemical control have not been developed. The use of chemicals would necessitate applications during the blossom period; such a practice may destroy insect pollinators working in the fields.

Cultural control

Cultural practices are the only recommended method of controlling the clover seed chalcid. Control by cultural practices requires community cooperation. If all recommended cultural practices were applied, the extent of damage could be substantially reduced. The recommended cultural practices are: (1) grow either first or second crop alfalfa seed in the same area (Lieberman and Knowlton 1955); (2) grow only one type of host plant in an area (Wildermuth 1931); (3) manage the seed crop so that ripening is as uniform as possible (Lieberman and Knowlton 1955); (4) prevent all volunteer host plants from forming seeds (Lieberman and Knowlton 1955); (5) utilize badly infested seed crops as hay and remove from fields as soon as possible (Peairs and Davidson 1956); (6) eliminate all chaff stacks before the clover seed chalcids emerge in the spring (Sorenson 1930); (7) reclean all seed and destroy or feed screenings (Lieberman and Knowlton 1955); and (8) cultivate to bury infested seeds which have fallen to the ground (Sorenson 1930). In the Biennial Report of the Utah Agricultural

Experiment Station (1932) an example was cited where community control was practiced and the average annual infestation was reduced from 35.9 percent in 1929 to 5.53 percent in 1930. Since the average infestation for Utah was 6.29 percent less in 1930 than in 1929, the total difference was not all attributed to cultural practices.

Natural control

Ten known species of parasites which attack larvae and occasionally pupae of the clover seed chalcid are found in the United States. These parasites are closely related to their host, *Bruchophagus gibbus* (Boh.), in that they also belong to the superfamily Chalcidoidea. The ten species as reported by Butler and Hansen (1957) include: *Liodontomerus perplexus* Gahan, *Liodontomerus insuetus* Gahan, *Liodontomerus longfellowi* (Girault), *Amblymerus bruchophagi* (Gahan), *Trimeromicrus maculatus* Gahan, *Habrocytus medicaginis* Gahan, *Tetrastichus bruchophagi* Gahan, *Tetrastichus venustus* Gahan, *Eupelmella vesicularis* (Retzius), and *Eupelmus* sp. Gahan (1914, 1915, and 1917) did much of the work in classifying and describing new species of parasites. Many of the species described by Gahan were reared by Urbahns from seed samples collected in Arizona and California. Research workers at the Arizona Agricultural Experiment Station are presently engaged in a study to determine the role these parasites play in controlling clover seed chalcids.

The extent of parasitism varies from season to season and area to area. Peairs and Davidson (1956) reported that in warmer areas parasites are able to develop nearly as fast as clover seed chalcids; hence, parasitism is increased. Sorenson (1934a) observed from seed samples collected in 1932 that 116 chalcids emerged; of these 90.56

percent were clover seed chalcids, 1.88 percent were Eutelus bruchophagi Gahan [Amblymerus bruchophagi (Gahan)], and 7.56 percent were Liodontomerus perplexus Gahan. In samples collected in 1933, 9065 chalcids emerged into traps; of these 93.18 percent were clover seed chalcids, 2.67 percent were Eutelus bruchophagi Gahan [Amblymerus bruchophagi (Gahan)], and 4.15 percent were Liodontomerus perplexus Gahan. A few specimens of Eupalmella vesicularis (Retzius) and Habrocytus medicaginis Gahan were also reared from seeds.

Several parasites of the clover seed chalcid have been reported in Russia. Nikol'skaya (1932) listed the presence of ten species of parasites in Poltava which included: Tetrastichus tibialis Kurd., Tetrastichus brevicornis Nees, Tetrastichus roesellae Nees, Tetrastichus bruchophagi Gahan, Habrocytus medicaginis Gahan, Eupelmus microzonus Forst, Eupelmus astropurpureus Dalm., Eupelmus vesicularis (Retizius), Liodontomerus perplexus Gahan, and Eutelus sp. He also reported that parasitism in that area of Russia ranged from 23.8 to 80.9 percent and about 90 percent of the parasitism was done by two species, Habrocytus medicaginis Gahan and Tetrastichus bruchophagi Gahan.

Sorenson (1932) reported that benefits from parasitism result from reducing numbers of clover seed chalcids in succeeding crops rather than saving infested seeds, as damage has been done before parasitism occurs.

METHODS AND PROCEDURE

Preliminary Studies

In 1956 and 1957 preliminary studies of the chalcid problem in alfalfa seed were conducted by the Staff of the Utah Agricultural Experiment Station. Some of the preliminary studies were directed toward developing techniques and sound methods of sampling alfalfa seed for chalcid infestations. Seed samples were harvested from two alfalfa varietal plots during 1956 and also from several commercial seed-producing areas of the state to determine extent of chalcid infestations.

Most of the seed samples harvested during the preliminary studies were threshed with the Forsburg seed scarifier. These seed samples were scarified for 3 or 4 seconds, then screened with two small-seed screens of one-twelfth (1/12) and one-twenty-fifth (1/25) inch mesh, over a blank bottom pan (figure 1). Scarifying and screening was repeated two or three times on each sample to remove all seeds from their pods. After the seed samples were threshed, they were cleaned with a South Dakota seed blower. The samples were subsampled and the percentages of infested seeds calculated.

Broken infested-seed fragments were observed in several of the seed samples. A thresher was devised which broke fewer infested seeds. This implement was the homemade "rubbing board" illustrated in figure 2. The rubbing board consisted of two parts, a bottom base board and a hand operated crushing board. The top surface of the base board and

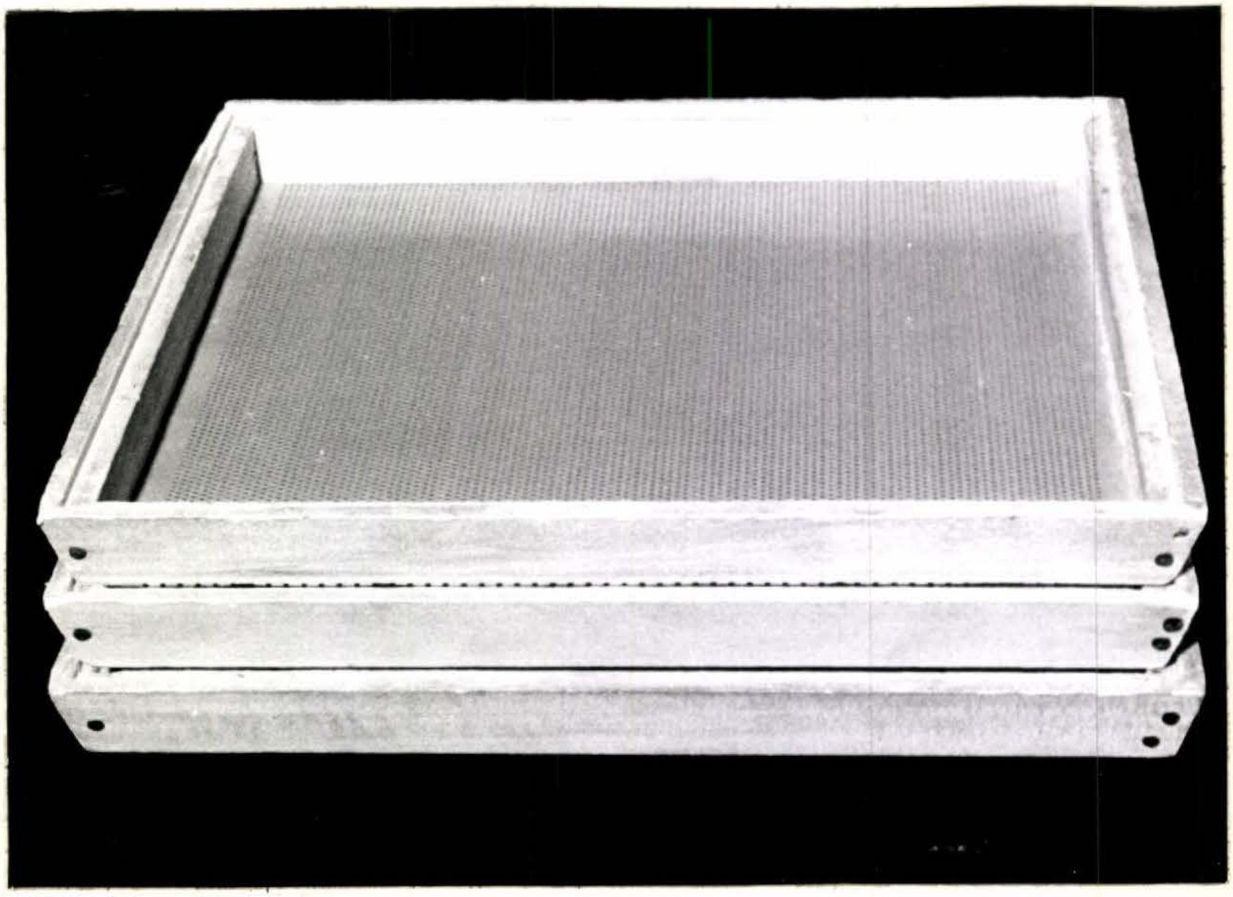

Figure 1. Small-seed screens used to separate the threshed seeds from uncrushed pods.

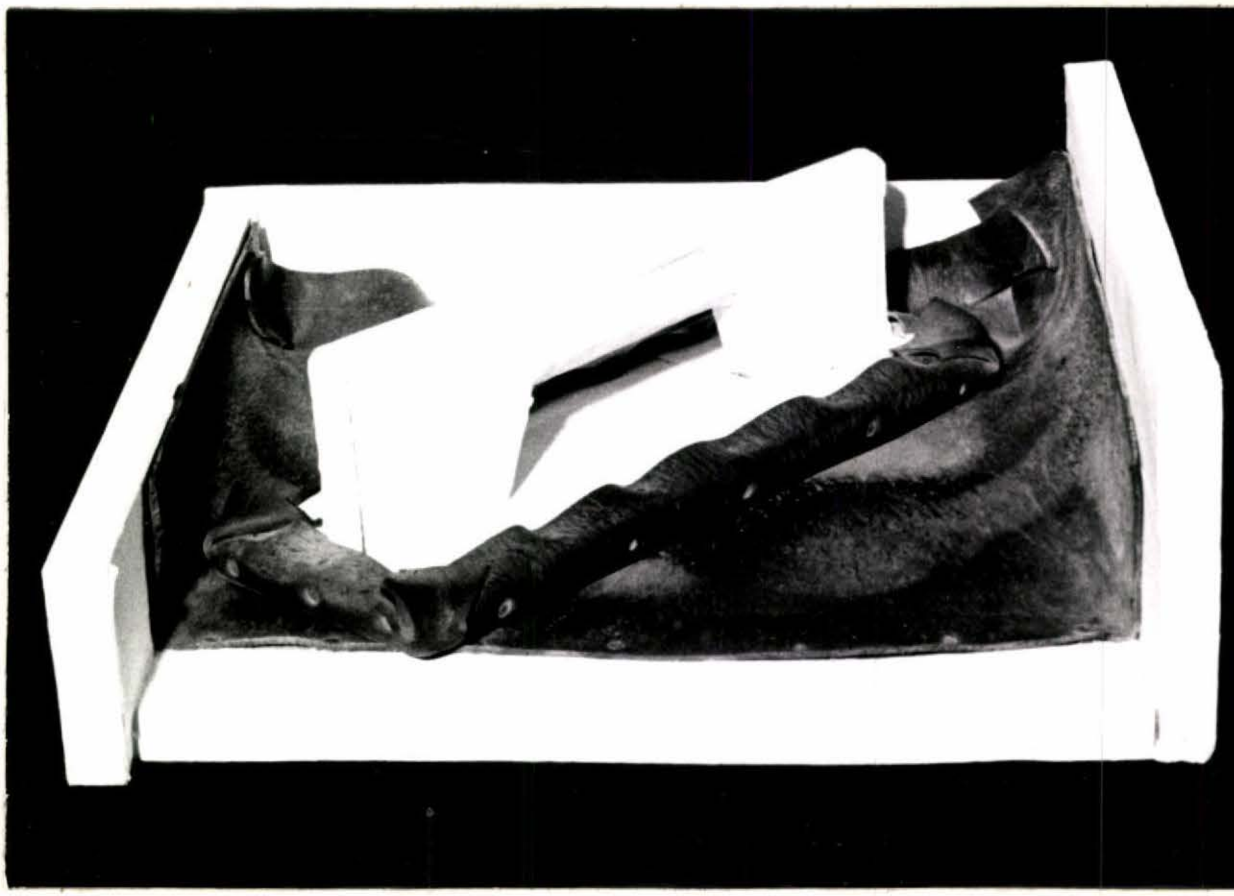

Figure 2. Rubbing board used in threshing small alfalfa seed samples.

the bottom surface of the crushing board were covered with pieces of rubber inner tube.

A sample of seed pods was placed on the base board and crushed lightly with the crushing board. The sample was then transferred to the small-seed screens where the crushed-out seeds were removed from the uncrushed pods. Uncrushed pods were returned to the base board where they were crushed with more force and again returned to the screens. Crushing and screening was repeated until all seeds were removed from their pods.

The two threshing implements, the Forsburg seed scarifier and the rubbing board, were compared for breakage of infested seeds. Five paired samples were threshed with both implements and the percentages of infested seeds in the samples calculated.

Since the South Dakota seed blower was not always available for cleaning seed samples, the "cleaning tray" (figure 3) was designed. The cleaning tray was made from a piece of heavy cardboard and was covered with flannelette cloth. The samples were cleaned by holding the cleaning tray at approximately a 45-degree angle, narrow end down so that it extended into a half-gallon container, and pouring the threshed samples onto the tray at the top. The round seeds rolled down the cleaning tray into the container and the chaff became lodged on the cloth-covered tray. Some seeds became lodged on the tray with the chaff; these were dislodged by shaking the tray. When all seeds had been carefully removed from the cleaning tray, the chaff was discarded. Seeds were returned to the cleaning tray as many times as was necessary to clean the samples.

The method of selecting subsamples from field samples included three steps: (1) placing the field sample on a piece of white paper

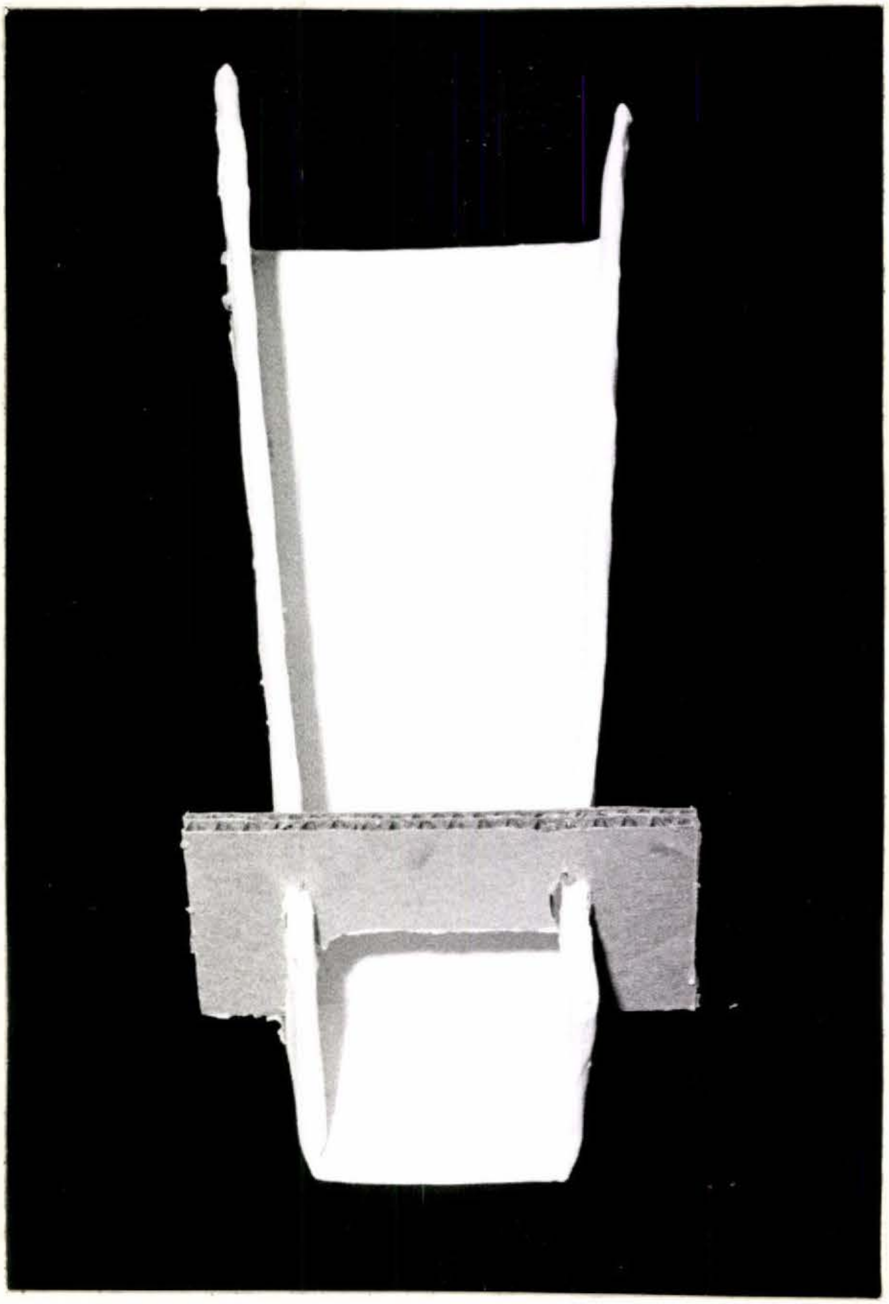

Figure 3. Cleaning tray used to remove chaff from small alfalfa seed samples

and thoroughly mixing; (2) heaping the seeds into a small pile and spreading them in a thin layer using a small flat-bottomed plate to press down on the pile in a circular motion; and (3) selecting a pie section of desired size from the spread sample. The exact numbers of seeds desired in the subsamples were counted from the pie sections and the numbers of infested seeds were tabulated.

In the preliminary studies 500-seed subsamples were drawn from the field samples. Counting small alfalfa seeds was tedious, time consuming, and expensive. Therefore, the possibility of using smaller numbers of seeds per subsample was explored. Subsamples of 500 seeds and 100 seeds were drawn from two seed samples of known infestations, 5 and 15 percent. The numbers of chalcid infested seeds were determined and the observed numbers analysed with the chi-square method.

Varietal Studies

Two alfalfa varietal plots were available for study during the 1958 season. One plot contained 8 alfalfa varieties (table 1) and the other plot contained the 8 varieties plus 32 more, a total of 40 varieties (table 2).

The 8 varieties

The 8 varieties were planted on the Evans Experimental Farm at Logan, Utah, on June 12, 1957, by R. C. Bunker, R. M. Taylor, and J. T. Knighton. The design was a randomized block with six replications. Each subplot consisted of four solid rows, 18 inches apart, and 9 feet long. The subplots were 18 inches apart. The side borders were planted to Ranger alfalfa in rows 9 inches apart. The side borders were five rows wide, and the top and bottom borders were 4 feet long.

Table 1. The 8 varieties of alfalfa used in the tagged raceme and bulk sample experiments with their indicated FC numbers, Logan, 1958

Variety no.	Variety	FC number
1	Buffalo	
2	Caliverde	32594
3	Ladak	
4	Nomad N B 51	
5	Ranger (Cert. R. A. 97)	
6	Rhizoma (Canadian Reg. 2299)	
7	South African	
8	Stafford	

Table 2. The 40 varieties of alfalfa used in the tagged raceme and bulk sample experiments with their indicated FC numbers, Delta, 1958

Variety no.	Variety	FC number
1	A-169	32075
2	A-224 Synthetic 1	
3	A-225 Northern Synthetic	24335
4	African (Arizona Com.) A4-35	
5	Arizona Chilean	23669
6	Atlantic	
7	B. Y. Strain (Borgeson-Santiquin)	
8	Buffalo	24190
9	Caliverde	32594
10	Cossack	24156
11	DuPuits	24340
12	Grimm	
13	Hairy Peruvian (Arizona Cert. '54)	
14	Kansas Common	24072
15	Ladak	
16	Lahontan (California Cert. '54)	
17	Meeker Baltic	23909
18	Narragansett	24333
19	Nemastan	
20	Nomad	24033
21	Ranger	
22	Rhizoma (Canadian Reg. 2299)	
23	Sevelra (Dickenson-Nampa, Ida.)	
24	South African N. K.	
25	Synthetic X A-249 O. P.	
26	Synthetic Y A-250 O. P.	
27	Synthetic Z A-251 O. P.	
28	Synthetic 4 clone	
29	Synthetic 7 clone	
30	Stafford	
31	Talent	32139
32	Terra Verde N. K.	
33	Turkish Wild O. P. (Bob Kneebone)	
34	Uruguay clone 10	23982
35	Vernal (Wisconsin Synthetic G.)	24790
36	Williamsburg	24152
37	919 (Nevada) N. K.	
38	919 (15) N. K.	
39	919 (20S) N. K.	
40	Common (Cameron Adams')	

No seed was harvested in 1957 from the 8 varieties. During 1958 first crop was cut for hay on June 4 and the second crop left for seed. Second crop is usually more highly infested with clover seed chalcids than is first.

Tagged racemes. One method of sampling the 8 varieties employed the use of small paper tags of different shapes and colors. When all varieties started to blossom, 10 tags of the same shape and color were tied to racemes of approximately the same blossom stage[1] on each subplot. Tags were tied to racemes on 10 dates, each Monday and Thursday during the blossom period from July 15 to August 21 with exceptions of August 11 and 14 when blossoms were not available.

When the alfalfa seed became ripe the 10 sets of 10 tagged racemes were harvested from each subplot, placed in labeled paper bags, and taken to the laboratory for analysis.

Samples from the tagged racemes were small, so threshing was done by rubbing the seeds out of the pods between the hands. After the samples were threshed they were cleaned with the cleaning tray and returned to labeled paper bags.

All seed samples were counted with the aid of a binocular dissecting microscope. Some of the infested seeds were unavoidably broken in the threshing process. These broken seeds were counted when at least one-half a seed was present or when a fragment contained a hilum.

Since the samples from the tagged racemes were small, all seeds in each sample were counted. Due to the small seed samples of variable size, data obtained from taggings could not be analysed as a split-plot design, as formerly planned. Instead, the data from

[1]Stage of blossom was defined as that condition when not more than 4 buds were present on the raceme and no flowers had fallen.

taggings were summed for each subplot and subplot percentages of chalcid infestation calculated. The infestation percentages were compared in an analysis of variance.

Bulk samples. In the second method of sampling the 8 varieties for chalcid infestation a bulk sample from each subplot was collected. After the tagged racemes were harvested, plant-stems from 1-foot areas of each of the four rows per subplot were cut and all pods removed. The pods were placed in labeled paper bags and taken to the laboratory for analysis.

The seed samples were threshed with the rubbing board and cleaned with screens and cleaning tray. Percentages of infested seeds were determined in two subsamples of 100 seeds which were drawn from each bulk sample and the data were analysed in an analysis of variance as a randomized block design.

Comparison of the two sampling methods. Percentages of chalcid infestations for tagged racemes and bulk samples were compared by analysis of variance to determine if the two methods gave similar results.

The 40 varieties

The 40 varieties were located on the Cameron Adams Farm, 1 mile north of Delta, Utah. They were planted on April 28, 1955 by Dr. D. R. McAllister, Dr. M. W. Pedersen, C. Adams, and K. Nielson. The basic design was a randomized block with four replications. Each subplot was 25 feet long and contained four rows, 8 inches apart. The subplots were 16 inches apart.

In 1958 permission was obtained to use the 40 varieties in the clover seed chalcid study. The first crop was cut for hay on June 12 and second crop was left for seed. On July 4 the entire plot was sprayed with a mixture of DDT and Parathion to control lygus bugs.

Tagged racemes. Ten racemes in approximately the same blossom stage were tagged within each subplot on four dates, at weekly intervals, between July 21 and August 12.

When the tagged racemes became ripe, the four sets of 10 tagged racemes were harvested from each subplot. The samples were threshed with the rubbing board and cleaned with screens and cleaning tray. A 100-seed subsample was drawn from each field sample and examined for infested seeds. The numbers of infested seeds were recorded and percentages calculated.

Because most samples were less than 20 percent infested, arcsin transformations were made on the sample percentages. The transformed data were analysed with the F test in an analysis of variance for a split-plot, randomized block design.

Bulk samples. Bulk samples were taken from the 40 varieties after the tagged racemes were harvested. Ten stems of alfalfa seed were selected at random from each subplot and all pods removed. The samples were threshed with the rubbing board and cleaned with screens and cleaning tray. Two 100-seed samples were drawn and counted. The numbers of infested seeds were recorded and infestation percentages calculated. The data were transformed to the arcsin percentages and analysed by analysis of variance.

Comparison of the two sampling methods. The two methods of sampling were compared in an analysis of variance for the 40 varieties.

Other Related Studies

Some other studies made in connection with the varietal studies as mentioned in the introduction were: chalcid emergence from infested seeds, use of sucrose to attract adult chalcids, correlation of adult chalcid populations with percentages of infested seeds, distributions of chalcid-infested seeds on plants, and distributions of infested seeds in fields.

Chalcid emergence from infested seeds

Adult chalcids were trapped in two types of cages: (1) large field-type cages which measured 35 inches long, 23 inches wide, and 29 inches high; were wooden framed; and were covered with dark brown denim cloth (figure 4); and (2) small seed-sample cages made from one-half gallon and 1 gallon ice cream cartons (figure 5).

From chaff and thresher screenings. Adult chalcids were collected in the large field-type cages from infested seeds in alfalfa chaff stacks (piles of chaff from threshing with a stationary thresher), thresher screenings, and chaff from a combine harvester in a field at Delta, Utah.

Three field-type cages were placed over chaff and thresher screenings on May 17 and 18, 1958. Each cage was checked two or three times weekly until emergence stopped, about June 26. Specimens were removed; counted; and the numbers of clover seed chalcid males, females, and other chalcids recorded. The cages were left over the chaff and screenings until late in the season and checked for chalcids several times, but none were observed after June 26.

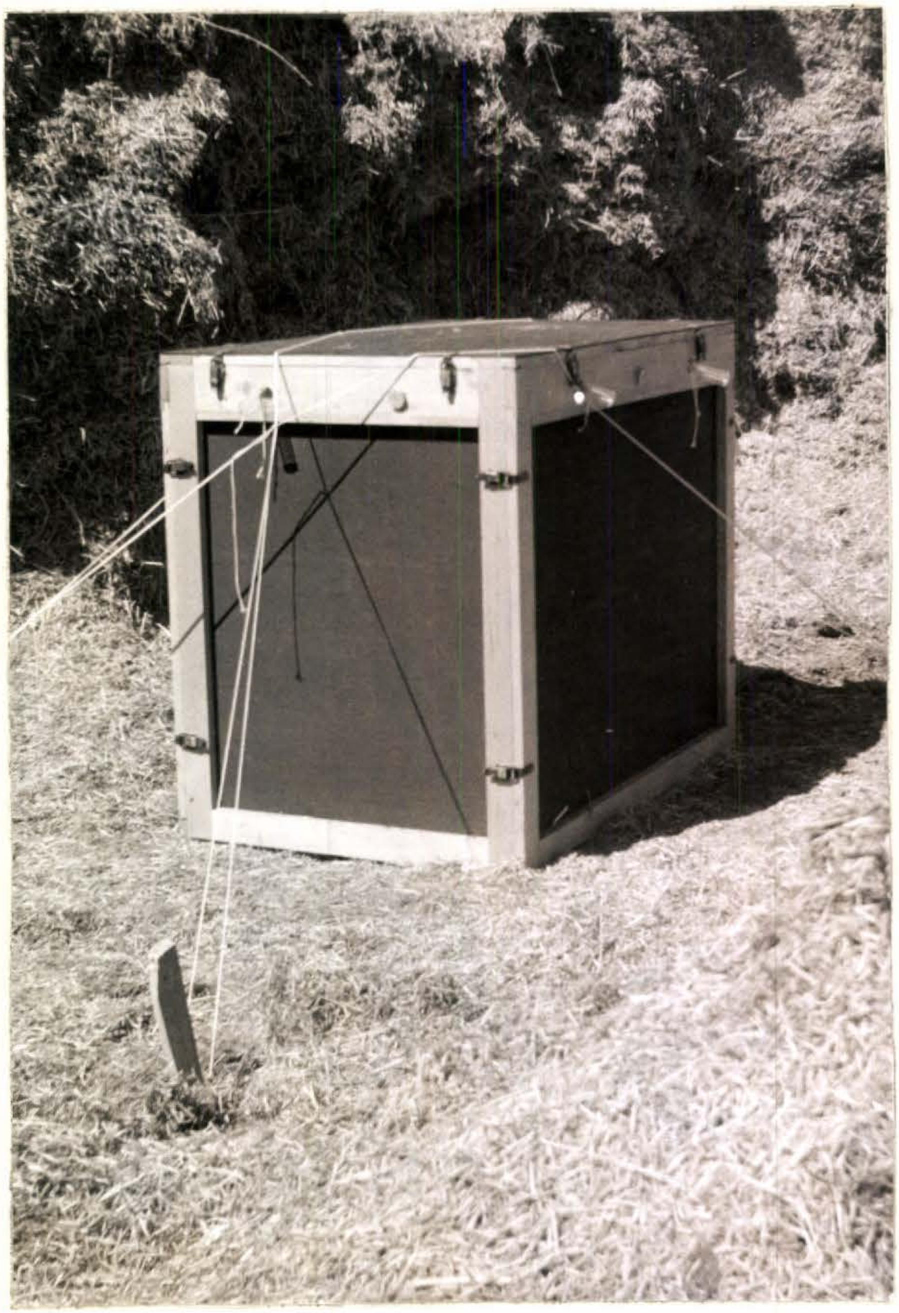

Figure 4. Large field type cage placed over thresher screenings to trap adult chalcids.

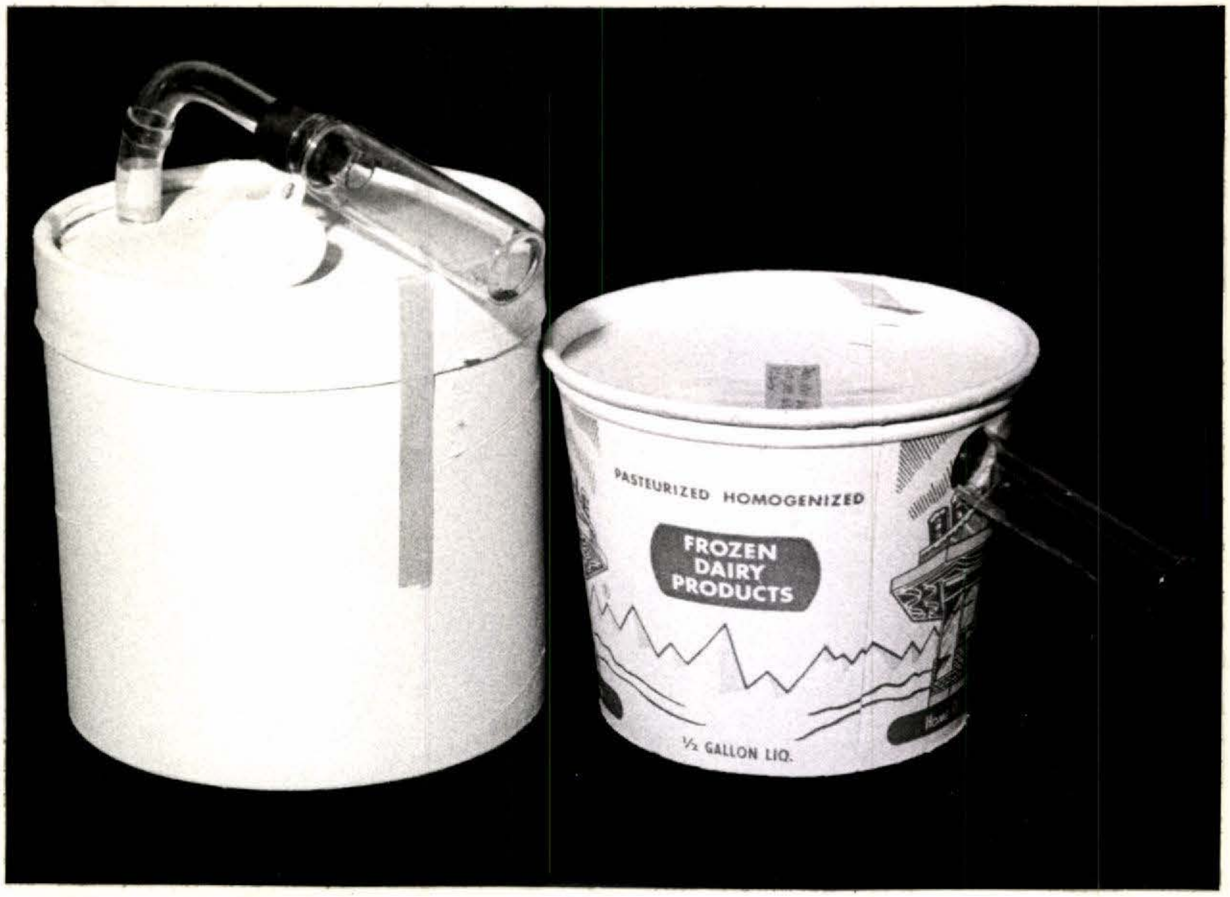

Figure 5. Small seed sample cages used to trap adult chalcids from field samples of alfalfa pods.

Three field-type cages were placed in a field over first crop chaff from a combine harvester. These were checked several times, but chalcids were not observed in them.

From pod samples. In determining the seasonal chalcid population in the Delta area, samples of seed pods were collected at about 15 day intervals and placed in the small cages. Six samples were harvested between July 15 and September 16, 1958, from first, second, and volunteer alfalfa seed crops. About 1 month after the pods were harvested the emerged chalcids were removed and counted.

Several samples were harvested in Cache Valley, however, these were not taken at regular intervals.

Use of sucrose to attract adult chalcids

This study was designed to explore the possibility of using a sucrose solution to attract adult chalcids to a particular area where the adults and immature forms could be destroyed. The attractant was 40 percent sucrose by weight in water. It was applied to selected racemes with a small atomizer. Applications were made on first crop blossoms and young seeds, June 25, 1958, at Delta, Utah.

On blossoms. This experiment included four sets of 10 paired racemes in the blossom stage. Each set consisted of 5 paired racemes on each of two plants. Paired racemes consisted of 2 racemes in about the same blossom stage on the same stem. One raceme from each pair was treated with the sucrose solution. All selected racemes were tagged and the tags labeled. Tagged racemes were harvested when ripe and bulk samples were taken from the same areas. The samples were threshed by hand and examined for chalcid infestations.

On young seeds. Twenty paired racemes in the young seed stage were selected and one from each pair was treated with the sucrose solution. When the seeds became ripe, they were harvested, threshed, and counted.

Correlation of adult chalcid populations with percentages of infested seeds

Sweepings were made at Delta with a standard 15-inch sweeping net in three alfalfa seed fields, two first and one second crop, while the fields were in the late blossom stage. The chalcids were captured and the numbers of clover seed chalcid males and females were recorded. When the seed became ripe, bulk samples were harvested, threshed, cleaned, and examined for infested seeds. Numbers of infested seeds were recorded and infestation percentages calculated. The total numbers of clover seed chalcids and the numbers of females per 10-sweep samples were each correlated with the percentages of infested seeds.

Distributions of chalcid-infested seeds on plants

The question arose as to where on plants seed samples should be taken. To answer this question, several alfalfa seed stem samples were harvested from the Delta area. The stem samples were cut at a height of 2 inches above the ground, tied, and taken to the laboratory for analysis. Each sample was cut into 3-inch sections from the top down. All pods were removed from each section and placed in a labeled paper bag. The samples were threshed and cleaned with the rubbing board, screens, and cleaning tray. Two 100-seed subsamples were drawn from each 3-inch section and examined for infested seeds.

Distributions of chalcid-infested seeds in fields

Five second crop alfalfa seed fields were sampled in the Delta area to determine the variation of chalcid infestations within and among fields. Small fields of approximately 16 acres or less were selected and each divided into six areas. Twenty stems were selected at random from each area and all pods were removed. The samples were threshed and cleaned with the rubbing board, screens, and cleaning tray. Two 100-seed subsamples were drawn and counted from each sample.

RESULTS

Preliminary Studies

Results of the alfalfa varietal plots in the 1956 preliminary studies suggested that chalcid infestations may not be uniform among varieties. Wide variations were observed in the data obtained and analyses of variance indicated high coefficients of variation, 47.6 and 106.5 percent. Some of the variations were probably due to losses of broken infested seeds in threshing.

The numbers of infested seeds observed in seed samples threshed by the two implements, the Forsburg seed scarifier and the rubbing board, indicated that fewer infested seeds were broken by the rubbing board. Two of the paired samples had nearly the same numbers of infested seed when threshed by both machines. Three of the samples threshed by the Forsburg seed scarifier yielded 11, 23, and 23 fewer infested seeds per 100 than their paired samples threshed with the rubbing board. The percentages of chalcid-infested seeds were high, approximately 60 percent, in four of the five samples.

Results of the preliminary investigation of subsample sizes indicated that subsamples of 100 seeds yielded nearly the same results of chalcid infestation as subsamples containing 500 seeds when drawn from the same field samples. The pooled chi-square value for subsamples of 500 seeds was 0.45, probability of 50 percent, and the chi-square value for subsamples of 100 seeds was 0.13, probability of about 70 percent. The low chi-square values indicate that the average variations of infested seeds observed within each subsample size were not great and might be expected in sampling from completely randomized seed samples.

Varietal Studies

The 8 varieties

Tagged racemes. Data from the tagged racemes indicated differences of chalcid infestion among the 8 varieties. These differences were significant at the 1 percent level of probability when analysed by analysis of variance. Mean percentages of chalcid infestation for the 8 varieties are ranked and Duncan's (1955) Multiple Range test applied as shown in table 3. The Duncan's test is used because the differences required for significance increase as means further apart in rank are compared. In the tagged raceme experiment of 8 varieties, mean percentages of chalcid infestation ranged from a high of 53.07 in Rhizoma to a low of 28.78 in Buffalo. Buffalo, Nomad, South African, Stafford, and Caliverde were not significantly different from each other, but were significantly lower than Ladak, Ranger, and Rhizoma.

Average percentages of chalcid infestation for the 8 varieties on 10 tagging dates are shown in figure 6. The averages for the 3 high and 5 low varieties are also indicated.

Bulk samples. Data from the bulk samples of 8 varieties indicated that significant differences of chalcid infestation exist at the 1 percent level. Mean percentages were analysed with the Duncan's test and the results are presented in table 4.

The range of means for bulk samples of 8 alfalfa varieties was from 61.40 percent in Rhizoma down to 37.82 percent in Caliverde. No significant difference was noted between Caliverde and Nomad or the other 3 varieties within the same range.

Table 3. Ranked mean percentages of clover seed chalcid infestations for tagged racemes of 8 alfalfa varieties, Logan, 1958

Variety	Mean percentage[a]	Least significant ranges[b] at the 1 percent level (Duncan's Multiple Range test)	
Rhizoma	53.07	|	
Ranger	50.82	|	
Ladak	45.97	|	
Caliverde	32.70		|
Stafford	32.48		|
South African	30.37		|
Nomad	29.70		|
Buffalo	28.78		|
$\bar{X}$	37.99		
F value for Replications	3.48*	$S_{\bar{x}}$	2.02
F value for Varieties	25.43**	C. V. percent	13.02

a Were calculated from the subplot totals of all taggings.
b A significant difference exists between any two means which are not found in the same range.
* Significant at the 5 percent level of probability.
** Significant at the 1 percent level of probability.

Table 4. Ranked mean percentages of clover seed chalcid infestations for bulk samples of 8 alfalfa varieties, Logan, 1958

Variety	Mean percentage	Least significant ranges[a] at the 1 percent level (Duncan's Multiple Range test)		
Rhizoma	61.40	|		
Ranger	56.96	|		
Ladak	51.84	|	|	
Nomad	43.00		|	|
Stafford	42.70		|	|
Buffalo	41.88		|	|
South African	38.88			|
Caliverde	37.82			|
$\bar{X}$	46.80			
F value for Replications	1.09	$S_{\bar{x}}$		3.03
F value for Varieties	8.45**	C. V. percent		22.41

a A significant difference exists between any two means which are not found in the same range.
** Significant at the 1 percent level of probability.

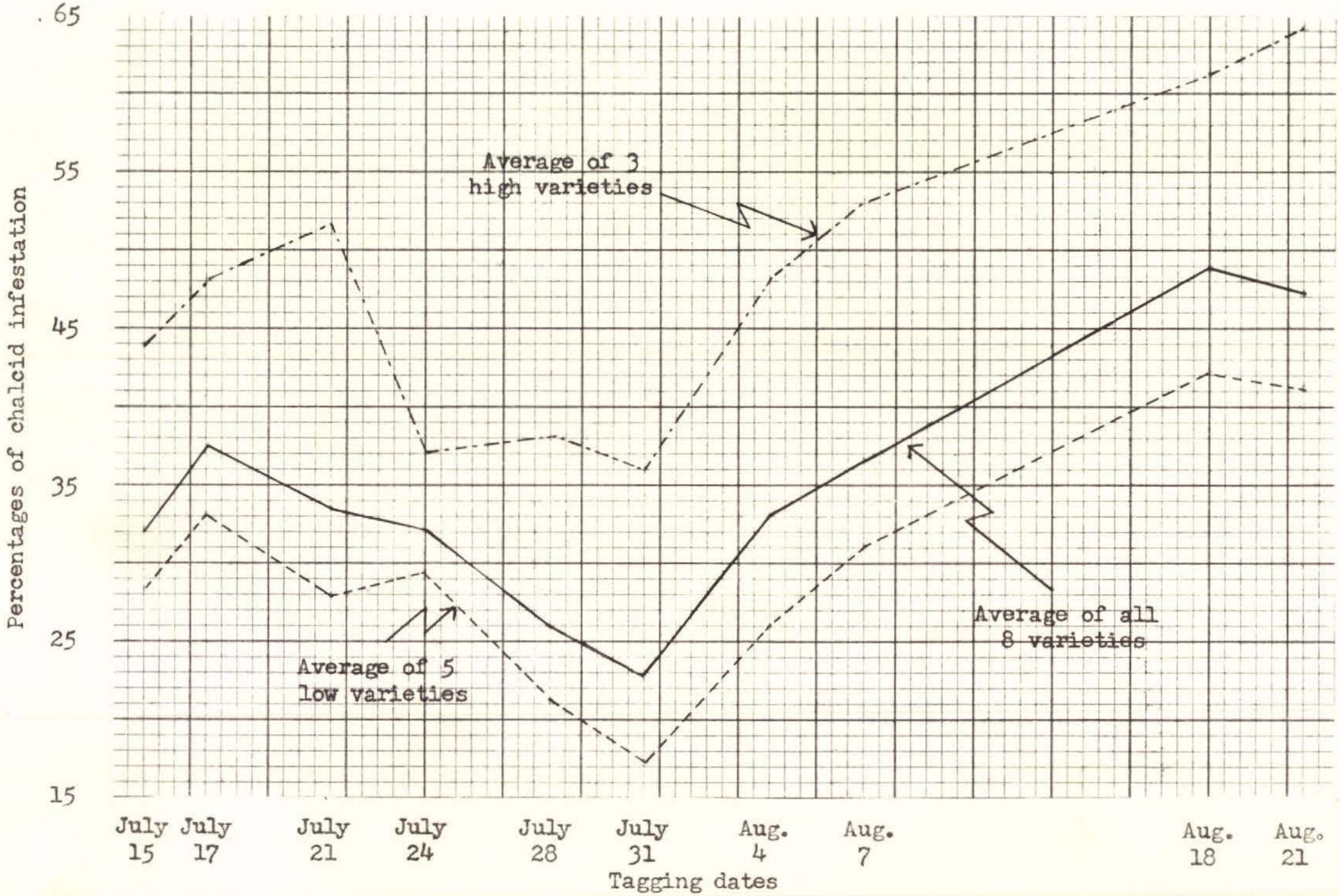

Figure 6. Histograms indicating average percentages of chalcid infestation on 10 tagging dates for 3 high varieties, 5 low varieties, and all 8 varieties, Logan, 1958.

Comparison of the two sampling methods. The analysis of variance performed on the data for two methods of sampling 8 alfalfa varieties indicated significance at the 1 percent level. Significance was not observed in the methods times varieties interaction.

The mean percentages of chalcid infestation for each method of sampling 8 alfalfa varieties are presented in table 5. The mean percentages obtained from bulk samples are all higher than those from tagged racemes.

The 40 varieties

Tagged racemes. Analysis of variance on data for tagged racemes of 40 alfalfa varieties indicated significance at the 1 percent level of probability. The Duncan's test was performed on the ranked mean arcsin percentages of chalcid infestation and the results are presented in table 6.

The lowest mean arcsin percentage occurred in Lahontan with 10.10 percent, 3.08 percent actual, and the highest occurred in Sevelra with 21.75 percent, 13.73 percent actual.

The analysis of variance indicated that taggings were highly significant at the 1 percent level. The actual mean percentages of chalcid infestation for the four taggings are: first, 1.20 percent; second, 3.66 percent; third, 8.81 percent; and fourth, 21.10 percent. The mean arcsin percentages indicate a near linear relationship, but actual percentages indicate a curvilinear relationship over the four tagging dates as shown in figure 7. Actual percentages on the last two dates indicate a rather rapid average increase in chalcid infestations during late blossom.

Bulk samples. The data obtained from the bulk samples for 40 varieties indicated significant differences of chalcid infestation at

Table 5. Mean percentages of chalcid infestation for two methods of sampling the 8 varieties, Logan, 1958

Variety	Mean percentages of infested seeds	
	Bulk samples	Tagged racemes
Buffalo	41.88	28.78
Caliverde	37.82	32.70
Ladak	51.84	45.97
Nomad	43.00	29.70
Ranger	56.96	50.82
Rhizoma	61.40	53.07
South African	38.88	30.37
Stafford	42.70	32.48
$\overline{X}$	46.80	37.99
F value for Methods	43.27**	
F value for Methods X Varieties	.70	
C. V. percent	15.50	

** Significant at the 1 percent level of probability.

Table 6. Ranked mean arcsin percentages of clover seed chalcid infestations for tagged racemes of 40 alfalfa varieties, Delta, 1958

Variety	Mean arcsin percentage	Least significant ranges[a] at the 1 percent level (Duncan's Multiple Range test)
Sevelra	21.75	
Vernal	21.02	
DuPuits	19.71	
Rhizoma	19.14	
Ladak	18.89	
Cossack	18.67	
Meeker Baltic	18.62	
Narragansett	17.65	
African	17.62	
Caliverde	17.20	
Terra Verde	17.10	
A-225 Northern Synthetic	17.05	
B. Y. Strain	16.96	
Talent	16.58	
Hairy Peruvian	16.52	
Synthetic X	15.92	
A-224 Synthetic 1	15.59	
Synthetic Y	15.34	
Grimm	15.33	
Common (Cameron Adams')	15.27	
Williamsburg	15.18	
South African	14.88	
Kansas Common	14.62	
A-169	14.48	
919 (20S)	14.36	
919 (15)	14.11	
Atlantic	14.09	
919 (Nevada)	14.05	
Synthetic 4 clone	13.99	
Synthetic 7 clone	13.86	
Stafford	13.36	
Turkish Wild	13.31	
Uruguay clone 10	13.23	
Nomad	12.88	
Arizona Chilean	12.74	
Buffalo	12.59	
Synthetic Z	12.37	
Ranger	11.88	
Nemastan	11.48	
Lahontan	10.10	
$\overline{X}$	15.53	
F value for Varieties	3.47**	$S\bar{x}$ .71
F value for Taggings	704.38**	C. V. percent 27.87
F value for Taggings X Varieties	1.15	

a A significant difference exists between any two means which are not found in the same range.

** Significant at the 1 percent level of probability.

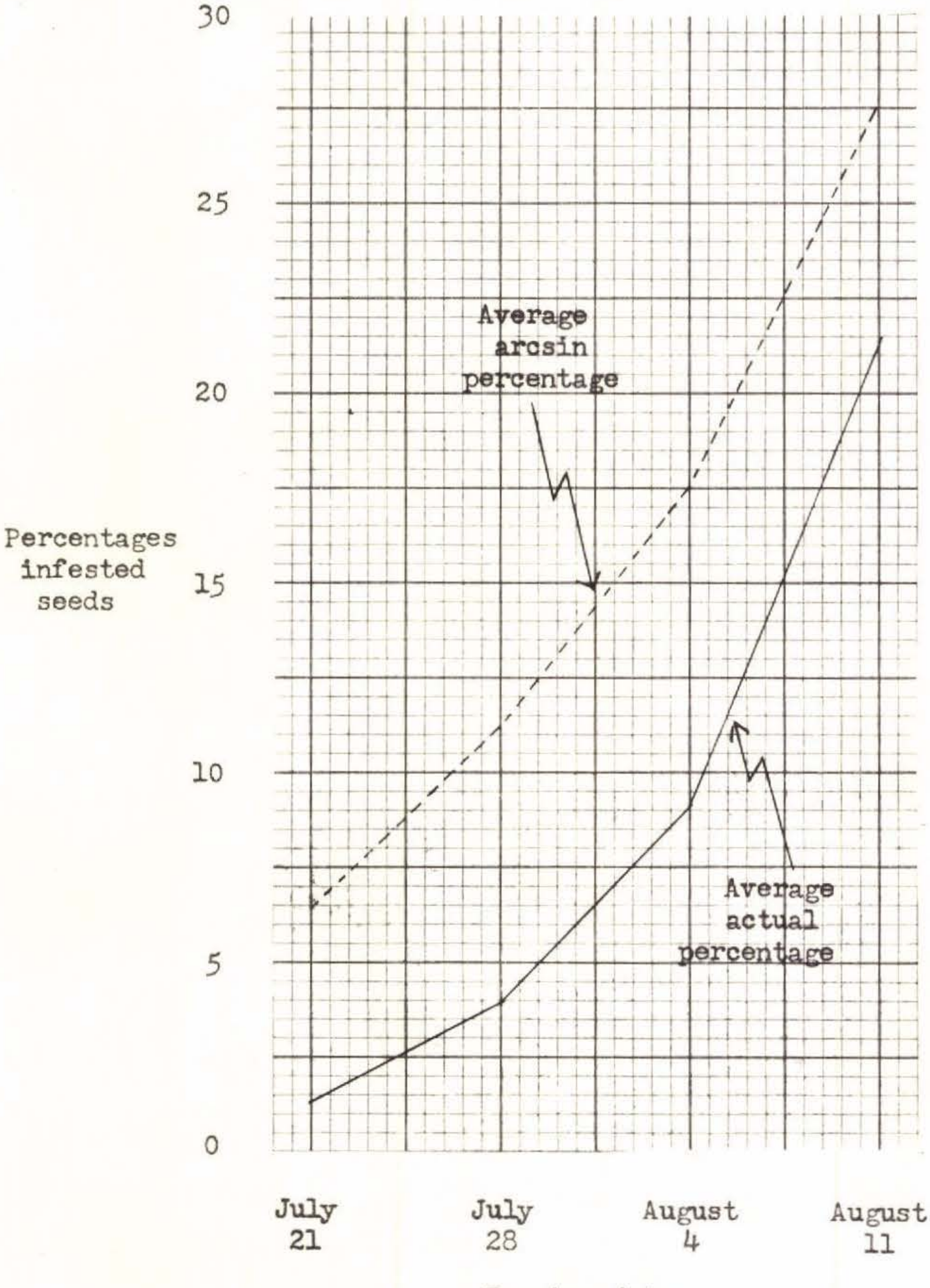

Figure 7. Histograms indicating average percentages of infested seed on 4 tagging dates for 40 alfalfa varieties, Delta, 1958.

the 1 percent level. The mean arcsin percentages were ranked and the Duncan's test performed. Results of the Duncan's test are presented in table 7.

The mean arcsin percentages ranged from a high of 22.87 in Rhizoma down to a low of 8.12 in Nemastan. These values are equal to 15.10 and 8.12 percent, respectively. Most significant ranges as illustrated in the Duncan's test are wide.

Comparison of the two sampling methods. Analysis of variance of the results obtained in the two sampling methods, tagged racemes and bulk samples, for 40 varieties indicated significant differences at the 1 percent level. Methods times varieties interaction was also significant at the 1 percent level. The significant interaction indicates that all varieties did not produce higher mean infestations by one sampling method. This is evident in table 8 where mean percentages for both methods of sampling are listed for the 40 varieties. However, mean percentages of chalcid infestation for tagged racemes are usually higher than for bulk samples.

Other Related Studies

Chalcid emergence from infested seeds

From chaff and thresher screenings. Infested seeds in chaff stacks and thresher screenings yielded 203 chalcids into cages between May 17 and June 26, 1958. The numbers of clover seed chalcid males and females and other chalcids with collection dates are listed in table 9. Chalcids were not observed in the traps after June 26. Females were more abundant than males in the cages. Large numbers of chalcids other than clover seed chalcids were collected from the

Table 7. Ranked mean arcsin percentages of clover seed chalcid infestations for bulk samples of 40 alfalfa varieties, Delta, 1958

Variety	Mean arcsin percentage	Least significant ranges[a] at the 1 percent level (Duncan's Multiple Range test)					
Rhizoma	22.87	|					
Vernal	17.21	|	|				
Terra Verde	17.12	|	|	|			
Uruguay clone 10	16.70		|	|			
Cossack	16.19		|	|	|		
Talent	16.06		|	|	|		
Synthetic X	15.95		|	|	|		
Narragansett	15.88		|	|	|		
Ladak	15.75		|	|	|		
919 (Nevada)	15.72		|	|	|		
Atlantic	14.96		|	|	|		
Synthetic Y	14.85		|	|	|		
Ranger	14.34		|	|	|	|	
Nomad	13.69		|	|	|	|	|
Meeker Baltic	13.65		|	|	|	|	|
Sevelra	13.46		|	|	|	|	|
A-224 Synthetic 1	13.39		|	|	|	|	|
African	13.26		|	|	|	|	|
Synthetic Z	13.08		|	|	|	|	|
Grimm	13.08		|	|	|	|	|
South African	13.00		|	|	|	|	|
Caliverde	12.94		|	|	|	|	|
DuPuits	12.92		|	|	|	|	|
919 (15)	12.82		|	|	|	|	|
B. Y. Strain	12.71		|	|	|	|	|
Kansas Common	12.52		|	|	|	|	|
Synthetic 7 clone	12.46		|	|	|	|	|
Synthetic 4 clone	12.35		|	|	|	|	|
919 (20S)	12.32		|	|	|	|	|
A-225 Northern Synthetic	12.32		|	|	|	|	|
A-169	12.25		|	|	|	|	|
Arizona Chilean	12.21		|	|	|	|	|
Stafford	12.16		|	|	|	|	|
Buffalo	11.40		|	|	|	|	|
Turkish Wild	11.31			|	|	|	|
Williamsburg	10.61				|	|	|
Common (Cameron Adams')	10.50				|	|	|
Hairy Peruvian	10.43				|	|	|
Lahontan	8.59					|	|
Nemastan	8.12						|
$\bar{X}$	13.53						
F value for Replications	3.91*	$S_{\bar{x}}$					1.29
F value for Varieties	4.11**	C. V. percent					27.03

a A significant difference exists between any two means which are not found in the same range.

* Significant at the 5 percent level of probability.

** Significant at the 1 percent level of probability.

Table 8. Mean percentages of chalcid infestations for two methods of sampling 40 alfalfa varieties, Delta, 1958

Variety	Mean percentages of infested seeds	
	Bulk samples	Tagged racemes
A-169	4.50	6.25
A-224 Synthetic 1	5.36	7.23
A-225 Northern Synthetic	4.56	8.60
African	5.25	9.15
Arizona Chilean	4.48	4.88
Atlantic	6.67	5.92
B. Y. Strain	4.88	8.51
Buffalo	3.91	4.75
Caliverde	5.02	8.74
Cossack	7.77	10.25
DuPuits	5.00	11.38
Grimm	5.12	6.99
Hairy Peruvian	3.27	7.99
Kansas Common	4.70	6.37
Ladak	7.36	10.48
Lahontan	2.23	3.08
Meeker Baltic	5.57	10.19
Narragansett	7.49	9.19
Nemastan	1.99	3.97
Nomad	5.60	4.97
Ranger	6.13	4.23
Rhizoma	15.10	10.75
Sevelra	5.41	13.73
South African	5.05	6.59
Synthetic X	7.56	7.58
Synthetic Y	6.58	7.00
Synthetic Z	5.12	4.59
Synthetic 4 clone	4.58	5.85
Synthetic 7 clone	4.65	5.75
Stafford	4.44	5.34
Talent	7.65	8.14
Terra Verde	8.66	8.65
Turkish Wild	3.85	5.30
Uruguay clone 10	8.26	5.24
Vernal	8.75	12.98
Williamsburg	3.39	6.86
919 (Nevada)	7.35	5.89
919 (15)	4.93	5.95
919 (20S)	4.56	6.15
Common (Cameron Adams')	3.32	6.94
$\overline{X}$	5.47	7.17

F value for Methods	80.18**
F value for Methods X Varieties	4.42**
C. V. percent	13.25

** Significant at the 1 percent level of probability.

Table 9. Relative numbers of chalcids collected in large field-type cages placed over chaff and thresher screenings, Delta, 1958

Dates	Clover seed chalcids		Other chalcids[a]
	Males	Females	
May 17 - May 29	15	18	53
May 30 - June 9	3	10	64
June 10 - June 26	0	3	37
Percentages	8.9	15.2	75.9

a Other chalcids are probably clover seed chalcid parasites, but specimens were not identified as to species.

cages; these were probably clover seed chalcid parasites, but were not identified.

Chalcids were not observed in the field-type cages placed over first crop chaff in the field following a combine harvester.

From pod samples. The results of chalcids collected in the small seed-sample cages are presented in table 10. In the samples harvested from the Delta area females were more numerous than males and the percentages of other chalcids were high. The sample collected on September 1 is the only sample from Delta which yielded more clover seed chalcid males than females and this sample has the smallest number of chalcids.

Ratios of males to females are quite variable in the samples collected from Cache Valley. The sample collected at Logan on July 15 contains the largest number of total chalcids and has the highest percentage of males.

Table 10. Relative percentages of chalcids collected in small seed-sample cages from samples harvested on several dates at various locations during 1958

Date	Percentages			Total number of all chalcids
	Clover seed chalcids		Other chalcids[a]	
	Males	Females		
		Delta, Utah		
July 15	23.1	25.2	51.7	389
July 31	21.0	24.3	54.7	148
August 15	5.0	6.6	88.4	483
September 1	13.1	4.3	82.6	46
September 4	8.1	9.4	82.5	149
September 16	9.8	11.1	79.1	398
Average percentages	12.5	14.0	73.5	
		Cache Valley		
July 15	35.2	57.0	7.8	655
Logan, Utah				
July 15	40.5	22.6	36.9	983
August 1	4.6	13.4	82.0	262
October 4	2.7	3.1	94.0	257
Richmond, Utah				
July 23	35.7	14.3	50.0	28
Average percentages	31.4	30.7	37.9	

a Other chalcids are probably clover seed chalcid parasites, but specimens were not identified as to species.

Use of sucrose to attract adult chalcids

On blossoms. The sucrose-treated blossoms yielded a total of 937 seeds, 401 occurred in the 40 treated racemes and 536 occurred in the 40 untreated racemes. Only one infested seed was found in the entire experiment. The one infested seed was observed in a sucrose treated raceme. Infested seeds were not observed in the bulk samples from the same area.

On young seeds. The total seed yield from the sucrose treated young seed experiment was 2077. The 20 treated racemes produced 928 seeds and the 20 untreated racemes produced 1149 seeds. Only 4 infested seeds were observed on 2 separate racemes. One infested seed was observed in a treated raceme and the other three were observed in an untreated raceme.

Correlation of adult chalcid populations with percentages of infested seeds

Seed samples from the two first crop seed fields indicated an infestation range of 0 to 1 percent. Due to the low infestation, correlations were not calculated.

The second crop seed field selected for this brief study was higher in chalcid infestation and the results of correlation are presented in figures 8 and 9. Females comprised approximately 83 percent of the collected clover seed chalcids. The correlation of total clover seed chalcids with percentages of infested seeds, figure 8, has a higher r value than the correlation of females with percentages of infested seeds, figure 9.

Distributions of chalcid-infested seeds on plants

Percentages of chalcid-infested seeds observed in the various 3-inch sections of 30 alfalfa-stem samples are presented in table 11.

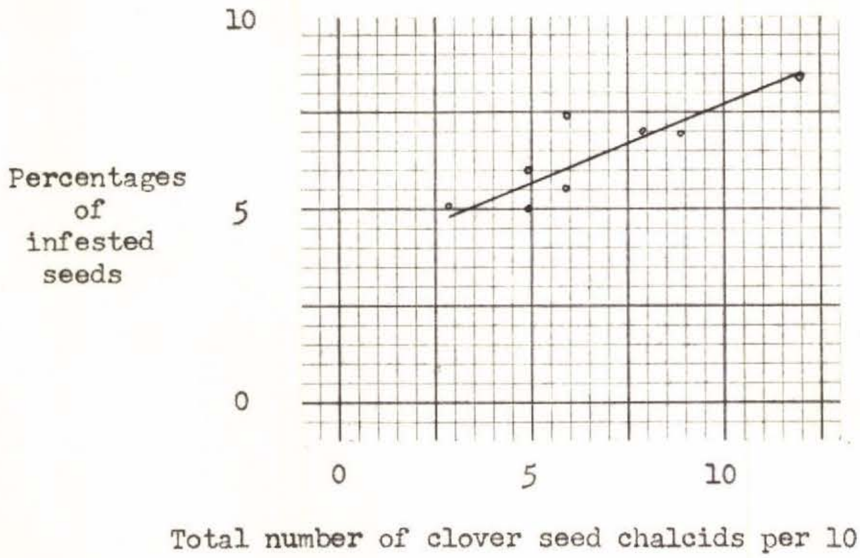

Figure 8. Scatter diagram for eight paired observations of the total numbers of clover seed chalcids per 10 sweeps with the percentages of infested seeds. r = .918 b = .414

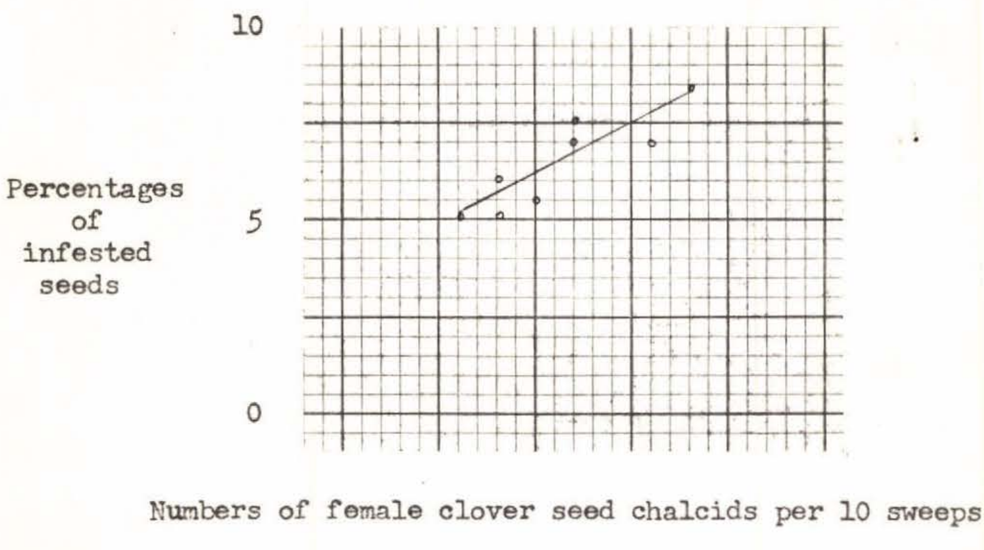

Figure 9. Scatter diagram for eight paired observations of the numbers of female clover seed chalcids per 10 sweeps with percentages of infested seeds. 4r = .889 b = .546

Table 11. Percentages of chalcid infestations for various 3-inch sections of 30 alfalfa-stem seed samples with the calculated chi-square values, Delta, 1958

Sample no.	Sections of stem samples from top down (inches)						Chi-square
	Top 3	3-6	6-9	9-12	12-15	15-18	
1	11.5	16.0	8.0	13.5			3.18
2	5.0	1.5	0.5	2.5	1.0	1.0	7.36
3	6.0	2.5	0.5	0.0			10.12*
4	5.5	1.5	1.5	1.0			5.68
5	1.0	0.5	1.0	1.5	0.5	1.0	0.78
6	11.0	11.5	8.0	5.0	10.0		3.41
7	20.0	20.0	18.0	15.0	10.5		4.66
8	9.0	8.0	6.0	6.0	1.5		5.80
9	18.0	7.5	10.5	1.0	1.5		27.76**
10	10.5	9.0	3.5	3.0	0.0		15.68**
11	0.0	12.5	7.0	6.0	5.5	3.5	15.76**
12	19.5	7.0	2.5	3.0	3.0		32.07**
13	7.5	7.0	4.0	1.5	2.0		7.30
14	4.5	6.0	14.5	14.0	6.5		10.96*
15	4.5	6.5	5.0	5.5			0.43
16	13.0	8.0	3.5	2.0	2.5		15.99**
17	3.5	5.0	2.0	1.0	2.0	3.5	3.76
18	3.0	5.5	2.5	2.0	1.5		3.44
19	3.5	4.0	7.0	6.5	2.5		3.37
20	2.5	5.0	6.0	2.5	7.0		3.81
21	5.5	3.5	3.5	3.5	7.0		2.32
22	2.5	1.0	1.0	4.5	4.0	1.5	4.96
23	6.0	7.0	3.5	2.0	1.5	2.5	7.03
24	3.5	2.5	2.0	2.5	3.5	1.0	2.03
25	15.5	19.0	18.5	14.0			1.24
26	13.5	16.5	11.0	10.5			2.02
27	14.0	13.5	14.5	11.5	14.0		0.47
28	5.0	3.5	5.0	7.0	1.0		4.80
29	10.5	8.5	7.5	7.5			0.77
30	5.0	3.5	5.0	7.0	1.0		4.81
$\overline{X}$	8.00	7.43	6.11	5.42	3.90	2.00	4.87

* Significant at the 5 percent level of probability.
** Significant at the 1 percent level of probability.

Seven of the 30 samples indicate significant variations at the 5 percent level and five of the seven are also variable at the 1 percent level. High chi-square values indicate that chalcid infestations are not uniformly distributed throughout plants.

The average percentages of chalcid infestation for 3-inch sections of all 30 samples form a uniform trend of high infestation in the top section to low in the bottom.

Distributions of chalcid-infested seeds in fields

Percentages of chalcid infestations for six areas of five alfalfa seed fields are presented in table 12 with chi-square values. The chi-square values for five fields indicate that variations of chalcid infestation among areas and within each field are not significant at the 5 percent level. However, fields number 1 and number 5 have high chi-square values which approach significance. The variations of field means gave a chi-square value of 5.60 with a probability of 22 percent. High chi-square values with low levels of probability indicate that chalcid infestations are not entirely uniform within and among fields. An infestation pattern was not apparent from the results, such as direction of infestation or proximity to infestation sources.

Table 12. Percentages of infested seeds in six sample areas of five alfalfa seed fields with the field means and chi-squares, Delta, 1958

Field no.	Sample areas 1	2	3	4	5	6	Field mean	Chi-square
1	4.5	12.0	4.5	10.5	5.5	11.0	8.00	8.42
2	5.5	9.0	11.5	9.5	5.5	10.0	8.50	3.92
3	2.0	1.5	4.0	4.0	3.5	1.5	2.75	2.58
4	4.0	2.5	3.0	1.0	5.0	1.5	3.00	3.26
5	1.0	8.5	9.5	2.5	4.5	5.5	5.25	10.43
Mean percentage for five fields							5.50	
Chi-square for five field means							5.60	

DISCUSSION AND CONCLUSIONS

Results of the varietal studies indicated that all 40 alfalfa varieties tested were not infested with clover seed chalcids to the same extent. Differences in chalcid infestations were significant at the 1 percent level in all varietal studies.

The 8 varieties at Logan had high mean percentages of infestation, ranging from 28.78 to 61.40 percent. This high degree of infestation suggests that none of the 8 varieties has a pronounced resistance to chalcid infestation even though significant differences were observed. Rhizoma, Ranger, and Ladak had high infestations in both methods of sampling and the other 5 varieties (Caliverde, Nomad, Buffalo, South African, and Stafford) had low, but the varieties were not consistant in rankings for both methods.

Sampling methods for the 8 varieties were significant at the 1 percent level, but the interaction, sampling times varieties, was not. Tagged racemes yielded lower mean infestation percentages than bulk samples in all 8 varieties.

Results of 40 alfalfa varieties at Delta indicated that certain varieties usually had high or low mean infestation percentages when compared by both methods of sampling. Rhizoma and Vernal ranked among the top 4 varieties in chalcid infestation for both methods and Lahontan and Nemastan were the 2 lowest varieties.

Arcsin transformations were made on the data obtained from both methods of sampling the 40 varieties. Arcsin transformations weight more heavily smaller percentages which have smaller variations and tend to give binomial populations a normal distribution. The range of mean arcsin percentages was 8.12 to 22.87 percent, this compares to 1.99 and 15.10 percent actual.

Several differences were noted in the ranking of means for 40 varieties when bulk samples and tagged racemes were compared. Analysis of variance for sampling methods indicated highly significant differences at the 1 percent level. Comparisons of the means for the 2 sampling methods indicated that tagged racemes were usually higher than bulk samples in the 40 varieties.

In comparing the two varietal plots, Ranger, which ranked in the high ranges of the 8 varieties, was observed in the medium to low ranges of the 40 varieties. Only 3 of the 8 varieties (Nomad, Ranger, and Rhizoma) found in both plots indicated a tendency for bulk samples to yield higher means than tagged racemes. No attempt was made to determine why all 8 varieties did not react the same when compared in both plots.

Coefficients of variation for the varietal experiments reported in this thesis range between 13.02 and 27.87 percent. In the preliminary varietal studies conducted in 1956, the coefficients of variation were 47.6 and 106.5 percent. Reductions in coefficients of variation indicate a marked improvement in reducing experimental errors.

Results of taggings indicate a close correlation of chalcid infestations with blossom stages and numbers of chalcids present within an area. Reference is made to figures 6 and 7, where average infestations are indicated at the times of tagging. During early blossom stages few seeds were available for chalcid infestation and uniform numbers of chalcids were present. High infestations were observed in the Logan plot during early blossom. These were probably the results of large numbers of chalcids in the area at that time. High infestations were not observed during the early blossom in the Delta plot. The numbers of chalcids in the fields during this period were low, also, first crop seed from the same area was extremely low which indicates low numbers of chalcids during the early blossom stage. Sorenson (1930) indicated that chalcid populations usually increase gradually through the season until cutting time. Therefore, when the plots were in full blossom, chalcid numbers had probably increased only slightly from early blossom and the numbers of seeds suitable for oviposition had increased greatly. This condition might have caused a decrease in chalcid infestation as noted in figure 6, or a continuous low infestation as noted in figure 7. During late blossom the chalcids should have increased to maximum numbers when the numbers of seeds suitable for oviposition were reduced. High percentages of chalcid infestation are probably the results of such a condition apparent in both figures 6 and 7.

Use of bulk samples to determine chalcid infestations appears to have a sound basis in areas where uniform conditions of plant growth prevail. However, when several varieties are grown within small areas, such as varietal plots, conditions of growth may not be uniform and bulk samples may be biased. Tagged racemes were employed to help standardize conditions of plant growth.

Differences have been observed among alfalfa varieties in the length of blossom periods, total amount of blossoms within periods, and when blossom periods occur. The number of chalcids is a constant at any one time, but varies from time to time within an area.

In small experimental plots where blossom periods are not uniform, assuming that varieties are not resistant to chalcid infestation, it is expected that chalcid populations may do either one of two things: (1) chalcids may become more or less uniformly distributed over a small area in blossom; or (2) they may become more or less uniformly distributed over young seeds suitable for oviposition. If the first theory is true, then those varieties with low numbers of blossoms on a given date should probably indicate high infestations for that date and those varieties with high numbers of blossoms should probably have low infestations. However, if the second theory is true, then a nearly uniform infestation might be expected to occur among varieties on any given date. Results from the tagged racemes are helpful in determining which theory might be true.

The F value for taggings times varieties interaction of the 40 varieties is not significant, but the F value for varieties is highly significant. This indicates that chalcid infestations for all varieties tended to change in the same direction through the season, but chalcid infestations among varieties were different. From this standpoint, it is suggested that either varieties which ranked low in chalcid infestation are resistant, or chalcids became distributed more or less uniformly over the entire plot and low infestations are probably the results of high numbers of blossoms throughout the season.

These suggested theories might be evaluated by tagging racemes in a varietal plot and estimating amounts of blossoms for each variety on tagging dates. Additional strength might be added to the test if bulk samples were taken and infestation percentages correlated to the total amount of blossoms through the season.

The results of using a sucrose solution to attract adult female chalcids for oviposition indicated that an experiment of this type should have been made in areas of known high chalcid populations.

Chalcid infestation percentages were closely correlated with numbers of adult chalcids collected in sweepings during the late blossom stage. In the observed field, both the total numbers of chalcids and the numbers of females were closely correlated with infestation percentages. The r value for correlation of total numbers of chalcids with infestation percentages is higher than the r value for females with infestations, but this correlation may change when percentages of females in the population change. Results observed in the correlation of females with infestation percentages should be more reliable for future correlations. The correlation lines appear to be nearly linear in this study, but a curvilinear relationship probably exists at the lower end.

Chalcid emergence from chaff stacks and thresher screenings probably occurs in early spring, as indicated by the results from the field-type cages. It is presumed that these early-emerged chalcids seek out volunteer alfalfa for the first oviposition in spring rather than first crop. This also seems likely since volunteer alfalfa seeds are often highly infested in areas where first crop alfalfa seed has low infestations. The field-type cages yielded more chalcids from thresher screenings than they did from chaff.

It seems likely that most chalcids in first crop seed have either emerged or have entered a state of aestivation before harvesting occurs, since chalcids were not observed in the field-type cages when placed over chaff in the field following a combine harvester.

Results of the adult chalcid population emerging from infested seeds in pod samples which were placed in small seed-sample cages indicate that ratios of adult males to females may remain quite constant or they may change throughout the season. Percentages of other chalcids seemed to increase with the season.

Samples of 3-inch sections of plants indicated that chalcid infestations were not uniform throughout plants. However, a uniform trend was observed in the mean percentages of highest infestation in the top 3-inch section to lowest in the bottom section. These results indicate that samples should be taken from all areas of plants. This might best be done by collecting bulk samples of stems and removing all pods.

Observed, variations of chalcid infestation within five fields suggest that chalcid populations are not uniformly distributed in all areas. Chalcid distributions did not seem to follow any apparent pattern.

SUMMARY

Preliminary studies of chalcid damage in alfalfa were made in 1956 and 1957. Results of these early studies indicated there might be differences in chalcid infestations among varieties and that better methods for studying the chalcid problem were needed. Methods of threshing and cleaning seed samples were developed and subsamples of 100 seeds were deemed satisfactory for determining chalcid damage.

In 1958, 40 alfalfa varieties were tested for extent of chalcid damage in a replicated plot at Delta; 8 of the 40 varieties were also tested in a replicated plot at Logan. Chalcid infestations were determined by two methods of sampling, bulk samples and tagged racemes. Bulk samples consisted of seeds harvested from selected plant stems; tagged-raceme samples included the seeds from uniform numbers of racemes tagged during the blossom stage at weekly and biweekly intervals. The two sampling methods were compared in analyses of variance. The observed differences of chalcid infestation in the two methods for both plots were significant at the 1 percent level.

Percentages of infested seeds in samples harvested by each sampling method in each plot were evaluated in an analysis of variance. Significant differences of infestation were observed at the 1 percent level for each varietal experiment. Mean infestation percentages of the 8 varieties were high 28.78 to 61.40 percent, which suggests that none of the 8 varieties might be considered resistant. Lower infestations were observed among the 40 varieties with Lahontan and Nemastan

having the lowest mean percentages. Infestations in Rhizoma and Vernal were consistantly high.

Average percentages of chalcid damage were determined for each tagging date. Infestations appeared to be correlated with blossom stages and chalcid populations throughout the season.

Adult chalcids were trapped from infested seeds in chaff, thresher screenings, and pod samples. Emergence from chaff and thresher screenings occurred in early spring. Chalcids collected from pod samples during the season at Delta indicated that female clover seed chalcids were slightly more numerous than males. The sex ratios were variable in the Cache Valley samples. Large numbers of other chalcids were observed among the trapped specimens; these were probably clover seed chalcid parasites, but were not identified as to species.

A sucrose solution was applied to blossoms and young seeds to attract adult chalcids into an area for possible control. Chalcid infestations were too low in the treated area to determine the effectiveness of sucrose treatments.

Adult chalcid populations and percentages of infested seeds were correlated. Adult chalcids were sampled by sweeping with an insect net in late blossom and percentages of infested seeds were determined from bulk samples. The correlations were close and appeared linear, but probably were curvilinear toward the lower extreme.

Distributions of infested seeds on plants were determined from 3-inch sections of stem samples. Chi-square analyses indicated that infestations of seven plant samples were significantly variable at the 5 percent level. Mean percentages of the 3-inch sections showed a general decrease of infestation from the top section to the bottom. These results suggest that infested seeds may not be uniformly distributed on plants and that samples from entire plants should probably be used to determine chalcid damage in fields.

Field distributions of chalcid infested seeds were estimated in six areas of five fields. Chi-square values indicated that chalcid infestations were not significantly variable within fields, however, infestations among areas of two fields approached significance at the 5 percent level. Variations among field means indicated a probability of 22 percent. These results indicated that chalcid infested seeds may not be uniformly distributed within and among fields. This suggests that several entire fields should be sampled to determine reliable estimates of average chalcid infestation within areas.

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