FIELD STUDIES ON THE ALFALFA SEED CHALCID, *BRUCHOPHAGUS RODDI* GUSS., IN SOUTHERN ARIZONA

by

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APPROVAL BY THESIS DIRECTOR

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INTRODUCTION

The alfalfa seed chalcid, *Bruchophagus roddi* Guss., is a small jet-black wasp which breeds and develops on alfalfa. This pest is a serious threat to alfalfa seed production in western United States and nearly all alfalfa producing areas report losses. The damage varies from year to year. From 2 to 85 per cent of the alfalfa seeds may be infested in any given crop, with losses being greater in late maturing than early maturing alfalfa plants. In 1927, losses in Arizona were estimated at 70 lbs. per acre (Wildermuth, 1931).

Chemical control of this chalcid has not yet been successful (Bacon *et al.*, 1959) for a number of reasons. The eggs and the developing larvae are well protected from the usual insecticides used on alfalfa. The adult wasps are most active in the fields during periods of bloom and seed set when bees and other pollinators are especially needed. An application of an insecticide at this time, even though effective against the chalcid, would be detrimental to such beneficial insects. Though there are numerous parasites of the chalcid, their usual low numbers contribute relatively little to lowering the populations of this pest. These limitations naturally direct research toward other possible control measures, including resistant varities of alfalfa, for a solution to the chalcid problem.

The present observations of the biology of the alfalfa seed chalcid were made in 1962 and 1963 and involved the following areas of study:

1. The adult emergence pattern.

2. The age of seed at which maximum chalcid infestation occurs.

3. The possibility of seasonal differences in the degree of infestation.

4. Observations of selected alfalfa varieties for possible resistance to the chalcid.

5. The nature and degree of parasitism of the chalcid by its natural enemies.

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LITERATURE REVIEW

Classification

The name *Bruchophagus gibbus* (Boheman) has for many years been incorrectly assigned to eurytomid seed pests of red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.). Recent investigations by Russian workers have revealed that three distinct species of *Bruchophagus* are involved. Each species is restricted to one genus of plants. Basing their argument on (1) host range and cross-infestation studies, (2) morphological differences, and (3) biological differences, they designated *B. gibbus* (Boh.), *B. kolovobae* Fed., and *B. roddi* Guss. as the chalcids associated with red clover, birdsfoot trefoil and alfalfa, respectively (Kolobova, 1950; Nikols'kaya, 1932; Fedoseeva, 1954, 1956). Until very recently the above classification was not adhered to in the United States (Strong, 1962a) even though Hansen (1955), and Neunzig and Gyrisco (1958) reported the host specificity of these eurytomids in this country, so that chalcids associated with alfalfa, red clover and birdsfoot trefoil were also restricted to the genera of *Medicago*, *Trifolium*, and *Lotus* respectively.

The alfalfa seed chalcid was first described in

Scandinavia in 1835 by Boheman as *Eurytoma gibba*. In 1879, Howard (1880) described the North American species as *Eurytoma funebris*. A. V. Gahan (in Nikols'kaya, 1932) showed that the two names were synonymous. Ashmead (1894) placed *E. funebris* in the genus *Bruchophagus*. He again erected the genus *Systolodes* with *S. brevicornis* as the type species. Girault (1916) however showed that *Eurytoma* and *Systolodes* were synonymous. Subsequent workers have used *Bruchophagus* as a result of the current practice provision of the International Code sanctions. The present name, *Bruchophagus roddi* Guss., has been assigned to eurytomids of this genus infesting alfalfa on the basis of recent work on the *B. gibbus* complex (Strong, 1962a).

Host Plants

Fourteen species of clover and alfalfa have been reported in the literature as host plants of the "clover seed chalcid" (Urbahns, 1920; Bridwell, 1923; Sorenson, 1930; and MacDonald, 1946).

Kolobova (1950) was the first to show that two distinct races of chalcids are involved in the infestation of clover and alfalfa. Host relationship studies by other Russian workers revealed the restricted nature of chalcid infestation, each species attacking a particular genus of plants. *B. roddi* Guss., the alfalfa seed chalcid, has been reported as capable of infesting only the seeds of alfalfa (*Medicago sativa* L. and *M. falcata*), while *B. gibbus* (Boh.) has been reported to be restricted to red clover (*Trifolium pratense* L. and *T. medium* L.) (Kolobova, 1950; Nikols'kaya, 1932; and Fedoseeva, 1954).

During a series of rearing and infestation experiments, Hansen (1955) demonstrated that eurytomids reared from red clover would not infest alfalfa or bur clover (*Medicago hispida*), while those reared from alfalfa would not infest species of *Trifolium*. Neunzig and Gyrisco (1958) also found out that chalcids reared from birdsfoot trefoil (*Lotus corniculatus*) failed to oviposit on seeds of alfalfa or red clover.

Life Cycle

Adult: The adult chalcid is a small, black insect which measures 2 mm. in length. The alfalfa seed chalcid overwinters as a fully developed larva within the seed; the adult emerges by gnawing a hole through the seed coat and pod (Sorenson, 1930). Emergence of the adult is influenced by temperature. Field and laboratory studies (Vinogradov, 1941) showed that the adult chalcids emerge when temperatures range between 18° and 20° C.

Adult survival studies (Strong, 1962b) indicate that optimum survival occurs when adults are fed and held at 50° F. At this temperature 50 per cent of the adult population survives for 38 days.

Female chalcids are active on warm, sunny days and it is under such conditions that most of the eggs are laid (Folsom, 1909; Strong, 1962b). In mid-summer, oviposition occurs a few days after emergence of the female adult chalcid. The ovipositing female bends its abdomen ventrally and forward, extrudes its ovipositor, and thrusts it into the pod and into the soft substance of the seed (Sorenson, 1930). The egg is deposited just beneath the integument of the developing seed, sometimes between the cotyledons, and frequently within the semi-fluid contents of the cotyledons (Urbahns, 1920; Sorenson, 1930).

<u>Egg</u>: The small, elongated egg is 0.2 mm. long and 0.08 mm. thick. One end terminates in a flexible point and the opposite end is drawn into a slender tube-like filament about 0.2 mm. long (Urbahns, 1920). Strong (1962b) observed that the mean hatching time at 30° C. was 42.5 hours.

The eggs are deposited in seeds before they reach the "dough" stage (Kolovoba, 1929; Sorenson, 1930; and Shull, 1944). Strong, (1962b) in an experiment conducted in a greenhouse at an average temperature of 79° F., found that seeds 8 to 10 days old appeared to be most favorable for oviposition. In general the lowest female-to-pod ratio produces the lowest infestation though the trend is frequently inconsistent (Strong, 1962b).

Sorenson (1930) dissected gravid females and reported them capable of laying from 24 to 66 eggs, with an average of 44 eggs. Strong (1962b) observed that 8 females caged on alfalfa racemes produced from 6 to 86 offspring; four females produced 46 or more offspring. Urbahns (1920) reported that parthenogenesis is known to occur in female chalcids.

<u>Larva</u>: The larva is grub-like in shape, 1.9 mm. long and 0.9 mm. thick when fully developed. Feeding takes place after the larva is one to two days old (Urbahns, 1920). By measuring differences in mandible length, Strong (1962b) revealed the presence of four larval instars. He reported the time required for development of the various instars at 24° to 28° C. as follows: first instar 2 to 7 days, the second 5 to 7 days, the third 6 to 9 days, and the fourth 7 to 17 days. The developmental period for larvae at 24° to 25° C. is 12 to 23 days. Strong found that larvae apparently do not defecate during the early instars. The mature larva may (1) continue its development by defecating and entering the prepupal stage or (2) enter into diapause. Larvae that have broken diapause can readily be distinguished by their white color and the absence of fecal material in their digestive tract.

Urbahns (1920) reported that diapause formation depends on the maturity of the seed and time of oviposition.

If the infested seeds mature before larvae are fully developed, the larvae enter into diapause. Temperature is also an important factor which contributes to diapause formation. Strong (1962b) found that diapause terminated slowly at room temperatures. This reaction, called the diapause-ending process, was hastened by chilling larvae. On chilling larvae at 30°, 40°, 50° and 60° F. for periods of 3, 6, 9 and 12 weeks at each temperature level, he found that the adult emergence was more rapid in larvae chilled at higher temperatures than those chilled at lower temperatures. The length of chilling time was inversely proportional to the time required for 50 per cent adult emergence.

Pupa: Adequate moisture content of seed, together with a sufficiently high temperature, is essential for the transformation of the fully developed larvae into pupae (Urbahns, 1914; Wildermuth, 1931). The pupa is 1.88 mm. long. A description of the various stages of the pupa has been reported by Strong (1962b). He describes the prepupa as a white, short, thickened quiescent larva. This stage lasts for a day. The pupa has four stages which are determined by the color of the eyes and body. The first stage pupa has white eyes and a white body, and lasts for a day. The second stage pupa, which lasts for 3.15 days, has pink to red eyes and yellowish body. The third pupal stage has brown eyes, black abdomen and yellowish body; the stage is completed in 1.82 days. The final stage pupa, which has brown eyes and a black body, lasts for a day. The average total time required for pupal development at 24° C. was determined to be 10.52 days. Wildermuth (1931) reported the pupal stage to last from 10 to 40 days under outdoor conditions.

Control Measures

1. Parasites

A brief description of ten chalcid parasites occurring in the United States has been given by Butler and Hansen (1958). Of this number, the following are found in Arizona: *Tetrastichus bruchophagi* Gahan, *T. venustus* Gahan, *Liodontomerus insuetus* Gahan, *L. perplexus* Gahan, *Habrocytus medicaginis* Gahan and *Trimeromicrus maculatus* Gahan. As far as abundance is concerned, *L. perplexus*, *T. bruchophagi* and *T. maculatus* are the most important.

Nikols'kaya (1932) reported that the rate of parasitism in the Poltava region of Russia ranged from 23.8 to 80.9 per cent. In the southwestern United States parasites of the seed chalcid do not usually occur in numbers great enough for dependable control of this pest on seed alfalfa.

2. Cultural Control

The principal sources of chalcid infestations are (1) infested seeds shattered from pods before or during harvest, (2) chaff stacks, (3) infested plant material blown into the air during threshing, (4) uncleaned seeds, and (5) volunteer alfalfa (Sorenson, 1930).

Methods of cultural control have been described by Urbahns (1914, 1920) and Sorenson (1930). Essentially these methods involve: (1) the cultivation of alfalfa fields in fall and winter, (2) destruction of volunteer alfalfa, (3) cleaning up of stacks, screenings and crop debris in waste places, (4) sowing clean seed, and (5) the adoption of a uniform date for harvesting the seed crop in each locality. Success of these control measures depends on the full co-operation of all farmers in a community.

3. Chemical Control

The internal location of the chalcid larva, coupled with the necessity for preservation of pollinators at alfalfa bloom periods, makes the use of insecticides somewhat difficult. Crothers and Haws (1961), after testing four systemic insecticides (dimethoate, Di-syston, demicron and Thimet) reported that yield of alfalfa seed was significantly higher in the dimethoate plots than in the other treated plots or the check plot.

4. Plant Resistance

Breeding of resistant varieties of alfalfa appears to be one of the promising avenues for the control of the seed chalcid. Studies conducted in Yuma and Mesa, Arizona have resulted in the detection of several sources of genetic material that may ultimately be used in developing alfalfa varieties resistant to the seed chalcid (Nielson, 1962).

Painter (1951, 1958) reported that chemical senses of insects and other senses, such as those concerned with the perception of surfaces or color, may play an important role in host plant selection.

Fronk (1962) located sense organs on the antennae, second trochanter, tarsi, third valvulae, and possibly on the ovipositor, of the alfalfa seed chalcid. This discovery may lead to the understanding of the mechanism which operates in attracting or repelling chalcids to or from alfalfa plants.

Approximately 120 chemicals are known to occur naturally in alfalfa. In olfactory studies of chemicals which occur naturally in alfalfa, Fronk (1962) found L-cystine, L-proline, DL-aspartic acid and beta-carotene to be most attractive. The most repellent were butyric acid, pyridoxine and coumarin. Itaconic acid, inositol and coumarin were narcotic in their effects.

In the field, Fronk (1962) found that seeds resulting from yellow or yellow-green flowers had heavier chalcid infestations than did seeds from darker colored flowers.

* * *

DESCRIPTION OF EXPERIMENTS - I

Adult Emergence Patterns

The study is based on alfalfa pod samples harvested in July 1962 from variety test plots on the University of Arizona Farm at Yuma, Arizona. Samples of pods were collected from each of six replicated plots of each of 10 alfalfa varieties. These 60 samples were stored in half-pint glass jars in the field laboratory at the Campbell Avenue Farm at Tucson until the spring of 1963.

a. Overwinter emergence pattern: On April 6, 1963, 200 infested seeds from each of the above jars were examined to determine the extent of adult emergence between harvest and the examination date. These observations, representing a total of 1,200 infested seeds from six replicates of each of 10 varieties, are summarized in Table 1. Results are expressed as percentages of non-emergence and are believed to represent largely the individuals which interrupted their development by entering into diapause.

b. Spring emergence pattern: On March 23-25, 1963, six-gram samples of alfalfa pods were taken from each of the 60 Yuma samples mentioned above for emergence observations, after first removing all previously emerged chalcids and parasites. These pod samples were placed in one gallon cardboard ice cream containers. Glass outlet tubes were fixed to the lids of the containers and vials were attached by means of one-hole stoppers to the outlet tubes for the collection of chalcids and parasites. The containers were kept on a rack in an open garage. Weekly inspections were made to count emerged chalcids and parasites. Only a few insects emerged although the weekly inspections were continued until June 14. At the end of the experiment, the pods in the containers were examined for chalcids and parasites that emerged but were unable to fly to the collecting vials. The total record of chalcids and parasites that emerged from these samples, from March 25 through June 14, 1963, is summarized in Table 2.

An explanation for the rather low emergence of chalcids and parasites can be inferred from the data in Table 1. This table represents the per cent infested seeds from which chalcids or parasites had not emerged by the end of winter 1962-63. The varieties of alfalfa used in this study were the same as those used for the spring emergence pattern determination. If therefore any emergence were to occur in the samples for the spring emergence pattern, it would have resulted from the per cent seeds from which chalcids or parasites had not emerged at the end of the winter. It can be inferred that the low population of emerged chalcids in spring, 1963, indicates that the remaining diapausing larvae should emerge in the spring of the second year (1964) except for those affected by disease or other mortality agents. This explanation apparently suggests the condition whereby, in exceptional cases, larvae continue in diapause into the second year (Urbahns, 1920).

Table 1

Non-emergence of alfalfa chalcids in April, 1963, from infested seeds from pods collected in July 1962.[a]

Alfalfa Variety	Per cent of Infested Seeds with Non-emerged Chalcids
Buffalo	35.8
New Mexico 11-1	35.7
Arizona Chilean	33.6
California Common	33.6
Moapa	29.1
Caliverde	26.6
Hairy Peruvian	26.1
Cody	23.6
Lahontan	21.0
African	20.6

[a]Each percentage figure is based on an examination of 1,200 seeds, representing 200 infested seeds from each of six replicate samples of each variety.

Table 2

Total numbers of chalcids and parasites which emerged between March 25 and June 14, 1963, from alfalfa seed pods collected in July, 1962.[a]

Variety	Total Emerged Individuals	
	Chalcids	Parasites
African	2	2
Arizona Chilean	2	1
New Mexico 11-1	5	1
Buffalo	3	3
California Common	1	2
Cody	2	1
Hairy Peruvian	2	1
Caliverde	1	2
Moapa	3	1
Lahontan	1	1

[a]These figures represent the total emergence from six-gram pod samples from each of six replicates of each variety.

DESCRIPTION OF EXPERIMENTS - II

Age of Seed at Which Maximum Oviposition Occurs

Several workers, notably Kolovoba (1929), Sorenson (1930) and Shull (1944), have reported the stage of alfalfa seed development at which chalcid infestation is greatest. They all agree that seeds that have not reached the "dough" stage of development are most preferred for oviposition. They failed, however, to indicate the exact age of seed preferred prior to the "dough" stage of development. Strong (1962b) made the first attempt to determine this exact age. His results showed that racemes containing seeds 8 to 10 days old appeared to be the most favorable for oviposition. Rowley (1962) reported that seeds were susceptible to oviposition from the fifth to the fourteenth day after tripping. In the following studies, an attempt was made to find the age of seed at which maximum oviposition occurred in the clonal selections Y-56-11, Y-56-225, Y-56-39, Y-56-183, Y-56-146, and Y-56-628.

Field plan: The Campbell Avenue Farm of the University of Arizona at Tucson was the location for the experiment. The field plot was planted to the following clonal cuttings of alfalfa provided by Dr. M. H. Schonhorst of the Department of Agronomy: Y-56-11, Y-56-225, Y-56-39, Y-56-183, Y-56-146, and Y-56-628. The cuttings were planted 18 inches apart in individual rows three feet apart and about 40 feet long.

Alfalfa seed chalcid rearing: Alfalfa pods infested with chalcids were collected during June and July, 1962, from the Overpass Farm of the University of Arizona at Tucson. The fresh pods were dried from 3 to 5 days on a metal screen raised about two feet above the laboratory floor. This was done to prevent the pods from becoming moldy. The dry pods were then put in one-quart, unwaxed "Dixie" containers, covered with transparent plastic lids, and put on a rack. The chalcids were allowed to emerge under room temperature conditions which during the summer were quite favorable. Emergence of chalcids occurred within one week to two weeks. Some adult chalcids died from entanglement in the space between the groove of the lid and the rim of the container although this did not greatly interfere with the availability of an adequate supply of adult chalcids for experimental purposes.

Collection of chalcids: The center portion of the bottom of a cardboard carton measuring approximately 24 x 12 x 12 inches was removed, leaving a margin of about 3 inches. The area removed was covered with a fine mesh, white nylon cloth. The box was placed by a window with the nylon portion facing the outside to allow light to enter the box. To maintain some amount of darkness, the remaining part of the box, including an access opening, was covered with a black cloth. Consequently, only the nylon portion of the box was illuminated. "Dixie" containers with alfalfa pods and emerged chalcids were opened two at a time inside the box. The chalcids were immediately attracted to the source of light and after flying about for a while, settled on the nylon. By means of an aspirator, ten female chalcids were sucked into glass tubes 3 inches long and five-eighths of an inch in diameter. The bottoms of the tubes were covered with a nylon organdy cloth of a mesh which made it impossible for the chalcids to escape. The open ends of the tubes were corked.

Preparation of alfalfa selections for infestation: Selected alfalfa plants in each test row were clipped for a regeneration of new growth. Developing racemes which appeared after the clipping were covered with paper bags and stapled to prevent pollination. The paper bags were removed after a week, at which time most of the flowers had opened up. The flowers were hand-tripped with a paper "boat." The paper "boat" was made from an index card by folding the card along the middle of the long side. An oblique cut was made with a pair of scissors about 2 inches from the end of the free side, to the tip of the line of fold. Thus on unfolding the index card the cut end formed an inverted "V." Tripping with a paper "boat" was a rather tedious operation which was not completely effective but was still the best technique available.

Infestation of alfalfa pods: After artificial tripping, the flowers were again covered with paper bags and stapled. Three, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16 and 18 days after tripping, the developing pods were infested with adult female chalcids. Ten females were placed on each single raceme in special cages which replaced the paper bags. Six cages, for each alfalfa selection, were used for each stage of seed development. The cages were similar to those used by Strong (1962b). They were 1 5/8 inches long and 1 1/8 inches in diameter and were made from "Lusteroid" centrifuge tubes. One end of the cage was covered with 32-mesh Saran screen and the other end was plugged with a disc made of foam plastic. The discs were cut from sheets of the material with a cork borer and were slightly larger in diameter than the inside of the cages. A slit, about one-half diameter, was made in each of the discs. The rachis of a raceme was inserted in this radial slit. A small corked hole at the side of the cage provided an aperture for the introduction of chalcids after the cage had been put in place. The chalcids were removed after a 24 hour period. The infested pods were harvested 20 to 24 days after tripping and stored in vials containing 70 per cent ethyl alcohol. The pods were later broken open and each seed was dissected under a dissecting microscope to determine numbers of infested and uninfested seeds.

Results are shown in Table 3. Because of the inefficiency of the hand-tripping technique, seed set was not uniformly high. No seed set was obtained from the flowers, the developing pods of which were to be infested 12 days after tripping. The results appear to show that seeds from the clones used are susceptible to oviposition from the fifth to the eleventh day after tripping.

Table 3

Per cent chalcid infestation in alfalfa seeds of different ages, Tucson, Arizona, 1962.

Selections	Age of Seeds (days)	No. of Seeds Examined	Per cent Infestation
Y-56-628	3	216	6.0
Y-56-11	3	282	4.2
Y-56-146	3	231	4.8
Y-56-183	3	286	9.4
Y-56-225	3	72	4.2
Y-56-39	3	85	4.7
Average			5.5
Y-56-628	5	52	32.7
Y-56-11	5	28	21.8
Y-56-146	5	18	38.9
Y-56-183	5	114	54.4

Table 3, continued

Selections	Age of Seeds (days)	No. of Seeds Examined	Per cent Infestation
Y-56-225	5	31	29.0
Y-56-39	5	27	59.2
Average			39.3
Y-56-628	6	109	48.6
Y-56-11	6	68	63.2
Y-56-146	6	247	48.6
Y-56-183	6	160	71.2
Y-56-225	6	11	72.7
Y-56-39	6	--	--
Average			60.9
Y-56-628	7	7	71.4
Y-56-11	7	40	82.5
Y-56-146	7	72	55.5
Y-56-183	7	32	75.0
Y-56-225	7	30	63.3
Y-56-39	7	243	70.8
Average			69.7
Y-56-628	8	134	80.6
Y-56-11	8	27	88.9
Y-56-146	8	--	--
Y-56-183	8	276	69.6

Table 3, continued

Selections	Age of Seeds (days)	No. of Seeds Examined	Per cent Infestation
Y-56-225	8	--	--
Y-56-39	8	42	69.0
Average			77.2
Y-56-628	10	39	61.5
Y-56-11	10	25	80.0
Y-56-146	10	109	79.8
Y-56-183	10	111	70.2
Y-56-225	10	75	61.3
Y-56-39	10	--	--
Average			70.6
Y-56-628	11	63	54.0
Y-56-11	11	38	68.4
Y-56-146	11	203	57.6
Y-56-183	11	--	--
Y-56-225	11	183	47.5
Y-56-39	11	104	63.5
Average			58.2
Y-56-628	14	292	11.3
Y-56-11	14	378	15.3
Y-56-146	14	352	6.5
Y-56-183	14	261	0

Table 3, continued

Selections	Age of Seeds (days)	No. of Seeds Examined	Per cent Infestation
Y-56-225	14	155	28.4
Y-56-39	14	167	13.1
Average			12.4
Y-56-628	16	310	0
Y-56-11	16	97	0
Y-56-146	16	250	0
Y-56-183	16	144	0
Y-56-225	16	62	0
Y-56-39	16	--	0
Average			0
Y-56-628	18	231	0
Y-56-11	18	231	0
Y-56-146	18	133	0
Y-56-183	18	206	0
Y-56-225	18	143	0
Y-56-39	18	151	0
Average			0

DESCRIPTION OF EXPERIMENTS - III

<u>Seasonal Differences in the Degree of Chalcid Infestation</u>:

Alfalfa pods from the clonal selections Y-56-183, Y-56-146, Y-56-628, Y-56-225, Y-56-39, and Y-56-11 (the same material as described in Section II) were harvested on August 4, 16, and 28, 1962. The samples were placed in one-half pint paper cups with tightly fitting translucent lids and stored in the laboratory under room temperature conditions. In March, 1963, the emerged chalcids and parasites were separated from the pods and counted. The pods were then threshed and the seeds were cleaned. The cleaned seeds were put in vials containing 70 per cent ethyl alcohol to which a few drops of a detergent "Triton B-1956" (a Rohm and Haas product) had been added. The addition of the detergent was to facilitate penetration of the alcohol into the seeds. After soaking for a week, the seeds were dissected to determine numbers of infested and uninfested seeds.

Results of this study are shown in Table 4. It can be seen that pods harvested on August 16, had the highest degree of infestation. With the exception of selections Y-56-11 and Y-56-628, the degree of infestation declined in the pods harvested on August 28.

Table 4

Per cent infestation of alfalfa seeds and parasitization of chalcids from alfalfa harvested on August 4, 16, and 28, Tucson, 1962.[a]

Selection	Date of Harvest	Per cent Infestation	Per cent Parasitization
Y-56-183	August 4	58.7	19.0
Y-56-628	"	56.5	10.0
Y-56-39	"	52.0	15.8
Y-56-146	"	57.4	20.3
Average		56.1	16.3
Y-56-183	August 16	74.2	27.4
Y-56-146	"	74.8	21.6
Y-56-628	"	58.0	38.5
Y-56-225	"	45.2	12.3
Y-56-39	"	76.8	13.7
Y-56-11	"	35.6	20.7
Average		60.1	22.4
Y-56-183	August 28	49.4	15.9
Y-56-146	"	52.1	8.9
Y-56-628	"	62.2	8.1
Y-56-225	"	43.8	16.9
Y-56-39	"	42.6	11.1
Y-56-11	"	64.3	22.5
Average		52.6	13.9

[a]Percentages are based on the totals of two replicates.

McAllister *et al*. (1962) indicated that chalcid population densities are closely associated with the bloom habits of alfalfa plants. Chalcids occur in greatest numbers a few days after the peak of bloom. Under the summer conditions, the author noted that alfalfa pods started drying up 18 to 23 days after tripping. If it is assumed that the pods harvested were approximately three weeks old, it can be inferred that the flowers which gave rise to pods that were harvested on August 16 were in bloom during the last week of July. As the seeds from these pods had the highest infestation of the three harvesting dates, it could be assumed that there was a much higher density of chalcids during the final week of July, with periods two weeks earlier and later having lower population levels. This may explain the observed pattern of infestation.

DESCRIPTION OF EXPERIMENTS - IV

Observations of Selected Alfalfa Varieties for Possible Resistance to Chalcids

These studies were made with samples of alfalfa varieties which were collected at the University of Arizona Farms at Mesa and Yuma, Arizona.

Yuma varieties: The Yuma field consisted of 54 entries of alfalfa planted in October, 1960, and arranged in a randomized complete-block design with six replicates. Each block was made up of six rows of plants 40 inches apart and ten feet long. Pods from replicated plots of 12 varieties including African, Buffalo, Caliverde, Hairy Peruvian, Cody, Lahontan, Moapa, New Mexico 11-1, California Common, Sirsa #9, Arizona Chilean, and Vernal were harvested on July 30, 1962, and brought to Tucson for study.

Mesa varieties: The Mesa field consisted of 48 entries of alfalfa planted in February, 1962, and arranged in a randomized complete-block design with seven replicates. Each entry was planted in a one-row plot $4\frac{1}{2}$ feet long and 3 feet apart. Pods of 21 of these varieties and selections, including Sirsa Composite 4, M-56-10, M-56-11, M-56-225, M-56-628, Atlantic, Buffalo, Zia, New Mexico 11-1, African, Hairy Peruvian, Fruile, Tuna, Du Puits, Hardigan, Cody, Talent, Sirsa #9, Chilean 21-5, Moapa and Lahontan were harvested on August 20, 1962, and brought to Tucson for study.

Per cent infested seeds as an index to alfalfa resistance to chalcids: Samples of the following Yuma varieties, which had been stored in the laboratory since harvest, were divided into "quarters" on April 6, 1962: African, Buffalo, Caliverde, Hairy Peruvian, Cody, Lahontan, Moapa, New Mexico 11-1, California Common, and Arizona Chilean. Opposite "quarters" were taken and threshed with a hand thresher. The hand thresher consisted of a rectangular wooden structure 18 inches long by 6 inches wide. Three sides of the equipment projected vertically to a height of about ½ inch. This projection enclosed a flat area or platform which was covered with corrugated rubber. For a scrubber, an ink pad-shaped block covered with corrugated rubber was used. The alfalfa pods were put on the platform of the rectangular block and by gentle forward and backward movements with the pad-like scrubber, the pods were broken open to liberate the seeds. The seeds were cleaned of pod trash by elutriation, using a column blower. The cleaned seeds were soaked for a week in 70 per cent ethyl alcohol to which a few drops of "Triton B-1956" had been added. Two hundred seed samples from six replicates of each of the 10 varieties of alfalfa were dissected to determine the numbers of infested and uninfested seeds.

Resistance is assumed to be correlated with the percentage of seeds that are infested; the lower the percentage, the higher the resistance and vice versa. The original data were transformed to $\sin^{-1}\sqrt{X}$* (Table 5). An analysis of variance* was applied to the transformed data and differences among the means demonstrated, using Duncan's Multiple Range Test* at the 5 per cent level of probability. The analysis of variance showed significance at the one per cent level. The range test revealed that at the 5 per cent level there was no significant difference in the per cent infestation of seed of Caliverde, Moapa, New Mexico 11-1, California Common, Lahontan, Cody, Buffalo, or Arizona Chilean. The per cent infestation of the varieties Hairy Peruvian and African differed significantly from the other varieties listed above with the exception of Arizona Chilean. Arizona Chilean tended to bridge the two levels of infestation. Further studies are needed to reveal the true position of this variety.

If any resistance is to be assigned to the varieties studied, Caliverde, Moapa, New Mexico 11-1, California Common, Lahontan, Cody, and Buffalo could be regarded as

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*Statistical procedures used are described by Steel and Torie (1960).

Table 5

Ranked means[a] showing per cent seed chalcid infestation in alfalfa seed (based on 200 seeds per entry), Yuma, 1962.

Rank	Entry	Per cent Infestation	Multiple Range Test[b] at 5 per cent level
1	Caliverde	15.7	a
2	Moapa	15.8	a
3	New Mexico 11-1	16.0	a
4	California Common	16.8	a
5	Lahontan	18.4	a
6	Cody	20.4	a
7	Buffalo	20.5	a
8	Arizona Chilean	22.2	ab
9	Hairy Peruvian	27.2	b
10	African	27.4	b

F value for entries : 4.51**

$S\bar{x}$: 2.08

C. V. per cent : 25.40

[a]Means represent arcsine or angular transformed data.

[b]Duncan's Multiple Range Test. Entries or varieties not having common letters are significantly different at the 5 per cent level.

**Significant at the 1 per cent level.

the more resistant varieties.

<u>Chalcid emergence from infested seeds as an index to resistance in alfalfa plants</u>: Fifty-pod samples of the Yuma varieties - African, Buffalo, Caliverde, Hairy Peruvian, Cody, Lahontan, Vernal and Sirsa #9 - were put in half-pint Kerr "self sealing" jars on July 21, 1962, one day after harvest. They were stored under unheated room temperature conditions until spring 1963 when the room was heated to a temperature ranging from 90° to 95° F. with a thermostat regulated radiant heater. Light from a 120 watt bulb was on throughout night and day to accelerate the emergence of chalcids and parasites. In May, 1963, the emerged chalcids and parasites were sieved with a 9-mesh "U. S. Standard Sieve Series No. 10" and counted. In these counts chalcid parasites were regarded as chalcids on the assumption that if the chalcids had not been parasitised, they could have emerged.

Results are shown in Table 6. Ignoring factors such as continuation of diapause, disease, and other mortality causative agents which may prevent the emergence of chalcids and parasites, it can be assumed that the level of emergence of chalcids and parasites from infested alfalfa seeds is correlated with the level of infestation. Levels of infestation in turn give an indication of the degree of resistance present.

Table 6

Ranked mean populations[a] of alfalfa seed chalcids in eight varieties of alfalfa (based on 50-pod samples per entry), Yuma, 1962.

Rank	Entry	Mean Population	Multiple Range Test[b] at 5 per cent Level
1	Cody	2.90	a
2	Lahontan	3.26	a
3	Vernal	4.10	b
4	Buffalo	4.13	b
5	Caliverde	4.59	bc
6	African	4.90	c
7	Sirsa #9	4.99	c
8	Hairy Peruvian	5.75	c

F Value for entries : 2.44*

$S\bar{x}$: 0.19

C. V. per cent : 33.95

[a]Means represent square root transformed data.

[b]Duncan's Multiple Range Test. Entries or varieties not having common letters are significantly different at the 5 per cent level.

*Significant at the 5 per cent level.

The original data were transformed to $\sqrt{(X + 0.5)}$.* An analysis of variance was applied to the transformed data and differences among the means of the varieties demonstrated by Duncan's Multiple Range Test at the 5 per cent level of probability. The analysis of variance indicated significance at the 5 per cent level. The range test showed that no significant differences occurred within the following groups: (1) Cody and Lahontan; (2) Buffalo and Caliverde; (3) Caliverde, African, Sirsa #9 and Hairy Peruvian, although significant differences existed between the groups 1 and 2, and between groups 2 and 3. Caliverde tended to bridge groups 2 and 3. Further tests will reveal the true position of this variety.

Lahontan progeny test: Samples were collected from an experimental area at Mesa, Arizona, of a completely randomized design with six replicates. The progenies under study were C-902, C-901, C-900, C-89, and C-84, with Lahontan certified as a check. Fifty-raceme samples from pods harvested on August 20, 1962, were stored in half-pint paper cups with tightly fitting translucent plastic lids. They were kept in the laboratory under similar conditions as in the previous experiment. Emerged chalcids and parasites were separated from the racemes and counted.

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*X represents the total number of chalcids and parasites for each replicate.

The original data (Table 7) were transformed to $\sqrt{(X + 0.5)}$.* An analysis of variance was made on the transformed data. The analysis of variance showed no significant difference among the means. In Lahontan parent and progeny studies by Nielson in 1961, he found that C-89 and the progeny from it had the lowest mean chalcid population. Tests repeated in 1962 showed that the parent clone C-89 continued to have the lowest population of chalcids but the progeny had the highest chalcid population. In the present study the author found the highest chalcid populations in C-89 progeny which confirms Nielson's results. Nielson explained that the change in chalcid population of C-89 progeny might be attributable to contamination of the field by the presence of volunteer alfalfa which grew from seeds that shattered during the previous season. This condition would tend to mask the response among entries since the volunteer alfalfa plants represented a heterozygous mixture resulting from open pollination. Since the author's samples were taken from the same field Nielson used for his 1962 test, his explanation for the unusually high chalcid population in C-89 progeny also holds for this test.

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*X represents the total number of chalcids and parasites for each replicate.

Table 7

Mean populations[a] of alfalfa seed chalcids on five Lahontan progenies, Mesa, 1962.

Entry	Mean
Lahontan Certified	100.0
C-901	115.1
C-900	117.6
C-902	118.9
C-84	128.4
C-89	143.6
F value for entries :	less than 1 N.S.[b]

[a]Means represent square root transformed data.

[b]Not significant.

Per cent uninfested seed as an index to alfalfa resistance to chalcid: If the assumption that the percentage of seeds infested by chalcids is correlated with the amount of resistance present in an alfalfa plant is true, then the corollary also holds that the percentage of uninfested seeds is likewise an index of resistance.

Samples collected from varieties and selections grown at Mesa in 1962 were stored in half-pint "Dixie" cups with tightly fitting translucent lids. Conditions under which they were stored were the same as in previous experiment. In May, 1963, emerged chalcids and parasites were separated from the pods with a 9-mesh "U. S. Standard Sieve Series No. 10" and counted. As the samples were not uniform, counts had to be made of uninfested seeds after the pods had been threshed and the seeds cleaned. Infested seeds do appear normal at times (Wildermuth, 1931); hence, the technique of counting uninfested seeds was not altogether satisfactory. Lygus bug damage was severe and this also made counting of seeds difficult.

The original data were transformed to $\sin^{-1}\sqrt{X}$ (Table 8). There was no significant difference among the varieties and selections when an analysis of variance was determined on the transformed data. Lack of significance in this study may possibly be attributed to the technique used. The high level of lygus bug damage may also have influenced the results.

Table 8

Mean[a] percentages of uninfested[b] seeds of selected alfalfa varieties collected at Mesa, Arizona, 1962.

Entry	Per cent uninfested seeds
Moapa	86.4
Hardigan	86.2
Zia	86.0
Sirsa Composite 4	85.3
M-56-11	84.9
Hairy Peruvian	84.5
New Mexico 11-1	84.3
Chilean 21-5	82.1
Talent	81.8
Atlantic	81.8
M-56-225	81.2
Fruile	80.9
Lahontan	80.8
M-56-628	80.7
Buffalo	80.7
M-56-10	80.3
Tuna	80.1
African	79.6
Cody	79.2

Table 8, continued

Entry	Per cent uninfested seeds
Sirsa #9	79.0
Du Puits	77.0

F value for entries : less than 1 N.S.[c]

[a]Means represent arcsine or angular transformed data.

[b]An emerged chalcid was taken to represent one infested seed. There was a number of replicates without chalcid emergence; hence, to make data analysis possible, per cent uninfested seeds were taken.

[c]Not significant.

DESCRIPTION OF EXPERIMENTS - V

Parasitization of Chalcids

Data for this study were obtained from all preceding studies in which chalcid and parasite counts were made by recording the total number of all the parasite species that emerged. This study was directed towards the finding of the following: (1) the degree of parasitism, (2) varietal influence on parasitization, and (3) seasonal influence on parasitization. No attempt was made to identify the parasite species observed in this study.

The rate of parasitism in these studies was based on counts of emerged chalcids and parasites. The per cent parasitism was calculated from the numbers of parasites and the total number of insects (parasites and chalcids) that emerged. Nikols'kaya (1932) reported that the rate of parasitism was in the range 23.8 to 80.9 per cent. The rates of parasitism in the present studies were generally low (Tables 4, 9 and 10).

In the study of varietal influence on the rate of parasitism, only the data from the Lahontan progeny test (Table 11) could be analysed statistically. In the other varietal studies, parasite emergence in most cases was nil, hence the data obtained from them were not analysed. In the Lahontan progeny test, the original data were transformed to $\sin^{-1}\sqrt{X}$. An analysis of variance was applied to the transformed data. Differences among the means were not significant.

The degree of parasitism in alfalfa pods harvested on August 4, 16 and 28, 1962, from the clonal selections appear to follow the levels of infestation (Table 4). This condition is not very obvious from the per cent parasitism of the individual selections but the average rate of parasitism for each harvesting date indicates that pods harvested on August 16, which had the greatest infestation, also had the grestest rate of parasitism. This tends to show that the parasite population density is influenced by chalcid population density. This agrees with Butler's (1959) finding that there is a positive correlation between chalcid and parasite abundance.

Table 9

Per cent parasitization[a] of alfalfa seed chalcids in seeds of alfalfa, Mesa, 1962.

Entry	Parasites	Total chalcids and Parasites	Per cent Parasitism
Sirsa Composite 4	27	43	62.8
M-56-10	53	89	59.5
M-56-11	17	29	58.6
Moapa	11	20	55.0
Du Puits	98	186	52.7
Hairy Peruvian	22	44	50.0
Zia	7	14	50.0
Cody	50	106	47.2
M-56-628	66	154	42.8
Chilean 21-5	26	61	42.6
Fruile	39	96	40.6
African	41	102	40.2
Sirsa #9	47	128	36.7
Buffalo	24	66	36.4
M-56-225	33	93	35.5
Tuna	49	150	32.7
New Mexico 11-1	13	42	30.9
Hardigan	8	29	27.6
Atlantic	13	51	25.5

Table 9, continued

Entry	Parasites	Total Chalcids and Parasites	Per cent Parasitism
Lahontan	16	80	20.0
Talent	9	82	11.0

[a]Per cent parasitization is based on totals of seven replicates.

Table 10

Per cent parasitization[a] of alfalfa seed chalcids in alfalfa seeds (50-pod samples) harvested on July 30, 1962, Yuma, Arizona.

Variety	Parasites	Total Chalcids and Parasites	Per cent Parasitism
African	15	150	10.0
Vernal	10	105	9.5
Lahontan	4	70	5.7
Buffalo	5	107	4.7
Caliverde	6	135	4.4
Hairy Peruvian	9	202	4.4
Sirsa #9	6	165	3.6
Cody	1	78	1.3

[a]Per cent parasitization is based on totals of six replicates.

Table 11

Mean per cent parasitization[a] of alfalfa seed chalcid in infested seed of five Lahontan progenies, Mesa, 1962.

Entry	Per cent Parasitism
C-902	33.4
Lahontan Certified	33.2
C-901	30.3
C-84	30.4
C-900	30.1
C-89	21.1
F value for entries :	less than 1 N.S.[b]

[a]Means represent arcsine or angular transformed data.

[b]Not significant.

SUMMARY

The present observations of the biology of the alfalfa seed chalcid were made in 1962 and 1963 and involved the following areas of study: (1) the adult emergence patterns, (2) the age of seed at which maximum chalcid infestation occurred, (3) the possibility of seasonal differences in the degree of infestation, (4) observations of selected alfalfa varieties for possible resistance to chalcids, and (5) the nature and degree of parasitism of the chalcid by its natural enemies.

Emergence of chalcids in the spring of 1963 was rather low. The low emergence was attributed to the possibility of the remaining diapausing larvae emerging in the spring of the second year (1964) in cases where failure to emerge was not due to death by disease or other agents.

Though seed set, in the experiment to determine age at which alfalfa seed was most susceptible to infestation, was not high for certain age groups, the results indicated that the alfalfa seed chalcid prefers young seeds. Maximum oviposition occurred within seeds ranging from five to eleven days old. Younger seeds were less preferred for oviposition.

There appeared to be a relationship between the bloom pattern of alfalfa plants and the level of chalcid infestation. Though the bloom pattern of alfalfa was not studied as such, there is some reason to believe that the low level of infestation of alfalfa seeds harvested on August 6 and 28, 1962, might have been influenced by a low bloom or a lull period in plant bloom.

Of the varieties of alfalfa studied to determine relative resistance to seed chalcid infestations, Caliverde, New Mexico 11-1, Cody, Vernal and Buffalo appeared to have some degree of resistance. The relative resistance shown by Caliverde and Cody was erratic.

The observed degree of parasitism of the alfalfa seed chalcid was low compared with figures that appear in the literature. Statistical information could not be obtained on the correlation between the relative resistance of alfalfa varieties and the degree of parasitism because emergence in some varietal samples was nil. There appears however, to be some relationship between chalcid population density and the rate of parasitism.

LITERATURE CITED

Ashmead, W. H. 1894. Description of new parasitic Hymenoptera (Papre No. 1). Trans. Am. Ent. Soc. 21:318-344.

Bacon, O. G., W. D. Riley, V. E. Burton, and A. V. Sarquis. 1959. Clover seed chalcid in alfalfa. Calif. Agr. 13 (7):7 and 11.

Bridwell, J. C. 1923. The occurrence of the clover seed chalcid of Astralagus. Jour. Wash. Acad. Sci. 13:260.

Butler, G. D., Jr. 1959. Insect parasites in relation to reducing chalcid injury to alfalfa seed crops. Progress Report for 1959, Western Regional Project W-74.

Butler, G. D., Jr., and H. L. Hansen. 1958. The parasites of the clover seed chalcid in the United States. Pan-Pacific Ent. 34:223-229.

Crothers, W. C., and A. B. Haws. 1961. Studies of four systemic insecticides for the control of the clover seed chalcid (Abs). Utah Acad. Sci. and Let. Proc. 38:117.

Fedoseeva, L. I. 1954. On species of Bruchophagus living in seeds of Leguminosae (in Russian). Muskov. Univ. Vestinik. 9 (3):113-118. (Cited in Strong, F. E. 1962a, original not seen).

---------------- 1956. New species of the genus Bruchophagus Ashm. (Hymenoptera: Chalcidoidea) developing upon leguminous plants. Doklady Akad. Nauk SSSR 111:491-493. (Cited in Strong, F. E. 1962a, original not seen).

Folsom, J. W. 1909. Insect pest of clover and alfalfa. Ill. Agr. Exp. Sta. Bul. 134:113-196.

Fronk, W. D. 1962. Orientation of seed chalcids to physical and chemical factors. Progress Report for 1962, Western Regional Project W-74.

Girault, A. A. 1916. Descriptions of and observations on some chalcidoid Hymenoptera II. Can. Ent. 48:337-344.

Hansen, H. L. 1955. The host relationships of the seed chalcid Bruchophagus gibbus (Boheman), Hymenoptera: Eurytomidae. Unpublished PhD Thesis. University of California, Berkeley, California. 96 pp.

Howard, L. O. 1880. Report of the entomologist. U. S. Dept. Agr. Report for the year 1879. p. 196.

Kolovoba, A. N. 1929. A contribution to the study of pest of lucerne (in Ukranian). Trans. Poltava Agric. Expt. Sta. Mo. 82, 15 pp. (Abstracted in Rev. Appl. Ent. (A) 19:369. 1931).

--------------- 1950. The clover and lucerne races of the seed pest Bruchophagus gibbus Boh. (Hymenoptera, Eurytomidae). Ent. Obozr 31 (1-2):63-70. (Abstracted in Rev. Appl. Ent. (A) 41:164-165. 1951).

MacDonald, H. A. 1946. Birdsfoot trefoil (Lotus corniculatus L.) its characteristics and potentialities as a forage legume. N. Y. Agr. Exp. Sta. Mem. 261.

McAllister, D. R., B. Austin Haws, W. A. Rowley, and G. D. Minion. 1962. Seasonal abundance of the alfalfa seed chalcid (Bruchophagus roddi.). Farm and Home Science. Utah Agr. Exp. Sta. 23:10-11, 28.

Neunzig, H. H., and G. G. Gyrisco. 1958. Host relationships of seed chalcids reared from birdsfoot trefoil. Jour. Econ. Ent. 51:409-410.

Nielson, M. W. 1962. Alfalfa seed chalcid resistance studies. Progress Report for 1962, Western Regional Project W-74.

Nikols'kaya, M. N. 1932. The clover seed chalcid (Bruchophagus gibbus Boh.) in alfalfa seeds in U. S. S. R. (in Russian). Plant Prot. 1932 1:107-111. (Abstracted in Rev. Appl. Ent. (A) 21:578. 1933).

Painter, R. H. 1951. Insect resistance in crop plants. The Macmillan Co., New York. 520 pp.

-------------- 1958. Resistance of plants to insects. Ann. Rev. Ent. 3:267-290.

Rowley, W. A. 1962. The entomological aspects of seed chalcid, Bruchophagus roddi Gussakovsii resistance in alfalfas. Unpublished M. S. Thesis. Utah State University, Logan, Utah. 67 pp.

Shull, W. E. 1944. Idaho recommendations for insect control. Idaho Agr. Exp. Sta. Bul. 252:15.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York. 481 pp.

Sorenson, C. J. 1930. The alfalfa seed chalcis-fly in Utah 1926-29, inclusive. Utah Agr. Exp. Sta. Bul. 218. 33 pp.

Strong, F. E. 1962a. Studies on the systematic position of the Bruchophagus gibbus complex (Hymenoptera: Eurytomidae) Ann. Ent. Soc. Am. 55:1-4.

--------------- 1962b. Laboratory studies on the biology of the alfalfa seed chalcid Bruchophagus roddi Guss. (Hymenoptera: Eurytomidae) Hilgardia. 32 (3):229-249.

Urbahns, T. D. 1914. Chalcis-fly in alfalfa seed. U. S. Dept. Agr. Farmers Bul. 636. 10 pp.

--------------- 1920. The clover and alfalfa seed chalcis-fly. U. S. Dept. Agr. Bul. 812. 19 pp.

Vinogradov, V. P. 1941. The clover-seed chalcid, Bruchophagus gibbus, and its distribution in the clover areas of the U. S. S. R. (in Russian). Bull. Plant. Prot. 1:20-26 (Abstracted in Rev. Appl. Ent. (A) 30:375. 1942).

Wildermuth, V. L. 1931. Chalcid control in alfalfa production U. S. Dept. Agr. Farmers Bul. 1642. 14 pp.