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**LABORATORY STUDIES OF THE BIOLOGY
OF THE ALFALFA SEED CHALCID,
Bruchophagus roddi Guss.
(Hymenoptera: Eurytomidae)**

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The alfalfa seed chalcid, *Bruchophagus roddi* Guss., a small jet-black wasp which infests *Medicago*, has caused alfalfa seed losses of up to 83 per cent in the western United States. Experiments conducted on the seed chalcid (actually a seed eurytomid, but commonly called a chalcid) revealed that:

- Infestation was maximal when the ratio of adults to pods was 1:1, when seeds were 8 to 10 days old, when adults were fed, and when temperatures at time of oviposition were 28° to 30° C.
- Although previous studies had indicated that there were no definite larval instars, this investigation revealed four larval stages, distinguished by both body and mandible lengths.
- Larvae chilled at slightly below room temperature emerged more rapidly than those held at room temperature or those subjected to lower temperatures.
- The greatest number of adults survived when they were fed and held at 50° F.
- The species is more prolific than previously suspected, as demonstrated by the production of 86 offspring by one female during a 13-day period.

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LABORATORY STUDIES OF THE BIOLOGY OF THE ALFALFA SEED CHALCID, *Bruchophagus roddi* Guss. (Hymenoptera: Eurytomidae)¹

FRANK E. STRONG²

INTRODUCTION

THE ALFALFA SEED CHALCID, *Bruchophagus roddi* Guss., is a small, jet-black wasp which breeds in and develops on species of *Medicago*. This cosmopolitan insect is a major pest of seed alfalfa. In the western United States, seed growers have sustained losses that range from 5 per cent to 83 per cent, as a result of infestations (Urbahns, 1920; Sorenson, 1934; Bacon, *et al.*, 1959). Similar losses have been reported from Russia (Kolobova, 1950).

Although *Bruchophagus roddi* is commonly called a seed chalcid, it should more properly be referred to as a seed eurytomid, for it belongs to the family *Eurytomidae*. Despite this technical discrepancy, the term chalcid will be used in this paper because of its generally accepted usage. For years this insect has been referred to as the clover seed chalcid, *B. gibbus* (Boh.). In the last decade, a series of papers by a group of Russian workers offered evidence, based on morphological studies, to show that *B. gibbus* is in reality three distinct species: *B. roddi*, *B. gibbus* and *B. kolobovae* (Kolobova, 1950; Nikolskaya, 1952; Fedoseeva, 1954). Strong (1961) has shown that these three species can be separated on the basis of female genitalia.

Both Sorenson (1934) and Urbahns (1920) have reported on the biology of *Bruchophagus roddi* as observed in the field. Strong (1960) reported on the variance encountered when sampling small plots for seed chalcid damage. Hansen (1955) investigated the host relationships of *B. gibbus*. Neunzig and Gyrisco (1959) studied the parasites associated with *B. roddi* and related species in New York State. Bacon and Strong (unpublished data) have studied the seasonal occurrence of *B. roddi* in California. The flight habits of *B. roddi* are currently being investigated and will be reported elsewhere.

This paper describes a series of laboratory investigations designed to provide information on the general biology of *Bruchophagus roddi*. The life cycle of this species can be summarized briefly as follows: the females oviposit

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in green alfalfa seeds. The eggs hatch in 1 to 3 days, and the larvae feed within the seed for 8 to 12 days. Another 8 to 12 days are spent in the pupal stage within the seed. The adult emerges from the pupa, cuts the typical exit hole and emerges. There may be several generations per season. This insect overwinters as a mature larvae in the seed.

Fig. 1. Plastic cage used to confine adult seed chalcids, *Bruchophagus roddi* Guss., to individual raceme.

GENERAL METHODS

The alfalfa plants utilized throughout these studies were propagated in a greenhouse. The plants, originally raised from seed, were of the varieties Caliverde, Narragansett, Vernal and a clone designated as C-175. The latter was used most frequently because it bloomed profusely and produced large racemes (15 to 20 pods with 4 to 5 well-developed seeds per pod). Several cuttings were propagated from a Vernal plant which also possessed these attributes. The plants were maintained under 17 hours of light daily, which provided continual bloom. All blooms were hand tripped in such a manner as to provide for cross-pollination. The plants were fumigated with methyl bromide to control other pests when necessary.

Two types of incubators were employed. Several precision incubators of

one type were equipped with three to seven daylight fluorescent lamps plus a 40 watt incandescent bulb. A time switch was wired into the lighting circuits. Each precision incubator had a temperature range of 0°C to 50°C and a tolerance of $\pm 0.5^{\circ}\text{C}$. The second type of incubator was a temperature box having no light and capable of maintaining temperatures within 2°F of its setting.

Fig. 2. Cage used to permit adult chalcids, *Bruchophagus roddi* Guss., access to several racemes simultaneously.

In many tests, individual females were caged on single racemes for oviposition. These cages were 1 to 1½ inches long, 5/8 inch in diameter and were made from clear plastic centrifuge tubing. One end was covered with a section of material cut from women's nylon hose; the other was plugged with a disc cut from a ¼-inch thick, synthetic, sponge-like packing material. The rachis of the raceme was inserted in a radial slit in the plug. The cage was then slipped over the raceme and onto the plug (fig. 1). A small stoppered hole in the wall of the cage provided a means of introducing insect adults after the cage was in place.

Several other types and sizes of cages were tested. Most were suitable for caging individual females, but increased oviposition always occurred when the area of confinement was small and there was close juxtaposition of chalcid and raceme.

In a few tests, a large number of racemes were to be infested simultaneously. In this case, the racemes from several plants were placed in a single half-gallon ice cream carton. The stems were inserted through longitudinal slits in the carton's side. The carton was then covered with a polyethylene film and several hundred adults were introduced through a small opening (fig. 2).

PRELIMINARY STUDIES ON OVIPOSITION

Since adults can be induced to emerge from infested seed throughout the year, it is not necessary to rear the chalcids in order to insure a continuous supply of test organisms.

It is usually necessary, however, to rear an insect to study its life cycle. Various workers have reported difficulty in rearing this insect in the laboratory. The stumbling block to laboratory success was apparently the inability of the experimenters to induce the females to oviposit sufficiently to create a high per cent infestation in test plants. Thus, several preliminary experiments were organized to investigate some of the factors which may influence oviposition.

Effect of Age of Seed on Oviposition

One of the first factors investigated was the effect of the age of seed on its suitability as an ovipositional site. This experiment was conducted in a greenhouse with an average temperature of 79°F. Racemes of one large Vernal plant were tripped intermittently over a 23-day period. At least six racemes were tripped on each selected date. When the oldest seeds were 28 days old, five female chalcids were caged on each raceme and left for 48 hours. Twenty days after exposure, all racemes were harvested and each seed dissected. The numbers of infested seeds are shown in table 1.

TABLE 1
EFFECT OF AGE OF ALFALFA SEED ON
CHALCID OVIPOSITION

Age of seed at time of oviposition	Number of seeds examined	Number infested	Per cent infested
3 days.....	103	0	0.0
6.....	128	15	11.7
8.....	94	42	44.6
9.....	94	54	57.5
10.....	308	128	41.5
13.....	90	13	14.4
17*.....	215	54	25.1
19.....	23	2	8.7
23.....	38	0	0.0

* There is reason to believe that contamination occurred here.

According to these results, racemes containing seeds 8 to 10 days old appear to be most favorable for oviposition. Seeds of this age were therefore used in all subsequent tests. It is recognized, however, that the results may have been affected in part by several factors other than age of seed.

Effect of Feeding on Oviposition

A second factor investigated was the effect of adult feeding on the numbers of eggs laid. This was tested in two experiments. Adults for both experiments were collected as they emerged from infested seed and given no food or water at that time. In the first experiment, seven females were caged individually on single racemes. Three of them were then fed a 5 per cent honey

solution administered by dabbing it on the screened end of the cage. The remaining four were given no food. In the second experiment, using the same technique, thirteen adults were used, seven fed and six not fed. After the adults were introduced into the cages, the plants were placed in a precision incubator (temperature = 25°C). Forty-eight hours later the insects were removed and the plants transferred to a greenhouse. Twenty days after exposure, all racemes were dissected and the seeds observed for infestation. Although the number of test organisms was small, the results, shown in table 2, indicate that a five-fold increase in oviposition was achieved by feeding the adults.

TABLE 2
TOTAL NUMBER OF CHALCID OFFSPRING FROM INDIVIDUAL ADULTS
WITH AND WITHOUT ACCESS TO FOOD

Experiment number	With food				Without food			
	Number of adults	Total seeds tested	Number infested	Per cent infested	Number of adults	Total seeds tested	Number infested	Per cent infested
1.....	3	113	40	35.4	4	144	10	6.95
2.....	6	261	144	55.2	7	288	36	12.50
Totals.....	9	374	184	49.3	11	432	46	10.67

Relation of Temperature to Oviposition

A third factor pertaining to rearing was investigated in a preliminary manner only; this was the relation of temperature to oviposition. Six racemes with long rachises were harvested when 9 days old and cut ends were placed individually in small vials of water; each raceme was then enclosed in a small plastic cage. Five such series of six racemes each were prepared and numbered in a comparable manner. Three adult female chalcids were placed in each cage of the first series and were held at 10°C in a precision incubator under constant lights. At the end of 6 hours, the females from the first series were transferred to the same numbered cage of the second series and held 6 hours at 15°C. This transferring process was continued until all five series had been used. Five days later, all seeds were dissected and the presence of any larvae recorded. The per cent infestation observed at 10°, 15°, 22°, 28°, and 30°C was 0.0, 2.8, 10.4, 21.2, and 17.6 per cent, respectively. These data suggest that the higher temperatures are more favorable for oviposition than the lower temperatures.

Miscellaneous Experiments

Several preliminary experiments were performed to gain information on other factors which may influence oviposition. Of these, two will be mentioned.

1) A study was conducted to see if there is an optimum female-to-pod ratio for oviposition. Cages were placed on racemes bearing 12 to 14 pods each. Chalcids were introduced at female-to-pod ratios ranging from 9:11 to 2:1. Exposure time was uniform throughout. In general, the lowest ratios

produced the lowest infestations, but the trend was not consistent. One female was frequently responsible for most of the offspring taken from a single raceme. Thus the ratio 1:1 was selected for testing purposes where an infestation of 75 to 95 per cent was desired. A raceme with 10 pods would then be exposed to 10 females. Experience has shown that at least 1 of these 10 females will produce copiously.

2) Another study was conducted to see if females oviposit in darkness and if light of different wave lengths markedly inhibits or facilitates oviposition.

Different wave lengths of light were produced by covering clear plastic cages with colored cellophane. The colors red, blue and green were tested. In addition, one cage was covered with aluminum foil to produce total darkness, while another was left completely uncovered. Ten females were placed

TABLE 3
PER CENT INFESTATIONS PRODUCED BY CHALCID ADULTS
OVIPOSITING IN VARIOUS COLORED CAGES

Test 1	Color sequence.....	Clear	Red	Dark	Green
	Per cent infestation.....	5.2	17.5	26.3	14.8
Test 2	Color sequence.....	Green	Dark	Clear	Blue
	Per cent infestation.....	5.9	8.7	11.1	5.9

in the cage covered with clear plastic on a single raceme and left for 36 hours. They were then transferred to the red colored cage and left another 36 hours. This was continued until all colored cages had been utilized. Using a second group of 10 females, another color sequence was run. Twenty days after exposure, the seeds were dissected and larvae recorded. The results are shown in table 3.

This simple experiment demonstrates that female chalcids will oviposit in complete darkness and under different wave lengths of light. No attempt was made to ascertain whether the light within the colored cages was monochromatic or what each wave length was.

STUDIES OF THE LARVAL STAGES

Determination of Number of Instars

Sorenson (1934) mentioned that he had observed no definite larval instars of the alfalfa seed chalcid, and expressed the opinion that the larvae molted irregularly until mature. As this is not in accord with the accepted knowledge of insect development, a study was initiated to determine the number of larval instars of the seed chalcid. The general scheme was to examine larvae of several known ages and then to plot frequency histograms to see if the measurement data would fall into groups.

Approximately 35 racemes of a large Vernal plant were all tripped on the same day. Nine days later, adults were permitted ovipositional access for 3 days, according to the method shown in figure 2. Three racemes were har-

Fig. 3. Mandibles of third-instar chalcid larvae (*Bruchophagus roddi* Guss.). Measurements made between A and A' yielded the clearest class distinctions among the four instars.

vested on each of the third, fourth, fifth, sixth and seventh days after removing the adults. The seeds were dissected and all live larvae were measured for length and then mounted in Hoyer's medium. The width of the first abdominal spiracle and the length of the mandible from its tip to the base of the tooth were measured for each of these larvae (fig. 3). The data given in table 4 were plotted in frequency histograms (fig. 4).

TABLE 4
BODY LENGTH AND MANDIBLE LENGTH OF SEED CHALCID
LARVAE AT FOUR INSTAR STAGES

Instar	Mandible (in microns)			Body (in mm)		
	Number of larvae	Mean $\pm$ Standard length deviation	C.V.*	Number of larvae	Mean $\pm$ Standard length deviation	C.V.*
1.....	5	7.64 $\pm$ 1.22	15.96	6	0.324 $\pm$ 0.054	16.80
2.....	9	15.18 $\pm$ 1.26	8.32	13	0.705 $\pm$ 0.096	13.71
3.....	12	23.03 $\pm$ 1.57	6.82	18	1.179 $\pm$ 0.120	10.17
4.....	20	35.72 $\pm$ 2.54	7.11	11	1.924 $\pm$ 0.716	3.72

* C.V. = coefficient of variation = (standard deviation/mean) $\times$ 100.

In figure 4, the measurements of both body length and mandible length fell into four groups, each of which is considered to be an instar. The abdominal spiracle was too variable to permit segregation. The mandible character was the least variable (see coefficient of variation, table 4) and yielded the clearest class distinctions. For general laboratory purposes, however, the length of living larvae appeared to be satisfactory even though second and third instar larvae sometimes may be confused.

Additional racemes were harvested from this plant every day for 14 days. No larvae were observed to exceed the live length range for the fourth instar. Pupating larvae were noted on the twelfth day.

Fig. 4. Frequency histograms of measurements of alfalfa seed chalcid larvae (expressed in arbitrary units) indicating four instars

Time Required for Development

Eggs. Several racemes, 8 days old, were harvested with long rachises, placed in vials containing water, and caged individually with small plastic cages, as shown in figure 1. Several females were introduced into each cage and allowed a 3-hour period in which to oviposit. At the end of this period, the racemes were placed in a precision incubator set at 20°C. At varying intervals thereafter, the racemes were removed and dissected eggs observed for

hatch. No hatching was observed among 25 eggs at the end of 121.0 hours (5 days). This experiment was repeated at a temperature of 30°C. At this temperature, 29 eggs were observed, and the mean hatching time was 42.5 hours.

The egg is milky-white when laid. It then gradually clears until, just prior to hatching, it is almost translucent. The mandibles of the embryo can be observed only a few hours prior to hatching. If a green seed has been infested, the location of the egg can be determined by observing the "sting." This is a brown scar left by the ovipositor, which remains visible for a week or so after the egg is laid.

Instars 1 to 4. An experiment was performed in an attempt to determine the number of days necessary for the development of individual instars. Twenty racemes were infested by the procedure shown in figure 2. The females were allowed to oviposit for 38 hours. Each day thereafter, one raceme was harvested, and all larvae were dissected from the green seeds. The larval instars were determined by body length measurements. This test was conducted in a greenhouse where the average temperature for the entire test was 24° to 28°C.

Since the racemes were harvested beginning one day after removal of adults, additional data concerning the hatching time of eggs were obtained. The mean number of days required to attain a given instar was computed as follows: the products of the number of larvae in any particular instar and the day-number on which these larvae were removed were summed for every day that the particular instar was present. This sum was then divided by the total number of those larvae under consideration. It is realized that this method leaves much to be desired, but it is a commonly used method when one is actually unable to observe the developing larvae. The results, shown in table 5, suggest that the fourth instar is attained in slightly over 12 days, at 24°C.

TABLE 5
NUMBER OF DAYS REQUIRED FOR DEVELOPMENT OF SEED
CHALCID THROUGH VARIOUS STAGES AT AN AVERAGE
TEMPERATURE OF 24° TO 27° C

Stage	Number of chalcids	Cumulative days to attain indicated stage	Range in days (inclusive)
Egg incubation.....	20	1.75	1-3
1st instar.....	31	3.93	2-7
2nd instar.....	17	5.83	5-7
3rd instar.....	12	6.58	6-9
4th instar.....	55	12.23	7-17

Mature Larva to Adult

A chalcid larva apparently does not defecate during its early instars. Once the larva is mature, it can follow one of two paths: 1) continue its development and become a pupa, or 2) enter diapause. If path 1 is followed, the larvae defecate and enter the prepupal stage. If they enter diapause, defecation does not occur until diapause is terminated. Larvae in diapause can

thus be classified by observing the presence or absence of the feces within the body.

To determine the length of time necessary for development of the pupal stages, mature larvae that had not defecated were removed from green seeds and placed in individual gelatin capsules which were supported upright on end in a styrefoam block. The larvae were held in a precision incubator set at 25°C, in which the relative humidity measured 50 to 65 per cent. The following key was utilized to facilitate identification of the various stages of the pupae and prepupae.

Key	Stage of Development
E	Mature larva; gray; has not defecated; exhibits movement
D	Mature larva; white; has defecated; exhibits movement
C	Prepupa—a short, thickened larva; white; no movement
H	Pupa—eyes white; body white
G	Pupa—eyes pink to red; body yellowish
I	Pupa—eyes brown; abdomen black; thorax yellowish
J	Pupa—eyes brown; entire body black
*	Adult

The developmental sequences are listed in order above. Each larva was observed daily and the time required to pass from stage to stage recorded.

TABLE 6
NUMBER OF DAYS REQUIRED FOR THE DEVELOPMENT OF THE MATURE
CHALCID LARVAE THROUGH VARIOUS PUPAL STAGES AT 25°C

Stage of development	Number of larvae	Mean days $\pm$ standard deviation
D — Defecated larva.	4	2.25 $\pm$ 1.49
C — Prepupa.	6	1.0 $\pm$ 0.0
H — Pupa—eyes white.	13	3.15 $\pm$ 0.375
G — Pupa—eyes red.	17	1.82 $\pm$ 0.528
I — Pupa—eyes brown; abdomen black.	7	1.0 $\pm$ 0.0
J — Pupa—entirely black.	20	3.55 $\pm$ 0.456
Total (Stage D to adult)		12.77 days

The data recorded in table 6 indicate that defecation is completed in about 2 days. The prepupal stage lasts less than a day thereafter. The eyes begin to change color about 3 days after the formation of the pupa. As soon as the eyes have become completely brown, the body rapidly turns black, a process that requires less than a day. The adult emerges about 3.5 days after the body turns black.

The total length of time for development from egg to adult at 24° to 25°C can be summarized as follows: Egg incubation = 1.75 days; first through fourth instar = 12.23 days; defecating larvae until adult emergence = 12.77 days; total = 26.75 days.

Effect of Age of Seed on Rate of Development

It was originally supposed that the age of the seed at the time of oviposition affects the rate of development. This hypothesis was tested as described

in the following experiment. Fifteen racemes on a single plant were all tripped on the same day. Seven days later, fifteen more racemes from the same plant were tripped. When the youngest racemes were 8 days old (and the oldest 15 days old), each raceme was caged, and 15 to 20 females placed in each cage. The chalcids were allowed to remain on the racemes for 6 hours.

The racemes from each group were harvested on two separate dates. On each date, all dissected larvae were then computed. To facilitate this computation a number was arbitrarily assigned the various developmental stages as follows:

Stage of Development	Arbitrary number
Egg	0
First instar larva	1
Second instar larva	2
Third instar larva	3
Fourth instar larva	4
Mature larva; gray; has not defecated	5
Mature larva; white; has defecated	6
Prepupa	7
Pupa—eyes white	8
Pupa—eyes pink	9
Pupa—eyes brown	10
Pupa—all black	11

The arbitrary numbers corresponding to the immature chalcids removed on each date were then averaged, to indicate the average developmental stage.

There was a significant difference (based on Student's *t*) between the larval instars taken from the two groups of racemes on each date. The larvae removed on January 16 from seeds which were 26 days old were third instars on the average, while the average larvae removed from seeds 33 days old on the same date had just begun to defecate. These figures are shown in table 7.

TABLE 7

AVERAGES OF NUMBERS ASSIGNED TO INDIVIDUAL CHALCIDS TO INDICATE THEIR STAGE OF DEVELOPMENT WHEN NURTURED ON RACEMES OF TWO AGES (Eggs Laid on December 28, 1960)

Date harvested	Date of trip and age of seed at harvest			
	December 13—33 days		December 20—26 days	
	Number of chalcids	Mean instar $\pm$ standard deviation	Number of chalcids	Mean instar $\pm$ standard deviation
January 16.....	37	6.00 $\pm$ 1.47	23	3.04 $\pm$ 0.77
January 9.....	88	2.68 $\pm$ 0.49	25	0.84 $\pm$ 0.55

A possible explanation of these results is that when an egg is laid in a young seed, there are not sufficient nutrients to promote optimum growth of the larvae. A seed which is approaching maturity, however, has stored nutrients which are readily available to the larvae.

This experiment was performed during a prolonged cloudy, foggy period. The plant was maintained on a window sill in the laboratory, and because of the short winter day and lack of direct sunlight, the seed pods were growing very slowly. Normally, an 8-day-old pod on a vigorously growing plant will have two full turns, and the developing seeds will be readily apparent as bulges in the pod; if the seeds are dissected, the cotyledons will be deep green, firm and well developed. On this plant, however, the 8-day-old pods, at the time of infestation, had but one turn. A few seeds were dissected and found to be whitish-green and mostly fluid, a state of development usually observed in a 3- to 4-day-old seed.

Fig. 5. Typical emergence pattern of alfalfa seed chalcid adults from seed collected in the fall and held at room temperatures

Emergence of Adults from Diapaused Larvae

When infested seeds are collected from the field in late fall (about October 15) and stored at room temperatures, adults emerge as shown in figure 5. There is an initial emergence of adults that have developed from nondiapausing larvae, which represents about 35 per cent of those that will ultimately emerge. After this, no adults emerge for about 6 weeks, because the larvae are in diapause. About mid-December, the diapause begins to terminate and adults soon emerge again. They continue to do so, until about the first week in April.

The induction and termination of diapause have been investigated for several species (Lees, 1956), but apparently not for *Bruchophagus roddi*. It is generally conceded that low temperatures are often responsible for breaking diapause, a mechanism more aptly termed the "diapause-ending

process" by Schneiderman and Horwitz (1958). The effect of temperature on the diapause-ending process was therefore investigated.

Seeds collected in the fall were held in the laboratory until those adults developing from nondiapausing larvae had emerged. Thirty-gram lots of this seed were then placed in pint ice cream cartons each of which had a $\frac{3}{8}$ -inch hole bored in the side, 1 inch from the top. A 6-dram vial was screwed into the hole, and the lid of the carton affixed in place. As adults emerged, they collected in the vials, since this was the only light source inside the carton.

TABLE 8
TOTAL NUMBERS OF ADULTS EMERGING FROM THREE LOTS OF SEED
CHILLED AT DIFFERENT TEMPERATURES FOR VARIOUS
TIME INTERVALS

Chilling time in weeks	Temperature			
	30° F	40° F	50° F	60° F
	Number of adults			
3	779	702	735	754
6	809	848	802	818
9	814	758	736	743
12	771	740	822	495

	Chilling time in days at 10° F					
	1	3	4	8	16	32
Number of adults	718	559	658	526	547	638

Twelve cartons of seed were placed in each of the following temperature cabinets: 30°, 40°, 50°, and 60°F. Three additional cartons of seed were held at room temperature to serve as controls. At the end of 3, 6, 9 and 12 weeks of chilling, three cartons were removed from each cabinet, placed in the laboratory (mean temperature, 74°F) and observed for emergence until less than 2 or 3 adults were collected in a week. The total number of adults that emerged from the three cartons removed on a given date is shown in table 8. Although the design was a 4×4 factorial replicated three times, these totals were not analyzed statistically since they did not appear to vary significantly. Adult emergence was plotted on probability paper and, from the eye-fitted line, the 50 per cent emergence time was read graphically.

The results of this experiment indicated that: 1) If the diapausing larvae were left at room temperature, the adults emerged very slowly for a period of about 120 days, requiring 68 days for 50 per cent emergence (fig. 5). Thus, the diapause-ending process can occur at room temperature. 2) As the chilling time increased, the number of days required for 50 per cent emergence decreased for all temperatures considered. (See table 9 and fig. 6.) This fact suggests that the diapause-ending process is hastened by exposure to lower temperatures. The relationship between periods of chilling and emergence times was not linear (fig. 6). Chilling for 6 weeks caused a propor-

TABLE 9
NUMBER OF DAYS FOR 50 PER CENT ADULT EMERGENCE AFTER
TERMINATION OF CHILLING

Weeks of chilling	Temperature				Mean
	30° F	40° F	50° F	60° F	
	Number of days				
3.....	52	49	48	49	49.5
6.....	33	31	29	29	30.5
9.....	29	24	23	20	24.0
12.....	25	20	18	16	19.7
Mean.....	35.5	32.7	30.2	29.2

Fig. 6. The effect of chilling diapausing larvae for various lengths of time on adult emergence in alfalfa seed chalcid

tionately greater reduction in emergence time than did 3 weeks of chilling; likewise, a lesser proportionate reduction occurred when larvae were chilled for 9 or 12 weeks.

These two observations are not quite consistent with the hypothesis propounded by Schneiderman (1957) to explain the diapause-ending process in *Mormoniella vitripennis* (Walker), namely, that two reactions are involved. The primary reaction favored by lower temperatures leads to the synthesis and accumulation of some substance which, upon reaching threshold

levels, enables the insect to moult after it is returned to high temperature. The secondary reaction is a breakdown of this substance at high temperatures (that is, above 15°C). If this hypothesis is to be extended to *Bruchophagus roddi*, one must conclude that the secondary reaction is not complete, thus permitting a slow accumulation of the diapause-ending substance at higher temperatures. Schneiderman and Horwitz (1958) stated that "if low temperatures act by effectively promoting some synthetic reaction . . . this reaction . . . ought to be inhibited when an insect's metabolism is decreased." If *Mormoniella* larvae are chilled at very low temperatures, they demonstrate inhibition, as evidenced by the fact that the diapause-ending process failed to occur at -6°C.

TABLE 10
MEAN EMERGENCE OF ADULTS FROM DIAPAUSING
LARVAE HELD AT 10°F FOR VARYING
LENGTHS OF TIME

Days chilled	Days to 50 per cent emergence after:		Total number of days emergence occurred
	Termination of chilling	First adult emergence	
1.....	51	51	83
3.....	51	51	83
4.....	48	48	80
8.....	48	48	72
16.....	45	39	62
32.....	37	30	45

A second experiment was conducted to test the applicability of this hypothesis to *Bruchophagus roddi* by placing 18 cartons in a freezer at 10°F and removing 3 cartons at the end of each of the following days of exposure: 1, 3, 4, 8, 16 and 32 days. The removed cartons were then held at room temperature and adult emergence recorded as described above. From table 10, it is evident that the longer the diapausing larvae of *B. roddi* were subjected to a temperature of 10°F (-12°C), the shorter the adult emergence time, a fact in direct contrast to the above hypothesis.

TABLE 11
NUMBER OF DAYS FOR 50 PER CENT EMERGENCE DATING
FROM FIRST EMERGENCE

Weeks of chilling	Temperature				
	30° F	40° F	50° F	60° F	Mean
	Number of days				
3.....	50	47	46	47	47
6.....	30	27	23	26	26
9.....	20	14	7	5	11
12.....	16	11	8	6	10
Mean.....	29	25	21	21	

Two additional observations can be recorded here. The effect on reducing the adult emergence times by chilling was not influenced appreciably by the temperature when chilling occurred for 3 or 6 weeks, as shown in figure 7. When chilled for 9 or 12 weeks, however, the larvae subjected to a temperature of 60°F produced adults more rapidly than those chilled at 30°F. The explanation for this may be that there is an overlap of the critical temperatures for the diapause-ending process and for morphogenesis. Morphogenesis occurs slowly at 16°C. When the larvae were returned to room temperatures (24°C) they were already partly developed, thus reducing the time required to complete development. Table 11 lists the number of days necessary for 50 per cent adult emergence after emergence first began.

Fig. 7. The effect of chilling diapausing larvae at different temperatures on adult emergence in alfalfa seed chalcid

Induction of Diapause

The effects of temperature and photoperiod on the induction of diapause were investigated in another group of experiments. Adults, pupae, and mature larvae were subjected to various temperatures and photoperiods. The results to date, however, have been too erratic and inconsistent to justify reporting at this time. It is not known as yet whether adults lay eggs destined to produce diapausing larvae or if diapause is induced in the larval or pupal stages.

STUDIES OF THE ADULTS

Longevity

A test was conducted in an attempt to determine how long adults live at various temperatures. Fifty adults (sex ignored) were transferred to a small cage of the type shown in figure 1. This cage was then placed in a Dixie cup sealed with an air-tight plastic lid. Two lots of eight such cups each were prepared. In one lot the adults were fed a 10 per cent honey solution by placing a drop of the solution on the screened end of the cage. The insects in

TABLE 12
LONGEVITY OF ADULT *BRUCHOPHAGUS RODDI* HELD AT
VARIOUS TEMPERATURES

Temperature	No food		Food	
	LT ₅₀ *	LT ₁₀₀ †	LT ₅₀	LT ₁₀₀
<i>Fahrenheit</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>
30.....	10.5	33	22.0	30
40.....	14.5	49	25.5	48
50.....	13.8	50	38.5	52
60.....	6.2	17	26.0	52
70.....	4.7	11	18.0	24
80.....	3.0	5	6.0	17
90.....	1.4	4	4.2	12
100.....	0.8	3	2.3	9
	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>
110.....	117	240	..	..
112.....	33.8	90	66.5	160
114.....	14.2	27	18.7	45
116.....	7.0	15	7.0	13
118.....	3.1	5	..	..
120.....	1.9	3.5	..	..
122.....	1.1	2	..	..
124.....	0.6	1.0	..	..

* LT₅₀ = time necessary for 50 per cent of the population to die.

† LT₁₀₀ = time necessary for 100 per cent of the population to die.

the other lot received no food. A cup from each lot was then placed in eight different temperature cabinets ranging from 30° to 100°F. The insects were examined each day thereafter, and the number and sex of dead adults recorded. The results were plotted on log-probability paper and the time necessary for 50 per cent mortality (LT₅₀) was read graphically. These readings are shown in table 12.

The time mortality of adults, both fed and not fed, at temperatures from 110° to 124°F was determined by placing 50 adults in one of the small cages into which the bulb of a thermometer was inserted. The cage and adults were then transferred to an incubator equipped with a glass top. Counts of downed insects were made at varying intervals by direct observation. The length of time necessary for 50 per cent knockdown was again determined graphically. These results are included in table 12.

The death rate of the sexes was recorded separately. Males were much shorter lived than females. Although the LT_{50} 's expressed in table 12 are based on both sexes, the females lived, on the average, three times longer than males at all temperatures tested.

These results indicate that, as the holding temperature was decreased to 50°F , adult longevity was increased. A further reduction in temperature reduced the life span of adults. Hence, the temperature of maximum longevity was approximately 50°F (fig. 8).

Fig. 8. The longevity of adult alfalfa seed chalcids held at different temperatures with and without food

Adults that were fed a 10 per cent honey solution lived more than twice as long as nonfed adults. There is some question whether food *per se* (in this case, honey) was responsible for the observed increase in longevity, or, if water was the relevant factor. Two separate tests were conducted to bear on this question. Three groups of 50 adults each were caged. The first group was fed 10 per cent honey; the second, water; and the third was not fed. These insects were then placed in an 80°F cabinet. No differences were observed between the LT_{50} 's of the first and second group in either test. The third group had a significantly shorter LT_{50} . Thus, at higher temperatures, it appeared that nutrients themselves did not increase longevity. Insects given neither food nor water probably had a shorter LT_{50} due to desiccation. Whether the adults held at low temperatures starved or desiccated was not determined.

Preliminary experiments on cold-hardiness indicated that 5 minutes exposure to a temperature of 0°F results in 100 per cent mortality. At 20°F ,

adults entered a permanent cold stupor after 2.5 hours of exposure. The lack of suitable low temperature cabinets precluded a critical assessment of cold-hardiness.

Ovipositional Capacity and Duration of Oviposition Period

Sorenson (1934) reported that females of *Bruchophagus roddi* were capable of laying 27 eggs, based on dissection of gravid females. During the course of these studies, it was often observed that one female produces more offspring than this. Eight females were caged individually on single racemes (fig. 1). Six of these females in cages were transferred to a new raceme every other day while the remaining two were transferred daily. Transfers were

TABLE 13
PRODUCTION OF OFFSPRING BY INDIVIDUAL FEMALE CHALCIDS
(*BRUCHOPHAGUS RODDI* GUSS.) ON INDICATED DAYS

Female	Days														Total
	1	2	3	4	5	6	7	8	9	10	11	12	13		
	Number of offspring														
A.....	0	..	12	..	8*	..	..	..	..	..	..	..	..	20	
B.....	26	..	16	..	7	..	..	..	15	..	21	..	1*	86	
C.....	0	..	11	..	12	..	8	..	11	..	10	..	0*	52	
D.....	1	..	8	..	0	..	0	..	0	..	0	..	0*	9	
E.....	0	..	14	..	5	..	8	..	5	..	..	..	0*	49	
F.....	0	3	1	0	1	4	2	20	15	..	17*	..	0	46	
G.....	0	0	0	0	0	6	0	0	0	..	..	..	..	6	

* Indicates female was dead at the completion of the time period.

continued until the females died. The tests were run in a greenhouse where temperatures averaged 80° ± 2°F. Each female was fed every time its cage was transferred. The number of offspring produced on different days, as determined by seed dissection 14 days after each transfer, is shown in table 13.

The range of these 8 females is considerable, but of interest is the fact that 4 females produced 46 or more offspring. Female B oviposited continually during a 13-day period, producing 86 offspring in all.

SUMMARY

A series of preliminary experiments were conducted to investigate methods of handling *Bruchophagus roddi* in the laboratory. It was found that a maximum infestation was produced when a 1:1 ratio of adults to pods existed, when adults were fed 5 per cent honey, given 8- to 10-day-old seeds in which to oviposit, and held at 28° to 30°C while ovipositing.

Measurements of mandibles and body lengths of larvae indicated that this species has four larval instars. Larval development was completed in 12.23 days at 24°C. At the completion of development, the larvae either defecated and pupated, or entered diapause. When development continued, 2 days were required for defecation, 1 day for the formation of the prepupa and 9.5 days

for complete pupal metamorphosis. The rate of development of larvae was found to be influenced by the physiological age of the seed.

Diapause terminated slowly at room temperatures. This reaction, called the diapause-ending process, was hastened by chilling the larvae. The length of the chilling time was inversely proportional to the time required for 50 per cent adult emergence. Larvae chilled at the higher temperatures emerged more rapidly than those chilled for equal times at lower temperatures.

Adult survival studies indicated that the optimum survival conditions occurred when adults were fed and held at 50°F. Unfed adults lived only one half as long as those fed. Fifty per cent of the adult population survived 38 days at 50°F.

In studies on reproduction, the maximum number of offspring from a single female was found to be 86, which were produced over a 13-day period.

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