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Biology of the Trefoil Seed Chalcid, *Bruchophagus kolobovae* Fedoseeva (Hymenoptera: Eurytomidae)

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Biology of the Trefoil Seed Chalcid, *Bruchophagus kolobovae* Fedoseeva (Hymenoptera: Eurytomidae)[1]

INTRODUCTION

LARVAE OF THE seed chalcid *Bruchophagus kolobovae* Fedoseeva develop in seeds of the two cultivated species of birdsfoot trefoil — *Lotus corniculatus* Linnaeus, broadleaf or upright trefoil, and *L. tenuis* Waldstein and Kitaibel, narrowleaf or prostrate trefoil. California growers and seedsmen have been concerned about this insect because almost nothing was known about its economic significance in this state.

The purpose of the present study was to obtain detailed information on the life history and ecology of *B. kolobovae* in California. Attention was given to characters that distinguished adults of this species from those of the closely related species *B. roddi* Gussakovskii—an important pest of seed alfalfa (*Medicago sativa* Linnaeus), which infests also the seeds of bur clover (*M. hispida* Gaertner). Cross-mating between these two chalcids was attempted.

Laboratory experiments were focused on oviposition by *B. kolobovae*—its duration, the choice of host, the age of acceptable seed, and the influence of light and temperature. Five larval instars were identified by head-capsule measurements, and the duration of each instar was determined under laboratory conditions.

Seasonal population trends were studied in the field and correlated with cultural practices. Data on diapause were obtained from seedpods collected weekly and held for the observation of emergence patterns. Studies of the important parasites covered their identification, seasonal histories, and significance for the control of *B. kolobovae*.

HISTORICAL REVIEW

According to Burks (1957), 32 species have been referred to the genus *Bruchophagus* Ashmead, and three plant families serve as their hosts. Until recently seed chalcids developing on several species of *Lotus, Medicago,* and *Trifolium* were considered indiscriminately as a single species, *Bruchophagus gibbus* (Boheman), which was known previously by this and various other names—including *Systole platyptera* Walker and *Eurytoma funebris* Howard.

The *Bruchophagus gibbus* complex

Kolobova (1950) was the first to suggest that what was then called *B. gibbus* might be a complex of species. Hansen (1955) studied the host relationships of seed chalcids from alfalfa, red clover (*Trifolium pratense* Linnaeus), and birdsfoot trefoil. He found that each of these populations retained its host specificity and apparently would not interbreed with the others, but he

[1] Submitted for publication March 9, 1965.

felt that there was insufficient evidence to call them distinct species.

Strong (1962*a*) reviewed the systematic position of the *B. gibbus* complex, exchanged material with Fedoseeva, and concluded that the complex consisted of three distinct species, namely: *B. gibbus,* the clover seed chalcid; *B. roddi,* the alfalfa seed chalcid; and *B. kolobovae,* the trefoil seed chalcid. All three species are found in North America as well as in the Palearctic region. Peck (1963) gave a comprehensive list of references on the species of the complex and pointed out that some authors considered *Bruchophagus* as a subgenus of *Eurytoma.*

Claridge (1959) pointed out that *B. gibbus* is a synonym of *Systole platyptera.* The Entomological Society of America (Blickenstaff, 1965) has adopted the specific name *Bruchophagus platyptera* (Walker) for the clover seed chalcid and also the common names suggested by Strong (1962*a*)—clover seed chalcid, alfalfa seed chalcid, and trefoil seed chalcid.

Reports on *Bruchophagus kolobovae*

Mayr (1878) first associated chalcids with the seeds of *Lotus corniculatus,* but he mistakenly considered them parasites of the seed-eating weevil *Apion loti* Kirby. Crèvecoeur (1946) emphasized the phytophagous nature of the seed chalcid infesting *L. corniculatus* in Belgium and described its biology in some detail. He failed to extend his research because he considered his chalcid the same as the species on alfalfa that had been studied extensively by Urbahns (1920) under the name of *B. funebris* (Howard). Mac Donald (1946) reported on a heavy outbreak of a seed chalcid on *L. corniculatus* in New York in 1939. He called the chalcid *B. funebris* and observed it for several years before publishing. He concluded that it was of only minor economic importance at that time but was a potential threat to trefoil seed production.

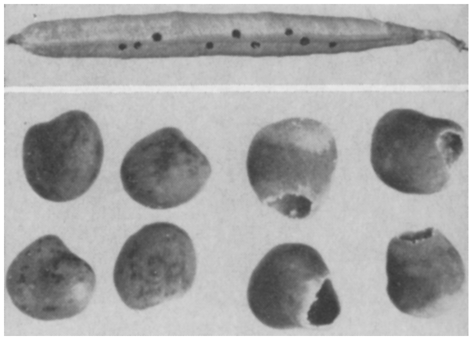

Fig. 1. *Above,* seedpod of *Lotus tenuis,* showing emergence holes made by *Bruchophagus kolobovae. Below,* seeds of *L. tenuis* at higher magnification, to show distortion and emergence holes: *left,* uninfested; *right,* infested by *B. kolobovae.*

Fedoseeva (1956) reported on chalcid specimens collected from seeds of *L. corniculatus* from various localities in the Soviet Union and dated as far back as 1936. She described this chalcid as a new species and named it in honor of A. N. Kolobova, who had recognized it in 1948 as distinct from the clover and alfalfa seed chalcids (Kolobova, 1950). Fedoseeva (1958) provided characters for distinguishing *B. kolobovae* from related species and constructed a key to 11 species of *Bruchophagus* that develop in seeds of leguminous plants.

Neunzig and Gyrisco (1955) reported what they called *B. gibbus* on *L. corniculatus* in New York, with 1 to 2 per cent infestation in most seed fields but with occasional damage as high as 17 per cent. They briefly described the life history of the insect and reported several parasites associated with it. After further study of host relationships, Neunzig and Gyrisco (1958) concluded that their chalcid was probably *B. kolobovae.* Also, they found five chalcidoid parasites associated with chalcid-infested seed of birdsfoot trefoil in New York (Neunzig and Gyrisco, 1959).

PLANT INJURY BY *BRUCHOPHAGUS KOLOBOVAE*

Under field conditions the only damage caused by *B. kolobovae* is the loss of the infested seeds. Usually a single egg is inserted into the endosperm of an immature seed, so that the larva comes to lie between the seed coat and the developing plant embryo. It feeds at first on one of the growing cotyledons and forms a cavity on that side, visible through the thin wall of the seed coat and giving a window effect. An individual larva infests only one seed. It consumes all of the contents and pupates inside the hollow seed coat. The mature infested seeds are somewhat distorted and usually appear larger than uninfested seeds. Their seed coats lack the glossiness of the healthy seed, and surface spots are less distinct (Crèvecoeur, 1946). After the adult wasp emerges from the pupal skin it chews a small circular escape hole through the seed coat and another through the side of the mature pod (fig. 1). The emergence holes in the seedpods indicate chalcid infestation.

DESCRIPTIONS OF STAGES

Adults

In the adult stage, *B. kolobovae* is a tiny, jet-black wasp (fig. 2). Fedoseeva (1956) wrote the original description of this species in Russian. A translation follows:

Bruchophagus kolobovae Fedoseeva sp. n., on *Lotus* sp.

Female. Length 1.5–1.8 mm. Black. Basal segments of palps entirely black. Segments of antennae slightly tapered; club curved. Tips of femurs brown; claws and anterior tarsi brownish. Hairs on wing dark; fringe of wing quite long. Wing veins dark brown; marginally and postmarginally diverging veins alike; radial vein rounded distally (fig. 1, 1) [in Fedoseeva's paper]; intermediate segment of micronet not longitudinally thickened. Middle of back humped so that the height of thorax, in profile, is greater than half its length. Abdomen slightly shorter than thorax, rounded, its end not turned up. Tergite IV nearly as wide as tergite III; tergites V and VI much narrower.

Male. Length 1.0–1.2 mm. Color and sculpture as in female. Basal segments of palps slightly wider in the middle than those of female. Segments of antennae constricted at both ends. Abdomen with stalk nearly as long as the posterior portion.

The species is similar to the alfalfa seed chalcid, but distinguished from it by smaller body, more hunchbacked (gibbous) dorsum, rounded tips of radial veins, and longer wing fringe.

Specimens have been obtained from seeds of trefoil, *Lotus corniculatus* L., collected from various localities: Pyatigorsk (25 VII 1954); Poltava (A. N. Kolobova, VI 1953); Kanev, Ukranian SSR (A. F. Kryshtal, 31 VII 1936); vicinity of Moscow (VIII 1953).

California specimens. The characters of specimens from both *Lotus corniculatus* and *L. tenuis* in California are in general agreement with the original description of *B. kolobovae.* Specimens were measured with an ocular micrometer to compare their size with that given by Fedoseeva. Living individuals —emerged in the spring from fall-collected seeds of *L. tenuis*—were chilled on foil-covered ice cubes. The mean body length (excluding antennae) was 1.42 mm ± 0.12 for 25 females and 1.38 mm ± 0.09 for 25 males. Mean length of the mesothoracic wing was 1.37 mm ±

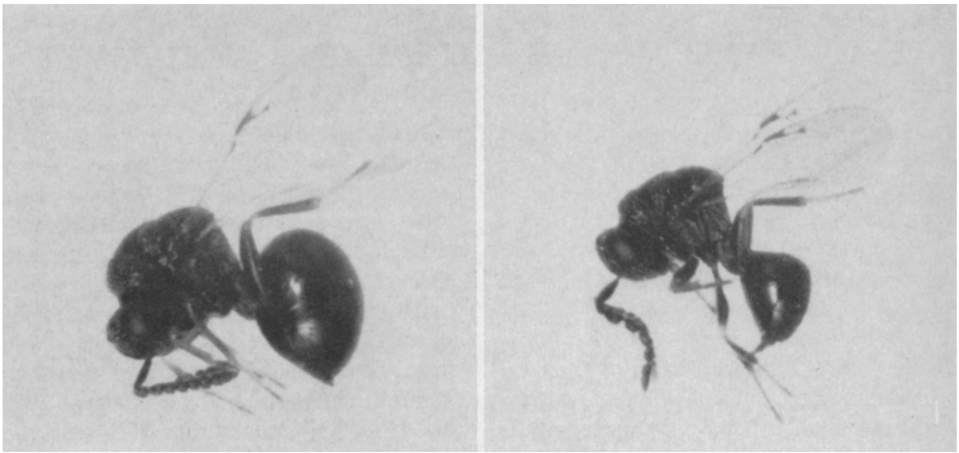

Fig. 2. *Bruchophagus kolobovae. Left,* adult female; *right,* adult male.

0.08 for the same 25 females and 1.32 mm ± 0.09 for the 25 males.

Species identification. For the validity of this research it was essential to distinguish *B. kolobovae* from the closely related species in the genus. There is no problem of identification when the host plant is known, because of the host specificity of these chalcids. However, adults of *B. roddi* (fig. 3) occurred in some field collections with those of *B. Kolobovae,* because the host plants *Medicago hispida* and *Lotus tenuis* occasionally grow together in pastures or along roadsides.

Strong (1962*a*) distinguished the three related species by measurements of the ovipositor, using the terminology of Michener (1956). The present study uses Strong's method but with a slightly different interpretation of the structures and a more complete labeling. The structure that Strong designated as the second ramus is here considered as the first ramus. The angle measured is ABC (fig. 4), where A is the tip of the second valvifer and B is the base of the second valvula. The line BC is drawn along the anterior edge of the sclerite between the first ramus and the first valvifer. This angle was measured on both sides of the ovipositors of 44

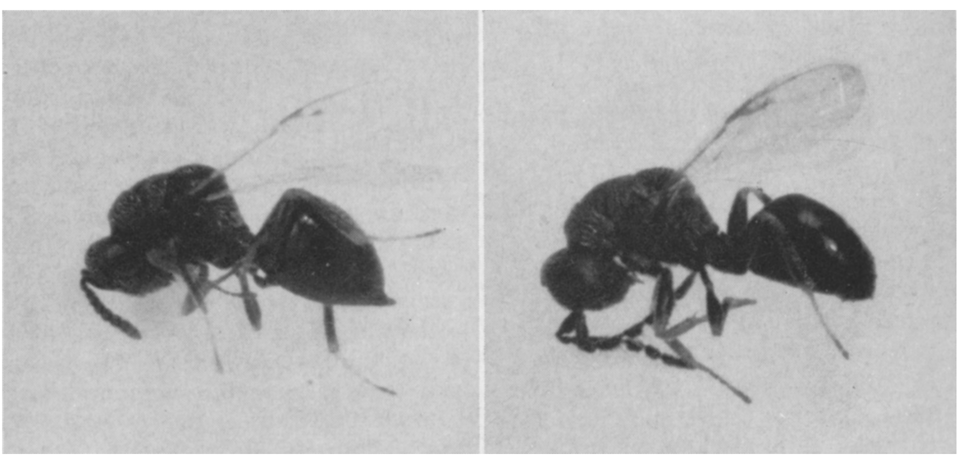

Fig. 3. *Bruchophagus roddi. Left,* adult female; *right,* adult male.

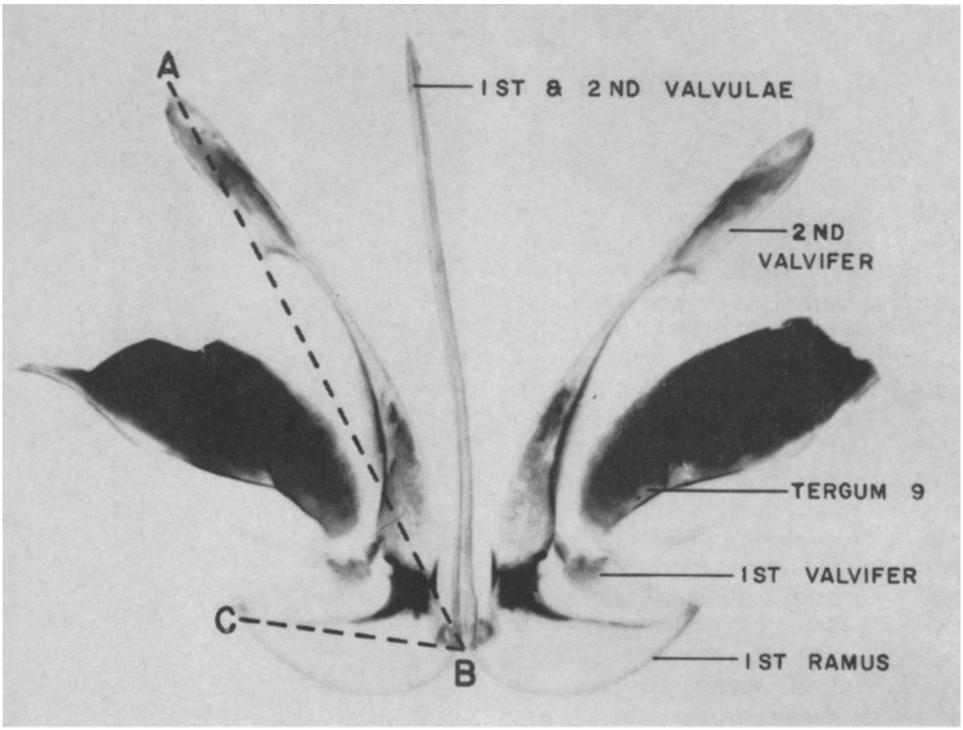

Fig. 4. Ovipositor of *Bruchophagus kolobovae* mounted in clear Hoyer's medium. Angle ABC is a useful character in distinguishing this species from related species of *Bruchophagus*.

TABLE 1
PROPORTIONS OF THE THORAX IN TWO RELATED CHALCIDS
Ratio of height to length (CD/AB in figure 5)

Item	*Bruchophagus kolobovae**		*Bruchophagus roddi*†	
	Female	Male	Female	Male
Mean ratio CD/AB......	0.4869	0.4440	0.4027	0.3681
Standard deviation....	0.0277	0.0322	0.0325	0.0288
No. of specimens........	51	45	26	21

* Adults from seeds of *Lotus tenuis*.
† Adults from seeds of *Medicago sativa* and *M. hispida*.

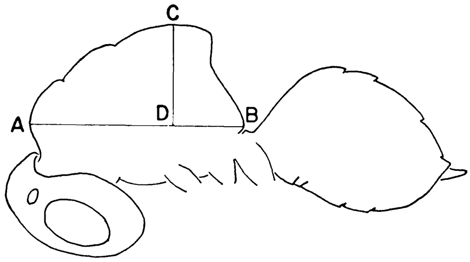

Fig. 5. Profile of *Bruchophagus kolobovae* indicating measurements of thorax length, AB, and thorax height, CD. Drawn from actual specimen, after Fedoseeva (1958).

freshly killed females (*B. kolobovae* from seeds of *L. tenuis*). The measurements for all of the right sides gave a mean similar to the mean for all of the left sides. All measurements combined gave a mean of 47.6° ± 7.5 for angle ABC. Strong reported an angle of 50.1° ± 4.1 for *B. kolobovae*, 25.3° ± 5.1 for *B. roddi*, and 2.1° ± 2.0 for *B. gibbus* (now *B. platyptera*).

It is not practical to use the ovipositor angle for rapid identification of specimens in bulk samples in ethanol, because the mounts for this measurement are made most easily with freshly killed specimens and their preparation is time-consuming. Most of the distin-

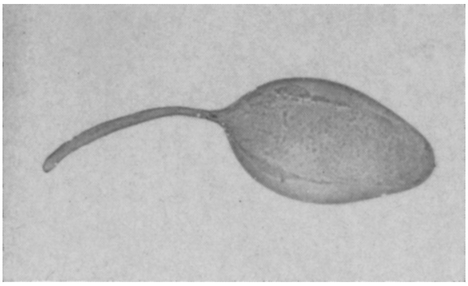

Fig. 6. Egg of *Bruchophagus kolobovae* mounted in clear Hoyer's medium.

guishing characters provided by Fedoseeva (1956 and 1958) are quite subtle. However, the overall shape of the thorax in profile is a valuable recognition character, and the measurements can be made easily under a dissecting microscope with ocular micrometer. Fedoseeva stated that the height of the thorax in *B. kolobovae* was greater than half its length—i.e., the ratio of CD to AB in figure 5 was greater than 0.5—and in *B. roddi* the ratio was less than 0.5. For California specimens (table 1) the ratios are less than 0.5 in both species, but the difference between the two species is highly significant. As the male of each species has a lower ratio than the female of the same species, comparisons should be made only between individuals of the same sex. Most of the specimens can be distinguished in this way. Doubtful specimens were identified by other characters, including those of the female genitalia.

Egg

The egg of *B. kolobovae* is spindle-shaped, with a long, tubular filament at the anterior end and a very short filament at the posterior end. The preparation in figure 6 shows only the long filament. Measurements of 11 eggs gave the following size ranges: length of egg with long filament, 0.52 to 0.57 mm, mean 0.54 mm; length of long filament 0.28 to 0.33 mm, mean 0.30 mm.

When the egg is laid the yolk mass is opaque white and the rest of the egg is

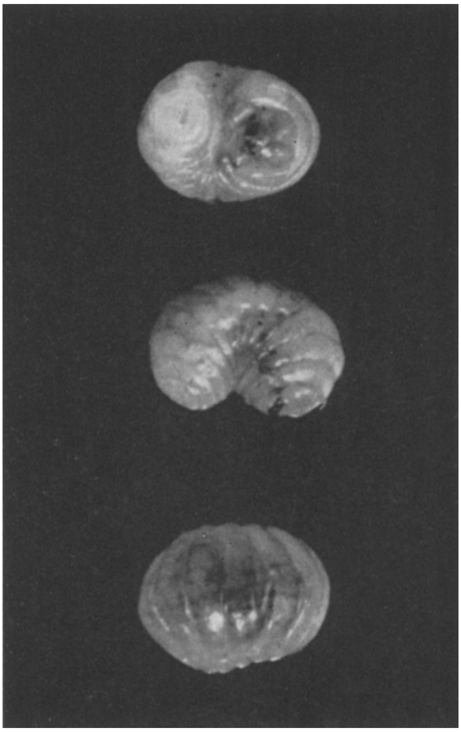

Fig. 7. Fifth-instar larvae of *Bruchophagus kolobovae*. *Above,* ventral view; *middle,* lateral view; *below,* dorsal view.

transparent. As the embryo develops it is clearly visible through the shell. Mandibles and body segmentation are discernible before hatching. The embryo does not enter the long filament, but its head develops at that end of the egg.

Kolobova (1950) pointed out that the eggs of the three related chalcids could be distinguished on the basis of overall shape. No attempt was made to confirm her observations.

Larva

The larva of *B. kolobovae* (fig. 7) is a white, legless grub with a well-developed head and a body of 13 segments. The head is rigid and lacks coloration except for the prominent pair of dark-amber sclerotized mandibles. In instars II through V each mandible has a well-developed tooth.

No spiracles have been found in the first instar, even with a phase-contrast microscope. In the second instar the mesothoracic spiracles are well developed and the next three pairs are small; posteriorly the spiracles are still less developed. Larvae of the third, fourth, and fifth instars have nine pairs of well-developed spiracles—on the mesothorax, the metathorax, and the first seven abdominal segments. The first-instar larva has bands of minute setae on each segment, but later instars have only a few setae on each segment.

Number of instars. There are five distinct larval instars, similar in general appearance but distinguished clearly by the measurements of three characters (table 2). The least variable character found is the total mandible length. Width and height of the head capsule are less distinctive measurements but they support the identifications. Each of the head parts measured grew in a geometric progression at each molt, according to Dyar's rule as interpreted by Wigglesworth (1953).

The larvae measured were of known age, reared in seeds of *Lotus tenuis* on growing greenhouse plants. Details of handling the plants and the insects are given under Rate of development (p. 451). As the heads of larvae contain the structures best suited for distinguishing the instars (fig. 8), specimens were oriented on slides so as to show the undistorted frontal aspect. A living larva was punctured with a sharp needle so that the body contents flowed out, leaving the nearly transparent cuticle. This cuticle was mounted in Hoyer's medium and flattened carefully under a cover slip, without crushing or rolling. Finally the slide was heated gently over an alcohol flame.

Several late-stage embryos were so oriented that it was possible to measure their mandibles inside the egg shells. The mandible lengths of these embryos

TABLE 2

MEASUREMENTS OF MANDIBLES AND HEAD CAPSULES OF *BRUCHOPHAGUS KOLOBOVAE* IN ITS FIVE LARVAL INSTARS*

Instar	Mandible				Head capsule				Head capsule			
	Specimens	Mean length	C.V.†	G.F.‡	Specimens	Mean width	C.V.†	G.F.‡	Specimens	Mean height	C.V.†	G.F.‡
	no.	$\mu \pm$ SD			*no.*	$\mu \pm$ SD			*no.*	$\mu \pm$ SD		
I (in egg)	3	16.50 ...	...	...	0	... ...	...	...	0	... ...	...	...
I	76	17.29 ± 0.81	4.69	...	49	98.56 ± 6.90	7.01	...	48	80.11 ± 4.50	5.61	...
II	26	30.58 ± 1.20	3.92	1.76	24	155.55 ± 11.06	7.11	1.58	24	119.66 ± 7.80	6.52	1.49
III	17	44.97 ± 2.23	4.96	1.47	15	224.76 ± 17.17	7.64	1.44	14	161.91 ± 7.98	4.93	1.35
IV	30	65.43 ± 3.76	5.74	1.45	26	322.04 ± 26.93	8.36	1.43	25	212.43 ± 18.18	8.56	1.31
V	75	96.90 ± 3.90	4.02	1.48	70	387.62 ± 19.54	5.04	1.20	70	254.60 ± 14.20	5.58	1.20

* Specimens of known age, reared in greenhouse in seeds of *Lotus tenuis*, August 29–September 23, 1963. The structures measured are shown in figure 8, diagram V. Wherever possible all characters were measured on the same specimens. Because of distortions in some of the preparations, only one or two characters could be measured on those specimens.
† C.V. = coefficient of variation = (standard deviation/mean measurement) × 100.
‡ G.F. = growth factor = mean measurement/mean measurement for preceding instar.

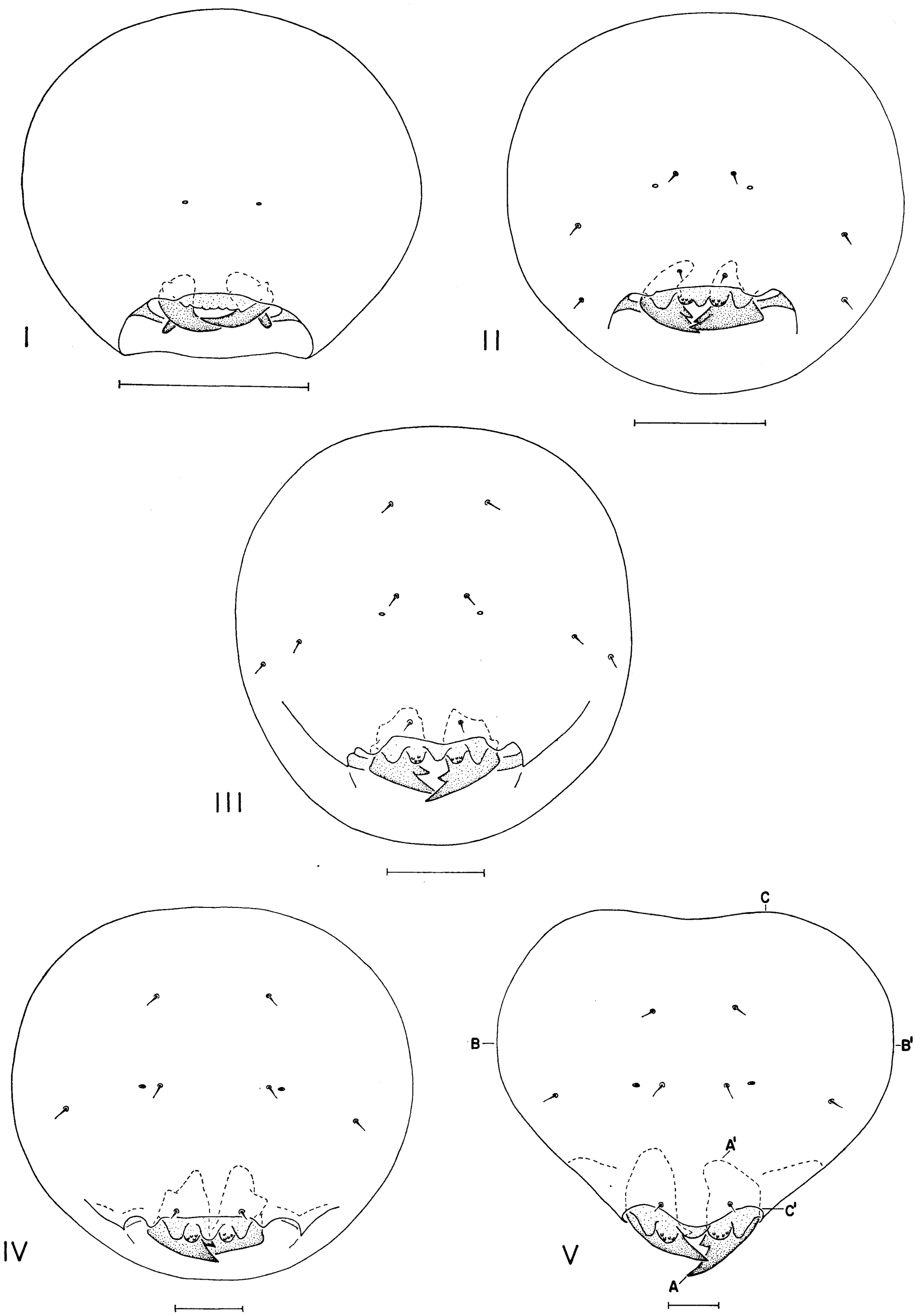

Fig. 8. Head capsules of *Bruchophagus kolobovae* at larval instars I to V. The structures measured for identifying the different instars are indicated on diagram V: AA′, mandible length; BB′, head-capsule width; CC′, head-capsule height. SCALE: The line under each diagram represents 50 μ.

came within the range of measurements made on the smallest larvae and confirmed the identification that these larvae were in the first instar. An additional character—the lower margin of the head capsule—is very distinct in the first instar, and the width of the capsule at this point was of the same magnitude as in embryos.

Exuviae of the first four larval instars were never found because of their small size and transparency. However, mounted specimens of each of these instars in preparation for molting showed the developing mandibles of the next instar within the loosened cuticle.

Strong (1962*b*) identified four larval instars in *B. roddi* on the basis of mandible and body measurements. The length of the mandible from its tip to the base of the tooth gave the clearest distinction between instars. *B. kolobovae* has no mandibular tooth in the first instar, but in instars II through V the measurements of the mandible from tip to tooth were similar to those reported by Strong for the four larval instars in *B. roddi*. This suggests that *B. roddi*, also, might have a small, unnoticed first-instar larva.

Pupa

The pupa of *B. kolobovae* is exarate. It is completely white at first, changes gradually to a very light amber with distinctly red eyes, and becomes jet black before the adult emerges. Strong (1962*b*) described similar changes in *B. roddi*. The pupa commonly retains the shed cuticle of the fifth-instar larva at its posterior end; the skin is shed as a unit.

The sexes can be distinguished in the pupal stage by the length of the antennae, which can be seen through the pupal covering. The antennae are longer in the male and extend posteriorly beyond the tips of the wings and of the prothoracic tarsi. In the female the antennae do not extend to the tips of the wings or of the prothoracic tarsi.

Emergence of adult

When the wasp is fully developed it frees itself from the pupal skin—all except the portion that covers the antennae. It uncovers the antennae after it has emerged from both the seed and the pod. The process as described by Crèvecoeur (1946) was verified in this study.

LABORATORY AND GREENHOUSE STUDIES

Materials

Greenhouse conditions

Experiments were run in the greenhouse between June 14 and October 11, 1963. During this period temperatures ranged from nighttime lows of 59°–70° F to daytime highs of 73°–93°, and relative humidities ranged from daytime lows of 45–76 per cent to nighttime highs of 66–100 per cent. Figure 9 shows the daily maximums and minimums from a continuous hygrothermograph record. A mean value for the temperature or the humidity during any given period was calculated by averaging all the daily maximums and minimums. A mean calculated in this way probably approximates fairly well to the mean value that would be obtained on the basis of hourly records. For the entire four months, the calculated mean temperature was 74.8° F and the calculated mean relative humidity was 69.3 per cent. Mean values for shorter periods varied only slightly from these overall mean values.

A 15-hour photoperiod favored the development of trefoil pods and was used in the greenhouse throughout the growth of the plants as well as during experiments. The term photoperiod is

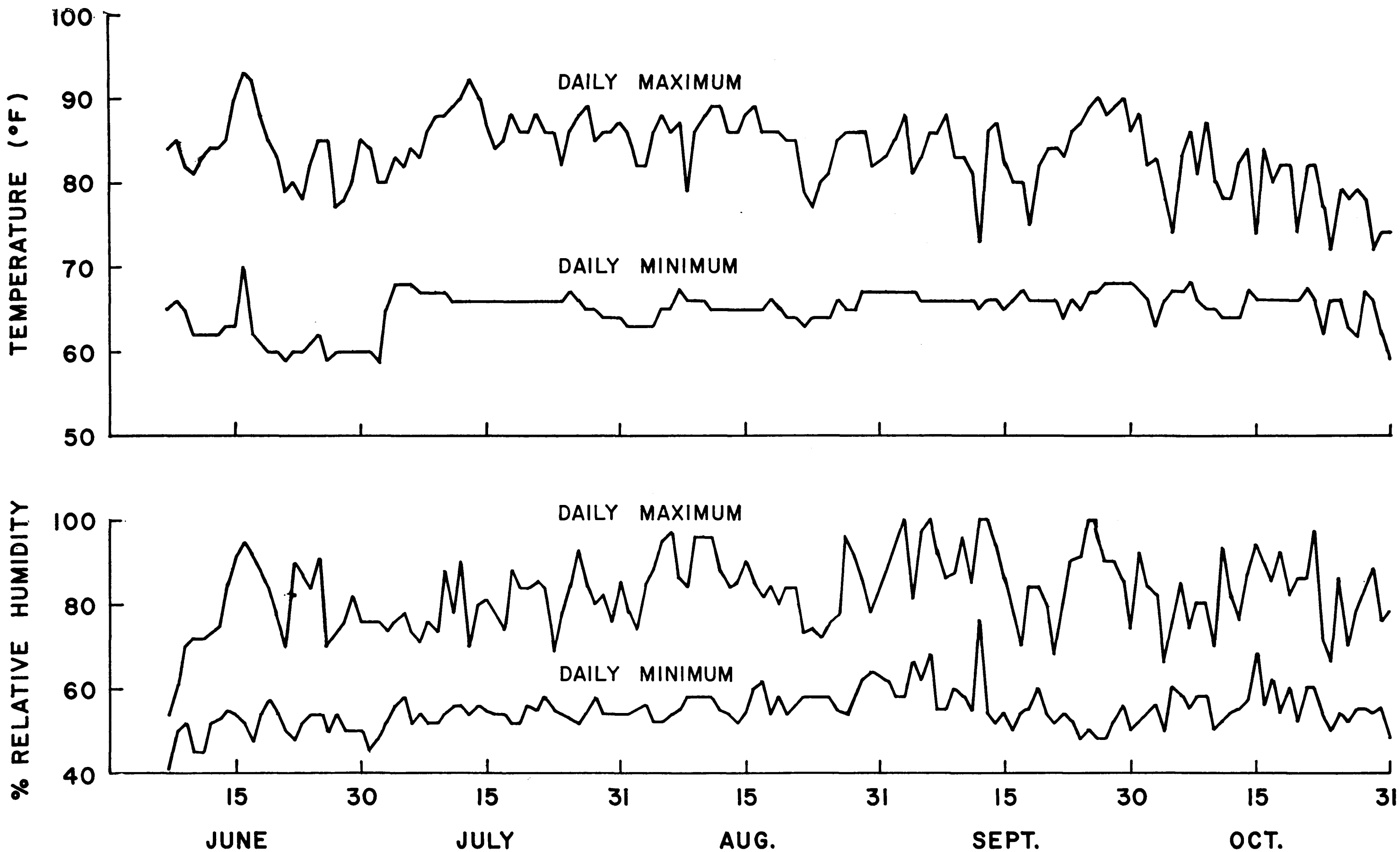

Fig. 9. Temperature and humidity in greenhouse during biological experiments with *Bruchophagus kolobovae*, including periods of seedpod development. Davis, California, 1963.

Fig. 10. General view of greenhouse area, showing trefoil plants with experiments in progress.

used here as defined by Henderson and Henderson (1960) to mean the duration of daily exposure to light.

To control greenhouse pests, the plants were fumigated with methyl bromide shortly before starting an experiment but not after the introduction of chalcids. One treatment was sufficient, and only light pest populations developed before the end of an experiment.

Host plants

Plants of *Lotus corniculatus* and *L. tenuis* were taken from commercial field plantings. Alfalfa plants were started either from seeds or from cuttings. All were grown in individual 6-, 8-, or 10-inch clay pots (fig. 10). Plants of these three species bloomed well and, after hand-pollination, produced adequate numbers of pods with sound seeds. To standardize the description of material used in experiments, the age of seeds or of seedpods is counted from the time of pollination.

Plants of *Lotus heermannii* (Durand and Hilgard) Greene were dug from an undisturbed mountain area, and plants of other native species of *Lotus* and *Medicago* were started from field-collected seeds. All were identified by the keys of Munz and Keck (1959). All bloomed and set pods in abundance without hand-pollination. The seedpods of these plants were used only for experiments on host preference, where it was not essential to know the exact age of the seeds.

Equipment for controlled environments

Cabinets. Three insulated cabinets were available for experiments under controlled conditions. Each was approximately 20 inches wide, 18 inches deep, and 33 inches high, and had two 15-

Fig. 11. Plastic-dish cage containing oviposition experiment.

watt, 15-inch, cool-white fluorescent lights and one 15-watt incandescent light on the ceiling. A time switch controlled all the lights, to give a 15-hour photoperiod during all experiments. Heat, from two 100-watt metal-enclosed heating elements, was regulated within approximately ± 2° F by an adjustable bimetallic switch with relay. A fan ran constantly, to equalize the temperature throughout the cabinet.

A 42° F room housed the three controlled-temperature cabinets. The utility light was operated by a hand switch, but the room was dark most of the time. Temperature was maintained within approximately ± 1° F.

The 70° F room was a multipurpose room, with a 15-hour photoperiod. The room was not used for experiments that required rigid light control, as no precautions were taken to prevent brief interruptions of the dark cycle. Temperature was maintained within approximately ± 2° F.

Only one hygrothermograph was available for the two rooms and the three cabinets. However, the records were consistently uniform, and frequent checking of thermometers revealed no appreciable temperature variations during the experiments.

Humidity control. There was no humidity control in the controlled-temperature rooms and cabinets, but seedpods and insects were protected from excessive drying by providing water in an open dish when needed. Actual humidity control was provided in chambers of two kinds, each used in only one experiment. They are described in connection with those experiments: the influence of humidity on emergence and the influence of temperature on oviposition.

Cages

Emergence cartons were the containers used routinely for bulk supplies of seedpods when they were first brought in from the field. Each was a 1-gallon ice cream carton with a 3-dram screw-cap glass vial inserted in a hole near the top. When adult insects emerged from the pods they flew into the vial, because it was the only source of light. They were transferred into holding cages three times a week.

Holding cages for adult chalcids were made from locking-cover plastic dishes of about 3 inches diameter. A large hole was cut in the top and bottom of each dish and screened with fine-meshed nylon organdy, cemented in place. A small hole in the upper screen of the inverted dish, with a gummed reinforcement ring and a cork or a cotton plug (fig. 11), served for inserting or removing the insects.

Plastic-dish cages were the same as holding cages. The lid of the inverted dish, supported on brackets over a bowl of water, formed the floor of the cage. The threads of the nylon screen could be spread apart while the peduncle of a detached umbel was pushed through

Fig. 12. Plastic-dish cage, used for confining insects over detached seedpod umbels with their peduncles extending to water below.

the weave (fig. 12). Then the threads were eased back to fit closely around the peduncle. When the dish was assembled with the peduncle in the water below the cage, the seedpods remained unwilted for a day or longer at moderate temperature.

A plastic-tube cage (Strong, 1962*b*) was used for confining test insects over an umbel of one or more seedpods on a growing plant (figs. 13 and 14). These cages were cut from 5/8-inch plastic centrifuge tubing in lengths of approximately 1½ inches. Specimen holes in the tubing were made with a paper punch. Fine-meshed nylon organdy was cemented over one end of each cage. A circular plug for the other end was cut from 1/8-inch-thick foam-rubber matting material with sealed surfaces. By a radial slit in the foam rubber, from the edge to the center, the plug could be fitted closely around the peduncle of the umbel.

Tube cages on platform. Three or four plastic-tube cages were attached by spring clips to the lid of a holding cage, set over a dish of water (fig. 15). These smaller cages were used to provide replicates in some experiments with umbels or with detached seedpods. Individual *Lotus* seedpods are slender enough so that one seedpod can be pushed through the screen from below without disturbing the insects in the cage. When an umbel of two or more seedpods is used, the peduncle is placed in water and the seedpods remain attached. One seedpod can be exposed to the insects inside the cage while the others are held outside of the cage, under the screen, in reserve for a later exposure.

Fig. 13. Small plastic-tube cage, used for confining insects over seedpod umbel on growing plant.

Insect supply

Insects for greenhouse and laboratory experiments were obtained from the bulk lots of *Lotus tenuis* seedpods collected weekly in 1963 and used primarily for studies based on field populations. No chalcids from *L. corniculatus* seedpods were used in any experiment. The seedpods were picked by hand in the vicinity of Davis, California. Brown seedpods attached to the plant by green peduncles were selected because they were nearing maturity and contained fifth-instar larvae or early pupae. The seedpods were placed in emergence cartons in the 70° F room. When insects emerged from the seedpods, the survey data were recorded and then *B. kolobovae* individuals were transferred to holding cages and stored

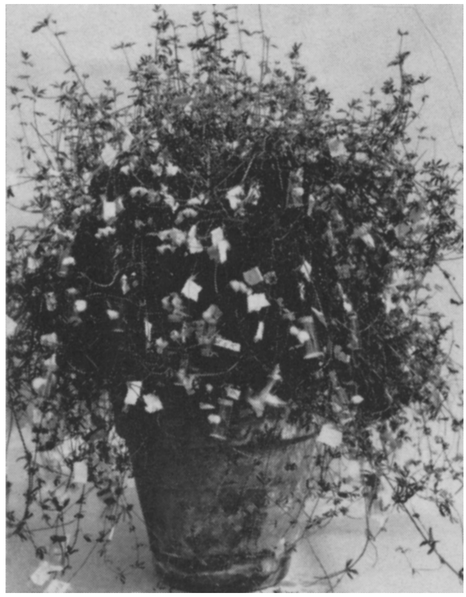

Fig. 14. *Lotus tenuis* plant with pollination-date labels and plastic-tube cages.

in the 70° room until they were used in experiments.

The age of insects used in most of the experiments was not considered. The use of large numbers of insects in the experiments presumably compen-

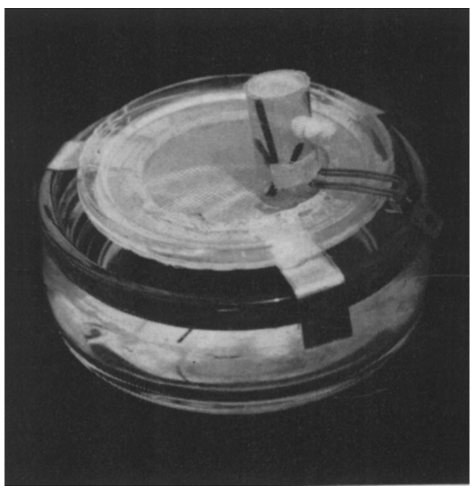

Fig. 15. Plastic-tube cage supported on nylon platform, used for confining insects over a detached seedpod umbel with its peduncle in water below.

sated for any inadequacies due to age or previous egg-laying as well as for other, individual differences in egg-laying behavior.

Unmated adults, newly emerged, were required for experiments on cross-breeding, egg output, and life-span. They were obtained from infested seeds of *L. tenuis* held individually in gelatin pill capsules. However, only small numbers of adults of *B. kolobovae* were recovered from large numbers of infested seeds in these capsules. Parasites killed a high percentage of the larvae, and drying of exposed seeds probably killed many others.

Biological Experiments

Influence of humidity on emergence

Three wooden humidity chambers—glass-topped and with fans—were used in this experiment. Each was 20 inches long, 12 inches wide, and 12 inches high. Humidities were regulated by saturated salt solutions and were measured with a dew cell at two-day intervals during the experiment. The relative humidity values remained consistent throughout, though they differed somewhat from those that O'Brien (1948) reported for the three salts used.

Nine samples—three at each humidity—were tested for 11 weeks in the 70° F room. Each sample consisted of 50 infested seeds, taken at random from mature seedpods of *Lotus tenuis* collected on August 14, 1963. Each sample was held in a container made by removing the bottom from a 3-dram glass vial and the top from its threaded bakelite cap and replacing both ends with fine-meshed nylon organdy.

Insects were removed from the vials three times a week, as they emerged. In 11 weeks only 237 adult insects—118 trefoil seed chalcids and 119 parasites—emerged from the 450 infested seeds (table 3). Nearly three times as many adults of *B. kolobovae* emerged at the highest humidity as at the lowest. The greatest number of each species of parasite emerged at the intermediate humidity. The number of seeds from which either *B. kolobovae* or one of its parasites emerged was practically the same at the two higher humidities but was much less at the lowest humidity.

Seedpods for this experiment were taken from an isolated area not used for other collections in this study. *Liodontomerus perplexus* Gahan, the predominant parasite in this lot of seeds, was rarely found associated with trefoil in other areas studied.

Mating and reproduction

Males of *B. kolobovae* appear to mate readily with females of the same species—in large or small cages, either on trefoil plants or without plants. The male climbs onto the back of the female, stands with his tarsi on her thorax and folded wings, and leans forward and backward rhythmically, touching his antennae to hers. After a period of this activity the male shifts directly backward, curves the tip of his abdomen under, and attempts to insert the aedeagus into the female. It is not certain how often the observed attempts were successful. Firmly attached pairs were never seen. The females appeared nonaggressive in mating, but males attempted mating repeatedly for many days. At least one male attempted to mate 16 days after he emerged as an adult.

Crossbreeding test. *Bruchophagus roddi* was selected for an attempt at crossbreeding with *B. kolobovae*, because specimens were readily available and because the female genitalia of

TABLE 3

ADULT TREFOIL SEED CHALCIDS AND PARASITES THAT EMERGED FROM INFESTED SEEDS OF *LOTUS TENUIS*

150 seeds at each humidity, held at 70° F ± 2° for 11 weeks starting August 15, 1963

Insect species	R. H. 23.3%* over lithium chloride	R. H. 41.6%* over magnesium chloride	R. H. 52.5%* over calcium nitrate	Total from 450 seeds
	no.	*no.*	*no.*	*no.*
Bruchophagus kolobovae	21	36	61	118
Tetrastichus bruchophagi†	7	12	8	27
Trimeromicrus maculatus†	0	10	1	11
Liodontomerus perplexus†	25	33	23	81
Totals				
B. kolobovae	21	36	61	118
Parasites	32	55	32	119
No emergence	97	59	57	213

* Mean relative humidity, from dew-cell measurements made in each chamber at two-day intervals.
† Parasite of *B. kolobovae.*

these two species are more nearly alike than are those of *B. platyptera* and *B. kolobovae.* All insects were unmated at the start of the experiment—that is, they had been isolated in gelatin capsules since before their adult emergence. Three separate runs were made, as material was not available at one time. In each case, the test insects were caged for 72 hours on living plants in the greenhouse. The exposed seedpods were held in the same cages for seven weeks after oviposition, to allow for the development of offspring.

Each female had a separate cage, either alone or with one or two males. Unmated (i.e., isolated) females of either species produced only male offspring—an indication of arrhenotokous parthenogenesis (Doutt, 1959). Previously unmated females of either species likewise produced only male offspring when they were caged with males of the other species—an indication that no successful cross-fertilization took place. When they were caged with males of their own species, females of either species produced both male and female offspring in approximately equal numbers. Although only small numbers of unmated insects were available for this experiment, the results (table 4) confirm the distinctness of the two species, because there is no reason to suspect that any actual cross-breeding took place.

Conditions affecting oviposition

An egg-laying female of *B. kolobovae* walks back and forth on a trefoil seedpod, tapping its surface with her antennae. While she is locating a seed for oviposition she usually claims her territory by driving off any other female that comes in close proximity on the seedpod. When she finds a suitable seed she lowers the tip of her abdomen so that it touches the pod, with the under side of the abdomen nearly at a right angle to the pod. The valvulae of the ovipositor are withdrawn from the ovipositor sheath and inserted into the plant tissue while the abdomen returns slowly to a position parallel to the surface of the pod. After a few seconds the valvulae are withdrawn completely from the seedpod, then snap back into the sheath. The actual passage of an egg into the seed was not observed.

Seedpod requirement. Carrillo and Dickason (1963) indicated that females of *B. roddi* deposited eggs readily on the glass walls of their cage but that these eggs all failed to hatch.

I started an experiment on August 9, 1963, to find if females of *B. kolobo-*

TABLE 4
CROSSBREEDING TESTS WITH TWO SPECIES OF *BRUCHOPHAGUS**

Parents				Host plant	Adult offspring	
Species		Individuals				
Female	Male	Female	Male		Female	Male
		no.	*no.*		*no.*	*no.*
B. roddi†	*B. roddi*	4	8	*M. sativa*	33	36
B. roddi	*B. kolobovae*	4	8	*M. sativa*	0	75
B. roddi	None	3	0	*M. sativa*	0	32
B. kolobovae‡	*B. kolobovae*	8	8	*L. tenuis*	24	19
B. kolobovae	*B. roddi*	8	8	*L. tenuis*	0	28
B. kolobovae	None	8	0	*L. tenuis*	0	50

* Previously unmated insects were caged for 72 hours over growing seedpod umbels in the greenhouse. Each cage contained one female with seedpods of the plant species on which she had developed and either one or two males, if any. Seedpods were allowed to mature on the plants and were held in the same cages, still in the greenhouse, for seven weeks after oviposition.
† Adults of *B. roddi* from field-collected seedpods of *Medicago sativa.*
‡ Adults of *B. kolobovae* from field-collected seedpods of *Lotus tenuis.*

vae would oviposit on glass or cloth when no seedpod was available. Newly emerged adults—10 females and 10 males—were held in a rectangular glass cage, similar to one used by Carrillo and Dickason. The cage was made of four microscope slides glued together at right angles along their long sides. The ends were screened with fine-meshed nylon organdy. Droplets of undiluted honey and droplets of water were supplied twice daily, as needed, on the nylon. The cage was kept in a controlled-temperature cabinet with a 15-hour photoperiod, at 70° F for the first 12 days and thereafter at 80°. All sides of the cage were examined twice daily, with magnification, during the lifetime of all the insects—a total of 49 days. Frequently one female (always the same individual) was seen trying to probe the glass with her ovipositor in the way she would probe a seedpod, but no eggs were found in the cage—either on the glass or on the nylon. I inspected holding cages, also, on several occasions, without finding eggs. Sometimes these cages contained several hundred adult chalcids for as long as two weeks.

Importance of seed stage. Strong (1962*b*) found that egg-laying females of *B. roddi* preferred alfalfa seeds approximately 8 to 10 days old (meaning days after pollination). However, the important factor is not the chronological age of the seeds but their stage of development, which depends on the growing conditions. The total length of the plant embryo cannot be measured accurately because the hypocotyl turns under the cotyledon during growth. Therefore two other measurements were used to indicate the physiological age of seeds—the length of the developing cotyledon and the overall length of the seed. The ratio of cotyledon length to seed length gives an index of the amount of development, with values from zero to somewhat less than one. This method does not indicate the growth stage of a seed before the cotyledon appears (index zero), although females sometimes probe such young seeds and occasionally oviposit in them.

In two experiments, trefoil seedpod umbels from flowers pollinated on different days were cut from the plant and exposed for 24 hours to oviposition by *B. kolobovae*. Only one cage was used for each experiment, so that the egg-laying females had a choice of pods at different stages. All of the seeds were dissected and measured after the exposure. Both experiments indicated

TABLE 5
SEED-PROBING AND OVIPOSITION BY *BRUCHOPHAGUS KOLOBOVAE* IN RELATION TO AGE OF SEEDS
Test No. 1*

Seeds				Eggs laid		Cotyledon length/seed length‡
Age at start	Exposed	Probed†		Total output	Per seed exposed	
days	*no.*	*no.*	*per cent*	*no.*	*av. no.*	*ratio (mean)*
4	116	0	....	0	....	0.000
5	110	5	4.5	0	....	0.000
7	71	28	39.4	10	0.14	0.000
9	42	35	83.3	21	0.50	0.000
10	52	48	92.3	47	0.90	0.008
12	30	27	90.0	59	1.97	0.118
16	15	5	33.3	0	....	0.666
17	8	3	37.5	0	....	0.608

* Seedpod umbels from one plant of *Lotus tenuis*, hand-pollinated on different days, were cut on July 26, 1963, and placed together in one plastic-dish cage with their peduncles in water. The seedpods were exposed to egg-laying by 150 females from *L. tenuis* (with 80 males) for 24 hours at 80° F, with a 15-hour photoperiod.
† All seeds that were probed one or more times, with or without egg-laying.
‡ An index of the physiological age of the seeds. All seeds were dissected and measured immediately after the oviposition period.

that the egg-laying females selected seeds at a particular stage of development (tables 5 and 6, figs. 16 and 17) and rejected seeds at earlier or later stages, beyond a fairly restricted range. In both experiments the preference was for seeds 11 or 12 days old, with an index of development between 0.10 and 0.15. However, the females in these tests laid eggs in seeds from 7 to 15 days old, with indexes of development from 0.0 to 0.47, and probed seeds from 5 to 17 days old. In other tests females probed seeds as old as 23 days.

The drop in oviposition in Test No. 2 was associated with a lack of turgidity

TABLE 6
SEED-PROBING AND OVIPOSITION BY *BRUCHOPHAGUS KOLOBOVAE* IN RELATION TO AGE OF SEEDS
Test No. 2*

Seeds				Eggs laid		Cotyledon length/seed length‡
Age at start	Exposed	Probed†		Total output	Per seed exposed	
days	*no.*	*no.*	*per cent*	*no.*	*av. no.*	*ratio (mean)*
7	42	17	40.5	14	0.33	0.000
8	35	26	74.3	28	0.80	0.007
9	68	51	75.0	37	0.54	0.007
10	49	44	89.8	21	0.43	0.027
11	31	29	93.6	37	1.19	0.102
12	38	36	94.7	48	1.26	0.150
13	56	30	53.6	19	0.34	0.256
14	24	3	12.5	1	0.04	0.480
15	23	4	17.4	2	0.09	0.473

* Seedpod umbels from one plant of *Lotus tenuis*, hand-pollinated on different days, weie cut on August 5, 1963, and placed together in one plastic-dish cage with their peduncles in water. The seedpods were exposed to egg-laying by 113 females from *L. tenuis* (with 49 males) for 24 hours at 80° F, with a 15-hour photoperiod.
† All seeds that were probed one or more times, with or without egg-laying.
‡ An index of the physiological age of the seeds. All seeds were dissected and measured immediately after the oviposition period.

found in all of those particular 9- and 10-day-old seeds. Presumably this seed condition was due to some unfavorable growing condition. The insects probed these seeds extensively but did not lay large numbers of eggs in them.

It seems probable that a female selects, tentatively, a seedpod that appears suitable and probes it, but that the condition on the seed influences whether or not she oviposits in that seed. In these experiments, the high ratio of eggs to seeds at the preferred stage of development was related to the high ratio of females to seeds at that stage. In field collections, on the other hand, there was seldom more than one egg in any seed.

Selection of host species. Egg-laying females of *B. kolobovae* from seeds of *Lotus tenuis* were given a choice among different legume species in a plastic-dish cage. Seedpod umbels of *L. tenuis* and seedpod racemes of either *Medicago sativa* or *M. hispida* were available in each of four tests. Other potential hosts tested were five wild species of *Lotus* and two cultivated varieties of *L. corniculatus*. An attempt was made to provide seedpods as nearly as possible at a suitable physiological stage, i.e., comparable to the stage of development preferred for seeds of *L. tenuis*. Seedpods of species that were not hand-pollinated were selected for the experiment by comparing them with dissected samples.

The insects laid relatively large numbers of eggs in seeds of the two domesticated trefoils, *L. tenuis* and *L. corniculatus*. In tests where seeds of the two host species were of the same age (tables 7 and 8), the insects laid more eggs per seed in *L. corniculatus* than in *L. tenuis*. In one test (table 9), where seeds of *L. corniculatus* were only seven days old, the insects laid more eggs in the eight-day-old seeds of *L. tenuis*. However, no conclusion is drawn on the relative suitability of the two species for *B. kolobovae,* because of the lack of replication and the lack of criteria on suitability of seeds of different species in relation to their age.

In general, the egg-laying females avoided the wild species of *Lotus*. They laid a few eggs in seeds of *L. purshianus* (Bentham) Clements and Clements in two tests (tables 8 and 10) but did not even probe older seeds of that species (table 9). *L. purshianus* was tested again, with negative results, in an experiment on host specificity (p. 451). In *L. micranthus* Bentham, one egg was found in a seed of undetermined age (table 8), but seeds of apparently suitable age in two other tests (tables 9 and 10) were not even probed. No eggs were found in seeds of *L. subpinnatus* Lagasca y Segura: five seeds of that species were probed in one test (table 10) but older seeds were not probed (table 9). No eggs were found in seeds of *L. heermannii* in two tests or in seeds of *L. humistratus* Greene in one test. There were no probings in either species of *Medicago*.

Influence of temperature. This experiment used the controlled-temperature cabinets at 70°, 80°, and 90° F. At each temperature there were three plastic-tube cages supported on a nylon platform held on brackets over water in the bottom of a 1-gallon glass jar. This was the second kind of humidity chamber. The jar had a screw top and a small fan. The motor for the fan was outside the jar, with a shaft through the lid. Each of the nine cages held one 10-day-old seedpod of *Lotus tenuis* and 25 females of *B. kolobovae* from *L. tenuis*. Three 3-seedpod umbels were used, with a total of 130 seeds. To minimize the effect of possible seedpod variations, one seedpod of each umbel was used at each temperature. The seedpods were detached from the umbels and did not touch the water under the platform. They remained in good condition because the exposure was short and the atmosphere inside the jar was saturated with water vapor.

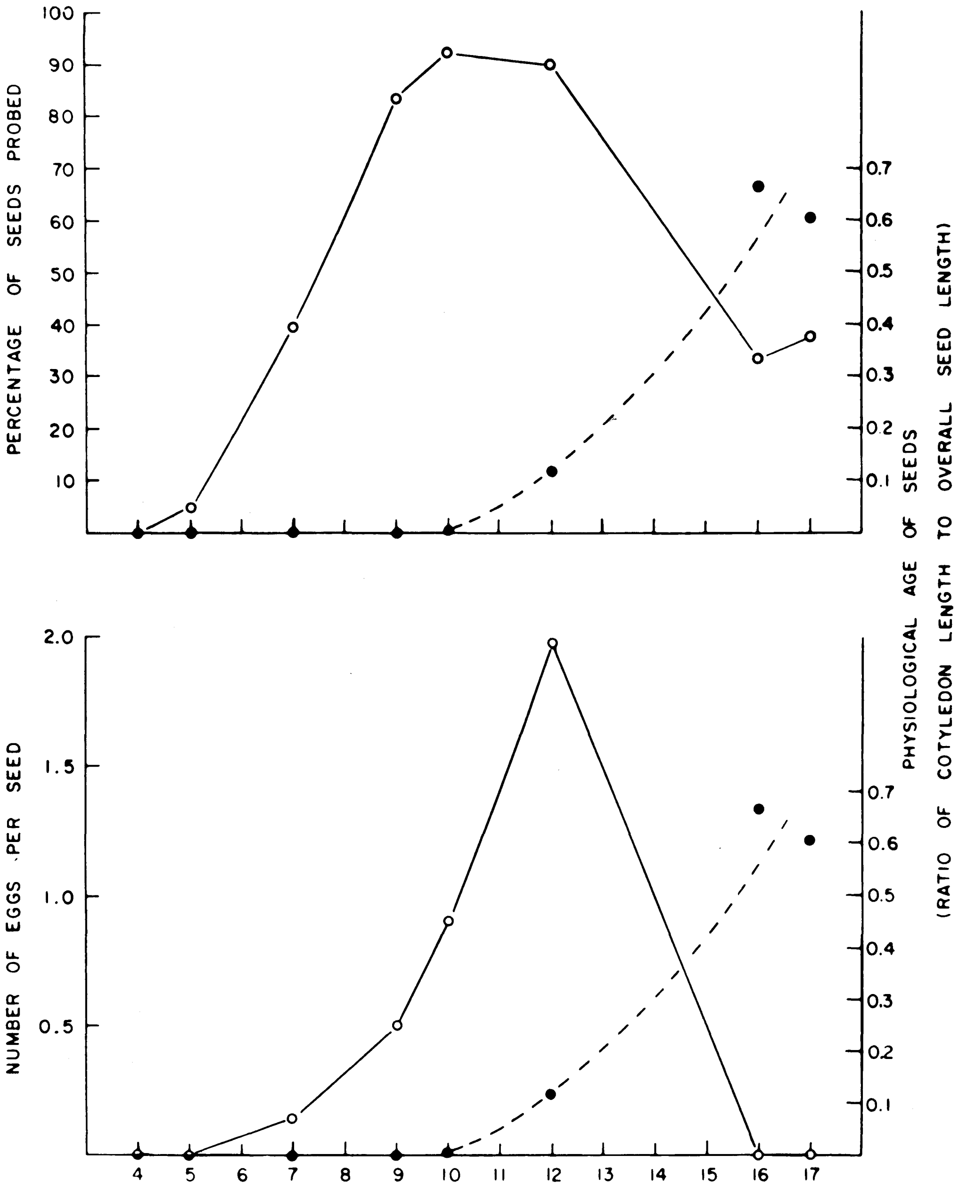

Fig. 16. Seed-probing and oviposition by *Bruchophagus kolobovae* in relation to age of seeds. Test No. 1.

This experiment was performed on September 7, 1963, and the eggs were counted after an oviposition period of one hour. Thirteen eggs were laid in the three cages at 80° F and 25 eggs at 90°, but none at 70° in this experiment. However, eggs have been obtained at 70° in other experiments, where more time was allowed for oviposition. No determination was made in terms of total egg output in relation to temperature.

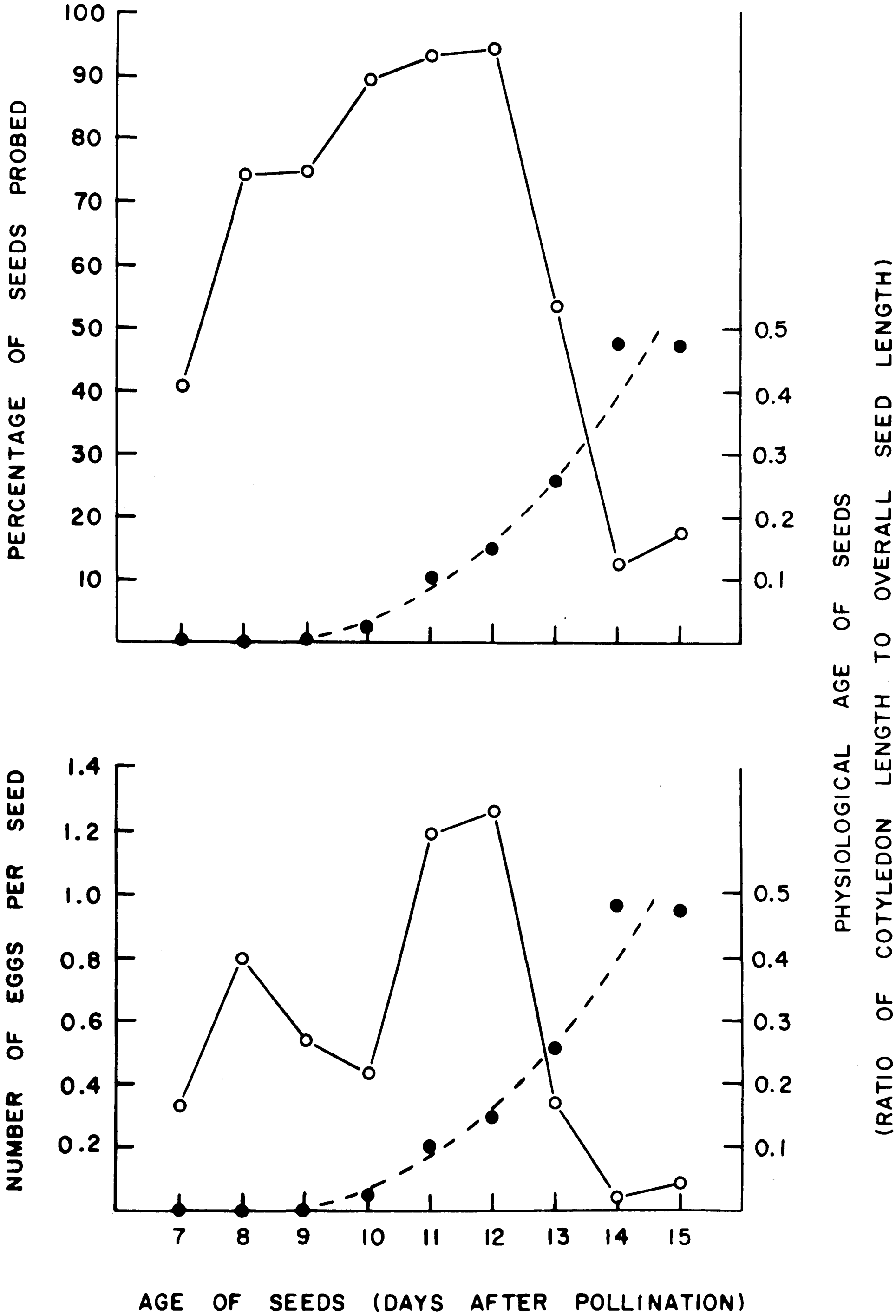

Fig. 17. Seed-probing and oviposition by *Bruchophagus kolobovae* in relation to age of seeds. Test No. 2.

TABLE 7
HOST SELECTION BY *BRUCHOPHAGUS KOLOBOVAE*
Oviposition Data*

Plant species	Seeds available	Eggs laid	Eggs/seed	Seeds probed		Cotyledon length/seed length
	no.	*no.*	*av. no.*	*no.*	*per cent*	*ratio (mean)*
Lotus tenuis	16	5	0.3	13	81.3	0.006
L. corniculatus 'Viking'	33	64	1.9	28	84.9	0.005
Medicago sativa 'Ranger'	80	0	...	0	....	0.016

* In one cage, 100 females and 100 males (all from *L. tenuis*) were confined over the seedpods for 24 hours at 80° F under a 15-hour photoperiod. There were two seedpod umbels or racemes of each plant species, all nine days old when the test started. All seeds were dissected and measured at the end of the test. July 16–17, 1963.

TABLE 8
HOST SELECTION BY
BRUCHOPHAGUS KOLOBOVAE
Oviposition Data*

Plant species	Seeds available	Eggs laid	Eggs/ seed
	no.	*no.*	*av. no.*
Lotus tenuis	13	4	0.31
L. corniculatus 'Viking'	7	13	1.86
L. corniculatus 'Empire'	44	80	1.82
L. humistratus	33	0	....
L. micranthus	42	1	0.02
L. purshianus	31	3	0.10
L. heermannii	20	0	....
Medicago hispida	39	0	....

* In one cage, 50 females from *L. tenuis* were confined, without males, over the seedpods for 24 hours at 80° F under a 15-hour photoperiod. There were 3–13 seedpods of each plant species. The seeds of *L. tenuis* and of *L. corniculatus* (both 'Viking' and 'Empire') were eight days old when the test started. Cotyledon and seed lengths were not measured. June 22–23, 1963.

Influence of light. Strong (1962*b*) studied oviposition by *B. roddi* under lights of four different colors and found that females of that species deposited eggs under all lights tested and also in darkness. The following experiment (table 11) shows that females of *B. kolobovae,* on the contrary, deposited eggs only during exposure to light and that they responded to the light factor rather than to time of day. Colored lights were not tested, nor were light thresholds.

The experiment was conducted in two parts. In the first part I was testing the rates of oviposition during light and dark phases in the usual 15-hour photoperiod. Two series of insects, A and B, were provided with fresh seedpods for each of three consecutive exposures in one 80° F cabinet. Series A was provided with seedpods in the sequence light-dark-light and Series B in the sequence dark-light-dark. The insects to be tested were taken from holding cages in the 70° room. They were counted out into eight tube cages and were placed in a 70° F cabinet at 11 A.M. on August 18. They were held in that cabinet for rigid photoperiod control both before the first exposure and during part of the storage interval between the two parts of the experiment (fig. 18).

Each series used 60 females (15 in each of four replicates) in tube cages on a platform, with one seedpod of *Lotus tenuis* in each cage for each exposure. To minimize the effects of possible seedpod variations, which might influence the rate of oviposition, one umbel of three seedpods was used for each cage. For the first exposure of a sequence I inserted one seedpod into the cage, from below, and left the other two, still attached to the peduncle, under the platform in reserve for Exposures 2 and 3.

Each exposure lasted 8 hours and 45 minutes. The insects were conditioned to each change of phase for 15 minutes before a seedpod was inserted for a scheduled exposure. For the dark phase the seedpods were inserted under re-

TABLE 9

HOST SELECTION BY *BRUCHOPHAGUS KOLOBOVAE*

Oviposition Data*

Plant species	Seeds available	Eggs laid	Eggs/seed	Seeds probed		Cotyledon length/seed length
	no.	*no.*	*av. no.*	*no.*	*per cent*	*ratio (mean)*
Lotus tenuis..........................	26	21	0.81	18	69.2	0.000
L. corniculatus 'Viking'..............	11	4	0.36	6	54.6	0.000
L. micranthus.........................	19	0	....	0	....	0.198
L. purshianus.........................	11	0	....	0	....	0.510
L. subpinnatus........................	23	0	....	0	....	0.482
Medicago hispida......................	31	0	....	0	....	0.645
M. hispida var. *confinis*............	16	0	....	0	....	0.572

* In one cage, 100 females and 100 males (all from *L. tenuis*) were confined over the seedpods for 24 hours at 80° F under a 15-hour photoperiod. There were 3–12 seedpods of each plant species. When the test started, seeds of *L. tenuis* were eight days old, seeds of *L. corniculatus* were seven days old. All seeds were dissected and measured at the end of the test. July 11–12, 1963.

duced light. Because of the difference in length of the light and dark phases in the 15-hour photoperiod, the insects were held without seedpods for the last six hours of each light phase.

The second part of the experiment was the fourth exposure of each series and used the same insects. For this test the light and dark phases were reversed in relation to the time of day. I was then comparing the oviposition of the Series A insects, which were provided with seedpods during the daytime but in darkness, with that of the Series B insects, which were provided with seedpods during the night with the lights on. A conditioning period, partly at 70°, preceded this second part of the test. There was no interruption in the dark phase before the A-4 exposure, because both the transfer of cages from 70° and the insertion of pods into the cages were made in total darkness. Likewise the light phase preceding Exposure B-4 was not interrupted in the transfer of cages from the 70° holding cabinet.

The seedpods for Exposures A-4 and B-4 were randomized between the two series. Four two-seedpod umbels were used—one seedpod of each umbel used in Exposure B-4 and the other seedpod held in reserve for Exposure A-4, which followed immediately.

TABLE 10

HOST SELECTION BY *BRUCHOPHAGUS KOLOBOVAE*

Oviposition Data*

Plant species	Seeds available	Eggs laid	Eggs/seed	Seeds probed		Cotyledon length/seed length
	no.	*no.*	*av. no.*	*no.*	*per cent*	*ratio (mean)*
Lotus tenuis..........................	63	64	1.0	59	93.7	0.007
L. micranthus.........................	25	0	...	0	....	0.101
L. subpinnatus........................	20	0	...	5	25.0	0.275
L. purshianus.........................	14	3	0.2	4	28.6	0.267
L. heermannii.........................	9	0	...	0	....	0.031
Medicago hispida......................	23	0	...	0	....	0.281

* In one cage, 130 females and 65 males (all from *L. tenuis*) were confined over the seedpods for 24 hours at 80° F under a 15-hour photoperiod. There were four to seven seedpods of each plant species. Seeds of *L. tenuis* were nine days old when the test started. All seeds were dissected and measured at the end of the test. July 25–26, 1963.

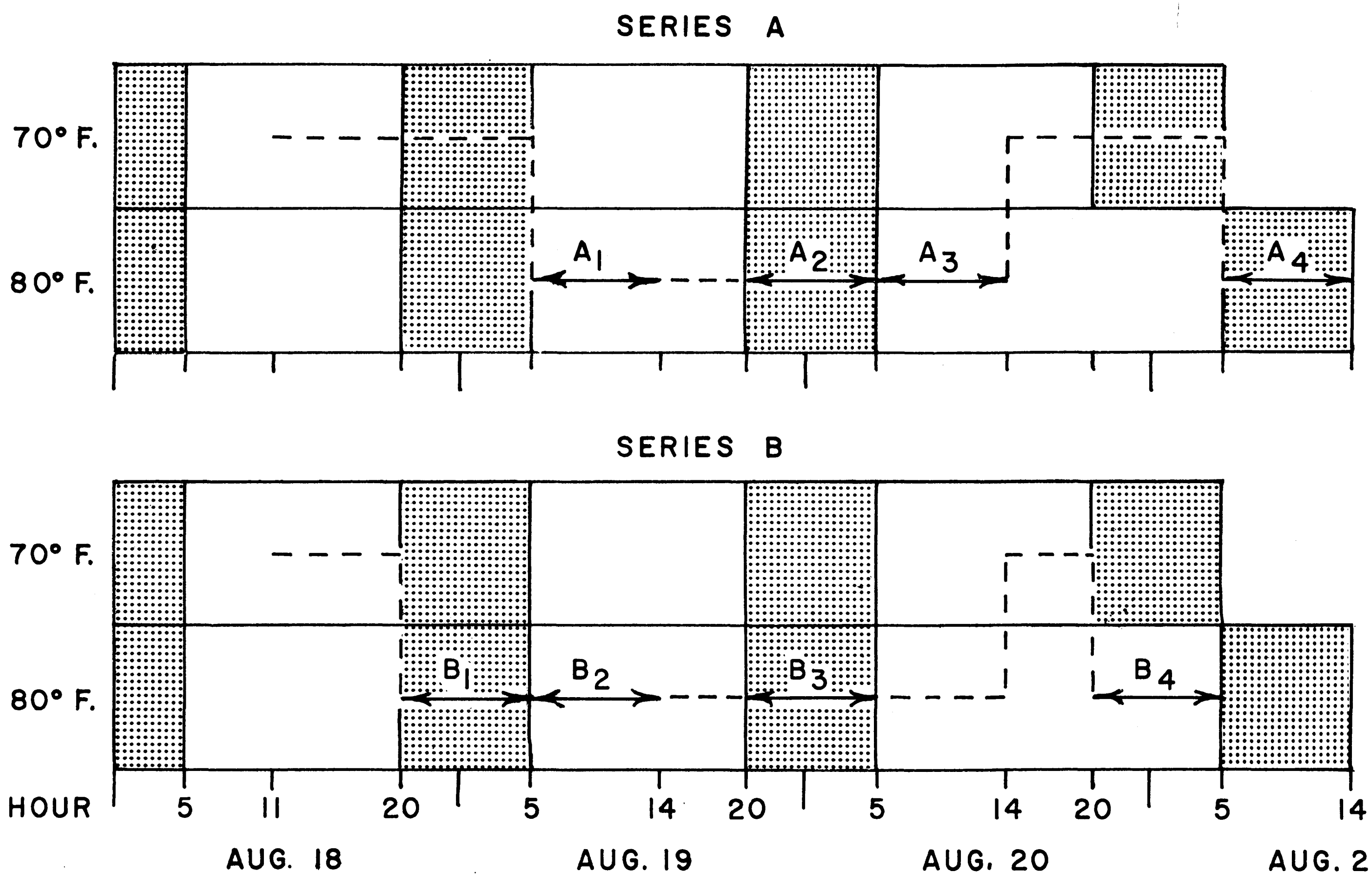

Fig. 18. Light and temperature conditions of holding *Bruchophagus kolobovae* in oviposition experiment (table 11) before and during exposures of seedpods, using two controlled-temperature cabinets with 15-hour photoperiod. Stippled areas indicate dark phases. Dotted lines indicate periods of holding insects caged without seedpods; solid lines indicate periods when seedpods were exposed to oviposition. Sixty females (15 in each of four replicate cages) were used in each series.

TABLE 11
OVIPOSITION BY *BRUCHOPHAGUS KOLOBOVAE* ON *LOTUS TENUIS* UNDER CONDITIONS OF LIGHT OR DARKNESS*

Treatment			Seeds available in four cages	Eggs deposited	Eggs per seed
Exposure	Time	Lights			
			total no.	*total no.*	*av. no.*
A-1	Day	On	47	85	1.8
A-2	Night	Off	32	0	...
A-3	Day	On	43	49	1.1
A-4	Day	Off	52	0	...
B-1	Night	Off	46	0	...
B-2	Day	On	68	81	1.2
B-3	Night	Off	39	0	...
B-4	Night	On	49	59	1.2

* Each exposure period was 8 hours and 45 minutes at 80° F. The photoperiod was 15 hours. Figure 18 shows the schedule of the exposures in both series and the reversal of the photophases for Exposures A-4 and B-4.
For each series (A and B) there were four replicate cages, each containing 15 females from *Lotus tenuis*. The same 60 insects in the four cages were used for all four exposures of the series, but a fresh seedpod was supplied in each cage for the period of each exposure.

Host specificity

Lotus corniculatus 'Viking' was tested in the greenhouse as a suitable host for the offspring of *B. kolobovae* parents from field-collected seeds of *L. tenuis.* Ten females and five males were confined for 24 hours in a tube cage over each umbel, using four umbels on July 28 and five umbels on August 11, 1963. The 19 seedpods thus exposed were allowed to mature on the plants and were held in the same nine cages in the greenhouse until October 16, 1963. During that time, 24 offspring (13 females and 11 males) developed and emerged as adults. The reverse test, using egg-laying females from seeds of *L. corniculatus* confined over seedpods of *L. tenuis,* was not made.

Lotus purshianus, also, was tested in the greenhouse as a suitable host for *B. kolobovae.* Fourteen seedpods, each in a tube cage with 10 females and 0 to 5 males, were tested between July 31 and August 25, 1963. The exposure was either 24 or 48 hours. The seedpods were allowed to mature on the plants and were held in the same cages in the greenhouse until October 16. No offspring emerged, and examination of the mature seeds gave no indication of larval feeding.

In field surveys during three summers, *B. kolobovae* has not been found on any native species of *Lotus.* However, an undetermined species of *Bruchophagus* — distinctly different from *B. kolobovae*—was found infesting seeds of *L. purshianus* in an uncultivated area near Davis and in one near Roseville, California. Females that emerged from these field-collected seeds were confined over seedpods of *L. purshianus* growing in the greenhouse, using 14 seedpods, each exposed to two to five females for periods ranging from one hour to three days. Thirteen adult offspring were produced in these tests. As there were no other insects associated with the seedpods in the greenhouse test, it was concluded that the larvae of this undetermined *Bruchophagus* are seed-feeders, not insect parasites.

Rate of development, by stages

Observations on the development of *B. kolobovae* required host seeds on growing plants in the greenhouse, because the seeds did not develop normally on plants growing under the

TABLE 12
INCUBATION TIME OF *BRUCHOPHAGUS KOLOBOVAE* EGGS IN GROWING SEEDS OF *LOTUS TENUIS**

Time after oviposition	Eggs unhatched	Larvae	Hatch
days	*no.*	*no.*	*per cent*
2.0	18	0	....
2.5	27	0	....
3.0	20	0	....
3.5	32	0	....
4.0	8	0	....
4.5	24	2	7.69
5.0	11	2	15.38
5.5	7	10	58.82
6.0	6	11	64.71
6.5	1	12	92.31
7.0	1	7	87.50

* On August 25, 1963, 25 females were caged for six hours over each of eight 12-day-old seedpod umbels in the greenhouse. Sample seedpods were removed and dissected every 12 hours, from two to seven days after oviposition.

controlled conditions available. *Lotus tenuis* was used as the host plant for these studies.

Egg. Eight 12-day-old umbels (16 seedpods) on two plants were exposed to oviposition for one hour, with 25 females caged over each umbel. Sample seedpods were removed and dissected twice daily, starting two days after oviposition. Not more than one seedpod was taken from any umbel at one sampling, but single seedpods from one, two, or three of the umbels were taken as needed, to make up an adequate sampling of eggs. The eggs hatched over a period of about three days, beginning between four and four and a half days after oviposition (table 12). The mean time required for hatching was about five and a half days, but the hatch did not reach 100 per cent in the seven days that the pods lasted. The same hatching pattern was found in the study of larval instars, below. In that study, also, unhatched eggs were found up to seven days after oviposition.

Larval instars. Flower umbels on four plants were pollinated on two successive days, and 19 umbels (48 seedpods) 11 and 12 days old were used. Each umbel was exposed for two hours to oviposition by 20 females. Single seedpods from one or more umbels were removed and dissected daily, from the fifth to the twenty-fifth day after oviposition. Eggs and larvae dissected out from the seeds were mounted in Hoyer's medium and used as material for the measurements and identification of larval instars (see p. 433). Table 13 gives the daily findings at each stage.

The following calculations gave an estimate of the period required for development of each larval instar: For each day when a particular instar was found, the percentage of the population at that instar was multiplied by the day number. The sum of the above products was divided by the sum of the population percentages for each occurrence of that instar. The results indicated peaks of abundance as follows: first larval instar approximately 7.7 days after oviposition; second, 10.5 days; third, 13.0 days; and fourth, 14.8 days. The eighteenth day after oviposition—when all of the larvae found in seeds had reached the fifth larval instar—was used as the peak of abundance of that instar. These peaks indicate that larval molts occurred at intervals of two to three days in the greenhouse.

At the end of the experiment, on September 23, 1963 (25 days after oviposition), none of the larvae had defecated or shown other evidence of pupating. The larvae were mature and the seeds were completely hollowed out, with only their hardened brown coats remaining. It appears that all of these larvae were in diapause.

Because of crowding in this experiment, many of the seeds contained two or more eggs and a few contained two or more young larvae, in separate feeding cavities. Usually only one larva (not always the more advanced) survived after the cavities merged. Apparently the larva that first breaks into the adjoining cavity kills the other larva by puncturing it. There was some indica-

TABLE 13

RATE OF LARVAL DEVELOPMENT OF *BRUCHOPHAGUS KOLOBOVAE* IN SEEDS OF *LOTUS TENUIS* ON GROWING GREENHOUSE PLANTS*

Day after oviposition	Number of individuals found							Per cent of the day's population					
	Egg	Instar I	Instar II	Instar III	Instar IV	Instar V	Total	Egg	Instar I	Instar II	Instar III	Instar IV	Instar V
5	30	11	0	0	0	0	41	73.2	26.8	....	....	....	....
6	2	23	0	0	0	0	25	8.0	92.0	....	....	....	....
7	2	24	0	0	0	0	26	7.7	92.3	....	....	....	....
8	0	34	1	0	0	0	35	....	97.1	2.9	....	....	....
9	0	9	6	0	0	0	15	....	60.0	40.0	....	....	....
10	0	0	12	2	0	0	14	....	....	85.7	14.3	....	....
11	0	2	8	5	0	0	15	....	13.3	53.3	33.3	....	....
12	0	1	2	7	0	0	10	....	10.0	20.0	70.0	....	....
13	0	3	4	6	7	0	20	....	15.0	20.0	30.0	35.0	....
14	0	0	0	9	9	0	18	....	....	....	50.0	50.0	....
15	0	0	0	2	9	2	13	....	....	....	15.4	69.2	15.4
16	0	0	0	1	8	10	19	....	....	....	5.3	42.1	52.6
17	0	0	0	3	3	9	15	....	....	....	20.0	20.0	60.0
18	0	0	0	0	0	12	12	....	....	....	....	....	100.0
19	0	0	0	0	0	16	16	....	....	....	....	....	100.0
20	0	0	0	0	0	8	8	....	....	....	....	....	100.0
21	0	0	0	0	0	10	10	....	....	....	....	....	100.0
22	0	0	0	0	0	9	9	....	....	....	....	....	100.0
23	—	—	—	—	—	—	—	—	—	—	—	—	—
24	0	0	0	0	0	4	4	....	....	....	....	....	100.0
25	0	0	0	0	0	2	2	....	....	....	....	....	100.0
Total	34	107	33	35	36	82	327						

* On August 29, 1963, 20 females were caged for two hours over each of nineteen 11- to 12-day-old seedpod umbels. Sample seedpods were removed and dissected daily from five to 25 days after oviposition, except for the twenty-third day. Larval instars were identified by head-capsule measurements (table 2 and fig. 8).

tion that larval development was retarded if the cavities did not merge; most of the late-developing larvae of the first, second, and third instars were found in multiple infestations. The most advanced development found in a multiple infestation was two fourth-instar larvae in one seed.

Diapause and pupation. For many years it has been known that fully grown larvae of seed chalcids may undergo a condition of arrested development (Urbahns, 1920). This condition is now considered a facultative larval diapause, but its mechanisms are not well understood. In diapause, mature fifth-instar larvae of *B. roddi* retained the solid wastes in the gut and remained inactive for an extended period of time (Strong, 1962*b*). When diapause ended the larvae defecated and pupated in the usual way. The diapause in *B. kolobovae* appears similar to that in *B. roddi*. Its seasonal occurrence was studied with field-collected material.

When pupation begins, development is normally uninterrupted and continues directly to adult emergence. The pupal stage lasts approximately 8 to 11.5 days at a constant temperature of 70° F.

Egg to adult. Nondiapausing individuals of *B. kolobovae* developed from egg to adult in approximately 33 days in growing seeds of *Lotus tenuis* in the greenhouse. The time required for development of 26 females was 30 to 36 days, with a mean of 33.0 days ± 1.4. The time for 34 males was 29 to 36 days, with a mean of 32.7 days ± 1.9.

Adult life-span

Seven females and seven males of *B. kolobovae,* all newly emerged in gelatin capsules, were confined as pairs in plastic-tube cages over suitable seedpod umbels of *Lotus tenuis* in the greenhouse. Each pair or the surviving female was transferred daily to a fresh umbel. At each transfer a drop of undiluted honey was placed on the nylon screen of the cage. Strong (1962*b*) found that adults of *B. roddi* fed readily on diluted honey and that either the feeding or the liquid extended their life-span. In the present experiment the plant tissue probably supplied any needed liquid.

The seven females lived from 17½ to 43½ days, with a mean of 25.9 days ± 9.4 and the seven males lived from 6½ to 23½ days, with a mean of 12.6 days ± 5.6 (table 14).

In the experiment on Seedpod requirement (see p. 442), the 10 females lived from 29½ to 49 days, with a mean of 37.3 days ± 6.0 and the 10 males lived from 14½ to 26½ days, with a mean of 20.0 days ± 4.1.

Reproductive potential

Following the above experiment on life-span, all of the seedpods were allowed to mature on the plants and were then cut off and held in their cages in the greenhouse. Daily records of all emergences provided detailed data on the offspring of each pair of insects. Pods and seeds were dissected 49 days after the last emergence, to count any remaining offspring. Although the actual numbers of eggs laid may have been somewhat greater than the numbers of offspring recovered, the additional information wanted from this experiment could not have been obtained if seeds had been dissected before the adults emerged.

The period of oviposition varied considerably in relation to the age of an individual insect. The preoviposition period of Female A was less than three days and that of Female F was 33 days (table 14). It was not determined whether those females that began to oviposit late were incapable of ovipositing earlier or whether they retained their eggs because of undetermined factors. In spite of the fact that Female F started oviposition 25 days after the death of her mate, she produced female

TABLE 14
REPRODUCTION BY *BRUCHOPHAGUS KOLOBOVAE* IN SEEDS OF *LOTUS TENUIS* GROWING IN THE GREENHOUSE

Parents*				Offspring				
Pair	Age of female during oviposition	Life-span		Adults		Larvae		Total
		Females	Males	Females	Males	Diapausing	Dead	
	days	*days*	*days*	*no.*	*no.*	*no.*	*no.*	*no.*
A..........	3–23	31.5	6.5	2	3	19	0	24
B..........	....	25.5	12.0	0	0	0	0	0
C..........	7–17	17.5	23.5	26	20	9	1	56
D..........	....	17.5	11.5	0	0	0	0	0
E..........	....	26.5	14.5	0	0	0	0	0
F..........	33–42	43.5	7.5	2	2	10	2	16
G..........	11–15	19.0	13.0	0	28	1	0	29

* Each pair caged separately and transferred daily to a fresh 9- to 12-day-old seedpod. Experiment started July 19, 1963.

offspring—which indicated that some of these late eggs were fertilized by sperm from a mating at least 25 days earlier.

There was no apparent correlation between the age of the female at the time of oviposition and either the sex of her offspring or the proportion of diapausing larvae. The four females with offspring produced both diapausing and nondiapausing larvae from eggs laid on one day in the seedpods of one umbel and, in at least one case, within the seeds of a single seedpod.

Numbers of offspring. Only four of the seven females tested produced offspring (table 14). There was no evidence that the other three laid any eggs, though they all lived between 17 and 26 days. The four fertile females produced a total of 125 offspring, from 16 to 56 per female.

In *B. roddi,* also, there was a considerable range in the numbers of offspring produced by individual females. Strong (1962*b*) obtained from 6 to 86 larvae from eggs laid by individual females. Carrillo and Dickason (1963) dissected field-collected females of *B. roddi* and counted the ova and oocytes that they contained. The highest count for one individual was 95.

FIELD STUDIES

Both *Lotus corniculatus* and *L. tenuis* are grown in California as commercial seed crops and as permanent pasture crops (Peterson, Jones, and Osterli, 1953). Escape plants of both species, especially of *L. tenuis,* are abundant along roadsides and irrigation ditches.

Usually the commercial seed growers prevent early seed production and control weeds at the same time, either by grazing livestock heavily in the fields or by cutting the plants for hay during the first part of the season. This tends to make the seed-setting more uniform later in the season. When grazing is stopped the growers irrigate frequently, to produce large quantities of seed over a short period of time and to maintain moisture in the maturing seedpods. Abundant water and early harvesting help to reduce the loss of seeds from dry and dehiscent seedpods.

Trefoil plantings used solely for pasture are often grown adjacent to seed fields. The trefoil may come into bloom very early and produce seed throughout the growing season. Usually, however, the pastures are grazed to such an extent that they produce only small quantities of seed at any given time.

Most of the roadside plants remain undisturbed and produce large quantities of seed throughout the season, if water is sufficient.

Methods of sampling

Populations of *B. kolobovae* in the three ecological situations were studied from 1961 through 1963 by collecting from four seed fields, one pasture field, and three uncultivated roadside areas. The pasture field sampled was adjacent to Seed Field 4 and was similar to it except in cultural handling. It was a very weedy field, but was otherwise a pure stand of trefoil. It was irrigated less and was grazed during a longer season than were the seed fields. The following samples were taken weekly, in their seasons: Adult populations; immature seedpods for counts of larvae; and bulk lots of maturing seedpods for data on emergence and diapause, sex ratios, and parasites. Seed samples were taken at harvest time, also, to determine the overall seed loss caused by *B. kolobovae*.

Adult populations in the field were sampled with a D-Vac® suction machine (fig. 19), that sucked up the insects from plants over a ground area equivalent to 1 square foot (Dietrick, Schlinger, and van den Bosch, 1959). Insects from 100 such areas, collected in one net, constituted one sample. The insects were anesthetized lightly with carbon dioxide while the samples were transferred to a modified Berlese funnel. Heat was applied to the top of the funnel; a light below the funnel attracted the insects to a specimen jar, which contained 60 per cent ethanol. Adult chalcids in each sample were counted with a dissecting microscope. The density of the population was expressed as the number of individuals per 100 square feet.

Seed Field 1 (*Lotus corniculatus* 'Empire') and Seed Field 2 (*L. corniculatus* 'Viking') were sampled weekly during the summer of 1961, Seed Field 3 (*L. tenuis*) during the summer of 1962, and the other five locations from May 1, 1962, through May 28, 1963—though less frequently from mid-October through April. On every sampling date one sample (material collected from 100 1-square-foot areas) was taken from Roadside Area 1 (*L. tenuis*), one from Roadside Area 2 (*L. tenuis*), two from Roadside Area 3 (*L. tenuis*), two from the pasture field (*L. tenuis*), four each from Seed Fields 1 and 2, and three each from Seed Fields 3 and 4 (both *L. tenuis*). Figure 20 shows the data from these collections of adults, together with data on the populations of larvae (next paragraph).

Populations of larvae. Samples of immature seedpods were taken on each vacuum-sampling date during the period of seed development in the summer of 1962. This period was longest in the uncultivated areas. No seedpod samples were taken from Seed Fields 1 and 2, studied in 1961, but casual inspection of seeds from these fields during the season revealed only very few infestations. In the other six locations, one 10-pod sample was collected from the general vicinity of each 100-square-foot vacuum sample. The percentage of infested seeds in each 10-pod sample was determined by dissection. This information supplements the data on adult populations by indicating the amount of egg-laying activity. However, the percentage of infested seeds is not a good index of the actual numbers of larvae in the field, because this percentage varies inversely with the abundance of acceptable seeds. The suction samples, on the other hand, were more indicative of actual population changes because they were based on unit areas.

Maturing seedpods. In 1962 and 1963, large numbers of seedpods of

Fig. 19. Sampling adult insect populations by suction. Photo from Hilgardia 35(1):3.

Lotus tenuis were collected weekly for studies on emergence and diapause. Selection of seedpods with green peduncles made for uniformity in the age of the insects, with a range of probably not much more than a week. The same collections provided data on sex ratios and parasites. Most of the newly emerged adults in an emergence carton came to the collecting vial, but still many remained among the seedpods. This was discovered too late in 1962 to use the additional insects for that season's data, but in 1963 the cartons were opened at every observation and all living insects that were found were removed and counted. No dead insects were counted, because of possible confusion about the time of emergence in relation to diapause.

Bruchophagus kolobovae adults from the 1963 seedpod collections were used for laboratory and greenhouse experiments after all of the above data were recorded.

Harvest samples. Mature seeds were collected from three seed fields at harvest time. In 1961, seeds of *Lotus corniculatus* from the combine harvester in Seed Field 2 were sampled before cleaning. No infested seeds were found, but some may have been lost in harvesting. Two fields of *L. tenuis* were sampled just before the 1962 harvest, by the following method: All of the plants were gathered from a ground area of 1 square foot. The seedpods were removed from these plants and hand-threshed. Three such areas were collected from Seed Field 3 and nine such areas from Seed Field 4. Samples of each seed lot were analyzed by a technique devised by Strong (1960).

Population dynamics

The highest infestations were found consistently in the uncultivated areas. Three or possibly four overlapping generations of seed chalcids may develop there in one summer, because of the long season of seed production. It is believed that uncultivated areas and possibly some pasture fields provide the principal means for early buildup of seed-chalcid populations and that adults migrate from there into adjacent seed fields. Seed Fields 1, 2, and 3 were isolated from uncultivated trefoil areas that might allow the buildup of seed-chalcid populations, and the surveys of these three fields indicated very little infestation at any time. Seed Field 4, where definite infestation was found, was close to uncultivated trefoil areas and also to trefoil pastures.

Strong, Bacon, and Russell (1963) found that *B. roddi* was capable of traveling in the air to distances of at least 4,100 feet. Probably it travels much farther, especially under suitable wind and host-plant conditions. Adults of *B. kolobovae,* also, are capable of strong flight, but no distance studies were made. It seems likely that *B. kolobovae* could travel easily from uncultivated areas into nearby seed fields.

Seasonal trends. Adults of *B. kolobovae* from the overwintering population began to appear in uncultivated areas about May 1 (fig. 20), at about the same time that trefoil blooms and sets seed if undisturbed. This suggests that the seasonal cycle of *B. kolobovae* is synchronized closely with the availability of seedpods. The percentage of infestation was high in the small numbers of early seeds, but it dropped rather soon—obviously because egg-laying did not keep pace with the rapid increase in actual numbers of seeds in these areas. When the adult populations in uncultivated areas increased in July following the May and June egg-laying, the percentage of infested seeds there rose again and remained fairly high the rest of the summer.

In the seed fields there were no seedpods available for oviposition before about July 1, and the adult populations of seed chalcids remained very low through July. However, the earliest seeds in Seed Field 4 showed fairly high percentages of infestation, probably because of the migration of egg-laying chalcids. As in the uncultivated areas, the percentage of seed infestation dropped when more seeds became available but rose again in August as a result of egg-laying by adults which developed from the July eggs.

The major peaks of adult population occurred in August in all infested areas. Populations were very high in two of the uncultivated areas. Somewhat smaller peaks, possibly caused partly by migration, occurred in Seed Field 4 and in the pasture field.

Although the harvest samples were relatively small, the data (table 15) are consistent with the weekly samples taken from the four seed fields. The only appreciable infestation found at any time was in Seed Field 4. The harvest samples show that losses caused by *B. kolobovae* in the seed fields studied were not of much economic significance.

Sex ratios. For the 5,664 adults of *B. kolobovae* collected by suction in the field in 1962 and 1963, the overall sex ratio was 1 male to 1.50 females. The greater longevity of females would be a factor here. For the 5,026 adults that emerged from seedpods collected in 1962 and 1963, the ratio was 1 male to 1.44 females. If the males are, in general, weaker individuals than the females, the artificial and relatively dry laboratory conditions may have affected this ratio. In the combined data from those greenhouse tests where females of *B. kolobovae* were given opportunity to mate (tables 4 and 14 and the offspring reared on *L. corniculatus*, p. 451), the sex ratio for the 150 offspring was 1 male to 0.81 females.

Seasonal occurrence of diapause. Little detailed information is available concerning the seasonal occurrence of diapause in seed chalcids. Urbahns (1920) presented a diagram of the relative abundance over the year of each stage in the development of *B. roddi.* His data showed that "resting larvae," relatively uncommon in summer, constituted the overwintering survival stage. Strong (1962*b*) showed, also in *B. roddi,* that low-temperature treatments appeared to accelerate the diapause-ending process.

In 1962, I made weekly collections of maturing seedpods of *Lotus tenuis* from the three roadside areas and from Seed Field 4, when available. The seedpods were held in emergence cartons under room conditions (average about 72° F) and insects that emerged were removed three times a week. All seedpods were held until June 21, 1963, to allow for any delayed emergence. Adults of *B. kolobovae* that emerged during the first three or four weeks after collecting were considered as non-diapausing individuals; those that

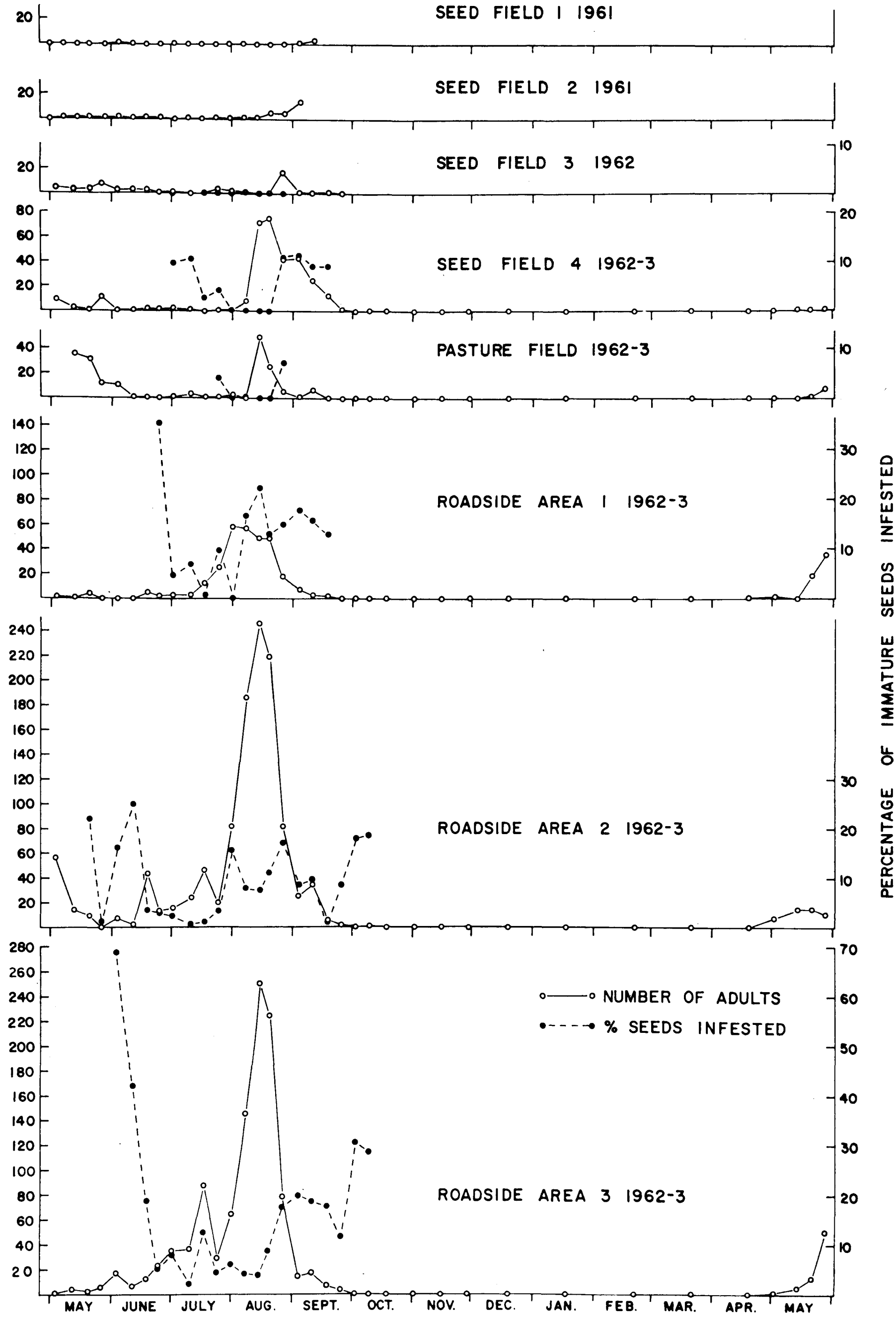

Fig. 20. Population trends of *Bruchophagus kolobovae* in vicinity of Davis, California, 1961–1963. *Lotus corniculatus* was growing in Seed Fields 1 and 2, *L. tenuis* in the other areas sampled.

TABLE 15
HARVEST SURVEY OF *BRUCHOPHAGUS KOLOBOVAE* INFESTATION IN COMMERCIAL SEED FIELDS NEAR DAVIS, CALIFORNIA*

Seed field	Plant species	Collection date	Seeds Examined	Seeds Infested	
			no.	*no.*	*per cent*
2	*Lotus corniculatus*	9-4-61	490	0	0.00
3	*L. tenuis*	9-3-62	3,966	1	0.03
4	*L. tenuis*	9-10-62	8,810	61	0.70

* Sample from Seed Field 2 taken from combine harvester before cleaning. Samples from Seed Fields 3 and 4 collected by hand shortly before harvest.

TABLE 16
SEASONAL OCCURRENCE OF DIAPAUSE IN *BRUCHOPHAGUS KOLOBOVAE* IN FIELD-COLLECTED SEEDPODS OF *LOTUS TENUIS**
Davis, California. 1962

Collection date	Adults emerged from each week's collection: Without diapause	After diapause	
	no.	*no.*	*per cent*
6/3	333	0	....
6/11	53	0	....
6/18	48	0	....
6/24	56	0	....
7/1	20	0	....
7/10	77	0	....
7/17	135	0	....
7/24	115	0	....
7/31	149	0	....
8/7	107	0	....
8/14	57	0	....
8/19	26	4	13.3
8/26	66	23	25.8
9/3	37	41	52.6
9/10	23	47	67.1
9/18	2	61	96.8
9/25	0	33	100.0
10/2	0	92	100.0
10/9	0	37	100.0

* Seedpods held in emergence cartons under room conditions (average 72° F) until June 21, 1963. Insects were removed from the glass collecting vial three times a week.

emerged after a pronounced delay were considered as having undergone diapause.

There was only one period of emergence from seedpods collected between June 3 and August 14, 1962. All emergences occurred within four weeks after collecting the seedpods. There were two distinct periods of emergence from seedpods collected between mid-August and mid-September—an early emergence, without diapause, and a delayed emergence, following diapause. There were only a few diapausing individuals in the late-August seedpod collections, but the proportion of diapausing to nondiapausing individuals increased in each succeeding week's collection, and the collections made after September 18 contained diapausing individuals exclusively, with no early emergence (table 16). Diapause ended spontaneously at room temperature, after differing intervals. The ensuing emergence started gradually, with the first few individuals appearing two months or more after the end of the initial emergence period.

In 1963, I collected two cartons of maturing seedpods of *L. tenuis* every week from Roadside Area 3. Each sample of seedpods was held in an emergence carton in the 70° room for an initial emergence period of five weeks from the time of collecting, chilled in the 42° room for the next three months, and then returned to the 70° room until the experiment ended on April 13, 1964. The 70° room had a 15-hour photoperiod but the 42° room was kept dark most of the time. In both rooms the dark period may have been interrupted occasionally. Relative humidity averaged about 48 per cent in the 70° room and about 87 per cent in the 42° room.

All of the cartons in the 70° room were inspected three times a week, both before and after chilling. The initial emergence from each sample of seedpods in 1963 was completed well within the time allowed. There was no emergence during the three months at 42°. The final emergence started almost exactly six weeks after the seedpods were returned to 70°—in all samples that contained diapausing individuals—and was completed about three weeks later. Apparently the prolonged chilling stimulated the subsequent ending of diapause, even in January. Six weeks is longer than the usual pupation time and probably included a considerable period for the diapause-ending process.

Cumulative data on the rate of emergence were plotted for each of the 16 seedpod collections of 1963. Figure 21 gives four of the curves, which are representative of the others in showing the development of early-season populations without diapause followed by a gradual increase in the incidence of diapause after the mid-August collections. Also the curves show the effect of chilling on the termination of diapause. Table 17 gives data from the 1963 collections in terms that can be compared with the 1962 data for *B. kolobovae*. The patterns of the two years are in close agreement—considering variations inherent in field sampling and the different conditions of holding the collected seedpods. Comparison of data from the chilled seedpods of 1963 with those from the seedpods held at room temperature in 1962 suggests that the emergences started much more uniformly after chilling and were condensed over a shorter period of time but were not necessarily earlier than emergences from seedpods held at room temperature.

Overwintering stage. All indications are that in *B. kolobovae* the diapausing larval stage, protected inside the hollowed seed, carries the insect over the winter. This does not preclude the possibility that other stages also, either pupa or adult, might survive the winter—although there is no evidence for either possibility.

Fifty fifth-instar larvae of *B. kolobovae,* removed from maturing pods of *Lotus tenuis* collected on September 16, 1963, were placed individually in Plexiglas cells within plastic petri dishes. They were held in a controlled-temperature cabinet at 70° F ± 2°, with a 10-hour photoperiod during the first six months and a 16-hour photoperiod during the last two months. The insects were observed two or three times each week until the experiment was terminated on May 16, 1964.

Mortality was high in this experiment, because of parasitism and apparently, also, because of the desiccation of larvae removed from seeds. Only eight adults were obtained from the 50 larvae. Each of these surviving individuals had remained for an extended period as a diapausing larva and had defecated just before pupating. The pupal stage lasted from 10 to 17 days and was not prolonged in any specimen. The first adult emergence took place on January 16 and the eighth on May 16.

Parasites. Four hymenopterous parasites were found associated with *B. kolobovae* in the Davis area. The relative abundance of each species varied with the season as well as with the year and the locality. In almost every collection the numbers of parasites were considerably higher than the numbers of seed chalcids. Identifications made by the keys of Butler and Hansen (1958) were confirmed by B. D. Burks of the U. S. National Museum, Division of Parasitology (personal communication, 1963). Neunzig and Gyrisco (1959) found three of these species parasitizing *B. kolobovae* from trefoil. The fourth species, *Liodontomerus perplexus* Gahan, is a common parasite of *B. roddi* in alfalfa but had not been reported previously from *B. kolobovae.*

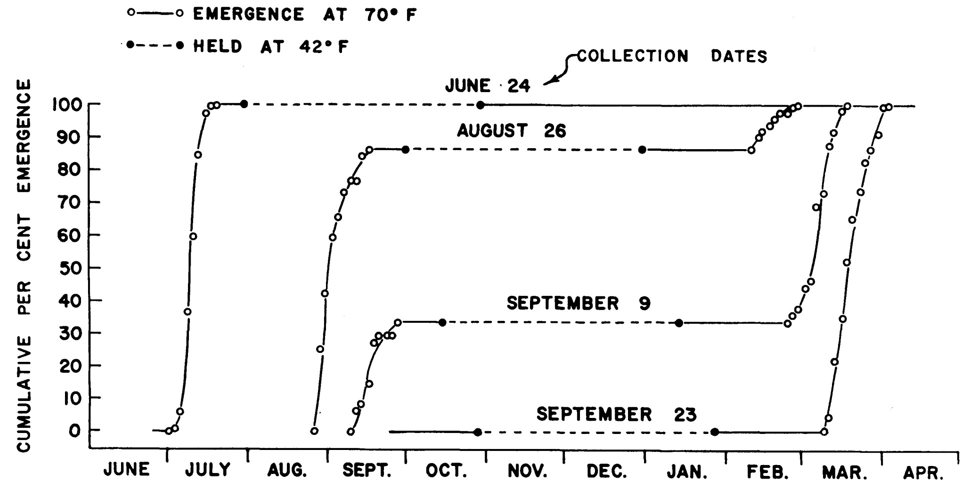

Fig. 21. Emergence of *Bruchophagus kolobovae* from bulk samples of maturing seedpods of *Lotus tenuis* collected in 1963 on four different dates. Freshly collected seedpods were placed in the 70° F room, to permit the emergence of nondiapausing adults, shown at left in the first three curves. Five weeks after collecting, the seedpods were moved to the 42° room and chilled for three months, then returned to 70° until April 13, 1964, the end of the experiment. Emergence of adults after diapause is shown at right in the last three curves.

TABLE 17

SEASONAL OCCURRENCE OF DIAPAUSE IN *BRUCHOPHAGUS KOLOBOVAE* AND IN THE PARASITE *TETRASTICHUS BRUCHOPHAGI* IN FIELD-COLLECTED SEEDPODS OF *LOTUS TENUIS**

Roadside Area No. 3, Davis, California. 1963

Collection date	Adults emerged from each week's collection					
	B. kolobovae			*T. bruchophagi*		
	Without diapause	After diapause		Without diapause	After diapause	
	no.	*no.*	*per cent*	*no.*	*no.*	*per cent*
6/17	8	0	...	15	0	...
6/24	771	0	...	1,085	1	0.1
7/1	634	0	...	900	1	0.1
7/8	269	0	...	664	1	0.2
7/15	220	0	...	514	2	0.4
7/22	72	1	1.4	300	0	...
7/29	78	0	...	191	0	...
8/5	206	7	3.3	446	7	1.5
8/12	601	28	4.5	1,125	195	14.8
8/19	284	13	4.4	705	124	15.0
8/26	45	7	13.5	534	111	17.2
9/2	16	11	40.7	146	196	57.3
9/9	16	32	66.7	138	511	78.7
9/16	9	22	71.0	83	471	85.0
9/23	0	23	100.0	29	130	81.8
10/1	0	11	100.0	2	19	90.5

* All seedpods were held in emergence cartons at 70° F for the first five weeks after collecting, at 42° for the next three months, and again at 70° until April 13, 1964. All living adult insects were removed three times a week, both from the glass collecting vial and from among the seedpods.

TABLE 18

BRUCHOPHAGUS KOLOBOVAE AND ITS PARASITES, FROM FIELD-COLLECTED SEEDPODS OF *LOTUS TENUIS**

Roadside Area No. 3, Davis, California. 1963

Collection date	All of adults emerged from each week's collection, with or without diapause				
	B. kolobovae	Parasites of *B. kolobovae*			
		Tetrastichus bruchophagi	*Trimeromicrus maculatus*	*Eupelmus allynii*	Parasitization
	no.	*no.*	*no.*	*no.*	*per cent*
6/17	8	15	0	0	65.2
6/24	771	1,086	1	0	58.6
7/1	634	901	0	0	58.7
7/8	269	665	0	0	71.2
7/15	220	516	1	2	70.2
7/22	73	300	0	0	80.4
7/29	78	191	0	1	71.1
8/5	213	453	0	0	68.0
8/12	629	1,320	13	0	68.0
8/19	297	829	10	0	73.9
8/26	52	645	2	1	92.6
9/2	27	342	1	1	92.7
9/9	48	649	0	3	93.1
9/16	31	554	0	0	94.7
9/23	23	159	0	1	87.4
10/1	11	21	0	0	65.6
Total	3,384	8,646	28	9	71.95

* Insects that emerged in diapause experiment (table 17). All seedpods were held at 70° F for five weeks after collecting, at 42° for the next three months, and again at 70° until April 13, 1964.

It was the most abundant parasite of *B. kolobovae* in one isolated uncultivated area approximately 20 miles north of the areas where regular collections were made for the field surveys. Only a few individuals of this species were found in trefoil seeds in the areas that were studied more regularly. The isolated area happened to be the source of seedpods used in the experiment on relative humidity (table 3). No external parasites were observed in the entire study, either on early larvae from immature seeds or on fifth-instar larvae from maturing seeds.

Tetrastichus bruchophagi Gahan was the predominant parasite in the areas that were surveyed regularly (table 18). Populations occurred at extremely high levels in the uncultivated trefoil areas, but in seed fields the numbers built up slowly in early summer. Emergences from the field-collected seedpods of 1962 and 1963 totaled 18,413 adults of *T. bruchophagi* and only 5,026 adults of *B. kolobovae*. However, the actual ratio of parasites to seed chalcids probably was somewhat lower than these figures indicate, because the parasites seemed better able than *B. kolobovae* to emerge from pods held under relatively dry conditions. Females predominated in every collection, but males were usually present also, in very low numbers, throughout the season. For the 18,413 specimens found during this study (all from *Lotus tenuis*), the sex ratio was 1 male to 43.37 females. Thus, deuterotokous parthenogenesis seems to be the usual form of reproduction in this species, at least when it parasitizes *B. kolobovae,* and this situation undoubtedly contributes to its efficiency as a parasite. Crèvecoeur (1946) reported the same parasite from trefoil seed chalcids in Belgium, where the populations likewise were composed predominantly of females. From alfalfa seed chalcids,

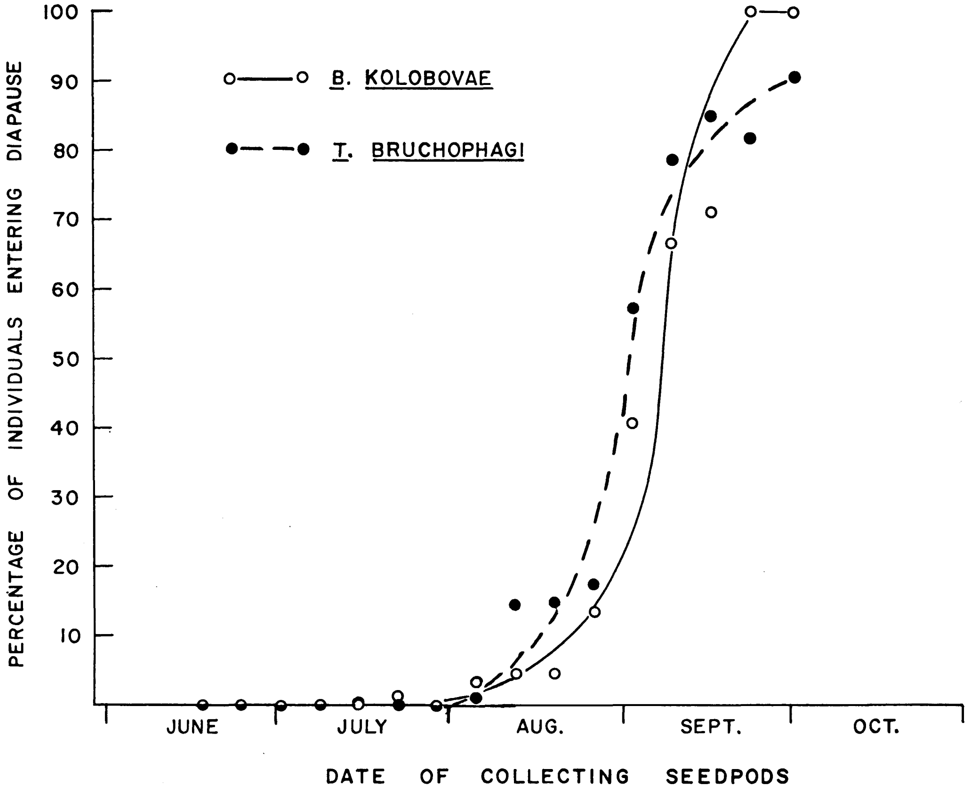

Fig. 22. Occurrence of diapause in *Bruchophagus kolobovae* and *Tetrastichus bruchophagi* in 1963, in relation to date of collecting seedpods of *Lotus tenuis*.

Urbahns (1917) found 1 male of *T. bruchophagi* to 4.94 females; in a later study he found 1 male to 6.92 females (Urbahns, 1919).

I observed the emergence of 27 larvae of *Tetrastichus bruchophagi* in two experiments, in which 82 fifth-instar larvae of *B. kolobovae* had been dissected out from maturing seeds. In most cases the host larva defecated shortly before the parasite emerged, when to all appearances the host was in diapause (overwintering stage, p. 461). The fact of host defecation at this time leads to speculation that it might be caused by mechanical disruption of the membrane between midgut and hindgut—either by increased pressure or by actual puncturing of the gut. The parasite larva was nearly as large as its host and emerged through the ruptured body wall. Usually the parasite emerged with its gut full.

Tetrastichus bruchophagi may undergo a diapause in the larval stage, similar to that of *B. kolobovae*. In the laboratory, after the full-grown parasite larva had emerged from the host larva it retained its gut contents and remained in diapause for an extended period of time. The diapause ended spontaneously, and defecation was followed by pupation. Pupation was completed in seven to nine days at 70° F, and adult emergence proceeded without further interruption.

The pattern of emergence from seedpod collections in 1963 (table 17 and fig. 22) indicated that the seasonal occurrence of diapause in *T. bruchophagi* was very similar to that of its host but with an apparent time lag at the end of the season. Nondiapausing parasites were present in late-fall collections in which all of the host larvae were diapausing. However, the proportions of these nondiapausing individuals diminished in the later collections. It is possible that some individuals in the parasite population fail to enter diapause at the end of the growing season.

Sex ratios were calculated for all of the parasites that emerged from field-collected seedpods of *L. tenuis*. Most of the specimens were obtained from the weekly seedpod collections made in 1962 and 1963. *Tetrastichus bruchophagi* is reported above. The other species occurred in very low numbers. For 138 individuals of *Eupelmus allynii* (French) the sex ratio was 11.5 males to 1 female; for 88 individuals of *Liodontomerus perplexus* it was 1 male to 1.5 females; and for 54 individuals of *Trimeromicrus maculatus* Gahan it was 1.6 males to 1 female.

CONCLUSIONS AND SUMMARY

Experiments

These investigations have given confirmatory evidence that *Bruchophagus kolobovae* is a distinct species—first by its anatomical differences from *B. roddi,* second by host specificity, and third by reproductive isolation. Females of *B. kolobovae* laid no eggs in seeds of alfalfa or bur clover, which are the important hosts of *B. roddi.* Attempts at breeding *B. kolobovae* females and males with *B. roddi* individuals of the opposite sex gave no indication that any fertile cross-mating took place. Arrhenotokous parthenogenesis occurred regularly in both species.

Five larval instars of *B. kolobovae* were distinguished by measurements of the mandibles and head capsules. Under greenhouse conditions the mean time for egg hatching was 5½ days after oviposition, larvae molted at two- to three-day intervals, and the peak of adult emergence occurred about 33 days after oviposition.

For oviposition the females of *B. kolobovae* preferred seeds of the two cultivated species of *Lotus* almost exclusively and required also seeds at a particular stage of development, within a rather narrow range. They required light for oviposition and deposited eggs at night when light was provided. The period of oviposition by individual females was quite variable in relation to their ages. The greatest number of offspring produced by one female was 56, but the potential is probably higher.

Seven females on a greenhouse plant lived an average of 25.9 days and seven males an average of 12.6 days. In a glass cage with only honey and water, 10 females lived an average of 37.3 days and 10 males an average of 20.0 days. Females outnumbered males in field collections of adults and in emergences from field-collected seedpods.

Adults of *B. kolobovae* had difficulty in emerging from very dry seeds. Nearly twice as many emerged from seeds held at 53 per cent relative humidity as from those held at 23 per cent.

Diapausing fifth-instar larvae, practically nonexistent in the field in early summer, appeared in maturing seedpods collected about the middle of August. These were the overwintering individuals. Their percentages increased markedly in each collection during the next five weeks and reached 100 per cent by late September. When seedpods were held at room temperature, emergence of adults following diapause took place irregularly and over a long period—from three to seven months after collecting. When seedpods were chilled for three months, following the emergence of all nondiapausing individuals, the emergence following diapause took place uniformly during the seventh to ninth weeks after the termination of chilling, i.e., during the sixth month after collecting the seedpods.

Parasites of *B. kolobovae,* belonging to four hymenopterous species, emerged from field-infested seedpods of *Lotus tenuis.* The important species, *Tetrastichus bruchophagi,* was very prevalent throughout the season and in emergence records outnumbered *B. kolobovae* 18,413 to 5,026. The fact that its populations are mainly females undoubtedly contributes to its efficiency as a parasite. The patterns of its life history follow those of its host and are closely synchronized with them. Most if not all of its population enters diapause in the fall, inside the seed coat but outside of the cuticle of the host larva.

Practical considerations

It so happens that the present methods of trefoil-seed production in California favor control of *B. kolobovae* and that this chalcid causes no significant crop losses, even in the more heavily infested of the seed fields studied. Cutting or grazing, continued up to mid-June, prevents early seed development. Heavy watering in July and August stimulates production of a large amount of seed over a short period of time. Early harvesting, intended primarily to prevent the loss of seeds by dehiscence from dry pods, serves also to forestall the occurrence of the overwintering stage of the chalcid—provided the seeds mature early and are harvested before the middle of August. If harvest is later than this an increasing proportion of seeds will contain diapausing larvae of *B. kolobovae,* and some of these seeds are apt to remain in the field, either because of dehiscence or as discards from the combine. In such a way, the seed fields may contribute to the carryover of seed chalcids and, though probably to a lesser extent, of their parasites.

The principal source of seed-chalcid infestation is in uncultivated roadside areas, where escaped and volunteer trefoil plants favor a population buildup. Three or possibly four overlapping generations develop there during the long growing season, and the diapausing larvae may overwinter without hindrance.

Population patterns obtained from field surveys over three summers in the vicinity of Davis, California, indicate that adults of *B. kolobovae* from overwintering populations begin to appear in infested roadside areas about the first of May. Trefoil growing undisturbed blooms and sets seed at about the same time, and these early seeds become infested promptly. As soon as seeds start to develop in the seed fields, in late June and early July, adults fly there from nearby roadside areas and start a new infestation. However, there is time for only one or two generations of seed chalcids to develop in seed fields before harvest. In trefoil pastures the situation usually shares some of the features of the other two areas. The growing season may be long, but grazing usually limits the quantity of seed. Although the seed loss itself is unimportant, a significant seed-chalcid buildup would contribute to the general problem—especially as some seeds are usually left in pasture fields for reseeding.

Females of *B. kolobovae* from seeds of *Lotus tenuis* laid eggs readily in seeds of *L. corniculatus* as well as of *L. tenuis,* and the next generation developed normally in either host. They laid no eggs in seeds of alfalfa or bur clover. In native species of *Lotus,* oviposition in the laboratory was extremely rare. Moreover, no native species of *Lotus* and no species of *Medicago* were found infested by *B. kolobovae* in the field. Hence, only the two cultivated species of trefoil appear to be significant in the biology or control of *B. kolobovae.*

Larvae of *B. kolobovae* may be consumed by parasitic larvae—especially those of the chalcid *Tetrastichus bru-*

chophagi, which attacks *B. roddi* also. The parasites outnumbered the seed chalcids in emergence from field collections, and it is obvious that they must exert a very appreciable control over the populations of *B. kolobovae,* especially in uncultivated areas. Although parasites do not prevent a considerable buildup in numbers of seed chalcids during a summer, they undoubtedly keep a biological balance from year to year.

In terms of controlling the trefoil seed chalcid in seed fields, a grower should manage his crop so that seeds are set early and over a minimum period of time. He should harvest as early as possible, preferably by mid-August, both to avoid chalcid buildup with seed loss in the present crop and to prevent the development of overwintering larvae. If further control measures should become necessary, the reduction of volunteer trefoil plants in uncultivated areas may be helpful. However, this measure carries a considerable risk unless allowance is made for the imporance of the parasites, which overwinter mostly in uncultivated areas and make their early buildup there. If widespread destruction of the roadside plants were possible, it would probably eliminate most of the parasites that now hold down the populations of *B. kolobovae.* The few remaining seed chalcids might build up very destructive infestations before their natural enemies could return to check them.

Bruchophagus kolobovae is a potential economic pest and a threat to trefoil seed production. It might become important if the present balance should be changed in its favor. However, the total losses are minor at present, because of the cultural control obtained by the commercial seed-growing methods in current use and also because of the very effective action of parasites.

It is suggested that some of the principles and cultural measures that have kept the trefoil seed chalcid under reasonable control might be investigated for their possible application to seed production in alfalfa, where losses caused by the related species *B. roddi* are great and where no satisfactory control measures have been developed.

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