tory, however, yielded no parasites, presumably because the parasitized aphids had migrated and disappeared into crevices. The infestation completely disappeared within 10 days.

Although the early artificial infestations had been unattended by ants, by the middle of May an increase in the highly localized ant-attended natural infestations occurred. The increase in ant-attended natural infestations was correlated with a gradual seasonal increase in daily temperatures and rainfall, the daily temperature range being from 37° to 70° F. during March and 80° to 90° F. during July.

An ant-attended natural infestation of the black citrus aphid was noted May 11 on two "water sprouts" on a lemon tree. A sticky barrier was placed around the base of each sprout, and during the next 48 hours this infestation was observed to be visited by two adults of a yellow braconid (probably *Trioxys*), and two species of probable hyperparasites, a black and a yellow cynipid.

As the season advanced with increasing temperatures and humidities, the proportion of hyperparasites and predators in relation to the primary parasites increased. Three species of coccinellids were observed feeding on the aphid populations. This relation appears to be correlated with an increase in the honeydew-collecting activity of ants. Hyperparasites such as *Aphidencyrtus*, and predators such as lacewing larvae, are less readily disturbed by the ants attending the aphids than are the primary parasites. It is noteworthy that ants were observed to attend the predatory lacewing larvae in the same manner that they attended the aphids.

Ants tend to perpetuate aphid colonies by cleaning up the honeydew in which young aphids may be trapped. They were also observed to collect the cast exuviae of aphids and place them together in "refuse dumps."

The new growth on tangerine is generally free of aphids. However, several natural colonies of the black aphid, which were observed on tangerine June 20, were soon eliminated by the action of the slate-grey "pill-bug" larvae of *Cryptogonus orbiculus* (Gyll.) and the jumping larvae of a cecidomyid. It is noteworthy that an adult of a minute blood-sucking fly was observed feeding on aphids.

Predation by the larvae of *Cryptogonus orbiculus* appears limited to aphids. They were found associated with the sugarcane aphid, *Oregma lanigera* Zhnt. (Lopez 1931). The species is oriental ranging from Ceylon to Japan (Kapur 1948). During the University of California Department of Biological Control 1948–51 program of introducing from China into California the natural enemies of citrus pests, four of the shipments contained live specimens of *C. orbiculus*.

Thirty shipments of parasitized citrus aphids were shipped by air mail from China to California during the period of February 1954 to May 1955. The junior author received these shipments and reared out of them the following endoparasites: 110 *Aphelinus chaonia* Walk., 250 *Aphelinus* sp. (near *toxopteraphidis* Kurdj.), 21 *Trioxys* sp., and 15 *Lipolexis* sp. One parasitized aphid collected in April 1955 from citrus at Shatin, N. T., yielded a primitive undescribed species of Aphidiinae. (The writers are indebted to their colleagues Harold Compere and E. I. Schlinger, and to C. F. W. Muesebeck, for the identification of the aphid parasites.)

The specimens of *Lipolexis* consisted of 9 females and 6 males. These reproduced for one generation on nearly mature *Aphis gossypii* infesting squash seedlings. On this host the life cycle was 14 days at temperatures between 78° and 80° F.

BIOLOGY OF *Aphelinus chaonia* WALK.—*A. chaonia*, which attacks the black citrus aphid, is a biparental species similar in appearance and habit to *A. mali* Hald. In California this species was propagated on *Aphis spiraecola* Patch., an important pest of citrus in North and South America. In the life-history studies of *Aphelinus* conducted in China, the artificially infested citrus branches were removed and the aphid infestations subjected to parasitization in the laboratory. The female readily oviposits in immature aphids and between ovipositions feeds on their body fluids. At 70° F. oviposition requires about 1 minute; the host-feeding process requires about 3 minutes for puncturing and about the same time for feeding (ingesting).

When about to oviposit, the parasite slowly approaches an aphid and takes a stance a short distance from it with antennae folded vertically to the plant surface. After a few seconds the parasite reverses its position, bends the distal half of its wings upward and forward against the other half, extends its ovipositor horizontally, and inserts it in the undisturbed aphid. During the entire oviposition process, the parasite touches the aphid only with its ovipositor. Seven days after the eggs are oviposited, the larval progeny are full-fed, completely filling the body cavity of the aphid. It is prior to attaining this condition that the aphid migrates, becoming negatively phototropic and positively thigmotropic. The full-fed larva of the endoparasite secretes a substance that fastens the blackened host body to the substrate.

Aphelinus sp. (near *toxopteraphidis*) has a similar biology and was produced in California in considerable numbers on *Myzus persicae* infesting potato sprouts. Propagation in the insectaries at Riverside and Albany from 1954–57 resulted in the colonization of 250 *Aphelinus chaonia* in Orange County against *Aphis spiraecola* on citrus, and 11,800 *Aphelinus* sp. (near *toxopteraphidis*) in Monterey and Ventura counties against *Myzus persicae*, *Aphis spiraecola*, and *Toxoptera aurantii*.

Proper evaluation of effectiveness of these colonizations was precluded by subsequent heavy incidence of disease among the aphids.

Six of the shipments from New Territories included 300–400 specimens of *Aphidencyrtus* sp. A note sent with the first shipment stated that this species might be secondary in habit. Subsequent tests under quarantine showed that such was the case. *Aphidencyrtus* oviposits in the live aphid while standing on its back "head to tail." The ovipositor is inserted in the *Aphis* for about 1 second. The full-fed hyperparasite fastens the aphid to the substrate and covers the inner wall of the aphid's body with a white silk. Usually, in the process of spinning this cocoon the body of the aphid is ruptured. The minimum life cycle of this hyperparasite at 80° F. is less than 18 days, that of its host (*Aphelinus* sp.) is 15 days.

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A Chalcid[1] Parasitizing Spotted Alfalfa Aphids and Greenbugs in Kansas[2]

R. G. SIMPSON, C. C. BURKHARDT, F. G. MAXWELL and E. E. ORTMAN,[3] *Kansas State College, Manhattan*

Rapid and widespread infestation following the establishment of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in the United States in 1954 has emphasized the necessity for in-

[1] *Aphelinus semiflavus* Howard, Family Eulophidae.

[2] Contribution No. 729, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. Accepted for publication February 25, 1959.

[3] Graduate Research Assistant, Assistant Professor, Graduate Research Assistants, respectively. Kansas State College, Manhattan.

creased investigations in biological control. The Agricultural Research Service of the United States Department of Agriculture and the University of California have been cooperating in a continued search for natural enemies in foreign countries, especially parasites of the aphid, and have been rearing them in large numbers. Mass parasite production of *Aphelinus semiflavus* Howard and other parasite species was initiated at Albany and Riverside, Calif., and at Moorestown, N. J.

The *A. semiflavus* released in Kansas originated in Israel and France, but were reared and shipped from the laboratory at Moorestown, N. J.[4] A total of 55,250 individual parasites was released in east-central and north-central Kansas, specifically: two sites in Riley county; three in Geary county; and one in Marion county. Releases were at eight different intervals from May 25 to June 18, 1956. A few specimens of *A. semiflavus* were recovered from the release sites during the fall of 1956.

Previously, however, this chalcid was collected as a native insect in Kansas. The hosts were listed as: *Anuraphis viburnicola* (Gill.); *Capitophorus rosarum* (Kltb.); *Chaitophorus viminalis* Monell; *Macrosiphum cornelli* Patch; *M. granarium* (Kby.); *M. sanborni* Gill.; *Myzus persicae* (Sulz.); *M. sp.* on *Aquilegia; Rhopalosiphum poae* (Gill.); *R. pseudobrassicae* (Davis); and *Toxoptera graminum* (Rond.) (Muesebeck *et al.* 1951).

Field collections of the spotted alfalfa aphid used for greenhouse cultures and later for biological studies became parasitized by *A. semiflavus* during midJuly, 1958. By August 17, the cultures were severely threatened by the chalcids. Collections were identified by B. D. Burks of the United States Department of Agriculture Insect Identification and Parasite Introduction Laboratories. It was stated that these specimens could not be associated, with certainty, with either the eastern North American or the Middle East strains. The collection contained both macropterous and brachypterous forms. These forms were previously described by Gahan (1924).

A second group of parasites on cultures of spotted alfalfa aphids was collected from another greenhouse September 16. These specimens contained *A. semiflavus* as well as an Encyrtid wasp, *Aphidencyrtus aphidivorus*. Burks stated that the latter species was thought to be a secondary parasite. According to Muesebeck *et al.* (1951) this species has not been reported previously from Kansas.

Cultures of the greenbug, *Toxoptera graminum* (Rond.), in an insectary had become heavily parasitized by *A. semiflavus* during September and October. The parasites presumably had been introduced by parasitized spotted alfalfa aphids on alfalfa cuttings.

The above three infestations gradually increased in their respective greenhouses and insectaries and later threatened to completely destroy both spotted alfalfa aphid and greenbug cultures.

It is thought that the species of *A. semiflavus* native to Kansas is of the eastern North American strain. At present authorities are unable to differentiate between the eastern North American and the Middle Eastern strains. Therefore, there is no positive way of knowing whether the infestations reported here were caused by the native species or by a buildup of the released species of the parasite.

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[4] The efforts and assistance of James K. Holloway, Albany, California, and D. W. Jones, Moorestown, New Jersey, are appreciated.

Rapid Collection of Insect Blood[1]

JAMES STERNBURG and JOHN CORRIGAN, *Department of Entomology, University of Illinois, Urbana*

The collection of blood from insects for biochemical and physiological studies has always been a time-consuming process. Withdrawing blood by means of capillary tubing yields, at best, microliter amounts and only a fraction of the total volume present can be removed. Thus, manual expression of blood from the cut ends of antennae and legs of American cockroaches (*Periplaneta americana*) (L.)) was used by Sternburg & Kearns (1952). The blood was frozen as it was collected to prevent clotting and darkening. By this method one man could collect about 2 ml. of blood a day at the rate of 50 female or 100 male American cockroaches per ml.

During an investigation of biochemical changes in the blood of DDT-poisoned insects, it became necessary to collect comparatively large samples of cockroach blood. For this purpose a method was developed involving low speed centrifugation of cockroaches which has the advantage of rapidly yielding a large percentage of the hemolymph present.

To prevent contamination of the blood by the contents of the digestive tract, the mouth and anus must be sealed. This is done by dipping the lower part of the head and the tip of the abdomen into melted paraffin at a temperature between 65° and 100° C. The paraffin solidifies quickly around the mouth parts and the terminal abdominal processes and, due to the irregular contours of these structures, adheres to form an effective seal. One dipping is sufficient since it is unnecessary to build up a heavy layer of paraffin. Following sealing of the body openings each leg is severed across the coxa, the antennae clipped short and the cockroach placed head down upon a perforated disc inserted about 40 mm. below the top of a centrifuge tube. One tube can accommodate 8 to 10 cockroaches at a time. The tubes are placed in a centrifuge and spun for 5 to 10 minutes. The blood is thrown down through the disc as a clear serum with the cells massed in a layer on the bottom of the tube. The cell-free serum can be decanted off or, by gentle swirling, the cells can be resuspended in the serum.

The centrifuge used was an International Clinical model, with a four-place angled head holding four 50-ml. metal shields. The tubes were 40-ml., conical, ungraduated Pyrex brand glass centrifuge tubes with a heavy wall and an inside diameter of 25 mm. The centrifuge setting for optimal yield of blood was about 2200 r.p.m., giving a relative centrifugal force of about 650 X gravity. At this setting most of the blood was collected after 5 minutes, although we usually centrifuged 10 minutes to insure sedimentation of the hematocytes. Twenty adult females gave about 1 ml. of blood while 20 adult males yielded about 0.5 to 0.7 ml. of blood.

The tubes used for blood collecting were prepared in the following manner. The tubes were softened by heating them in an oxygen-gas flame and four slight indentations were made with a pointed tool at equal distances around the tube wall and about 40 mm. from the top of the tube. These concave impressions served as supports for a plastic disc, through which small holes were drilled to allow blood to run into the tube.

By using a six-place angled head with six heavy duty, 12-ml., conical graduated Pyrex brand glass centrifuge tubes, it was possible to collect blood from individual cockroaches. The 12-ml. tubes were prepared in a different manner than the larger ones, by holding them horizontally and rotating them evenly in an oxygen-gas flame placed about 40 mm. from the top of the tube. The tube wall softened and gradually thickened, forming an internal constriction of uniform circumference. Careful manipulation prevented any change in the shape of the external tube wall. A small perforated plastic disc was placed in the tube and

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