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UNIVERSITY OF CALIFORNIA DIVISION OF AGRICULTURAL SCIENCES

I. The genus *Coccophagoides* (Hymenoptera: Aphelinidae) is revised herein and two new species are described. These are *Coccophagoides comperei* Doutt and *Coccophagoides utilis* Doutt. Both *C. utilis* and another new species, *Anthemus inconspicuus* Doutt (Hymenoptera: Encyrtidae), are primary parasites of *Parlatoria oleae* (Colvée). They were collected in Pakistan and have been imported to California to control this olive pest.

II. *Coccophagoides utilis* is arrhenotokous. The females develop as internal primary parasites of *Parlatoria oleae* whereas the males develop adelphoparasitically on the prepupal and pupal stages of their own species. A significant aspect of the life history of *C. utilis* is a mechanism of retarded development in certain female progeny which ensures that the males on emerging meet with females.

(Continued, inside back cover.)

THE AUTHORS:

R. L. Doutt is Professor of Biological Control and Entomologist in the Experiment Station, and Chairman of the Division of Biological Control, Berkeley. S. W. Broodryk is Entomologist at the Plant Protection Research Institute, Pretoria, Republic of South Africa. C. E. Kennett is Associate Specialist in the Experiment Station, Berkeley. C. B. Huffaker is Professor of Entomology and Entomologist in the Experiment Station, Berkeley. G. L. Finney is Specialist in Biological Control in the Experiment Station, Berkeley.

S. W. Broodryk and
R. L. Doutt

II. The Biology of *Coccophagoides utilis* Doutt (Hymenoptera: Aphelinidae)[1]

INTRODUCTION

IT WAS necessary to conduct detailed investigations into the biology of *Coccophagoides utilis* Doutt because nothing was known previously of the developmental habits of any member of this genus, and manipulation to obtain the utmost efficiency of a natural enemy requires that certain aspects of its biology be well understood.

When these studies were started, *Coccophagoides utilis* was being produced in large numbers in the insectary of the Division of Biological Control, University of California, Albany, for colonization and establishment in the olive groves of the San Joaquin Valley. The natural host, *Parlatoria oleae* (Colvée), was used as the insectary host and was reared on potato tubers according to a method described by Finney (1966).

Male and female parasites of all ages were freely available from this insectary stock throughout the duration of this work. These were generally used except where specialized conditions required the employment of other techniques and the study of individuals with specifically known histories. Some aspects of the biology were studied in the insectary, where the temperature was maintained at 79° F ± 1° and the humidity at 67 per cent. Illumination in the insectary was continuous and was provided by warm white fluorescent tubes.

In some studies, where particularly constant temperatures were required, two thermostatically controlled cabinets were used. The inside dimensions of these cabinets are: length, 35 inches; width, 28 inches; and height, 23½ inches. Heating is provided by an element built into the side of each cabinet, which heats the air before it is fed into the cabinet by a fan. Both cabinets share the same refrigeration system, which comes into operation automatically whenever required. These cabinets maintained a high degree of accuracy, and one was kept at 68° F ± 0.5° (cabinet A), while the other was kept at 76° F ± 0.5° (cabinet B).

Humidity was provided by placing two large enamel pans with water inside each cabinet. In cabinet A the relative humidity was kept at 72 per cent ± 5, while it was kept at 55 per cent ± 5 in cabinet B.

No illumination was provided in addition to the indirect sunlight entering the room.

Besides the cabinets and the insectary itself, three bioclimatic chambers, primarily in operation for studies on *Therioaphis maculata* (Buckton), were also used in this work. The description of this equipment is as follows (Flitters and Messenger, 1953):

The chambers are of the walk-in type, of sectional construction and heavily insulated with fiberglass and plywood.

[1] Submitted for publication October 6, 1964.

TABLE 1

CONDITIONS MAINTAINED IN THE BIOCLIMATIC CHAMBERS DURING STUDIES OF *COCCOPHAGOIDES UTILIS* DOUTT

Chamber	Temperature*			Rel. humidity		Photoper. hrs.
	Night	Day	Av.	Night	Day	
	°*F*	°*F*	°*F*	*per cent*	*per cent*	
I	40	62	51	90	40	12
II	50	70	60	90	40	12
III	60	90	75	90	33	12

* Lowest temperatures were reached at 6:00 A.M., and the peaks at 2:00 P.M. to 3:00 P.M. daily.

The interior is lined with stainless steel, and all the seams are sealed. The inner chamber is 6 feet square and 6½ feet high, giving a working space of 36 square feet. One refrigerator-type door, of similar construction to that of the walls, opens outward from the chamber into an anteroom. The anteroom measures 4 feet square, inside dimensions, and the height and construction are the same as those of the main chamber. The anteroom permits entry without influencing the conditions being maintained within.

Temperature and humidity are controlled by means of cams, which through tracer points transmit to the control system the exact conditions required inside the cabinets. Each cabinet is capable of controlling temperature from −5° F to 125° F with an accuracy of ± 1° F on a dry-bulb thermometer. Relative humidity can be controlled through a range of 20 to 95 per cent at temperatures from 35° F to 125° F. All chambers have sufficient capacity to raise the temperature 40° F and to raise or lower the relative humidity 60 per cent, each within 60 minutes.

A blower circulates fresh air through the chambers at the rate of 20 cubic feet per minute and the air flow can be controlled by dampers.

Light is provided by white fluorescent tubes and infrared incandescent lamps, and the photoperiod is regulated by time clocks.

Conditions prevailing in the bioclimatic chambers during the work on *Coccophagoides utilis* are shown in table 1.

The method of studying the life history of *C. utilis* was as follows: Potatoes infested with scales, seven to nine weeks old, were exposed for six hours in a battery jar to a heavy density of parasitoids from the insectary stock. After exposure, the potatoes were kept in the battery jars in the insectary (temperature 79° F ± 1°) for the duration of the development time required by *C. utilis.* From the first day after oviposition, host scales were dissected daily in order to follow closely the development of the parasites.

The individuals do not all mature at the same rate, for a certain portion of the population was found to be retarded in development. Because of this phenomenon, average developmental times are apt to be misleading. Therefore the figures given in this paper represent the time elapsed between one developmental stage and the first appearance of individuals in the next stages of development.

Microscopic observations were made by means of wet mounts in distilled water; and where cover glasses were used, they were always supported with fragments of glass obtained by breaking a second cover glass.

LIFE HISTORY OF *Coccophagoides utilis*

The egg stage. *Coccophagoides utilis* is an arrhenotokous species, the male of which develops only as a hyperparasite on the subterminal immature stages of its own species.

The unmated female lays eggs which give rise to male progeny only. She lays these eggs on prepupae and pupae of her own species, and each such male-producing egg is secured to the outside of the host's integument by means of a stalk or pedicel (fig. 1). The egg is elongate, with both ends equally rounded; it is translucent, and the stalk is generally less than one third as long as the egg itself. At 36 to 46 hours after oviposition, the average dimensions of 10 male eggs were: length 0.144 ± 0.0082 mm, and width 0.049 ± 0.0065 mm.

The first male eggs hatched after six days under insectary conditions, and towards the time of eclosion the white tissues of the developing larva could be seen.

The mated female lays eggs which produce female progeny only. The eggs in this case are deposited in the body fluids of the scale *Parlatoria oleae,* usually at the rate of one egg per scale. The female-producing egg at time of oviposition is generally elongate (fig. 2), with both ends equally rounded, but infrequently it may either be thickened equatorially or tapered toward one of the poles. It is translucent, and the contents appear to be granular. From oviposition to the time of hatching, the egg shows a considerable increase in size (table 2).

The first female eggs hatched after seven days under insectary conditions.

In about 2 per cent of the eggs inspected from mated females, there occurred a well-developed pedicel. These eggs were found in the body fluids of the host scales, and unfortunately their further history could not be followed.

Dissection of the ovaries of unmated females on three occasions yielded two to three eggs of uncommonly large dimensions. The average length of six such eggs was 0.221 mm, and the average width was 0.066 mm. The significance of these eggs is not clear.

The larval stage. The number of molts in larvae of both sexes was determined by means of daily observations on the development. At least 10 scales were dissected daily; and when 70 per cent or more of the larvae inspected were found to be molting on that particular day, this was considered to be representative of the population. Three definite waves of molting were observed in the female larvae, while the male larvae showed only two waves. A possible error exists, however, because of the differences in the individual rates of development.

The female first-instar larva (fig. 3) floats free in the host body fluids. The body is semitranslucent white, and the gut is not discernible before the larva starts feeding. The head is well developed, and the thorax forms the widest part of the body. The abdomen tapers caudally to a bluntly rounded apex. The integument over the whole body is wrinkled except in the head region. Segmentation is not clear. External spiracles do not occur, although the two lateral tracheal trunks with one anterior commissure are well developed. The body is slightly curved ventrally. Cendaña (1937) considers larvae of this description to have the typical coccophagine larval form.

The first-instar female larval stage lasts three to four days, and at this time the host may or may not show the first visible reaction to the larva in the form of an accumulation of opaque granular material under the integument in the median region.

The second-instar female larva has the typical scarabaeoid body shape evident in many hymenopterous larvae. This instar lasts three days. The third-instar larva has the same body shape,

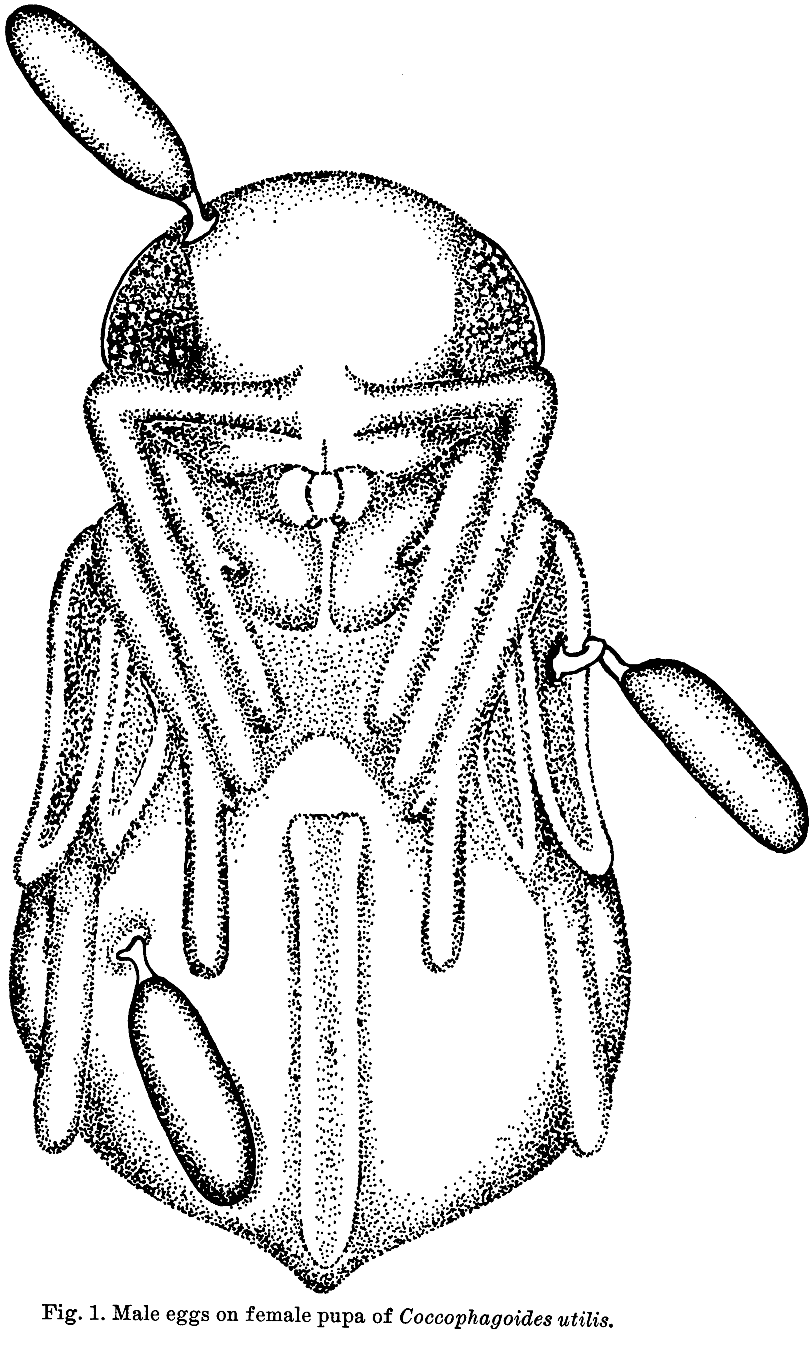

Fig. 1. Male eggs on female pupa of *Coccophagoides utilis.*

and molts within two days to become a fourth-instar larva.

During the course of these events the scale contents turn completely opaque-white. The integument is at first colorless and transparent, and the parasitoid is visible inside. Then it turns a light brown color. Toward the end of the larval stage, the host integument turns from light brown to progressively deeper hues of brown, but remains transparent to some degree.

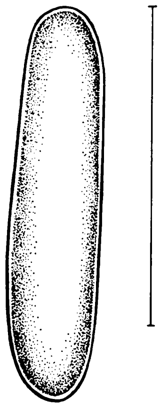

Fig. 2. Female egg of *Coccophagoides utilis*.

TABLE 2
INCREASE IN SIZE OF FERTILIZED EGGS OF *COCCOPHAGOIDES UTILIS* FROM OVIPOSITION TO TIME OF HATCHING

Egg age	Length	Width	No. measured
	mm	*mm*	
6 hours..	0.1231 ± 0.0039	0.0243 ± 0.0000	20
48 hours..	0.1387 ± 0.0077	0.0418 ± 0.0034	20
192 hours..	0.2487 ± 0.0195	0.0628 ± 0.0043	20

The fourth-instar female larva (fig. 4) is curved ventrally and is almost as long as the longitudinal axis of its host scale. The body width is about one-third of the length. The head is to some extent retractable into the first thoracic segment, and the mouthparts are extremely difficult to distinguish. Apart from the head, there are 13 body segments. The tracheal system is well developed, and spiracles occur on segments two through ten. The alimentary canal is filled with brown host material. The integument is transparent and shiny.

The larva in this instar will complete the consumption of the scale tissue, so that only the brown host integument is left and the larva remains in this shell to become a prepupa. Where scale eggs inside the host have attained a certain point in their development, the larva is not able to consume them, but these soon become desiccated and die.

The fourth and final female instar larval stage lasts two to three days, at the end of which the meconium is voided and a prepupa is formed. When the full-grown larva is ready to void the meconium, it comes to rest lengthwise on the longitudinal axis of the scale. The head may be either cephalad or caudad with reference to the host body. The abdomen is swung to one side, and half of the material is deposited; then the abdomen is turned to the opposite side, and the remainder of the material is deposited. The meconial substance is ejected in small clumps and consists of dark-brown granules in a viscous dark fluid. Two days after voiding, the fluid has dried away, and the meconium now is represented by three or more hard, dark-brown pellets lying on each side of the pupa against the lateral scale integument.

As soon as the meconium has been voided, most of the larval characteristics are lost and a prepupa is formed, which lies with its ventral aspect toward the scale's dorsum. Within 24 to 36 hours the prepupa becomes a pupa.

The male larva develops as an ectoparasite on its host. After eclosion it

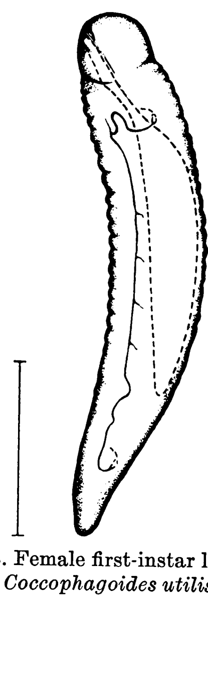

Fig. 3. Female first-instar larva of *Coccophagoides utilis*.

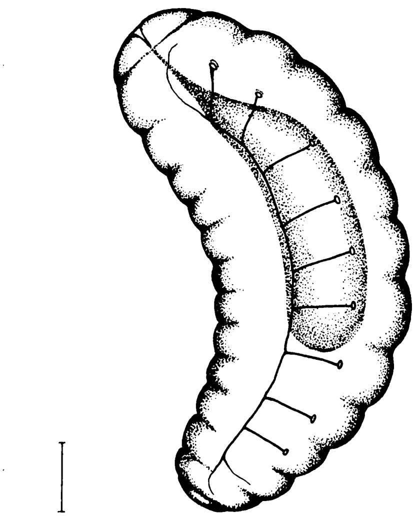

Fig. 4. Female fourth-instar larva of *Coccophagoides utilis.*

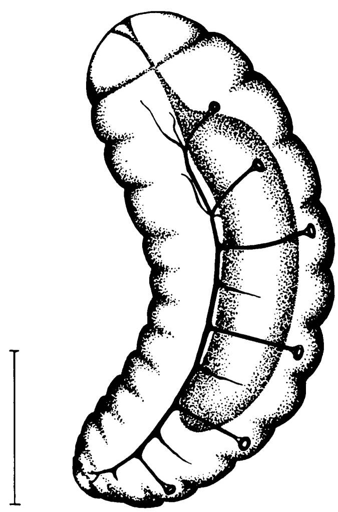

Fig. 5. Male final-instar larva of *Coccophagoides utilis.*

feeds in the immediate area where the egg was placed, but as it grows larger the larva feeds its way into the host tissue. The gut is filled with white material and the rest of the body is transparent to opaque white. The host tissue is also white, and this makes it difficult to distinguish the feeding larva.

Only two larval molts were observed, but the possibility of a third is not excluded. The first-instar male larva resembles its female counterpart in general features. This instar lasts three days, and, after molting, a larva with the scarabaeoid body shape appears. The second instar lasts three days, and the third instar two days.

The full-grown male larva (fig. 5) is almost as long as the longitudinal axis of the host, and its width is about one-third of the body length. The head is distinct, slightly retractable and is followed by 13 body segments. A well-developed tracheal system is present and the spiracles are found on segments II, III, IV, VI, VIII, and X. The spiracles are heavily sclerotized and are situated in the dorsoventral area.

The contents of the alimentary canal are light in color, and consequently the meconium is of a lighter color than that of the female. The presence of two sets of meconia inside the scale shell signifies the development of a male.

After the meconium has been voided, the prepupa appears, and within 36 hours gives rise to the pupa.

The pupal stage. The newly formed female pupa (fig. 6) is off-white; and as the time of emergence approaches, sclerotization of the body gradually proceeds. The female pupal stage lasts eight days, and at emergence the body is fully sclerotized.

The average length of 10 female pupae is 0.7875 ± 0.1625 mm, and the average width is 0.4725 ± 0.0207 mm.

The pupa lies with the ventral aspect facing the host's dorsum. The egg-laying apparatus is clearly visible on the abdomen. A female, when ready to emerge, pierces the scale integument with her mandibles and increases the size of this hole until it is large enough for the body to pass through.

The male pupa (fig. 7) is slightly smaller than the female pupa. The abdomen is slender and tapers caudally, but in most other respects the male pupa closely resembles that of the female. The male pupal stage lasts 12

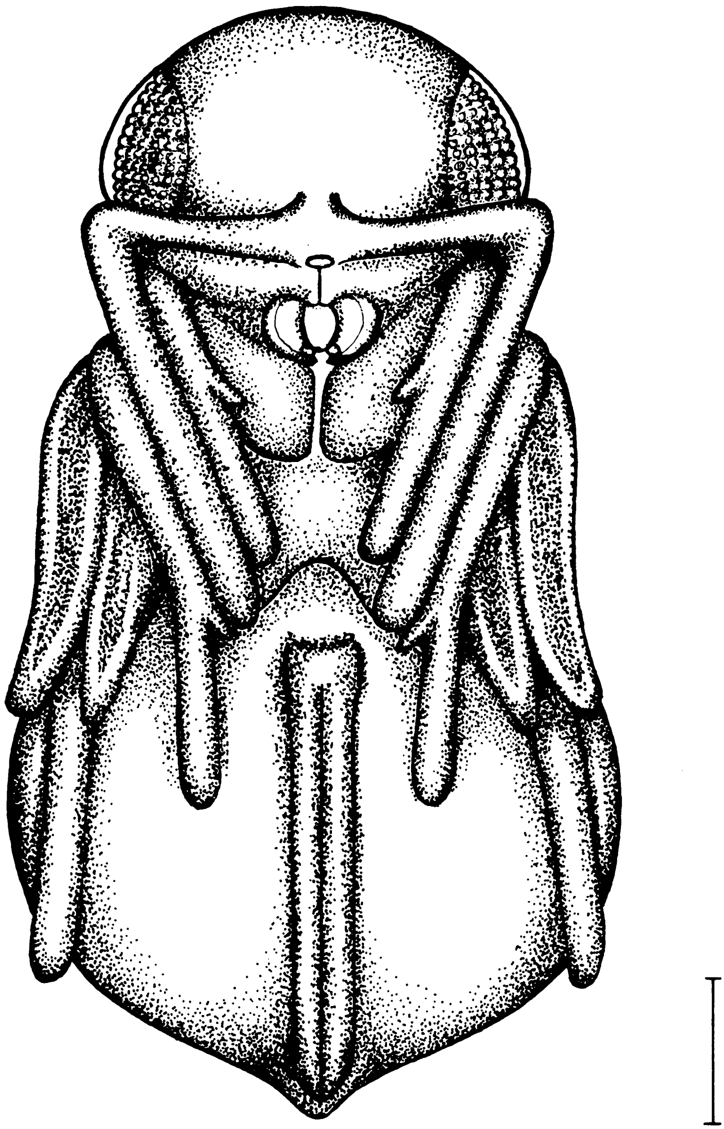

Fig. 6. Female pupa of *Coccophagoides utilis.*

days, after which the male emerges by making a round hole in the host's integument with its mandibles.

The adult stage. The adult female emerges 26 days after oviposition under insectary conditions. The average length of 20 females measured was 0.651 mm, the individual dimensions ranging from 0.500 to 0.800 mm. This stage has been described and its dimensions figured by Doutt (1966). The male is smaller than the female, for the average length of 20 males measured was 0.585 mm, and the individual range was from 0.437 mm to 0.650 mm.

The influence of temperature on development. *Coccophagoides utilis* is exposed to a wide range of temperatures in the San Joaquin Valley of California, as well as in the locality (Parachinar, Pakistan) from which the stock was originally obtained. The effect of temperature on its development was tested by exposing olive scales on po-

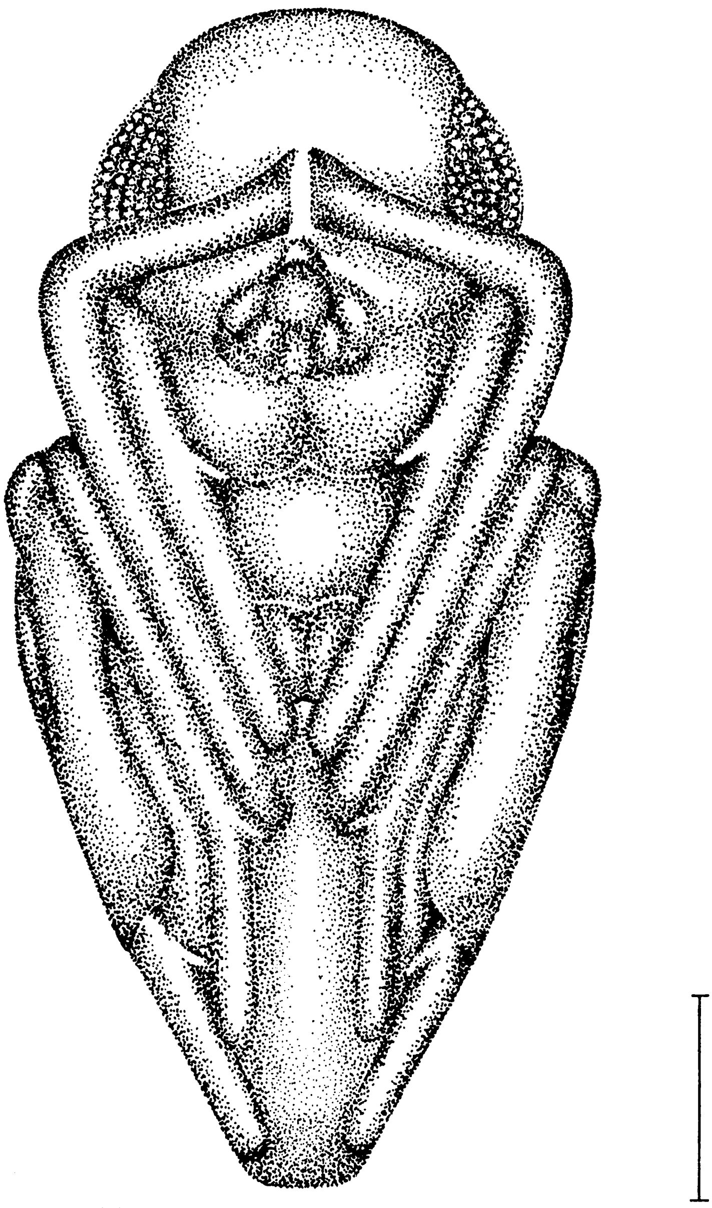

Fig. 7. Male pupa of *Coccophagoides utilis.*

tatoes to a heavy density of parasitoid adults for 24 hours at various temperatures. The adults were then removed, and the host scales were held at the test temperature. The developmental time for females was taken as the number of days elapsed from oviposition to the first emergence from the host scales. The male developmental time was taken as the time elapsed between the emergence of the first females and the first emergence of males.

Three development tests at each of the following temperatures were run: 51° av.; 60° av.; and constant temperatures of 68°, 76°, and 80°F. The two lowest figures represent the mean between the highest and lowest points in bioclimatic chambers I and II, respectively, where tests took place at fluctu-

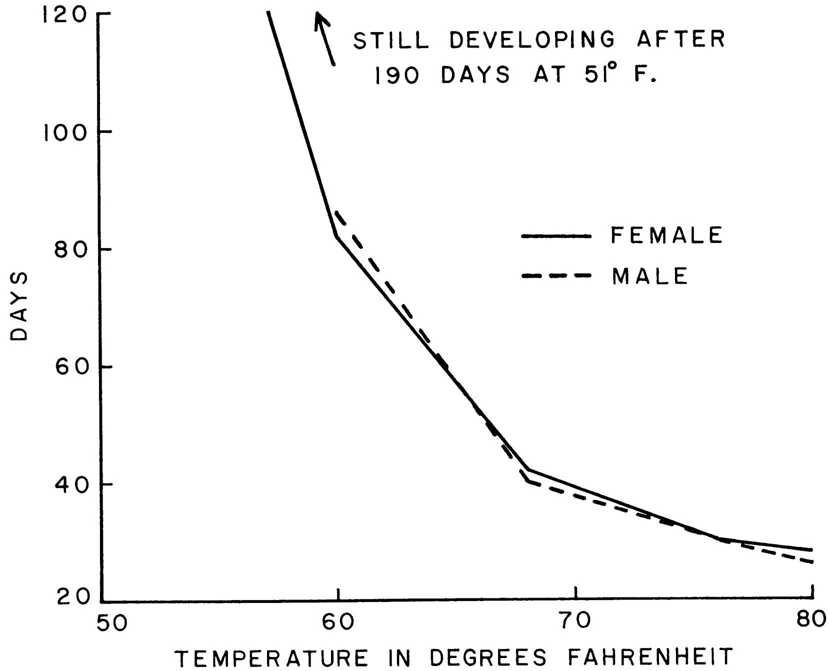

Fig. 8. Average developmental period for males and females of *Coccophagoides utilis.*

ating temperatures. The other tests were run at constant temperatures: those at 68°, and 76° in cabinets A and B; the test at 80° in the insectary.

The average developmental time at each temperature for both male and female *C. utilis* is given in figure 8.

The response to temperature appears to be the normal one of accelerated development at high temperatures and slower development at lower temperatures. The male and the female parasites respond in the same way.

At the temperatures maintained in bioclimatic chamber I (high 62°F, and low 40°F; av. 51°F), development is extremely slow; but it is not yet at a standstill after 190 days, as demonstrated by the population distribution (table 3). There is also at this low temperature no differential mortality between the host and its parasite.

The host at this low temperature (51°F) did not show any ill effects, even after prolonged exposure. Egg production by the hosts continued, but none of the eggs hatched, a factor which led to extremely crowded conditions under the scale armor.

The influence of host age on development. A range of host ages was obtained by infesting one potato per day with olive scale crawlers for 32 consecutive days. On the thirty-third day all the scales were exposed to a heavy density of parasites for 24 hours; next, each potato was put into a separate glass jar, which was then closed with fine organdy mesh. The scales were kept under insectary conditions for observation (temperature 79°F ± 1°), and from 25 days after oviposition until the end of this experiment each potato was inspected every three days for parasite emergence.

The number of days from oviposition to first emergence from scales of each age was then plotted on a graph ordinate, and the scale age was plotted on the abscissa (fig. 9).

According to the information thus obtained, the female parasite may attack the host scale within 24 hours after the scale has ceased the typical "crawler"

TABLE 3
AGE DISTRIBUTION OF PROGENY OF MATED FEMALES OF *COCCOPHAGOIDES UTILIS* DOUTT SHOWING SLOW DEVELOPMENTAL RATE AT LOW TEMPERATURES

Stage of development	Distribution (per cent)	
	Bioclim.ch.I (Av. 51° F)	Bioclim.ch.II (Av. 60° F)
Parasitoid visible, scale not discolored	5.7	15.0
Scale discolored but contents still fluid	34.8	35.5
Scale contents dry or consumed, meconium not yet voided	7.2	8.5
Prepupa	19.5	10.5
Pupa	32.8	26.5
Adults emerged	0	4.0

activity and settled down. Furthermore, development of the parasite in scales younger than 12 days is delayed. The maximum extent of the delay is about 12 days, and in later dissections of young scales the delay apparently occurs in the first larval instar.

Host scales between 13 and 32 days old give rise to adult parasites 35 to 38 days after oviposition, and within this range of host age there are no differences.

Three development trials similar to the one described above (see page 241) were run at 76°F ± 0.5°F, with scales of the following ages: 2 days, 1 week, 2, 3, 4, 5, 6, and 7 weeks. The average results of these three tests were plotted (fig. 10). The data again showed that the host under 14 days of age was attacked but the development of the parasite was retarded. There is, however, little difference between the developmental times of the parasite in scales from two to seven weeks old.

It can be concluded that the host age above 12 days does not influence the development of the parasite significantly. If the host is attacked on or before the twelfth day after settling, parasite development is delayed and the adults emerge 10 to 12 days after those from the older scales at the temperatures tested.

The development of the male parasite did not show any differences when females of different ages were used as hosts in the insectary. The host in this case, however, is susceptible to attack for a period of less than 14 days. This fact could possibly account for the absence of host age effect in the development of the male.

The influence of host sex on development. Emergence observations in the insectary demonstrated the fact that scales of both sexes could serve as hosts for *Coccophagoides utilis*. This aspect was further investigated by exposing scales of known ages to parasites and noting the host sex when emergence eventually takes place.

Two potatoes were used in each age class of scales. The age classes were: 9, 11, 13, 15, 17, 21, 25, and 29 days. All the scales were exposed on the same day to a heavy density of parasites for 24 hours in the insectary, after which each potato was put into a separate glass jar closed with fine organdy mesh. The jars with potatoes were then transferred to a constant-temperature cabinet maintained at 68°F ± 0.5°F. After 35 days, daily checks were made for parasite emergence. The number of adults emerged and the host sex from which they emerged were recorded. The results of this test are presented in figure 11.

The emergence data indicate that emergence from scales up to 17 days old at time of oviposition is almost exclusively from male scales. The male scale insect develops rapidly and is ahead of its female counterpart. This may be the reason why the early male host is chosen in preference to the early female stages for oviposition.

When the host scales are 21 days old, considerably more females are selected than at scale ages below 18 days. In this case also, the development of the parasite is more rapid in the male scale than in the female.

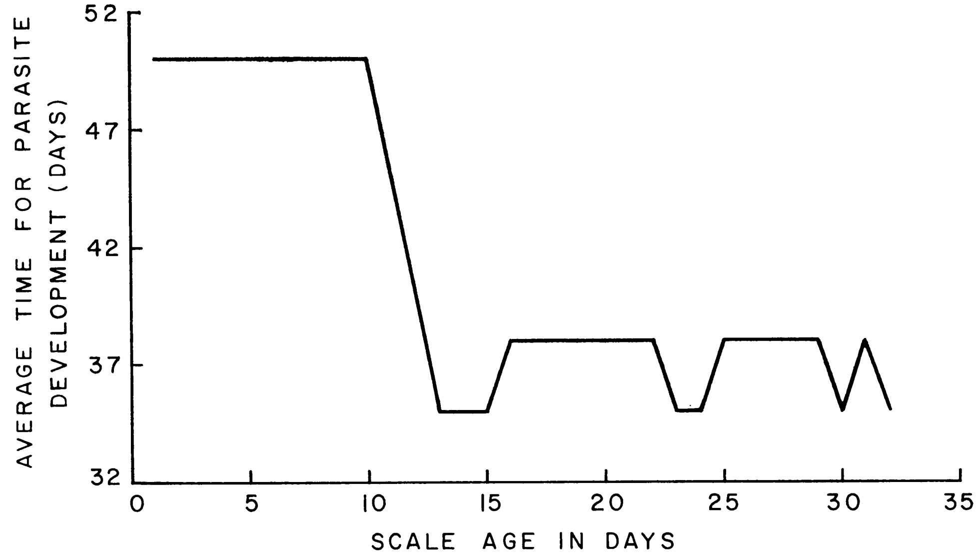

Fig. 9. Number of days from oviposition to first emergence of *Coccophagoides utilis* from scales of different ages.

Emergence patterns from host scales 25 days of age or older show emergence predominantly from the female scales. The ovipositing parasite appears clearly to favor the female host at this age. Possible reasons may be that the male scales are too far along in their development to be suitable hosts, or that the female scales after this age have accumulated nutrients or other stimulants sufficient to make them more attractive hosts than the males. The latter explanation is the more feasible, since developmental time of the parasite in scales 25 and 29 days old is shorter inside a female host than in a male host. Furthermore, at these ages of the host, the developmental time of the parasite from oviposition to emergence of the adult is shorter with the female host than with a male host of any age during these tests.

The conclusions drawn from the information given are that the male scale up to about 17 days of age is the preferred host sex for oviposition and also the more suitable for immediate development, judging by the developmental time of the parasite. At the age of about 25 days the female scale becomes the preferred host and then offers the parasite a shorter developmental time.

Mating. Mating brings about a profound change in the behavior of the female parasite. Before copulation, the female searches for a hymenopteran host, of her own species, to which she attaches an egg. The larva from this egg consumes the individual host, which is usually of a different sex; and after pupation the larva gives rise to a male parasite. After copulation has taken place, the same female searches for a host of another taxonomic order, namely a homopterous diaspine scale. She pierces the integument of the scale with her ovipositor to lay an egg, which floats free in the host's body fluid. The larva from this egg will either consume the scale and pupate within its dried integument to emerge as an adult female, or will be utilized as a source of nutrition for yet another male of its own species.

Copulation may take place at any time and lasts from five to eleven seconds. The male finds the female by means of a scent. The scent is present only in unmated females and appears within 12 hours after emergence. Fe-

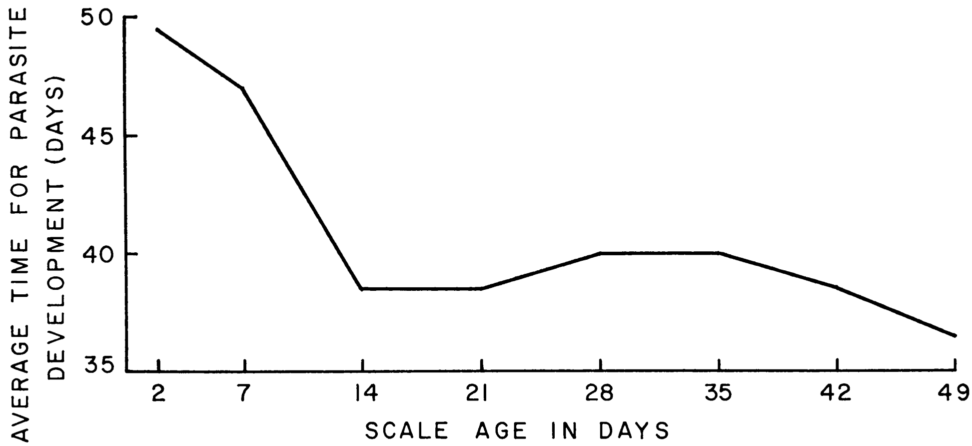

Fig. 10. Developmental time of *Coccophagoides utilis* as affected by host age.

males attracted males even if they had been isolated from host material since emergence; therefore it appears that production of the scent is not triggered by oviposition activity. Observations showed that the male is attracted to the female from a distance of at least 10 cm. When he is in the immediate proximity of the female, the male follows her around until contact is made; then the male climbs onto the back of the female and copulation takes place.

The mating capacity of the male was tested in the following way: Six virgin females were put in the same container with a male, 6 hours old, in the insectary. After 24 hours the females were each given a potato with scales, 35 days of age, and each potato was put into a separate container. These were left in the insectary; and when the adult parasites started to emerge, the jars were inspected daily. The same test was replicated once. In both the first test and the replicate, the male was able to mate more than once. In the first test, adult parasites emerged from five potatoes, and in the replicate all six potatoes yielded adult parasites. Males used in testing reproductive longevity were still capable of mating after 15 days.

The female parasite may start ovipositing in scales 60 to 90 seconds after mating.

The time lapse between emergence of a female parasite and production of the attracting scent may be sufficient to ensure the oviposition of a number of male eggs before the male is actively attracted. A mechanism like this would tend to ensure that a number of males will always be produced.

Oviposition by the unmated female. Within 60 minutes after emergence, the unmated female starts searching for a suitable host. The insect runs over the host substrate, rapidly tapping the area in front of her with vertically-held antennae. When a scale insect is detected, she pauses a moment to inspect it closely with the antennae. Finally, she gets onto the scale armor and either oviposits or abruptly breaks the sequence and moves on. Probing with the ovipositor without consequent oviposition was very rarely observed. Oviposition may take from four to nine minutes.

A suitable host of this unmated female is normally a female parasite in the prepupal or pupal stage. After the parasite has pierced the scale armor and dried integument, the egg is attached to the outer integument of the host by means of a pedicel. Eggs have been found on all parts of the body; no specific area is favored. Male eggs are not laid in a fluid environment; therefore the female prepupal stages and pupal stages are the only suitable host stages.

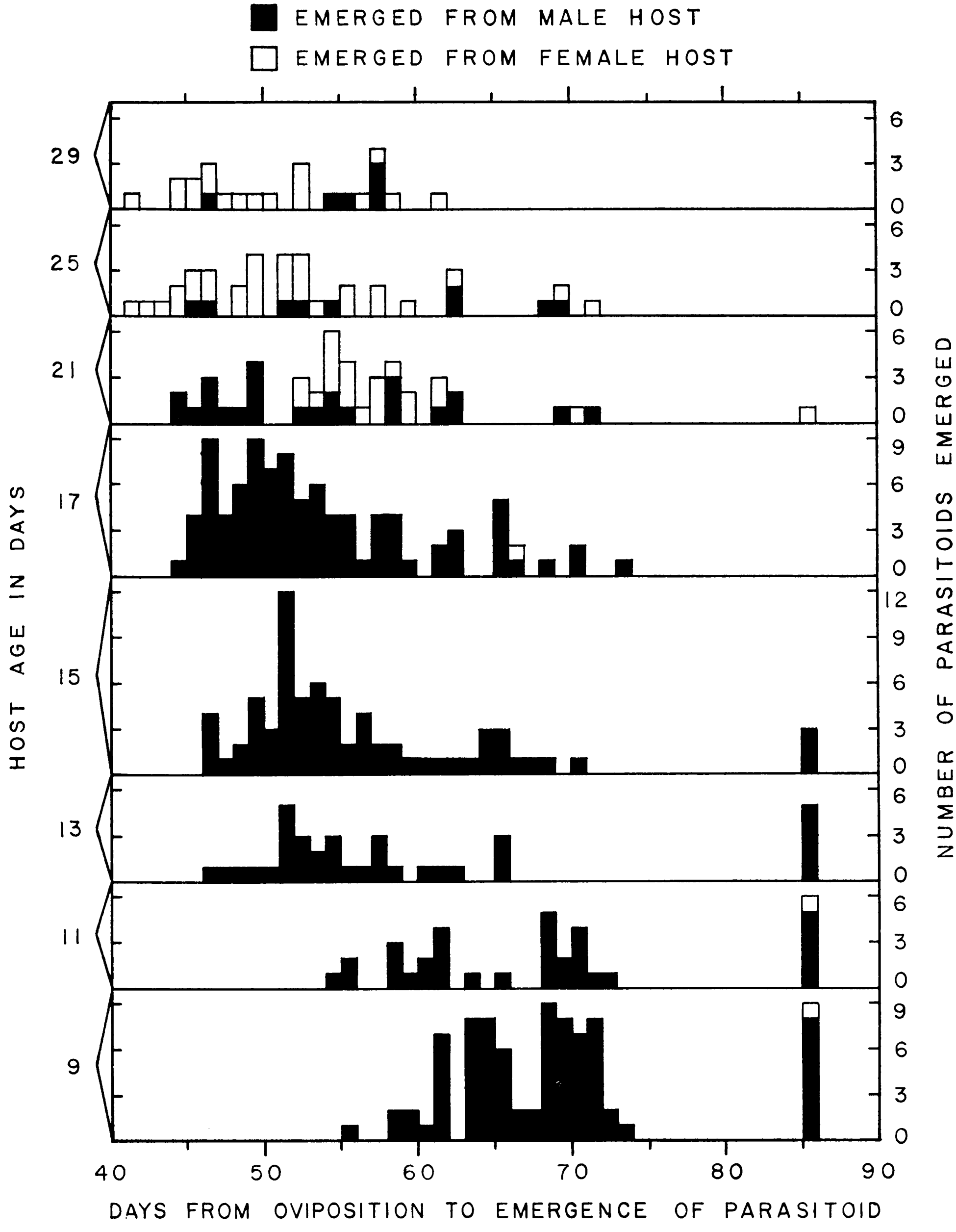

Fig. 11. Influence of sex of host on development of *Coccophagoides utilis.*

Eggs were often, on the other hand, found on pupae which were fully sclerotized and had been close to emergence. Males from these eggs were nevertheless able to complete their development, and all but the black exoskeleton was consumed.

The host pupa is inactivated by the ovipositing female. The affected pupa remains well preserved until the male has consumed it. It is not clear whether the pupa is killed by venom from the female or merely paralyzed.

When 10 host pupae without scale

armor, but inside the dry scale integument, were offered to five virgin females on filter paper in a petri dish, oviposition occurred on three pupae within 24 hours in the insectary. The scale "shell" was held onto the filter paper with a minute quantity of petroleum jelly, and the dorsal area was directed upwards. The same test was repeated two more times with no oviposition in the one case and five eggs laid in the other. Thus a female in the unmated condition accepts a host which lacks the usual stimuli and appearance, in an uncharacteristic environment; this indicates that there is a considerable pressure on the virgin females to oviposit.

Pressure to oviposit is also the likely cause of superparasitism, which was frequently encountered. Individual host pupae were often found with up to five eggs attached to various sites on the body. In these cases the penetration of the host integument at more than one point caused the host to desiccate and harden before it could be utilized, resulting in death of all the feeding males.

On rare occasions male larvae were found developing on male pupae. The host sex was determined by the size of the abdomen and the presence of two sets of meconial pellets.

The situation regarding the unmated female can be summarized as follows: The unmated female utilizes a hymenopteran host of her own species, to which she externally attaches an egg by means of a pedicel. The host is inactivated by the female, possibly by means of venom. The egg that has been laid gives rise to an ectoparasite which, when it has matured, is the male of the species.

Oviposition by the mated female. The mated female starts searching for a suitable host soon after copulation. The antennae are held vertically; and as the parasite moves along, it taps likely objects and inspects them with the tips of the antennae. When a suitable host is found, the female may either climb on top of the scale armor or back up to it and then drill through the armor with the ovipositor to deposit an egg inside the host.

A suitable host in this case is the scale insect *Parlatoria oleae,* in almost any stage after settling. The egg is deposited in the body fluids of the host and usually has no pedicel. The host suffers no apparent ill effects due to oviposition itself, and in rare instances scales still produced eggs up to 12 hours after having been attacked. The parasite did not oviposit in scales from which the armor had been removed.

Superparasitism occurred frequently in confined conditions. When 1,000 parasites were confined in a glass jar with about 400 scales on one potato for 24 hours in the insectary, up to 19 eggs per scale were found in subsequent dissections. Where superparasitism occurred, only one larva developed beyond the first instar, while the others died in the egg stage or soon after. These dead parasite eggs and larvae show a granular consistency. In the light of this information it appears that the first larva to hatch liberates an agent which diffuses through the scale haemolymph to destroy any other eggs or larvae which would ultimately compete for the same limited food supply. The first larva must be immune to this substance, but its influence on the host is not clear. On one occasion, out of thousands of scales inspected, two fully mature normal pupae were found in the same scale shell. The eliminating mechanism on this rare occasion for some reason had been ineffective.

Where there was an abundance of host material per female available, as in the oviposition tests discussed below, the same host was never attacked more than once.

Dissection of five newly emerged virgin females yielded the following ovarian egg counts: 48, 27, 31, 28, and 19, giving an average of 30.6 eggs per female. The duration of ovipositional activity and daily rate of oviposition were

TABLE 4
DAILY OVIPOSITION OF MATED FEMALES OF *COCCOPHAGOIDES UTILIS*

Number of eggs laid by female:						Day no.
	No. 1	No. 2	No. 3	No. 4	No. 5	
	11	21	19	28	12	1
	0	0	6	1	0	2
	2	2	0	1	3	3
	1	0	0	2	1	4
	0	1	4	0	1	5
	1	1	0	0	0	6
	0	0	1	2	0	7
	1	0	2	1	0	8
	0	1	0	0	1	9
	2	0	0	0	0	10
	0	0	0	1	1	11
	Dead	Dead	0	1	0	12
	..	..	Dead	0	Dead	13
	..	..	..	Dead	..	14
Total	18	26	32	37	19	
Average number of eggs per female: *26.4*						

tested by providing a newly emerged, newly mated female with fresh scales every 24 hours in the insectary and dissecting the scales removed to count the number of eggs laid by the parasite. The number of scales that had to be dissected daily was reduced by exposing only a flat slice of potato peel with about 100 scales to the female each day. This slice was roughly 6 mm thick and was sealed onto a microscope slide with paraffin wax (melting point, 127°F to 131°F). It was readily accepted for oviposition. The results of five replicates are given in table 4. During these tests, food in the form of undiluted honey was freely available.

The results of these tests indicate that the average number of eggs laid is approximately 26 per female. The distribution of oviposition in time is important in that, on the average, 18 of the 26 eggs are laid within the first 24 hours. After the first three days, the females in the tests showed decreasing interest in the scales offered, and after a brief inspection of the scales they started wandering around inside the jar. During this time, egg-laying was erratic and limited.

The egg from a mated female is deposited in the body fluids of a scale host as stated, and gives rise to a larva which is an endoparasite and which will eventually consume its host, pupate, and emerge from the scale integument as an adult female of the species.

The effect of relative humidity on oviposition. Wide fluctuations in relative humidity levels occur in nature. In order to ascertain how these changes may influence oviposition, tests were run in the following way: A suitable container to hold both the host and the parasites was made by covering the open end of the lid of a half-gallon ice-cream carton with fine organdy mesh. An oval hole (18.75 sq. cm. in area) was cut in the opposite end, and through this hole the anaesthetized parasites were introduced. Host scales were provided by plugging the hole with an infested potato. This potato was then sealed into the hole with heated paraffin wax. This whole unit was then placed in a desiccator jar containing a supersaturated aqueous salt solution in the bottom. The jar, in turn, was put into a cabinet maintaining 68° ± 0.5°F for the duration of the trial.

Supersaturated aqueous solutions of salts were used to obtain the desired relative humidities above the solutions in the closed jar. The following salts were used: lithium chloride ($LiCl.H_2O$) for 15 per cent RH; sodium bromide ($NaBr.2H_2O$) for 58 per cent RH; and ammonium chloride ($NH_4Cl.H_2O$) for 79.50 per cent RH. Pure distilled water was used to obtain 100 per cent RH. The lid of the desiccator jar was sealed with petroleum jelly during the tests.

Host scales used were between 40 and 50 days old, and the parasites were up to 48 hours old. This particular arrangement was due to the fact that the importance of the first day of oviposition was not realized at the time these tests were conducted and also because

TABLE 5

LONGEVITY OF *COCCOPHAGOIDES UTILIS* DOUTT UNDER A VARIABLE TEMPERATURE WITH A MEAN OF 75°F

Category	Day																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	*average cumulative mortality (per cent)**																			
Males, fed	0	10	10	10	10	10	10	10	10	10	10	20	35	50	75	85	100	..	..	..
Males, unfed	0	0	30	85	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Females, fed, hosts present	0	0	0	0	0	0	0	0	0	0	40	80	100	..	..	..	..	..	..	..
Females, fed, hosts absent	0	0	0	0	0	0	0	0	0	0	0	5	7.5	7.5	10	25	40	62.5	85	100
Females, unfed, hosts absent	5	20	45	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

* Twenty individuals in each category were used per trial, and each trial was repeated two times.

parasites were needed in large numbers and the insectary stock was collected every two days.

After each test all the scales were dissected and the parasite eggs were counted. The parasites were anaesthetized, counted, and sexed after each test, because the number to be introduced could not be controlled beforehand.

Tests were repeated three times at each humidity level, and the results were converted to eggs laid per female at each level and then plotted (fig. 12).

According to these results, oviposition is extremely poor at 15 per cent relative humidity. However, as the humidity increases, the eggs per female also increase, and at 100 per cent relative humidity the highest number of eggs is laid.

Longevity. Tests of longevity were conducted in the following five categories:

1. Males, fed.
2. Males, unfed.
3. Females fed, hosts present.
4. Females fed, hosts absent.
5. Females unfed, hosts absent.

These tests were run in bioclimatic chamber III (min. 60°F, max. 90°F; and av. 75°F). Twenty individuals in each category were used per trial, and each trial was repeated two times. The insects were kept in 1-pint glass jars covered with fine organdy mesh during the tests; and where food was required, undiluted honey was streaked on the insides of the jars in such a way that fine droplets were formed.

The average cumulative mortality on each day is expressed as a percentage in table 5.

The mortality figures indicate that, in the absence of honey, the life span is seriously reduced in both sexes. Honey contains a high percentage of water, and in its absence death due to desiccation is very likely. The longest life span in the female was found where honey was present but the host was absent. When both hosts and honey were supplied, the life span of the females was shorter than when hosts were absent. It appears that the rigors of egg production make drastic demands on the physiology of the body and this in turn shortens life.

Establishment studies. Theoretically, a species such as *Coccophagoides utilis,* with a unique method of producing its males, would not be capable of spreading through a potential habitat very rapidly. The mated females are the only agents capable of spreading the species, since the unmated female must have developing parasite material already present on which to oviposit. The mated female, however, can give rise to female progeny only, which, without males, would also be the end of the line, assuming that males are not perhaps

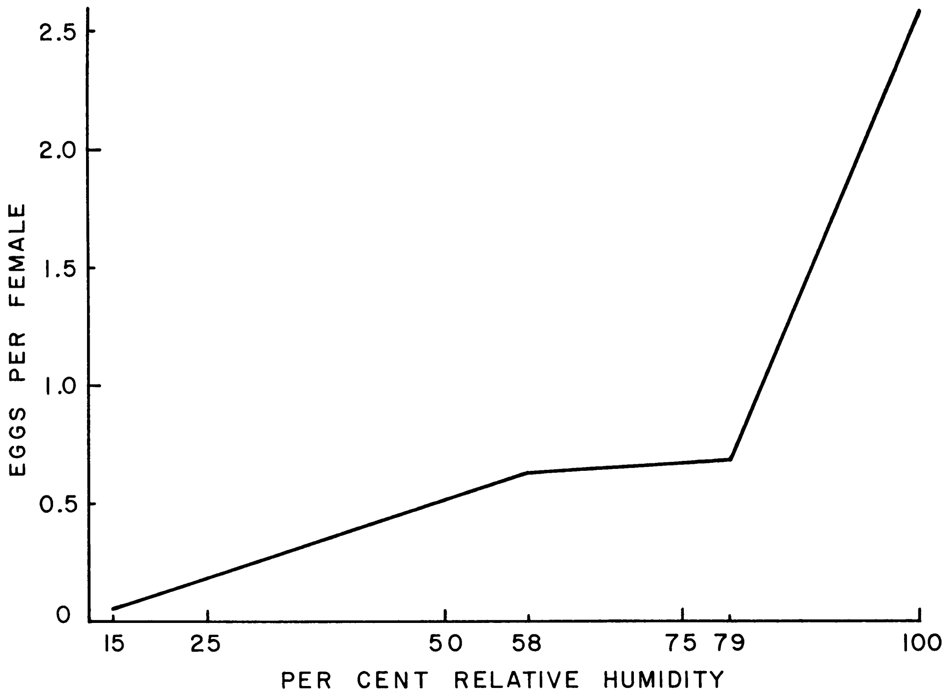

Fig. 12. Oviposition of *Coccophagoides utilis* as influenced by relative humidity.

emerging nearby. These females would die within 20 days after emergence, and their male progeny would emerge at least 10 days later. Nevertheless, from field information it appears that this species disperses rapidly, spreading through large olive groves in a matter of a few years (Kennett, Huffaker, and Finney, 1966).

An explanation was sought for this phenomenon in the laboratory. Four potatoes with scales six weeks old were exposed to about 300 parasites of mixed sex for 20 hours. After exposure the host material was kept in a glass battery jar covered with fine organdy mesh in bioclimatic chamber III (av. 75°F). As soon as the first emergence occurred, the jar was checked every four days for subsequent emergence. These adults were counted and sexed and the results were plotted (fig. 13).

The results indicate that female emergence occurs initially at a high rate, relative to that of males, and then drops to a lower level. The first emerging females oviposit on the female pupae of their own generation, and this may reduce emergence to some extent. The first males emerge about 30 days after the first wave of females. At the same time there is an emergence of females that had been retarded in their development, and this emergence coincides with the availability of males. Thus the sexes meet and the population can perpetuate itself in that locality.

The males also emerge over an extended period and are therefore available when the progeny of the females and earlier males emerge and require fertilization. The emergence time of these males may be extended merely because their parent females emerged at different times, or because a retarded development mechanism is also active in developing males.

When the males emerge, an abnormal abundance of this sex is produced at the expense of female numbers. However, this may be because, during the test, the females were confined to the host vicin-

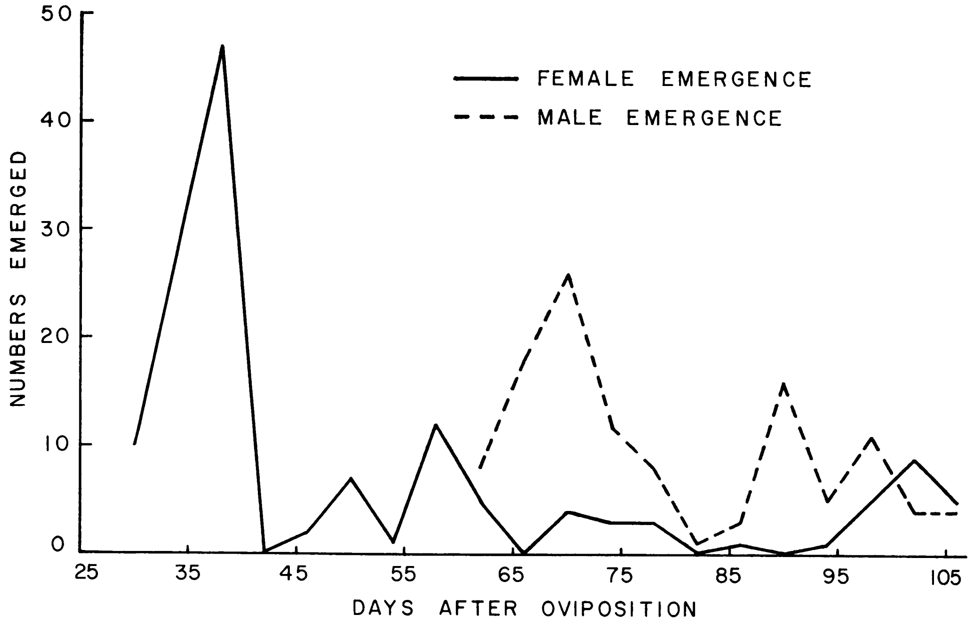

Fig. 13. Comparative emergence of *Coccophagoides utilis* by sex from hosts exposed to the parasites for only a 20-hour period.

ity, a situation which does not arise in nature.

A mechanism of retarded development is also clearly demonstrated by the distribution of developmental stages shown in table 3. The eggs in this case were all deposited in the short period of 24 hours, yet there was a differential in development which led to large ultimate differences in emergence time.

The above information suggests two possibilities. The inherent ability to lay slow-developing eggs could be present only in some individuals of the population; it is thus a population characteristic. In this case a certain number of individuals would have to be present in a new locality to extend the development time of a fraction of the population over a period long enough to ensure emergence of both sexes at the same time. The other possibility is that variable development is genetically fixed in every individual of the species. If this is indeed the case, it would ensure that the presence of merely one mated ovipositing female guarantees the meeting of sexes and so leads to the establishment of the species in a suitable new area.

The two possibilities were investigated in the following way: Five newly emerged mated female parasites were confined with one host-bearing potato tuber for their full life span, and upon emergence their progeny were counted and classified. The same was done with two females per potato and one female per potato. The potatoes were each put into a separate 1-pint glass jar and kept in bioclimatic chamber III (av. 75°F) for the duration of the trial. Four replicates were run in each case. The scale age was 42 to 49 days.

The results given in figures 14, 15, and 16 represent the total emergence in the four tests, not the average emergence per test. Tables 6, 7, and 8 contain the actual data.

Where five females were present per scale population, males and females emerged simultaneously in each of the four replicates; that is, the sexes met in each case. Where two females were present per host-bearing potato, the sexes met in two of the four cases. When

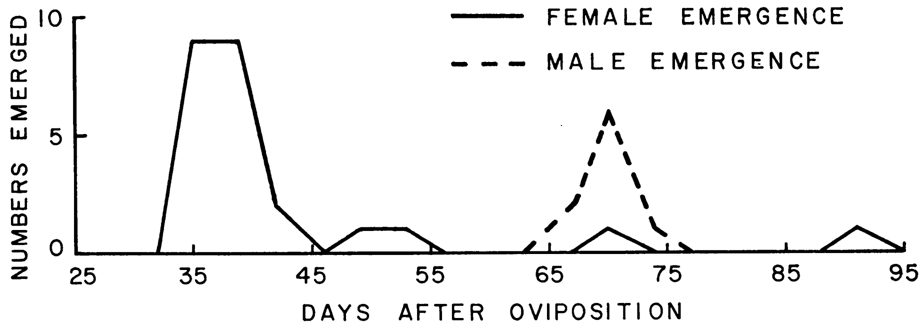

Fig. 14. Total emergence of progeny of a single mated female *Coccophagoides utilis* confined to one host-bearing potato tuber. (Four replicates.)

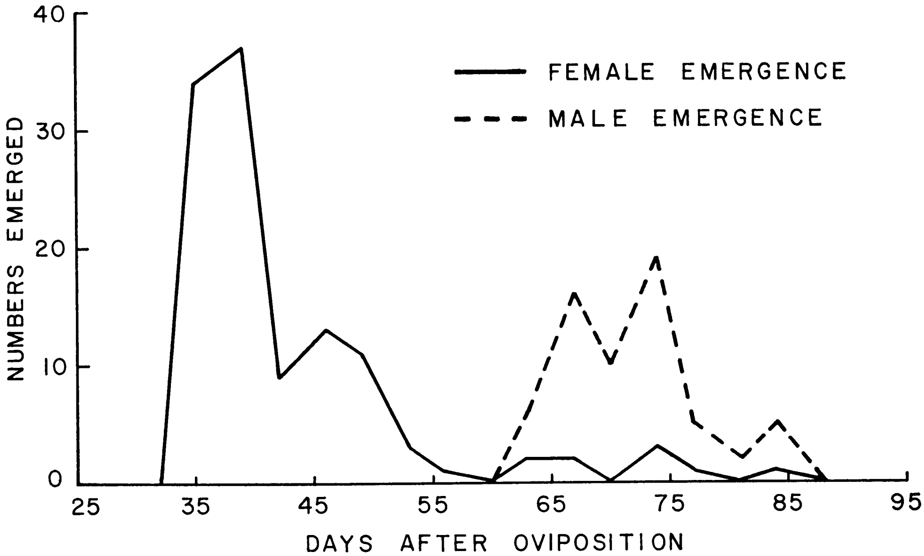

Fig. 15. Total emergence of progeny of 2 mated female *Coccophagoides utilis* confined to one host-bearing potato tuber. (Four replicates.)

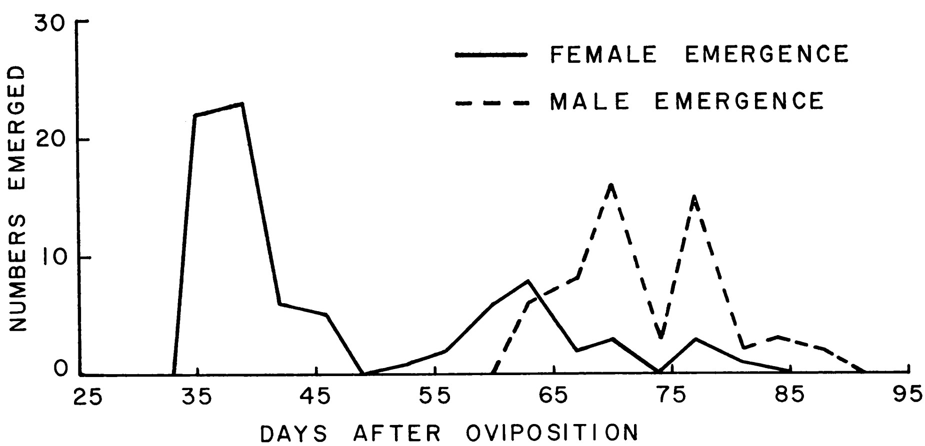

Fig. 16. Total emergence of progeny of 5 mated female *Coccophagoides utilis* confined to one host-bearing potato tuber. (Four replicates.)

TABLE 6
PROGENY OF *COCCOPHAGOIDES UTILIS* WITH ONE FEMALE PER SCALE POPULATION

Days after oviposition	Potato A		Potato B		Potato C		Potato D		Total	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
	Number of progeny									
32	..	..	..	..	..	..	..	..	..	..
35	4	..	4	..	..	..	1	..	9	..
39	8	..	..	..	1	..	..	..	9	..
42	..	..	1	..	1	..	..	..	2	..
46	..	..	..	..	..	..	..	..	..	..
49	1	..	..	..	..	..	..	..	1	..
53	..	..	1	..	..	..	..	..	1	..
56	..	..	..	..	..	..	..	..	..	..
60	..	..	..	..	..	..	..	..	..	..
63	..	..	..	..	..	..	..	..	..	..
67	..	..	..	1	..	..	..	1	..	2
70	1	4	..	1	..	1	..	..	1	6
74	..	1	..	..	..	..	..	..	..	1
77	..	..	..	..	..	..	..	..	..	..
81	..	..	..	..	..	..	..	..	..	..
84	..	..	..	..	..	..	..	..	..	..
88	..	..	..	..	..	..	..	..	..	..
91	1	..	..	..	..	..	..	..	1	..

only one female was present per host-bearing potato, the sexes met in only one of the replicates. Erratic oviposition and confined conditions may affect the performance of a female, and it would be all the more apparent where only one female was used per test. The rate of oviposition, as seen in table 6, was extremely low in those tests with single females except in one case, and in this one case the sexes did meet. It is apparent that the lack of an adequate

TABLE 7
PROGENY OF *COCCOPHAGOIDES UTILIS* WITH TWO FEMALES PER SCALE POPULATION

Days after oviposition	Potato A		Potato B		Potato C		Potato D		Total	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
	Number of progeny									
32	..	..	..	..	..	..	..	..	..	..
35	1	..	10	..	18	..	5	..	34	..
39	17	..	15	..	..	..	5	..	37	..
42	8	..	1	..	..	..	..	..	9	..
46	8	..	..	..	..	..	5	..	13	..
49	8	..	..	..	..	..	3	..	11	..
53	..	..	..	..	..	..	3	..	3	..
56	..	..	..	..	..	..	1	..	1	..
60	..	..	..	..	..	..	..	..	..	..
63	..	..	..	..	..	5	2	1	2	6
67	..	2	..	1	1	10	1	3	2	16
70	..	2	..	1	..	4	..	3	..	10
74	..	3	..	1	2	10	1	5	3	19
77	..	1	..	1	1	..	..	3	1	5
81	..	..	..	1	..	..	..	1	..	2
84	..	..	..	3	1	1	..	1	1	5
88	..	..	..	..	..	..	..	..	..	..

TABLE 8
PROGENY OF *COCCOPHAGOIDES UTILIS* WITH FIVE FEMALES PER SCALE POPULATION

Days after oviposition	Potato A		Potato B		Potato C		Potato D		Total	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
	Number of progeny									
32	..	..	..	..	..	..	..	..	..	..
35	6	..	2	..	10	..	4	..	22	..
39	3	..	3	..	11	..	6	..	23	..
42	..	..	..	..	4	..	2	..	6	..
46	2	..	1	..	2	..	..	..	5	..
49	..	..	..	..	..	..	..	..	..	..
53	..	..	..	..	1	..	..	..	1	..
56	1	..	..	..	1	..	..	..	2	..
60	..	..	..	..	5	..	1	..	6	..
63	1	..	3	..	4	..	..	6	8	6
67	..	1	2	3	..	3	..	1	2	8
70	1	5	..	3	2	3	..	5	3	16
74	..	..	..	..	..	1	..	2	..	3
77	1	7	..	3	2	2	..	3	3	15
81	1	1	..	..	..	..	..	1	1	2
84	..	..	..	1	..	1	..	1	..	3
88	..	..	..	..	..	1	..	1	..	2
91	..	..	..	..	..	..	..	..	..	..

number of eggs to begin with will seriously hamper the establishing system. This is also confirmed by the fact that the males did emerge at the expected time but there were no females available.

It can thus be concluded that the ability to establish the species in a new locality may be present in a single mated female, but the chances of establishment are increased if the new locality is invaded by more than one mated female.

The presence of a delayed development mechanism provides two important safeguards to the population. First, it will cause females to be slow enough in development to emerge at the same time as the males and copulation can occur. Second, it ensures that, when the first females emerge, some of the same brood are still in the larval stage in a fluid environment which makes them unfit to be hosts for the male egg. Although each developing female has a susceptible period to face, it is likely that dispersal under natural conditions, together with the delayed development mechanism, will minimize the possibility of all developing females in a population being destroyed by males.

SUMMARY

Coccophagoides utilis Doutt of the family Aphelinidae is a hymenopterous parasite that attacks the olive scale, *Parlatoria oleae* (Colvée).

The species is arrhenotokous, and the mated female gives rise to female progeny only. The egg in this case is deposited in the body fluids of the host scale. The unmated female attaches her eggs externally to the prepupal and pupal stages of developing males and females of her own species, and these eggs give rise to male progeny. The male, therefore, is parasitic on its own species.

Development is accelerated at high temperatures and slowed at low temperatures in both sexes. At an average tem-

perature of 51°F, development was extremely slow, but still proceeded.

The host age above 12 days has no marked influence on development of the parasite. Scale age below 12 days retarded development considerably.

Male scale insects are the preferred hosts up to about 17 days after settling, after which the female scale gradually becomes the preferred host sex in which to oviposit.

Mating changes the ovipositional behavior of the female entirely. Female parasites attract males by means of a scent. Males are capable of mating more than once.

Mated females laid an average of 26 eggs, of which the first 18 were laid on the first day.

Oviposition was poor at a low relative humidity level but improved as the humidity increased.

Longevity was much reduced in the absence of honey and other nutrients, probably because of desiccation. Fed females lived the longest in the absence of hosts.

A mechanism of retarded development ensures that the males on emerging meet with females. It also prevents all the developing female parasite individuals from being parasitized by their own males. The mechanism seems to be genetically fixed, and the progeny of a single female may provide individuals of both sexes at such a time as to ensure mating and the establishment of the species in a new area.

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III. The olive scale, *Parlatoria oleae,* was first found in California in 1934 and has since become a major pest. Attempts to control this insect by biological means began with the introduction of the external parasite, *Aphytis maculicornis,* from Iran in 1952. Inconsistent control by *A. maculicornis* led to the introduction of two additional parasites from Pakistan in 1957. One of these, *Coccophagoides utilis,* became established in California.

Coccophagoides utilis is an internal parasite which attacks both scale generations which *P. oleae* produces each year. Adult female *C. utilis* which have been mated deposit female eggs only. Unmated females deposit male eggs only. Field results show *C. utilis* capable of destroying up to 50 per cent of each host generation. The two species of parasites working together have exhibited the ability to give excellent control of olive scale.

IV. The competitive population interactions between *Aphytis maculicornis* and *Coccophagoides utilis* were analyzed in order to determine their roles in controlling olive scale, *Parlatoria oleae,* in California olive groves. There is strong evidence that the two parasites working together give better control of olive scale than does *A. maculicornis* working alone. Conclusions are based on observations of parasite populations at selected groves over a period of five years.

K-values for various factors affecting olive scale mortality were developed in order to measure and assess the controlling effects of these two parasites on olive scale from generation to generation.

V. In 1961 the large-scale production of the aphelinid parasite *Coccophagoides utilis* was initiated. During the seasons of 1962 and 1963, over four million were made available for release against the olive scale, *Parlatoria oleae,* in colonization sites throughout California.

The factors involved in the production of *P. oleae* and *C. utilis* are briefly discussed and the methods and equipment used in the insectary are described and illustrated.

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