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Studies of Two Parasites of Olive Scale, *Parlatoria oleae* (Colvée)

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R. L. Doutt

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

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C. E. Kennett, C. B. Huffaker, and G. L. Finney

IV. Biological Control of *Parlatoria oleae* (Colvée) Through the Compensatory Action of Two Introduced Parasites

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V. The Culture of *Coccophagoides utilis* Doutt, a Parasite of *Parlatoria oleae* (Colvée)

G. L. Finney

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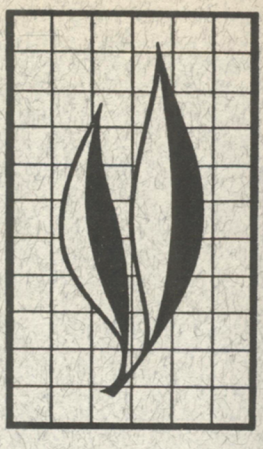

I. The genus *Coccophagoides* (Hymenoptera: Aphelinidae) is revised herein and two new species are described. These are *Coccophagoides comperei* Doutt and *Coccophagoides utilis* Doutt. Both *C. utilis* and another new species, *Anthemus inconspicuus* Doutt (Hymenoptera: Encyrtidae), are primary parasites 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.)

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G. L. Finney

V. The Culture of *Coccophagoides utilis* Doutt, a Parasite of *Parlatoria oleae* (Colvée)[1]

INTRODUCTION

IT BECAME apparent early in 1961 that the aphelinid parasite *Coccophagoides utilis* Doutt had definitely become established on olive scale, *Parlatoria oleae* (Colvée), in certain San Joaquin Valley colonization sites as the result of only a few relatively small releases. These releases had been made coincident with the mass-rearing and release of *Anthemus inconspicuus* Doutt, another parasite of olive scale, and had consisted of the parasites produced over and above those needed for a minimum stand-by stock then being maintained in the insectary.

The emphasis of the project, therefore, was shifted from the mass-rearing of *A. inconspicuus* to that of *C. utilis* and the insectary effort was directed toward the development of techniques and equipment for rearing this latter species in large numbers.

Some of the rearing units used in the mass production of *C. utilis* were similar or identical to those used in the previous production of *Aphytis maculicornis* (Masi) (Huffaker, Kennett, and Finney, 1962). However, because of the peculiarly involved and complicated reproductive habits of *C. utilis* as described by Broodryk and Doutt (1966) and discussed by Kennett, Huffaker, and Finney (1966) there were some changes and modifications in methods and equipment.

Unlike *A. maculicornis, Coccophagoides utilis* is highly specific to its natural host, *P. oleae,* and does not recognize as a host other species of diaspine scales commonly utilized in insectary work, such as *Hemiberlesia lataniae* Signoret or *Aspidiotus hederae* (Vallot).

It seemed highly desirable to obtain within a 24-hour period well-distributed infestations of olive scale on potato tubers (fig. 1). Compared to most other commonly used diaspine scales, *P. oleae* is rather ill-adapted to conventional mass-rearing techniques because of certain behavioral characteristics. The young "crawlers" are but mildly phototactic and so do not respond well to the "shadow-trap" method of collection, as do those of *H. lataniae* or *Saissetia oleae* Bernard. Furthermore, the young tend to wander excessively before settling down, a factor which renders rather inefficient the "drop" method so successfully used with *H. lataniae* in producing *Aphytis maculicornis.* Also, because of this wandering habit there were not at any given time enough young aggregating on a source "mother" potato to make the "brushing" technique practical in a large-scale operation. However, such "brushed-on" scale crawlers would tend to remain on the tuber if it was placed immediately in total darkness for 24 hours.

[1] Submitted for publication October 8, 1964.

INFESTING THE POTATOES

The unit that was finally adopted for infesting the potatoes with *Parlatoria oleae* consisted of a cardboard box (fig. 2) 17½ in. long, 16 in. wide, and 3½ in. deep, having a cardboard cover with 2-in. sides that fitted down over the outside of the units. An aperture 4½ in. by 2 in. was cut in the center of the cover running parallel to the long side of the box. As shown in figure 2, the "source" potatoes rested one and sometimes two on a cardboard triangle, which in turn rested on a block of wood 4 in. square and 1½ in. high. Four such blocks were arranged in the box, one at the mid-point of each side. The potato to be infested was placed "center stage."

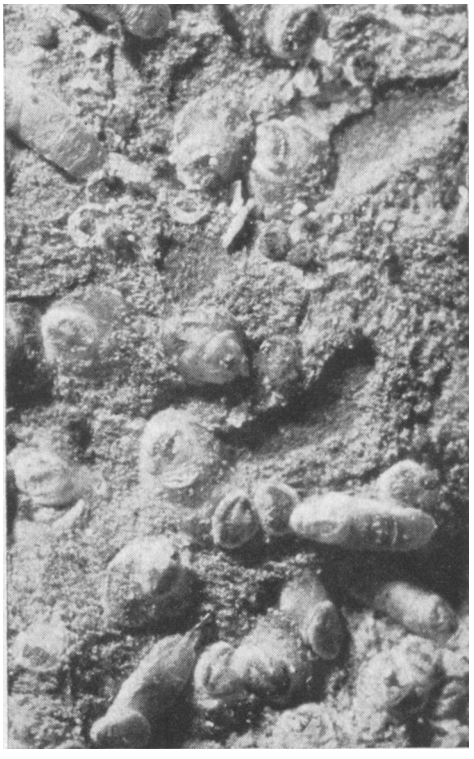

Fig. 1. Olive scale, *Parlatoria oleae,* on a potato tuber. The elongate forms are male scales. Many of the female scales are nearly obscured by the surface matrix peculiar to the Russet-type potato under which the armor of the scale is spun.

The triangles were so adjusted that the tips of each rested on the adjacent side of the potato tuber.

In operation, the box with the cover in place was set in series with other such units on a table with a reflector-mounted fluorescent light hung about 3½ ft. above. The apertures in the box tops were positioned at right angles to the lamp. The young scales moving toward the central light dropped from the "source" potatoes, crawled across the triangular "bridge" onto the potato at the center, where they congregated and finally settled down and became established.

The potatoes were prepared for infesting by first being washed. While they were still wet, each was wrapped with a section of good quality cheesecloth (38 × 44 mesh). The cloth was pressed tightly to the surface of the tuber. After drying, the cloth would adhere to the potato and, if not handled roughly, it remained securely in place. During the 24-hour infesting period the scale crawlers would penetrate the mesh of the cloth and settle on the covered portion of the potato. The cloth thus encouraged establishment of the young scales, yet it tended to discourage undesirable crowding.

Each day the infested potato was replaced by another, and the one removed was held in a light-proof container for 24 hours, during which time any unsettled scales would become established. The cheesecloth was removed a day or two later. Each day's group of infested potatoes was placed in a screen tray labeled with the infestation date and stored in a dimly lighted room having a temperature of 75°–78° F and 60 per cent relative humidity until the scales had developed to the stage appropriate to their particular use.

The potatoes in the storage room were periodically desprouted to relieve

unnecesary drain on the vitality of the tubers. The shelves of the storage racks, as well as the room itself, were maintained as clean as possible. The potatoes were spot-checked from time to time for signs of contaminant insects such as mealy bugs and tubermoth; also for evidences of any unwanted species of scales or parasites.

Particular care had to be observed as to the type of potatoes obtained for use in this phase of the work. The success of the program depended basically on the ability of the potatoes to bear an increasingly heavy burden of scales for as long as 180 days. The standard specifications were that they should be Grade A Russet (netted) variety, having a very light matrix; that they be smooth and of uniform shape, weighing from 6 to 8 ounces each; that they had been grown in a relatively disease-free and tubermoth-free region such as Oregon or Idaho; and that neither the tubers nor their containers had been treated with a pesticide. The White Rose variety is a better host than the Russet, but since the former is more susceptible to bacterial rot it could not be used safely over the long period of rigorous stresses to which the host is subjected in this project.

Scales destined to become source material in the infestation units were held until the second generation had matured and the young were being produced in considerable numbers. This second generation of scales would accumulate slowly, and in most cases the tubers were completely covered with scales of all stages. This brood of scales would produce young over a period of several weeks.

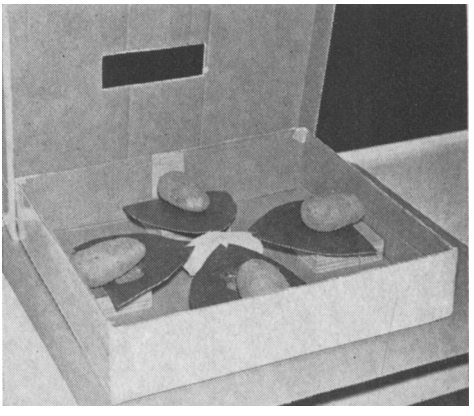

Fig. 2. The infesting unit with the top lifted back so as to show the interior arrangement of the components.

INITIATING PARASITIZATION

When a single introduction of mated female *Coccophagoides utilis* is used to initiate parasitization, the survival of the colony is very precarious, and at best a considerable time would elapse before any concurrent emergence of the sexes would be brought about. The mated females would deposit only female eggs within the body of the scales, where they would develop as primary parasites. These, upon emergence, being virgin females, would deposit only male eggs on the nearly mature larvae of late-developing females. Because of the scarcity of such male-producing host material, only a few males would be produced. These males after emergence would then mate with any surviving virgin females and parasitization of scales would be resumed.

To bring about a maximum production of parasites in a minimum period of time and to make secure the establishment and perpetuation of a productive stock, a series of introductions was made over a period of at least 20 days. This provided for constant parasite activity in the unit and offered a continuity of host material for the development of males and hence a high constant level of production in the unit rather than a critical decline of the stock.

The first adult females emerged in about 34–36 days; the first males appeared around 29–30 days later. Con-

currrent emergence of male and female parasites would be established in another 30–40 days.

Potatoes with scales approximately 25 days old were selected for the initial exposure to *C. utilis*. These were arranged somewhat loosely in hardware-cloth trays 15 in. by 11½ in. in size. The trays were placed two each in a parasitization unit (fig. 3). This unit was a pine box with inside measurements 25 in. by 16½ in., and 3½ in. deep, with a slot ½ in. deep and 1/32 in. to 1/16 in. wide cut in the center of the top edge of the box and extending continuously around.

Strips of brown waxed paper ½ in. wide, covered on one side with honey droplets serving as food for the parasites, were slipped edgewise between the rows of potatoes.

About 1500 *C. utilis* adults, anaesthetized briefly in a glass vial, were distributed by tapping out a few on each potato in the box. The unit was then immediately covered with a sheet of bleached muslin secured snugly in place by shoving the cloth into the slot by means of an instrument such as a dull putty knife with rounded corners. The boxes were placed on the shelves of a rack draped on each side with brown (kraft) wrapping paper so as to provide dim nondirectional lighting on the inside.

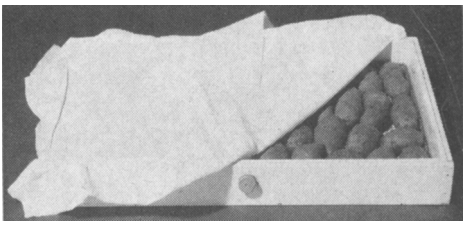

Fig. 3. The parasitization unit. Used only for initiating parasitization of scales on potatoes thereafter transferred to the production units.

Four more introductions of 1500 parasites were added to each unit at 5-day intervals. This was done each time by partially inactivating the parasites in the unit with CO_2, lifting back the cover, and introducing the additional stock, as was done before. Thirty-five days after the unit was initiated, each box was filled with CO_2-ether gas and the trays were transferred to production units.

THE PRODUCTION UNIT

The production unit (fig. 4) was a box made of ¾-in. pine strips having no attached floor or cover. The walls of the unit were 18 in. long, 15 in. wide, and 17 in. high. The openings on the longer side were 13 in. long and 4½ and 7 in. high, top and bottom, respectively; on the short sides they were 11 in. long and the same 7 in. in height. Sponge rubber weather stripping was glued to the bottom edge of the box and also around the underneath edge of the ¾ in. plywood cover.

Suspended from the cover was a "stirrup" rack made of bronze rods welded together to form a support for four screens of potatoes, each above the other. The weight of the trays of potatoes was brought to bear on the weather-stripping gaskets, making the box an insect-tight unit.

The openings of the unit were covered on the inside surface with nylon organdy held in place with masking tape. Handles were attached to the cover so that the entire load could be lifted from the unit when necessary. Each unit rested on a sheet of kraft paper, which extended at least 1 in. beyond the base on all sides.

The production units were checked periodically for concurrent emergence of males and females, which usually began about 30 days after transfer to these units. Collection for field releases was begun when both sexes were present in considerable numbers.

The parasitization units served only

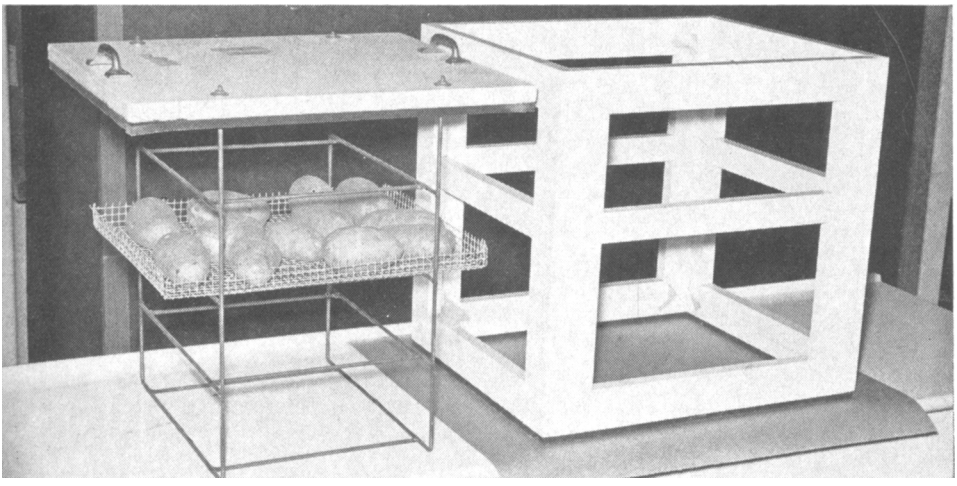

Fig. 4. The production unit. The cover and the attached rack have been removed so that the construction could be clearly observed. The nylon organdy covering has been removed from the openings of the unit.

to initiate effectively and evenly the distribution of parasite activity on the potatoes destined for the production units. Once these were loaded, the parasitization units were stored for the rest of the season. Any potatoes in the production units that became rotten, or any on which all the scales had been utilized by emerged parasites, were removed and replaced by potatoes supporting scales of the proper stage. Thus, the production units became ecosystems that yielded parasites without interruption throughout the colonization season.

COLLECTING THE PARASITES

The parasites were collected by use of anaesthetization. Two "cover boxes" made of ¼-in. plywood were designed to provide gas-tight enclosures around the production units while the parasites were being anaesthetized (fig. 5). The inside measurements of these boxes were slightly greater than the outside measurements of the production units, including the handles on the tops. A sponge-rubber seal was glued to the open edges of the box so as to give a gas-tight seal between it and the table. A 1-in. hole used for the injection of the gas was drilled in the wall of the box at a spot approximately opposite the center of the upper window of the production unit when it was in an operating position. A cork stopper was used to close this aperture.

The anaesthetizing was begun by placing the "cover" boxes over the first two production units of the series of twenty. The cork was removed from the injection aperture, and CO_2-ether gas at about 3½ lbs. pressure was allowed to flow into the first unit for 1½ min. The hose was then transferred to the second

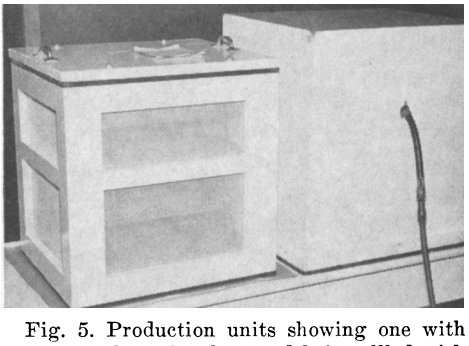

Fig. 5. Production units showing one with the "cover box" in place and being filled with CO_2-ether gas, and another fully assembled production unit awaiting anaesthetization of *Coccophagoides utilis*.

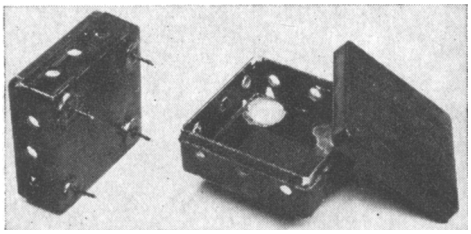

Fig. 6. Parasite-cleaning units.

box and the cork stopper replaced in Unit I. In about 1 min. the first box was lifted from the first unit and placed over the third. Some of the anaesthetized parasites would lodge on potatoes in their fall toward the paper base. These were removed by blowing across each tray through the nylon-covered windows. The unit was then lifted from the paper and placed on a similar paper on another table. The paper with the inactive *Coccophagoides utilis* was lifted up by taking hold of the opposite sides, which were then brought together so that the parasites slid to the center. The trough of paper thus formed was tipped so that the insects would pour into a cleaning unit (fig. 6) and could be placed in a covered jar for recovery from the anaesthetization effects.

The above operation could be done in about two minutes or less. In the meantime the second "cover" box had been removed from the second unit and had been placed over the fourth. This was repeated progressively until all twenty units had been worked. With a little practice and skill the day's collection would be finished in less than 40 minutes. The parasites were collected three times a week—usually on Mondays, Wednesdays, and Fridays.

The cleaning unit referred to above consisted of a plastic specimen box 1¾ in. square and ¾ in. high, including the snug-fitting slip-on lid. Four thumb tacks were glued to the base of the box, one at each corner. Three holes 3/16 in. in diameter were located in each side ¼ in. from the base. The outside of the box was painted with black enamel.

The function of the cleaning unit was to separate the parasites from the debris, etc., that dropped with them to the paper when they were anaesthetized. About 20–30 min. after the cleaning unit had been put in the glass jar, most of the parasites had revived and emerged from the unit, leaving the debris behind.

The parasites were measured out in the calibrated stem of a small glass funnel cut to a length of about 3 in. They were transported to the field in ½-pint paper food cartons (fig. 7). Each carton contained a bit of excelsior to provide "roosting" space for the parasites, and a strip of brown waxed paper was attached to the side of the carton and covered on one side with small honey droplets, which served as food for the parasites. As many as 5000 *C. utilis* were shipped in each carton.

During the 1962 and 1963 seasons over 4,000,000 *C. utilis* were produced for field release.

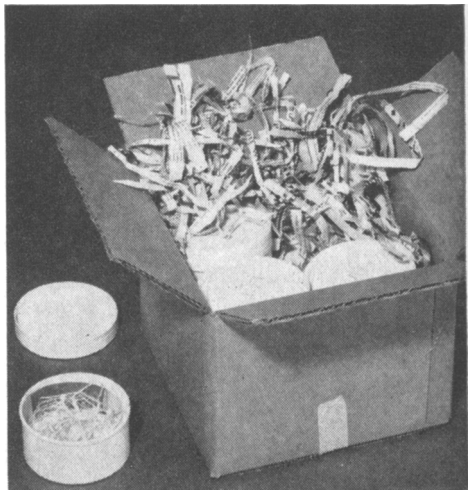

Fig. 7. Method of shipping *Coccophagoides utilis* for field colonization.

ACKNOWLEDGMENT

Grateful acknowledgment is made to F. E. Skinner for the photographs which comprise figures 1–7.

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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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