VOL. 4 JUNE, 1930 NO. 16

HILGARDIA

A Journal of Agricultural Science

PUBLISHED BY THE

California Agricultural Experiment Station

CONTENTS

Mass Production of Egg Parasites
of the Genus Trichogramma

STANLEY E. FLANDERS

UNIVERSITY OF CALIFORNIA PRINTING OFFICE
BERKELEY, CALIFORNIA

HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 4 JUNE, 1930 No. 16

MASS PRODUCTION OF EGG PARASITES OF THE GENUS TRICHOGRAMMA[1]

STANLEY E. FLANDERS[2]

INTRODUCTION

Of the comparatively small number of entomophagous insects whose habits and host relations appear to make them usable in biological control work, the hymenopterous egg-parasite, *Trichogramma,* has probably received the widest attention. Attempts have been made to utilize it in every phase of biological control, by protection through proper cultural practices, by introduction to establish it in new habitats, and by production in quantities for mass liberations. The liberation of *Trichogramma* in great numbers may prove to be a practical method for the control of several species of injurious insects.

The following account gives the origin and development of the mass production[3] of *Trichogramma* and the methods of utilizing it.

Trichogramma minutum Riley was used in this work because it is the most common representative of the genus in California. The characteristics that adapt it for mass production are probably generic.

CHARACTER OF *TRICHOGRAMMA*

The genus *Trichogramma* was established by Westwood[(25)] in 1833. The type specimens were found on an oak leaf in Epping Forest. There are few distinct species. The species most frequently

[1] The material for this paper was collected, for the most part, while author was employed by the Saticoy Walnut Growers' Association, Saticoy, California.

[2] Parasite Collector, Citrus Experiment Station, University of California.

[3] The first, and so far only, successful application of the mass-production idea occurred in California. There a method was developed for utilizing *Cryptolaemus montrouzieri* Muls., an introduced coleopterous predator of the citrophilus mealybug.[(23)]

encountered in Europe and America are *T. evanescens* Westw. and *T. minutum* Riley. The latter is not well differentiated morphologically from *T. evanescens.* In America the genus was first recorded when Lintner[13] reared *Trichogramma minutum* from saw-fly eggs at Utica, New York, in 1866.

The adult parasite is less than 1 mm in length and is brightly colored. Its wings are refringent and appear in direct sunlight a brilliant purple, its eyes are bright red, and its body varies from dark brown to clear yellow.

This chalcid is widely distributed, and its hosts, according to Martin,[15] number well over one hundred and fifty species in the orders Lepidoptera, Coleoptera, Hymenoptera, Neuroptera, Diptera, and Hemiptera. It has been known to attempt oviposition in globules of okra juice, the swollen abdomen of the mite, *Pediculoides* sp., paper smeared with the hair covering of the egg masses of the brown-tail moth, and in the dry excrement of adult moths. The majority of its hosts, however, are lepidopterous, and its oviposition is usually confined to eggs in exposed places unprotected by a hard or sticky covering. Eggs on a sticky or very pubescent surface are relatively immune to attack. The highest number of individuals developing in a single hymenopterous host egg was noted by Severin and Severin[21] who found thirty pupae in an egg of *Cimbex americana* Leach. Patterson[17] reared thirty-seven adults from a single egg of *Coloradia pandora* Blake, a lepidopterous host.

According to Howard and Fiske[12] there are two "races" of the *minutum*-like form of *Trichogramma* which differ only in that one is thelyotokous and the other arrhenotokous. The former is reported only from Europe. Marchal[14] found, in addition, color differences and no interbreeding, and suggested that if observations were made from various hosts and localities a number of strains of *Trichogramma* would be found having the status of races or elementary species. Girault[7] states that undoubtedly "biological species" exist. The races of *Trichogramma* when reared under identical environmental conditions may possibly be differentiated by the length of their life cycles and amount of pigmentation in the adult females.

The life cycle from egg to adult varies according to the host and with the prevailing temperature. The shortest life cycle of *Trichogramma* observed by the writer occurred in the eggs of *Ephestia cautella* Wlk. At temperatures between 91°–95° F it completed its life cycle in 144 hours. The pupal stage was reached 72 hours after oviposition.

After about one-third of the time required for the completion of the life cycle has elapsed, the host eggs begin to turn dark and finally become more or less black. This change is brought about in the eggs of all host species by preparation of the larva for pupation. In flattened types of eggs, such as the eggs of *Alabama* and *Carpocapsa,* after the enclosed parasites have formed their pupae, the shell sometimes shrinks about them, leaving little oval cells indicating the position of the parasites. When ready to emerge the parasite cuts a hole in the shell just large enough for it to struggle through. *Trichogramma* does not void meconium until after emergence. At emergence it is sexually mature.

Light intensity appears to be the dominating factor in the activity of *Trichogramma.* Emergence from the host egg is stimulated by light; in confinement it is positively phototropic; an increase in light intensity appears to stimulate copulation, and a decrease in light intensity retards activity. Locomotion ceases when light intensity is low.

Howard and Fiske[12] found that *Trichogramma* can be reared from infertile eggs or eggs killed by cold storage, but not from eggs in which the embryological development has passed beyond a certain point prior to attack or from eggs very often stung.

ADAPTABILITY OF *TRICHOGRAMMA* TO BIOLOGICAL CONTROL WORK

Thirty-five years ago Enock[4] called attention to the possibilities in 'farming' *Trichogramma* on a large scale. Recent investigations show that it is adapted to this work in many ways. Some of the biotic characteristics that determine its adaptability are as follows:

1. It mates and oviposits readily in confinement.
2. It develops to maturity in the eggs of moths which feed in stored grain.
3. It has a short life cycle.
4. Its development extends throughout a greater temperature range than many of its hosts.
5. It has a great variety of hosts.
6. It accommodates itself as to number of generations according to the host it parasitized, as ascertained by Marchal.[14]
7. It develops throughout the year, temperature and food permitting.
8. It has few competing species and no known secondary parasites.

9. It maintains a concentration sufficient to effect a local reduction of the host. Dispersal is so localized that its effectiveness is measurable.

Factors that limit to some extent its adaptability are as follows:

1. It does not appear to be specific as to host location; it apparently finds its host by means of random movements, tending to climb upward and fly downward.
2. It will deposit more eggs in a host than can develop to maturity.
3. It will oviposit in eggs too far developed to enable the parasite to mature.
4. Under natural conditions while in the host egg it is prey to such predators as ants, ladybird beetles, and mites.

ABUNDANCE OF *TRICHOGRAMMA* AS AFFECTED BY FARM PRACTICES

Cultural practices such as the burning of sugar-cane trash(11) and the flooding of cranberry bogs(6) appear to reduce the abundance of *Trichogramma* the following spring. Experiments in the maintenance of *Trichogramma* in the sugar-cane fields of Louisiana through the conservation of cane trash have been conducted during the past ten years, but proof of its hibernation in cane trash is lacking. *Trichogramma* dies within its host when the latter remains submerged in water. During the submergence of its host, however, it may develop to the late pupal stage.

Clean cultivation, by reducing the number and variety of the food plants of the hosts of *Trichogramma*, acts as a check on the natural abundance of the latter. In southern California there is a noticeable difference between the degree of parasitism of the codling moth in orchards and on fruit trees in dooryard situations where food plants of moths are present throughout the year. Properly managed cover crops may aid parasitism under orchard conditions.

As a rule, the hosts of *Trichogramma* are widely distributed general feeders and adaptive successful insects that have a large excess of progeny. As a group, they are benefited by the attack of this parasite, since it tends to prevent them from completely using up their food supply. Under natural conditions a very high degree of parasitism aften occurs late in the season when the host is abundant. Since the excess of progeny is greatest in plant environments as modified by man, *Trichogramma* may be of value in maintaining such pest populations below the economic zero.

EARLY METHODS OF INCREASING PARASITE POPULATION

Owing to the extreme variability of its food supply the appearance of *Trichogramma* under natural conditions is seasonal and irregular. Organized production as a means of overcoming this handicap captured the imagination of entomologists in Europe and America. The objective of this early work was to advance the date of effective abundance by accelerating the natural accretion with liberations made early in the season.

In 1909 Howard and Fiske[12] liberated a great many thousands against the brown-tail moth. The parasites were held in cold storage in brown-tail moth eggs. These eggs had been collected in the field the previous summer and then parasitized.

The habit of the brown-tail moth of depositing its eggs in masses prevents control by *Trichogramma,* for the parasite can attack only the outer eggs. Howard and Fiske concluded, however, that since it is an efficient parasite on numerous other hosts it would, at some future time, be possible to utilize it. Mokrzecki and Bragina[16] in 1913 found that the number of *Trichogramma* which it is possible to rear in the laboratory is theoretically unlimited. Mokrzecki, however, cautioned the Russian fruit growers against exaggerated expectation in the results of the artificial production of this parasite and its use against the codling moth.

The earliest method of laboratory host production was that used by Pospelow[18] who reared larvae from the winter imagos of *Euxoa segetum* (Schiff.) on sprouted wheat and slices of potato. Later Portchinsky[18] suggested collecting large quantities of overwintering larvae or pupae of a prolific host of *Trichogramma,* forcing their early maturity and oviposition, and parasitizing their eggs with *Trichogramma* carried over for that purpose from the preceding season. He advocated the use of *Phalera bucephala* L., since the overwintering pupae could be purchased cheaply.

Later it was found that the eggs of *Ephestia kuehniella* Zell. were suitable for the reproduction of *Trichogramma* and were more readily obtained. In Japan *Ephestia* has been used recently to produce *Trichogramma* for use against the rice moth. The susceptibility of *Ephestia* to larval parasitism and the webbing habit of the larva tended to limit its use in quantity production.

Another method proposed by Vuillet[24] and also by Harland[8] was that of increasing the abundance of hosts on the windward side of infested plantings. For example, *Mamestra brassicae* L. was suggested as a preliminary host adjacent to a vineyard infested with *Polychrosis botrana* Schiff. Den Doop[3] states that the spread of the parasite is most probably due to the midday winds. If such is the case, such a method has possibilities.

A natural condition somewhat similar occurred in southern California in 1926 when the codling moth was found to be heavily parasitized as early as the middle of May. The butterfly, *Vanessa cardui* L., had occurred in great numbers during March and April and had deposited quantities of eggs on weeds everywhere. It appears probable that this butterfly served as a preliminary host to *Trichogramma.* Whatever the preliminary host may have been, two hundred out of three hundred codling moth eggs on one walnut tree were found to be parasitized prior to May 25.

INITIATION OF MASS PRODUCTION

In April, 1925, Harry S. Smith had suggested to the writer that *Trichogramma minutum* was the most adaptable egg parasite with which to attempt the biological control of the codling moth since it "is well adapted to this purpose and breeds with extreme rapidity." Consequently, when its natural effectiveness was manifested so strikingly in 1926, the possibility of using it in control work was given serious consideration.

The failure of other workers to accomplish results indicated that success could be attained only by the low-cost production of great quantities of *Trichogramma* so that it could be made effective in the field through sheer force of numbers.

With this idea in mind experimentation began August 11, 1926. Ten females reared from tortricid eggs secured in the field on walnuts were allowed to oviposit in the eggs of *Ephestia kuehniella.* The second to fourth generations were reared on the lichen moth, *Illice nexa* Boisd., and the potato tuber moth, *Phthorimaea operculella* Zell. The fifth generation was reared on *Sitotroga cerealella* Oliv., which was selected for further experimentation because it was available in large numbers and had the convenient habit of ovipositing in narrow crevices, forming an egg mass of one layer. The use of this moth as laboratory host made possible the solution of the problem of the mass production of *Trichogramma.*

The eggs of *Ephestia cautella* were tested by the writer in Mexico but proved to be unsuitable for mass production, although *Tricho-*

gramma in several instances showed a marked preference for them. The larvae from eggs escaping parasitism spun a dense webbing over the adjacent eggs and also fed on them.

During October about a million silk-worm eggs were donated by the American Silk Factors, Inc., at San Diego for testing as a laboratory host of *Trichogramma*. Although the silk-worm egg proved to be attractive, the tough chorion prevented oviposition. Ten females were observed on one egg, each drilling vigorously in an effort to oviposit.

EFFECT OF HOST ON THE PARASITES

Directly or indirectly the host influences the size, longevity, and fecundity of the adult parasite. In an egg maturing only one parasite, the size of the mature parasite is determined by the size of the egg. When the egg is super-parasitized its size only determines the number of parasites developing in it. A single adult reared from a potato-tuber-moth egg has a larger body than an adult from a *Sitotroga* egg, but in length they are equal. In the field where one to four parasites develop in a codling-moth egg the single parasite may be twice the length of the smallest of the four parasites. The larger the parasite the more young it is capable of producing. *Trichogramma* reared on *Sitotroga* eggs were allowed to oviposit in the large eggs of an arctiid moth. Two of their offspring produced 240 progeny on *Sitotroga*, whereas the maximum progeny per female in successive generations on *Sitotroga* was 45.

It is doubtful, however, if large eggs will prove to have any advantage in artificial production over an equal number of small eggs because of super-parasitism. A *Sitotroga* egg rarely yields more than one *Trichogramma*. Larger eggs which allow the development of more than one parasite may produce under laboratory conditions parasites smaller and weaker than those from *Sitotroga* eggs. If the shell of the host egg is relatively hard they may be unable to emerge.

The longevity of parasites reared on the eggs of *Ephestia cautella* appears to be much shorter than those reared on *Sitotroga*.

A dependable source of an enormous amount of host material is necessary for maximum production. On October 1, 1928, the writer secured three female parasites from codling moth eggs in the Lloyd-Butler orchard at Saticoy. At the end of seven weeks the progeny of these, composing the sixth generation, amounted to about 300,000. The supply of moth eggs available was inadequate to maintain such multiplication.

ADAPTABILITY OF *SITOTROGA* TO THE MASS PRODUCTION OF *TRICHOGRAMMA*

Sitotroga cerealella proved to be peculiarly adapted to the quantity production of *Trichogramma*. Numerous generations can be reared in rapid succession from stored grain under regulated conditions.

The life cycle of *Sitotroga* in the laboratory is about 28 days and its fecundity at least 50 eggs per female. A high rate of reproduction can therefore be obtained.

The habits and tropisms of *Sitotroga* and their value in the mechanical manipulation of production are as follows:

The newly hatched larvae are negatively phototropic and positively geotropic so that corn in bulk can be easily infested. The feeding and the pupal stages occur within the kernels of grain so that the interspaces are not clogged with frass and webbing. The newly emerged moths therefore have free egress from the deeper layers of grain. Incidentally, this grain has a higher sales value after being used than grain matted with webbing and excreta.

The newly emerged moths are as flexible in their movements as are larvae and at first are negatively geotropic and thigmotropic so that they all issue from compactly arranged tilted bins. They return to the corn to oviposit but do not remain there. No dead moths are found when the bins are emptied.

The moths mate promptly after emergence and their pre-oviposition period, as Simmons and Ellington[22] observed, is less than 24 hours under laboratory conditions. Maximum oviposition occurs within 60 hours after emergence, so that the moths can be handled in crowded egg-deposition cages wherein the adult life is shortened to two or three days.

During the daytime moths tend to crawl upward and come to rest in positions of positive thigmotropism and negative phototropism. Loose boards resting against vertical surfaces serve as 'traps.'

The oviposition responses of the females facilitate the collection of eggs. Crevices about 0.23 mm or 0.01 inch in width stimulate egg deposition. By crowding the bottom of the egg cages with moths their bodies form crevices that stimulate egg deposition and the constant shifting causes the eggs to drop through the 20-mesh screen into a trough below.

The chorion of a *Sitotroga* egg is relatively tough so it can be handled in mass as readily as grains of rice. The egg is of a size that prevents, as a rule, the development of more than one *Trichogramma.* In mass production the average size of the parasites probably is greater than would be the case if either larger or smaller host eggs were used.

Under normal conditions *Sitotroga* oviposits only in crevices but the pressure from a strong current of air directed against a 20-mesh screen can be substituted. The positive anemotropic females come to rest where the air velocity is optimum, thrust their ovipositors through the mesh against the air current, and extrude their eggs, which either adhere to the screen or fall free. Egg deposition is thus obtained by a forced draft. The eggs may be prevented from hatching and held for long periods before being parasitized. According to Back(1) their exposure to 1° F for 24 hours prevents hatching.

Sitotroga has relatively few enemies; bacterial and fungoid diseases are rare. In common with other insects, however, it suffers from the ravages of the mite *Pediculoides ventricosus* Newport. A large mite, *Tyroglyphus* sp., feeds to some extent on the eggs, the flat grain beetle, *Cryptolestes pusillus* Schon., will attack the newly hatched larva, and *Dibrachys boucheanus* Ratzeburg parasitizes the pupae. *P. ventricosus,* however, was the only one of these to interfere with production in this experiment.

THE CHRONOLOGICAL DEVELOPMENT OF REARING METHODS[4]

The development of rearing methods progressed rapidly because of the favorable habits and tropisms of the moths.

In August, 1926, about 500 pounds of infested corn was secured from a neighboring grower and placed in shallow bins. In this preliminary work the moths were collected in small glass vials, about a half-dozen to the vial. Since the female normally oviposits only in crevices, two slips of cardboard held together by metal clips were placed in each vial. The eggs were deposited in masses of a single layer which adhered to the cardboard. Each day the cardboards were removed and placed in vials containing newly emerged parasites.

The outside surfaces of the bins and the entire inside surface of the rearing room was painted green in order that the moths at rest on

[4] The writer is indebted to P. F. Wright, Laboratory Assistant during May, June, and July, 1927, for many helpful suggestions.

them could be easily observed. Early in the experiment the amount of infested corn was increased to nearly 2 tons and was placed in nine bins built one above the other 5½ inches apart (fig. 1). Each bin measured 12 feet long, 3 feet wide, and 3 inches deep. During October, 1926, in order to handle the increase in moth production, 1-gallon battery jars were adopted for egg deposition and halves of petri dishes inverted on square pieces of stiff cardboard for parasite cages.

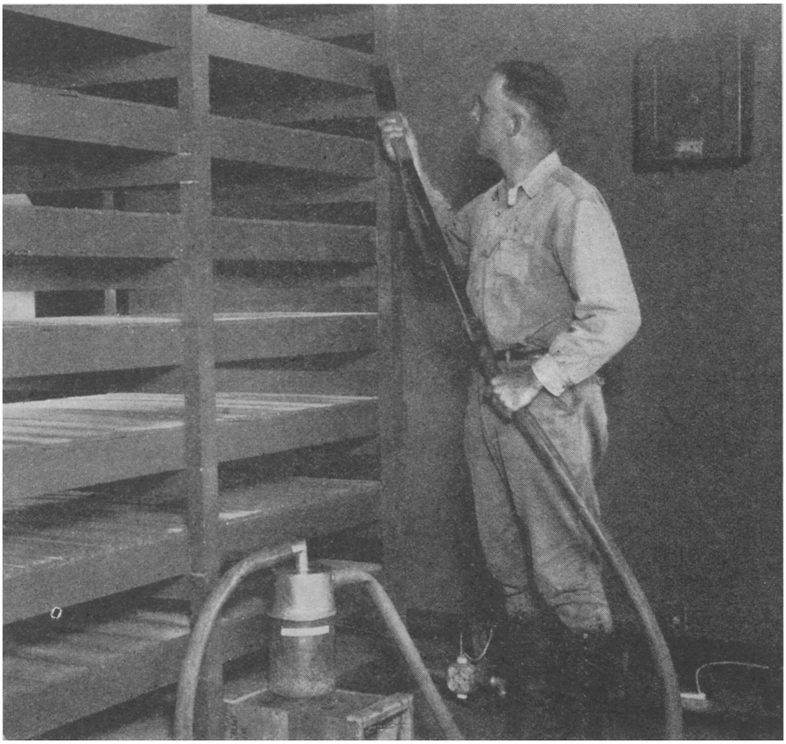

Fig. 1. Type of corn bins used in 1927. Note the heating unit at the rear. (Photo from Los Angeles Times.)

By the first of May, 1927, the daily production of *Trichogramma* amounted to about 25,000. The equipment and method of handling them in use was as follows:

1. 30 egg-deposition cages consisting of
   (*a*) 30 1-gallon cylindrical glass battery jars (numbered consecutively).
   (*b*) 30 celluloid cones fitted to corresponding jars and also numbered. (The numbers were written with ink and covered

thinly with shellac.) The cones were made from sheet celluloid and held in shape with celluloid cement. Each cone was about 6½ inches in height and open at each end, the diameter at the small end being 1 inch and that of the large end equaling the inside diameter of the jar.

(*c*) 30 circular celluloid grates 3½ inches in diameter. Each was formed from a celluloid disk by cutting out seven narrow strips ¼ inch in width to within ¼ inch of the circumference.

(*d*) 30 pieces of unbleached sponge to place in the small end of each cone.

2. A large quantity of circular smooth egg-cards 3½ inches in diameter.
3. A large quantity of blotting paper in strips (⅝ inch × 8 inches).

In assembling an egg-deposition cage a grating was fastened to a circular cardboard with metal clips so that there was no appreciable space between the two disks. This egg-card was then inserted in a tongue slit cut in the celluloid cone about 2½ inches from its base so that the egg-card was held in a vertical position at right angles to the base. The cone was then placed in its jar, point first, touching the glass only with its base, the cone being held in position by its base being wedged at the opening of the jar. When the cones had been in use several days the blotting-paper strips were employed to help bind the cones in place. A single strip was wedged in between the cone and the jar.

The moths on the room surfaces and on the under-surfaces of the bin covers were gathered a few at a time in a large vial and quickly dumped into an egg-deposition cage through the interior of the cone until about 400 were trapped in the cage. As each cage was stocked a sponge saturated with water was placed in the small opening of the cone to provide the moths with moisture and to prevent them from gaining access to the interior of the cone, which formed the exterior of the cage. The cage was then inverted and set aside for 24 hours.

When thirty egg-deposition cages were used they were handled as follows:

Twenty cages were kept stocked continuously. Each morning the moths in these were transferred to the ten reserve cages. After the used cages were taken apart, cleaned, and reassembled, ten of them were restocked with new moths, leaving ten cages in reserve for the following morning.

The transfer of the moths was accomplished by removing the cone and strip, placing a sheet of paper over the jar to retain the moths, inverting the jar over a freshly made up cage, removing the paper and blowing forcibly between the narrow space between the jars.

The moths deposited an average of 1,000 eggs on the egg-card beneath the celluloid grate and quantities on the strip at the base. All loose eggs deposited in the moth debris were collected, sifted, and scattered evenly over egg-cards covered with shellac.

This equipment was too cumbersome and it was decided to decrease the equipment and at the same time increase production.

A suction cap connected with a vacuum pump was designed which fitted over the jars and enabled the operator to collect moths with a great saving of time. Glass shelves were substituted for the cardboard bases of the parasite cages and eliminated a source of trouble.

The fact that the moths also oviposited in debris at the bottom of the cages led to the development of a greatly simplified method of obtaining eggs. In May, 1927, the battery jars were replaced by two cardboard cylinders capped on each end with 20-mesh brass sieves. Sufficient moths were collected and dumped in these cages to cover the bottom. The crevices formed by their bodies stimulated egg deposition. By this means free eggs were obtained. This was desirable for several reasons. The free eggs could be evenly distributed in a single layer over cards of uniform size and thus afford a fairly accurate means of measuring production. When thus fastened to the egg-card the danger of a larva hatching from an unparasitized egg and accidentally destroying parasitized eggs around it was negligible. A moth ovipositing in a crevice deposits her eggs in a horizontal position in a compact mass. The larva emerging at the cephalic pole of the egg may pass through a number of parasitized eggs.

Collection of the moths was facilitated by placing light boards across the top of each bin so that they were in contact with the corn. Each board measured 3 feet × 5 inches × ¼ inch. The moths gathered in numbers on the ventral surface of the board. When collecting the moths each board was removed and the moths scooped up with the collecting hose.

With these changes in method parasite production increased to 200,000 daily in July, 1927. In order to increase the capacity of the insectary for 1928 production, a more compact type of corn bin was designed which permitted the use of at least three times the amount of corn possible with the open bins in the same amount of space.

A description of the equipment used in 1928 follows:

The insectary of the Saticoy Walnut Growers' Association, where *Trichogramma* production was initiated (fig. 2), was divided into two compartments: the laboratory-office and the rearing room. The rearing-room ceiling and walls were lined with plaster board and the floor was of wood. The inside dimensions were: length 17 feet, width 15½ feet, height 8 feet. Two windows, 24 inches high, extended the length of each side. Circulation of air was obtained by means of a rotary ventilator which operates whenever the wind velocity exceeds

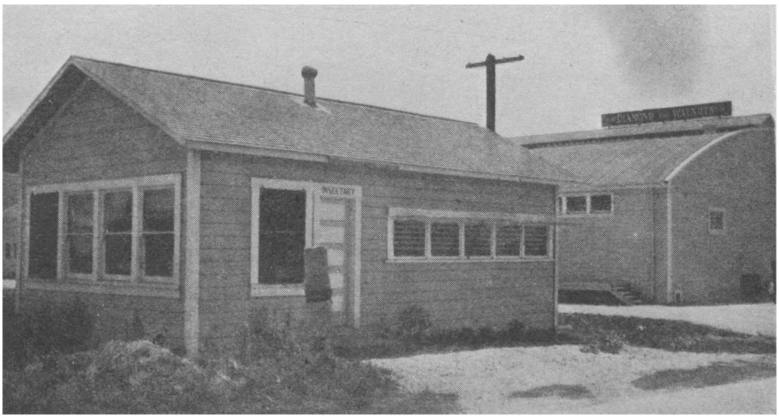

Fig. 2. Insectary of the Saticoy Walnut Growers' Association, where mass production of *Trichogramma* originated.

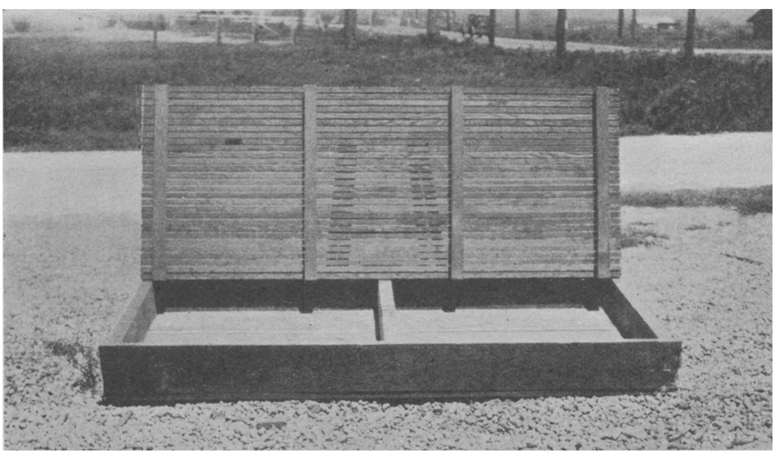

Fig. 3. Type of bin unit adopted in 1928.

two miles an hour. The air passed down through floor openings and upward through an 8-inch pipe that extended above the roof. The room was heated by eight electric heating units controlled by a thermostat.

The grain containers (fig. 3) were shallow bins with covers made of strong slats set 1/8 inch apart. Each bin was 5 feet long, 2 feet wide, and 4 inches deep, with all outside surfaces smooth. The slats were 3/4 inch wide and 3/4 inch thick and surfaced on three sides. The rough

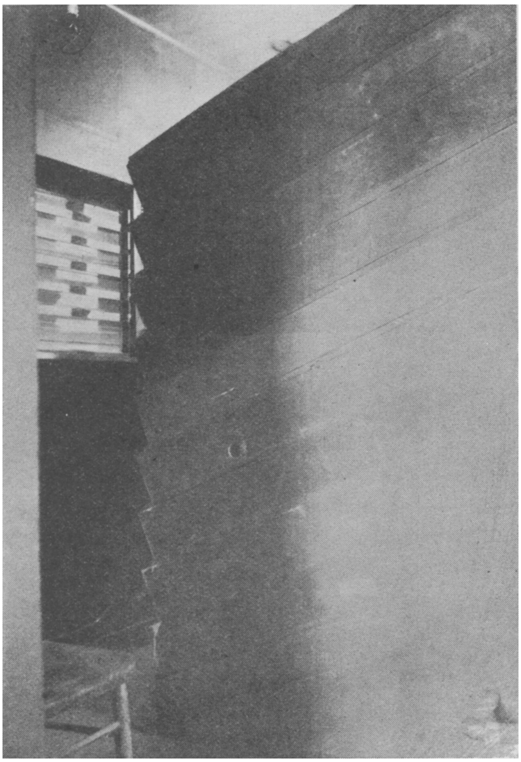

Fig. 4. Side view of bins in position.

side formed the inner surface of the cover. To save construction costs the bins were not constructed to permit filling and emptying without removing from the stacks.

As the bins were placed in the rearing room they were filled with corn and the covers fitted into place and fastened by dowels. The bins were stacked 10 high crosswise of the room (fig. 4), forming two rows lengthwise of 60 bins each. The inner end of each row rested on a baseboard 22 inches high and the outer end on the floor, so that the bins were set at an angle with the floor of about 25 degrees. If this angle is 22 degrees or less bin covers are not needed to hold the corn in place. The bin stacks and enclosures occupied a floor space 13 feet by 12 feet. A solid wooden cover was placed on the top bin of each stack.

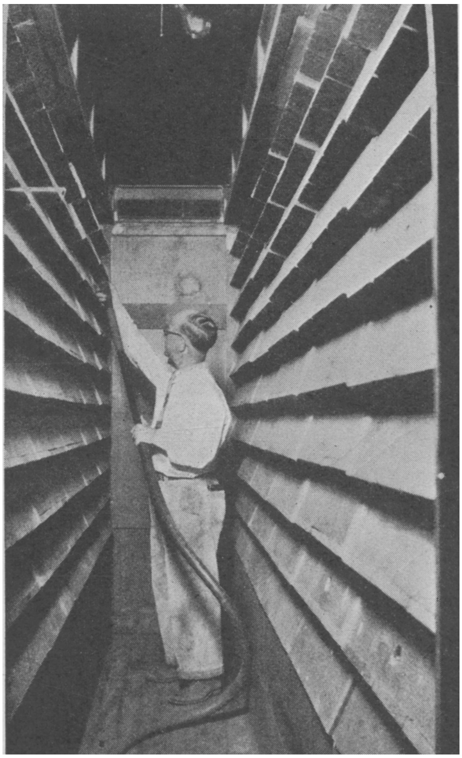

Fig. 5. Moth enclosure between stacks of bins.

The moth enclosure (fig. 5) was 13 feet long, 26 inches wide, and 8 feet high. The air entered the enclosure through ventilators and passed downward between the bins into the surrounding room. All the moths emerging from the grain accumulated in the moth enclosure. Crevices other than those leading into the grain were filled with putty.

The moths were drawn into a 1½-inch hose 17 feet long through a soft-nosed nozzle inserted in the end of the hose. The nozzle used was a standard vacuum-cleaner attachment, one end of which is compresed to make an opening 2 inches long and ¼ inch wide. The hose led into a receptacle (fig. 6) consisting of a gallon battery jar containing an interiorly directed truncated celluloid cone and a tin cover 6 inches in diameter and 4 inches high. The cover was provided with an inlet terminating a short distance above the smaller end of the cone and below a horizontal wire screen above which was an outlet that connected with the source of partial vacuum. The moths were prevented from passing out of the receptacle through the outlet by the wire

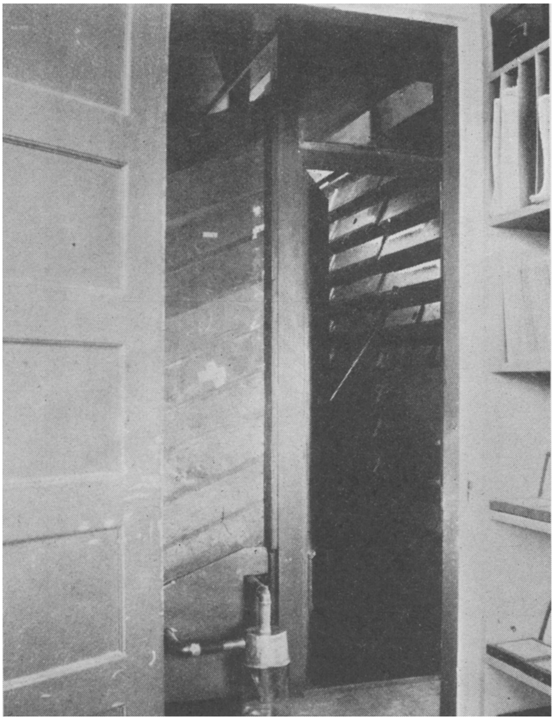

Fig. 6. Entrance to moth enclosure and moth trap in position.

screen and were retained in the jar by the cone. The wire screen also served to hold the inlet tube in position. The jar could be removed from under the metal cover without loss of moths.

After removal the jar was shaken sharply to cause the moths to settle in the bottom. The moths were then dumped into the egg-deposition cages (fig. 7). A strong current of air passing across the top of the cage tends to inhibit the flight of the moths. The 20-mesh screen will allow many of the males to escape.

The egg-deposition cages consisted of smooth cardboard cylinders capped on each end with 20-mesh brass sieves. Enough moths were placed in each cylinder to cover the bottom screen so as to prevent any light from filtering through. The cages were then set over a truncated trough (fig. 8) beneath which was placed a sheet of paper to catch the eggs dropping through. A mild current of air was circulated beneath the cages to carry away the scale dust.

The equipment for preparing the eggs for parasitism (fig. 10) consisted of 18- and 30-mesh wire strainers, moth-rinsing jar, filter

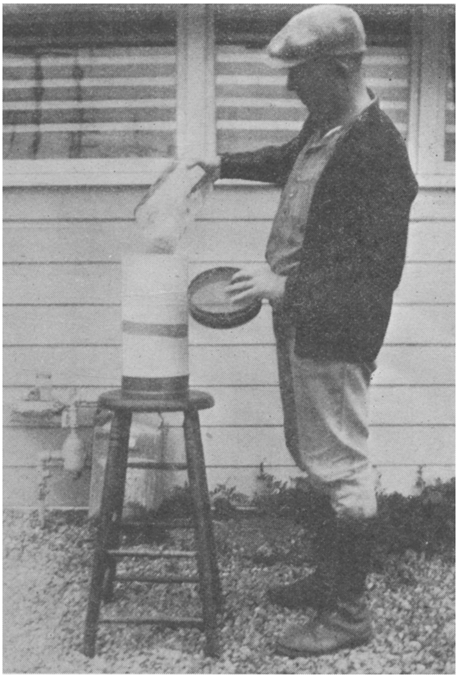

Fig. 7. Emptying moths from trap into egg-deposition cage.

paper and funnel, shellac, brushes, and cardboard disks or egg-cards. The diameter of each egg-card was 3½ inches; that of the aperture in the center ¾ inch. When the eggs were evenly distributed over the card the approximate number of eggs could be determined by multiplying the number of eggs visible in the low-power field of the binocular microscope by the number of times the area of the card exceeded that of the microscope field. The cards were placed on spindles (fig. 11) for storage and shipment in mailing tubes.

The inner face of each window in the rearing room was lined with a tier of six plate-glass shelves 5 inches wide (fig. 12). The shelf capacity was sufficient for 600 petri-dish cages. These were selected for their even edges so that when inverted on plate glass they would make contact at every point and prevent the escape of the parasites. The inside diameter of the petri-dish cages was 3¾ inches and they

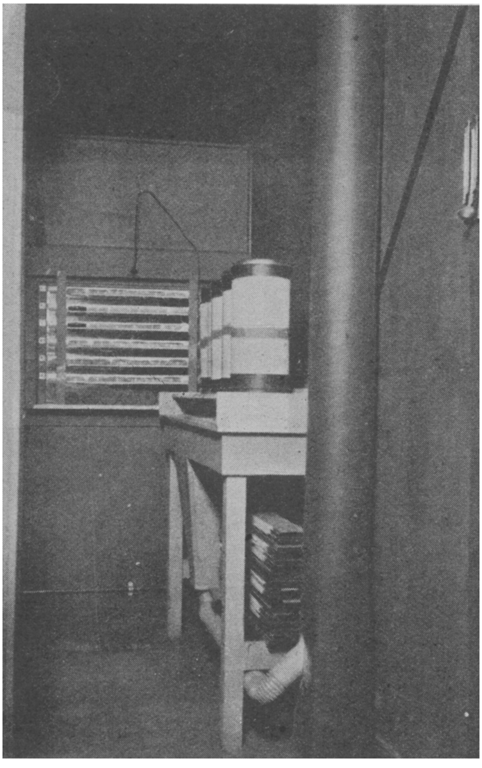

Fig. 8. Egg-deposition cages in position over trough.

easily covered the 3½-inch egg-cards. With this type of cage the egg-cards can be quickly removed and replaced with practically no loss of parasites.

An electric refrigerator was used for the cold storage of the parasitized eggs. The freezing unit was "de-iced" daily in order that the refrigerator might be used continuously. The dehydrating effect of the freezing unit in operation can be minimized by placing the parasitized eggs in small air-tight containers in the storage compartment.

Fig. 9. Separating eggs from moth debris before winnowing. (Photo from Los Angeles Times.)

Fig. 10. Equipment for separating eggs and preparing egg-cards.

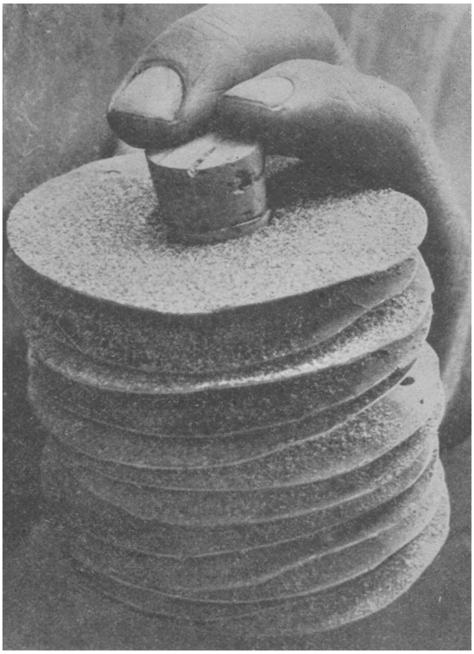

Fig. 11. Egg-cards on spindle for convenience in handling. (Photo from Los Angeles Times.)

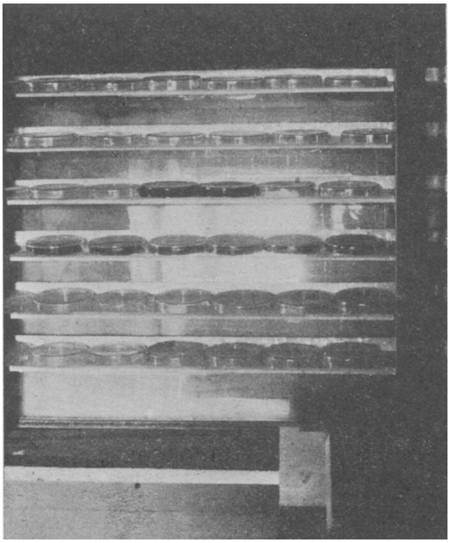

Fig. 12. Parasite cages in position on plate-glass shelves.

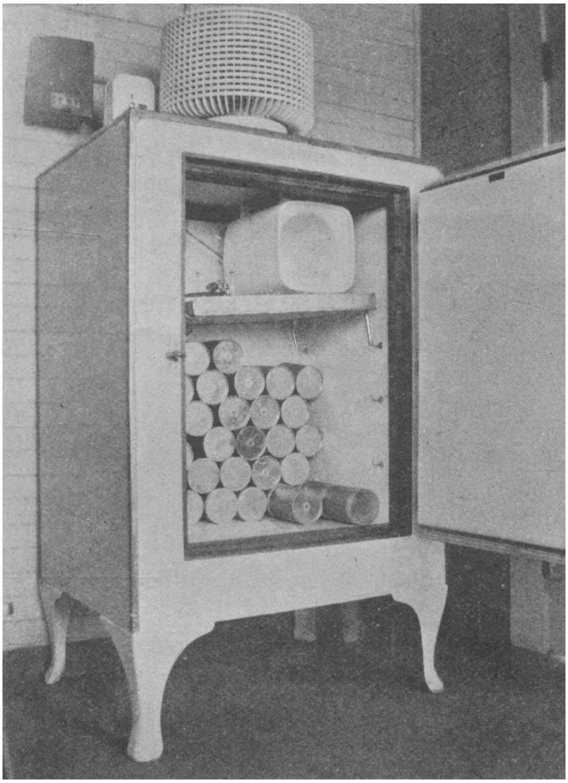

Fig. 13. Mailing tubes containing parasitized eggs in cold storage.

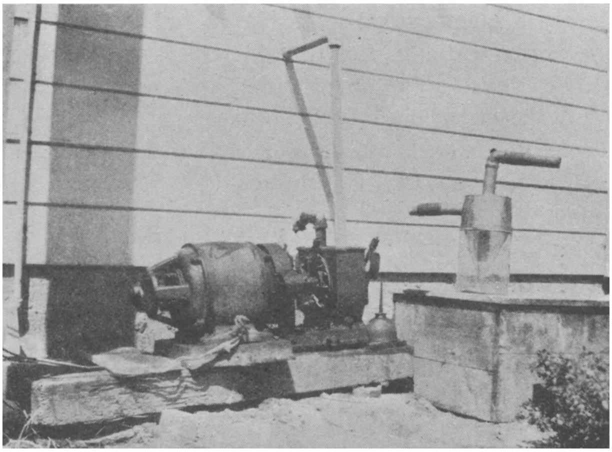

Fig 14. Lammert compressor as a source of suction for moth collection. On the box is the type of trap used.

The Lammert compressor (fig. 14) was connected with the moth trap by 17 feet of 1-inch pipe. It was operated by a 1½ H.P. motor, and the full amount of suction when used with a 1¼-inch collecting hose did not injure the moths.

A Royal dryer can be used instead of the compressor. It has the advantage of being portable. It can be fastened to the suction cap as in figure 17.

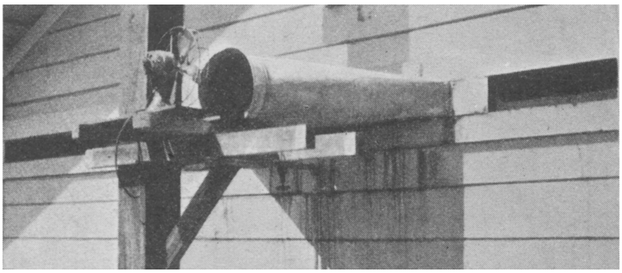

Fig. 15. Humidifier on outside of insectary.

The humidifier (fig. 15) was set in operation when the humidity in the moth enclosure dropped below 55 per cent. This apparatus consisted of a flattened sheet-iron funnel about 30 inches in length. A very small stream of water under high pressure was injected through the bottom of the funnel. This was broken into a spray and vaporized by passing it through an 80-mesh screen. A fan forced the moisture laden air into the moth enclosure through the ventilator. Two wire screen 4 inches apart in the large end of the funnel served to collect the excess water in the air and pass it outward. The inner screen, which is next to the spray inlet, was made of ordinary fly screening, and the outer one of 20-mesh screen. It is preferable to place the humidifier in the rearing room and recirculate the room air. A more uniform vaporization is obtained.

REARING EXPERIENCES IN 1928

In preparation for the 1928 production ½ ton of white corn was vacuum-fumigated with carbon disulfide for 24 hours and then placed in one of the rooms of the county insectary August 10, 1927. The temperature of this room was maintained at 80° F. Beginning several days later thousands of *Sitotroga* eggs were placed on the corn daily for a period of 20 days. Moths appeared at the end of 24 days.

By the first of October the old infested corn had been removed from the rearing room and sold for stock feed. Pending the resumption of moth production a million parasitized eggs were placed in cold storage in the local meat market.

Upon completion of the new bins the first of November the half ton of newly infested corn and an additional ton of uninfested corn were placed in the rearing room. Unfortunately, the precautions taken to prevent an infestation of the black weevil, *Sitophilus oryza* L., had not been sufficient, for several specimens were found in the bottom of the containers. These became so numerous by March as to be troublesome during the collection of the moths.

On December 7 the remaining bins were filled with 7½ tons of newly harvested corn. Some of the corn, which at the time felt heavy with moisture when held in the hand, later developed mold to the extent that infestation by the moths was prevented in eighteen of the bins. Moths began to emerge from the rest of the corn the first week in January. One hundred kernels from the upper part of one bin showed an infestation of 30 per cent on January 30.

At this time parasite production recommenced. By the first week in February 150,000 eggs were collected daily and parasitized. The number of moths then began gradually to decrease.

In order to bring about an increase, all of the eggs collected during the first 5 days in March were blown back into the bins. As a result there was a marked increase on March 28, and during the first week in April an egg production of 400,000 was attained. This peak, however, was followed by a rapid drop in production until by the middle of April the daily production was less than 50,000. During a 3-day period ending April 15 all of the eggs were again replaced.

On April 20 it was found that the corn contained only about 6 per cent of moisture. The rapid circulation of air maintained to prevent the corn from molding had evidently been continued too long. An indicator that should have received recognition was the foreign grain beetle, *Cathartus advena* Waltl. This fungus feeder was very abundant while the mold was developing on the corn but about the middle of March it suddenly disappeared.

In order to rapidly raise the moisture content of the corn the entire 9 tons was removed, run through a fanning mill, sprinkled with quantities of water, and then replaced. This procedure occupied 5 days beginning April 24 and raised the moisture content of the corn about 3 per cent.

The percentage of infestation in the corn was ascertained at this time. A count of the emergence holes in the kernels showed that the upper or inner portions of the bin averaged 27 per cent, the middle 6 per cent, and the lower or outer portion 11 per cent. Probably most of this infestation took place during the months of December, January, and February.

On the thirteenth day after the replacement of the corn an egg production of 150,000 was again reached. This occurred 28 days after the second replacement of eggs. After this peak, however, production rapidly declined to zero.

Many moths emerged but hardly had their wings expanded when they dropped to the floor and succumbed to some obscure ailment. The floor was swept twice daily, yet 6 hours after each sweeping it would be covered with moths showing no sign of injury or wear. Many of the moths died in copula and the females were turgid with eggs.

A microscopic examination of the sweepings revealed the presence of the mite, *Pediculoides ventricosus,* in great numbers. It was found in greater abundance on the top surfaces of the bin stacks. It attacked both the egg and the adult. The mite *Tyroglyphus* also disappeared with the advent of *P. ventricosus.*

The last full egg-card to be prepared was found to be infested with many mites. An examination of egg-cards prepared only a week earlier, however, showed no infestation.

This rapid rise in abundance of mites indicates that conditions prior to the removal and replacement of the corn had not been optimum for their development. The high humidity maintained after the installation of the humidifier on April 28 may have been the immediate cause of the outbreak. The relative humidity was maintained at an average of approximately 70 per cent, 30 per cent higher than was maintained before the installation of the humidifier.

The mites, however, had probably been present for a considerable time, for dead moths had been observed on the floor in March. It is possible that mites instead of lack of moisture were responsible for the earlier reduction in moth production.

The mites are very susceptible to sulfur fumes. On May 19 the 9 tons of corn were fumigated with 12 pounds of liquid sulfur dioxide. For several days thereafter it was impossible to find any mites, but a week later they were again plentiful. Sulfur dioxide apparently acts as a repellant and also as a poison to the black weevil. It caused great numbers of them to leave the bins and their audible gnawing of the corn ceased almost entirely. Ten more pounds of sulfur dioxide were applied late in June against the mites, greatly reducing their numbers.

All of the corn was again removed July 17. On this occasion it was noted that all of the black weevils in many of the bins were dead.

From the beginning conditions in the rearing room were more favorable to the development of the *Sitotroga* than for either *Ephestia keuhniella* or *Plodia interpunctella*, for although the latter were present in small numbers early in the season they disappeared almost entirely. Since *Sitotroga* larvae occasionally attack each other, they may have been the cause of the disappearance of the other moths.

The destructive weevil can be eliminated by the sterilization of the rearing room and vacuum fumigation of the new stock of grain prior to its inoculation with *Sitotroga.*

In September the used corn was sold for $1.95 a hundred pounds and 5 tons of new corn purchased. This was fumigated in a vacuum for 48 hours with hydrocyanic-acid gas and for 48 hours with carbon bisulfide. The rearing room was cleaned and then fumigated with liquid sulfur dioxide and hydrocyanic-acid gas. On October 7 the upper halves of the bins were filled with a total of 9,300 pounds of corn. During the following month almost a million moth eggs were placed on the corn. The eggs were obtained from a half ton of infested corn at the county insectary. The room was kept heated artificially until the heat of infestation was sufficient to keep the temperature in the bins at 70° to 95° F, which according to Back[1] is high enough for rapid development. With production under way it is desirable that the air into which the moths emerge should be cooler than the corn. The moth-rearing room should, therefore, be separate from the room used for moth egg-deposition and parasitism. As Schulze[20] found, a temperature of 81° F is optimum for *Trichogramma* propagation.

The daily collection of moths for egg production commenced December 10. Production increased until on January 20 the collection of eggs reached 600,000 daily. A month later, however, *Pediculoides* stopped egg production entirely. It is possible that insects such as the cadelle beetle and flat grain beetle are carriers of this mite and were responsible for its introduction to the rearing room.

The mite hazard could probably be reduced to a minimum by building up an infestation in eight weeks to a level at which egg production is sufficient for economical parasite propagation, i.e., 100,000 eggs daily per ton of corn used, maintaining this rate of production for about four weeks, and then fumigating the corn and reinfesting it or renewing with fresh corn.

To insure uninterrupted production auxiliary rearing rooms are necessary.

DAILY PROCEDURE IN PRODUCTION IN 1928

The procedure followed in the daily routine of production was as follows:

Once every 24 hours the eggs in the trough beneath the egg cages were collected, screened, and winnowed of moth appendages and scales. The cages were lightly shaken to dislodge eggs adhering to the moths.

The accumulated eggs were then poured onto freshly shellacked cardboard disks and all of the eggs not adhering were shaken off. The shellac at the moment of applying the eggs must be sticky enough to hold the eggs but not so fluid as to engulf them. It is best applied with a small brush.

After allowing the cards to dry for about half an hour or until the alcohol in the shellac has evaporated, they were placed in the parasite cages for a period of 24 hours with night illumination.

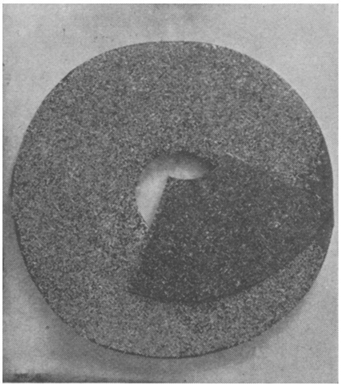

Fig. 16. A fresh egg-card and segment of emergence card. (Photo from Los Angeles Times.)

Before the introduction of the new egg-cards, one-third of a card (fig. 16), from which parasites had begun to emerge, was placed in the parasite cages to provide the stock required to impregnate at least 90 per cent of the new eggs.

Each emergence card was used for two days so that a six-fold increase was obtained.

At the end of 24 hours the new egg-cards were removed and suspended on hooks to aid the negatively phototropic and positively geotropic larvae from unparasitized eggs to leave the cards. Three days later the parasitized eggs had turned black, an indication that the parasites were in the prepupal stage. The cards were then placed in cold storage (fig. 13), one-sixth being retained for emergence cards.

After the collection of eggs and the preparation of the parasite cages, the day's crop of moths was gathered by means of the vacuum collector and dumped into the egg-deposition chamber.

After remaining in the egg-deposition chamber 2 days the moths were poured into a wire strainer and rinsed under water pressure to remove the adhering eggs. These eggs settled to the bottom of the container and the debris rising to the surface was drained of. The eggs were then separated out by means of filter paper or an 80-mesh brass sieve from which, after drying, the eggs were easily brushed off. Thousands of eggs were conserved in this manner in addition to those collected daily from beneath each cage.

When mailing cans were used for parasite cages the egg-cards were made in oblong sheets that fitted closely to the inside surface of the cages. These cards were pinned in position. Each card carried on one side about 150,000 eggs, or 6 eggs to each square millimeter. This type of cage was used in the same manner as the petri-dish cage. When inverted on the plate-glass shelves the metal bottom of the container reflected the light from below. Mercury vapor tubes 24 inches long fastened to the ventral surface of the glass shelves may be used for illumination.

TRICHOGRAMMA PRODUCTION IN MEXICO

In the spring of 1929 the writer, at the request of Señor Diego Redo, initiated the production of *Trichogramma* on his sugar plantation on the west coast of Mexico. The native strain of *Trichogramma* was used. The initial stock was obtained the last of February from parasitized eggs of the milkweed butterfly, *Danaus menippe* (Hbn.). A great quantity of corn, shelled and on the ear, stored in bulk in the plantation warehouse, was found to be heavily infested with many kinds of grain pests, but chiefly *Sitotroga.* The burlap method was devised for collecting the moths. Burlap sacks were laid out evenly on the surface of the corn. Moths congregated on the ventral surface in numbers. Once or twice a day each sack was lifted clear of the grain and looped to form a funnel, the lower end encircling a cylindrical receptacle made of 20-mesh brass screen about 8 inches in diameter. Several sharp blows on the burlap precipitated the moths into the screen container. This container was then used as an egg-deposition cage. It was placed in a housing so constructed that when a strong current of air was directed on it, oviposition was stimulated. Eggs not adhering to the outside surface of the screen dropped into a trough beneath. The outside of the cylinder was often white with eggs which were easily brushed off.

By the first of June the laboratory operator reported a daily production of 400,000.

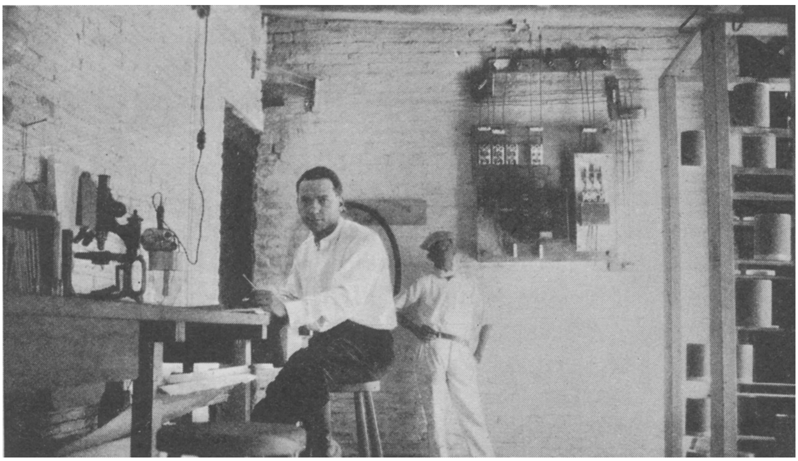

Fig. 17. Interior of the Redo y Cia Insectary at Eldorado, Mexico. Note the plate-glass shelves and the mailing tubes used as parasite cages.

The equipment used in handling the host eggs and the parasites was as follows:

FOR HANDLING EGGS

2 20-mesh screen cylinders (8 × 18 inches) with removable cap.
1 electric fan.
1 26-mesh wire strainer.
1 18-mesh wire strainer.
1 roll of mechanics pattern paper for egg-cards.
1 pair of shears.
1 gallon of white shellac.
1 brush for applying shellac.
1 brush for removing eggs from egg-deposition cylinders.
1 16-mesh strainer (6 inches in diameter in which to wash dead moths.
1 80-mesh brass sieve to strain out eggs washed from moths.
1 brush to remove eggs when dried.
2 glass gallon jars (6 inches in diameter).

FOR COLLECTING MOTHS

A number of burlap sacks.

1 Royal electric dryer mounted on a suction cap fitted to a glass gallon jar and having a 1¼-inch hose 5 feet in length attached.

FOR PARASITIZING EQUIPMENT

50 feet of plate glass 4 to 5 inches in width.

2 dozen selected glass petri dishes (4 inches in diameter).

12 dozen mailing tubes (5 × 3½ inches) with metal bottoms.

Several sheets of emery paper to smooth down rims of the mailing tubes used as parasite cages.

6 dozen rolls of fine wire.

STORAGE OF *TRICHOGRAMMA*

The optimum conditions for the cold storage of *Trichogramma minutum* have yet to be worked out. Fiske[10] kept parasitized eggs successfully for about ten months at temperatures of 28° to 30° F, placing them in cold storage the moment they began to turn black. He found[12] that if the young parasites are subjected to a low temperature their development is delayed many days even though exposed later to continuous high temperatures.

Mokrzecki and Bragina[16] were able to maintain *Trichogramma semblidis* in cold storage for ten months. They reported that hibernation sets in at 38° to 39° F. Zorin[26] found that the larvae of *T. evanescens* when kept at a temperature of 50° and 51.8° F enter the diapause and do not pupate.

Trichogramma reached maturity in 32 days when the average mean daily temperature for the period of development was 55.4° F. Twenty-six days were required at 58° F. The life cycle as determined by Mokrzecki was 38 to 43 days at temperatures from 48° to 52° F.

In March, 1928, a well-parasitized egg-card was sent to E. J. Newcomer at Yakima, Washington. He was notified that the parasites should emerge beginning March 19. This they did, but as he had no host eggs he placed the card in the cellar where the temperature was from 45° to 55° F. A month later he was considerably surprised to find many of the parasites alive.

The cold-storage facilities on trans-Pacific steamers made it possible to successfully transmit several thousand parasitized eggs to Australia in August, 1927.

In two local strains of *Trichogramma* there is a noticeable difference in coloration between the females developing at high temperatures and those at low temperatures. The bodies of the former are light yellow, while the latter are as dark as the males. The strain from a cool moist coastal region turn dark at a higher temperature than the strain from a hot, dry interior valley.

PROCEDURE IN FIELD LIBERATIONS

The procedure in liberation of the *Trichogramma* in the field was as follows:

The egg-cards were placed in the field just as emergence began. The cards were cut into sections of 10,000 eggs and a fine wire several inches in length was attached to each section.

Each section was suspended from the lower portion of the food plant of the host. The parasites are usually negatively geotropic. An egg-card suspended by the fine wire is protected to a greater degree from attack by predators than when in contact with the plant.

In timing the sequence of field liberations an increase or decrease of one degree in the mean daily temperature was considered as shortening or lengthening the developmental period one day.

REACTION OF *TRICHOGRAMMA* TO FIELD CONDITIONS

Complete emergence in the field required usually from 3 to 6 days. After 6 days the cards were examined to determine the amount of emergence. The fact that the males remain on the card, attracted by the emerging females, insures reproduction in the field, for unmated females produce only males. Male parasites may be found on the cards long after the females have left.

The rate of dispersion from the point of liberation varied directly with the daytime temperature and light intensity and inversely with the excessive air movement and surface moisture.

For a period of 8 days many parasites were found on the ventral surface of leaves in the immediate vicinity of an egg-card when the mean daily temperature had been 58° F. If the weather was cool and cloudy the parasites crawled up the wire in search of protected ventral surfaces.

In the field optimum temperature conditions for the increase of *Trichogramma* may be at a lower level than that which is optimum for the development of its host. For any increase to take place, however, it is essential that the distribution of the host eggs be dense enough to permit the parasite population bridging the gaps between them. The rate of increase tends to vary inversely as the square of the average distance separating the hosts.

At Saticoy where the normal mean temperature in summer is about 65° F a high degree of codling-moth parasitism is obtained by the liberation of *Trichogramma*. The comparatively low temperature and corresponding high humidity prolongs the life of the parasites, increasing the amount of ovulation and the chance of finding a larger number of host eggs than may occur when temperatures are higher and host eggs more abundant. Schulze[20] found that the life of the adult seldom exceeded one day at 89.6° F but at 77° F they lived an average of 13 days. Hase[9] kept adults alive with an abundance of food and at room temperature for 30 days. At high temperature and low humidity the activity of *Trichogramma* appears excessive and aimless. Mokrzecki and Bragina[16] found that in the laboratory temperature of 95° to 100° F were fatal.

According to Barber[2] *Trichogramma* appears to parasitize the European corn borer most effectively in cool seasons. He states that "the probable longer periods of life of adults [of the corn borer], caused by low temperatures, served to spread oviposition over a relatively longer period, which was favorable for the development of the egg parasite *Trichogramma minutum* Riley, and the relatively fewer eggs deposited per female probably served to increase the importance of the parasite." That is, in the cooler seasons the more uniform supply of host eggs insures the increase of *Trichogramma* and the smaller number of eggs results in a higher percentage of parasitism. The most important effect, however, is the lengthening of the incubation period of the host egg, exposing it to attack for a longer period. The temperatures at which parasitism takes place range from about 60° to 100° F. The developmental range of the parasite is from about 40° to 100° F. It is therefore probable that in some of its host relations the sphere of activity of each parasite and the percentage of parasitism is greatest when the daily temperatures are somewhat below the optimum for the increase of its host. The actual amount of parasitism, however, is determined by the host population.

EFFECT OF LIBERATIONS

In determining the effect of liberations the natural parasitism must be taken into consideration.

The earliest record of codling-moth parasitism in the vicinity of Saticoy was made in 1924. On a walnut tree in the center of a 70-acre grove 348 eggs were found to be 5 per cent parasitized. Most of this parasitism was due to *Prospaltella* sp.[5] In 1926 on a walnut tree 461 eggs were found to be 49 per cent parasitized.

TABLE 1

NATURAL AND ARTIFICIAL PARASITISM DURING A THREE-YEAR PERIOD ON ONE TREE NEAR THE CENTER OF A 30-ACRE WALNUT GROVE

		Weekly inspection								
		June		July					August	
1926 Natural parasitism	Live eggs	68	61	154	196	131	48	35	86	1
	Hatched eggs (no record)	....	....	....	....	....	....	....	....	....
	Black parasitized	0	5	20	29	38	15	12	5	0
1927 Artificial parasitism	Live eggs	....	....	....	50	54	11	3	7	0
	Hatched eggs	....	....	....	0	5	34	14	0	0
	Black parasitized	....	....	....	0	26	51	20	17	4
1928 Artificial parasitism	Live eggs	....	64	279	156	131	171	....	107	....
	Hatched eggs	....	20	35	19	119	116	....	105	....
	Black parasitized	....	2	3	53	184	52	....	23	....

In 1927 40,000 *Trichogramma* were liberated one week prior to the first inspection.
In 1928 9,000 *Trichogramma* were liberated at the first inspection.
Black parasitized eggs appear the second week after liberation.
Natural parasitism on the walnut codling moth in 1927 and 1928 was very light.

In 1927[(5)] and 1928 the natural parasitism of codling-moth eggs on walnut trees was very light. Hundreds of walnut trees were closely examined each year by four trained inspectors. The highest number of parasitized eggs found on any walnut tree since 1926 was 12 and these were all due to *Prospaltella*.

In 1928 it was found that the parasitism of codling-moth eggs on dooryard pears and apples was in some cases nearly 50 per cent.

[5] All the known species in this genus of parasites attack armored scales or white flies. That its attack on the codling-moth egg is accidental is borne out by the fact that so far only males have been obtained. It is a factor, however, in the evaluation of natural parasitism. Unlike the sac-like larve of *Trichogramma*, the larva of *Prospaltella* is eel-like and more active and voids solid meconium prior to pupation. The presence of this excrement indicates *Prospaltella* as having parasitized the egg.

Several hundred of the parasitized eggs were examined and it was found that 30 per cent of the parasitism was due to *Prospaltella.*

Observations were made of the parasitism on one walnut tree for three successive years (table 1). Each year codling-moth eggs were found on this tree in greater numbers than on any other tree in the vicinity. Parasitism on surrounding trees was very light.

In 1927 many thousands of *Trichogramma* were liberated in the walnut groves of the Association but accurate checks upon the percentage of parasitism were made only on six trees (table 2). The percentage of parasitism was first ascertained about two weeks after the first liberation when the parasitized eggs turn black. The effect of the release of *Trichogramma* was to increase the percentage of parasitism in three weeks from less than 1 per cent up to as high as 52.4 per cent on the most highly infested trees.

TABLE 2

PARASITISM RESULTING FROM THE LIBERATION OF APPROXIMATELY 100,000 *Trichogramma* ON SIX WALNUT TREES JUNE 27 AND JULY 6, 1927

Tree	A	B	C	D	E	F	Average
Number liberated	25,000	15,000	10,000	15,000	10,000	25,000	16,666.6
Hatched eggs	127	68	124	94	88	109	101.6
Parasitized	140	26	30	66	41	47	58.3
Per cent parasitized	52.4	27.6	19.5	41.2	31.8	30.1	36.4

Natural parasitism was less than 1 per cent.

The cards were placed on the trees 1 day prior to emergence. The amount of emergence was not determined.

In 1928 fewer parasites were available owing to the destruction of the laboratory host by *Pediculoides.* Cold-storage parasites, however, were liberated on one highly infested walnut tree and also on nine apples and pears on the Lloyd-Butler Ranch. As in 1927 a marked increase in parasitism followed the liberation (table 3). A comparison was made between the artificial parasitism obtained on the pears and apples on the Lloyd-Butler Ranch and the natural parasitism on check pear and apple trees located 3 miles distant. The parasitism on these check trees was the highest of any trees examined for use as checks. The highest natural parasitism was 45.7 per cent and the highest artificial parasitism was 72.9 per cent. The parasitism on each tree in the test plot was higher than 45.7 per cent, the maximum natural parasitism on the check trees. Because of the natural parasitism the influence of the liberated parasites on the total parasitism was probably greatly reduced.

In May, 1928, the writer sent 40,000 parasitized eggs to C. H. Alden in Georgia. In June he reported that the parasites emerging from them were very effective; over 200 codling-moth eggs were parasitized in approximately 220 eggs under observation.

TABLE 3

NATURAL PLUS ARTIFICIAL PARASITISM IN 1928 ON DOOR-YARD APPLE AND PEAR TREES DURING FIRST-BROOD EGG DEPOSITION

Summary of observations made each week from May 25 to June 29 on pear

	P1*	P2	P3	P4	P5	P6	P7	C1†	C2	C3
Number liberated	1,200	3,800	4,500	1,200	2,500					
Date of liberation	May 5	May 5	May 13	May 5	May 5					
Hatched	63	145	154	105	49	45	22	157	110	139
Parasitized	170	161	257	267	67	72	19	75	60	117
Per cent	72.9	52.6	62.5	71.7	57.5	61.5	46.3	32.3	35.3	45.7

Summary of observations made each week from June 1 to June 29 on apple

	A1*	A2	A3	A4	†C4
Number liberated	3,000	3,800	2,500	3,500	
Date of liberation	May 13	May 5–13	May 13–20	May 5–15	
Hatched	56	47	92	60	103
Parasitized	121	80	156	83	18
Per cent	68.3	63.0	62.9	58.0	14.8

* All trees marked *P* and *A* form a small door-yard orchard on the Lloyd-Butler Ranch.
† All trees marked *C* are located three miles west of trees *A* and *P*.
Cards were placed on the trees the day emergence began.

POSSIBILITIES IN THE USE OF *TRICHOGRAMMA*

The possibilities in the practical use of *Trichogramma* in biological control work deserves full investigation, since the production of this parasite in large quantities is now feasible.

Within the last two years over a score of entomologists have turned their attention to *Trichogramma*, and are investigating its use against the codling moth, the European corn borer, sugar-cane borer, the pecan nut case-borer, the corn ear worm, celery leaf-tyer, tea tortrix, oriental peach moth, rice moth, and cabbage butterfly.

In case of field-crop insects such as the European corn borer, early mass liberations when host eggs are fairly abundant and there is no natural parasitism may result in the building up of a parasite population early in the season. In such a case success is dependent on the natural accretion.

To employ this *accretive method* in the field when host eggs are comparatively scarce is useless. If liberations are delayed until the host eggs become abundant the acceleration of the natural parasitism probably would not be affected by the addition of more parasites. The saturation point of *Trichogramma* is not likely to be advanced since its survival potential decreases as its density of population increases above a certain point. The saturation point appears to be determined by unknown environmental complexes. For the parasite to be of value its population at this saturation point must be dense enough to prevent the host from becoming economically injurious. The most likely means by which the degree of parasitism may be built up earlier in the season is through utilizing egg-concentration centers. In the case of night-flying hosts having females which are in some degree positively phototropic, egg deposition may be concentrated in the vicinity of lighted points in the infested area. *Trichogramma* could then be colonized at such focal points and an early increase in parasitism started.

In the control of orchard insects, such as the codling moth or orange tortrix, parasites should be liberated each week or twice a week during the first part of the egg-deposition period of the moth, including the peak of egg deposition, possibly 10,000 to 50,000 being placed on each tree during the season. This type of control is analogous to spraying and dusting in that a greater amount of lethal material is used than is actually effective, that repetition may be necessary, and that the effect is more or less immediate. This might be called the *inundative method.*

In the control of the codling moth on apple trees the first effort will be to substitute *Trichogramma* for the cover-spray applications of lead arsenate and thus eliminate the arsenical residue problem.

The basis for commercial control by means of this parasite is mass production at a low cost. It is probable that in the near future improved methods in rearing will reduce the cost of *Trichogramma* production to less than $10.00 per million.

LITERATURE CITED

[1] BACK, E. A.
1922. Angoumois grain moth. U. S. Dept. Agr. Farmers' Bul. **1156**:6–7.

[2] BARBER, G. W.
1925. A study of the decrease of the European cornborer. Ecology **6**:39–47.

[3] DEN DOOP, J. E. A.
1918. De verspreiding van *Trichogramma,* den eiparasiet van *Heliothis obsoleta* Fabricius, ter Oostkust van Sumatra. Mededeelingen van het Deli Proefstation **19**:213–220.

[4] ENOCK, F.
1895. [Remarks on *Trichogramma evanescens* Westw. recorded by the Secretary of the South London Entomol. and Nat. Hist. Society.] The Entomologist **28**:283.

[5] FLANDERS, S. E.
1927. Biological control of the codling moth. Jour. Econ. Entomol. **20**:644.

[6] FRANKLIN, H. J.
1915. Report of Cranberry Substation for 1914. Massachusetts Agr. Exp. Sta. Bul. **160**:110–112.

[7] GIRAULT, A. A.
1911. Synonymic and descriptive notes on the Chalcidoid family *Trichogrammatidae* with descriptions of new species. Trans. Amer. Entomol. Soc. **37**:43–55.

[8] HARLAND, S. C.
1916. Notes on *Trichogramma minutum* (Pretiosa). West Indian Bul. **15**:168–175.

[9] HASE, A.
1925. Beiträge zur Lebensgeschichte der Schlupfwespe *Trichogramma evanescens* Westw. Arb. Biol. Reichsanst. Land-Forstw. Berlin **14**:171–224.

[10] HOLLOWAY, T. E.
1913. Some methods of handling minute hymenopterous parasites. Jour. Econ. Entomol. **6**:341–344.

[11] HOLLOWAY, T. E., W. E. HALEY, and U. S. LOFTIN.
1928. The sugar-cane moth borer in the United States. U. S. Dept. Agr. Tech. Bul. **41**:40–63.

[12] HOWARD, L. O., and W. F. FISKE.
1911. The importation into the United States of the parasites of the gipsy moth and the brown tail moth. U. S. Dept. Agr. Bur. Entomol. Bul. **91**:256–260.

[13] LINTNER, J. A.
1882. On an egg parasite of the currant saw-fly (*Nematus ventricosus*). Psyche **4**:48–51.

[14] MARCHAL, P.
1927. Contribution a l'etude genotypique et phenotypique des Trichogrammes. Les lignees naturelles de Trichogrammes. Compt. Rend. Acad. Sci. France **185**:489–493, 521–523.

[15] MARTIN, C. H.
1928. Biological studies of two hymenopterous parasites of aquatic insect eggs. Entomol. Americ. **8**:105–131.

[16] MOKRZECKI, S. A., and A. P. BRAGINA.
1916. The rearing of *Trichogramma semblidis* Aur. and *T. fasciatum* P. in the laboratory and temperature experiments on them. Salgir Exp. Pomological Station, Simferopol, Crimea. (Text Russian.) (As abstracted in Rev. Appl. Entom. ser. A **5**:155–156. 1917.)

[17] PATTERSON, J. E.
1929. The Pandora moth, a periodic pest of western pine forests. U. S. Dept. Agr. Tech. Bul. **135**:1–19.

[18] PORTCHINSKY, I. A.
1913. *Phalera bucephala* L. and its importance for the artificial breeding of *Pentarthron* (*Oopthora*) *semblidis* in winter. Memoirs Bur. Ent. Sci. Committee. Central Board of Land Adm. and Agr. **10**:1–16, illus. (Text Russian.) (As abstracted in Rev. Appl. Entom. ser. A **1**:317–318. 1913.)

[19] RILEY, C. V.
1885. Fourth report of the United States Entomological Commission, U. S. Dept. Agr. **1883–5**:102–104.

[20] SCHULZE, H.
1926. Über die fruchtbarkeit der Schlupfwespe *Trichogramma evanescens* Westwood. Zeitschr. Morph. u. Oekol. Tiere **6**:553–585, 2 figs., 11 refs.

[21] SEVERIN, H. C. M., and H. H. P. SEVERIN.
1908. Habits of the American saw-fly (*Cimbex americana*) with observations on its egg parasite (*Trichogramma pretiosa*). Trans. Wis. Acad. Sci. Arts and Letters **16**:61–76.

[22] SIMMONS, PEREZ, and G. W. ELLINGTON.
1924. Biology of the Angoumois grain moth—progress report. Jour. Econ. Entomol. **17**:41–45.

[23] SMITH, H. S., and H. M. ARMITAGE.
1920. Biological control of mealybugs in California. California State Dept. Agr. Monthly Bul. **9**:104–158.

[24] VUILETT, A.
1914. Utilization de certains insectes phytophages dans la lutte contre les ennemis des plantes cultivées. Revue Scientifique **52**:526–530.

[25] WESTWOOD, J. O.
1833. Descriptions of several new British forms amongst the parasitic hymenopterous insects. The London and Edinburgh Philosophical Magazine and Journal of Science **2**:444.

[26] ZORIN, P. V.
1927. A method of rearing *Trichogramma evanescens* Westw. Defense des Plantes **4:316–319** (Leningrad). (Text Russian.) (As abstracted in Review of Applied Entomology).

The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.
4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.
5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.
7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.
8. Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.
9. Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.
10. The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.
11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.
12. The Effect of the Plant on the Reaction of the Culture Solution, by D. R. Hoagland. November, 1923.
13. Some Mutual Effects on Soil and Plant Induced by Added Solutes, by John S. Burd and J. C. Martin. December, 1923.
14. The Respiration of Potato Tubers in Relation to the Occurrence of Blackheart, by J. P. Bennett and E. T. Bartholomew. January, 1924.
15. Replaceable Bases in Soils, by Walter P. Kelley and S. Melvin Brown. February, 1924.
16. The Moisture Equivalent as Influenced by the Amount of Soil Used in its Determination, by F. J. Veihmeyer, O. W. Israelsen and J. P. Conrad. September, 1924.
17. Nutrient and Toxic Effects of Certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and Ph of the Medium, by H. S. Reed and A. R. C. Haas. October, 1924.
18. Factors Influencing the Rate of Germination of Seed of Asparagus Officinalis, by H. A. Borthwick. March, 1925.
19. The Relation of the Subcutaneous Administration of Living Bacterium abortum to the Immunity and Carrier Problem of Bovine Infectious Abortion, by George H. Hart and Jacob Traum. April, 1925.
20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Relationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.