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SOME METHODS OF HANDLING MINUTE HYMENOPTEROUS PARASITES¹

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A year or so ago the writer had some experience in rearing and shipping the hymenopterous egg parasites, *Trichogramma minutum* (*pretiosa*) Riley and *Telenomus* sp. (probably *heliothidis* Ashmead). An account of this work has not been published, and the suggestion² has been made that the writer prepare a paper on his methods of dealing with these small insects. Though *Telenomus* is more difficult to rear than *Trichogramma*, the methods given below which were devised for the work with the latter species were found to be suitable for the former also.

At the beginning of his work the writer had the advantage of some suggestions which Mr. W. F. Fiske made to Dr. L. P. de Bussy, who transmitted them in the following words:

"He (Mr. Fiske) handles the insects in very narrow tubes (2-3 mm. in width and $\frac{1}{2}$ -1 inch long); in this way it is very easy to study them with a lens, to see the antennæ and to know the sex.

"*Trichogramma* breeds parthenogenetically for long generations, if necessary. Mr. Fiske thinks it parasitizes any soft-shelled eggs, but probably only when the larva inside has not yet developed too much. Infertile and dead eggs (after several months of cold storage) will be infested too, and perhaps also ovarian eggs, although this has not been studied.

"An egg will not give any *Trichogramma*, when it is very often infested by one or more females. The best way is to give 25 to 50 host eggs to one female *Trichogramma*. Once, as many as 163 *Trichogramma* have been bred from two females.

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² This suggestion was made by Mr. Geo. N. Wolcott, assistant entomologist of the Board of Commissioners of Agriculture in Porto Rico, who has done much work in rearing and shipping *Trichogramma* and other parasites.

"Therefore a very large quantity of host eggs is a principal question. Mr. Fiske had infested eggs in cold storage (28-30° F.) for about ten months, after which time the adults emerged normally. It is necessary to put the infested eggs in the cold temperature at the moment when they are beginning to turn black."

The easiest and almost the only way of collecting very small egg parasites is to collect eggs parasitized by them, and to rear the parasites from the eggs. The collected eggs should be separated as well as possible from the leaves or corn silk (in the case of *Heliothis* eggs) on which they were deposited. They should then be put in glass tubes about 8 mm. wide by 24 mm. in length, about ten or fifteen eggs to a tube. A piece of fine cotton batting is very good for use as a stopper. The eggs which are not parasitized will usually hatch before the parasites emerge from the other eggs. The larvæ from the unparasitized eggs should be removed from the tube.

The parasites may be expected to emerge within a short time after the eggs turn black. Sometimes several adults of *Trichogramma* emerge from one egg, so that quite a number of parasites may be found in the tube. A very weak solution of granulated sugar and water should be made and this should be given to the parasites. A good way to feed these minute insects is to moisten the pointed end of an insect pin with the sugar solution, and to insert it into the tube, around the cotton stopper but without removing the stopper. Some of the sugar solution should be made to adhere to the side of the tube, and then the end of the pin should be withdrawn. Only a small amount of the sweet solution is necessary for the parasites. The amount should be so little, in fact, that it cannot be observed very well except with the aid of a pocket lens. If much liquid is given to the parasites they will usually be caught in it and will be unable to free themselves. Naturally, the liquid should not be so thick as to be sticky. A sugar solution which is merely sweetened water is best.

Copulation will occur soon after both males and females have emerged from the eggs in the tube. The experience of the writer indicates, however, that it is best to leave the females with the males for about twenty-four hours before giving them host eggs for oviposition. If a female is given host eggs within a few hours after emergence it seems that there is some possibility that she may escape fertilization.

When it is desired to have the parasites oviposit, they may be introduced into a tube (size 8 mm. by 24 mm.) in which a number of fresh host eggs, or host eggs which have been kept in cold storage, and a minute particle of the sugar solution have been placed. (The writer did not use the very small tubes recommended by Mr. Fiske because he could not obtain them readily.) The manner of transferring the

insects from one tube to another will be described below. In placing the parasites with fresh eggs the males may be left out of consideration. In a tube where the females are ovipositing the males walk about aimlessly and do not disturb the females. The important thing is to get some females in the tube with the fresh eggs. The females of *Trichogramma* may easily be distinguished by the antennæ, though the living individuals usually move so rapidly that it is difficult to determine the sex in any way whatever. The action of the different sexes in the presence of the fresh eggs is positive evidence, however. While the males will pay no attention to the eggs, the females—as soon as they have become accustomed to their new location—will busily examine a number of the eggs, crawling over them and tapping them with the antennæ, and finally selecting some for oviposition. One egg after another will then be parasitized. The writer found it most convenient to place two or three females in a tube containing about fifty eggs. One female to about twenty-five host eggs may be correct.

In transferring the parasites from one tube to another, it was found best to place the tubes on a smooth white surface so that the insects might easily be observed. A plate of glass five by seven inches, with a piece of white paper glued to the underside, was used for this purpose. The plate of glass was placed on a table before the worker. At the worker's right hand was a very fine camel's hair brush, a fine insect pin, and a watch glass containing the sugar solution. The parasite tubes were placed at the left of the worker. A few parasites were allowed to come out on the glass plate, and they were then made to walk or jump into the proper tube, which was held open before them. If necessary, the parasites were touched *very slightly* with the camel's hair brush so as to make them go in the right direction. Occasionally, a parasite would escape, but after the operator acquired some experience there was little loss.

Another way to transfer the parasites from tube to tube is to open the two tubes and hold them mouth to mouth. The tubes should be of the same size, and the mouths should be held firmly together. If there are a large number of parasites in one of the tubes, however, too many will usually go into the new tube. In this work advantage should be taken of the fact that the parasites will ordinarily go toward the source of light. The tubes should be arranged so that the parasites will proceed from the old tube to the new one in the direction from which the light comes.

The tubes should be kept in a suitable tray on a piece of white paper, so that the parasites may be observed. A small label bearing a num-

ber may be glued to each tube so that a record may be kept of the work.

For shipment, the tubes containing parasitized eggs may be packed in pasteboard mailing cases. In the work with which the writer was connected, a little cotton batting was placed in each tube to prevent the eggs from shaking, but Mr. S. Leefmans, who received the eggs in Holland, suggested that pieces of very thin paper be used instead of cotton, as the parasites on emerging were liable to become entangled in the cotton. The writer does not know whether the thin paper was more satisfactory than the cotton. Each tube should be packed in cotton in a tube that is somewhat larger, and the larger tubes should be packed in cotton in the case. If cold storage is used—and it is desirable for long shipments, such as across the Atlantic—care should be taken not to remove the insects from the cold storage room at destination and to subject them suddenly to a much higher temperature. The mailing cases should be transferred from the cold storage room to thermos jars, in which they may be carried to the laboratory. They should be left in the thermos jars for about twenty-four hours, in which time they will gradually have come to the temperature of the laboratory. The temperature of the cold storage rooms on the ships used by the writer was said to be 38° F. The freshly parasitized eggs, packed as above, were sent by ordinary mail from Brownsville, Tex., to New York, where they were supposed to be placed in cold storage immediately by the steamship company. The several days during which they were on the road between Brownsville and New York gave the eggs time to turn black before being placed in cold storage.

Perhaps the most important point to be realized by an entomologist who undertakes to rear *Trichogramma* and other egg parasites is that such minute insects require special treatment because of their small size. The cages designed for them, the methods of feeding, shipping, etc., must all be considered with due regard to this factor.

Several entomologists of the writer's acquaintance are now engaged in work in connection with the control of certain injurious insects by the introduction or the artificial propagation of small hymenopterous parasites, and it is hoped that these and other workers may find some helpful suggestions in this article.