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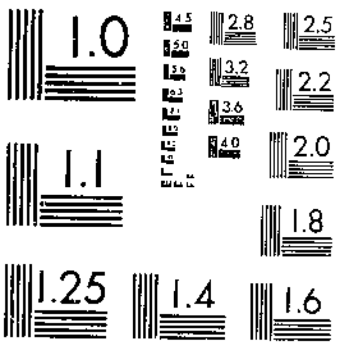

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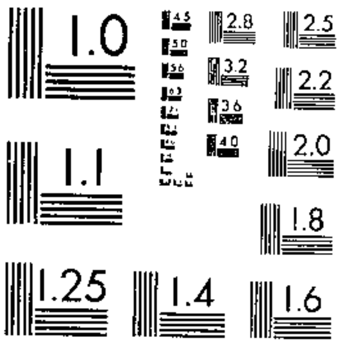

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TECHNICAL BULLETIN No. 215 NOVEMBER, 1930

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

A BIOLOGICAL STUDY OF TRICHOGRAMMA MINUTUM RILEY AS AN EGG PARASITE OF THE ORIENTAL FRUIT MOTH

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CONTENTS

INTRODUCTION

For three seasons the writer devoted considerable time to investigating the life history and habits of *Trichogramma minutum* Riley as a parasite of the eggs of the oriental fruit moth, *Laspeyresia molesta* Busck, and of the codling moth, *Carpocapsa pomonella* Linné. The investigations were conducted largely at Riverton and Moorestown, N. J. This bulletin summarizes the most important observations on the life history and habits of the parasite, and the relationship existing between environmental temperatures and the rate of development. A few notes on field tests and observations are also recorded.

The writer believes that two species or distinct strains of Trichogramma occur in New Jersey. In 1928 it was noted that during the warm weather of June, July, and August there existed two kinds of females: One, the more common variety, of a light lemon-yellow color, particularly noticeable on the thorax and abdomen; the other, less common variety, with a metallic olivaceous-brown thorax and abdomen. It was also noted that the late fall and early spring individuals of the commoner yellow variety had the thorax and abdomen colored metallic olivaceous brown like the less common midsummer strain. The two kinds would not interbreed. No copulation was

[1] Resigned Aug. 31, 1928. The writer is greatly indebted to G. J. Haeussler, A. C. Hodson, and R. L. Coffin for assistance in conducting this investigation. Mr. Haeussler made many of the field observations during 1927 and 1928 and Mr. Coffin took numerous photographs.

observed when males and females of the two kinds were placed together, although they were so placed some 50 different times, and the progeny were always males, indicating that the females had not been fertilized. The two kinds also differed in their rate of development. The more common kind, having yellow females during warm weather, completed its development in less time, by one to several days, than the variety having dark-colored females. The foregoing facts seem to indicate the existence of two species; however, this may be questioned because the writer has been unable to discover any morphological difference in the two kinds. Whether the two are morphologically distinct species or, possibly, biological or physiological species remains to be determined.[2]

The majority of the facts discussed in this bulletin deal with the species or form that has lemon-yellow colored females in the summer and is commonly found in many sections of the United States. The hosts used chiefly in this study were the eggs of the oriental fruit moth and of the codling moth. In the course of this investigation insect eggs of numerous species were exposed to females, and many of them were parasitized. The principal limiting factors appeared to be the condition of the egg coating and the stage of development of the host egg. Eggs with thick, tough, or hard shells, or eggs covered with some protective secretion, setae, or scales, were more or less free from attack. It was also found that eggs having well-developed larvae within could not be parasitized. Offhand it appears that almost any insect egg may be parasitized by Trichogramma provided these unfavorable conditions do not exist.

METHODS

The life-history study was conducted in a large screened insectary in a peach orchard near Riverton, N. J., in 1927, and near Moorestown, N. J., in 1928. (Fig. 1.) In this study all the insects were reared in glass vials (1 by 5 inches or one-half by 2 inches) covered with muslin or stoppered with cloth-covered cotton plugs. The vials were kept for the entire season in a large wooden tray (fig. 2) in a wooden rack near the center of the screened portion of the insectary and about 5 feet above the ground, where there was plenty of indirect light but no direct sunlight.

Each day throughout the season one or more fresh lots of from 10 to 20 newly emerged males and females of Trichogramma were placed in 1 by 5 inch vials and given from 50 to 150 newly deposited eggs of the oriental fruit moth on pear foliage. After 24 hours the eggs were replaced with other unparasitized eggs. This was repeated each day until no more females remained alive in the respective daily lots.

Experience shows that it is most satisfactory to make use of eggs deposited on pear or apple foliage or upon smooth paper. If pieces of peach foliage bearing eggs are inserted into 1 by 5 inch vials with adult parasites the insects will die within a few hours. This is also true to a limited extent with some varieties of plum and cherry. Apparently some gas, possibly a cyanide compound of some description, is given off in sufficient quantity to be toxic to the insect.

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[2] PETERSON, A. HOW MANY SPECIES OF TRICHOGRAMMA OCCUR IN NORTH AMERICA? Jour. N. Y. Ent. Soc. 38: [1]–8. 1930.

When host eggs are placed out of doors those located on pear foliage are more satisfactory than those located on peach foliage because pear foliage does not drop off the cut twigs as readily as peach foliage.

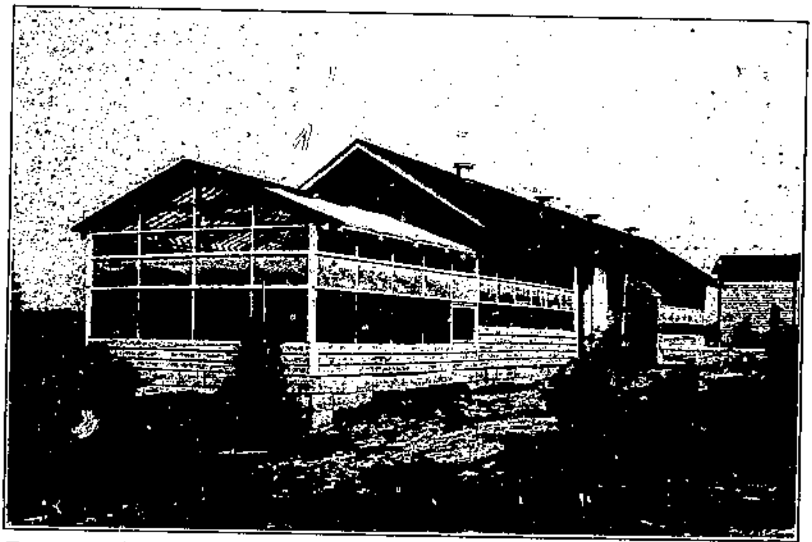

FIGURE 1.—Outdoor screened insectary, 18 by 75 feet, where the life-history studies were conducted

After the eggs had been parasitized they were placed in clean 1 by 5 inch vials, or the leaves or paper on which the eggs were deposited were cut into smaller pieces and placed in one-half by 2

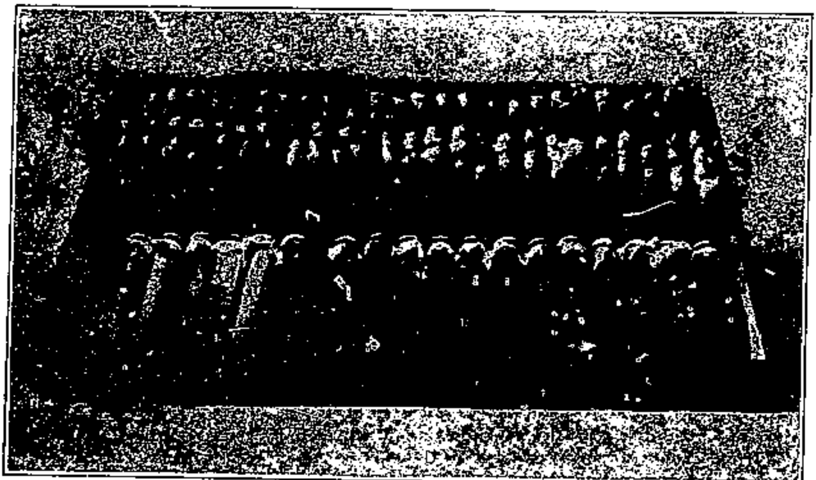

FIGURE 2.—Wooden tray, 18 by 30 inches, which held rearing vials, one-half by 2 inches and 1 by 5 inches in size

inch vials which were stoppered with cloth-covered cotton plugs. The mortality in handling material in this manner is low; in most instances it did not exceed 10 per cent.

The handling of *Trichogramma minutum*, particularly the kind having yellow-colored females, is very simple because they are attracted to sunlight or artificial light; as a rule, they crawl and do not fly readily. By shaking them out onto a piece of white paper before a light they may be transferred from point to point by placing small pieces of paper in their paths and permitting them to crawl on these. The species or kind having females which are naturally dark colored in the summer are more active and have a strong tendency to fly when disturbed or placed on a white paper.

A self-recording thermograph and maximum and minimum thermometers were employed in the insectary for ascertaining temperatures. All of the daily temperature averages mentioned in this bulletin were ascertained from 12 readings (2-hour intervals) per day from the thermograph records and checked against the records kept at the Japanese-beetle laboratory.

LIFE HISTORY

WINTER SEASON

There is little or no definite information on the natural wintering of *Trichogramma minutum* in North America. It is highly improbable that the adults of this species survive the winter, particularly in climates similar to that of New Jersey. Experience indoors has shown that parasitized eggs can be held at a constant temperature in cold storage for one to several months, but such a condition is far different from that prevailing during a variable winter out of doors. Observations of the writer show conclusively that the species passes the winter as partly developed individuals within darkened host eggs.

During the winter of 1927–28 this species was successfully wintered in the eggs of the oriental fruit moth and of the Mediterranean flour moth, *Ephestia kuehniella* Zeller. The dark-colored parasitized eggs were kept outdoors in an open screened insectary (fig.1) in 1 by 5 inch shell vials covered with muslin. All of the individuals which wintered successfully were parasitized after October 12. Under orchard conditions it is probable that few or no parasites pass the winter naturally in the eggs of the oriental fruit moth or codling moth, because few or no eggs of these hosts are present in the orchard as late as the middle of October. Insect eggs of other species must be utilized by this parasite, such as eggs of insects which normally pass the winter in the egg stage.

On warm days in December and also in January some adults may emerge out of doors. These adults probably are unable to find nonparasitized eggs unless they happen to emerge from a cluster of eggs where only a portion of the eggs are parasitized. In such an event they might parasitize the unparasitized eggs of the cluster and their progeny continue to develop and successfully pass the winter season.

ACTIVE SEASON

In 1928 in an outdoor screened insectary the spring-brood emergence of parasites (over 200 adults) took place between April 3 and May 4, the peak of emergence occurring April 18. It is possible that this emergence might have taken place a week or 10 days earlier if the material had been in a warmer location, such as the ground under peach trees in an orchard. The overwintering individuals for 1928–29 died, probably because they were moved from Moorestown, N. J., to Columbus, Ohio, by mail.

In 1927 the season's work started with the finding of dark parasitized oriental fruit-moth eggs in a small peach orchard near Riverton, N. J., on May 12, from which parasites emerged May 30. In 1928 the season's investigations started April 3, the first emergence date for spring-brood adults. From the above dates, for each season, until late in December (and early in January in 1928) adult parasites continued to emerge. In 1928 the maximum number of consecutive reproductions or generations was 13, while in 1927 there were 11 after June 1. Probably there had been in the field one complete generation, and a partial second, by June 1, which means that there were 12 or 13 generations in 1927.

The length of time required for the life cycle of an individual varies with the temperature and is discussed under temperature and moisture studies. There was little or no difference in the rate of development of the parasite when developing in the eggs of the oriental fruit moth or in those of the codling moth.

The number of individuals which may develop in one egg is largely dependent upon the size of the host egg. Four individuals from one oriental fruit-moth egg and seven individuals from an egg of the codling moth are the greatest number the writer has reared in the eggs of these hosts. All of these adults were decidedly subnormal in size. Where host eggs of these species are subjected to many parasites, there are commonly one or two parasites in each egg of the oriental fruit moth, and two to four in each egg of the codling moth. In other host eggs, particularly in large eggs of certain species of Lepidoptera, 15 to 25 adults have been reared from one egg.

Individual female parasites in the tests produced a maximum of 131 individuals. Tables 1 and 2 show what isolated females (54 and 30) produced in two seasons, 1927 and 1928, when ample host material was furnished. The average per season for 1927 and 1928 was 40.2 and 35.9, respectively.

Unfertilized females produced as numerous progeny as fertilized females; however, the progeny from unfertilized females were always males so far as the writer's observations went. Approximately two-thirds of the progeny from fertilized females were females (for 1928, 63 per cent). Table 2 shows the variation in the proportion of males and females from 30 isolated fertilized females. In five cases the males outnumbered the females (Nos. 3, 7, 8, 12, and 17).

Adults, particularly females, will live in 1 by 5 inch vials with a green pear or apple leaf for 1 to 22 or more days. The average length of life of females is somewhat greater than that of males. Tables 1 and 2 show the average length of life for isolated females to be 6.4 and 4.8 days for 1927 and 1928, respectively. Under insectary conditions, when the weather is cool in the spring or fall of the year, the adults live longer than in hot summer weather.

TABLE 1.—*Progeny from 54 isolated fertilized or unfertilized females of Trichogramma minutum and the length of their lives when reared in the eggs of the oriental fruit moth in an open screened insectary, 1927*

Female No.	Date of emergence	Adults emerging from eggs deposited by each female on specified days										Total	Longevity of females
		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth		
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Adults*	*Days*
1	June 28	4	([1])	18	7	1						30	7
2 [1]	do	17	([1])									17	3
3 [2]	do	22	12	1	([3])	1	7	([3])	([3])	1	[4] 15	59	15
4	do	20	10	9	5	([3])						44	9
5	do	17	8	2	([3])	([3])						27	8
6	do	18	([1])									18	7
7 [2]	June 29	16	19	22	([3])							57	6
8	July 1	17	13									30	7
9 [2]	do	11	15	8								34	4
10	July 7	21	22	6	13	([1])	19					81	10
11	do	10	10									20	7
12 [2]	do	14	18	7	7	([3])	6	2				54	10
13 [2]	do	16	21	11	11	([3])	([3])	6				05	9
14	July 8	11	10	3	3	1						28	6
15 [2]	do	22	3	([1])	3	([3])	15					43	10
16	July 15	29	17	14	([1])	6	([1])	1				67	10
17	July 18	15	4									19	7
18 [2]	do	57	([3])	13								70	4
19 [2]	do	43	([3])	14	4	4	5					70	10
20	July 22	39	33	16	25	([1])	18					131	13
21	July 23	38	([1])	([3])	([3])							38	5
22	July 30	51	9	([3])	([3])	5	7	6				78	10
23	do	45	17	([3])	8	([3])	11	([3])	7	([3])	4	92	11
24	July 31	31	12	([3])	([3])	8	11	([3])	11			73	13
25 [2]	Aug. 6	34	([1])	7	([3])	1						42	7
26	Aug. 11	7										7	9
27	Aug. 13	29										29	2
28	Aug. 15	31										31	2
29 [2]	Aug. 17	14	([3])	17								31	5
30	do	20										20	1
31	Aug. 18	31	([3])	13	([3])	11						55	8
32	Aug. 20	([1])	8									8	3
33 [2]	Aug. 22	20										29	3
34 [2]	Aug. 25	14	11									25	3
35 [2]	Aug. 27	12	17									29	9
36 [2]	Aug. 29	28										28	1
37	Sept. 2	31										31	1
38	do	39	([3])	11								50	10
39	Sept. 3	30										30	7
40	Sept. 10	17										17	2
41	Sept. 12	25	([3])	([3])								25	3
42	do	48	([3])	([3])	([3])							48	9
43	Sept. 15	68										68	1
44	Sept. 18	13	([3])	([3])	([3])	([3])						13	5
45	Sept. 19	62	([3])	([3])	([3])	([3])	([3])	([3])	([3])			62	7
46	Sept. 23	22	([3])									22	2
47	Sept. 25	24	([3])	4	([3])							28	4
48	Sept. 26	23	([3])	([3])								23	6
49	Sept. 30	38										38	5
50	do	15	([3])	([3])	([3])	([3])						15	7
51	Oct. 2	22	([3])	([3])	([3])							22	7
52	Oct. 3	33	([3])	([3])	([3])	([3])						33	5
53	Oct. 5	43	([3])	([3])	([3])	([3])	([3])					43	7
54	Oct. 8	25	([3])	([3])	([3])	([3])	([3])	([3])	([3])			25	8
Total												2,172	350
Average												40.2	6.4

[1] Observations made, but no eggs were parasitized.
[2] Unfertilized females. Progeny exclusively males.
[3] Exact number unknown; however, some were parasitized and are included in the record of the previous day.
[4] 3 of these were deposited when female was 12 days old and 5 were deposited when female was 13 days old.

TABLE 2.—*Progeny from 30 isolated fertilized females of Trichogramma minutum and the length of their lives when reared in the eggs of the oriental fruit moth in an open screened insectary, 1928*

Female No.	Date of emergence	Number of adults emerging from eggs deposited by each female on specified days																		Total adults			Longevity of females (days)
		First		Second		Third		Fourth		Fifth		Sixth		Seventh		Eighth		Ninth					
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Both	
1	May 16	5	8	0	7	3	4	0	3	2	7	1	4	0	2	0	2	0	1	11	38	49	13
2	May 18	2	18	0	12	0	7	0	4	2	7	0	2	0	3	1	0			5	53	58	11
3	May 24	18	2	1	1	1	0	0	0	1	0	0	1	2	0	5	0	2	0	30	4	34	9
4	May 29	5	18	1	3	2	3	4	13	5	13	2	6	4	6	5	0	9	0	37	62	99	10
5	May 30	4	13	2	5	1	7	6	13	2	6									15	44	59	9
6	June 4	5	13	4	5	2	9													11	27	38	11
7	June 8	7	4	4	8	3	9	3	4	7	0	5	0	8	0					37	25	62	7
8	June 13	16	22	18	8															34	30	64	3
9	June 15	3	1	5	8															8	9	17	3
10	June 19	3	12	2	5	0	1													5	18	23	7
11	do.	4	8	7	5	0	2													11	15	26	5
12	June 20	12	10																	12	10	22	6
13	June 24	6	12																	6	12	18	2
14	June 26	5	12																	5	12	17	1
15	do.	5	10																	5	10	15	2
16	June 27	3	5	3	5															6	10	16	3
17	do.	11	12	9	5	8	5	1	2											29	24	53	4
18	June 28	7	8																	7	8	15	1
19	June 29	6	8	7	12	1	12	0	8	1	5	0	2							15	47	62	8
20	June 30	5	5	3	5	3	3													11	13	24	5
21	July 1	12	21	1	0															13	21	34	3
22	July 7	6	15																	6	15	21	1
23	July 10	10	16																	10	16	26	1
24	July 11	5	10																	5	10	15	1
25	July 12	6	12	3	4															9	16	25	2
26	do.	13	18	10	13	4	5	3	8											30	44	74	4
27	July 14	2	5	7	9	2	9	1	1											12	21	36	4
28	July 15	1	3	5	10															6	13	19	2
29	July 16	10	17	2	3															12	20	32	3
30	July 17	7	8	0	9	1	0													8	17	25	3
Total																				411	667	1,078	144
Average																				13.7	22.2	35.9	4.8

The female parasite deposits most of her eggs the first few days after she emerges, provided ample host material is present and the temperature is above 70° F. For example, if a female lives seven days she will deposit all (or most all) of her eggs the first two or three days.

A number of individual females have been carefully watched for several consecutive hours, and the following is typical of what occurs: A female will crawl around on a leaf or any object and constantly touch with her antennae the surface on which she is crawling. When she happens to cross an insect egg she stops immediately and examines it carefully for several or many seconds. In most instances, if she is ready to oviposit, she moves forward and then stands in a somewhat upright position as she pushes her ovipositor through the eggshell. It requires two or three minutes to complete oviposition. When oviposition is completed the female will sometimes place her mouth parts on the oviposition puncture and apparently consume any liquid which has exuded.

So far as observed, there is no indication that a female can detect the presence of an oriental fruit-moth or codling-moth egg at a distance greater than one-fourth to one-half inch. At times females will pass by eggs of the oriental fruit moth when they are less than one-fourth of an inch away.

A female may continue to deposit in 20 to 25 or more host eggs without stopping for any length of time. For example, one female on a warm July day parasitized 22 oriental fruit-moth eggs in one hour. This same female parasitized 39 eggs between 2 and 4.30 p.m., and parasitized 54 eggs altogether within 48 hours. It is evident that under insectary conditions females will deposit most of their eggs within 24 to 48 hours after they emerge if there is sufficient host material, if the temperature is close to 80° F., and if the humidity is fairly high. There is strong evidence in the writer's investigations to show that more eggs are parasitized on bright days than

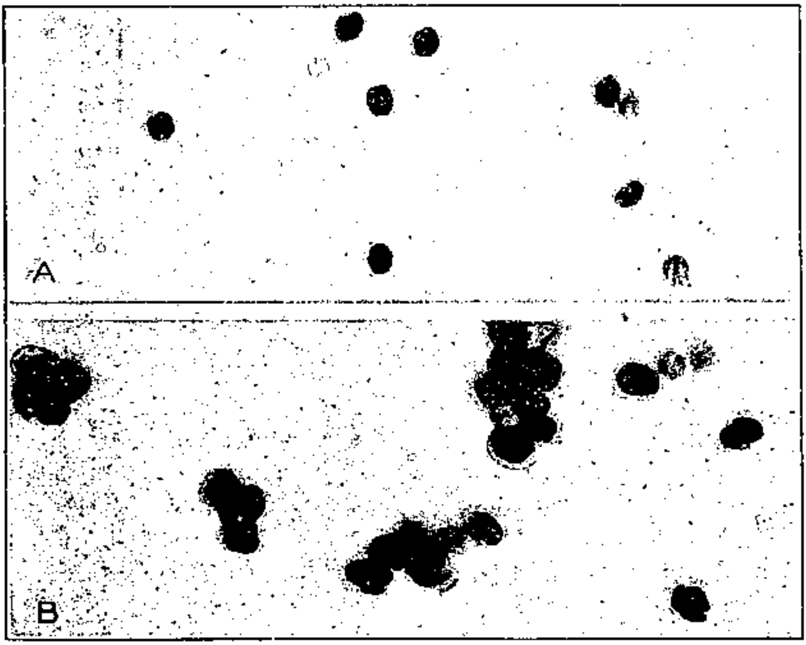

FIGURE 3.—Parasitized eggs in the late black stage with from one to three parasites (*Trichogramma minutum*) in each egg: A, Oriental fruit moth; B, codling moth. × 3

on dull cloudy ones. In artificial production the correct intensity of light is important.

A parasitized egg of the oriental fruit moth or codling moth can not be distinguished from a normal egg by the naked eye until the darkened stage is reached. (Fig. 3.) The parasite within an oriental fruit-moth egg becomes distinctly black when its development is approximately two-fifths to one-half completed. (Fig. 4, I and J.) A normal host egg of the oriental fruit moth or codling moth shows a small dark spot (becomes black-spotted) from 24 to 48 hours before hatching, this black spot being the darkened head (fig. 5, S) of the first-instar larva. If oriental fruit-moth eggs are parasitized some time during the first 24 hours of their existence, the darkened parasitized condition appears about the time that a normal egg shows a dark spot or is ready to hatch. The darkening of parasitized eggs varies with the host. Bagworm eggs, *Thyridopteryx ephemeraeformis* Haw., will darken from 12 to 24 hours after they are parasitized.

By means of a microscope a parasitized oriental fruit-moth egg can be distinguished before it darkens by the presence of one or more eggs or larvae of the parasite within the host egg. The egg of the parasite is very small and difficult to locate, while the larva is more readily visible, especially if the host eggs have been deposited on glass slides. Figure 4 shows what can be seen of the development of a living parasite in an egg deposited on a glass slide and observed under a compound microscope.

In warm weather the parasite egg hatches within a few hours and the small, active, maggotlike parasite larva can be observed moving about within the host egg. (Fig. 4, A, B.) A constant stream of egg plasma can be seen flowing toward the somewhat pointed anterior end of the larva. The parasite rapidly increases in size (fig. 4, C, D, E, F) and soon becomes large and motionless, except for the extreme anterior end which is constantly taking in food. When the larva is nearly full grown, it and the surrounding tissue gradually turn dark, obscuring further observation on the changes that occur. During this change distinctly pigmented and irregularly arranged ringlike structures appear. (Fig. 4, G, H.) As the host egg darkens these unidentified structures disappear. The darkly pigmented parasitized egg (fig. 4, I, J) shows a faint lighter colored line across the rounded area. This line marks approximately the division between the head and thorax of the pupa or the adult.

When the parasite has completed its development and is ready to emerge the adult bites an exit hole in the eggshell. In case there are several parasites in one egg, all may use the same exit hole. (Fig. 4, L.) The adults usually emerge early in the morning during midseason.

Parasitism by Trichogramma produces an immediate cessation of the normal embryonic development of the host egg. The parasite is able to stop the development of an oriental fruit-moth or codling-moth egg at any stage previous to the time when a distinct host larva with a well-developed head appears. (Fig. 5, R.) The more important changes that take place in the developing embryo are shown in Figure 5. All of the stages are subject to successful attack by Trichogramma except those shown by R and S. No attempt is made to explain in detail the embryonic changes through which the normal host goes. The age of each stage and some of the striking changes are noted in the legend. All observations were made from living eggs deposited on microscopic glass slides. Day and night temperatures were between 75° and 85° F.

The stages in the development of a normal oriental fruit-moth egg which Trichogramma can not attack appear about the time the incubation period is two-thirds completed. For example, if an egg requires six days to hatch, it is susceptible to parasitism for approximately four days. Female parasites may puncture eggs that are about to hatch, but this seems to have no effect on the well-developed larva within. It has also been observed that females will puncture

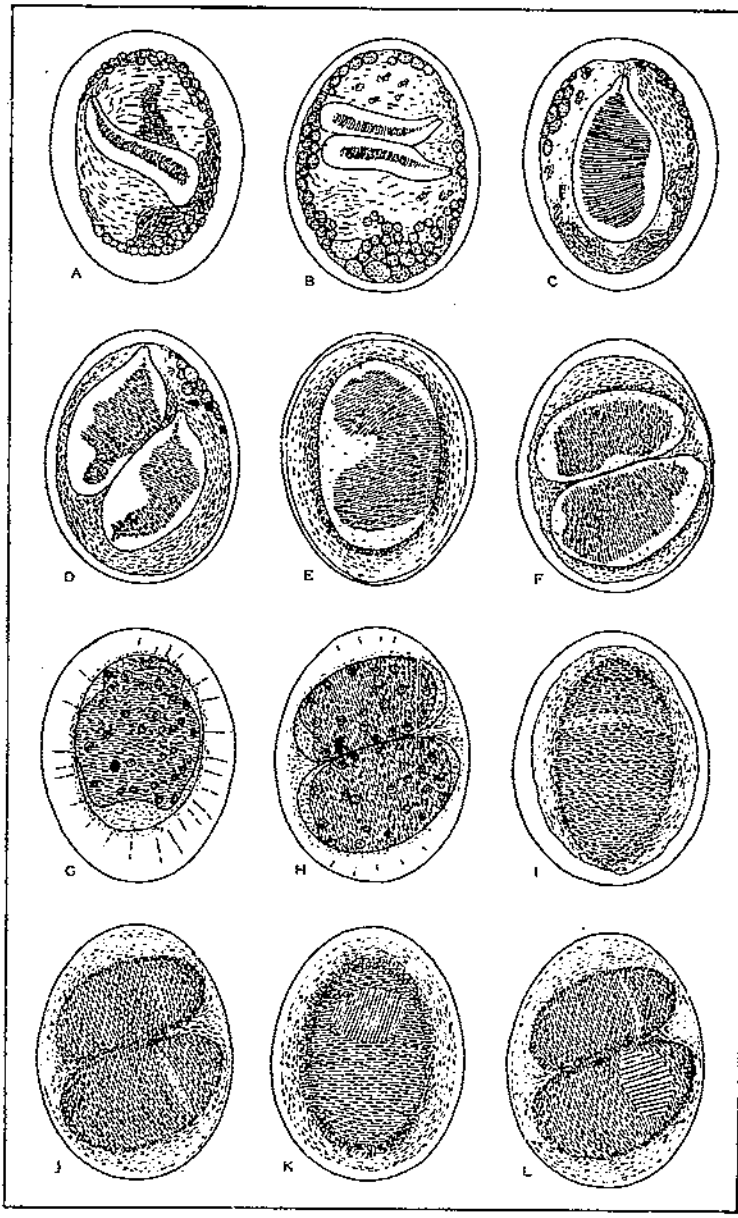

FIGURE 4.—Oriental fruit-moth eggs parasitized by *Trichogramma minutum:* A, C, E, G, I, and K show development of one parasite in an egg, while B, D, F, H, J, and L show two parasites in one egg. The approximate age of each parasite stage is as follows: A and B, 24 hours, larvae quite active at this stage; C and D, 28 hours, larvae motionless at this stage except the area near pointed end where food is taken in; E and F, 48 hours, larvae motionless and tissue starting to darken about them; G and H, 54 to 60 hours, larvae distinctly more opaque and with dark ringlike unidentified structures scattered over surface; I and J, parasite larvae 100 or more hours old and distinctly black except for a lighter streak near anterior portion; K and L, parasites hatched after 200 or more hours. One exit opening in L served for both parasites. × 35

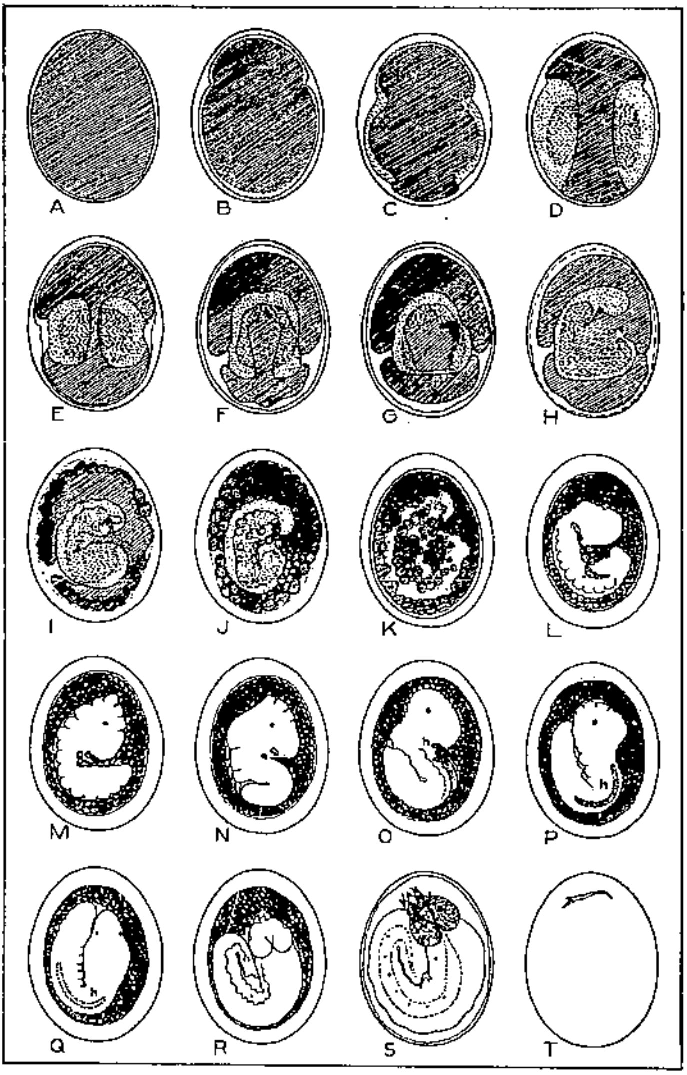

FIGURE 5.—Semidiagrammatic drawings of the more important stages in the embryonic development of a normal oriental fruit-moth egg. The approximate age of each stage is as follows: A, 2 hours, no differentiation in content; B, 4 hours, first indication of a constriction; C, 4½ hours, two constrictions showing; D, 5 hours, a distinct differentiation in color and consistency of the protoplasm; E, 5½ hours, symmetrical dumb-bell-like differentiation of protoplasm; F, 6 hours, a distinct break on the median line of the anterior and posterior portions; G, 6½ hours, first indication of asymmetry; H, 6¾ hours, asymmetry and invagination showing; I, 7½ hours, first cellular differentiation in yolk; J, 8 hours, yolk showing complete cell-like differentiation; K, 13 hours, migration of yolk cells toward center of embryo and their disintegration; L, 27 hours, metameric differentiation in embryo first noted; M, 31 hours, eye spot first noted; N, 32 hours, embryo starting to rotate; presence of thoracic embryonic legs noted; O, 34 hours, start of separation of dark body (*h*) along dorsal aspect; this may be the embryonic heart; P, 35 hours, dark organ (*h*) distinctly separated; Q, 37 hours, dark organ (*h*) faintly visible; R, 56 hours, larva showing distinct nonpigmented head with mandibles, also spiracles and trachea; no dark organ (*h*) visible; larva at this stage consumes yolklike material about its body by means of its mouth parts; this stage is not subject to attack by Trichogramma; S, 72 to 84 hours, fully developed larva ready to emerge; T, 90 hours, a hatched egg showing exit hole. × 25

hatched eggs. Whether or not they deposit eggs under these conditions is unknown.

TEMPERATURE AND MOISTURE

The time required for the completion of a life cycle of Trichogramma in oriental fruit-moth and codling-moth eggs ranges from

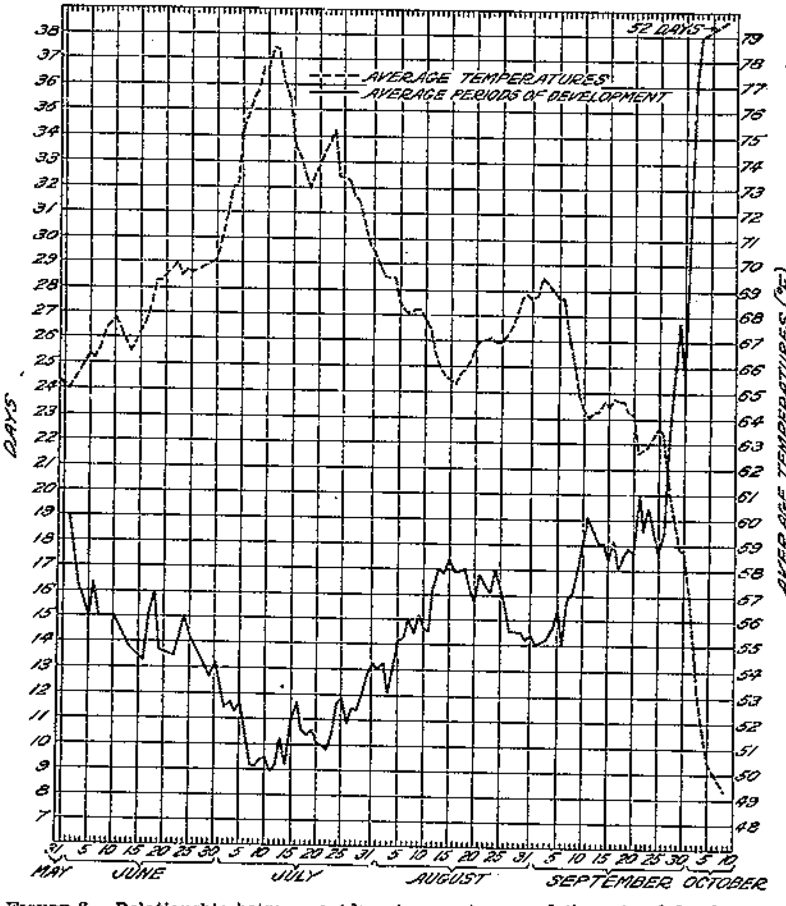

FIGURE 6.—Relationship between outdoor temperatures and the rate of development of *Trichogramma minutum* during 1927 in oriental fruit-moth eggs. The upper line shows the average temperature during the average period of development for each daily lot of parasites, 25 to 200 or more individuals; the lower line shows the average number of days required to complete the development of each daily lot

7 to 75 or more days. This variation is largely due to variations in the average temperature of the environment.

In Figures 6 and 7 the average time required for the completion of the life cycle of daily lots of material for two seasons, 1927 and 1928, is shown. These figures also show the average temperature during the average period for each daily lot. The curves show clearly the influence of average temperatures on the rate of development of Trichogramma for each season in general. As the temperature rises the number of days required to complete development is reduced, and vice versa.

Each point on the day curve is the average time of development required for all of the individuals started on a given date, while a point on the temperature curve is the average temperature for all of the days required to complete the development of a given daily lot. The spacing on the chart is such that 1° in temperature equals one day. This ratio does not exactly equal the ratio of the development of the insect. The changes in the temperature curve are greater than the changes in the day curve. Upon computing the ratio for the insect it was learned that within a range of average

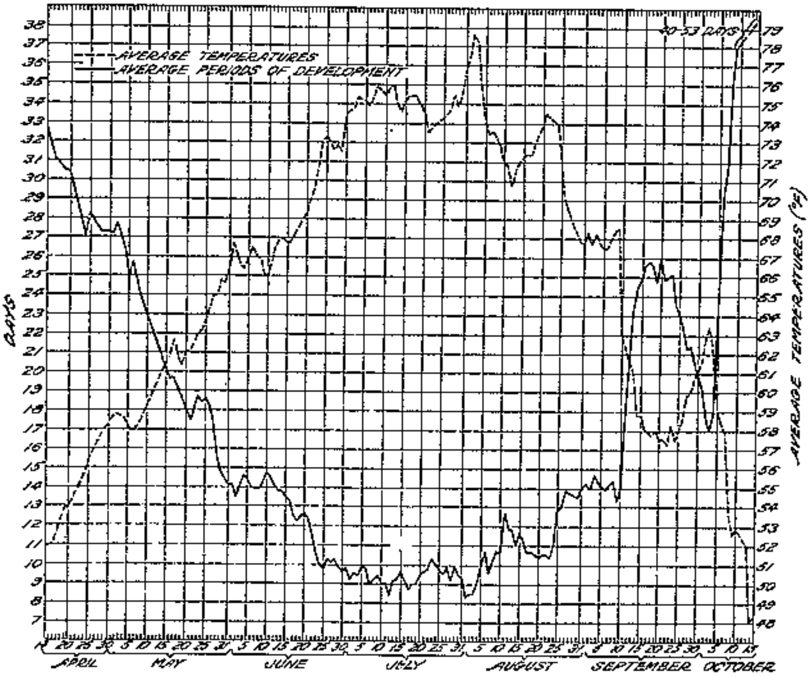

FIGURE 7.—Relationship between outdoor temperatures and the rate of development of *Trichogramma minutum* during 1928 in oriental fruit-moth eggs. The upper line shows the average temperature during the average period of development for each daily lot of parasites, 25 to 200 or more individuals; the lower line shows the average number of days required to complete the development of each daily lot

temperatures approximating 60 to 75° F. the changes in the rate of development equaled an increase or decrease of one day for each daily average decrease or increase of 1.25°. In other words, if the average temperature was increased 1.25°, the time required for development was reduced one day, and vice versa.

Figure 8 shows a comparison between the ratio, 1 day equals 1.25°, and the rate of development of Trichogramma for the two seasons 1927 and 1928. Line A—B is the ratio line of 1 day equals 1.25°. The points on the 1927 and 1928 curves are determined as follows: For example, to determine the average number of days required for the 2° range 68 to 69.9° F., expressed on the curve as 69°, all of the daily lots for the season showing average temperatures for the completion of their life cycles within this range were assembled. There were 13 daily lots, and these showed variations from 12.2 to 14 days, or an average of 13.3 days. The odd numbers, 49 to 79°, represent the 2° ranges, such as 48 to 49.9, 50 to 51.9, 51 to 52.9, etc.

The curves (fig. 8) for the two seasons do not coincide. The 1928 curve is below the 1927 curve at all points. The writer believes this is due to a distinct difference in the location of the thermograph for the two seasons. In 1928 the thermograph was adjacent to the life-history material, whereas in 1927 it was 10 feet away and 2 feet lower than the parasite material. The 1928 curve probably expresses the truth more closely than the 1927 curve.

A study of the plotted lines shows that in the upper right-hand portion of the chart the curves are above the line A—B where temperatures are low and the number of days exceeds 20 to 25. This is probably due to two factors, one of which is very likely more important than the other. The less important factor is probably the difference in the ratio of the rate of development to temperature at low average temperatures as compared with the rate at temperatures between 60° and 75° F. This has not been proved for Trichogramma, although it has been shown to be true for other insects. The writer believes that the more important factor which helps to explain the position of the curves above the A—B line is the fact that at low temperatures, particularly below 50°, little or no development takes place, and no corrections were made for these temperatures in formulating the curves for the two years.

In the lower left-hand corner of the chart the two curves also are above the A—B line to some extent. This may be due to the fact that in extremely high temperatures, probably 85° and above, the rate of development is retarded. In formulating the curves no corrections were made for the possible influence of extremely high temperatures.

Further study of the temperature relationship shows that the average temperature for the completion of a parasite during a given season is above 50° to 52° F. Any temperatures averaging less than 50° to 52° for several weeks usually produce wintering conditions. At Moorestown, N. J., temperatures which average 50° to 52° for a period of several consecutive weeks do not occur until October 10 to 15 and later in the average season. Adults continue to emerge on warm days throughout October and November, and to some extent in December and January, yet all of these adults are developed in eggs parasitized before the average temperature for their life cycle becomes less than 50° to 52° F.

It is probable that temperature conditions in the late fall and during midwinter play an important part in the relative abundance of the parasites early in the following active season. If high temperatures occur in the late fall or during the winter months, adults are apt to emerge from parasitized eggs which, under average conditions, would survive the winter. Most of these adults probably do not find eggs in which to oviposit, perhaps none of them do; consequently the number of individuals to survive until host eggs appear in the early spring, when temperatures are sufficiently high to permit continuous activity on the part of the female, is likely to be greatly reduced and may be almost negligible.

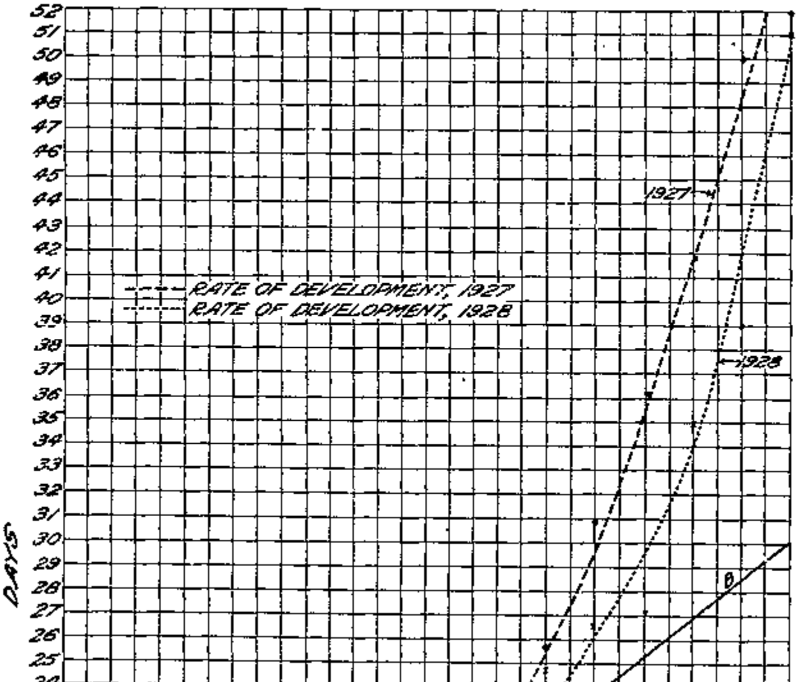

FIGURE 8.—The two plotted curves show the average rate of development of Trichogramma for all the specified 2° ranges of temperature in 1927 and 1928. These curves are computed from the average number of days and the average temperatures associated with the various daily lots, irrespective of the time of year the parasites may have developed. The straight line A—B represents the computed ratio of development of 1.25° equals 1 day. This ratio is approximately correct for average temperatures between 60° and 75° F.

If Glenn's [3] idea of the influence of effective degrees is applied to the development of Trichogramma, using a range of 50° to 85° F., the average number of effective day-degrees for all individuals for 1928 is 239. Whether or not the range of 50° to 85° is correct is unknown. So far as the writer's observations go it appears to be nearly correct.

The general color of the females and, to a slight extent, of the males is determined by the temperature of the environment. During midseason when temperatures are relatively high the adults, particularly the females, have a distinct yellowish cast, especially on the abdomen and thorax, which are lemon yellow, while in the early spring or late fall, when temperatures are comparatively low, the general color is dark with a brownish metallic cast. This change in color can be brought about by subjecting parasitized eggs to low temperatures. This is particularly true of eggs parasitized in September and early October. Adults emerging from eggs held in cold storage may be dark. Spring-brood males and females are dark, and in the fall of the year, late in September or early in October, when the outdoor temperature averages 62° F. or lower, dark-colored females appear. All adults emerging after November 1 are dark.

Moisture is important in rearing parasitized eggs and keeping adults alive, particularly in bulk rearing. In all stages of development the parasites seem to thrive best in humidities that average from 70 to 80 per cent. Parasitized host eggs will show the greatest emergence if they are not allowed to become too dry. This is particularly true of parasitized eggs on paper. Eggs on living plant tissue are not influenced greatly by dry air so long as the plant is alive and transpiring normally.

To maintain a satisfactory condition of moisture in small vials or bottles fresh seedless raisins may be used. If a fresh seedless raisin is placed on an insect pin, moistened, and inserted into the center of the small end of a cork and the cork is placed firmly into the open end of the vial or small bottle, the mosture content of the air within may be kept fairly high. When the raisin shows signs of becoming dry it should be removed, dipped into water, and then returned to the vial. This may be necessary every 24 to 48 hours, especially if the vials are kept in a room where the humidity is low (25 to 35 per cent) and the temperature averages 70° F. By this method the writer has kept adult Trichogramma alive during winter months for 30 days and has had progeny from adults that were 15 to 20 days old. He has also successfully shipped living adults in vials containing raisins, 500 to 1,000 miles. This method has also been very satisfactory for keeping many other species of adult parasites alive for several weeks and in some instances for several months.

FIELD AND LABORATORY OBSERVATIONS

In 1927 the first parasitized field-collected eggs of the oriental fruit moth were found in a peach orchard at Riverton, N. J., on May 12, and in 1928 on May 16 in the same orchard. The last eggs of

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[3] GLENN, P. A. CODLING-MOTH INVESTIGATIONS OF THE STATE ENTOMOLOGIST'S OFFICE, 1915, 1916, 1917. Ill. Nat. Hist. Survey Bul. 14: 219–289, illus. 1922.

the oriental fruit moth to be parasitized for the season were placed in a peach orchard at Riverton on October 28, in 1927, and on October 12, in 1928. These eggs were parasitized some time after these dates. They were found to be black on November 18, 1927, and on November 10, 1928. It is probable that if oriental fruit-moth eggs had been available in November, in 1927 and 1928, and had been placed in the orchard, they would have been parasitized.

For several years observations have been made on the abundance of Trichogramma in oriental fruit-moth eggs in peach orchards. Field collections show that in general in April and May few or no parasitized eggs can be found, while in June, July, and August few to many may occur, and in September and early October a moderate number to many are likely to be present. Table 3 shows the relative abundance of parasitized eggs in a small 7 to 8 year old peach orchard at Riverton for 1927 and 1928. Each day, during the entire active season of 1927 and 1928, three lots of newly laid oriental fruit-moth eggs were placed in a peach orchard in three widely separated trees. In 1927 the observations showed parasitized eggs in 55 of the daily collections, whereas in 1928 they were found in only 27. This decided difference in the number of times (days) parasitized eggs were found in this orchard is probably due to the fact that in 1928 the number of peach trees in the orchard had been reduced at least two-thirds; furthermore, the orchard was well cultivated the entire season, whereas in 1927 there was only one cultivation, weeds were abundant, and the branches of the trees interlocked. This probably produced a greater insect population in the orchard, which in turn produced a greater number of insect-host eggs for Trichogramma. Native insect-host eggs probably play an important rôle in building up the Trichogramma population in almost any environment.

TABLE 3.—*Number of daily field collections in which eggs normally parasitized by Trichogramma minutum were found in any one or all of the three lots of oriental fruit-moth eggs on pear foliage placed daily in widely separated peach trees in an orchard at Riverton, N. J., during 1927 and 1928*

Semimonthly periods of daily collections	Number of collecting days	Number of daily collections in which parasitized eggs were found		Semimonthly periods of daily collections	Number of collecting days	Number of daily collections in which parasitized eggs were found	
		1927	1928			1927	1928
May 1 to 15	15	0	0	Sept. 1 to 15	15	11	0
May 16 to 31	16	5	7	Sept. 16 to 30	15	7	5
June 1 to 15	15	6	1	Oct. 1 to 15	15	4	0
June 16 to 30	15	3	0	Oct. 16 to 31	16	1	0
July 1 to 15	15	5	1	Nov. 1 to 15	15	0	0
July 16 to 31	16	0	4	Nov. 16 to 30	15	1	0
Aug. 1 to 15	15	0	7				
Aug. 16 to 31	16	3	2	Total	214	55	27

In 1928, in a large 7-year-old peach orchard near Riverton, 13 peach trees adjacent to one another and arranged as shown in Figure 9 were selected for the purpose of determining the natural parasitism in the orchard. A pole, 2 by 4 inches in size and 6 feet long, was placed in the center of each of these trees in such a manner that the low, flat, wooden container on top of each pole was located among the leaves of a large branch so that the cut twigs bearing oriental fruit-moth eggs would be adjacent to, or in contact with, the foliage on the trees. From July 14 to September 8, on Mondays, Wednesdays, and Fridays of each week, bottles containing pear foliage bearing a known number of eggs (an average of 60 per twig for the season) were placed on top of these poles. The eggs were permitted to remain in the orchard until they showed parasitism, or until the normal larvae were ready to hatch.

About 20,000 eggs were used in this test, and Table 4 shows the results obtained.

TABLE 4.—*Data relating to egg parasitism by Trichogramma minutum in a field test in which 13 distinct lots of oriental fruit-moth eggs (averaging 60 eggs per lot) on pear foliage were placed in 13 peach trees on three days each week from July 14 to September 8, 1928*

Collection No.	Date eggs were placed in trees	Number of parasitized eggs found in tree No.—													Total number of eggs found parasitized	Number of lots of eggs found parasitized
		1	2	3	4	5	6	7	8	9	10	11	12	13		
1	July 14	----	----	10	----	23	33	----	20	26	28	11	24	2	177	9
2	July 16	----	4	----	4	6	1	----	34	----	----	----	----	9	58	6
3	July 18	43	----	42	7	----	----	----	----	----	3	----	42	34	171	6
4	July 20	25	----	----	5	12	----	22	2	----	----	----	4	----	70	6
5	July 23	17	2	----	2	2	----	----	54	18	9	28	6	4	142	10
6	July 25	----	----	5	----	12	----	18	14	6	14	13	8	13	103	9
7	July 27	----	7	24	46	7	----	----	41	----	12	----	9	23	169	8
8	July 30	----	16	----	3	----	----	----	----	----	7	10	3	----	39	5
9	Aug. 1	----	----	34	23	----	----	1	11	----	13	----	13	----	95	6
10	Aug. 3	----	----	----	----	5	----	1	12	7	52	----	9	12	98	7
11	Aug. 6	----	4	----	6	----	----	----	----	----	----	----	----	----	10	2
12	Aug. 8	21	----	8	23	7	----	10	19	10	18	----	2	----	118	9
13	Aug. 10	7	28	5	16	----	10	10	6	4	1	----	5	----	92	10
14	Aug. 12	14	----	12	3	----	3	----	5	----	----	----	----	----	37	5
15	Aug. 15	----	----	4	3	----	----	----	12	----	----	----	3	----	22	4
16	Aug. 17	----	----	----	27	----	----	3	16	----	13	----	----	----	59	4
17	Aug. 20	----	16	----	32	----	----	----	12	47	13	17	21	----	158	7
18	Aug. 22	44	11	4	7	2	----	1	6	----	24	4	25	79	207	11
19	Aug. 24	----	25	28	31	----	----	5	----	11	----	21	28	61	210	8
20	Aug. 27	----	----	----	29	----	----	----	28	17	----	----	----	----	74	3
21	Aug. 29	3	----	6	13	----	9	15	7	5	16	----	1	8	83	10
22	Aug. 31	----	----	----	34	----	----	----	----	----	----	----	----	----	34	1
23	Sept. 3	57	11	1	1	----	----	34	8	1	60	----	40	24	237	10
24	Sept. 5	----	----	----	----	----	----	----	----	----	----	----	----	----	0	0
25	Sept. 8	101	50	----	18	4	----	10	45	----	----	10	----	25	263	8
Total		332	174	183	333	80	56	130	352	152	283	114	243	294	2,726	----
Number of times parasitized eggs were found		10	11	13	21	10	5	12	19	11	15	8	17	12	----	164

For the entire period 14 per cent of all the eggs used were normally parasitized, and in the individual lots actually showing some parasitism the parasitism of the eggs averaged 26 per cent. In the table the numbers recorded in the main portion are the actual number of eggs parasitized in each tree for each collection date. For the entire season 50 per cent of the twigs having eggs and placed in the orchard showed some parasitism. On no date were all of the lots parasitized. On August 22, 11 out of 13 twigs bearing eggs showed parasitism, and at one collection, September 5, no parasites were found. Eggs placed in some trees were more frequently parasitized than those in others. For example, tree No. 4 showed 21 lots parasitized out of 25 tests, and tree No. 6 showed 5 lots out of 25 tests.

It is probable that the exact location of the poles in the trees had something to do with this difference; however, the poles were in similar positions as nearly as possible. This method of determining the presence and particularly the percentage of parasitism of Trichogramma in a peach orchard is open to considerable criticism. More work needs to be done on the matter of technic in determining percentage of infestation under orchard and field conditions.

Some preliminary tests were made during June and July in 1928 to determine the distance liberated parasites would fly and parasitize host eggs in an orchard. An old peach orchard was found near Moorestown which showed little or no parasitism by Trichogramma. Twice a week a number (100 to 200) of newly laid eggs of the oriental fruit moth were placed in several adjacent trees in the orchard, and in others some distance away. Several hundred fertilized female parasites (300 to 1,000) were liberated in a given tree about twice a week during June and July. In all of the tests, except one or two, parasitized eggs were found only in the trees where the adults were liberated. This preliminary test indicates that Trichogramma liberated in a given tree in numbers less than 1,000 will not spread from tree to tree. All of the liberations were made in the morning and on days that were bright and not very windy.

Considerable work needs to be done on the matter of distribution and frequency of liberation. The writer believes that in well-cared-for orchards it will be necessary to make frequent and large liberations to obtain satisfactory results for the control of an orchard pest. Possibly it may be necessary to liberate in every tree. It is also probable that several liberations made early in the season would be more beneficial than liberations made late in the summer.

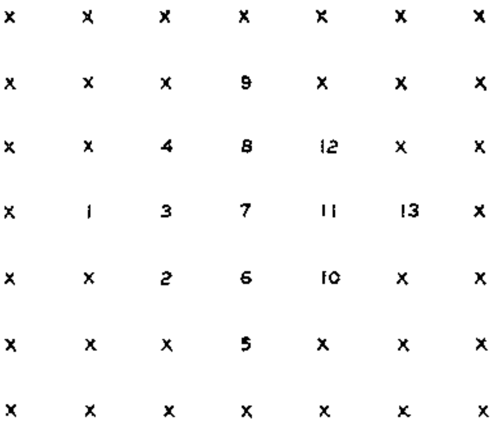

FIGURE 9.—Diagram showing the arrangement of the 13 peach trees in a commercial orchard near Riverton, N. J., where egg-parasite experiments were conducted in 1928. Adjacent trees are indicated by X

In some orchards Trichogramma undoubtedly plays an important rôle in the control of the oriental fruit moth. For example, in Burlington County, N. J., *Macrocentrus ancylivora* Roh., a parasite of the larvae in the twigs, is very important early in the season in reducing the infestation, yet late in the season, when the larvae of the oriental fruit moth enter the fruit, this parasite is not effective. At this season of the year Trichogramma is usually abundant, and parasitism of field-collected eggs may run as high as 60 per cent or more. In this manner the two parasites reduce the number of larvae which would normally go into the ripening peaches.

During 1927 an attempt was made to produce *T. minutum* on a large scale. A good start had been made with the host eggs of the

Angoumois grain moth (*Sitotroga cerealella* Oliv.) when a serious infestation of *Pediculoides ventricosus* Newp. appeared, and almost all of the host material was destroyed before it was learned how to control the mite with sulphur. By the time control was effective the season was nearly over. In the meantime some of the biological studies reported in this bulletin were conducted. In the course of these studies it was found that the Mediterranean flour moth was a very satisfactory host. In some respects it is superior to the Angoumois grain moth, especially in respect to the size and number of eggs produced. It is also a satisfactory host in which the parasite may winter, as mentioned previously. The eggs of the common bagworm. *Thyridopteryx ephemeraeformis* Haw., may also be used to carry over the parasite during dormant periods. Parasitized bagworm eggs kept outdoors during December, January, and February, at Columbus, Ohio, have produced adults in March. Also parasitized bagworm eggs have been kept dormant in refrigerator at 40° to 45° F. for several months.

Some studies were made in the use of artificial media for the production of these parasites. There is some indication that females may deposit eggs in agar-agar droplets or similar media. This phase of the investigation needs further study. The writer believes that there are possibilities in the use of artificial media; however, the technic may be extremely difficult to master.

SUMMARY

Trichogramma minutum Riley is a common and an important egg parasite of *Laspeyresia molesta* Busck and *Carpocapsa pomonella* Linné in New Jersey. Early in the season it may be scarce, while late in the summer it is usually abundant. Late in the season it undoubtedly plays an important rôle in reducing the number of larvae of the above hosts, some of which would enter the fruit.

Two species or strains of Trichogramma apparently occur in New Jersey in the hosts named. The important biological facts presented in this bulletin deal almost exclusively with the species which has distinct lemon-yellow females during the warm part of the active season.

The parasite has been carried through the winter (out of doors) in the eggs of the oriental fruit moth, the Mediterranean flour moth, and the common bagworm. Trichogramma out of doors emerges early in April, and adults of succeeding generations continue to emerge until late in the fall, November, and occasionally December and January.

In New Jersey there appears to be a maximum of 13 generations. From one to several individuals may develop in the eggs of the oriental fruit moth (1 to 4) and codling moth (1 to 7). Fertilized females produce more females than males, 63 per cent being produced in 1928. Unfertilized females produce males only. Adult parasites live only a few days (1 to 12) during midseason, and they deposit most of their eggs the first day or so if host material is available. One female parasitized as many as 131 eggs; the average for two seasons was 40.2 for 1927 and 35.9 for 1928. Oriental fruit-moth and codling-moth eggs whose development is two-thirds completed are not susceptible to attack by Trichogramma.

Temperature is an important factor in determining the length of the life cycle, egg to adult stage, of the parasite. For individuals the range is 7 to 75 days or longer, while with daily average lots, as shown in Figures 6 and 7, the range is from 8 to 53 days for two seasons. During most of the warm summer weather the time required is from 9 to 16 days. The ratio of change in the life-cycle period at average temperatures of from 60° to 75° F. is approximately 1 day increase or decrease for each 1.25° decrease or increase in the average temperature.

For maximum parasite production the temperature should average close to 80° F., the humidity should be fairly high, 70 to 80 per cent, an abundant and readily accessible supply of suitable host material should exist, and plenty of artificial light or indirect sunlight should be present.

A distinct change in color takes place in the adults, especially the females, when the average outdoor temperature falls below 62° F. During warm weather when daily average temperatures exceed 62° the females have a distinct lemon-yellow color while in the early spring or late fall when daily average temperatures are below 62° the females are distinctly metallic brown.

Preliminary field tests in a peach orchard indicate that small liberations of from 300 to 1,000 adults per tree are not sufficient to produce parasitism among eggs in adjacent trees.

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