The biology and rate of reproduction and the morphology of the immature stages of *Apanteles angaleti Muesebeck*

(Hymenoptera: Braconidae)

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(With 30 Figures)

Introduction

Platyedra gossypiella Saunders, the pink bollworm, is a very serious pest of cotton wherever it is grown. It is cosmopolitan in its distribution and is found damaging cotton bolls in all the cotton growing belts of the world. In spite of the several parasites that attack the pink bollworm in its various stages of growth and development, the moth assumed the status of a very major pest, threatening cotton-growing both in the old and new worlds. Though several larval and pupal parasites have been reared from the pest, none has been found to be effective in the biological control of the pest either in India or abroad. Very recently, a braconid parasite was reared from the pink bollworm infesting the cotton bolls in the farm of the Indian Agricultural Research Institute. This was the first record of the parasite and Muesebeck (1954) described it as *Apanteles angaleti*. A large number of parasites were successfully bred in the laboratory on the alternate host *Corcyra cephalonica Stainton* by Narayanan, Subba Rao, Angalet and others (1953) by developing a new technique. Several thousands of these parasites were shipped to U.S.A. by Mr. George W. Angalet, Entomologist, U.S.D.A., to be released against the pink bollworm in the cotton belts of that country. It was thought essential to study the biology and the rate of reproduction of this parasite which may prove to be of some potential importance for the control of the pest. During the course of these investigations the behaviour of the parasite in relation to the host has been carefully studied as also the exact stadia of the host larva which it prefers for parasitization. The morphology of the various instars of the parasite and the physiology of respiration have been studied in detail, for, besides the morphology, there is a gap in our knowledge of the physiology of respiration and the organs concerning this vital function.

I. The biology and rate of reproduction

Material and methods

The parasite has been initially reared from the pink bollworm from field-collected cotton bolls and further bred in large numbers on the alternate host *Corcyra cephalonica St.* For the study of various stages of development the breeding of the parasite was carried out in a room where-in the temperature was maintained at 27° C. and relative humidity at about 70%.

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Technique of breeding of parasites

The technique of breeding the parasite, though conforming with the original description in principle, has been improved by effecting certain changes to make the mass breeding more efficient and productive. The following is the technique adopted for the mass rearing of the parasite (Fig. 1).

Two glass troughs of about 6″ diameter and 4″ depth were obtained and one was filled with a little water and was covered with a piece of fine wire gauze, held tight by means of india rubber bands. A thin piece of muslin was used to cover the wire gauze. A dish containing freshly hatched *Corcyra* larvae in a thin film of flour was placed on the muslin cloth and the other trough was inverted over the first. The mated parasites were liberated in the upper trough for oviposition. The parasites were fed on fresh cut raisins. The idea of keeping water in the lower chamber was to increase the humidity in the upper chamber to about 90%, which after considerable experimentation was found to be ideal for efficient mass rearing of the parasite. The dish containing the larvae thus exposed was removed after 24 hours and a new dish with freshly-hatched unparasitised *Corcyra* larvae was replaced. The parasitised larvae were then transferred to large trays where plenty of crushed jowar with a little yeast was available for further growth and development. The larvae completed their development and the parasite grubs emerged out from the host larvae as the latter were just about to prepare their cocoons for pupation. The adult parasites in due course emerged from the trays.

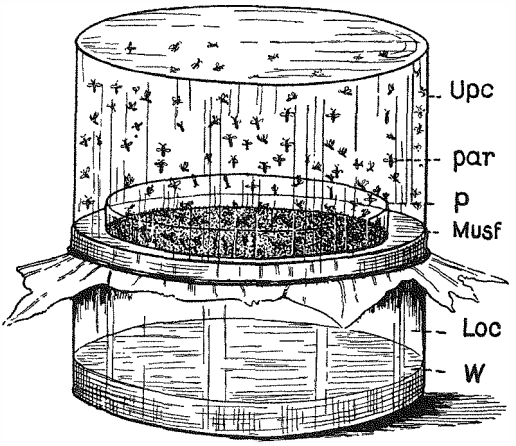

Fig. 1. Technique of breeding *Apanteles angaleti Mues.*
Loc — Lower chamber; Musf — Muslin covering; P — Petri dish; Par — Parasites; Upc — Upper chamber; W — Water

Oviposition

The females, as soon as they come near the host larvae, vibrate their antennae and locate the exact position of the host and thrust their ovipositor in quick succession. Oviposition is completed within a few seconds and the egg is laid within the body of the host. In the majority of the dissections that were carried out, it was observed that only one egg was deposited per host. However, it was not uncommon to find superparasitised hosts. The following table 1 will indicate the number of eggs dissected out from each larva.

The table shows that under normal conditions of laboratory breeding a host may contain eggs varying from one to five, though in the majority of the host larvae only one egg is laid.

The eggs are generally deposited in any part of the body of the host except the head and the 10th. abdominal segment. Investigations were

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made to find out the exact portion where the egg is normally deposited in the host body. The following table 2 gives a fair indication of the body segments of the host which the parasite prefers.

Table 1. The number of eggs dissected from each host

	The number of eggs					
	1	2	3	4	5	6
No. of hosts	1155	267	59	23	6	—

Table 2. The position of egg in the body

Head	Thorax			Abdomen									
	1	2	3	1	2	3	4	5	6	7	8	9	10
No. of eggs	3	14	23	30	34	31	16	11	20	13	27	3	—

Incubation period of egg

The incubation period for the egg of *A. angaleti* was investigated under four different temperatures, the humidity remaining constant. The following table gives the duration for the incubation of eggs under different temperatures.

Table 3. The incubation period of the eggs of *A. angaleti*

Temperatures (70% R. H.)	Incubation period in hours		
	Minimum	Maximum	Average
25° C.	28	35	32.2
27° C.	27	33	30.2
30° C.	22	27	26.3
35° C.	23	29	24.9

The results indicate that with the rise in temperature, the incubation period is reduced. However, it is interesting to note that in the case of 35° C. though the eggs hatched out after 24 hours, the larva could not develop and survive at this temperature.

The Larva

The larva of *Apanteles angaleti* moults three times during the course of its growth and development. The first two moults take place within the body of the host and the third moult occurs before transformation into pupa within the cocoon.

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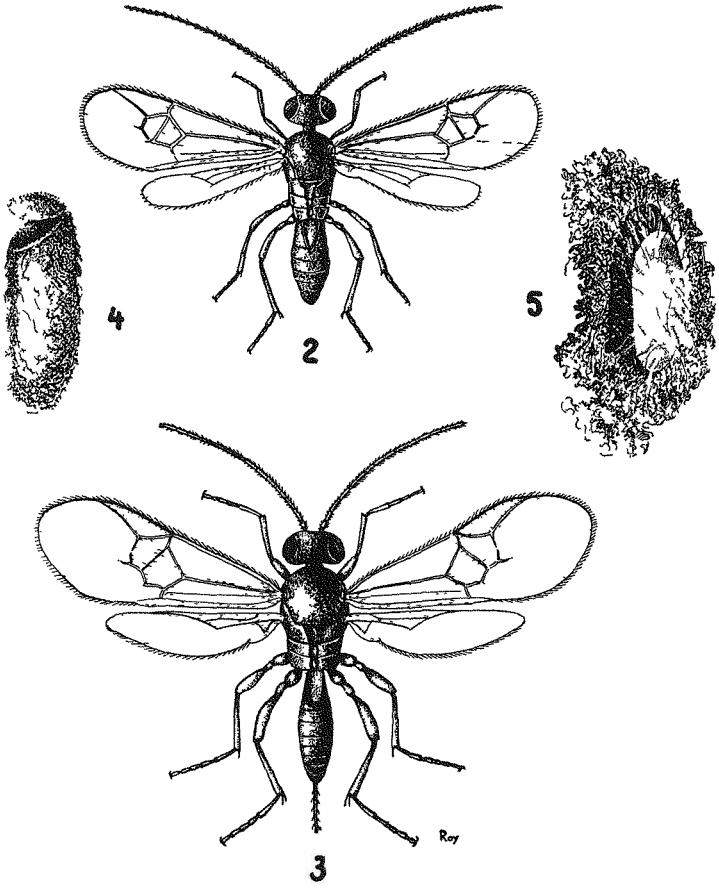

Fig 2 *Apanteles angaleti Mues* , male — Fig 3 *A angaleti*, female — Fig 4 Cocoon of *A angaleti* — Fig 5 Parasite cocoon within the host cocoon

First Instar Larva
(Fig. 8 & 9)

The egg, soon after it is deposited, begins to grow in size and just before hatching the cephalic end becomes swollen The fully developed embryo may be seen before hatching (Fig. 7). The larva cuts its way out from one side of the egg and the egg shell is left behind as a crumpled structure The larva soon floats in the body fluid of the host larva and feeds on it. When observed under the high power of a binocular the larva is seen constantly to bend its body so that the anal appendage and the head come together and then separate This sudden release from the tension gives momentum to the larva to move forward. It is possible that in this way

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the larva is capable of movement even in a limited space of the body cavity of the host.

In the majority of dissections, the first instar larva was observed in the sixth and seventh abdominal segments of the host larva. When more

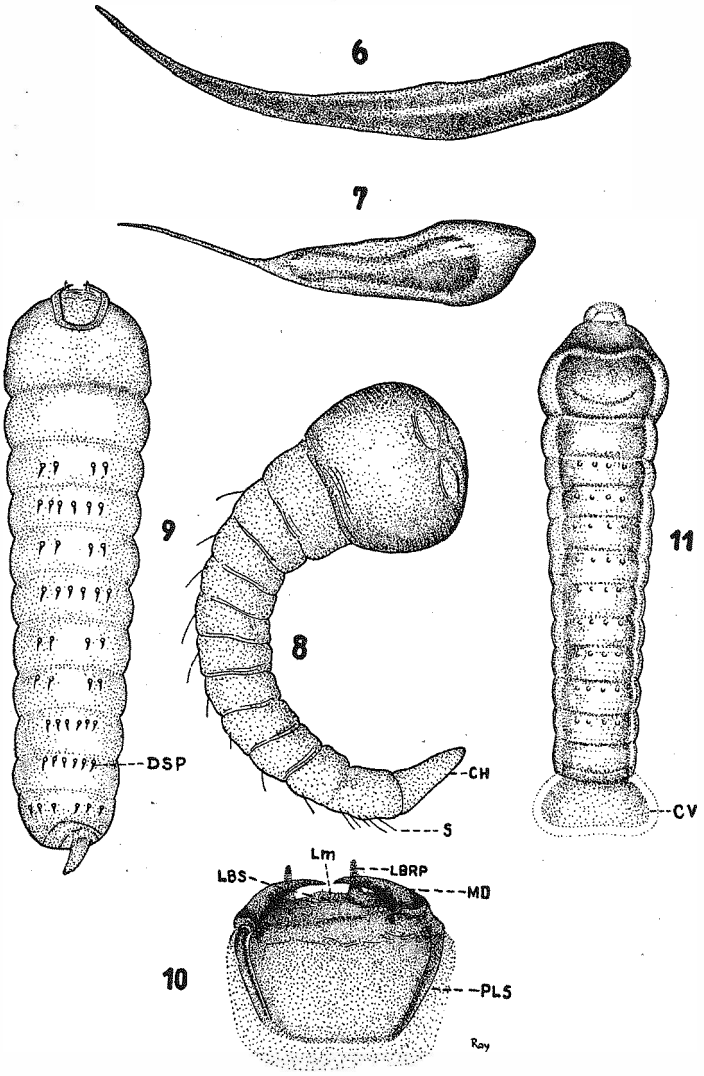

Fig. 6. Egg of *Apanteles angaleti Mues.* — Fig. 7. Egg of *A. angaleti* fully developed. — Fig. 8. Freshly hatched larva of *A. angaleti.* — Fig. 9. Late first instar larva of *A. angaleti.* — Fig. 10. The head of first instar larva of *A. angaleti.* — Fig. 11. Early second instar larva of *A. angaleti.*

CH — Caudal horn; CV — Caudal vesicle; DSP — Dorsal spines; LBRP — Labral process; LBS — Labio-stipites; LM — Labium; MD — Mandible; PLS — Pleurostoma; S — Setae

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than one egg is deposited in a single host it is common to find that the stronger one killed the supernumerary eggs or larvae with its mandibles. It is, therefore, certain that the first instar larva is capable of some movement within the host body.

The duration of the first instar larval period was investigated by means of regular dissections of the host larvae. The first-instar period lasted from 15.8 days to 20 days at 30° C. and 70% R.H. The larva which is very conspicuous by its caudal horn cannot be mistaken for the second instar larva which has a caudal vesicle. As soon as it moults from first to second the caudal horn disappears.

The second instar larva
(Fig. 11 & 12)

The second instar larva differs from the first instar very much in shape, size and mouth parts. The body becomes deeply segmented. The head which is rectangular in the first instar now assumes a rounded shape. The sickle-shaped mandibles disappear. The larva is found in the host body longitudinally with its head directed towards the head of the host. Usually the larva is found dorsal to the alimentary canal of the host. When observed under a microscope the caudal vesicle is seen to contract and expand almost imperceptibly. As in the case of first instar larva the second instar period was also investigated by regular dissections. It was observed that the second instar period lasted from 8.5 days to 13.2 days at 30° C. and 70% R.H.

Towards the end of the second instar the larva is seen to be enveloped in an outer skin and the mandibles of the third instar are also visible. The larva which is broad and flat in the early stages now becomes roundish and tapers anteriorly. The caudal vescicle is also greatly reduced.

The third instar larva
(Fig. 14)

When the second instar larva of the parasite is fully grown within the host body, the host caterpillar stops feeding and constructs a cocoon. When the cocoon is fully constructed the host larva remains quiet and. motionless. This is an indication of the emergence of the parasite larvae Observations made so far show that the parasitic grub emerges from the host body either on the same day of the construction of the cocoon or the next day. The second moult actually occurs while the larva is emerging from the host body. One very interesting feature of the emergence of the parasite grub from the host is that when the grub has completed emergence from the host body upto its last segment, it turns round and makes a second cut on the host body with its powerful mandibles and starts sucking the body fluid. After sometime it completely extricates its body from the host remains, but continues to feed till the host remains become a crumpled mass.

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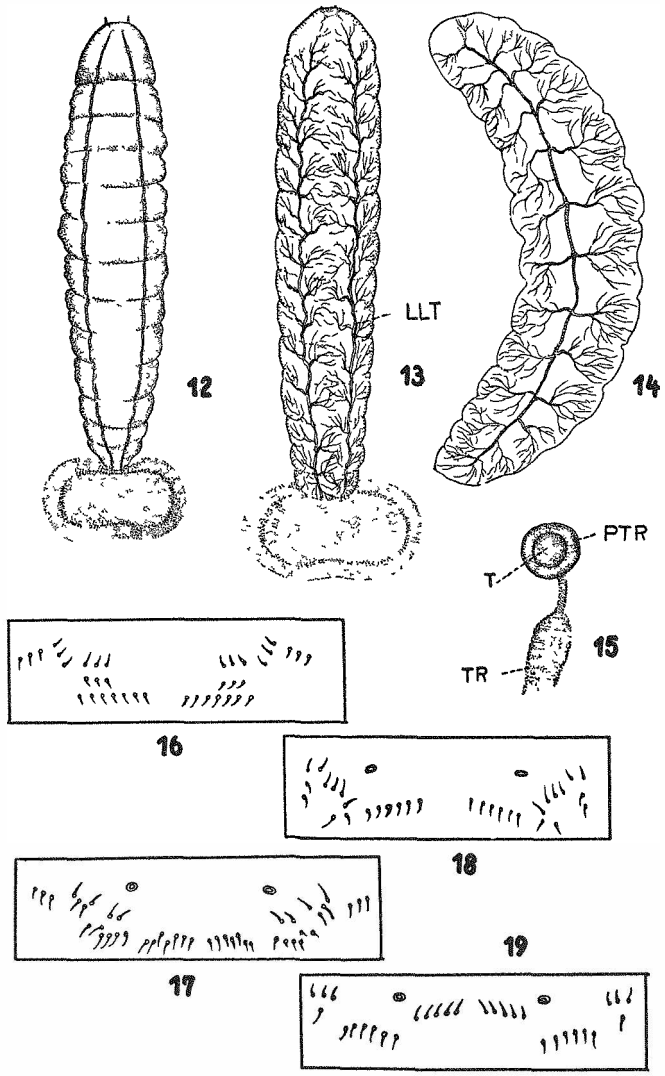

Fig. 12. Second instar larva of *A. pantels angaleti Mues.* — Fig. 13. Tracheal system of second instar larva. — Fig. 14. Tracheal system of third instar larva. — Fig. 15. The spiracle of third instar larva. — Fig. 16. Arrangement of setae on the prothoracic segment. — Fig. 17. Arrangement of setae on the mesothoracic segment. — Fig. 18. Arrangement of setae on the first abdominal segment. — Fig. 19. Arrangement of setae on the second abdominal segment. — LLT — Lateral longitudinal trunk; PTR — Peritreme; T — Tracheal opening; TR — Trachea

The majority of grubs during the course of these investigations emerged from the first to fourth abdominal segments of the host larva. It took from 7 to 18 minutes for the larva to extricate its body from the host. But

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feeding on the host remains continued from 35 minutes to 3 hours. It was also observed that whenever the host larva had not constructed its cocoon, the parasite grubs either failed to emerge or failed to construct its own cocoon for pupation. After a short duration the shining parasitic larva becomes pale and dry due to exposure.

Construction of cocoon

Soon after the feeding is over the third instar larva starts spinning its cocoon. As the cocoon of the host larva is always there to provide the base, the grub actively commences the spinning. Compared to the parasite cocoon the host cocoon is very thin and transparent and hence observation on the mode of spinning was possible.

The parasitic grub starts spinning a cocoon by passing silken threads on the sides of the host cocoon. The stretching of the silken threads from one side to the other is done by moving only the head. To start with, only the anterior portion is spun first and the larva reverses its position to complete the posterior half. After completing a thin layer it will continue to spin again to make the cocoon thick and tough. A number of observations were made with regard to the time taken for the construction of the cocoon and it was observed that the larva spent nearly 1 to 12 hours in the construction.

The cocoon
(Fig. 4 & 5)

The cocoon of *Apanteles angaleti* is enclosed by the host cocoon and it measures from 3.5 mm. to 5 mm. in length and 1.2 mm. to 1.54 mm. in breadth. The female cocoon always measures more than the male cocoon. The cocoon is of shining white colour and is very tough.

The prepupa

The prepupal stage starts from the time the parasitic larva stops feeding and spinning of the cocoon is started. The larva, after constructing the cocoon, rests for a while and excretes the meconium. After 24 hours, it changes into pupa by casting off the larval skin.

The adult
(Fig. 2 & 3)

The pupal period lasts for only 4 to 6 days at 30° C. temperature and 70% R.H. A little time before the emergence of the adult some movement is noticed within the cocoon. A circular lid-like portion is cut open at the anterior end with the help of the sharp mandibles, which are seen actively moving in a circular direction. When a little portion is cut the antennae are thrust out and by constant movement of the antennae the circular cut is deepened. After some time the lid-like portion is removed and the adult emerges out. It takes about 25 minutes to complete the cutting of

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the cocoon and the final emergence. The adult parasite now rests for a while, cleans its antennae, legs and abdomen and flies off.

Copulation

Immediately after emergence the female parasites are ready to mate if males are available. Normally the mating lasts for 38 to 90 seconds. Once the female is impregnated it will not allow the male to mate with it again for a second time. However, the male is capable of mating more than once.

Oviposition

Normally the females are ready to oviposit soon after the mating is over. Under laboratory methods of breeding, the female, as soon as it comes in the vicinity of its host, shows great excitement by vibrating its antennae and locates the host. Soon the ovipositor is lifted up and is thrust inside the host body and the deposition of the egg is accomplished. The oviposition is very quick and it takes hardly 3 to 12 seconds. It is not uncommon to see that the female thrusts its ovipositor in quick succession several times under mass rearing conditions. Generally the females are not able to distinguish between the parasitised and non-parasitised host larvae.

Fecundity

To determine the rate of reproduction in *Apanteles angaleti*, 10, 15, 20 and 30 *Corcyra cephalonica* larvae were offered to a single mated female every day till death. Six replications were made at a time. After 24 hours the host larvae were dissected and the number of eggs contained in them counted. The following table shows the result of these experiments.

Table 4. The fecundity of *Apanteles angaleti Muesebeck*

Replication No.	Total Number of eggs laid with each host density			
	10 hosts	15 hosts	20 hosts	30 hosts
1	66	33	49	62
2	24	51	170	130
3	93	160	58	52
4	56	76	104	31
5	90	97	111	127
6	42	34	113	116
Average	61.83	75.16	100.83	86.33

It is seen from the above table that the total number of eggs laid in the six replications increased with the increase in the number of hosts. However, beyond certain density of host, once again the total number of eggs laid decreases.

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Longevity

The adults are very delicate and are easily susceptible to temperature fluctuations. Under laboratory conditions (30° C. and 70% R.H.) the females when fed with freshly cut raisins lived from 7 to 12 days and the males 2 to 8 days.

Sex-ratio

The sex-ratio of parasite was studied by exposing 25 larvae to a single mated female daily until death. Four replications were carried out. After the emergence of the adults the sex-ratio was recorded. The following table shows the results of the experiment.

Table 5. The sex-ratio of *Apanteles angaleti* under laboratory conditions on *Corcyra cephalonica* host

Replication No.	Date of amergence of female	Date of death of female	No. of hosts exposed	Progeny	
				Male	Female
1	22. 5. 54	1. 6. 54	250	20	28
2	22. 5. 54	29. 5. 54	175	9	18
3	22. 5. 54	27. 5. 54	125	2	6
4	22. 5. 54	28. 5. 54	150	—	10
Total			700	31	62

It is seen from the table that the sex-ratio is 2:1, the females predominating.

The sex-ratio was also studied under mass rearing conditions and field conditions. In the case of field studies affected bolls were brought to the laboratory and kept in the wire-gauze cages. After the emergence of the adults the sex-ratio was recorded. The following two tables summarise the data obtained from the laboratory and field studies.

Table 6. Sex-ratio of *Apanteles angaleti* under mass rearing conditions

Total no. of emergence	Adults		Sex-ratio
	Male	Female	
1618	602	1016	1:16

Table No. 6 and 7 show that there is not much difference in the sex-ratio both under laboratory and field conditions.

Apanteles angaleti oviposits even without mating. But the parthenogenetically reproduced progeny consists of only males.

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Effect of parasitism on the host-larva

After hatching, the parasite grub feeds on the body fluid of the host. During the later stages of development the larva feeds also on the fat bodies of the host. Dissections made at different stages of the development have shown that the parasite grubs never attack any vital parts of the host.

Table 7. Sex-ratio of *Apanteles angaleti* under field conditions

Total No. of emergence	Adults		Sex-ratio
	Male	Female	
201	84	117	1:12

In the very early stages of parasitism it is possible to distinguish a parasitised larva by its deep pale colour from the healthy one which is creamish white. In the case of heavily superparasitised larvae, the hosts look yellowish and sickly. However, as the development proceeds it is very hard to distinguish a healthy larva from a parasitised one. But there is visible difference in the growth and development of the parasitised and non-parasitised larva.

The difference in growth and development is marked with regard to moulting, size and weight of the parasitised larvae. The following tables show the number of moults, size and weight of the parasitised and healthy larvae.

Table 8. The number of moults undergone by parasitised and healthy *Corcyra* larvae

Host	Number of moults undergone in each case											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Parasitised larvae	4	7	6	6	4	6	2	2	2	2	3	2
2. Healthy	Undergoes only 8 moults											

Table 9. The measurements of parasitised and healthy larvae

Stage of parasite in the host	Parasitised		Healthy larva of the same age	
	Average length	Average breadth	Av. length	Av. breadth
1. Host larva with fully developed 1st. instar parasite grub.	4.5 mm	0.7 mm	6.1 mm	0.8 mm
2. Host larva with early second instar parasite grub	5.5 mm	0.8 mm	6.6 mm	1.0 mm
3. Host larva with fully developed 2nd. instar parasite grub.	6.3 mm	1.0 mm	8.1 mm	1.5 mm

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It can be seen from the tables 8—10 that the weight, measurements and the number of moults undergone by the healthy and parasitised larvae differ greatly.

Table 10. The weight of the parasitised and healthy larvae of the same age

Average weight of parasitised larva	Average weight of healthy larva
0.005997	0.015686

Discussion

Apanteles angaleti Muesebeck is a new record as parasite of *Platyedra gossypiella* (*Saunders*) in India. The distribution of the parasite is almost well spread throughout India.

The parasite has been mass bred on an alternate host *Corcyra cephalonica Stainton* in the laboratory. Perhaps it is the first time that the genus *Apanteles* has been mass reared on an alternate host. It is well known that *Apanteles* is a shy parasite, and attempts to breed it under laboratory conditions on an alternate host have always been unsuccessful. Hence the success achieved in this country has overcome one of the main difficulties in the biological control of pink bollworm both in India and other parts of the world.

The egg of *Apanteles angaleti* is a narrow elongate structure with a short petiole. It has been observed that the eggs are usually deposited in the anterior region of the host body. Eearlier workers like MUESEBECK (1918) in *A. lacteicolor* CROSSMAN (1922) in *A. melanoscelus* PARKER (1935) in *A. solitarius* CHATTERJEE (1939) in *A. machaeralis* HAFIZ (1947) in *A. ruficrus* recorded that the eggs could be laid in any part of the body of the host. The observations made by us on the distribution of eggs within the host body do not support the view held by the above mentioned authors. However, it must be mentioned that most of the *Apanteles* spp. that have been studied in great detail are gregarious. But *A. angaleti* is a solitary parasite. It was also observed that *A. angaleti* laid more than one egg in a host and the maximum number recorded in a host was 8, while two in a host was a common phenomenon. However, only one completed its development and growth and attained maturity. The supernumerary eggs or larvae were destroyed in actual combat and the strongest survived.

The study of the incubation period at different temperatures showed that the period prolonged as the temperature decreased. The dynamics of development was thus faster at higher temperature. It was also observed that 35° C. was fatal to the freshly hatched parasitic larvae. The first instar parasitic grubs were mostly dissected out from 6th. and 7th.

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abdominal segments. This observation was not made by FULTON (1940) in *A. congregatus* and CROSSMAN (1922) in *A. melanoscelus*, as they mostly dissected the parasitic larvae from the posterior region of the host.

The duration of the larval instar seems to vary from species to species and with temperature and humidity conditions. The movement of the larva, or the locomotion in the parasitic grub is a matter of great controversy. The present studies have to some extent cleared this point. It was observed that the eggs in the superparasitised host larvae were not always laid in any particular segment. In such cases the early hatched larva or the strongest of the group moves within the host body to reach its rivals and destroy them. It is clearly seen in dissections that the first instar larva constantly whips its anal segment from side to side. It is quite possible that this activity, aided by the presence of long spines, helps the larva in its locomotion.

The third instar larva of *A. angaleti* has a characteristic way of feeding on the host body fluid after its emergence from the host. In fact the larva does not extricate its body fully but allows the anal segment to remain and act as a plug to the emergence hole. It then applies its mouth to the body of the host at a separate point and completely sucks the host blood. It is only then that the anal segment is withdrawn from the remains of the host. This curious habit has not been studied by earlier workers in any other solitary species of *Apanteles*. This habit has a definite bearing on the formation of the cocoon. It has been experimentally observed that when the larva was pulled out of the host body by force the host body fluid flooded the area on which the grub was to spin its cocoon. The task of spinning and completing the construction of the cocoon was always made impossible as even the slightest moisture prevented the construction of the cocoon and the parasite larva invariably died. It is therefore obvious that the last abdominal segment acts as a plug to the emergence aperture.

Careful observations made during the cocoon formation show that the parasite after emergence from the host is unable to form a cocoon unless it is within the host cocoon. In the absence of host cocoon the parasitic grubs were given soft paper, cotton wool, tissue paper, etc., but they failed to spin a cocoon. It is possible that the parasite which lives in a liquid medium, when it comes out to a dry environment cannot face the situation unless it is well protected. The only possible explanation for the death of the parasitic grub when it is not in its host cocoon is that it dies of desiccation. FALLIS (1942) has also observed in case of *Apanteles carpatus* that the grubs failed to construct cocoons when they were taken out of their host's cocoon.

The study of the fecundity of the endoparasites is always confronted with many difficulties. There are very few records of actual dissections of the parasitised larvae and of counting the eggs daily. However, this gives only an indication of the possible fecundity of the species studied,

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and in the field conditions it is probable that the females are capable of laying more than the number which they normally lay under laboratory conditions on an alternate host. However, the present studies have indicated that *A. angaleti* has a high fecundity.

The sex-ratio of the parasite has been recorded under experimental, mass breeding and field conditions and it was found to be 1:2, females predominating. *A. angaleti* is capable of parthenogenetic reproduction. But the progeny is always only males. However, there are instances of parthenogenetic reproduction in other species like *A. thompsoni* and *A. carpatus* quoted by VANCE (1931) and FALLIS (1942) in which males are unknown.

The effect of parasitisation on the host is not very easy to observe in the case of internal parasites. Though, physically both the healthy and parasitised larvae look alike, physiologically they differ very much. This is evidenced in the number of moults, colour, weight and sometimes size. As the age of the parasitised host larva advances it loses its apetite altogether, which is indicated by the absence of food particles in the crop and the alimentary canal. The larva comes to the top of the grain and prepares itself for the ensuing death.

II. The morphology of the immature stages

Material and methods

The immature stages required for the study were obtained from the breeding stock of the parasite. For the study of eggs and immature stages the host larvae were dissected out in 1% salt solution. Immature stages were first fixed in Bouin's fluid, washed in 70% alcohol and then dehydrated in the alcohol series. Material was embedded in paraffin wax and sections were cut at the required thickness. Sections were stained in Haematoxylin. Tracheal system was studied in the living condition after making temporary mounts in glycerine.

Description of immature stages

1. The egg
(Fig. 6 & 7)

The egg of *Apanteles angaleti* when freshly laid in the body cavity of the host is somewhat glistening-white and semi-transparent. It measures 0.3 mm to 0.48 mm in length and 0,04 in width. The cephalic end is rounded and the caudal end is drawn into a tail like structure. The chorion is smooth. Embryonic development is very rapid and the embryoformation takes place within 12 to 15 hours after oviposition. Just before hatching the fully formed embryo could be clearly seen under a low power of the microscope. At this stage the egg measures 0.4 to 0.65 mm length and 0.08 to 0.12 mm in breadth. The growth and development during the incubation period amounts to one and one quarter times in length and about four times in breadth.

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2. The larva

First instar (Fig 8 & 9)

Newly hatched larva has a translucent watery appearance and is very delicate. The head (Fig. 10) is the largest part of the larva, square in shape and appears to be composed of two segments, the second being a false one. The head is followed by three thoracic segments and seven abdominal segments. The seventh abdominal segment which represents the last three segments of the fully developed grub is fused and the segments cannot be distinguished easily. The last segment carries a caudal horn (Fig. 8, CH) on the ventral side. The larva as soon as it hatches measures on an average 0.24 mm in length and 0.05 mm in breadth.

Two of the posterior thoracie segments and all the abdominal segments bear a row of transverse sharp spines dorsally (Fig. 9, DSP). The first six rows of transverse spines are arranged across the middle of the segment, whereas the last three rows are more towards the anterior part of each of the segments. The distance between the spines also varies from segment to segment. The arrangement of spines is as follows:

Second thoracic segment	— 4 spines in groups of 2.
Third thoracic segment	— 6 spines arranged at equidistance
First abdominal segment	— Same as in the second thoracic segment.
Second abdominal segment	— Same as in the third thoracic segment.
3rd & 4th abdominal segments	— 4 spines in groups of 2.
5th & 6th abdominal segments	— 6 spines arranged at equidistance.
7th abdominal segment	— 6 spines in groups of 3.

After a period of feeding and growth, though still in the first instar, the larva changes considerably in shape and appearance and possesses a head, 3 thoracic segments and 9 abdominal segments, the last two abdominal segments being added as a result of the division of the 7th. abdominal segment. The larva now looks more slender whitish and possesses a small anal vesicle (Fig. 11, CV). The newly formed segments are devoid of spines. The caudal horn which is a very conspicuous appendage in the early first instar is now pushed off laterally due to the development of the caudal vesicle. Just before the larva moults into the second stage the caudal appendage disappears.

The mouth parts appear as a ventrally projecting lobe of the head (Fig. 10) and are well sclerotised. The labium (Lm) is clear. The mandibles are pointed and sickle-shaped with their bases broad. It measures 0.616 mm in length. The bases of the mandibles are supported by rod-like structures which are homologous with the pleurostoma (PLS) of other ectoparasitic

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hymenopterous insects that have a well defined first instar larval mouth parts. The labio-stipites (LBS) carry two small protruberances called the labral processes (LBRP) which project forward over the mouth opening. Just prior to the first moult the late first instar larva measures 0.54 mm to 0.9 mm in length with an average of 0.66 mm, the average head width being the same as 0.18 mm indicating that no moult has taken place. The body segments become deeply constricted and the anal vesicle becomes enlarged. The head loses its shape and becomes oblong, sligthly tapering towards the mouth. The dorsal spines tend to disappear. The larva at this stage has no tracheal system.

The Second instar larva

The second instar larva as soon as it moults from first to second almost resembles the first instar with the exception that the mandibles which were sickle-shaped and prominent in the first instar disappear. The larva possesses 13 body segments (Fig. 11) excluding the head. The head consists of 2 segments, the second being a false one as in the case of first instar. The larva now measures 1.0 mm to 1.9 mm in length and is devoid of dorsal spines. The mouth parts are not visible except for two fleshy lobes which perhaps represent the well developed earlier mandibles of the first instar. The early second instar is translucent, but as it develops it becomes opaque. The larva appears flat and the caudal vesicle is very much broader than the body segments.

Anatomy of the Second instar larva

Alimentary canal: — (Fig. 23). A thorough study of the serial longitudinal sections of the larva reveals that the alimentary canal consists of a short, slender, oesophagus which enlarges posteriorly into mid intestine (Fig. 23, MI). The mid-intestine is a long tube of almost equal breadth, lying in the centre of the haemocoel. The mid-intestine is blind at its posterior end. Transverse sections at the junction of the caudal vesicle and the abdominal segment shows that the intestine is closed (Fig. 24, PMI). The epithelium of the mid-intestine consists of a single layer of big cells with large nuclei. The peritrophic membrane separates the food from the epithelial layer.

Heart: — The heart (HT) is seen occupying a large space in the caudal vesicle where it is almost globular in shape. In the anterior region the heart is but a narrow tube. The transverse sections of the caudal vesicle at its origin (Fig. 24) show that the hind intestine is closed and the heart occupies a small area dorsal to it. The malpighian tubules are clearly seen ventral to the intestine and their lumen is empty (Fig. 24, MPG). As the sections pass through the posterior region of the caudal vesicle it is seen that the heart occupies the major portion of the vesicle. Actually the heart occupies more than 2/3rds of the total area. Near the

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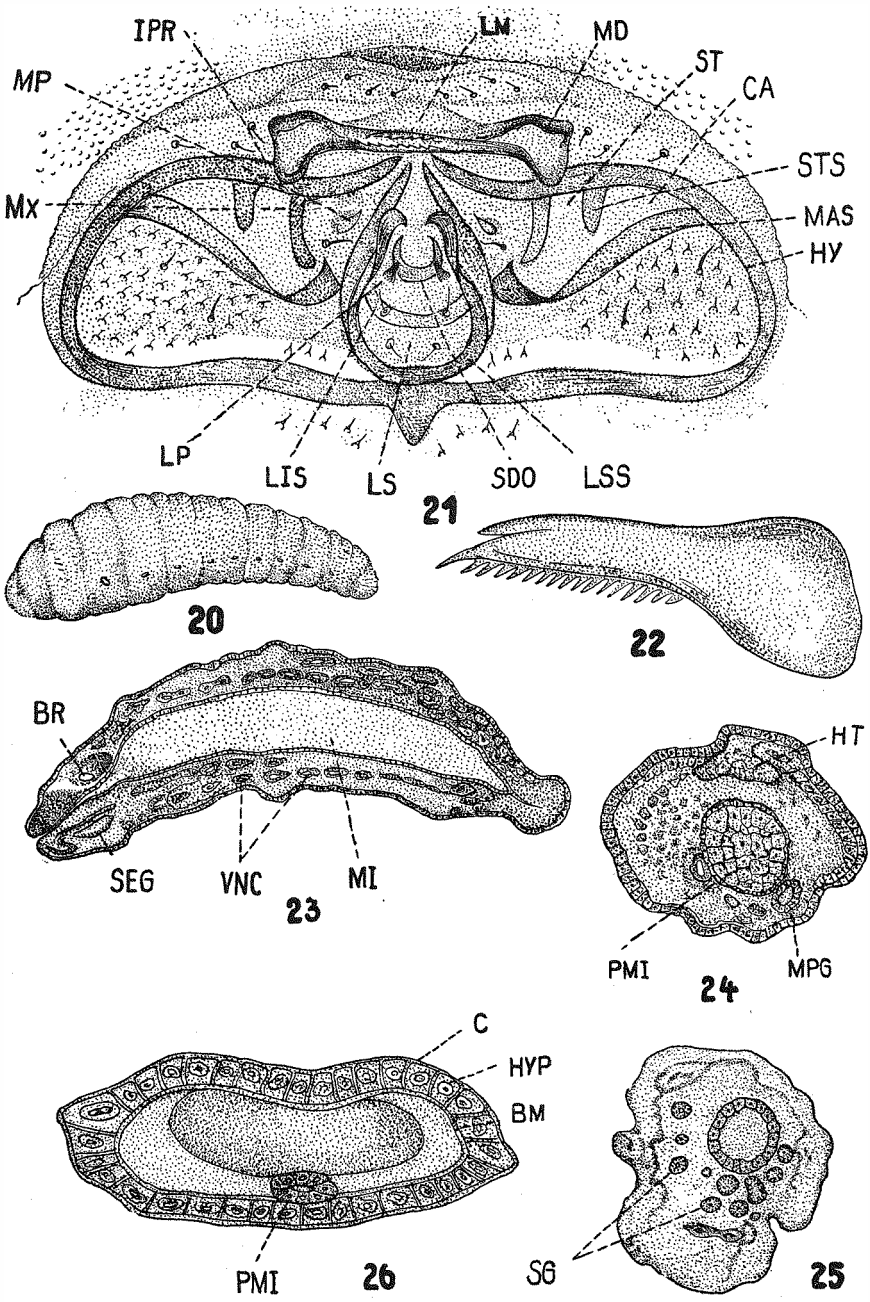

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posterior end the heart occupies the entire area. The wall of the caudal vesicle is made up of a single layer of large cells (Fig. 26, HYP) with large nuclei. The cells do not appear compact. A little inter-cellular space is left between the edges of the cells. The cellular layer is bounded externally by a thin layer of cuticle (C) and internally by a basement membrane (BM). The inter-cellular spaces render the wall of the caudal vesicle permeable to gaseous exchange (Fig. 27, HYPC).

Nervous system: — The nervous system is composed of a big bilobed brain (Fig. 23, BR) and a sub-oesophageal ganglion (SEG). There are 3 thoracic and nine abdominal ganglia (VNC). The ninth abdominal ganglion is the largest and it is a composite structure.

Silk glands: — The paired silk glands which are united at their anterior end traverse in the posterior direction and reach up to the point where the caudal vesicle begins. The glands are much convoluted and occupy a major area of the body cavity. The cross section (Fig. 25, SKG) shows that the silk glands lie around the mid intestine. The silk gland is made up of a single layer of columnar cells with large nuclei and their lumen are filled with the secretory products.

Respiratory system: — The tracheal tubes which are so conspicuous and well developed in the late second instar are totally absent in the early stages. When it is about the middle of the second instar the tracheal system appears and consists of two lateral longitudinal trunks (Fig. 13, LLT) that extend along the sides of the body. In each body segment two branches are given off from the lateral trunk, a dorsal and a ventral. These branches further divide and sub-divide into finer branches in such a way that they form a very fine dense net work which appears to extend to the cuticle. However, the spiracles are absent. The tracheoles do not extend to the caudal vesicle. Just before the end of the second instar the tracheal system undergoes a change. The fine net work of tracheoles disappears and only the main branches persist.

Fig. 20. Third instar larva of *Apanteles angaleti Mues.* — Fig. 21. Mouth parts and endoskeleton of third instar larva of *A. angaleti* — Fig. 22. Mandible of third instar larva of *A. angaleti.* — Fig. 23. Longitudinal section of the second instar larva. — Fig. 24. Transverse section of the second instar larva passing through the end of mid intestine. — Fig. 25. Transverse section of the second instar larva passing through the mid intestine. — Fig. 26. Transverse section of the caudal vesicle

BM — Basement membrane; BR — Brain; C — Cuticle; CA — Cardo; HT — Heart; HY — Hypostoma; HYP — Hypodermis; IPR — Inferior pleurostomal ramus; LIS — Ligular sclerome; LM — Labium; LP — Labial palpus; LS — Labial stipites; LSS — Labio-stipital sclerome; MAS — Maxillary sclerome; MD — Mandible; MI — Mid intestine; MP — Maxillary palpus; MPG — Malphigian tubule; MX — Maxilla; PMI — Posterior mid intestine; SDO — Opening of the salivary duct; SEG — Sub-oesophageal ganglion; SG — Silk glands; ST — Stipes; STS — Stipital sclerome; VNC — Ventral nerve cord

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Towards the end of second instar the larva becomes tapering anteriorly. The caudal vesicle becomes reduced and the third instar mouth parts appear. At this stage the larva measures from 2.6 mm to 4.16 mm.

The third instar larva

The fully grown larva which moults from second to third instar as it bores out its way from the host is elongate and of the typical braconid type. It is creamish white in colour and measures from 4.8 to 4.9 mm in length and 1.0 to 1.16 mm in breadth on the 6th and 7th abdominal segments. The larva taper slightly towards its end (Fig. 20).

The head is somewhat brownish and the facial rods are well sclerotised. The anal vesicle is completely retracted within the body. Morphologically the anal vesicle gives rise to the 10th abdominal segment which is globular and shiny, still showing the form of caudal vesicle within it. The hypopleural swellings are from 1st. to 8th. segment and are very prominent. The head and the body are studded with minute spicules. The spicules are triangular in shape and have a convex base. The first 12 body segments bear each a transverse row of posteriorly directed spines. The last segment is devoid of spines. The larva has 8 pairs of open spiracles. The first pair is situated on the anterior margin of the mesothorax and the remaining seven pairs on each of the first abdominal segments.

The Head: — The head of the third instar larva is partly telescopic and can be withdrawn into the prothorax. The mouth parts are sclerotised and well developed (Fig. 27). The lower edge of the labium is somewhat raised and extends back into the mouth opening. The mandibles are bifurcated at the tips and bear a row of 16 saw-like teeth on the dorsal edge of the blade. The mandible measures from base to tip 0.12 mm and is slightly pigmented. The ventral edge of the mandible articulates with a narrow pigmented bar, the inferior pleurostomal ramus (Fig. 21, IPR). The superior pleurostomal rami are not visible; probably they are absent. Hence the mandibles articulate only at one point. The hypostoma (HY) extends laterally from the inferior pleurostomal ramus and after a slight upward curve makes a semicircle ventrally when viewed anteriorly. A short stipital sclerome (STS) projects ventrally from the point where the hypostoma first curves dorsally. At its base the stipital sclerome is almost free. A pigmented U-shaped labio-stipital sclerome (LSS) borders the labio-stipites (LS) — laterally and ventrally. At the mid-ventral part the sclerome is enlarged slightly. It is somewhat hexagonal in shape with its two dorsal angles rounded and somewhat indistinct. Dorsally between the two free ends of the labio-stipital sclerome is situated the mouth opening. Ventral to the mouth opening a U-shaped salivary duct opens (SDO). Below this opening lies the ligular sclerome (LIS).

The labio-stipites project beyond the labio-stipital sclerome and form a pointed apex. On either side of the ligular sclerome is present a labial

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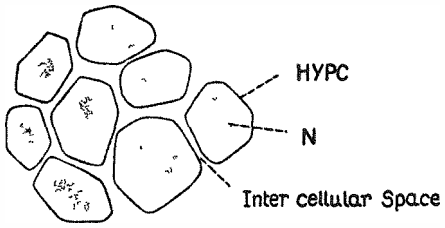
palpus (LP) which is a small conical projection from labio-stipites. Two pairs of labial setae are present ventral to the labial palpi, which are very characteristic of this species. A maxillary sclerome (MAS) extends on either side from the labio-stipital sclerome and passes laterally into the



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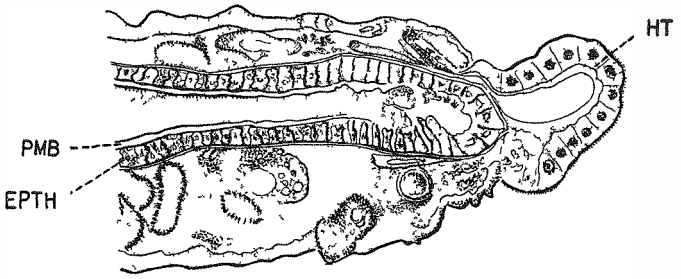

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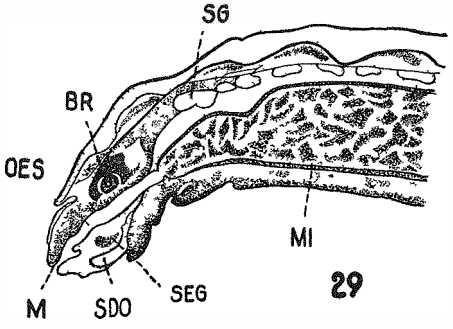

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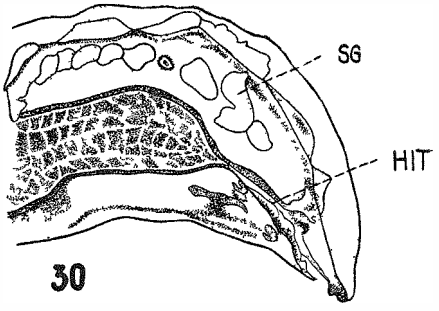

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Fig. 27. Longitudinal section of the wall of the caudal vesicle. — Fig. 28. Longitudinal section of the hind region of the second instar larva. — Fig. 29. Longitudinal section of the anterior half of the third instar larva. — Fig. 30. Longitudinal section of the posterior half of the third instar larva

BR — Brain; EPTH — Epithelium; HIT — Hind intestine; HT — Heart; HYPC — Hypodermal cell, M — Mouth, MI — Mid intestine, N — Nucleus, OES — Oesophagus; PMB — Peritrophic membrane, SDO — Opening of the salivary duct, SEG — Sub-oesophageal ganglion; SG — Silk glands

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area enclosed by the semicircular hypostoma and articulates with the hypostoma on either side of the head, thus forming a fulcrum on which the labio-stipital sclerome swings. This sclerome is a stout rod and at the point of articulation with the labio-stipital sclerome is spoon-shaped.

The maxillae (MX) are bound dorsally and laterally by the hypostoma and ventrally by the maxillary sclerome. The stipital sclerome which projects from the hypostoma partially divides the maxilla into a cardo (CA) and a stipis (ST). The maxillary palpus is located on the stipis (MP). One maxillary seta is present ventral to the maxillary palpus. No epistoma is visible. Four pairs of setae are present on the labial area dorsal to the mandibles and three pairs of setae are situated lateral to the bases of the mandibles on the frontal area. A pair of setae are present on the labial base.

Anatomy of the third instar larva

Alimentary canal: — The longitudinal sections of the larva (Fig. 29 & 30) show that the alimentary canal consists of the mouth (M) which opens into a short, slender oesophagus (OES) which in its turn opens into the mid-intestine (MI). The mid-intestine is a swollen tube and occupies much of the space within the body cavity. The hind-intestine (HIT) is a narrow tube. The two large silk glands ooeupy much of the body cavity. The short common salivary duct opens on the labium.

Tracheal system (Fig. 14): — The tracheal system is well developed in the third instar larva and consists of two longitudinal trunks, one an either side of the body. The head is supplied with two anterior branches from each longitudinal trunk. From each longitudinal trunk a dorsal and a ventral branch are given off in each of the thoracic and the first nine abdominal segments. These branches terminate in a fine net work. In the last abdominal segment the main trunk itself branches into fine tracheoles. A pair of spiracles are situated latero-dorsally on the mesothoracic segment and a pair each on the first seven abdominal segments. The spiracle is a rounded structure surrounded by black lines. The spiracular opening is surrounded by a dark chitinised ring and the spiracular trachea is a small spiral tube which soon enlarges into a bulb-like structure (Fig. 15, Ptr, T, TR).

Chaetotaxy of the third instar larva: — The larva possesses a number of spines which are arranged systematically one each segment of the body. The ventral side is devoid of the spines. The number and arrangement of the setae varies on pro- and meso-thorax. The prothorax has 38 setae whereas the mesothorax has 44. Though both first and second abdominal segments vary in the arrangement of setae the number of setae present is the same (Fig. 16—19).

The nervous system is the same as described in the second instar.

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Discussion

Apanteles angaleti Muesebeck is a potential parasite of the pink boll worm *Platyedra gossypiella Saunders* in India. The egg of *Apanteles* is slender and long with a short tail like structure. Braconid egg of this shape has been described by FALLIS (1942) in the case of *Apanteles carpatus*. In other species of *Apanteles*, only a short peduncle or petiole has been observed by various workers. Of the three larval instars in the genus *Apanteles* the first instar larva has been a matter of great controversy since SEURAT (1899) studied the genus *Apanteles* for the first time. The number of body segments has varied from species to species, ranging from 10 to 11 segments. As a matter of fact the freshly hatched larva is so small and the segmentation so weekly demarcated that it is very difficult to determine the number of segments in the posterior region. In *A. angaleti* there are present 3 thoracic and seven abdominal segments excluding the head. However the presence of 8th. and 9th. abdominal segments is indicated by false segments beyond the 7th. But as the larva develops to late first instar, segmentation becomes very clear and the abdominal segments number 9. CROSSMAN (1922) observed only 11 segments even in the late first instar of *A. melanoscelus*, the seventh abdominal segment being divided into two. The caudal horn persists until the larva moults. Hence, this stage of the larva can be correctly determined as long as the caudal horn persists.

Even the second instar larva has been a matter of controversy with regard to the number of segments. In *A. angaleti*, there are three thoracic segments and 10 abdominal segments besides the head. CROSSMAN (1922) in *A. melanoscelus* and *A. flavipes* has shown the caudal horn in the second instar also. It is evident that he has taken the late first instar stage as a second instar. TOWER (1915) could count only eleven body segments in *A. militaris* and TOTHILL (1922) confirmed the same.

The caudal vesicle: — Within recent years the physiology of respiration in endo-parasitic Hymenoptera has attracted the attention of many insect physiologists. It is needless to emphasise that the first instar larva of ectoparasites and the first and second instar larvae of endoparasites do not possess any functional tracheal system. In both the cases the oxygen intake is by means of feeding on the host blood. It is, however, true that little is definitely known with respect to the oxygen carrying capacity of insect blood, but the available evidence strongly indicates that it is from the latter source that the endoparasites first obtain their oxygen content.

This specialised method of respiration is facilitated by the presence of a caudal appendage or vesicle, which are present in diverse groups of parasitic Hymenoptera. These structures are found only in the early instars and becomes gradually retracted as growth proceeds and the spiracular respiration becomes established. The absence of caudal vesicle in all

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ectoparasitic forms is a fairly clear indication that they exercise a respiratory function. However, there is a good deal of controversy regarding the function of the caudal vesicle.

There are two schools of thought on the function of caudal vesicle and according to one which consists of LATZEBURG (1844), MUESEBECK (1918) and TOTHILL (1922) it is chiefly respiratory in function. SEURAT (1899), WEISSENBURG (1909), TOWER (1915) and GILMORE (1938) has put forward the theory that the caudal vesicle functions purely as an excretory organ. However, WEISSENBURG and TOWER are of the opinion that though excretion is the primary function of the caudal vesicle it has also a respiratory function though it is only secondary. MUESEBECK, though supporting the essentially respiratory function of the caudal vesicle, is impressed by WEISSENBURGs findings of its excretory function to some extent.

The present studies indicate that the caudal vesicle functions as a respiratory organ. In *A. angaleti* as in other endoparasitic Braconids the mid intestine is blind and does not communicate with the hind intestine which is in the form of a caudal vesicle in the early instars. The dorsal blood vessel or the aorta as it reaches the caudal vesicle becomes enlarged and occupies a major area in the vesicle. The anal vesicle of a live larva when observed under the microscope, is seen to contract and expand faintly at regular intervals, indicating that it is pulsating. This strongly suggests that the caudal vesicle is intimately associated with the circulatory system and that its chief and probably only function is the purification of the blood or, in other words, it is a blood-gill connected with a distinct respiratory function. To support this view the morphology of the caudal vesicle comes to our aid. The wall of the vesicle is composed of a single layer of hypodermal cells with a cuticular outer lining and an inner basement membrane. The hypodermal cells are not compact. It is obvious that this arrangement renders the wall permeable to gases and an exchange of gases between the impure blood of the parasite and the comparatively purer blood of the host. Another point that seems to favour the bloodgill theory is that the caudal vesicle is withdrawn and ceases to function soon after the emergence of the parasite larva from the host body.

In all the endoparasitic larvae there is no through passage between the hind intestine and the exterior, an arrangement which ensures against the contamination of the host blood by the faecal matter. It is only at the last moult, just prior to pupation the direct communication between mid and hind intestines become established and the contents of the intestine are discharged. However, in the early stages the waste products of metabolic activity may be thrown out in the form of gaseous exchange which is facilitated by the permeable wall ot the caudal vesicle. It is only in this limited sense that the caudai vesicle may be called an excretory organ also.

Oxygen is actually absorbed by the caudal vesicle as well as by the antire body wall surface, which is strongly suggested by the subcuticular

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net work of tracheoles. THORPE (1932) demonstrated this fact by the use of biological indicators. It is clear that even when the vesicle is at its maximum development it is of little importance when compared with the rest of the body surface. THORPE further suggests that where the vesicle is large and supplied with a good blood circulation, it can only supply one third of the oxygen requirements of the larva. It is undoubtedly an organ of respiration but not the sole, nor even the most important organ of respiration, since exchange of gases takes place over the whole body surface.

The mouth parts of the third instar larva definitely show specific difference. The two pairs of setae present on the labiostipites in case of *A. angaleti* is not shown in other species which were described by VANCE (1931) and BROWN (1946).

Summary

1. *Apanteles angaleti Muesebeck* has been recently found to parasitise the larvae of *Platyedra gossypiella* (*Saunders*), a serious pest of cotton in India and other cotton growing regions of the world.

2. The parasite was mass bred in the parasite laboratory of the Indian Agricultural Research Institute on an alternate host, *Corcyra cephalonica Stainton.*

3. The life history of the parasite, including the incubation period of the egg, duration of different larval stages and the mode of emergence from the host body has been studied. The life history under controlled conditions from egg to adult occupied 29 to 44 days.

4. The rate of reproduction, the longevity and fecundity have been studied.

5. The effect of parasitisation on the host results in the retardation of growth, irregular and less number of moults and also loss of weight.

6. The morphology of the immature stages has been described. *A. angaleti* has 3 instars of which the 3rd instar lasts only for a few hours.

7. The anatomy of the caudal vesicle of the second instar has been studied. The functions of the caudal vesicle have been investigated and discussed. It is established that the caudal vesicle is more of a respiratory function.

8. The mouth parts, chaetotaxy and the morphology of the third instar larva has been studied.

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References

BROWN, M. C., Studies on parasites of the Spruce Bud Worm *Archips fumiferana* (Clem.) 1. Life history of *Apanteles fumiferanae* Viereck (*Hym.*: *Braconidae*). Canad. Ent., **28**, 121—129, 1946.

CROSSMAN, S. S., *Apanteles melanoscelus*, an imported parasite of the Gipsy Moth. U. S. Dept. Agric. Bull. 1028, pp. 25, Wahington, 1922.

FALLIS, A. M., The life-cycle of *Apanteles carpatus* (Say) (*Hym.*: *Braconidae*), *a* parasite of the Webbing Clothes Moth, *Tineola biselliella* Hum. Canad. J. Res., **20**, 13—19, 1942.

GILMORE, J. V., Notes on *Apanteles congregatus* (Say), a parasite of Tobacco Horn Worms. J. Econ. Ent., **31**, 712—715, 1938.

HAFIZ, M., The biology and life-history of *Apanteles ruficrus* Hal. (*Hym.*: *Braconidae*). Bull. Soc. Fouad Ier Ent., **31**, 225—249, 1947.

DOI: 10.21248/contrib.entomol.6.3-4.296-320

Muesebeck, C. F. W., Two important introduced parasites of the Brown Tail Moth. J. Agric. Res., **14**, 191—206, 1918.

— Some Braconid parasites of Pink Boll Worm, *Pectinophora gossypiella*(Saunders). Boll. Lab. Zool. gen. agric. Portici, **33**, 1954.

Narayanan, E. S., Angalet, G. W., Subba Rao, B. R. & D'Souza, G. I., A technique for mass breeding of *Apanteles* n. sp. (*Braconidae*: Hym.). Curr. Sci., **22**, 121, 1953.

Parker, D. L., *Apanteles solitarius* (Ratz.), an introduced Braconid parasite of the Satin moth. U. S. Dept. Agric. Techn. Bull. 477, pp. 17, Washington, 1953.

Ratzeburg, I. T. C., Die Ichneumonen der Forstinsecten, **1**, Berlin, 1844.

Seurat, L. G., Contributions à l'étude des Hymenoptères entomophages. Ann Sci. Nat. Zool., (8), **10**, 1—159, 1899.

Tower, D. G., Biology of *Apanteles militaris*. J. Agric. Res., **5**, 495—508, 1915.

Tothill, J. D., The natural control of the Fall Webworm (*Hyphantria cunea*, Drury) in Canada, together with an account of its several parasites. (Canad. Dept. Agric. Bull. (N. S.) 3 (Ent. Bull. 19), pp. 107, Ottawa, 1922.

Thorpe, W. H , Experiments upon respiration in the larvae of certain parasitic Hymenoptera. Proc Roy. Soc. Lond., Ser. B, **109**, 450—471, 1932.

Vance, A. M., *Apanteles thompsoni* Lyle, a Braconid parasite of the European Corn Borer. U. S. Dept. Agric., Techn. Bull. 233, pp. 28, Washington, 1931.

Weissenberg, R., Zur Biologie und Morphologie endoparasitisch lebender Hymenopterenlarven (Braconiden und Ichneumoniden). Sitzber. Ges. naturf. Freunde, Berlin, 1909, pp. 1—28, 1909.

Die Erzwespe *Tetracampe diprioni Ferrière* als Eiparasit der Kiefernblattwespe *Neodiprion sertifer* (*Geoffr.*)

(*Hym.*: *Chalcidoidea* — *Hym.*: *Tenthredinidae*)

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