

STUDIES ON THE BIOLOGY OF THE PARASITES OF THE PEA LEAF MINER *PHYTOMYZA* *ATRICORNIS* (MEIGAN)

BY E. S. NARAYANAN, M.A., PH.D. (LOND.), D.I.C., F.R.E.S., F.E.S.I., F.A.Sc.,
B. R. SUBBA RAO, B.Sc. (HONS.), PH.D., ASSOC.I.A.R.I. AND
R. B. KAUR, M.Sc.

(Division of Entomology, Indian Agricultural Research Institute, New Delhi)

Received July 11, 1956

INTRODUCTION

THE leaf miner *Phytomyza atricornis* (Meigan) is a serious pest of several crops of economic importance. The host range is considerable and Ahmad and Gupta (1940) have listed twenty-nine species of plants, comprising fourteen families. The leaf miner is cosmopolitan in distribution and is widespread throughout Europe including Russia, United States of America and other countries of the old and new world. Lefroy (1906) was the first to record the pest on cruciferous plants in India. A review of published literature shows that the biology of no parasite of any leaf miner has been studied in India so far. This is not surprising because the problem is very difficult and beset with many difficulties. Even outside India there are only very few contributions on the biology of the parasites of leaf miners. We may now briefly review this work.

The investigations of Voukassovitch (1928) and Cohen (1936) on the parasites of the leaf miner *Phytomyza atricornis* are outstanding. Ahmad and Gupta (1941) while studying the biology of *Phytomyza atricornis* recorded *Solenotus* sp. as an endoparasite of the pest which is not actually the fact. In this paper the bionomics and biology of *Solenotus* sp. new an ectoparasite and *Rhopalotus* sp. new* (Eulophidæ: Hymenoptera) an endoparasite are described in detail. The fascinating problem on the parasite complex involving multiparasitism between the chalcid *Rhopalotus* and the braconid (to be identified) and its effect on the development of the latter is also discussed.

MATERIAL AND METHODS

The biology of the parasite was studied under room temperature and humidity. The mean maximum and minimum temperature being 75.5 and 79° F. during the period from fifteenth February to twenty-fourth March when investigations were carried out on the biology of the parasite. Leaves

* The description of the new species is being published elsewhere.

infested with leaf miners were collected and reared for parasite emergence. The parasites were offered with fresh field collected hosts in small tubes and after parasitisation the leaves containing the parasitised hosts were removed to petri dishes which were kept moist by keeping wet filter-paper at their base. The tracheal system was studied by temporary mounting in glycerine.

BIONOMICS OF *Solenotus* SP. NEW

The adults of *Solenotus* sp. make their appearance in the field from the second week of February to the middle week of March, attacking the leaf miners on pea and lucerne. From April onwards the parasite population decreases. It is probable that the peak of adult emergence occurs between the first and second week of March.

Host Selection

The female parasite actually flies about the leaf until she locates the suitable stage of the host larva in the tunnel of the leaf. Generally the parasite selects only well-developed larva for parasitisation. As soon as the parasite locates the host she pushes her ovipositor through the membranous covering of the leaf that protects the larva, stings and paralyses the host and deposits generally only one egg on the body. Occasionally up to three eggs are laid. But this is rather rare. The parasite larva after hatching is so tiny that it cannot be seen on the body of the host except under the low power objective of a microscope. In all the dissections carried out the host larvæ were invariably dead even though the parasite larva had apparently just hatched. Even under superparasitised conditions the parasite larvæ of which only a maximum of three were observed during the course of these investigations, completed their development and emerged as adults. Pupation takes place within the mine of the host but always away from the remains of the host body.

Development

During the growth and development of the immature stages of *Solenotus* sp., in all there are five instars. The main points of difference between the various instars are size, shape of mandibles, the tracheal system and the number of spiracles.

(a) *Egg* (Plate VIII, Fig. 5).—The egg is transparent and oval elongate in shape. The egg measures 0.35 mm. in length. The chorion is very smooth without any reticulations. The egg is deposited externally on the body of the host. The percentage viability of the egg is very high. The incubation period varies from fifteen to twenty-four hours.

(b) *First instar* (Plate VIII, Fig. 6).—The freshly hatched larva is composed of a head and body segments of which twelve are clearly differentiated. The thirteenth and fourteenth segments are not at all clearly defined. The larva is translucent in colour with a tendency to light green in older individuals. The shape is subcylindrical with maximum breadth about the middle, tapering anteriorly into a bluntly rounded structure and posteriorly into a bifurcated papillæ. Spines are present on all the body segments except the first two and the last segments. All segments are devoid of spicules.

The respiratory system makes its appearance at this stage itself. There are two lateral longitudinal trunks with spiracles only in the segments fourth, sixth, seventh, eighth and the ninth. So in all there are five pairs of spiracles in the first instar unlike most other chalcids which usually have only four pairs. The mandibles are sharp and slightly curved. They are pale brown in colour and overlap each other at their tips. The first instar larva measures about 0.35 mm. in length.

(c) *Second instar*.—The second instar larva is similar to the first instar except that it is slightly larger in size and without the caudal papillæ. As the thoracic segments have increased in length and width the head appears proportionately smaller. The individual body segments are more conspicuous and translucent and greenish white. The respiratory system is well developed. The two lateral longitudinal trunks are quite thick and prominent. The full complement of eight pairs of spiracles are present. The spines which were present in the first instar are absent in this instar. The mandibles are larger than in the first instar. The second instar larva measures 0.425 mm. in length.

(d) *Third instar*.—The third instar larva very much resembles the second except that it is again slightly larger than the second. It is greyish white in colour except at the region of the alimentary canal which is pale brown.

The respiratory system is more developed. Numerous lateral branches are given off to the internal organs and to the nerve cord. Mandibles are heavily chitinized, with the apex more closely set together than in the previous instars. Specimens referable to this instar have body length varying from 0.70 to 0.75 mm.

(e) *Fourth instar*.—The fourth instar closely resembles the previous stadium but can be distinguished from the third instar by the mandibular measurements. The alimentary canal is larger and its contents tend to give the larva a pale colour. The fat bodies appear at this stage.

The respiratory system is well developed with anastomosing branches. Setæ are absent. Mandibles are quite strong. Specimens referable to this instar measure from 1.05 to 1.075 mm. in body length.

(*f*) *Fifth instar* (Plate VIII, Fig. 7).—Final instar larvæ are light green in colour and the alimentary canal is pale brown. The head is a rigid hemispherical capsule, on which the antennæ are represented by a pair of conspicuous sensillæ. A few lesser developed sensillæ are also seen.

Numerous rows of spicules make their appearance in this instar. Except the head segment, spicules are arranged in regular rows in each segment. The ventrum is devoid of spicules. Mandibles are heavily chitinized and strong. The tracheal and spiracular trunks get more wide and prominent with numerous branches anastomosing and ramifying throughout the body.

The larva when full fed moves away from the host remains and pupate in the gallery made by the host. Specimens referable to this instar have a body length ranging from 1.25 to 1.30 mm. During the growth and development the larvæ exclusively feed on the blood of the host and do not attack the internal organs.

(*g*) *Pupa* (Plate VIII, Fig. 8).—Pupa is of the naked chalcid type. Initiation of the pupal phase is marked by the passing of fœcal pellets for the first time during the development. The body of the larva becomes cylindrical and rapid changes begin. The developmental changes can be observed closely under the low power objective of a microscope. This can even be seen to some extent with the naked eye. A slight constriction appears in between the head and the thorax and the thorax and the abdomen. The eyes and other appendages appear gradually. The early pupa is light green in colour with red eyes. As it grows older the colour of the body gets changed to a metallic lustre and the eyes become deep red and prominent. The thoracic region develops conspicuous imaginal wing buds. The average duration of the pupal phase is about five days at laboratory temperature and humidity. Specimens of pupa measure from 1.45 to 1.55 mm. in length.

(*h*) *The adult* (Plate VIII, Fig. 9).—The adult cuts a hole through the membranous gallery constructed by the host and emerges out. It rests for some time and flies away. The colour of the body is metallic green with red iridescence.

Length of the life-cycle.—The period from the egg stage to the adult of the parasite varies from ten days to fourteen at room temperature and humidity in the month of March. The incubation period of the egg varies from fifteen to twenty-four hours. The period from hatching of the egg to

pupation lasts from five to seven days and from pupation to emergence of the imago four to six days.

Longevity.—In an experiment with six females which were given freshly cut raisins as food, the length of life varied from fifteen to eighteen days under room temperature and humidity in the month of March.

Sex ratio.—Leaves infested with leaf miners were collected and the emergence of parasites were closely observed. Under field conditions the females predominated, their percentage being 90, males constituting just 10 per cent. of the total emergence.

Percentage of Parasitism

The percentage of parasitism is very high. 177 infested leaves were picked from the fields at random and the number of host miners counted on them. It was found that there were 396 hosts on these leaves. The parasites on them were allowed to emerge under room temperature and humidity, and the adults were counted every day. It was observed that the number of parasites that emerged were fairly high and counted 310. So the percentage of parasitism is very high. It was also observed that two other parasites, viz., *Rhopalotus* sp. and a braconid were also parasitising the host. Out of the total emergence *Solenotus* sp. constituted 90 per cent., *Rhopalotus* 9 per cent. and the Braconid 1 per cent.

Morphology of Immature Stages

Chaetotaxy (Plate IX, Fig. 1).—The spines are seen in the first instar and the spicules in the fifth. Spines are present on all the body segments except the first two and the last. Two longitudinal rows can be seen running from the third segment to the thirteenth, one on each side of the body. There are two transverse bands of very minute spines on the segment fifth and sixth. Each band consists of six spines. In addition to this there can be seen one pair on each side of the segments seventh, tenth and thirteenth. So in all there are eighteen pairs of spines present on the body of the first instar larva.

Numerous minute spicules make their appearance in the final instar. Except the head segment, these spicules are arranged in regular rows.

Mouth parts (Plate IX, Fig. 2).—Mandibles are extremely sharp and slightly curved. The tip is pale brown and the rest of the mandible being of a very pale yellow colour. They overlap at their tips. Labrum is provided with sensillæ. The epistoma is well developed and is a dome-shaped structure covering the mouth parts. The mandibles articulate with the supra and infra pleurostomal rami on either side. In the upper articulation the condyle

is on the ramus while in the lower the order is reversed, the condyle being a hollow pit on the pleurostoma. The tentorial rods give strength to the head capsule. Facial rods which are well developed in the case of other ectophagous parasites are not so well developed in this case and are much simplified. Morris (1935) has described similar mouth parts and facial rods in *Microplectron fuscipennis* Zett.

Respiratory system (Plate IX, Figs. 3 and 4).—The respiratory system consists of two lateral longitudinal trunks, united anteriorly but not posteriorly. There are eight pairs of spiracles unlike most other chalcids which have nine pairs. The spiracles are present in the segments third, fourth, sixth, seventh, eighth, ninth, tenth and eleventh. So they are regularly arranged from segment third to eleventh with the exception of fifth which forms a gap. They are attached to the lateral longitudinal trunks by means of coiled spiracular tubes.

In the second segment where the two trunks meet, five branches are given off on either side. In each spiracular segment with the exception of fifth, the traceales give off a pair of branches, one on either side of it, which in their turn bifurcate and further branch. Segment fifth, which is a non-spiracular segment, also receives these branches from the main trunk.

Digestive System (Plate IX, Fig. 5).—Mouth leads into a short, narrow pharynx, which in its turn leads into the stomach. The latter is quite large and occupies most of the body cavity. The mid-intestine becomes a distinct hind-intestine at the twelfth segment and the hind gut opens out as anus.

Salivary glands are well developed. But the silk glands as in the case of other ectophagous parasites, which spin cocoon, are not well developed.

The nervous system (Plate IX, Fig. 5).—The nervous system consists of a well-developed brain and a ventral nerve cord. There is a sub-oesophageal ganglion, three thoracic and six abdominal. The ventral nerve cord does not extend beyond the tenth segment. The last abdominal ganglion is a composite structure that represents the ganglia of the remaining four abdominal segments.

BIOLOGY OF *Rhopalotus* SP. NEW

Seasonal History

The emergence of adult *Rhopalotus* sp. commences in the second week of March and continues up to the last week. It is, however, probable that the peak of adult emergence occurs about March third week to last week, as during this period the population of the parasite is high in the field. *Solenotus* sp. makes its appearance first and *Rhopalotus* afterwards.

Host Selection

The female parasites move about over the leaf until they locate the suitable stage of host larva in its mine. Usually the parasite selects chrysalid stages of the pest but at times it attacks the last instar larva also. In the former case the development of the parasite is completed while in the latter case the developing grub of the parasite allows the host to form puparia whereas the parasite itself hibernates till the next spring. As soon as the parasite locates the host she pierces its ovipositor first through the membranous gallery of the host and then through the body of the host, and deposits one to six eggs at a time into the body. However, only one individual completes its development and emerges as adult. The supernumerary larvæ are killed by the strongest, which is usually the one that hatches first.

Development

The Egg (Plate X, Fig. 1).—The egg is crescent-shaped and translucent. The chorion is smooth and without any reticulations. Under high magnification a brown spot can be seen in the centre of the egg which marks the position of the developing embryo.

First instar (Plate X, Fig. 2).—Freshly hatched larva is opaque white in colour and is composed of a head and thirteen segments. The segmentation is deeply marked. It is crescent-shaped with the anterior end broader than the posterior. Head is hemispherical and broad. Under high magnification mandibles can be seen protruding out from the ventrolateral side of the head. The alimentary canal is prominent and is pale yellow in colour. On the extremity of the caudal segment can be seen eight cuticular spines that are conical in shape (Plate X, Fig. 3). All segments are devoid of setæ and there is no evidence of even a rudimentary respiratory system. Mouth parts are very simple. The mandibles are not so sharp as in the case of *Solenotus*. The tip is golden yellow and the rest colourless. Its length being 0.25 mm.

Second instar.—The second instar larva is somewhat crescent-shaped like that of the previous instar but is a little larger in size. The tracheal system is not seen in this instar.

Third, fourth and fifth instars.—All these instars are crescent-shaped and resemble each other closely except that the size goes on increasing with the age. Their anterior end is broad and rounded while the posterior end is narrow and rounded. Segmentation is quite prominent.

The respiratory system gets well established in the third instar. It consists of two lateral longitudinal trunks, united anteriorly but free posteriorly.

There can be seen seven pairs of spiracles in segments third, sixth, seventh, eighth, ninth, tenth and eleventh. Numerous anastomosing branches pass out to the internal organs.

Mandibles get more heavily chitinized. The body length of the final instar varies from 1.70 to 1.87 mm.

Pupa.—The larva pupates with in its last larval skin, which hardens in due course, but the parasite pupa is always found within the cocoon of the host. The freshly formed puparium is long and oval, and dark brown in colour, but with age becomes black in colour. The developmental stages can be observed easily. A slight constriction appears in between the head and the thorax, and the thorax and the abdomen. The eyes and the other appendages appear gradually. The eyes of the late pupa are quite prominent and scarlet red in colour. The early pupa is dark brown in colour but with age the colour gets changed to dark. The thoracic region develops the conspicuous imaginal wing buds.

The adult (Plate X, Fig. 6).—The adult comes out by breaking open the puparium with the help of its appendages. The freshly emerged *Rhopalotus* is brilliant metallic green.

Respiratory system (Plate X, Fig. 5).—The respiratory system of *Rhopalotus* sp. is well developed in the latter instars but is totally lacking in the first two instars. The intake of oxygen in the first and second instars is from the host blood on which it feeds. In the third instar the open tracheal system makes its appearance. The tracheal system consists of two lateral longitudinal trunks, united anteriorly but free posteriorly. There are seven pairs of spiracles, unlike most other chalcids which have the full complement of nine. The spiracles are present on segments third, sixth, seventh, eighth, ninth, tenth and eleventh. The spiracles are attached to the main trunk by means of spiracular stalks, which are longer as compared to that of *Solenotus* sp. The branching of the main tracheal trunk is just similar to that of *Solenotus* sp.

DISCUSSION

Very little work has been done on the parasites of the Dipterous leaf miners. Much of the work in the main relates only to the record of the parasites and to some extent meagre notes on their biology. *Solenotus* sp. as a parasite of the pea leaf miner *Phytomyza atricornis* was first recorded by Ahmad and Gupta (1941) as an endoparasite, which is totally unfounded. It is an ectoparasite which attacks only well-developed larvæ of the leaf miner. Cushman (1926) draws attention to the host preferences of *Symplicis* sp. which attacks Coleopterous, Dipterous and Hymenopterous leaf miners and

also develops as an external parasite of the egg of *Cimbex*, and he concludes that location rather than the type of the host is the determining factor in selection. This statement of his has no basis as it is always seen that the percentage of parasitisation is dependent on the availability of suitable host stages for parasitisation. The egg of the parasite is like that of any other chalcid. The number of body segments in the first instar larva is generally accepted as thirteen excluding the head. But in the case of *Solenotus*, the thirteenth segment is not clearly demarkated from the fourteenth, and the fourteenth is represented by a pair of papillæ which disappear in the later instars and the fourteenth segment becomes well established. The sensory setæ that are present only in the first instar are characteristically arranged on the dorsum of the larva. The tracheal system of the first instar is open and it consists of five pairs of open spiracles. The general accepted number, however, is only four pairs. Parker and Thompson (1925) in *Solenotus* sp. have shown only four pairs of spiracles.

Rhopalotus sp. is a fine example of what is known as the free living endoparasitic larva defined by Parker and Thompson (1925). The eggs of *Rhopalotus* sp. are laid within the larva or puparia of the host. It is interesting to record a larva of this type as it represents a transitional type between the ecto- and endoparasitic larvæ. This type of larva is strikingly different from conventional endoparasitic type by not possessing a caudal appendage or tail. The first instar stage is apneustic. Such a type has been described by Parker and Thompson (1925) in the case of *Pteromalus puparum* L.

Superparasitism in *Rhopalotus* is very common as one to six eggs are laid within the body of the host. However, only one individual completes its development and emerges as adult. The supernumerary larvæ are killed by the strongest, which is usually the one that has hatched first. The most interesting aspect of this study is the occurrence of multiparasitism among *Rhopalotus* sp. and a braconid, both being endoparasitic. Several hundreds of dissections of the host larvæ have always revealed that the braconid is the one that succumbs. It is surprising to see that the braconid larva which is several times larger and endowed with a pair of powerful sickle-shaped mandibles succumbs to the effect of multiparasitism. The general belief that some toxic products secreted by one species necessarily kills the other does not hold good in this particular case.

Parker and Thompson (1925), who described the transitional type of endoparasitic larva, have not described the mode of intake of oxygen, when the larva is devoid of both the caudal appendage and the open tracheal system. It is probable that the first and second instars of *Rhopalotus* sp. derive their

oxygen from the host blood, as they feed on them, and by the time the blood becomes scanty the open tracheal system gets established. The braconid gets its oxygen from the host blood through the integument and the caudal appendage only when the host is living. Hence, the moment the host larva which is already parasitised by the braconid is again parasitised by *Rhopalotus*, the braconid immature stages are killed due to lack of oxygen rather than through any other means. One more supporting observation is that the majority of dissections of the host have always revealed the first instar living braconid larvæ and a number of eggs of *Rhopalotus* sp. When the dissections revealed first instar *Rhopalotus* larva the braconid was invariably dead.

The sex ratio of both *Solenotus* and *Rhopalotus* is very high and the percentage of parasitism is also very high. Cohen (1926) recorded 93.3 per cent. parasitism by a braconid on *Phytomyza atricornis*. The fact that only a negligible per cent. of males are seen in *Solenotus*, it is probable that the chalcid is capable of normal reproduction even without mating.

SUMMARY

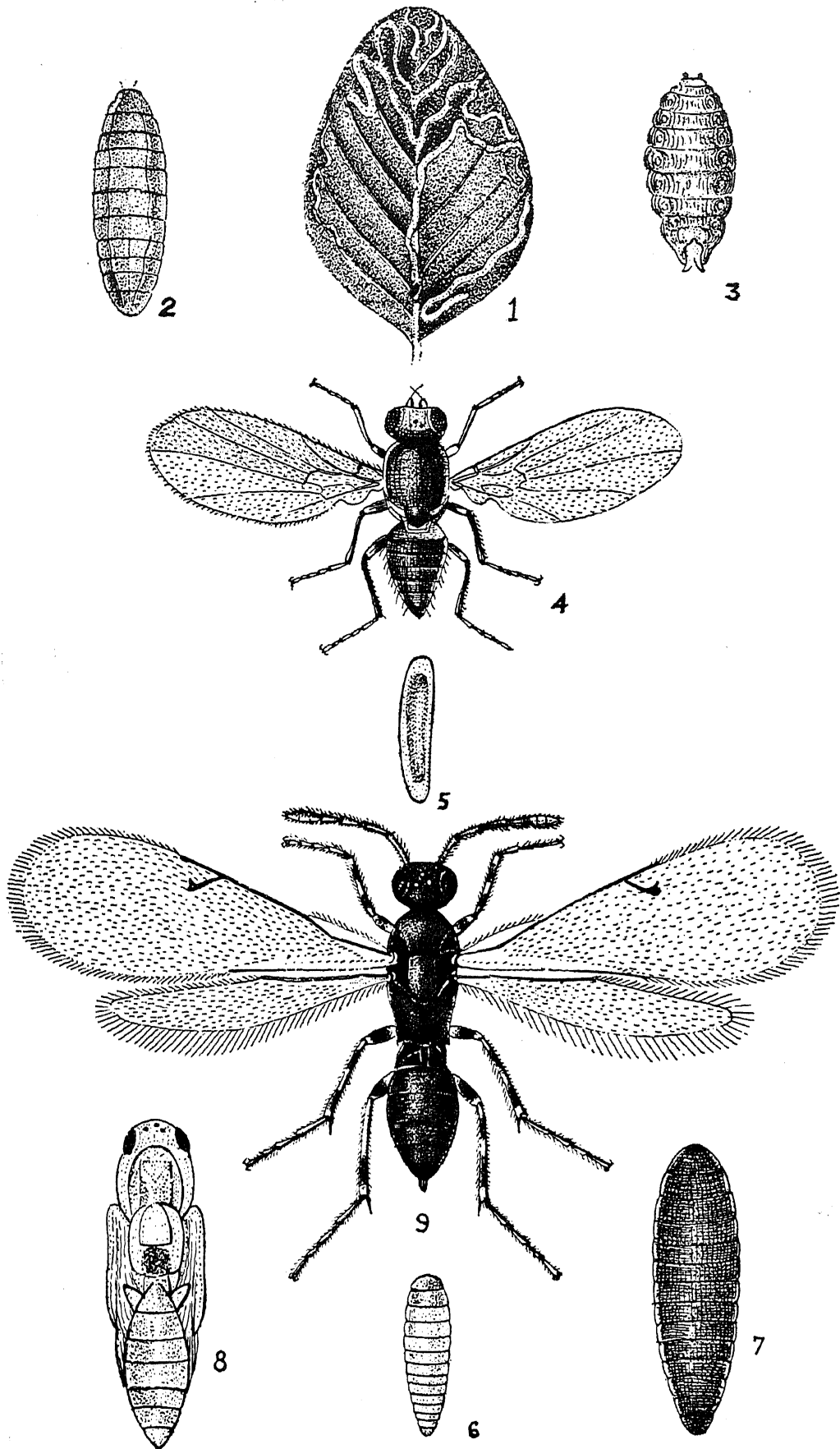
The biology and morphology of *Solenotus* sp. new and *Rhopalotus* sp. new have been studied in detail.

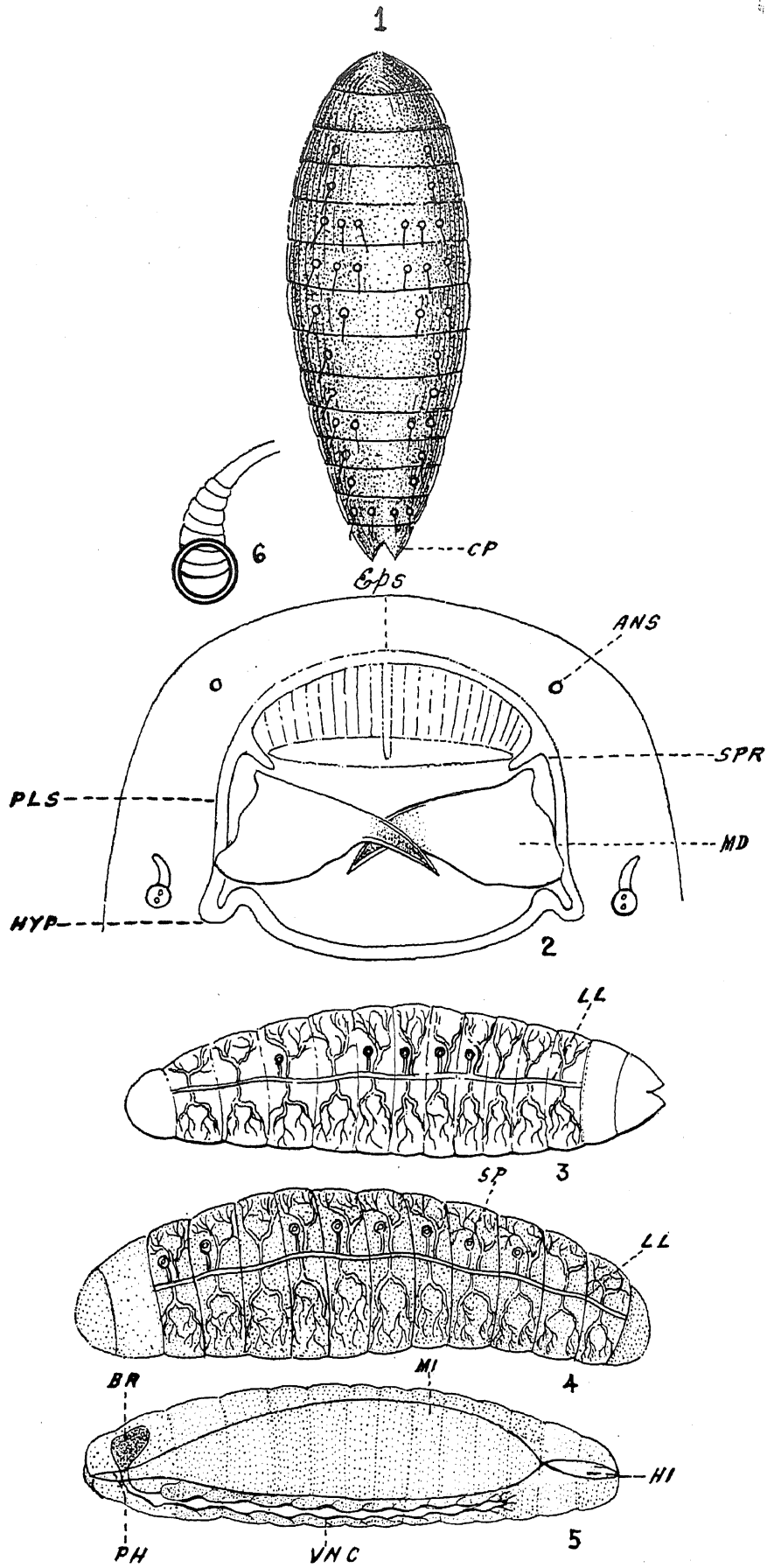
Solenotus sp. is an ectoparasite of the pea leaf miner *Phytomyza atricornis* (Meigan) and *Rhopalotus* sp. is an endoparasite of the same. One more internal parasite of the leaf miner is found to be a braconid. Multiparasitism occurs between the latter two in which *Rhopalotus* species survives and the braconid is killed in the early stages.

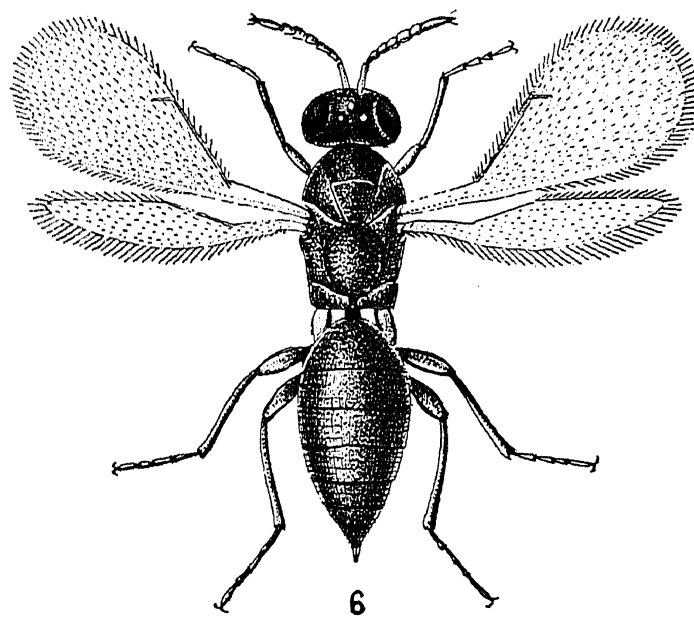
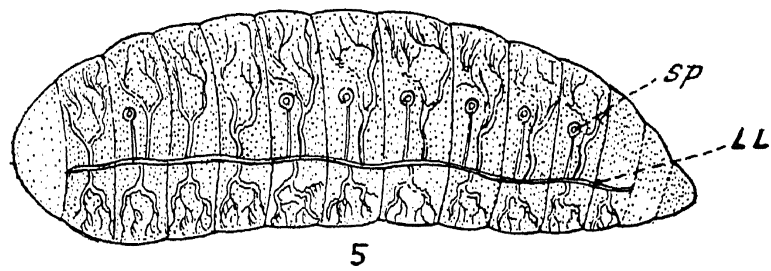
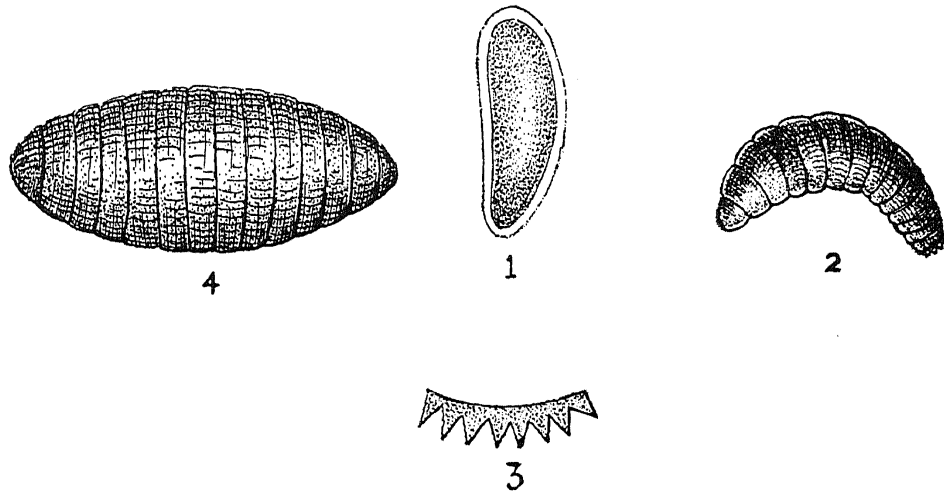
The respiratory system differs from other chalcids in having eight pairs of spiracles in *Solenotus* species and seven pairs in *Rhopalotus* species instead of the usual nine pairs. The early stage *Rhopalotus* larva represents a transitional stage between the ecto and endo type of parasitic larva.

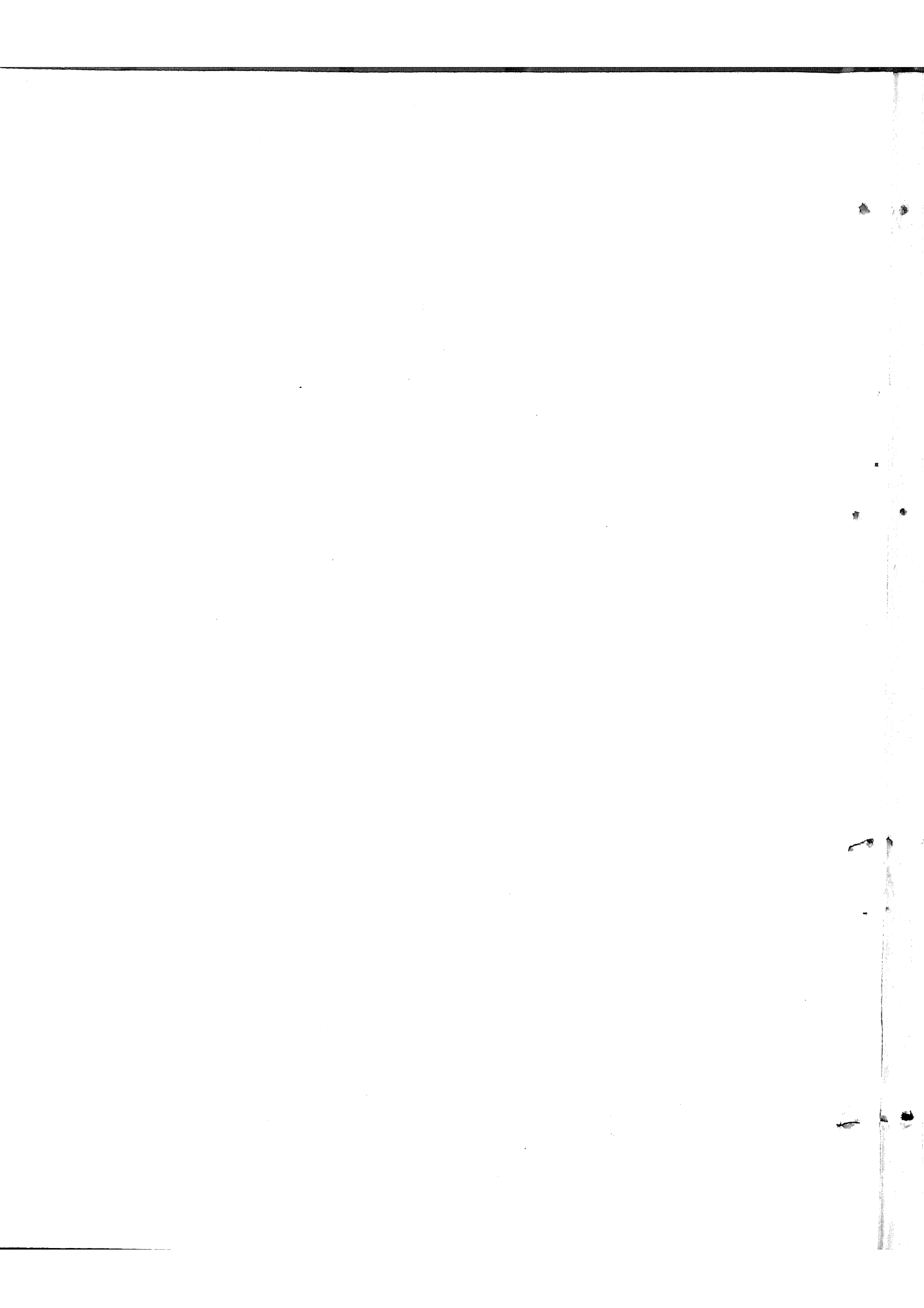
The life-histories of *Solenotus* and *Rhopalotus* are more or less similar. In both the cases there are five larval instars. *Solenotus* larvæ feed only on the blood of the host whereas those of *Rhopalotus* feed on the blood as well as on the internal organs. The pupa is naked in the former whereas it is not so in the latter. In both the cases the life-history is completed within ten to fourteen days at room temperature and humidity in the month of March.

Usually, *Rhopalotus* sp. selects chrysalid stage host larva for parasitisation but at times it attacks the last instar larva also. In the former case the development of the parasite is completed while in the latter case the developing grub of the parasite allows the host to form puparia, whereas the parasite itself hibernates till next spring.









REFERENCES

1. Cohen, M. .. "The biology of the chrysanthemum leaf miner *Phytomyza atricornis* (Meigan)," *Ann. App. Bio.*, 1936, 23 (3), 612-13.
2. Cushman, R. A. .. "Location of individual hosts versus systematic relations of host species as a determining factor in parasitic attack," *Proc. Wash. Ent. Soc.*, 1926, 128, 5-6.
3. Morris and Cameron, E. "The biology of *Microplectron fuscipennis* Zett., a parasite of the pine saw fly," *Bull. Ent. Res.*, 1935, 26, 407-418.
4. Parker, H. L. and Thompson, W. R. "Notes on the larvæ of the Chalcidoidea," *Ann. Ent. Soc. Amer.*, 1928, 18, 384-95.
5. Taskir Ahmad and Gupta, R. L. "The pea leaf miner *Phytomyza atricornis* (Meigan) in India," *Ind. J. Ent.*, 1941, 3, Pt. 1, 37-48.
6. Voukassovitch, P. .. "Sur deux chalcidiens, parasites de Dipteroïdes dont les larves minent les finillies des plants," *C.R. Soc. Biol.*, 1928, 98 (13), 1150-52.

EXPLANATION OF PLATES

PLATE VIII

FIGS. 1-9.—Fig. 1. A pea leaf showing the gallery made by the pest. Fig. 2. The larva of *Phytomyza atricornis* (Meigan). FIG. 3. Pupa of *Phytomyza atricornis*. FIG. 4. Adult fly of *Phytomyza atricornis*. FIG. 5. Egg of *Solenotus* sp. new. FIG. 6. First instar larva of *Solenotus* sp. FIG. 7. Last instar larva of *Solenotus* sp. FIG. 8. Pupa of *Solenotus* sp. FIG. 9. *Solenotus* sp. Adult female.

PLATE IX

FIGS. 1-6.—Fig. 1. Arrangement of spines on the body of first instar larva of *Solenotus* sp. Fig. 2. Mouth parts and facial rods of the last instar larva of *Solenotus* sp. Fig. 3. Tracheal system of first instar larva of *Solenotus* sp. Fig. 4. Tracheal system of last instar larva of *Solenotus* sp. Fig. 5. Diagrammatic figure showing the digestive and nervous system of *Solenotus* sp. Fig. 6. A spiracle of *Solenotus* sp.

PLATE X

FIGS. 1-6.—Fig. 1. Egg of *Rhopalotus* sp. n. Fig. 2. First instar larva of *Rhopalotus* sp. Fig. 3. The caudal papillæ of first instar. Fig. 4. Final instar larva of *Rhopalotus* sp. Fig. 5. Tracheal system of *Rhopalotus* sp. Fig. 6. *Rhopalotus* sp. Adult female.

EXPLANATION TO LETTERING

<i>ANS</i> = Antennal sensilla	<i>MD</i> = Mandible
<i>CP</i> = Caudal papillæ	<i>MI</i> = Mid intestine
<i>EPS</i> = Epistoma	<i>LL</i> = Lateral longitudinal trunk
<i>BR</i> = Brain	<i>PH</i> = Pharynx
<i>HYP</i> = Hypostoma	<i>SP</i> = Spiracle
<i>HI</i> = Hind intestine	<i>VNC</i> = Ventral nerve cord
<i>LB</i> = Labrum	