NOTES ON THE LARVAE AND PUPAE OF TWO FRUIT MOTHS, *GRAPHOLITA FUNEBRANA* TREITSCHKE AND *G. MOLESTA* BUSCK (LEPIDOPTERA : OLETHREUTIDAE)

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Introduction

The Oriental fruit moth, *Grapholita molesta* Busck is not known to be established in the United Kingdom, but its larvae are frequently encountered in fruit (mainly peaches) imported from southern Europe. The larvae of *G. funebrana* Treitschke also occur in imported fruit, and it has proved difficult to determine which species was present without breeding out the adult moths. The primary object of this study was to find means by which the larvae of the two species could be distinguished.

The two main works (MacKay, 1959 and Swatschek, 1958) on the larvae of the Olethreutidae do not distinguish between these species, as they do not occur together in either of the author's countries. Mature larvae of *G. funebrana* run down to *G. molesta* in MacKay's key. Balachowsky and Mesnil (1935) suggested that the mature larvae could be distinguished by the number of crotchets on the anal prolegs but, as Bovey (1937) pointed out, the numbers in the two species overlap. In this study an attempt has been made to find characters that would be distinctive in the earlier instars as well as in the mature larva, and some of the changes that take place in the external morphology of the larva of *G. funebrana* during its development are therefore described.

Two other species of Lepidoptera, *Laspeyresia pomonella* L. and *Anarsia lineatella* Zell., are commonly found in imported fruit, and characters for the separation of their larvae and pupae from those of *G. funebrana* and *G. molesta* are summarised.

Methods

The techniques described by Hinton (1956) were adapted for this study. To prepare the skins for microscopical examination, the head capsules of the larvae were removed and the skins cut longitudinally along the right hand side at about the line of the spiracles. They were then treated with dilute potassium hydroxide, stained in dilute chlorazol black in 70 per cent. alcohol and mounted in Salmon's polyvinyl alcohol type M.A.1 (Salmon, 1951). Skins of larvae that were too small to allow the use of scissors were cut with a needle on which a sharp edge had been ground. The needle was inserted into the body cavity and a pair of fine forceps rubbed gently against the cutting edge. With practice a fairly clean cut could be made in the skins of all but the smallest larvae. A foot-operated focusing movement on a stereoscopic binocular microscope was found to be a valuable tool in this work. Drawings were made with the aid of a microprojector or camera lucida whenever possible.

The nomenclature of the setae follows that used by Hinton (1946) and MacKay (1959).

Sources of Material

Larvae and pupae of *G. molesta* from Ontario, Canada were kindly provided by Miss M. R. MacKay of the Insect Systematics Unit of the Canadian Department of Agriculture. Further material was obtained from imported fruit in the form of

cast skins of the last instar and cast pupal cases from which the adults had been reared and their identity confirmed. Larvae of *G. funebrana* were collected from blackthorn (*Prunus spinosa* L.) in Hertfordshire. In addition, larvae found in imported fruit and attributable to one or other of these species were examined. Miss M. Gratwick of East Malling Research Station kindly supplied some first and second instar larvae of *Laspeyresia pomonella*, and Professor F. M. Summers of the University of California those of *Anarsia lineatella*.

Changes in the Morphology of the Larva of *Grapholita funebrana* During Development

General Colour Pattern

The head is dark brown to black in all instars, becoming lighter brown before pupation. The body is translucent white up to the last instar, which starts white and becomes bright pink as it matures, with muscle attachments *etc.*, standing out white.

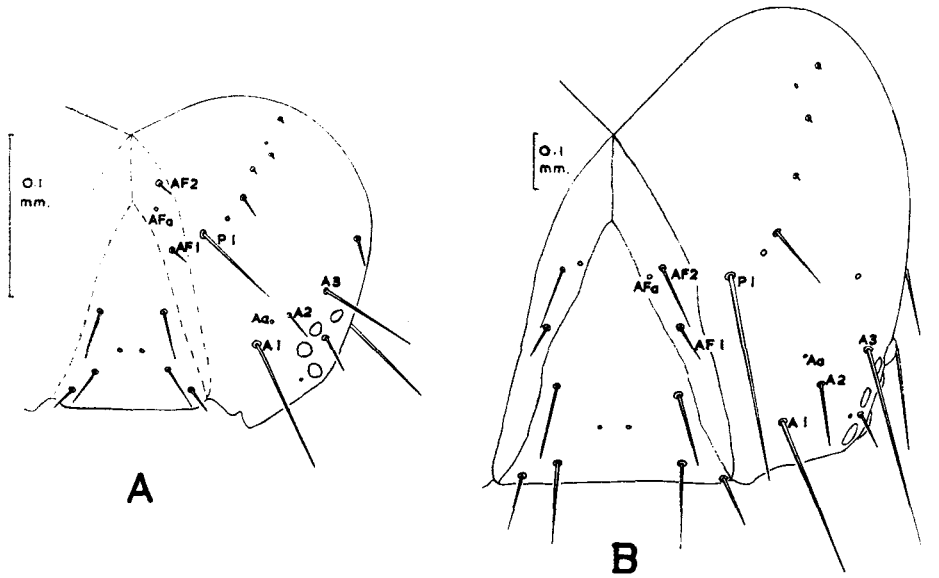

Fig. 1.—*Grapholita funebrana*, head capsules : (*A*) first instar larva ; (*B*) fourth instar larva.

The prothoracic and anal plates are grey-black in the early instars, with darker markings, and become pale brown with dark spots in the last instar. Similarly, other heavily chitinised parts such as setae and their basal plates, spiracles, spinules and legs are dark grey in the early instars and become progressively less heavily pigmented as the larva matures. The spinules appear as bright spots of pink in the mature larva.

Measurements

Overall length.—Too few specimens were available for statistical treatment of body measurements, but it was clear that these vary considerably with age within a particular instar. However, measurements were made of a few larvae extended in KAAD medium (Peterson, 1943) and preserved in 70 per cent. alcohol. The overall lengths (including head capsule) of instars 1–4 were 1·5, 3·0–4·0, 5·0–6·0 and 9·0–12·0 mm., respectively.

Head capsule widths.—The head capsule widths of the larvae examined fall into four groups, the means of which progress in a manner which approximates to

Dyar's Rule. The number of larvae measured was too small for any definite conclusion to be drawn, but the results suggest that four instars were present. The mean widths were as follows:

Mean width (mm.)	Instar	Number of larvae measured
0·26	1	9
0·44	2	3
0·68	3	9
0·99	4	12

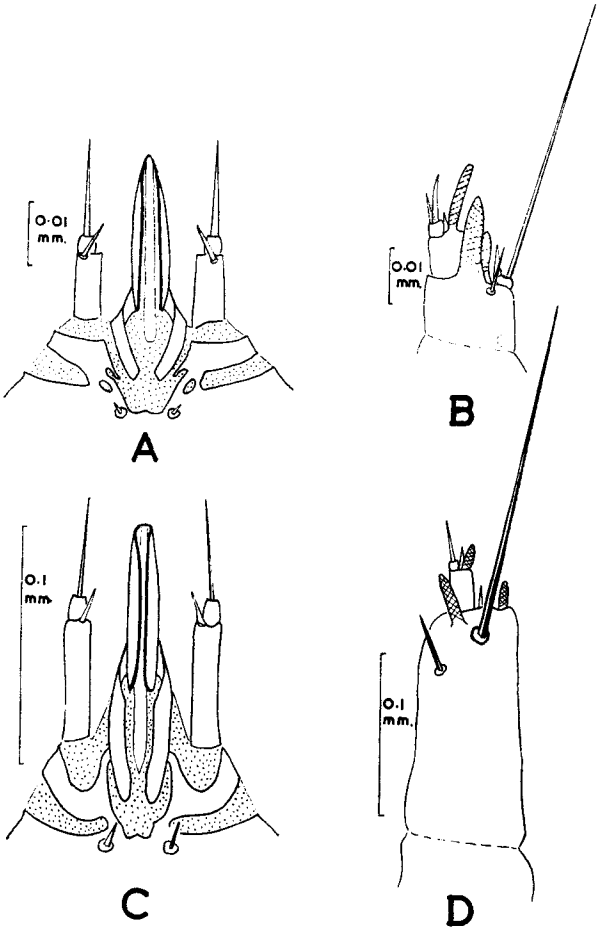

FIG. 2.—*Grapholita funebrana*. (*A–B*) first instar larva: (*A*) spinneret and (*B*) antenna (*C–D*) Fourth instar larva: (*C*) spinneret and (*D*) antenna.

The head capsule width of one larva was 0·58 mm., and it is possible that the number of instars may vary between four and five, as found by Peterson and Haeussler (1928) in *G. molesta*.

Setae and Other Features

Head.—In general, the setal arrangement (fig. 1) remains fairly constant throughout the development of the larva. The shape of the head changes slightly, *e.g.* the vertical angle is obtuse in the first two instars and becomes progressively more acute in the third and fourth. Of the macrosetae, the adfrontal group shows the greatest change. In the first instar AF2 is almost level with or slightly above the apex of

the clypeus and is further from P1 than is AF1. AF2 moves forward in the course of development until it is almost as far from the apex of the clypeus as it is from AF1, and both AF2 and AF1 are almost equidistant from P1. The punctures are the most unstable feature. Thus the angle A1–Aa–A2 varies from acute to almost 180°. The microsetae remain fairly constant. *Mouthparts:* the mandibles, maxillae and labrum show little change, except that some setae become longer relative to the remainder of the structure in the later instars. It is in the spinneret and antennae that the greatest changes in proportions take place (fig. 2). Thus, in the former, the length of the basal joint of the palps in relation to the length of the spinneret itself changes from less than one-half in the first instar to two-thirds in the last. Similarly, the second segment of the antenna is slightly wider than long in the first instar, whereas in the last instar it is at least twice as long as broad. The long seta on the antenna is five to six times the length of segment two in the first, but only one and a half times as long in the fourth instar.

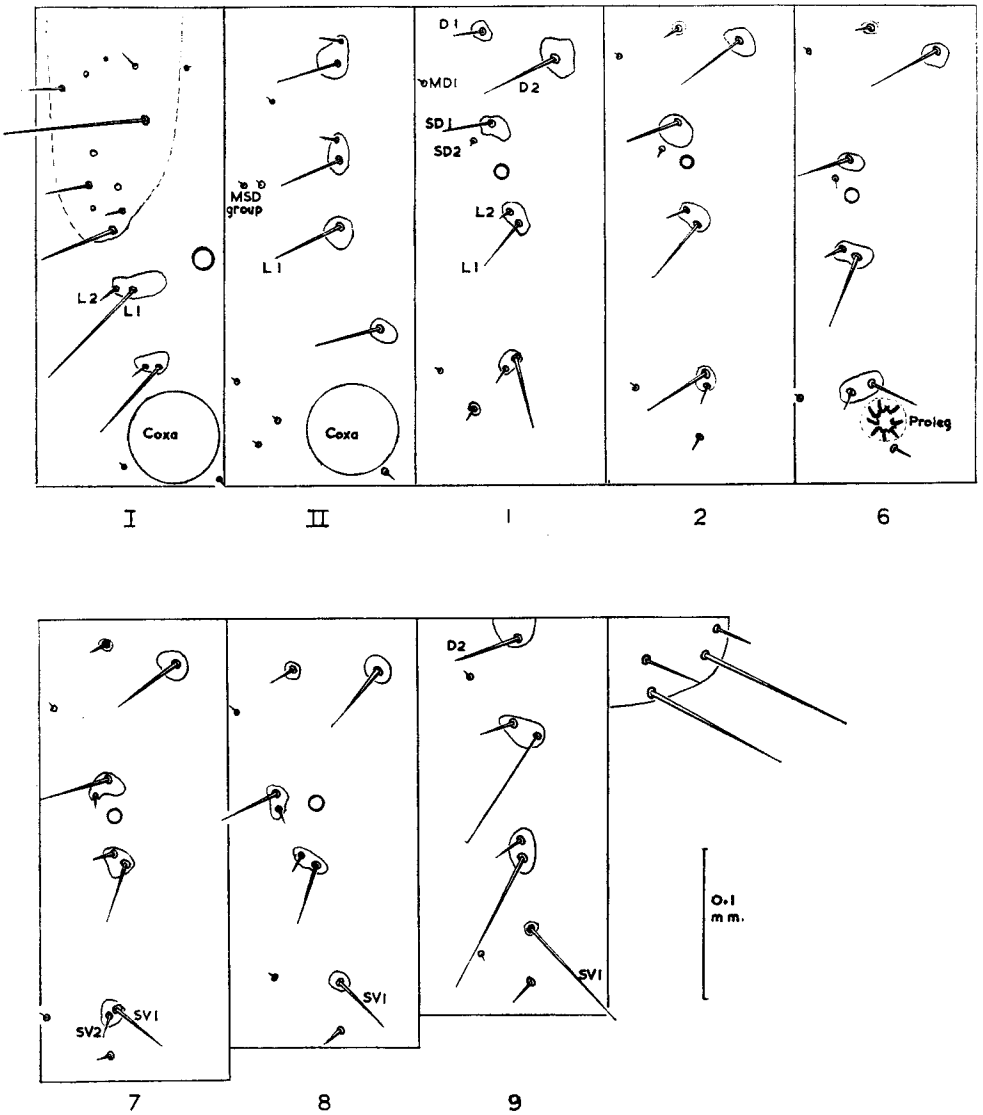

FIG. 3.—*Graphilota funebrana*, first instar larva: setal arrangement on thorax and abdomen.

Thorax.—Apart from the lateral group, the setal arrangement (figs. 3 and 4) remains fairly constant. In the first instar the lateral group contains two setae on the prothorax, and only one on the meso- and metathoracic segments. From the second instar onwards, there are three lateral setae on each of the thoracic segments. The setae on the thoracic legs appear relatively constant in number and arrangement through the instars. The proportions of the legs change slightly, the claw being larger relative to the rest of the leg in the early instars.

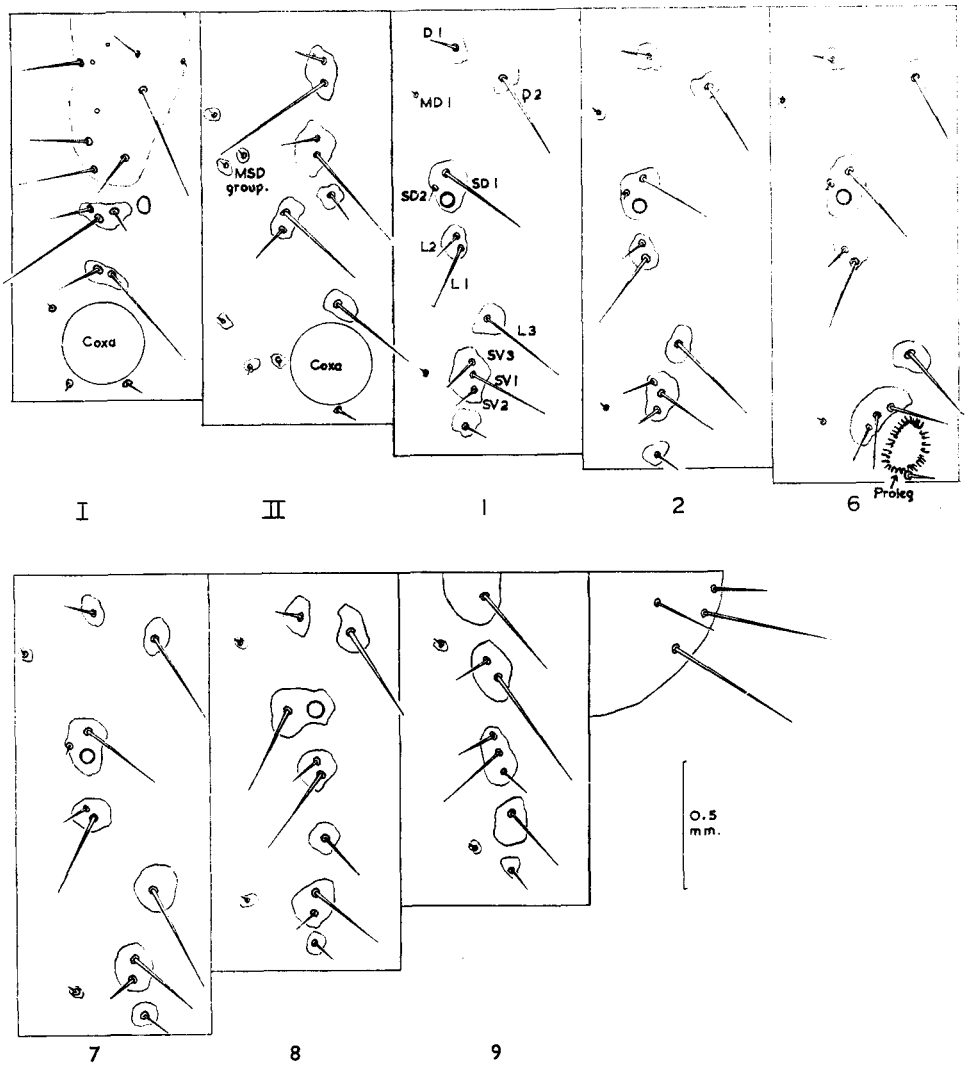

FIG. 4.—*Grapholita funebrana*, fourth instar larva : setal arrangement on thorax and abdomen.

Abdomen.—Only the lateral and subventral groups of setae show any appreciable change through the instars (figs. 3 and 4). Seta L3 is absent from all segments in the first instar, but is present in later instars. Likewise, SV3 is only present on segments one to six from the second instar onwards. On segment seven there are only two SV setae in all instars, and on segment eight there is only one SV seta in the first, and two in later instars. Segment nine has only one SV seta in all instars. The alignment of setae in relation to a vertical line through the spiracle, particularly in the lateral and subventral groups, is variable, as is the extent to which the basal

plates are joined to one another. This latter feature is especially evident in the lateral group on segment nine.

The number of crotchets on the prolegs increases from one instar to the next. Details are given in the table below :

TABLE I.—*Numbers of crotchets on prolegs*

Instar	Abdominal segment 3	4	5	6	last	Number of larvae examined
1st	7–10	9–10	8–10	8–9	6–8	4
2nd	14–17	13–15	14–16	12–13	9–10	1
3rd	17–23	15–25	14–24	16–24	9–14	3
4th	25–30	25–29	26–30	24–29	17–25	3

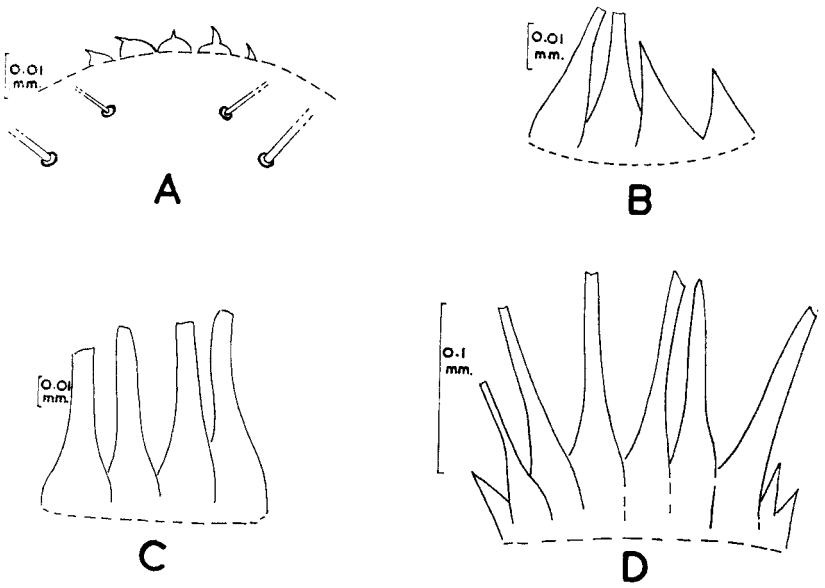

FIG. 5.—*Grapholita funebrana* larva, anal fork : (*A*) first instar ; (*B*) second instar ; (*C*) third instar ; (*D*) fourth instar.

The anal fork (fig. 5) first becomes clearly visible in the second instar. It may possibly be represented in the first by a group of large spinules under the anal plate. The number of prongs is variable, and the numbers observed were : second instar, 4 ; third instar, 4–5 ; fourth instar, 4–7, with 1–3 small additional prongs.

SEPARATION OF LARVAE OF *G. funebrana* TREITS. AND *G. molesta* BUSCK

Sufficient material of *G. molesta* was not available to make an assessment of the number of instars, but Peterson and Haeussler (1928) found four to five, depending on the rate of larval growth. So far as could be determined, the changes in morphology that take place during the growth of the larva are similar to those described for *G. funebrana*, though the coloration of *G. molesta* is somewhat different. In the material examined, the colour of the head capsule was variable in shade but in general it appeared to be somewhat lighter throughout development than that of *G. funebrana*. This was especially evident in the last instar. Some very pale specimens were found even in early instars, but one mature larva taken from imported fruit had a dark head capsule, though all the other characters agreed with those for *molesta*. The

mature larva of *G. molesta* never develops the brilliant red body colour found in *G. funebrana*, a dirty pink being the usual shade.

The close similarity of these two species made it difficult to find clear morphological differences between them. The number of crotchets on the anal prolegs does not provide a satisfactory character, for it increases at each moult and, as mentioned earlier, the numbers overlap in the two species. The spinules in *G. molesta* (fig. 6) are shorter and more numerous than in *G. funebrana*. This appears to be the case in all instars, though the considerable change in the numbers at each moult makes detailed comparison between the species difficult.

The following two characters were found to give a fairly reliable separation of the two species.

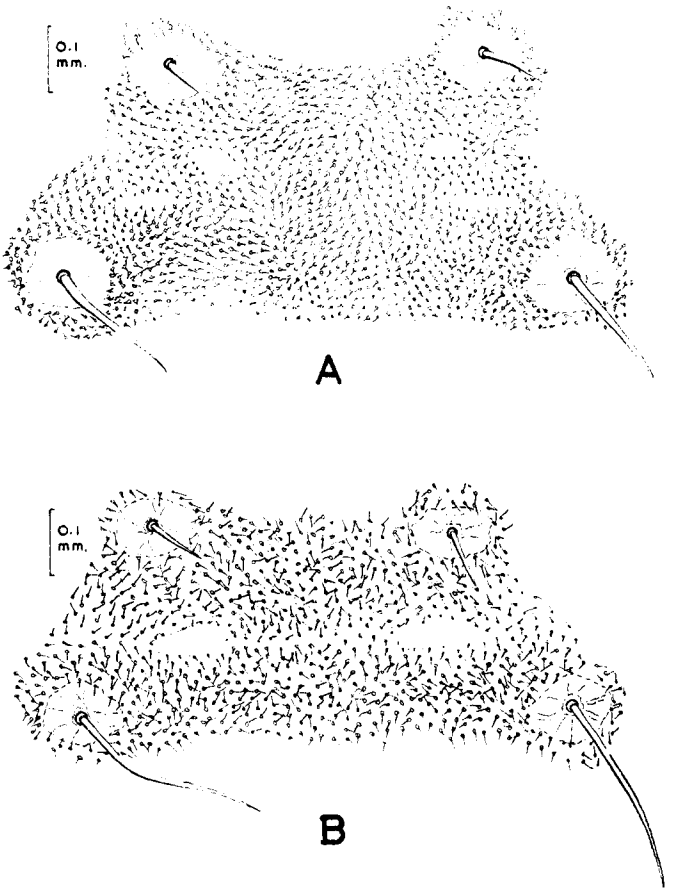

FIG. 6.—Arrangement of muscle origins within the dorsal group of setae on abdominal segment 4 in the mature larva: (*A*) *Grapholita molesta*. (*B*) *G. funebrana*.

(1) *The Arrangement of the Muscle Origins within the Dorsal Group of Setae on the Abdominal Segments* (fig. 6)

The muscles appear to be equivalent to the dorsal interior oblique 2 and the dorsal exterior oblique 1 of the larva of *Cecropia* described by Libby (1952). In *G. funebrana*, the origins of these two muscles are fused to form a single pair on most of the abdominal segments, usually as far back as segments 7 or 8. In *G. molesta*, the origins form two separate pairs on all the abdominal segments except the first. In mounted specimens the origins are clearly separated by an area of spinules, and

in the mature larva they appear white against the pink background and are thus easily visible under low power magnification.

The origins are visible in all instars except the first, where the spinules are too widely separated to show any well-defined clear areas of cuticle, and their arrangement in the early instars is similar to that found in the mature larva.

(2) *The Setae Dorsal to the Claws on the Thoracic Legs* (fig. 7)

In *G. molesta* these setae are flattened and almost parallel-sided for a large part of their length, in contrast with the more gradually tapered setae of *G. funebrana*. In the material available, the difference in shape could be seen in all instars, but was least evident in the third. The first instar of *G. molesta* has the setae clearly widened at the tip (fig. 7B). In the mature larvae, the setae of *G. molesta* are significantly

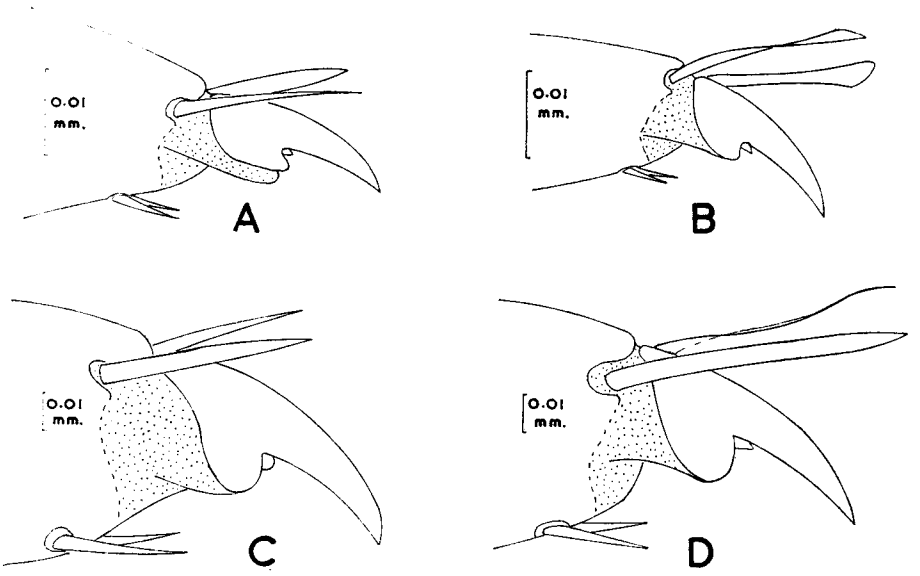

FIG. 7.—Setae dorsal to the claw on the prothoracic legs: (*A*) *Grapholita funebrana*, first instar; (*B*) *G. molesta*, first instar; (*C*) *G. funebrana*, fourth instar; (*D*) *G. molesta*, fourth instar.

longer in relation to the length of the claw than are those of *G. funebrana*. The length of the setae divided by the length of the claw varied from 1·03 to 1·27 in *G. molesta* (mean, 1·18; standard deviation, 0·10), and in *G. funebrana* between 0·58 and 1·00 (mean, 0·79; standard deviation, 0·03). Measurements on the few specimens available gave similar ratios for the third but not for earlier instars, in which the setae of both species appear relatively longer.

In addition to these two characters the two MSD setae on the mesothorax are usually on a single base plate in *G. molesta*, whereas they are frequently on separate plates in *G. funebrana*. However, this character is very variable in the latter species and is not easily visible in the whole larva. It can be used only in confirmation of the two main characters. The distribution of these three characters is given below.

> One pair of dorsal muscle origins, together with tapered setae dorsal to the claw, was found in 32 larvae of *G. funebrana* that were examined. Among these, the MSD setae on the mesothorax were arranged as follows: on single plates—3, on separate plates—11, asymmetrical larvae—10; they were not visible on eight larvae, being obscured by folds in the cuticle. No larvae of *G. molesta* were found with this combination of characters.

Two separate pairs of dorsal muscle origins on abdominal segment 4, together with flattened setae dorsal to the claw, were found in the eight larvae of *G. molesta* examined. On all the larvae the MSD setae on the mesothorax were on the same basal plate. No larvae of *G. funebrana* were found to have this combination of characters.

A mixed batch of 47 last instar larvae collected from fruit imported from southern Europe was also examined. The identity of the larvae had not been confirmed by breeding out adults, but their general characteristics placed them in one or other

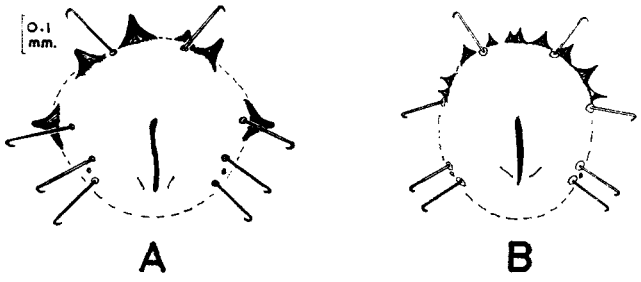

FIG. 8.—Terminal segment of pupa : (*A*) *Grapholita molesta* ; (*B*) *G. funebrana*.

of the species under consideration. The extent of association of the characters given above is evident from Table II.

TABLE II.—*Number of larvae with a particular association of characters*

Dorsal muscle origins on abdominal seg. 4		Setae dorsal to claw on thoracic legs	MSD setae on mesothorax: on single plates	on separate plates	asymmetrical larvae	not visible*	Totals
1 pair	+	flattened	0	0	0	0	0
1 pair	+	tapered	1	6	3	0	10
2 pairs	+	flattened	26	0	0	11	37
2 pairs	+	tapered	0	0	0	0	0
			27	6	3	11	47

* Usually hidden from view by a fold in the cuticle.

The numbers of larvae available for this study were too small to give any idea of the range of intraspecific variation, but the characters described would appear to provide a fairly reliable means of separating these two species.

The larvae of two other species of Lepidoptera are commonly found boring in imported fruit. These are the codling moth, *Laspeyresia pomonella* L. and the peach twig borer *Anarsia lineatella* Zell. The features distinguishing the larva of all four species may be summarised as follows :

1 Crotchets forming two groups on each anal proleg. D2 setae on abdominal 9 usually on separate plates. Seta SV2 absent on abdominal segment 1. Later instar larvae with secondary setae on anal plate. Body colour reddish-brown with white intersegmental membranes, giving banded appearance . . . . . **A. lineatella**

– Crotchets in single group on each anal proleg. D2 setae on abdominal 9 on single plate. Seta SV 2 present on abdominal segment 1. No secondary setae on anal plate. Body colour more uniformly dirty white or pink 2

2 Setae dorsal to the claws on the thoracic legs markedly unequal in length. No anal fork . . . . . **L. pomonella**
– Setae dorsal to the claws on the thoracic legs equal in length or nearly so. Anal fork present (except in first instar) 3
3 Only one pair of muscle origins within the dorsal group of setae on abdominal segment 4 (fig. 6). Setae dorsal to the claws on the thoracic legs tapered and usually shorter than the claw itself in late instars (fig. 7). MSD setae on mesothorax often on separate plates at least on one side of larva (variable) . . . . . **G. funebrana**
– Two pairs of muscle origins within the dorsal group of setae on abdominal segment 4 (fig. 6). Setae dorsal to the claw on the thoracic legs flattened and usually longer than the claw itself (fig. 7). MSD setae on mesothorax usually on a single basal plate . . . . . **G. molesta**

Pupae

Garman (1918) gives a key to the pupae of some North American fruit moths. As he points out, the pupa of *A. lineatella* is readily identified, but the pupae of *G. molesta* and *G. funebrana* are not easily distinguishable, either from one another or from those of *L. pomonella*.

The following key will serve as a guide to the pupae of these four species, but identification should always be confirmed by examination of the cast skin of the last instar larva present in the cocoon with the pupa :

1 Small pupa clothed in short hairs. No labial palps and no transverse rows of spines on the abdominal segments. Last 4 or 5 abdominal segments darker brown than thorax and anterior part of abdomen . . **A. lineatella**
– Pupa uniformly pale brown in colour, with labial palps and with prominent double transverse rows of spines on the dorsal surface of each abdominal segment. Only a few setae, no covering of short hairs 2
2 Spiracles on abdominal segment 2 slightly larger relative to length of pupa and more oval (diams. 0·11 × 0·07 mm. approx.) . . . . **L. pomonella**
– Spiracles on abdominal segment 2 relatively smaller and more nearly circular (diams. approx. 0·07 × 0·06 mm.) 3
3 Pupa with prominent dorsal arc of rather blunt spines on last abdominal segment. Length of larger spines usually about one-third to one-fourth distance between dorsal end of anal scar and base of dorsal spines. Number of spines variable (fig. 8*A*) . . . . . **G. molesta**
– Pupa with dorsal arc of spines on last abdominal segment composed of small sharply-pointed spines, but often only weakly developed. Lengths of larger spines usually less than one-fifth distance between dorsal end of anal scar and base of dorsal spines. Number of spines variable (fig. 8*B*) . . . . . **G. funebrana**

Discussion

Most of the published work on the identification of lepidopterous larvae has dealt with the last larval instar and authors have explicitly limited their keys to this stage. Almost all the characters described here have been used, especially the lateral and subventral groups of setae, the relative proportions of certain of the mouthparts and the presence or absence of the anal fork, but the way that they vary throughout the larval instars of *G. funebrana* shows some of the difficulties in adapting such keys or in constructing new ones to cover all instars.

The arrangement of the muscle attachment points on the cuticle is a character that has been neglected in the taxonomy of lepidopterous larvae and it may well provide useful interspecific differences in other groups.

Summary

Characters are described for the identification of larvae of *Grapholita funebrana* and *G. molesta* in all instars, keys are given to the larvae and pupae of four species of moths commonly found in fruit imported into the United Kingdom, and some of the changes in external morphology that take place during the development of the larva of *G. funebrana* are described.

Acknowledgments

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