ANATOMY AND HISTOLOGY OF THE MATURE LARVA OF THE AMERICAN COTTON LEAFWORM, *Alabama argillacea* (HÜBNER, 1818) (LEPIDOPTERA, NOCTUIDAE)

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ABSTRACT

The internal anatomy and histology of the different systems of the mature larva of *Alabama argillacea* (Hübner, 1818) have been studied and illustrated

INTRODUCTION

The American cotton leafworm, *Alabama argillacea* (Hübner, 1818) is an injurious insect pest of cotton plants. The larvae eat leaves, flowers, young bolls and other tender parts of the plant. They consume large quantities of leaf tissues, especially during the latter instars and may completely defoliate the plant. This insect is a migratory species, native of Central and South America. The time of its annual appearance in any locality depends upon its location. It occurs all over the New World countries (CREIGHTON, 1936; PARENCIA & RAINWATER, 1964).

The external morphology of *A. argillacea* was studied by OLIVEIRA (1935), VEGA (1939) and FREIBERG (1945). The biology by COSTA LIMA (1945), FREIBERG (1945), CALCAGNOLO (1965) and HABIB (1977a) CREIGHTON (1936), CALCAGNOLO (1965) and GLICK & GRAHAM (1965) studied the migration habits. Chemical control was studied by many authors, such as, FONSECA (1938), SNIPES (1940), IVY & EWING (1944, 1945), GALLO (1951), NICKEL (1958), ADKISSON (1958), ALMEIDA & CAVALCANTE (1965) and CALCAGNOLO (1965). The female sex pheromone was isolated by BERGER (1968) and the haemolymph was studied by HABIB (1977b), while a bacterial disease of the same insect was studied by HABIB (1978).

However, the anatomy and histology of the larva of *A. argillacea* have not yet been studied. Much work has been done on the anatomy of other caterpillars (PETERSON, 1912; SNODGRASS, 1934; SALAMA, 1956; EL–SAYED & ABDEL–RAHMAN, 1966; EL–MINSHAWY **et alii**, 1973; and WIGGLESWORTH, 1974). It is our aim, therefore, to give a picture on the internal anatomy and histology of the mature larva of *A. argillacea*. Both circulatory and respiratory systems were found to be similar to those of other noctuid larvae, so they will not be dealt with in this paper.

MATERIALS AND METHODS

To study the internal anatomy, the mature larvae were killed in hot water (55ºC) and preserved in 70% alcohol + 5% Bouin's solution. The larvae were slited longitudinally along the mid-dorsal or the mid-ventral surface, laid out flat and pinned in dissecting trays. The muscles were stained with eosin, while the nervous system was stained with haematoxylin.

For the histological studies, the larvae were starved for about 12 hours before being killed and fixed in hot Bouin's solution (50ºC) for two minutes. Cold Bouin was then added and the material was left in it for 24 hours. Dehydration was carried out in several ascending grades of ethyl alcohol (50, 60, 70, 80, 90, 95 and 100%) 2 hours in each. Aftewards, the larvae were put in a mixture of xylol and paraffin wax (1:1) for about two hours (55ºC). Embedding was done in paraffin wax (55ºC) for about 10 hours. Serial sections (eight μ thick) were prepared and stained with Ehrlich's haematoxylin and counter stained with eosin. The slides were mounted in Canada balsam.

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The study was based on permanent preparations and sections as well as newly killed and dissected specimens. Camara lucida drawings for various sections were prepared and are presented in the text.

RESULTS AND DISCUSSIONS

1. FAT TISSUES (Fig. 1, A)

Two types of fat tissues were encountered in the larvae of *Alabama argillacea*. The first, found between the body integument and the longitudinal muscles, is composed of a small number of fat cells. The second type, found in the body cavity surrounding the different systems and organs, takes the form of white lobulated bodies.

Microscopic examinations showed the oily nature of this tissue. The cells (FC) are filled with oily globules of fat (FD) and a homogenous cytoplasm with large dark nuclei (N). The fat cells are adherent and the external surfaces of cell masses are enveloped by a delicate connective membrane (CM). The lipid material, according to PERTERSON (1912) and WIGGESWORTH (1974), is stored for future use in metamorphosis.

2. ALIMENTARY CANAL (Fig. 1, B)

The alimentary canal occupies the greatest portion of the body cavity in the abdominal region and can be divided in the following principal divisions:

A. Stomodaeum:

This is the anterior ectodermal region of the alimentary canal, extending from the mouth cavity back to the metathoracic segment. It is differentiated into three regions, (1) the pharynx (PH), the smallest region of stomodaeum, is located in the head cavity. Some fibres were found arising from the muscular layer of the pharynx wall and attaching themselves to the head apodemas, (2) the oesophagus (OES), begins where the pharynx enlarges posteriorly and extends up to the prothoracic segment, and (3) the crop (C), occupies the whole length of the thorax. Its colour, shape and size depend on the amount of food in it.

Histologically (Fig. 1, C), in the pharynx, the intima (IN) is a thin cuticular layer enveloping the food content and separating it from the epithelial cells. The epithelium (Ep) is simple resting on a basement membrane (BM), and consists of flat cells with, somethimes, indistinct boundaries. The intima as well as the ephithelial layer possess some longitudinal folds, which according to SNODGRASS (1935) allow for expansion of the lumen as it becomes filed with food. The epithelium is coated externally with two types of muscles, an inner layer of longitudinal fibres (LM) and an outer one of circular fibres (CM). The latter does not attach to the epithelium.

B. Mesenteron (MES):

This is the endodermic region of the alimentary canal, appearing as a large undifferentiated tube and occupying the anterior six abdominal segments. The outer surface of the mesenteron is longitudinally divided into six areas by means of six fine longitudinal muscles with the following positions: one dorsal, one ventral, two dorso-lateral and two ventro-lateral. Between the crop and mesenteron there is a slight constriction which, according to SNODGRASS (1935) and WIGGESWORTH (1974), forms the so called "cardiac or stomodeal valve".

The food content, in a histological cross section (Fig. 1, D), appears to be clearly separated from the epithelial cells, indicating the presence of a thin membrane (PM) surrounding the food content. According to SNODGRASS (1935), there exists a delicate peritrophic membrane surrounding the food in the mesenteron region. This membrane, according to WIGGESWORTH (1974), is produced by and protects the epithelium. There are two types of epithelial cells. The first (DC), more abundant, corresponds to columnar or globlet cells responsible for digestion, secretion and absorption. The

second type (RC) is represented by small regenerative cells found either singly or in cluster between the bases of the first type cells. According to SNODGRASS (1935), the new digestive cells are formed from the special regenerative ones, which take no part in the other activities of the mesenteron. There exists a striated border towards the inner end of the digestive cells, as well as some cytoplasmic vacuoles. The epithelial layer rests upon a fine basement membrane (BM). The muscularis of the mesenteron includes outer longitudinal fibres (LM) and inner circular ones (CM).

C. Proctodaeum:

This is the posterior ectodermal region of the alimentary canal, extending from the caudal end of the mesenteron back to the anal opening occupying the posterior region of the abdominal cavity. It is divided in 3 distinct parts. (1) The pylorus (PL), is differentiated into an enlarged anterior part and a narrow posterior part. (2) The colon (COL) is a striated tube following the pylorus. It is the longest chamber of the proctodaeum and is constricted by many circular muscles. The colon wall is divided externally into six areas by means of six longitudinal fine bands of muscles. The rectal valve is located in the caudal end of the colon and is surrounded by bands of muscles. (3) The rectum (RE) is the terminal portion of the alimentary canal. It occupies the posterior part of the seventh abdominal segment and the whole cavity of the eighth. The walls of the rectum are thick and provided with few longitudinal striations.

Histologically, the epithelial layer (Ep) of the proctodaeum, lined internally by a thin cuticular intima (IN), shows a dark, unvacuolated and homogeneous cytoplasm, sometimes, without cell boundaries between the nuclei (Fig. 1, F). The epithelial cells rest upon a fine basement membrane (BM). The muscularis comprises inner circular fibres (CM) and outer longitudinal ones (LM).

3. MALPIGHIAN TUBES

The Malpighian tubes occur in two groups, each of three tubules (Fig. 1, B). The principal vessel of each group opens ventro-laterally into the lumen of the posterior part of the pylorus. This vessel branches into two arms: one arm is long (A), and the other is short and in turn bifurcates into two tubules (B, C). All of the tubules extend forwards adhering constantly to the mesenteronic wall up to the third abdominal segment. They, subsequently, return back until they reach the seventh segment, where they intermix forming a convoluted coiled mass. The terminal portions of these tubules extend out from this mass and run posteriorly to enter the rectal wall at its anterior part.

Histologically, the Malpighian tubules are dressed with a fine peritoneal membrane (PM) (Fig. 1, F). Transversal sections appear containing 5 - 8 epithelial cells with homogeneous vacuolated cytoplasm. These cells rest upon a basement membrane and their inner borders are striated by means of long cytoplasmic filaments. The outer wall of the tubule is provided with tracheal branches (TR) and some fine muscular fibres (MF).

4. GLANDS

A. Labial or silk glands (Fig. 1, G):

The small common silk duct opens at the base of the labium. The accessory glands (AG) are composed of two lateral groups, each consisting of numerous blade-like units. Each group opens laterally into the common silk duct. Posteriorly, this common duct ramifies into two fine silk ducts (SD). Each of these proceeds posteriorly through the head cavity beneath the suboesophageal ganglion (SOS. G.) up to the metathoracic segment. Then, each enlarges forming a silk gland (SG) which runs along the side of the lateral portion of the digestive tract till the seventh abdominal segment, where it terminates in the corresponding mass of Malpighian tubules.

B. Mandibular or salivary glands:

There are two white delicate tubes extending from the base of the mandibles posteriorly to the

metathoracic segment. The anterior part, in the head cavity, is very thin and adjacent to the lateral wall of the pharynx. The posterior part, in the thorax, is thicker and terminates in a mass of fat tissue marking the ventral line between the last two thoracic segments.

Histologically, both silk and salivary glands are similar in structure. A cross section shows large epithelial cells with striations towards the lumen border. The cell nucleus (N) is round or ovoid (Fig. 1, H) and located in the middle of the cell. The gland is coated by a peritoneal membrane (PM), while the lumen is surrounded by a smooth intima (IN).

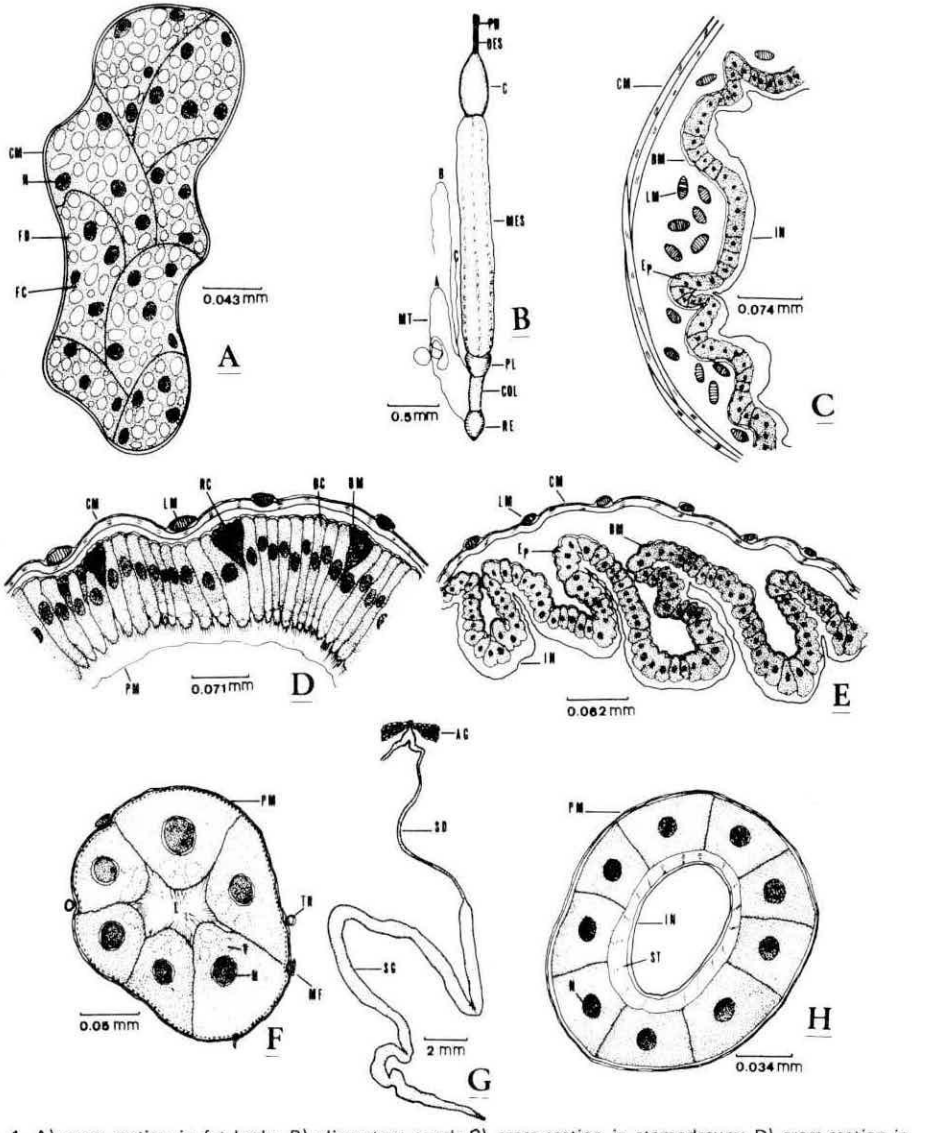

FIG. 1. A) cross section in fat body; B) alimentary canal; C) cross section in stomodaeum; D) cross section in mesenteron; E) cross section in proctodaeum; F) cross section in Malpighian tube; G) silk gland; H) cross section in silk gland.

5. MUSCULAR SYSTEM

The muscular system exists in a metameric arrangement in the body cavity. The musculature of the thoracic segment is complicated by the legs and cervix muscalature. The following muscles are encountered in both thoracic and abdominal segments:

A. *Large Dorso-longitudinal muscles,* are the large white fibres located along the sides of the heart.
B. *Small Dorso-longitudinal muscles,* are small fibres parallel and adjacent to the former type. Laterally, they are limited by an area free of muscles where the spiracles and the principal lateral tracheae are situated.
C. *Large Ventro-longitudinal muscles,* are located on the other side of, and ventrally to, the free area mentioned before and consist of large white fibres.
D. *Small ventro-longitudinal muscles,* are located ventrally on both sides of the ventral nerve cord.
E. *Dorso-ventral muscles,* are two groups of short fibres, extending between the tergum and sternum of each segment. The first group is located at the anterior part of the segment, while the second is at the posterior portion.

Histologically, a muscular fibre (Fig. 2, A) consists of various fibrillae ensheathed in a sarcolemma (Sm). These fibrillae are embedded in the sarcoplasm (Sp), and the nuclei (N) of the latter are located beneath the sarcolemma. The fibres join with the epidermis (Ep) whereas the fibrillae are directly connected to the cuticle (CT).

6. NERVOUS SYSTEM

A. Central nervous system:

This system consists of the brain (BR), the suboesophageal ganglion (SOS.G.) and the ventral nerve cord, the latter with its thoracic and abdominal ganglia. Three nerves originate on each side of the brain. The first is the optic nerve (O.N.) which inervates the ocelli. The second, the antennal nerve (AT.N.), goes to the antena. The third, called the labrofrontal nerve (LR.F.N.), divides into frontal ganglionic (F.G.N.) and labral (LR.N.) nerves (Fig. 2, B). Four pairs of nerves originate from the suboesophageal ganglion. These are the labial (LA.N.), maxillary (MX.N.), mandibular (MD.N.) and ventral (V.N.) nerves (Fig. 2, C). The ventral nerve cord contains 10 ganglia, three thoracic and seven abdominal. From each of these ganglia arise two pairs of lateral nerves (L.N.) on each sid. From the last abdominal ganglion, two lateral nerves and three additional ones are found on each side.

B. Sympathetic nervous systems:

The anterior part of this system consists of the frontal ganglion (F.G.), which is situated on the dorsal surface of the pharynx. It is connected to the brain by two nerves, one on each side. The recurrent nerve (R.N.) projects from this ganglion and extends backwards. The paired occipital ganglia (OC.G.), located beneath the pharynx, are each connected with the brain by an occipital ganglionic nerve (Fig. 2, B). The same ganglion connects with the recurrent nerve by another nerve (H). In addition, each occipital ganglion produces two nerves, one is directed to the mandibular muscles (M) and the other to the lateral surface of the crop (A). The corpora allata (Cor.A) are found adjacent to the posterior margin of the occipital ganglia.

The posterior part of the sympathetic nervous system contains the median nerves (M.N.), one of which extends backwards from each of the thoracic and abdominal ganglia (excepting the last abdominal ganglion). Each median nerve extends backwards for a short distance and then ramifies into two transversal nerves (T.N.). Each transversal nerve is connected with the lateral nerve of the subsequent segment by thin nerve fibres, plexus, (Px).

In a cross section, a ganglion of the ventral nerve cord exhibits an outer sheath called neurolemma (NM.), outer small imaginal nerve cells (IM.N.C.) and inner larger larval nerve cells (LV.N.C.). Some of the larval nerve cells extend to the middle of the ganglion (Fig. 2, E), thus separating two masses of medullary substances called neuropile (Np). Both the larval and imaginal nerve cells have a large nucleus.

7. REPRODUCTIVE SYSTEM

The gonads in both sexes are found laterally paired, adjacent to the heart in the fifth abdominal segment. These gonads are embedded in fat tissue which makes them difficult to localize. The gonads are supplied with fine tracheae.

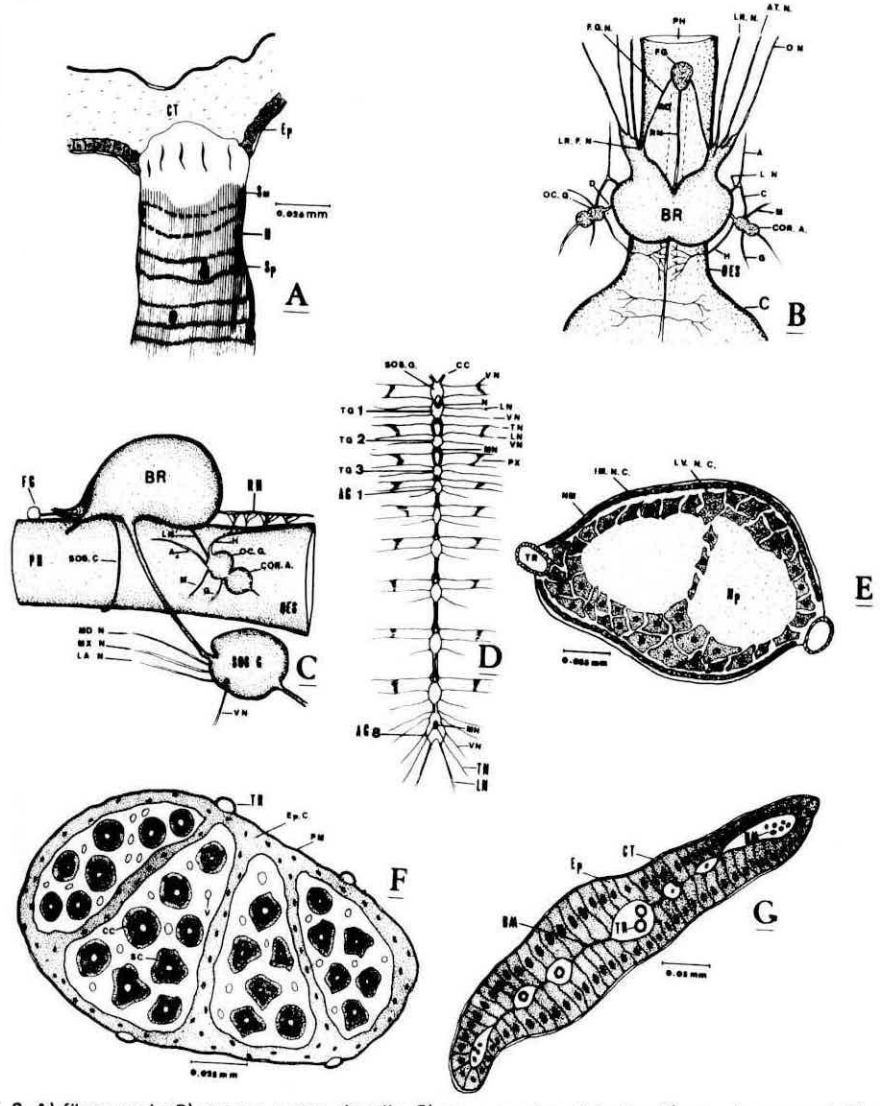

FIG. 2. A) fibre muscle; B) nervous system, dorsally; C) nervous system, laterally; D) ventral nerve cord; E) cross section in nerve ganglion; F) cross section in male gonad; G) cross section in wing bud.

A. Male gonads:

The male gonad is a relatively large kidney-shaped body, milky white in colour A fine thread-like duct extends form the posterior end of each gonad backwards to the eighth abdominal segment

parallel to the principal lateral trachea. There, it curves mesally ending in a small spherical body in the same segment.

Histologically, each gonad (Fig. 2, F) is coated by a peritoneal membrane (PM), followed internally by two layers of epithelial cell (Ep.C.). The epithelial cell boundaries are indistinct. The inner layer of epithelium forms the barriers between the four sperm tubes. Each sperm tube contains 5 to 10 primary sperm cysts (SC.), each coated by a delicate membrane. A primary sperm cyst cells (CC.) or sperm cells (spermatocytes, according to SNODGRASS, 1935).

B. Female gonads:

The female gonad is white in colour, spindle shaped and smaller than in the male. From the posterior end of each gonad, a fine duct extends backwards to the seventh abdominal segment, parallel to the principal lateral trachea, where it curves mesally to meet the corresponding duct from the other side. They fuse to form a small spherical body in the middle of the same segment.

8. WING BUDS

These are the imaginal buds of the adult wings. They are creamy white and spindle-shaped bodies located dorso-laterally in the mesothorax and metathorax.

Histologically, each wing bud (Fig. 2, G) is coated by a fine cuticular sheath (CT) and is composed of two epithelial layers of elongated cells. The epithelial cells (Ep) rest upon a collapsed tubular basement membrane (BM). This membrane is incompletely united, forming in some places small canals corresponding to the wing veins of the adult insect. These canals are provided with fine tracheae (TR) and haemocytes (HM).

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RESUMO

Anatomia e histologia do último estádio larval do "curuquerê do algodão" *Alabama argillacea* (Hübner, 1818) (Lepidoptera, Noctuidae)

A anatomia interna e a histologia de vários sistemas foram estudadas em larvas de último estádio de *Alabama argillacea* (Hübner, 1818).