MORPHOLOGY AND HISTOLOGY OF THE LARVAL DIGESTIVE

SYSTEM OF HELIOTHIS VIRESCENSE (FABR.),

HELIOTHIS ZEA (BODDIE), SPODOPTERA

FRUGIPERDA (J. E. SMITH), AND

SPODOPTERA ORNITHOGALLI

(GUENEE). (LEPIDOPTERA,

NOCTUIDAE)

By

CHE CHI

Bachelor of Science

National Taiwan University

Taipei, Taiwan

Republic of China

1970

Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE July, 1972

Thesis 1972 C532m Cop. 2

• .

.

FEB 5

MORPHOLOGY AND HISTOLOGY OF THE LARVAL DIGESTIVE SYSTEM OF <u>HELIOTHIS VIRESCENSE</u> (FABR.), <u>HELIOTHIS ZEA</u> (BODDIE), <u>SPODOPTERA</u> <u>FRUGIPERDA</u> (J. E. SMITH), AND <u>SPODOPTERA ORNITHOGALLI</u> (GUENEE). (LEPIDOPTERA, NOCTUIDAE)

Thesis Approved:

Thesis Adviser Dean of the Graduate College

Name: Che Chi

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE MORPHOLOGY AND HISTOLOGY OF THE LARVAL DIGESTIVE SYSTEM OF <u>HELIOTHIS VIRESCENSE</u> (FABR.), <u>HELIOTHIS ZEA</u> (BODDIE), <u>SPODOPTERA FRUGIPERDA</u> (J. E. SMITH), AND <u>SPODOPTERA ORNITHOGALLI</u> (GUENEE). (LEPIDOPTERA, NOCTUIDAE).

Pages in Study: 49 Candidate for Degree of Master of Science

Major Field: Entomology

- Scope and Method of Study: The morphology and histology of the alimentary canal is described and illustrated for the fifth instar larvae of four species of Noctuidae. Live fifth instar larvae were used for both gross dissection and tissue sections of the gut. Particular attention is given to the comparison between <u>Heliothis virescense</u> (Fabr.) and the three species. Detailed descriptions are made of the alimentary canal of <u>H. virescense</u> and only the differences of the other three species: <u>Heliothis zea</u> (Boddie), <u>Spodoptera frugiperda</u> (J. E. Smith), and <u>Spodoptera ornithogalli</u> (Guenne).
- Findings and Conclusions: The digestive system of the four species in this study consists of a pair of salivary glands and an alimentary canal. The species in the same genus are similar in their digestive system. However, there are some differences between the two genera. The salivary glands of <u>Heliothis</u> spp. are simple tubes, but in Spodoptera spp. there is a branch on each gland. The alimentary canal of the Heliothis spp. consists of foregut: esophagus, crop; midgut: anterior interstitial ring, ventriculus; hindgut; posterior interstitial ring, pyloric chamber, pylorus, ileum, colon and rectum. The esophagus has cuboidal epithelium. The crop has simple squamous epithelium. The regenerative cells, two types of simple columnar cells, and two types of goblet cells compose the midgut cellular lining. The pylorus and colon have cuboidal epithelium. The other parts of the hindgut have simple squamous epithelium with branching nuclei. The six malpighian tubules arise as two groups on the ventrolateral sides of the pylorus. The rectum forms the cryptonephridial system. The anterior ileum has six protuberances in Heliothis spp., but there are no distinct protuberances in the ileum of Spodoptera spp. Also, the hindgut epithelium of Spodoptera spp. is composed almost entirely of simple squamous epithelium. This is not true in the two species of Heliothis.

ADVISER'S APPROVAL William A. Snew

ACKNOWLEDGEMENTS

÷ 7

The author is sincerely grateful to Drs. William A. Drew and Jerry H. Young for their guidance and supervision throughout the course of this study and the preparation of the thesis.

Sincere appreciation is extended to the other member of the advisory committee: Dr. John R. Sauer for his advisement and critical review of this thesis.

Special thanks are extended to Dr. Milton R. Curd for his helpful suggestions and guidance. Appreciation and thanks are also expressed to Mr. Donald R. Molnar for his assistance with the photography. Special thanks are extended to Dr. Jakie A. Hair for his encouragement and advisement. Gratitude is expressed to Dr. R. C. Berberet for his suggestions and helpfulness.

Thanks are expressed to all the people in the Insectory for the supply of the specimens. Appreciation is also extended to Dr. D. E. Howell for the supply of materials in the experiment. Thanks are extended to Mr. John H. Pickle for his helpfulness and encouragement during this research.

Special recognition is extended to my parents for their understanding, encouragement, and confidence which have made the achievement of this study possible.

. . .

This thesis is dedicated to my parents.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
METHODS AND MATERIALS	-4
RESULTS AND DISCUSSION	5
<u>Heliothis virescense</u> (Fabr.)	5 12 12
Spodoptera frugiperda (J. E. Smith)	15
SUMMARY	17
BIBLIOGRAPHY	19
APPENDIX	21

.

.

LIST OF FIGURES

 \bullet , ε

1

Figur	e	Page
1.	The Alimentary Canals of <u>Heliothis</u> spp. and <u>Spodoptera</u> spp	. 23
2.	The Salivary Gland of <u>Heliothis</u> spp. and <u>Spodoptera</u> spp	. 29
3.	The Longitudinal Section of the Midgut and Hindgut and Four Types of Cells in Midgut Epithelium of <u>H</u> . <u>vire-</u> <u>scense</u>	. 33
4.	The Gastric Caecum and Midgut Wall of <u>H</u> . <u>virescense</u>	. 37
5.	The Longitudinal-Sections of the Hindgut and the Midgut of <u>H</u> . <u>virescense</u>	. 39
6.	The Cross-Sections of the Esophagus and the Pylorus of <u>H</u> . <u>virescense</u>	• 41
7.	The Cross-Sections of the Colon and Rectum of <u>H</u> . <u>vire</u> - <u>scense</u>	• 43
8.	The Cross-Sections of the pylorus and colon of <u>Spodoptera</u> <u>frugiperda</u>	• 45
· 4 ⁰⁰ . 9 ,	The Cross-Sections of the Pylorus and the Colon of <u>S</u> . <u>ornithogalli</u>	• 47
10.	The Cross-Sections of the Ileum of <u>H</u> . <u>virescense</u> , <u>S</u> . <u>frugiperda</u> and <u>S</u> . <u>ornithogalli</u>	• 49

v

• •

. s.i

INTRODUCTION

The purpose of this research was to compare the digestive systems of four species of Noctuidae.

The four species studied were: the budworm, <u>Heliothis virescense</u> (Fabr.); the corn earworm, <u>Heliothis zea</u> (Boddie); the yellow-striped armyworm, <u>Spodoptera ornithogalli</u> (Guenee), and the fall armyworm, <u>Spodoptera frugiperda</u> (J. E. Smith).

In this work <u>H</u>. <u>virescense</u> was used as a standard. The various regions of the alimentary canal were determined on the basis of cell type and muscular arrangement. The other three species were compared to <u>H</u>. <u>virescense</u>.

The <u>Heliothis</u> spp. are important cotton pests and have received much attention regarding economic control. However, little work has been done on the morphology and histology of the digestive system of these four species except the work of Chauthani and Callahan (1967), Standlea and Yonke (1968) on the morphology of the alimentary can**‡**1 of <u>H</u>. <u>zea</u>. There is not any histological work that has been done on these four species.

Chauthani and Callahan (1967) worked on the gross morphology of the alimentary canal of <u>H</u>. <u>zea</u>. Their work was corrected in some aspects by Standlea and Yonke (1968). However, Standlea and Yonke also neglected some points in the hindgut of the fifty instar larvae. They named the whole area behind the pylorus as the rectum. Actually it can be differentiated into ileum, colon, and rectum according to the

different cell type in this region.

Peterson (1912) worked on the anatomy which included external and internal morphology of tomato hornworm larva, <u>Protoparce quinquemacula-</u> <u>tus</u> Haworth (Lepidoptera, Sphingidae). According to his description, the gross structure of the alimentary canal of tomato hornworm is not much different from those of the four species of this work, except there is no crop in tomato hornworm.

Judy and Gilbert (1969, 1970) reported on morphological and histological research of the digestive system of <u>Hyalophora cecropia</u> (L.) (Lepidoptera, Saturniidae). They studied the gut structure at different developmental stages (1969) and discussed the cell types of the alimentary canal (1970). Their work emphasized the change during metamorphosis. Byer and Bond (1971) worked on the spinulation of the hindgut structures and their cell types. They concluded that the spines on the hindgut intima is to assist in breaking off the peritrophic membrane after it: exits from the midgut.

Anderson and Harvey (1966) conducted a detailed investigation on midgut epithelium of the fifth instar larvae of <u>Hyalophora cecropia</u> (L.). It has columnar cells and goblet cells, but no regenerative cells in the midgut epithelium. The midgut epithelium of female <u>Aedes aegypti</u> (L.) is simple columnar with regenerative cells (Hecker, Freyvogel, Briegel and Steiger, 1971). The midgut of <u>Dysdercus fasciatus</u> (Sign.) also has simple columnar cells with striated borders on the free surfaces. They are derived from regenerative cells which are grouped into nidi. The midgut epithelial cells are broken after starvation (Raqiq and Ford, 1962). Couch and Mills (1968) worked on the midgut epithelium of the American cockroach. The starved animals have a number of autophagic vacuoles in the midgut epithelium. If starvation is extended, vacuoles may empty into the lumen of the gut. They implied that the starved animals have a holocrine secretion. It has been postulated that both merocrine and holocrine secretion of digestive enzymes occur in the midgut (Pradhan 1940, Owsley 1946, Goodchild 1952, Chapman 1969). Shinoda (1927) believed that merocrine secretion occurs during normal conditions and holocrine occurs during starvation. Pradhan (1940) believed that the secretory mechanism in the phytophagous forms is merocrine; whereas, in carnivorous species the mechanism is of the holocrine type. In the four species of this work, the secretion seems to be a merocrine type.

The rectal complex of lepidopterous larvae has two sets of malpighian tubules: the outer layer and the inner layer which are separated by a double membrane (Ishimori 1924, Saini 1964). The result of this work does not show any sign of two sets of malpighian tubule in the rectal complex. Actually, in the four species of this work, the rectal complex has only one set of malpighian tubules between the rectal epithelium and a thin layer of membrane which is inside a sheet-like muscular layer.

METHODS AND MATERIALS

Fifth instar larvae were used to study the digestive system. The mature fifth instar larvae were reared on a synthetic diet (Burton, 1969) at room temperature in the Insectory of Oklahoma State University.

Morphological studies were carried out by dissection at the middorsal line of the fresh larvae under a stereo dissecting microscope. The salivary glands are located ventrad to the digestive tract and in order to study the salivary glands, it was necessary to carefully remove the digestive tract.

To test for the existence of a chitinous lining in the foregut and hindgut, the technique used by Campbell (1929) was employed.

Fresh specimens were also used for the histological studies. After dissecting out the entire gut, it was cut into five or six pieces: one with foregut and a small portion of midgut, several pieces of midgut, and one with hindgut and small portion of midgut. The midgut peritrophic membrane with its enclosed food was removed before fixing the tissue. Several fixatives were used. Bouin-Dubosco (Alcholic Bouin's) was the most satisfactory. Tissues were embedded in paraffin (56°). Longitudinal serial sections and cross serial sections were cut at eight *u* with a rotary microtome and stained with standard alum hematoxylin and triosin. In addition, two other stains were used on midgut epithelium: Lillie's azure A eosin B and Masson trichrome stain.

The drawings were made freehand. The photomicrographs were taken with 35 mm Kodak plus-x pan film.

RESULTS AND DISCUSSION

Heliothis virescense (Fabr.)

Morphological Studies

The digestive system of the fifth instar larva of budworm, <u>Heliothis virescense</u> (Fabr.), consists of a pair of salivary glands and a simple digestive tract.

The salivary glands are relatively long and extend two thirds the length of the body. They are white and swollen posteriorly. The anterior two thirds of each gland is very thin and contains an inner duct which extends to the prothoracic region where the two glands are slightly distended. Then the glands enter the hypopharynx and anastomose forming an oblong, sclerotized structure. The inner ducts join near the distal end of this sclerotized structure which empites into the spinneret (Fig. 2A).

The digestive tract of the fifth instar larva of <u>H</u>. <u>virescense</u> is simple. It is a typical insect digestive tract composed of foregut, midgut and hindgut. The midgut is the largest portion, with a greater length than the foregut and the hindgut combined (Fig. 1A).

Foregut. Anterior to the foregut is the buccal cavity which is surrounded by the labrum, the mandibles and the hypopharynx. In the budworm, the foregut is not differentiated into a pharynx and an esophagus. The duct anterior to the crop is the esophagus. The crop is an enlarged sac and is the most prominent structure in the foregut.

Midgut. The midgut is a long straight simple tubular duct. At the beginning of the midgut there is a series of small knobs protruding into the haemocoel. They form a ring which marks the separation of the foregut and the midgut. These knobs are gastric caecae or may be called the anterior interstitial ring. The midgut wall has transverse foldings along the entire length. These foldings give elasticity to the gut wall which allows a large amount of food to pass through it. Since the gut wall is expandable, sometimes there is an irregular outer shape to the gut because of the variation in the amount of food at different locations.

The hindgut begins at the posterior interstitial ring Hindgut, which separates the midgut and the hindgut. The calyx shaped pyloric chamber is next and is continuous with the pylorus. On the two ventrolateral sides of the pylorus, the malpighian tubules arise as two major ducts. Each duct gives rise to three branches. The branching point is not uniform. There are two types of branching. One has three branches split at the same point, in the other, one branch arises first, then, after a short distance the second and third branches arise. In the same species, some individuals have the first type of branching on both sides, some have the second type of branching on both sides, others have different types on either side. The malpighian tubules are attached to the midgut wall. They pass anteriorly about two thirds the length of the midgut, then they turn back and extend to the pyloric region. The posterior part of malpighian tubules convolve around the whole hindgut and finally terminate at the rectum. They penetrate through a sheet-like muscle layer and a thin membranous layer and then rest on the rectal epithelium. The malpighian tubules in this

part form a network between the epithelium and the membrane. This is a cryptonephridial excretory system.

Six protuberances surround the anterior ileum. The lumen of these protruberances are continuous to the major lumen of the ileum. The posterior ileum has a simple tube shape. Both anterior and posterior portions have circular muscle bundles internally and six bundles of longitudinal muscle externally. The longitudinal muscel bundles go between the protuberances and attach to the posterior end of the pylorus.

The colon is short. It cannot be distinguished from the posterior ileum externally. The colon has a thick circular muscle and six bundles of dilator muscle that attach to the outside of the circular muscle.

The rectum is the most posterior part of the digestive tract. It has a sphericle shape. The outer most layer is a muscle layer which is a sheet-like layer covering the whole rectum. Next is a layer of membrane with very large oval-shaped nuclei. Between the epithelium and membrane is a meshwork of convoluted malpighian tubules. Inside the epithelium is the chitinous intima.

Histological Studies

<u>Foregut</u>. The esophagus has cuboidal epithelial cells with rather large, more or less round, nuclei (Fig. 6D). Inside the epithelium is the chitinous intima, outside is the musculature. The epithelium has complicated longitudinal folds. Thus, the lumen has a branched tubular appearance with three major branches. Each branch has a secondary branch. Because of the epithelial folds, the epithelium attaches to the circular muscle only at certain points. Spaces exist between the circular muscle and epithelium. The musculature includes a rather thick layer of circular muscle but no longitudinal muscle. The dilator muscles attach to the esophagus at several places and penetrate through the bands of circular muscle and inserts on the epithelium. This is an efficient mechanism which controls the expansion of the esophageal lumen when the dilator muscles contract. Microspines spread on the intima in this anterior area.

The crop has squamous epithelium and a thin layer of circular muscle. The most inner part is a chitinous intima. The crop wall is very thin.

<u>Midgut</u>. The gastric caeca (anterior interstitial ring) have low columnar epithelium with small nucléi. The cytoplasm is neutral, so it has a pale appearance when stained. The anterior interstitial ring is composed of a series of small knob-like caeca.. The basement membrane of the ring projects out having small low columnar cells attaching to it which form the caeca.. The caeca are evenly distributed around the whole circle of the gut (Fig. 4A).

The midgut is composed of three major layers: peritrophic membrane, mucosa, and musculature. The peritrophic membrane is a thin membrane which surrounds the food particles. The mucosa is the cellular component of midgut. It consists of regenerative cells and midgut epithelium. The regenerative cell or nidus is located basally among the midgut epithelium. Nidi usually have a group of closely packed nuclei surrounded by a thin layre of unstained cytoplasm. The nuclei of nidi are small, spherical and have sparse chromatin. The midgut epithelium has two major varieties of cells: simple columnar and goblet cells (a type of columnar cell with a vacuole in the cytoplasm). Both of these are tall with a brushed border of microvilli on the free surface. There

· .:

are four types of cells within these two major varieties (Fig. 3B, 4C,D). Type one has a relatively small sphericle vesicular nucleus that is similar to the nucleus of a regenerative cell. The nucleus is basally placed with a large vacuole at the distal end. Type two has a larger oval shaped nucleus located in the middle of the cell and a vacuole above the nucleus. The vacuole is smaller than that of the type one. Type three also has a centrally located nucleus but no vacuole. The nucleus has a denser chromatin, especially on the distal part of the nucleus. Type four is without a vacuole and differs in appearance from the other cell. A large and dense chromatic nucleus is located at the distal end of the cell with scalloped microvilli attached to the thin layer of cytoplasm above the nucleus.

The question regarding whether the simple columnar cell and goblet cell in the insect midgut are the same kind of cell but in different physiological phases or whether they are a totally different kind of cell remained unanswered. (Anderson and Harvey, 1966). The four types of cells found in the midgut of <u>H</u>. <u>virescense</u> seem to be four different growing stages of the same kind of cell. The first stage would be the type one cell which has a small nucleus and a large vacuole and is generated by the nidi. As the cell grows, the secretion diffuses into the lumen through the microvilli. As the vacuole gets smaller, the nucleus increases in size and in chromatin and moves up to the middle part of the cell. The nucleus metamorphs to an oval shape. This is the second stage and would be the type two cell. The microvilli part at the middle and release the vacuole. After releasing the secretion which formed the vacuole, the microvilli move back to the original position and forms the cell of type three which has a centrally placed

ુ9

nucleus and no vacuole. The fourth and the last stage of growing is that in which the nucleus has moved up to the distal end, and forms the cell of type four. According to the secretory process, it appears to be the merocrine secretion.

The musculature of the midgut consists of inner circular and outer longitudinal muscles. There are spaces between the adjacent muscle bundles.

<u>Hindgut</u>. The posterior interstitial ring is made up of low columnar epithelium with small round nuclei and neutral cytoplasm (Fig. 5A). This epithelium is similar to that on the anterior interstitial ring except it is slightly taller. Chitinous intima starts at the posterior interstitial ring and ends at the anus.

The pyloric chamber has simple squamous epithelium which folds around the gut lumen. The pyloric chamber also has an inner circular muscle and an outer longitudinal muscle. (Fig. 5B).

The pylorus can be subdivided into three parts: the anterior, middle, and posterior parts. The pyloric wall consists of the epithelium, musculature and chitinous intima. The anterior part has thick squamous epithelium anteriorly followed by relatively large and irregular shaped cuboidal cells. The mucosa has four longitudinal folds in this anterior part (Fig. 5B, 6A). This part has the thickest musculature which is generally oriented so as to have an inner circular layer and an outer longitudinal layer, but they are poorly differentiated. Some longitudinal bundles are found among the circular layers. Also some transverse muscles run from the outer layer through the whole musculature and attach to the epithelium (Fig. 6A). The transverse muscle should be very effective in the control of the ex-

pansion of the gut lumen.

Epithelial folds flatten in the middle part producing a large and more or less oval shaped lumen. The middle part has squamous epithelium intervened with some large irregular cuboidal cells. In this part, the outer longitudinal layer penetrates through the spaces between the circular muscles and goes to the inside; thus, reversing the usual muscular arrangement. (Fig. 5B, 6B). The inner longitudinal muscle ends at the end of the middle part.

The posterior part has a smaller lumen with regular cuboidal epithelium. Only two or three layers of circular muscle exist in the posterior part (Fig. 5B, 6C). The musculature in the pyloric region gets thinner as it extends to the posterior end.

A pair of common malpighian ducts insert through the musculature on two ventrolateral sides of the pylorus. They rest in the folds of the mucosa between the muscular layer and epithelium. There are some sclerotized microspines projecting from the chitinous intima in the pyloric region.

The ileum has simple squamous epithelium (Fig. 5C, 10A). The epithelial cells have highly amorphous nuclei which are elongated and have several branches. Outside of the epithelium is the musculature. The circular muscle is inside and longitudinal muscle outside. They are not continuous, with spaces between the adjacent bundles. This arrangement of only the epithelium and basement membrane as continuous layers between the lumen and the open circulatory system might facilitate the reabsorption of water and ions.

The colon has large cuboidal epithelium which has complicated longitudinal folds (Fig. 5C, D; 7A, B). Because of the epithelial folds, the actual space of the lumen is very restricted and has a compound tubular appearance. There are three major folds, each has a secondary branch and half of the secondary branches have tertiary branches. Folds give the lumen a potential to expand when large amounts of food pass through. The circular muscle layer is one cell thick and six cells wide for each circle. Epithelium at the anterior colon attach to the circular muscle layer at six points which are the junctions of adjacent circular muscle cell. Large spaces occur between the circular muscle and epithelium because of the epithelial folds. Besides the circular muscle, there are six bundles of dilator muscle attached to the woutside of the corresponding sites of the epithelial attachment points (Fig.7A). Nearer the rectum, the colon circular muscle becomes twice as thick as they are anteriorly (Fig. 7B). The gut diameter gets smaller. Epithelial cells are crowded together. The tubular lumen becomes more narrow, but still has the same pattern as it has anteriorly.

The rectum has a sheet-like muscle and a thin membranous layer covering the convoluted malpighian tubules and the rectal epithelium (Fig. 5D, 7C). The rectal epithelium has simple squamous cells with branching nuclei which are like those of the ileum (Fig. 7D).

<u>Heliothis</u> <u>zea</u> (Boddie)

The structure of the digestive system is almost exactly the same as that of the <u>H. virescense</u>. The only difference is that in the posterior part of the pylorus the musculature is twice as thick as that in <u>H. virescense</u>.

Spodoptera frugiperda (J. E. Smith)

The salivary glands of fall armyworms, <u>Spodoptera frugiperda</u> (J. E. Smith), have some variations from those of <u>H</u>. <u>virescense</u>. The fall armyworm also has two salivary glands which join together at the anterior end and empty into the spinneret, but there is a branch bearing ten to twelve small branches on each gland which does not exist in <u>H</u>. <u>virescense</u>. (Fig. 2B).

The foregut is essentially like the foregut of H. virescense.

The midgut wall is composed of peritrophic membrane, mucosa, and musculature like that of <u>H</u>. <u>virescense</u>. The difference is that while there are longitudinal and circular epithelial folds throughout the midgut of <u>H</u>. <u>virescense</u>, in the fall armyworm only circular folds exist. The mucosa includes nidi and midgut epithelium. Nidi are the same as those in <u>H</u>. <u>virescense</u>, but the number of nidus is greater in the fall armyworm. Another difference is in the nuclei of the midgut epithelium which have more distinct nucleoli than those of the budworm.

The posterior interstitial ring and pyloric chamber are the same as those of <u>H</u>. <u>virescense</u>.

Most of the pylorus has squamous epithelium. The cuboidal epithelium appears at the posterior one third of the pyloric region. The cytoplasm has large basophilic granules. The pylorus of the fall armyworms can also be subdivided into three parts. There is a slight difference between the arrangement of the three parts in fall armyworms and those of budworms. The anterior part has simple squamous epithelium which has large and complicated longitudinal folds (Fig. 8A). The lumen is extremely small. It has a branched tubular appearance. The muscle: is extremely thick in this part. It is composed mostly of longitudinal muscle.

The middle part still has squamous epithelium, but the corresponding area in <u>H</u>. <u>virescense</u> has cuboidal cells intervened with simple squamous cells. The epithelial folds in the middle division flatten considerably, forming a lot of small longitudinal folds resulting in the largest space in the lumen of the pylorus. At the end of the middle section, the epithelial foldings invade to the center of the lumen to form four large folds (Fig 8B). This change restricts the lumen and produces smaller spaces and a tubular shape again. The middle region has two or three layers of circular muscle outside several bundles of longitudinal muscle and a few transverse muscle attaching to the circular muscle and epithelium.

The posterior part is the area with simple squamous epithelium and cuboidal epithelium which is at the tip of each epithelial folds (Fig. 8C); whereas, in <u>H</u>. <u>virescense</u> there are only cuboidal cells. The epithelium has three major folds and small secondary folds in this part. The longitudinal muscle exists on the outside of circular muscle in fall armyworm, but not in <u>H</u>. <u>virescense</u> in the posterior part of the pylorus.

The ileum has simple squamous epithelium with branching nuclei. There are several minute epithelial folds at the anterior end. They leave a relatively large lumen surrounded by the chitinous intima. At the posterior end of the ileum, epithelial folds become deep and follow the folds of the chitinous intima (Fig. 10B). The muscular arrangement is the same as that in <u>H</u>. <u>virescense</u>. No protuberance projects from the anterior part of the ileum. So there is no reason to differentiate an anterior and posterior ileum in the fall armyworm.

The colon is composed of intima, mucosa, and musculature (Fig. 8D). The mucosa is composed of simple squamous epithelium with irregular shaped nuclei. The cytoplasm of the epithelial cells has large basophilic granules. Three major longitudinal epithelial folds give rise to secondary and tertiary branches. At the tips of three major folds, the cells are thicker. They are more or less like low cuboidal cells. The peripheral part of the mucosa has typical squamous epithelium. The muscular arrangement is the same as that of the budworm. The main difference between the fall armyworm and budworm colon is in the epithelium. The fall armyworm has simple squamous epithelium and the budworm has cuboidal epithelium.

The rectum is similar to that of H. virescense.

Spodoptera ornithogalli (Guenee)

The salivary glands of the yellow-striped armyworm, <u>Spodoptera</u> ornithogalli (Guenee), are the same as those of the fall armyworm.

The foregut of the yellow-striped armyworm is the same as that of <u>H. virescense.</u>

The midgut is the same as those of \underline{H} . virescense.

The posterior interstitial ring and the pyloric chamber are the same as those of <u>H</u>. <u>virescense</u>.

The epithelium is simple squamous in most of the pylorus. Posteriorly the epithelium changes to a simple cuboidal epithelium (Fig. 9C). The musculature is undifferentiated anteriorly. The circular muscle, longitudinal muscle and transverse muscle intermingle at this point (Fig. 9A). After passing the mid-line, the muscle differentiates into an outer circular muscle and inner longitudinal muscle. Among them, several transverse muscle bundles exist (Fig. 9B). The inner longitudinal muscle is continuous to the end of the pylorus. This differs from <u>H</u>. <u>virescense</u> which does not have longitudinal muscle at the posterior end of the pylorus.

The ileum has a simple squamous epithelium, an inner chitinous intima and an outer musculature (Fig. 10C). There is not much difference between the yellow-striped armyworm and the budworm in this region, except that the yellow-striped armyworm does not have protuberances in the anterior ileum. The epithelial folds are more complicated toward the posterior end. It is quite similar to that of the fall armyworm.

The colon has simple squamous epithelium which becomes thicker in the posterior end (Fig. 10D). Since both the ileum and colon have simple squamous epithelium, they are distinguished by differences in the muscular arrangement and the different appearance of the cytoplasm of the cells. The colon circular muscle is three times thicker than that of the ileum. There are a lot of bluish staining basophilic granules in the cytoplasm of colon cells, but not any in the ileum cells. These characters differentiate the ileum and colon.

The rectum is the same as that of the budworm.

SUMMARY

The digestive system of the fifth instar larvae of <u>Heliothis</u> <u>virescense</u>, <u>H. zea</u>, <u>Spodoptera frugiperda</u>, and <u>S. ornithogalli</u> includes a pair of salivary glands and the alimentary canal.

The salivary gland of <u>Heliothis</u> spp. are a pair of simple tubes and those of <u>Spodoptera</u> spp. are branched.

The alimentary canal of Heliothis virescense consists of foregut: esophagus, crop; midgut: anterior interstitial ring, ventriculus; hindgut: posterior interstitial ring, pyloric chamber, pylorus, ileum, colon and rectum. Both the foregut and hindgut have chitinous intima. The esophagus has cuboidal epithelium which is surrounded by muscle. A layer of flat cells line the crop which is followed by the anterior interstitial ring. A series of small low columnar cells forms the anterior interstitial ring which separates the foregut and midgut. The midgut wall is composed of peritrophic membrane, mucosa, and musculature. The regenerative cells, two types of simple columnar cells and two types of goblet cells form the cellular lining of the midgut. The midgut muscle consists of circular muscle inside and longitudinal muscle outside. The posterior interstitial ring of low columnar cells is the beginning of the hindgut. It is followed by the pyloric chamber and pylorus. The six malpighian tubules arise as two groups on the ventrolateral sides of the pylorus. The epithelium of the phylorus is simple squamous anteriorly and cuboidal posteriorly. The muscle is thick in the pylorus. There are six protuberances on the

anterior ileum. The ileum has simple squamous epithelium which have branching nuclei. The colon has thick circular muscle which surrounds the cuboidal cellular lining. The globular shaped rectum forming the cryptonephridial system is the last part of the alimentary canal. The epithelium of the rectum is simple squamous with branching nuclei.

The alimentary canal of <u>H</u>. <u>zea</u> is similar to that of <u>H</u>. <u>virescense</u>. There are some minor differences in the digestive tract of <u>Spodop</u>-<u>tera</u> spp. The difference in the gross structure of the alimentary canal is that there are no distinct protuberances in the anterior part of the ileum in <u>Spodoptera</u> spp. The histological difference is that the epithelial lining in the hindgut of <u>Spodoptera</u> spp. is almost entirely simple squamous with only a small amount of cuboidal cells in the posterior part of the pylorus. However, in <u>Heliothis</u> spp., the pylorus and colon are composed mostly of cuboidal cells.

BIBLIOGRAPHY

- Anderson, E. and W. R. Harvey. 1966. Active transport by the Cecropia midgut. II Fine structure of the midgut epithelium. J. Cell. Biol. <u>31</u>: 107-134.
- Burton, R. L. 1969. Mass rearing the corn ear worm in the laboratory. USDA ARS-33-134, 8.
- Byers, J. R. 1971. Metamorphosis of the perirectal malpighian tubules in the meal worm <u>Tenebrio molitor</u> L. (Coleoptera, Tenebrionidae) I Histology and Histochemistry. Canadian J. of Zool. <u>49</u>: 823-830.
- Byers, J. R. and E. F. Bond. 1971. Surface specializations of the hindgut cuticle of lepidopterous larvae. Canadian J. of Zool. <u>49</u>: 867-876.
- Campbell, F. L. 1929. The detection and estimation of insect chitin; and the irrelation of "chitinization" to hardness and pigmentation of the cuticula of the American cockroach, <u>Periplaneta americana</u> L. Ann. Entom. Soc. Amer. <u>22</u>: 401-426.
- Chapman, R. F. 1969. The insects-structure and function. The English Universities Press LTD.
- Chauthani, A. R. and P. S. Callahan. 1967. Developmental morphology of the alimentary canal of <u>Heliothis zea</u> (Lepidoptera: Noctuidae). Ann. Entom. Soc. Amer. <u>60</u>: 1136-1140.
- Couch, E. F. and R. R. Mills. 1968. The midgut epithelium of American cockroach: acid phosphomonoesterase activity during the formation of autophagic vacuoles. J. Insect. Physiol. 14: 55-62.
- Galigher, A. E. and E. N. Kozloff. 1964. Essentials of practical microtechnique. Lea and Febiger, Philadelphia. 306-345.
- Goodchild, A. J. P. 1952. A study of the digestive system of the West African cacao capsid bugs (Hemiptera, Miridae). Proc. Zool. Soc. Lond. <u>122</u>: 543-572.
- Hecker, H., T. A. Freyvogel, H. Briegel and R. Steiger. 1971. Ultrastructural differentiation of the midgut epithelium in female <u>Aedes aegypti</u> L. (Insecta, Diptera). Imagines ACTA Tropica Separatum. <u>28</u>: 80-104.
- Humason, G. L. 1967. Animal tissue techniques. W. H. Freeman and Company, San Francisco, 2nd ed. 569 pp.

- Ishimori, Naoto. 1924. Distribution of the malpighian vessels in the wall of the rectum of lepidopterous larvae. Ann. Entom. Soc. Amer. <u>17</u>: 75-84.
- Judy, K. J. and L. I. Gilbert. 1969. Morphology of the alimentary canal during the metamorphosis of <u>Hyalophora cecropia</u> L. Ann. Entom. Soc. Amer. <u>62</u>: 1438-1446.
- Judy, K. J. and L. I. Gilbert. 1970. Histology of the alimentary canal during the metamorphosis of <u>Hyalophora cecropia</u> L. J. of Morphol. <u>131</u>: 277-300.
- Lillie, R. D. 1965. Histopathologic technic and practical historhemistry. New York, Blakiston Division, McGraw-Hill. 3rd ed. 162-165.
- Marshall, A. T. and W. W. K. Cheung. 1970. Ultrastructure and cytochemistry of an extensive plexiform surface coat on the midgut cells of a Fulgorid insect. J. Ultrastructure Research. <u>33</u>: 161-172.
- Owsley, W. B. 1946. The comparative morphology of internal structures of the Asilidae (Diptera). Ann. Entom. Soc. Amer. <u>39</u>: 33-68,
- Peterson, A. 1912. Anatomy of the tomato worm larva, <u>Protoparce</u> <u>carolina</u>.(Lepidoptera, Sphingidae). Ann. Entom. Soc. Amer. <u>5</u>: 246-269.
- Pradhan, S. 1940. The alimentary canal and pro-epithelial regeneration in <u>Coccinella septempunctata</u> with a comparison of carnivorous <u>Coccinellids</u>. Quart. J. Micr. Sci. 81: 451-478.
- Raqiq, K. M. and J. B. Ford. 1962. Studies on digestive enzyme production and its relationship to the cytology of the midgut epithelium in <u>Dysdercus fasciatus</u> Sign. (Hemiptera: Pyrrhocridae). J. Insect. Physiol. <u>8</u>: 597-608.
- Saini, R. S. 1964. Histology and physiology of the cryptonephridial system of insects. Trans. Roy. Entom. Soc. London. <u>116</u>: 347-392.
- Shinoda, O. 1927. Contribution to the knowledge of intestinal secretion in insects. II A comparative histo-cytology of the midintestine in various orders of insects. Z. Zellforsh. <u>5</u>: 278-292.
- Standlea, P. P. and T. R. Yonke. 1968. Clarification of the description of the digestive system of <u>Heliothis zea</u>. Ann. Entom. Soc. Amer. <u>61</u>: 1478-1481.

APPENDIXES

Figure 1. The alimentary canals of <u>Heliothis</u> spp. and <u>Spodoptera</u> spp.

A. The alimentary canal of <u>Heliothis</u> <u>virescense</u> (Fabr.).



Figure 1. Continued

B. The alimentary canal of <u>Spodoptera</u> <u>frugiperda</u> (J. E. Smith).



Figure 1. Continued

C. The alimentary canal of <u>Spodoptera ornithogalli</u> (Guenee).



Figure 2. The salivary gland of <u>Heliothis</u> spp. and <u>Spodoptera</u> spp.

A. The salivary gland of <u>Heliothis</u> spp.



Figure 2. Continued

۰.

B. The salivary gland of <u>Spodoptera</u> spp.



Figure 3. The longitudinal section of the midgut and hindgut and four types of cells in midgut epithelium of <u>H</u>. <u>virescense</u>.

A. The longitudinal section of the midgut and hindgut of <u>H</u>. <u>virescense</u>.



Figure 3. Continued

.

B. The four types of cells in the midgut epithelium.

n, nucleus; v, vacuole; m, microvilli.



ς Υ Figure 4. The gastric caecum and midgut wall of <u>H</u>. <u>virescense</u>.

- A. Gastric caecum and midgut epithelium. gc, gastric caecum; mep, midgut epithelium. Cross-section, stained with hematoxylintriosin. Scale equals 50 μ.
- B. Midgut wall, showing the muscle and epithelium. cm, circular muscle; 1m, longitudinal muscle; n, nucleus. Longitudinalsection, stained with hematoxylin-triosin. Scale equals 50 µ.
- C. Midgut epithelium, showing the type 1 and type 4 cells. mep 1, type 1 cell of midgut epithelium; mep 4, type 4 cell of midgut epithelium; mv, microvilli; n, nucleus. Cross-section, stained with Lillie's azure A eosin B. Scale equals 50 µ.
- D. Midgut epithelium, showing the type 2 and type 3 cells. mep 2, type 2 cell of midgut epithelium; mep 3, type 3 cell of midgut epithelium; nd, nidi. Longitudinal-section, stained with hematoxylin-triosin. Scale equals 50 µ.



Figure 5. The longitudinal-sections of the hindgut and the midgut of \underline{H} . virescense.

- A. Posterior interstitial ring. 1m, longitudinal muscle; mep, midgut epithelium; pir, posterior interstitial ring. Longitudinalsection, stained with hematoxylin-triosin. Scale equals 100 µ.
- B. Pylorus and part of the anterior ileum. ap, anterior pylorus; cm, circular muscle; il, ileum; lm, longitudinal muscle; mp, middle pylorus; pc, pyloric chamber; pp, posterior pylorus. Longitudinal-section, stained with hematoxylin-triosin. Scale equals 500 p.
- C. Posterior ileum and colon. cmc, circular muscle of colon; cmi, circular muscle of ileum; epc, epithelium of colon; epi, epithelium of ileum. Longitudinal-section, stained with hematoxylintriosin. Scale equals 500 µ.

D. Colon and rectum. ci, chitinous intima; cmc, circular muscle of colon, epc, epithelium of colon; epr, epithelium of rectum; mb, membrane; mt, malpighian tubule; sm, sheet-like muscle. Longitudinal-section, stained with hematoxylin-triosin. Scale equals 500 µ.



Figure 6. The cross-sections of the esophagus and the pylorus of <u>H</u>. <u>virescense</u>.

- A. Anterior pylorus, showing the irregular cuboidal cells and the thick muscle. cep, cuboidal epithelium; cm, circular muscle; lm, longitudinal muscle; tm, transverse muscle. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- B. Middle pylorus, which has an inner longitudinal muscle and outer circular muscle. The epithelium consists of both cuboidal and squamous cells. cep, cuboidal epithelium; cm, circular muscle; lm, longitudinal muscle; m, common ducts of malpighian tubules; sep, squamous epithelium. Cross-section, stained with hematoxylintriosin. Scale equals 250 µ.
- C. Posterior pylorus, which has only circular muscle. The epithelium consists of regular cuboidal cells. cep, cuboidal epithelium; cm, circular muscle; m, common ducts of malpighian tubules. Crosssection, stained with hematoxylin-triosin. Scale equals 250 u.
- D. Esophagus, showing the cuboidal epithelium, muscle and chitinous intima. cep, cuboidal epithelium; ci, chitinous intima; cm, circular muscle; dm, dilator muscle. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.



Figure 7. The cross-sections of the colon and rectum of H. virescense.

•

- A. Anterior part of the colon. cep, cuboidal epithelium; cm, circular muscle; dm, dilator muscle. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- B. Posterior part of the colon. cep, cuboidal epithelium; cm, circular muscle; dm, dilator muscle; 1, lumen. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- C. Rectal wall, showing the cryptonephridial system. ci, chitinous intima; mb, membrane; mt, malpighian tubule; sep, squamous epithelium; sm, sheet-like muscle. Cross-section, stained with hematoxylin-triosin. Scale equals 100 µ.
- D. Rectal epithelium, showing the branching nuclei. bn, branching nucleus; epr, epithelium of rectum; mt, malpighian tubule. Cross-section, stained with hematoxylin-triosin. Scale equals 100 µ.



Figure 8. The cross-sections of the pylorus and colon of Spodoptera frugiperda.

- A. Anterior pylorus, showing the highly complicated epithelial folds and the thick muscle layer. 1, gut lumen; ml, muscle layer; sep, squamous epithelium. Cross-section, stained with hematoxylintriosin. Scale equals 250 µ.
- B. Middle pylorus. cm, circular muscle; m, common ducts of malpighian tubules; sep, squamous epithelium; tm, transverse muscle. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- C. Posterior pylorus. cep, cuboidal epithelium, m, common duct of malpighian tubules; sep, squamous epithelium. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- D. Colon, showing the squamous epithelium and the restricted gut lumen. cm, circular muscle; dm, dilator muscle; l, gut lumen; sep, squamous epithelium. Cross-section, stained with hematoxylintriosin. Scale equals 250 µ.



Figure 9. The cross-sections of the pylorus and the colon of S. ornithogalli.

- A. Anterior pylorus. 1, lumen; ml, muscle layer; sep, squamous epithelium. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- B. Middle pylorus. ci, chitinous intima; cm, circular muscle; lm, longitudinal muscle; sep, squamous epithelium. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- C. Posterior pylorus. cep, cuboidal epithelium; cm, circular muscle; lm, longitudinal muscle. Cross-section, stained with hematoxylintriosin. Scale equals 250 µ.
- D. Colon. ci, chitinous intima; cm, circular muscle; sep, squamous epithelium. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.



Figure 10. The cross-sections of the ileum of <u>H</u>. <u>virescense</u>, <u>S</u>. <u>frugiperda</u> and <u>S</u>. <u>ornithogalli</u>.

- A. The ileum of <u>H</u>. <u>virescense</u>. ci, chitinous intima; cm, circular muscle; sep, squamous epithelium. Cross-section, stained with hematoxylin-triosin. Scale equals 250 μ.
- B. The ileum of <u>S</u>. <u>frugiperda</u>. ci, chitinous intima; cm, circular muscle; sep, squamous epithelium. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.
- C. The ileum of <u>S</u>. <u>ornithogalli</u>. ci, chitinous intima; cm, circular muscle; sep, squamous epithelium. Cross-section, stained with hematoxylin-triosin. Scale equals 250 µ.



VITA

Che Chi

Candidate for the Degree of

Master of Science

Thesis: THE MORPHOLOGY AND HISTOLOGY OF THE LARVAL DIGESTIVE SYSTEM OF <u>HELIOTHIS VIRESCENSE</u> (FABR.), <u>HELIOTHIS ZEA</u> (BODDIE), <u>SPODOPTERA FRUGIPERDA</u> (J. E. SMITH), AND <u>SPODOPTERA ORNITHOGALLI</u> (GUENEE). (LEPIDOPTERA, NOCTUIDAE).

Major Field: Entomology

Biographical:

Personal Data: Born in Peiking, China, February 6, 1949, the daughter of Mr. and Mrs. Yu-Ming Chi.

Education: Graduated from Provincial Taichung Girls' High School, Taichung, Taiwan, Republic of China, in 1966; received a Bachelor of Science degree from National Taiwan University in June, 1970; completed requirements for Master of Science degree in July, 1972.

Professional Experience: Research assistant in Department of Entomology, Oklahoma State University from January, 1971 to May, 1972.