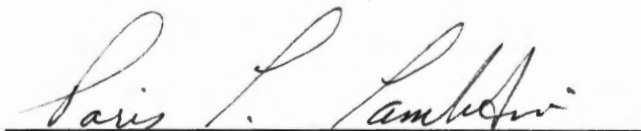


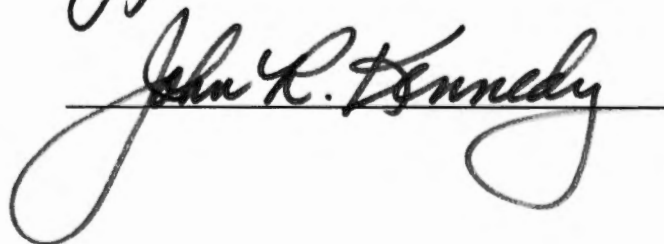
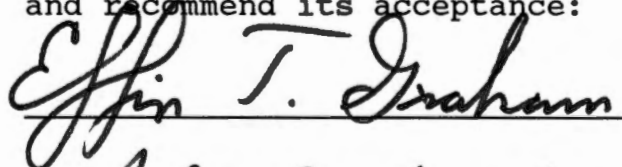
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COMPARATIVE ANATOMY OF THE DIGESTIVE TRACT BETWEEN
THREE SPECIES OF SCALE INSECTS (HOMOPTERA:COCCOIDEA)

A Thesis
Presented For The
Master of Science
Degree
The University of Tennessee, Knoxville

Robert Carleton Brown

May 1991

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ABSTRACT

This research attempted to examine and compare various areas of the internal anatomy in scale insects for relationship studies. Three major aspects of internal anatomy were examined: the digestive tract in three species of female scale insects, the cephalic region of males in two species of scale insects and the blood cell types in the female of a mealybug, Phenacoccus gossypii Townsend and Cockerell.

Species were collected and processed for histological microtechnique, examined with a brightfield light microscope and characters measured. Some of the specimens were examined with a computerized image analysis system coupled to a microscope. Comparative analysis of measurements was subjected to a non-pooled Students T-test for significance.

Comparisons between the females revealed significant differences in 5 of the 18 areas studied. Comparisons between the two male species revealed no significant differences, even though there was morphological contrast. Examination of the blood in the Mexican mealybug produced four blood cell types.

Results of this study revealed evidence that there is a gradient of characters, generalized in the Mexican mealybug through the Tuliptree scale and specialized by the Euonymous scale, based on anatomy of the digestive tract in the females. The study in the males produced similar results. Hemolymph in the Mexican mealybug revealed a generalized cell type pattern similar to other members of the same order.

TABLE OF CONTENTS

PART I: COMPARATIVE ANATOMY OF THE DIGESTIVE TRACT BETWEEN THREE SPECIES OF SCALE INSECTS (HOMOPTERA: COCCOIDEA) .

CHAPTER	PAGE
1. INTRODUCTION	1
2. MATERIALS AND METHODS	9
3. RESULTS	17
4. CONCLUSION	44
5. LITERATURE CITED	63

PART II: COMPARATIVE ANATOMY OF THE CEPHALIC REGION BETWEEN TWO SPECIES OF MALE SCALE INSECTS (HOMOPTERA: COCCOIDEA)

1. INTRODUCTION	49
2. MATERIALS AND METHODS	50
3. RESULTS	53
4. LITERATURE CITED	63

PART III: HEMOCYTE TYPES FOUND IN THE HEMOLYMPH OF
PHENACOCCLUS GOSSYPII TOWNSEND AND COCKERELL

CHAPTER	PAGE
1. INTRODUCTION	75
2. MATERIALS AND METHODS	78
3. RESULTS	80
4. CONCLUSION	83
LITERATURE CITED	85
VITA	88

LIST OF TABLES

TABLE

PAGE

**PART I: COMPARATIVE ANATOMY OF THE DIGESTIVE
TRACT BETWEEN THREE SPECIES OF SCALE INSECTS
(HOMOPTERA: COCCOIDEA)**

1. Mean Lengths and Widths for Digestive Tract45
2. Variation of Level Significance for Means46

**PART II: COMPARATIVE ANATOMY OF THE CEPHALIC REGION
BETWEEN TWO SPECIES OF MALE SCALE INSECTS (HOMOPTERA:
COCCOIDEA)**

1. Mean Lengths and Widths of Cephalic Region53

20. Pseudovitellus of Mexican Mealybug	34
21. Pseudovitellus and Follicles of Euonymous Scale	35
22. Malpighian Tubules of Mexican Mealybug	37
23. Brushborder of Malpighian Tubules of Mexican Mealybug	38
24. Malpighian Tubules of Tuliptree Scale	39
25. Fat Body of Mexican Mealybug	40
26. Adipose Tissue of Mexican Mealybug	41
27. Fat Body of Tuliptree Scale	42
28. Fat Body of Euonymous Scale	43

**PART II: COMPARATIVE ANATOMY OF THE CEPHALIC REGION
BETWEEN TWO SPECIES OF MALE SCALE INSECTS (HOMOPTERA:
COCCOIDEA)**

1. Cephalic Region of Mexican Mealybug	52
2. Cephalic Region of Euonymous Scale	52
3. Cephalic Ganglion of Mexican Mealybug	56
4. Cephalic Ganglion of Euonymous Scale	56
5. Antennae of Mexican Mealybug	57
6. Antennae of Euonymous Scale	58
7. Ocelli of Mexican Mealybug	59
8. Ocelli of Euonymous Scale	59

**PART III: HEMOCYTE TYPES FOUND IN THE HEMOLYMPH OF
PHENACOCCUS GOSSYPII TOWNSEND AND COCKERELL**

1. Prohemocyte of Mexican Mealybug	80
2. Plasmatocyte of Mexican Mealybug	81
3. Granulocyte of Mxican Mealybug	82
4. Spherulocyte of Mexican Mealybug	82

**PART I: COMPARATIVE ANATOMY OF THE DIGESTIVE TRACT
BETWEEN THREE SPECIES OF SCALE INSECTS (HOMOPTERA: COCCOIDEA)**

CHAPTER 1

INTRODUCTION

Insects in the superfamily Coccoidea (Homoptera) have been traditionally classified based on external morphology of the female. This reliance on female morphology stems from lack of any males in parthenogenetic species, the ephemeral nature of the males in heterosexual species, and the ease with which the females can be studied (Miller and Kosztarab 1979). Past work on internal anatomy primarily concentrated on description of organs and tissues of females (Balachowsky 1937). Research concentrating on the integumentary glands proved to be the most popular aspect. Dufour (1933) provided the basic framework for future work by studying gland types in members of the superfamily. This process was continued by Mayer (1892) and Berlese (1893, 1894, 1895) to expand the number of species and families examined. Visart (1894) did comparative studies on the integumentary glands of Dactylopius citri Risso and a species of Ceroplastes. In 1895, he conducted a study of the comparative structure of these glands between different orders of insects which included Dactylopius. The timely advent of improved techniques in histology during the early 1900's resulted in an increase in the amount of work in this area. Teodoro (1911a, 1911b, 1913, 1915) conducted studies on species of coccids with emphasis on

the glands and their secretions. Herberg (1918, 1922) described the structure of integumentary glands in Eriopeltis lichensteini Signoret, and reviewed the tracheal system in coccids. The use of improved techniques brought about work establishing a classification system for gland types and their respective secretions (Matheson 1923; Ferris 1928; Marshall 1929, 1930, 1935; Misra 1939, 1942). The mechanisms involved in wax secretion was determined for Icerya purchasi Maskell (Murdock 1928) and Quadraspidiotus perniciosus (Comstock) (Disselkamp 1954). Benassy (1961) provided a review on secretions of coccids. In 1967, Gianotti reported results on observations of the cellular formations by dorsal gland cells of the Black scale, Saissetia oleae (Bernard). The composition of scale insect honeydew was studied by Salama and Risk (1969). Later, Hague (1975a, 1975b) examined secretory cells of the Lac insect by electron microscopy. Subsequent use of the electron microscope fostered research into the ultrastructure of cuticular glands implicated in the secretion of waxes of the outer test (Foldi 1978, 1981, 1983, 1984, 1986; Gerini 1980; Waku and Foldi 1984; Foldi and Cassier 1985; Yasuda 1979; Young and Yao 1987 and Pesson and Foldi 1978). Moreno and Fargerland (1975) examined the ultrastructure of sex pheromone gland cells in the yellow scale, Aonidiella citrina (Coquillett).

Lubbock (1858), an early investigator of the alimentary canal, studied the system in the brown scale, Coccus

hesperidum Linneaus. Berlese (1893, 1894, 1895, 1895b, 1896) conducted an extensive examination of several scale insect families that included the alimentary canal. The histology and organology of the digestive tract was examined through the early 1900's (Teodoro 1911a; Childs 1914; Hough 1925b; Bochenska 1939; Pesson 1934, 1935, 1936, 1942, 1944; Colton 1944). Bielenin (1963, 1975) and Bielenin and Weglarska (1973) conducted a series of investigations on the post-embryonic development of female digestive system in Lecanium pomeranicum Kaweckii. and Q. ostreaeformis (Curtis). Also, an examination of the digestive tract was included in studies on the internal anatomy in Centrococcus insolitus Green by Srivastava and Sinha (1966b), and Yadava and Chandel (1968, 1969) for Saccharicoccus sacchari (Cockerell). Foldi (1973) examined the ultrastructure of the filter chamber for the mealybug, Planococcus citri (Risso).

Other aspects of internal anatomy in coccids were investigated that provided information on various organ systems or tissues (Leydig 1854; Claus 1860, 1864; List 1886; Berlese 1893, 1894, 1895, 1896; Kuwana 1907). Schmidt (1883) studied developmental anatomy in female Aspidiotus nerii Bouche, and Kreckler (1909) that of the eyes in male D. destructor. Johnston (1912) reported the results of research on internal anatomy of I. purchasi. Marshall (1935, 1935a) conducted a series of studies of the sense organs in S. oleae, Pflugfelder (1936) studied the ocelli in L. corni Bouche,

Poisson and Pesson (1938) made one of the few examinations of the circulatory system of scale insects in a species of Pulvinaria and Bochenska (1939) the anatomy in the first instar nymphs of the Polish cochineal, Porphyrophora polonica (Linnaeus). Sperm and the intricacies of meiotic systems in scale insects was investigated by Douthett (1949, 1952), Harris and Robinson (1973), Berlowitz in 1974 and Nur (1970, 1971, 1972). Endosymbiotes associated with scale insects have generated an increasing amount of interest among workers (Louis 1969, 1970; Iaccarino and Tremblay 1970; Houk and Griffiths 1980; Tremblay and Tripodi 1980). Examination of the reproductive system was conducted by several researchers (Colton 1944; Delavault and Royer 1966; Karenago and Okudai 1970; Ksinzkiewicz 1980; and Akopian and Magakian 1985). However, few attempts were made to examine the internal anatomy of representatives from different families. Thus, the objective of this study was to compare the internal anatomy between members of three families of scale insects. The alimentary canal of three different species was selected as a target organ system for study, as well as the cephalic region of the males in two species, the euonymous scale and the Mexican mealybug, and a study of the blood cell types in the female Mexican mealybug. The decision to use this organ/tissue system was made because the type of alimentary canal in the Homoptera is commonly retained by all its members and unique among insects in general, the head region for male

scale insects is important for mate selection and the study of hemocyte types aids in identification of tissue types in histological sections.

Hemipteroid insects differ from insects of other orders by possessing stylets formed by fusion of the maxilla and mandibles used to penetrate plant surfaces to suck the sap from phloem tissue (Weber 1930). Stylets of the Homoptera differ from those of the Hemiptera by being produced from beneath the head (Weber 1930). In Hemiptera, the stylet arises from the anterior aspect of the head (Snodgrass 1935). Internally, scale insects and other Homoptera possess an alimentary canal composed of a foregut with salivary glands, a midgut with a filter chamber, the hindgut with large proctodaeum, rectal area and the Malpighian tubules (Balachowsky 1937). Such a digestive system enables these insects to dispose of excess fluid which they procure in quantity (Foldi 1973). In this way, the fluid in the midgut is concentrated with sugar; thus the "honeydew" associated with members of the Homoptera. This fluid is rich in polysaccharides and nitrogenous compounds (Adamako 1972). Honeydew forms a substrate for fungi that cause black sooty mold on scale insect-infested plants. The establishment of the mold on the surface of the plant inhibits photosynthesis and may lead to stress or death.

The filter chamber is one of the structures of the alimentary canal that separates the Homoptera from other

insects (Wigglesworth 1950). No other structure like it exists in the insect realm. There is some variation in form and function among the Coccoidea (Snodgrass 1935).

General External Morphology of P. gossypii, T. liriiodendri, and U. euonymi:

Adult females of the Mexican mealybug, Phenacoccus gossypii Townsend and Cockerell, were oval, 2.50 mm-3.75 mm in length and 1.40 mm-2.10 mm in width (McKenzie 1967). These insects were covered with a thin "mealy" wax material. Specimens possessed four supramarginal openings on the dorsal surface, the ostioles, which function to secrete honeydew. Ventrally, the legs, antennae, eyes, vulva and rectum were well-developed and a prominent structure, the circulus, was located in middle of the second abdominal segment. The circulus aided in the adhesion of the insect to its substrate by secreting a substance that acted as a glue (McKenzie 1967). The surface of the insect possessed numerous minute pores that secrete the waxy filaments that impart the "mealy" consistency characteristic for this group of insects.

These insects had high reproductive rates, often with several generations together on the same plant. This species was often regarded as a common indoor pest. Occasionally these insects can become pests of outdoor crops (McKenzie 1967).

Adult females of the tuliptree scale, Toumeyella liriodendri (Gmelin), were 7.5 mm in length, 5.9 mm in width (Simpson and Lambdin 1983) possessed a convex, oval and reddish-brown, mottled pattern "test". Quinquelocular and other pores of the dorsal surface were involved in the secretion of the hard waxy material of the outer covering. Ventrally, the legs and antennae were reduced and the eyes lost. There was also a pair of deltoid anal plates on each anal lobe surrounding the anal opening. These insects possessed a high reproductive rate with eggs produced in August and September (Burns and Donley 1970, Simpson and Lambdin 1983). Several species of ants were known to be associated with this scale insect that were known to harbor and benefit from the coccids (Burns 1964, Simpson and Lambdin 1983). This insect was known occasionally as a pest of certain trees of the Magnoliaceae, particularly the Tuliptree, Liriodendron tulipiferae (Linnaeus).

Adult females of the euonymous scale, Unaspis euonymi (Comstock), were 0.5-1 mm in length and 0.2-0.4 mm in width (Dekle 1965). The test was dark brown and tended to be larger at the posterior end due to the deposition of wax and exuviae over time which appeared oyster-shell shaped (Dekle 1965). The eyes and legs were completely lost and the antennae reduced to one segment. Ventrally, prominent mouthparts are visible. Dorsally, the dorsal ducts and perivulvar pores are implicated in the secretion of waxes for the test and

protection of the eggs. Adult females became sedentary and could only move in their immature "crawler" stage. This insect was a primary pest of the golden euonymous ornamental bush, Euonymous japonicus Linnaeus (Dekle 1965).

CHAPTER 2

MATERIALS AND METHODS

Rearing Procedures: In 1988, three species of scale insects, P. gossypi, T. liriodendri, and U. euonymi were obtained from greenhouse or other host sources grown on the University of Tennessee Agricultural campus, Knoxville, TN. Hosts for the specimens were Coleus sp., for the Mexican mealybug, E. japonicus, for the Euonymous scale (both raised in a greenhouse), and L. tulipifera for the Tuliptree scale. The Tuliptree scale was obtained from hosts located at the tree plantation of the U.T. agricultural farm.

Specimen Preparation: Ten Coleus sp. plants were obtained from seeds sown in 18 X 40 cm flats. From these ten 3 cm plants of Coleus sp. plants were potted in a 50% peat/ 50% soil mixture in 10 cm diameter pots these plants were placed into two wood frame and nylon mesh cages (Figure 1). Once the plants reached 11 cm in height the insects were transferred onto these plants by microdissection spatula. Euonymous plants were obtained from clippings and grown under identical circumstances as for the Mexican mealybug with the exception that caging the plants was not required. Ten adult female Mexican mealybugs were removed from sources in the greenhouse by microdissection spatula, placed in a specimen vial and taken to the laboratory for identification. Identification of

the Mexican mealybug was made based on criteria established by McKenzie (1967) for live and slide-mounted specimens. Once identified the specimens were placed on isolated plants grown in cages (Figure 1). Five female mealybugs were placed onto host plants in cages by prodding the insects off the plants and directly transferring them by microdissection spatula to caged Coleus sp. plants. Through July the insects were monitored daily to insure that the specimens had established themselves on the plants and to note their rate of development. By August, there were sufficient numbers on these plants to transfer to plants outside the cages. The population size was considered sufficient for transfer when first instar "crawlers" were abundant. They were transferred

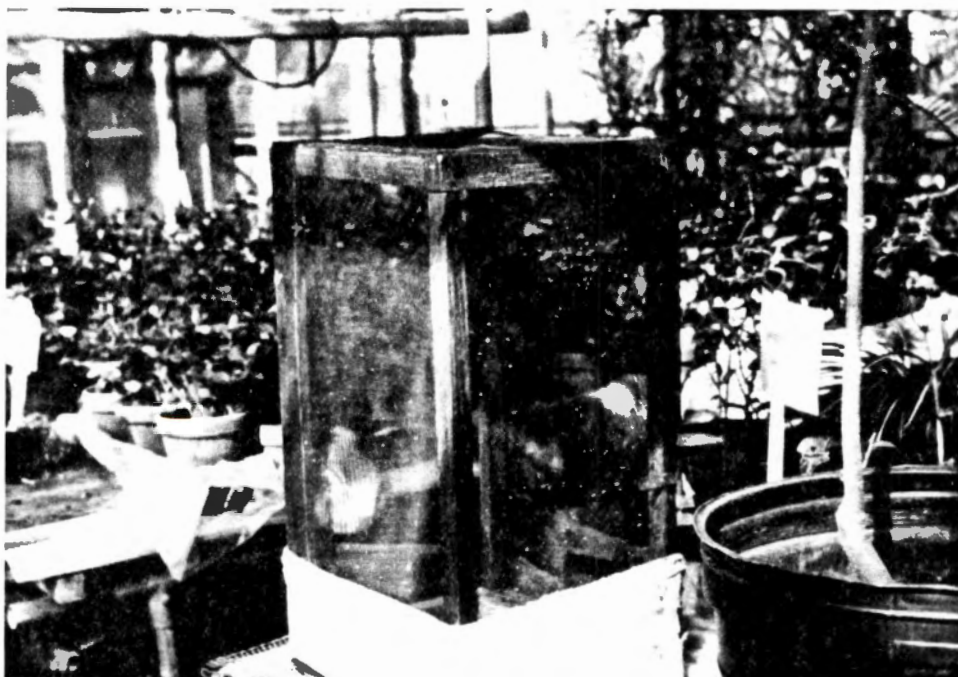


Figure 1. Colony of Caged Mealybugs. Cage is the type used to isolate the initial colony of P. gossypii.

outside the cages by taking the infested plants from the cages and placing them among uninfested plants in pots in flats. The uninfested Coleus sp. plants were grown in pots on flats to aid the spread of the crawlers. The plant stands averaged 11 plants per flat in small 10 cm diameter pots. Insect population sufficient for use were available by the beginning of the Fall semester. Plants for the study were grown from seed or from clippings, but were not allowed to flower to maintain their numbers.

Tuliptree scale specimens were obtained from sources outside the greenhouse on the U.T. Agricultural campus. These specimens were removed from clipped branches as encountered from April through August 1990. Adult females were most abundant during this period (Simpson and Lambdin 1983). Densities for this insect averaged 3 from a single twig or branch/tree. There was recovered only 24 specimens out of 100 trees examined. Late in the season, predation by the pyralid moth, Laetilia coccidivora (Comstock) was observed resulting in ca. 50% reduction in population number. Infested branches were brought into the laboratory and the insects gently teased-off by microdissection spatula and placed into a fixative and processed for histological microtechnique.

Specimens of the Euonymous scale were obtained from 4 cm long clippings of infested twigs and branches of their host plant, golden euonymous, E. japonicus. Clippings of infested and uninfested plants were grown in a 50% peat/ 50% soil

mixture. Plant densities were similar to Coleus sp. (Figure 2). Once a sufficient insect population was available plant leaves were taken into the laboratory where insects and leaf tissue were processed for histology. The leaf immediately around the insect was cut and placed into the fixative.



Figure 2. Flats of Euonymus Scale on Host Plants. Figure shows a flat of E. japonicus plants infestated by Euonymous scale.

Tissue Preparation: Histological techniques used were standard protocols of preparation through to paraffin embedding except a phosphate-buffered solution of 2.5% glutaraldehyde/ 2% formaldehyde, pH. 7.2, was used as the fixative (Hayat 1986, Humason 1979). Specimens were fixed for 3-4 hours under vacuum to allow complete penetration of the fixative through the hemocoel and tissues. This was accomplished by placing them under a vacuum bell fitted to an aspirator in a sink (Figure 3). The fixative was removed by Pasteur pipette and tissue rinsed three times in a phosphate buffer, pH 7.2.

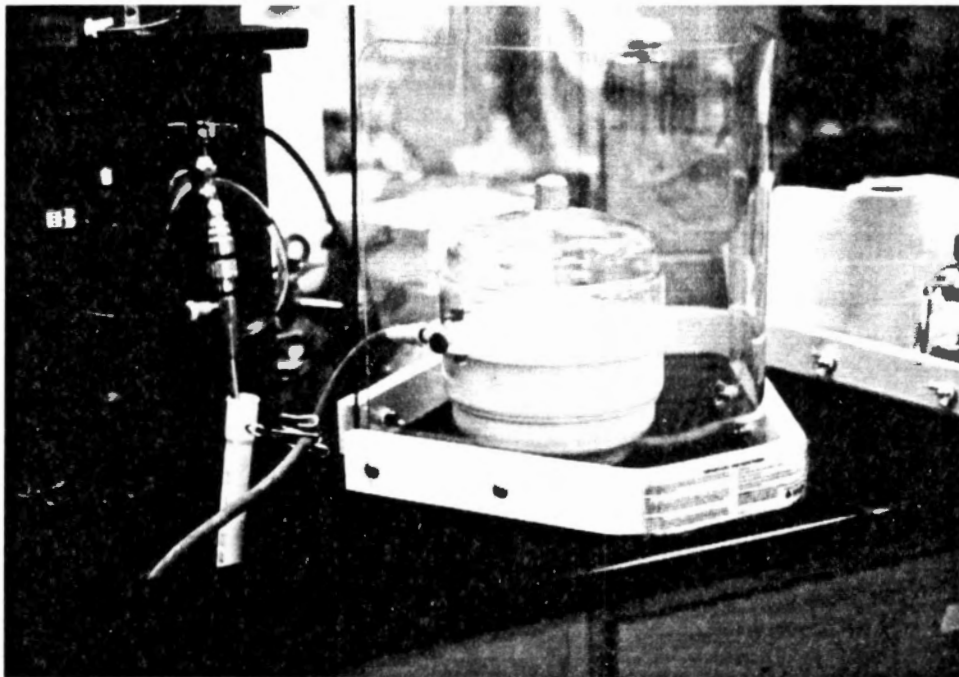


Figure 3. Aspirator Used in the Tissue Preparation. Infiltration of the fixative into the hemocoel and tissues of the specimens was accomplished when vials with the specimens were placed into the chamber (right) from vacuum produced with the fixture in the sink (left).

Dehydration through a graded alcohol series from 30% to absolute 2-propanol was made with a Pasteur pipette at 15 minute intervals. Specimens were then transferred through three washes of a 50% tertiary butanol/ 50% ethanol solution (Johansen 1939) for 15 minutes each after which they were placed overnight into liquid paraffin in an oven at 40° C. They were then removed from the liquified paraffin with an applicator stick and placed into metal molds, oriented, and allowed to solidify on a cooling plate. After removal from the molds, the blocks were trimmed and placed onto a microtome for sectioning. The specimens were sectioned on a LKB rotary, retracting microtome at 10 μ , placed on standard microscope slides and processed for Gill's hematoxylin (Gill et al. 1974) and eosin staining (Humason 1979). This protocol involved running the slides through a Histoclear series to dissolve the paraffin, rehydration through a graded series of alcohols from absolute ethanol to water, and staining with hematoxylin primary stain. Slides were then washed with tap water and stained in an eosin counter-stain for 20 minutes and then washed in 20 % ethanol. They were subsequently taken through a graded ethanol series from 30%-absolute ethanol for dehydration, transferred through three washes of Histoclear, mounted and placed on a slide warmer overnight to dry. The slides were then examined under oil immersion with a Leitz Laborlux D microscope.

Examination was augmented by the use of an image analysis

system that digitized the image from a microscope with a CCD camera. This camera converted the image from analogue to digital through an array of light sensitive semiconductor elements. The elements stored the charge collected by incoming photons from the microscope and transferred them to frame buffers through an analogue-to-digital converter. The frame buffer stored the image and processed it for the correct format to display on a monitor. The image was transferred to a monitor through a computer interface resulting in a black-and-white representation composed of a composite of 255 shades of gray. Comparing the black-and-white image with a "gray scale" index, also displayed on the screen, allowed for the establishment of particular thresholds for optical density of the specimen. Optical density was a function of the amount of light that entered the CCD camera making dense objects darker than those objects in the image that were less dense and allowed more light to continue through. The program allowed for the substitution of the gray image with "Pseudocolors" so that the observer could customize the shades of gray to a particular color. The image was enhanced by several means. Brightness and contrast was adjusted so that sharpness of the image was enhanced. A histogram of the images light intensity and shades of gray with the number of pixels that were receiving light of that particular shade established the optical density of the specimen. The histogram was also a way to measure the extremes of light, eg. too dark or light, and

a guide to modifying it for the best image. Adjustment was accomplished by adding or subtracting qualities (out of 225) for either the brightness or the contrast.

Experimental Design: The length and width of the digestive tract was measured in each of the species and compared. The areas examined were: foregut, midgut, filter chamber, hindgut and rectum. The quantitative analysis of the internal anatomy was obtained by using the image analysis system and measurements from an ocular micrometer on a microscope. Qualitative observations of tissue composition was made by direct observations from a microscope. Criteria based on standard light absorption of hematoxylin and eosin by tissue was used as the basis for descriptive analysis and involved description of the color of the cytoplasmic matrix and nucleus, positions of the nucleus within the cell, texture of the cytoplasm, the presence of specialized structures, eg. microvilli, large droplets within the cytoplasm, etc..

Measurements were made through the imaging system and saved to files. Measurements were set up as a completely randomized design (CRD) and subjected to a non-paired Student's T-test. There were 30 replicates per treatment, with the exception for T. liriiodendri which had 15.

CHAPTER 3

RESULTS

I. Gross Internal Morphology.

A. The Digestive Tract.

The stylet of the Mexican mealybug was twice the length of its body. The mouthparts originated in the head region between the forelegs. The deltoid mouthparts were composed of a rostrum, or beak, in front of a large plate-like structure, the clypeolabial shield. The rostrum was composed of three sclerotized segments. Internally, these structures were lined by a layer of epithelial cells, the hypodermis. The rostrum surrounded two neural processes from the protocerebrum to the subesophageal ganglion. Located lateral to the rings of the rostrum lie ducts of the salivary glands (Figure 4). There were a pair of these glands, both composed of an acinar gland with four to five cells connected to a common duct. The cells were large (30 μ), stained darkly, and grouped closely together. The nuclei were produced into squat forms at the basal region of the cells. The ducts lead to the pharyngeal region. From here, the foregut widened its breadth, with many large basal cells that produced villi that projected into the lumen (Figure 5). This region stained

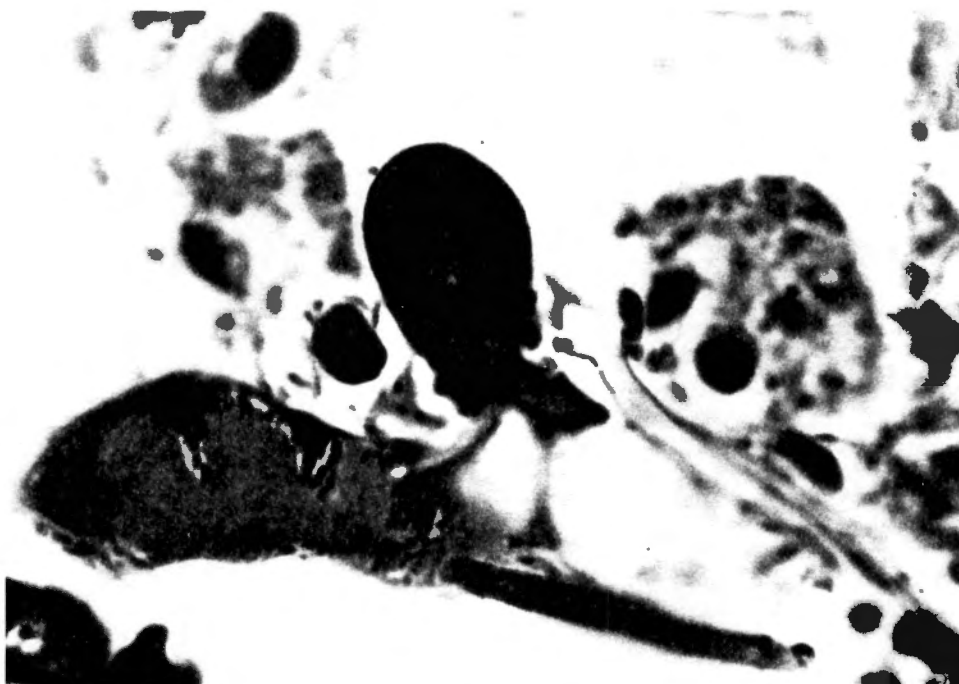


Figure 4. Salivary Glands of Mexican Mealybug. The salivary glands (dark body, center) are proximal to subesophageal ganglion (bottom left) (600 x).

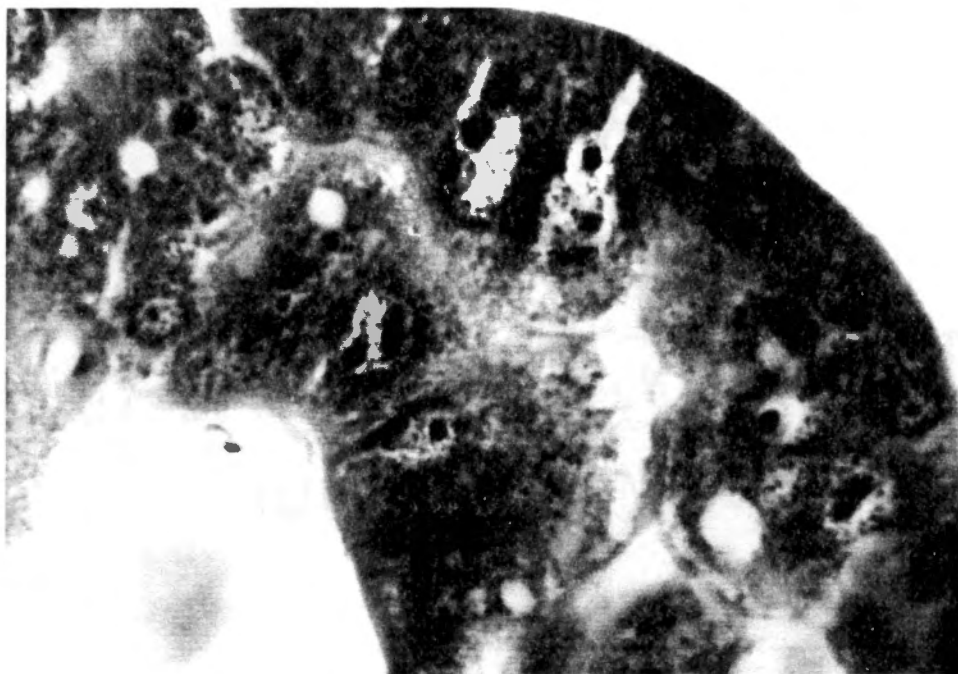


Figure 5. Foregut of Mexican Mealybug. Light micrograph (600 x)

darkly, the cells of which possessed prominent nuclei that were round, obvious nucleoli (Figure 5). The lumen was thin and squeezed into lateral crypts by the villus-like processes. There was no brushborder in this region. The foregut was surrounded by a mesothelial sheath contiguous with the outer cuticle. The breadth of this tube widened until eventually forced to constrict at the juncture with the filter chamber. The filter chamber was elliptical to oval in shape and quite complex in morphology (Figure 6).



Figure 6. The Filter Chamber of Mexican Mealybug. The filter chamber was surrounded by squamous epithelium and cuticle while the cortex was composed of cuboidal cells. Cuticle also invaginated the cortex (600 x).

It appeared to be a self-contained system of anastomosing small tubes. The tubes were composed of the same cuticular sheath as that of the outer mesothelial sheath of the foregut (Figure 6). There were many large cells that made up the central region of this organ. In addition, cells were located at the outer fringes of the organ that were squat and cuboidal in shape. The cells that made up this organ stained less well than those of the foregut and did not produce villus-like processes. The nuclei were prominent with heterochromatin and two nucleoli.

An anastomosing tubule system of the interior extended directly to the rectum (Figure 7). This tubule system was small, dark-staining, and ran the length of the abdominal region to the rectum. The midgut joined the filter chamber at a constricted orifice similar to that which linked the foregut to the same organ. This tube system was larger than the foregut and possessed numerous villi and cells (Figure 8). These cells stained similar to those of the foregut, but were larger and dikaryotic. The nuclear composition was light with prominent nucleoli. The lumen of the midgut was wider and more tube-like than the foregut. A brushborder appeared on the apical surface of the cells along the lumen in this region of the alimentary tract (Figure 8). This section of the digestive system was the longest and extended through the hemocoel in a serpentine manner. The midgut eventually merged with the hindgut at a juncture below the filter chamber. The

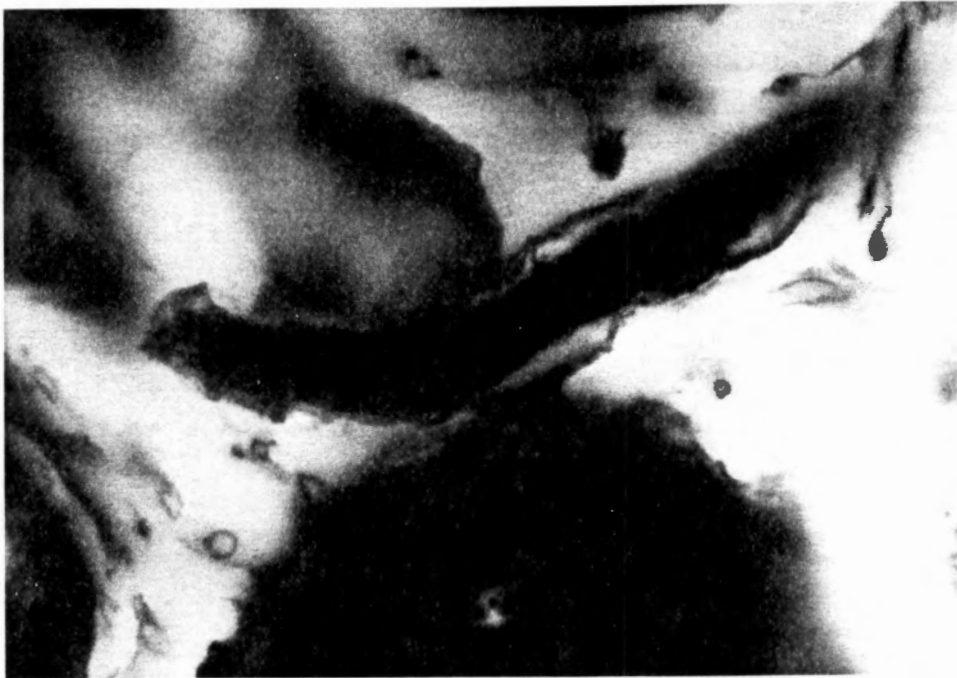


Figure 7. The Tubule System of the Mexican Mealybug. The tubule system (center) bypasses the midgut (bottom) (600 X).

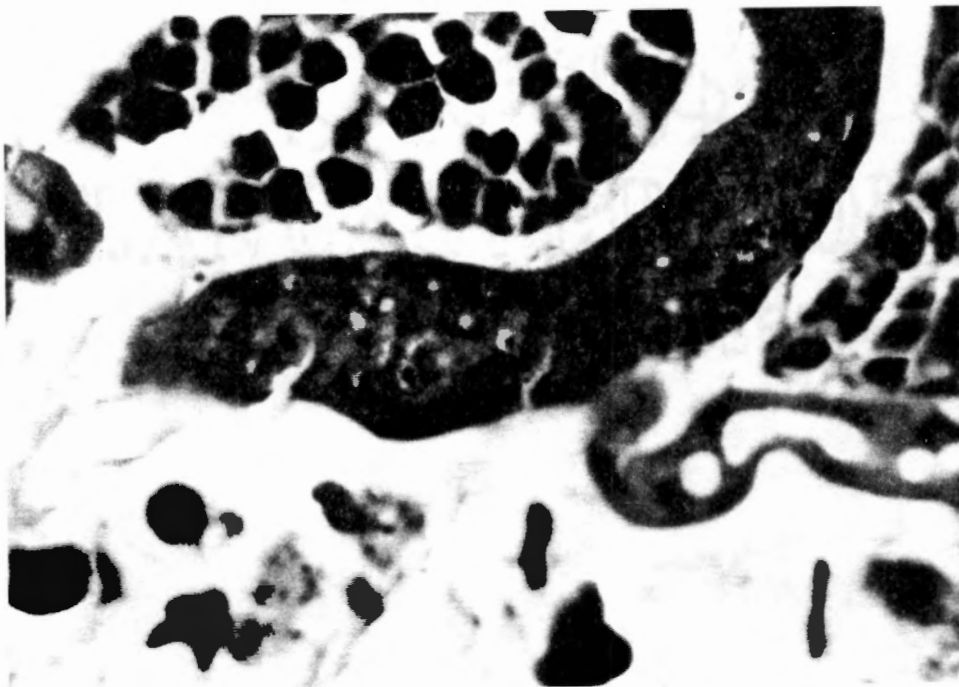


Figure 8. Midgut of the Mexican mealybug. The midgut (center) proceeds through the hemocoel in a serpentine manner (400 x)

two Malpighian tubules also arose at this point at very small constrictions. The hindgut (Figure 9) was essentially a cuticular tube with few cells. It occupied the majority of the ventral interior hemocoel to the rectum. The cells of the rectum stained darkly with Gill's hematoxylin and formed a papilliform network. There was a cuticular sheath that enveloped the outer mesothelial layer of this organ. The point of contact with the outer orifice was also lined with cuticle, surrounded by a complex of glands believed to secrete wax.

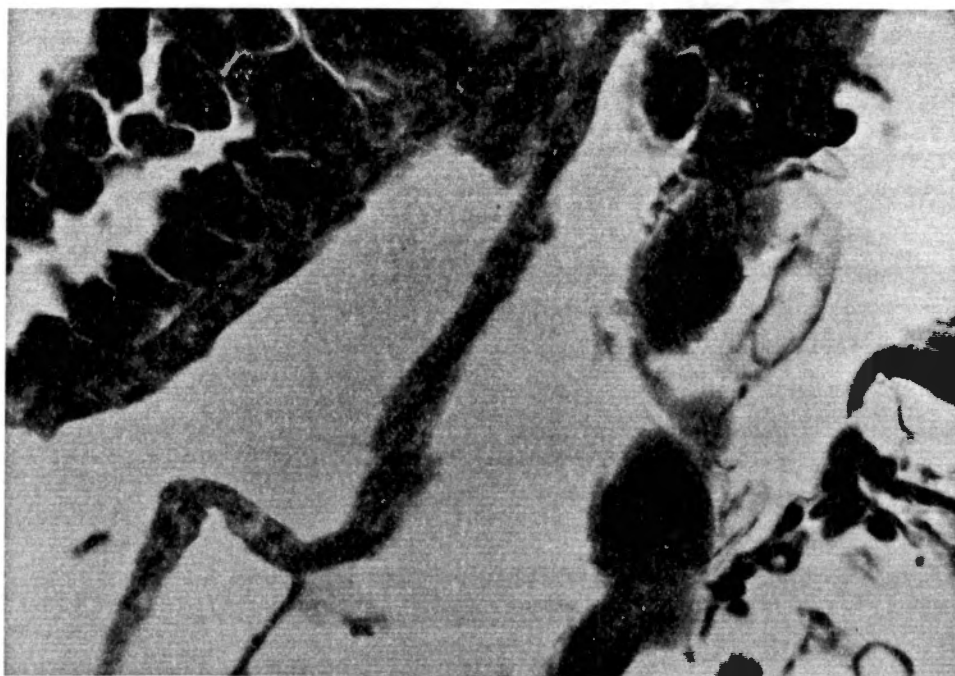


Fig. 9 The Hindgut of the Mexican Mealybug. The hindgut is basically a cuticular structure with a thin squamous epithelium lining its outer layer (400 x).

The labrum and clypeus made up the clypeolabial shield of the Tuliptree scale located at the cephalothorax between the forelegs (Williams and Kosztarab 1972). It was the exterior-most sclerotized segmentation of the outer mouthparts. The pharynx and foregut were joined at the interior base of this structure at apodemes and by skeletal muscle. The ducts of the salivary glands joined the pharynx at the medial aspect between the clypeolabial shield. The two salivary glands were located at the base between the cephalic and subesophageal ganglia. These glands were acinar in shape, dark staining, and composed of 5 cells located at the distal end of the organ. The nuclei were dark and distended at the basal area of the cells. The foregut (Figure 10) was made up of basophilic cells with prominent euchromatic nuclei. Many of the cells were dikaryotic. The lumen was large and cryptic. The mesothelial sheath was composed of cuticle intimately associated with the basal aspects of foregut cells. The juncture of the foregut and midgut occurred at a constricted tubule in which the cuticle of the mesothelia ended and was replaced by thin muscle tissue. Cells of the midgut were basophilic, large with prominent nuclei and possessed a well-defined brushborder along the lumen. Many of the cells were dikaryotic. The lumen was less well-defined than that of the foregut, smaller and more cryptic. The filter chamber (Figure 11) was a large elliptic organ intimately associated with the rectum.

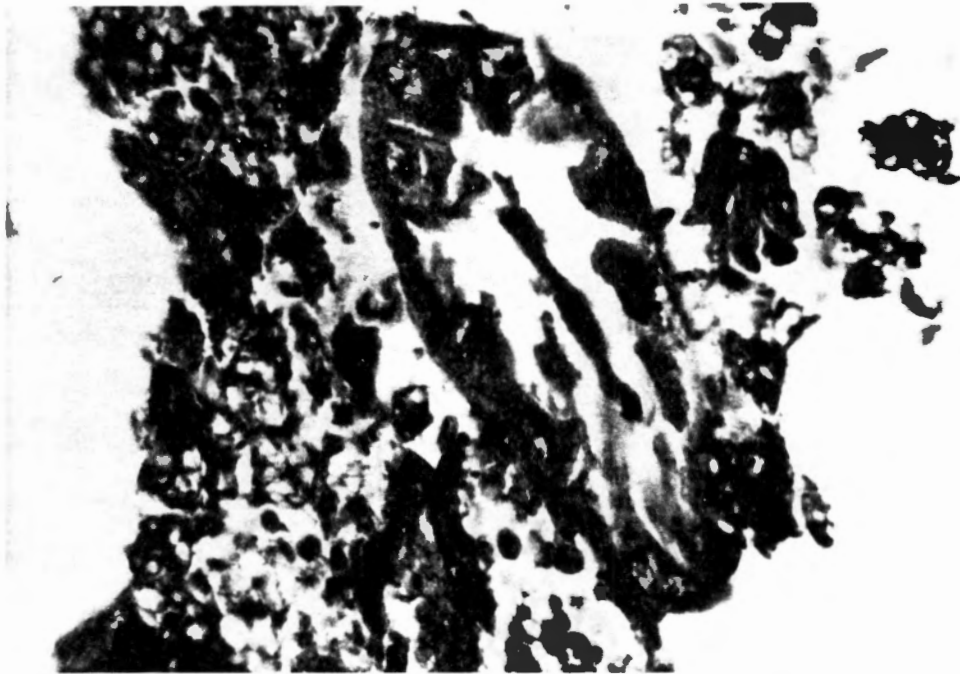


Figure 10. Foregut of Tuliptree Scale. The foregut is the oval structure (center right) (400 x).



Figure 11. Filter Chamber of Tuliptree Scale. The filter chamber is a large, elliptic organ (400 x).

The tubule system within the filter chamber did not anastomose as did the tubule system for the Mexican mealybug, but the tubules were much larger. The hindgut (Figure 12) was composed of a thin cuticular sheath on the luminal side with thin, squamous epithelia on the mesothelial side. The hindgut led to the rectum which was located on the ventral posterior aspect of the 8th abdominal segment.



Figure 12. The Hindgut of the Tuliptree Scale. The hindgut is center (open space) of the micrograph (400 x).

The alimentary canal of the *Euonymus* scale was composed of two major organ systems; the foregut and the hindgut. Linked and intercalated between these organs were the two Malpighian tubules. Externally, the segments of the rostrum and labium possessed the same sclerites as those of the Mexican mealybug and the Tuliptree scale. Internally, the rostrum was linked at the cuticle by a short tube that functioned as a flange for the attachment of the stylet to the vascular pump and the salivary glands in the pharynx. The internal apodemes of the rostrum were attached by muscle and connective tissue. The salivary glands were an amorphous mass of dense, basophilic cells with a duct system attached to the base of the esophagus. The esophagus widened abruptly into the foregut. The foregut (Figure 13) was essentially a large, round organ that ended as a blind sac. The cells that made up this organ were large, stained lightly with eosin and lacked a well-defined cell apex facing the lumen. The nuclei were large, light staining and euchromatophilic with two distinctive nucleoli. There was no evidence of dikaryotic cells in this organ. Proximal to the posterior-most mesothelial surface of the stomach was a ligament that attached this organ to the Malpighian tubules. The hindgut was a linear tube with cuboidal cells on its outer surface and a cuticular lumen (Figure 14). The hindgut was connected to the proximal area of the Malpighian tubules. The hindgut led to an orifice located on the eighth abdominal segment.

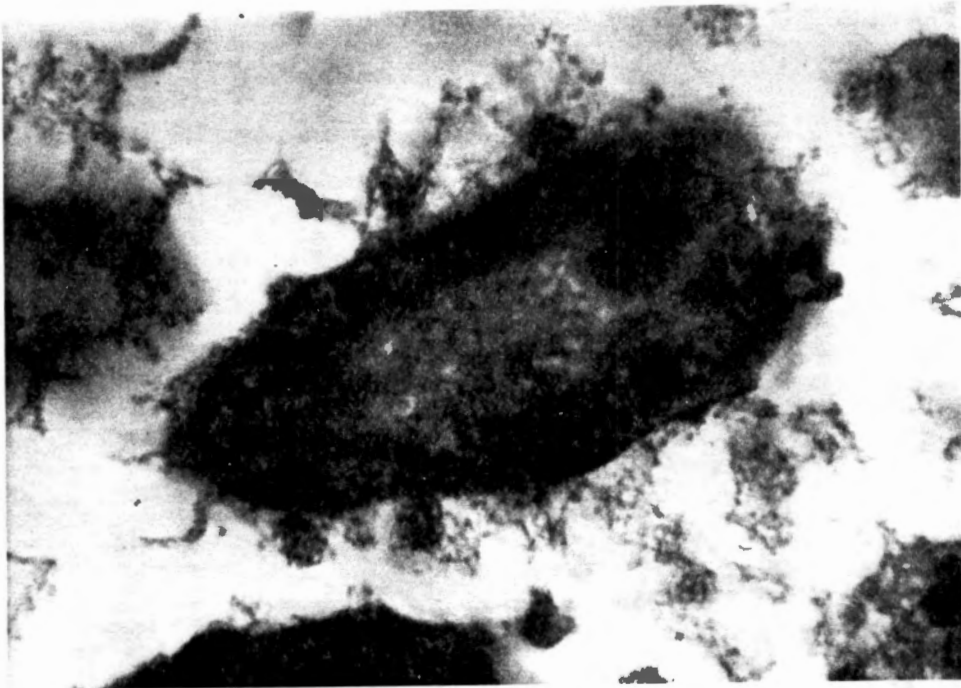


Figure 13. Foregut of Euonymous scale. The foregut (center) (1000 x).

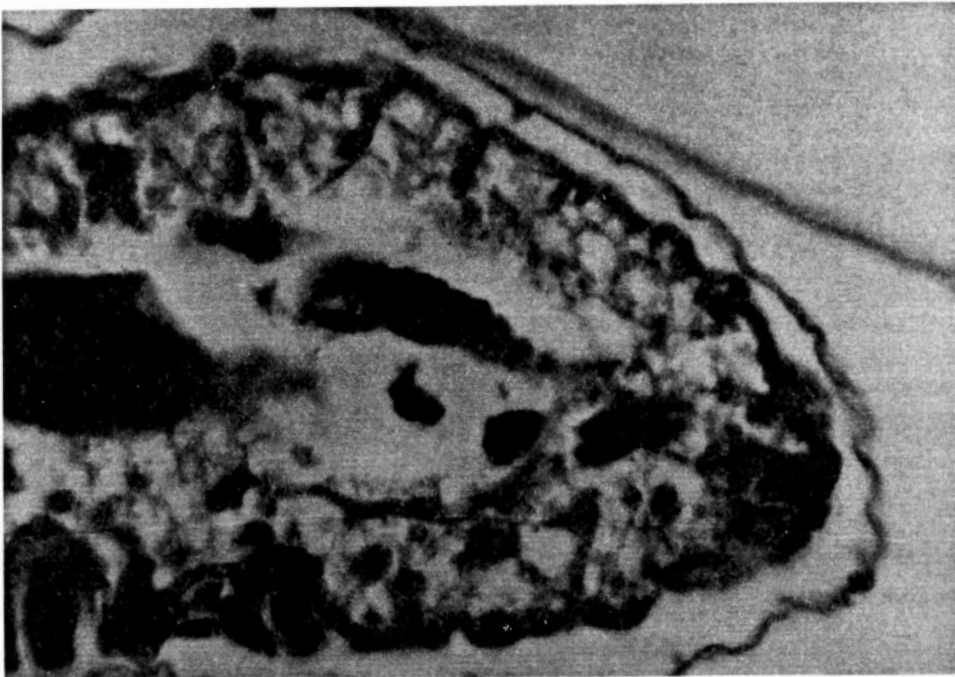


Figure 14. Hindgut of Euonymous Scale. The hindgut is the linear structure (center) (400 x).

B. The Nervous System.

The nervous system of the Mexican mealybug was composed of two major ganglia, the cephalic and subesophageal ganglia. No attempt was made to make an extensive study of the nerve processes that were produced from the main ganglia.

The protocerebrum (Figure 15) made up the bulk of the cephalic ganglion. This mass of cell bodies surrounded the centrally located nerve processes, mainly axons, found in the central aspect of the head. The organ was basically a lateral ellipse with four main processes arising from the apical and posterior areas, respectively. The apical processes arose from the lateral edges and supplied the two simple eyes and the antennae. The posterior processes supplied the subesophageal ganglion. These connectives ran the ventral aspect of the hemocoel through the rings of the rostrum. It was at this point that the connectives began to widen and spread out. Once away from the rostrum the processes merged with the subesophageal ganglion (Figure 16) located within the ventral thoracic cavity. This structure was a large, oval mass of cell bodies and their cellular processes. This mass made up a large portion of the ventral cavity of the thorax. Most the mass was located in between the mid and hind thoracic legs. Nerve processes proceeded from there to the legs and other areas of the thorax and abdomen.



Figure 15. Protocerebrum of Mexican Mealybug. The protocerebrum is composed of two lobes (center) (400 x).

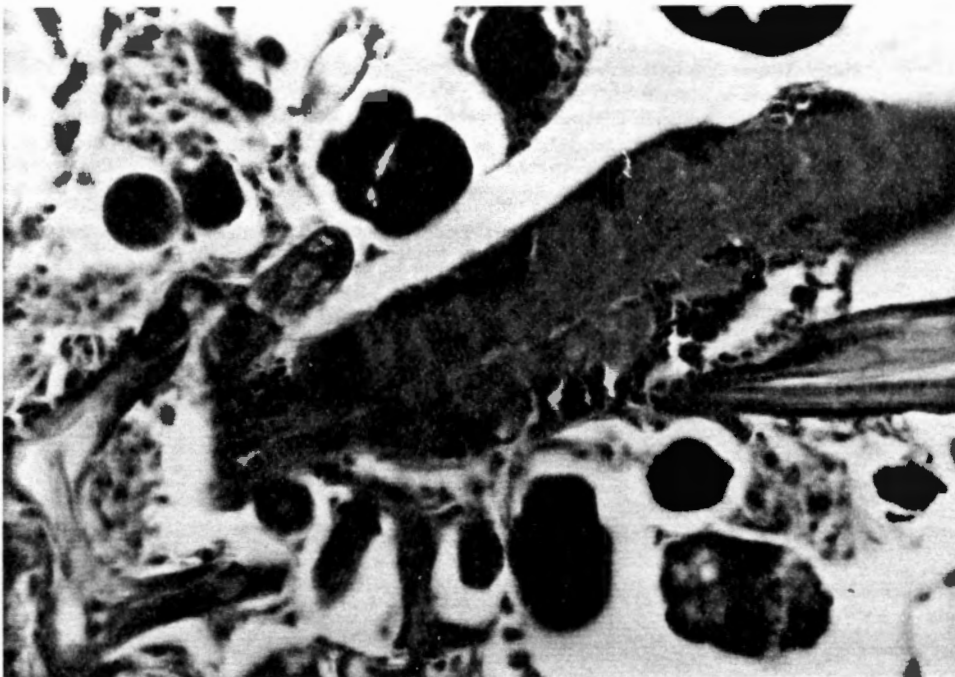


Figure 16. Subesophageal Ganglion of Mexican Mealybug. The subesophageal ganglion is the long structure (center) (400 x).

The nervous system of the Tuliptree scale was basically similar to that seen in the Mexican mealybug. The protocerebrum was a lateral ellipse with connectives arising from the apical and posterior aspects, respectively. The apical processes innervated the antennae while the posterior processes connected to the subesophageal ganglion. The subesophageal ganglion was a linear process with processes emanating from the posterior aspect and innervating the abdomen.

The nervous system of the Euonymous scale followed a structure similar to that of the Mexican mealybug. The cephalic ganglion (Figure 17) was a mass of cell bodies surrounding a mass of their processes in the center. In the Euonymous scale this mass was plainly divided into lateral lobes, the right and left lobes of the protocerebrum. However, this organ was smaller than that of the pseudococcid and the lobes were much more anterior-to-posteriorly oriented. The posterior processes to the subesophageal ganglion were more pronounced and widely separated. These connectives became widely separated and linked with the subesophageal ganglion (Figure 18) at extremes to the anterior of this organ. The subesophageal ganglion was a large mass more rectangular in shape than that of the Mexican mealybug.

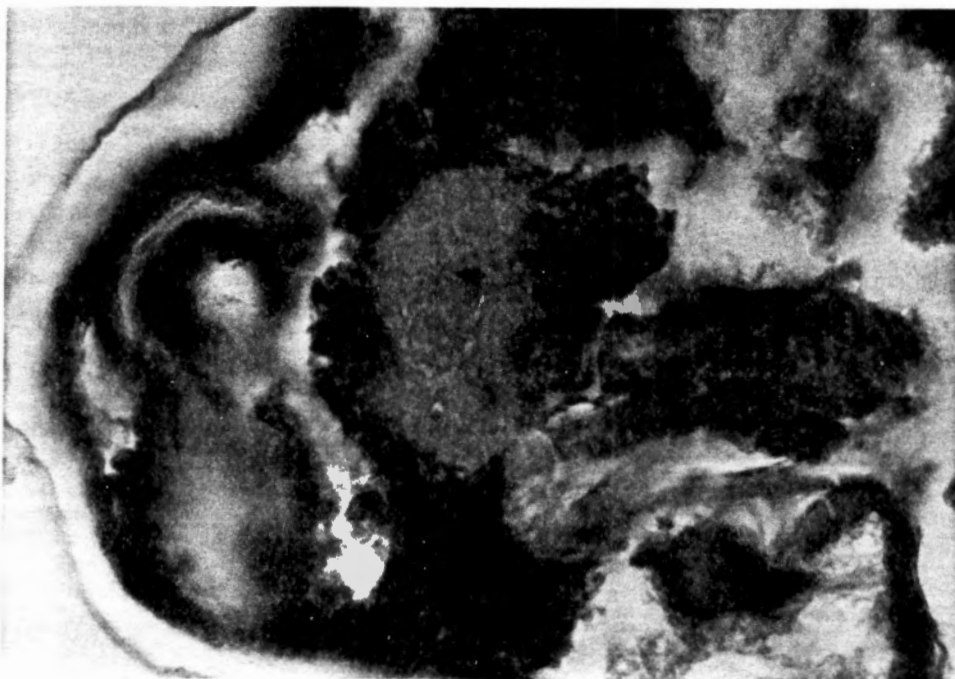


Figure 17. Protocerebrum of Euonymus Scale. The protocerebrum is the central mass (light area, with dark-staining cell nuclei in the periphery) (600 x).

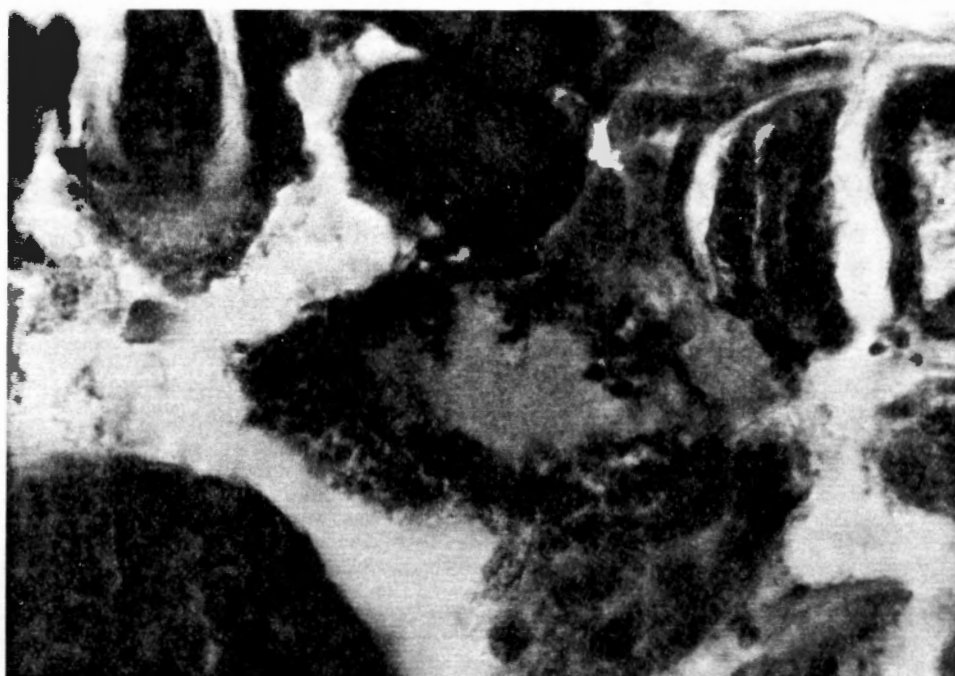


Figure 18. Subesophageal Ganglion of Euonymus Scale. The ganglion is center with cell bodies forming segmented lobes (600 x).

C. The Reproductive System.

The reproductive system of the Mexican mealybug was comprised of an exterior cuticular vulva, located between the eighth and the ninth abdominal segments, the vagina, which proceeded anteriorly within the seventh abdominal segment to merge with branching tubes, the paired oviducts, along with a third cuticular duct to the seminal receptical. This latter organ was located below the vagina at the seventh abdominal segment. The paired oviducts proceeded into the sixth abdominal segment and into the thorax. They branched off into the respective ovarioles and the pseudovitellus. The ovarioles were made up of many follicles (Figure 19) at various stages of development. The oocytes of the follicles possessed cytoplasmic bridges that connected these follicles to the nurse cells located proximally to one side (Ksiazkiewicz 1980). These bridges degenerate eventually and the fully mature oocyte is composed an outer layer of follicular epithelia with a large amount of ooplasm. The ooplasm was composed of many lipid and proteinaceous granules and droplets. Under the light microscope the ooplasm appeared as a heterogeneous mixture of clear vacoules, lipid droplets, and dark-staining matrix of small granules. The oocyte was actually one large cell, the nucleus of which was large, heterochromatic, with two distinctive nucleoli. The pseudovitellus was a large organ intimately associated

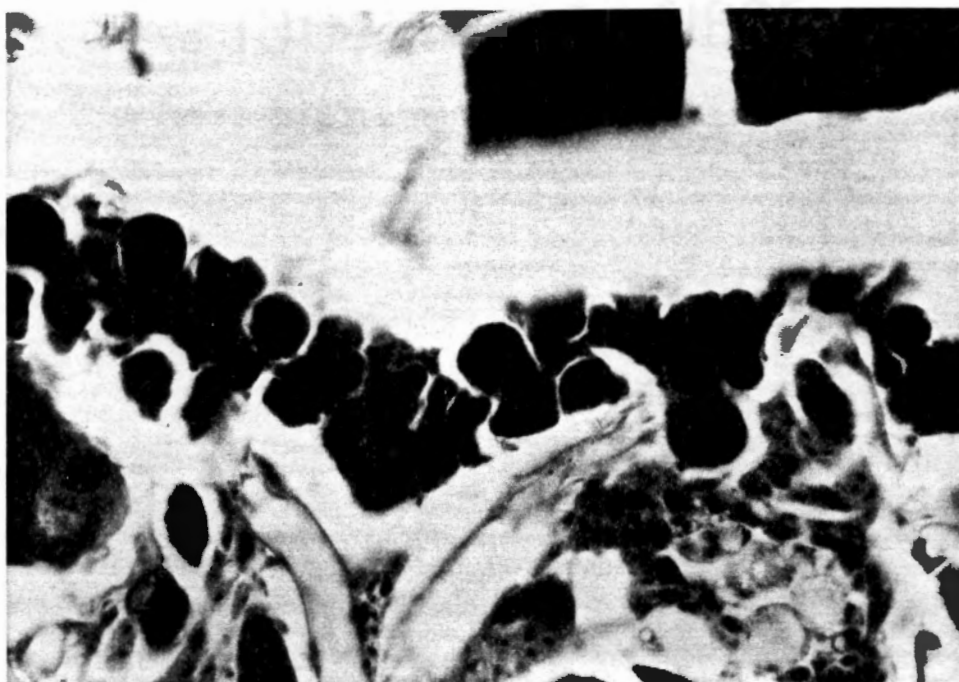


Figure 19. Follicles of Mexican Mealybug. Light micrograph of follicles (dark-staining bodies, center) (600 x).

with the ovarioles and seemed to be the site of meiotic and mitotic activity prior to formation of the follicles and nurse cells (Figure 20). It appeared to be the trophic area of germ cell formation. The cells stained intensely making distinction of organelles difficult. The whole organ encompassed the ventral aspect of the abdomen below the hindgut and penetrated the thorax.

For the Tuliptree scale, the reproductive system was similar to that seen in the Mexican mealybug. The vulva originated between the eighth and ninth abdominal segments. The vagina proceeded anteriorly into the sixth and fifth abdominal segments, diverged into a Y-shaped process which lead to the follicles. A well-developed pseudovitellus was intercalated within the abdominal cavity between the follicles.

The exterior portion of the reproductive system of the euonymous scale began on the ventral aspect of the eighth abdominal segment. Internally, there was a pronounced

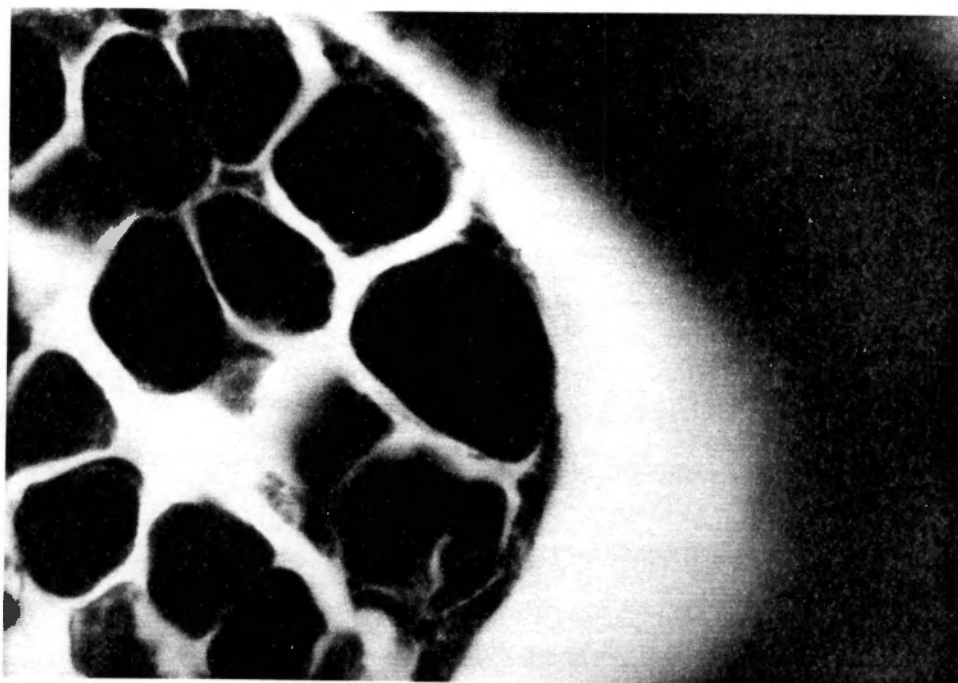


Figure 20. Pseudovitellus of Mexican Mealybug. Pseudovitellus with individual follicles (dark-staining bodies) (600x).

cuticular vagina that led to a pair of oviducts. These ducts then extended to the ovaries where the follicles were arranged around a common vestibule. The seminal receptical arose from the base of the "Y" of the paired oviduct. The follicles of the ovaries possessed dark-staining cells along with the oocytes (Figure 21). These were the nurse cells which also degenerate during the course of oocyte maturation. The oocytes of this insect tended to be smaller than those of the Mexican mealybug and the nurse cells smaller still. There was also a well developed pseudovitellus that occupied the majority of the abdominal cavity and stained darkly as with the Mexican mealybug.

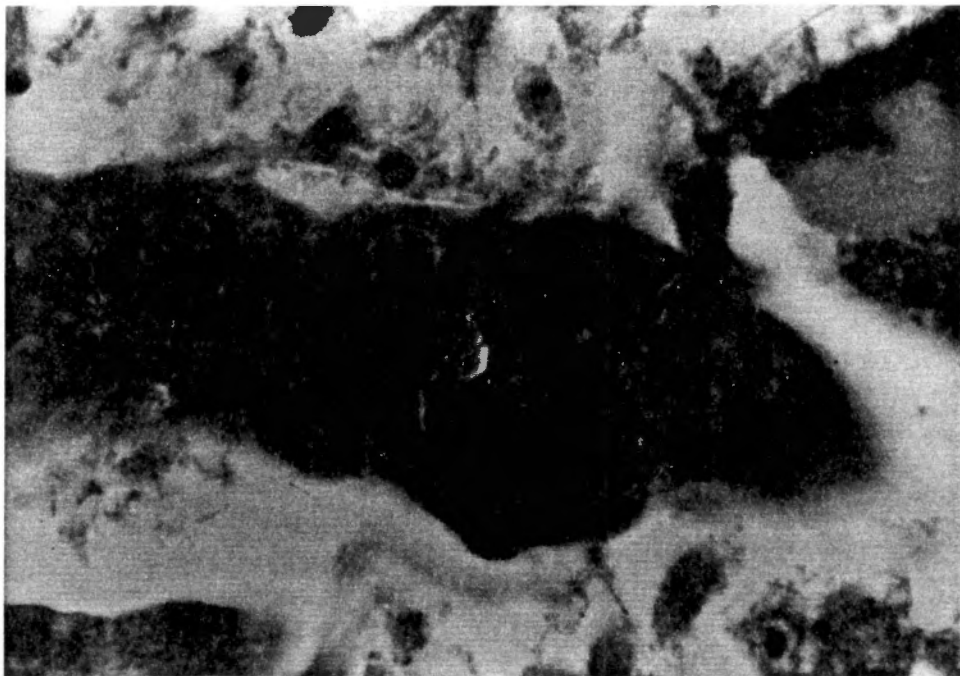


Figure 21. Pseudovitellus and Follicles and pseudovitellus of Euonymus scale. Located center (600 x).

D. The Respiratory System.

The respiratory system of the Mexican mealybug was composed of two thoracic spiracles located between the pro- and mesothoracic and the meso- and metathoracic segments, respectively. These apertures were closely associated with the legs of these segments. Internally, there were several main tracheae that departed from these openings. However, the major commissures made up the trunks that fed the anterior cephalic region, the interconnection between the two spiracles, the trunk that feeds the posterior regions of the abdomen, and the two trunks that connect the lateral trunks dorsally. There did not appear to be any interconnections at either the anterior or posterior ends. There were other major networks of these trunks that supplied various regions, particularly in the area of the pseudovitellus.

The Tuliptree scale possessed trachea that were larger than that of the other scale insects used in the study. This was a reflection of the larger overall size of this insect and the need for greater ventilation and gas exchange. The gross morphology was similar to that found in the Mexican mealybug. There were concentrations of smaller trachae within the cephalothorax in the area of the cephalic ganglion and the pseudovitellus.

The Euonymous scale possessed a spiracular and tracheal system that was just a small variation of that seen in the

Mexican mealybug and the Tuliptree scale. The basic differences were in the size of the spiracles, trachea and lack of association with the legs.

E. The Excretory System.

The Mexican mealybug possessed a single pair of Malpighian tubules. These were located within the abdomen and arose at the juncture between the midgut and the hindgut. From there they divided in anterior and posterior directions. These went as far as the thoracic region anteriorly. The cells were large, granular with large basophilic nuclei (Figure 22). Many were dikaryotic. The inclusions were dense and brown. The lumen was well defined, round and with a brush-border along the edge of the apical region of the cells (Figure 23).



Figure 22. Malpighian Tubules of Mexican mealybug. Tubules are center (600 x).

These cells were triangular-shaped and offset from each other so that in any one region of the tubule there was one cell that predominated that region. This gave the tubule a rather wriggly appearance.

The Malpighian tubules of the Tuliptree scale were much larger, 30 microns, possessing a crystalline texture of granulation in the cytoplasm (Figure 24). The nuclei were large, dark-staining, and dikaryotic. There was a brush border along the inner membrane of the lumen.

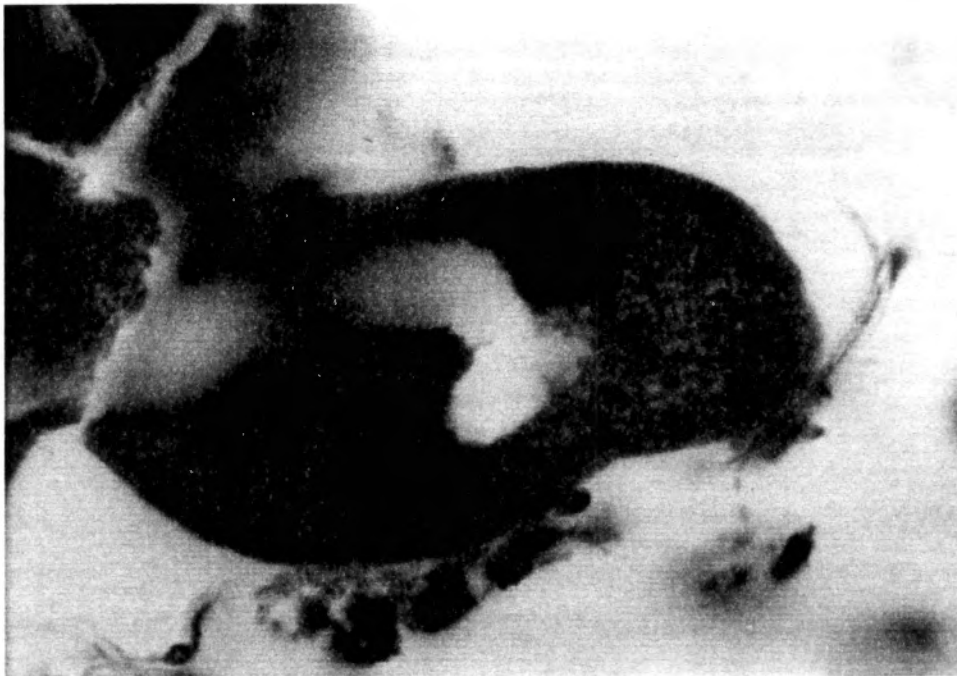


Figure 23. Brushborder of Malpighian Tubules of Mexican Mealybug. Light micrograph (600 x).

The Euonymous scale possessed a well-developed Malpighian tubule system. This organ of excretion was as large as that of the Mexican mealybug. This may be a reflection of the lack of midgut in this insect. That the Malpighian tubules of the armored scales must accomplish the same task as that of the midgut and its filter chamber has long intrigued some scientists (Childs 1914, Balachowsky 1939). The cells of this organ were large and granular. These cells possessed large, basophilic nuclei with prominent light nucleoli.



Figure 24. Malpighian Tubules of Tuliptree Scale. Crossection of Tubules (center) (400 x).

F. The Fat Body.

Two major cell types were found to make up the bulk of the fat body tissue in the Mexican mealybug. The majority of the tissue was located proximally to the alimentary canal. In the region just proximal to the digestive tract the cells were large, granular with many large vacoules (Figure 25). These cells possessed a small centrally placed nucleus with cytoplasm that stained basophilically. The nucleus was small and stained intensily. Located distally from the alimentary canal occurred an area of adipose tissue (Figure 26). The

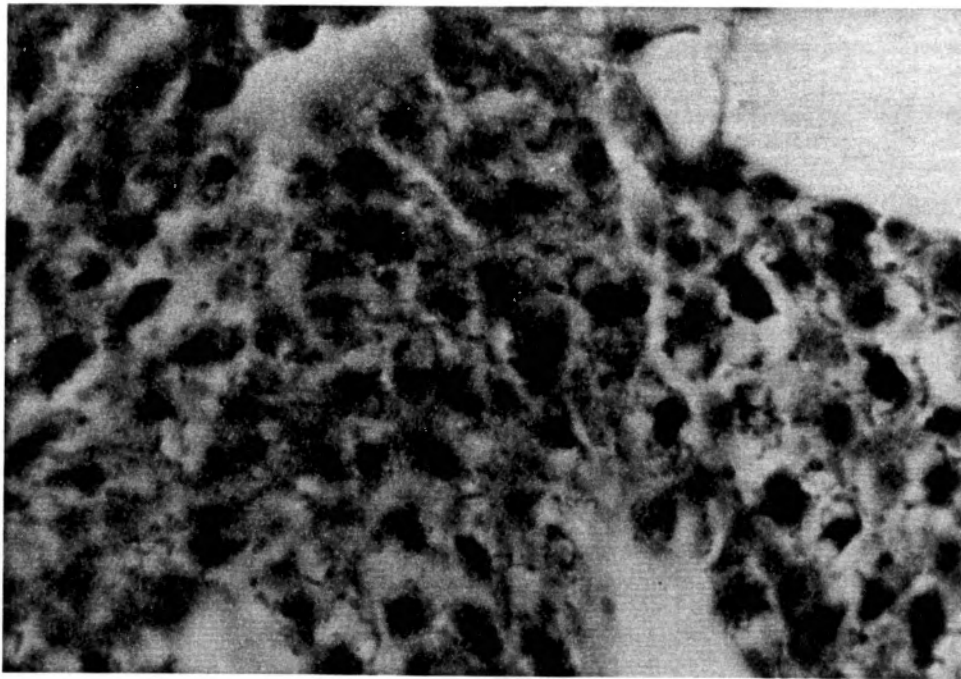


Figure 25. Fat Body of Mexican Mealybug. Tissue of the type found proximally to the alimentary canal and adipose tissue (600 x).



Figure 26. Adipose Tissue of Mexican Mealybug. Tissue found throughout the hemocoel distally from the alimentary canal in the Mexican mealybug (400 x).

cells were broadly oval, possessed a large central vacuole and dense, flattened nuclei at the outer edge of the unit membrane. The vacuoles were clear, thus, indicating a lipid composition dissolved away during treatment.

The fat body of the Tuliptree scale was composed mainly of dark staining cells (Figure 27). These cells were oval, vacuolated with small granules throughout the cytoplasm. The nuclei were centrally located and large. This tissue could be found throughout the hemocoel of the insect. There appeared to be a general lack of adipose tissue.

The fat body in the Euonymous scale was composed of adipose tissue similar to that seen in the Mexican mealybug (Figure 28). The adipose tissue was located more proximally to areas of the digestive tract than the Mexican mealybug.

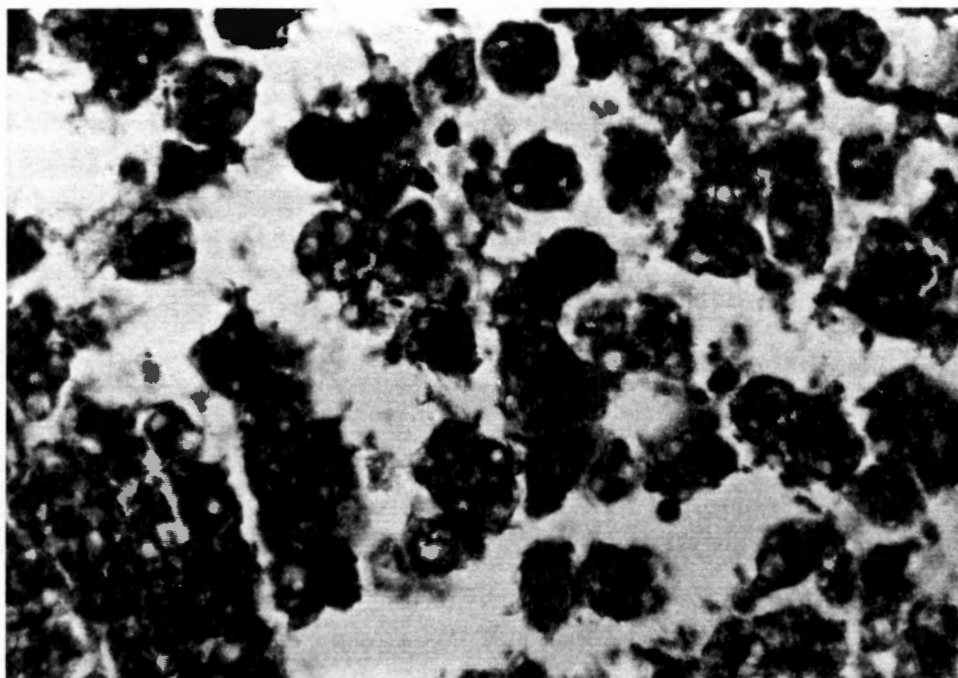


Figure 27. Fat Body of Tuliptree scale. This tissue is made up primarily of granular tissue (400 x).

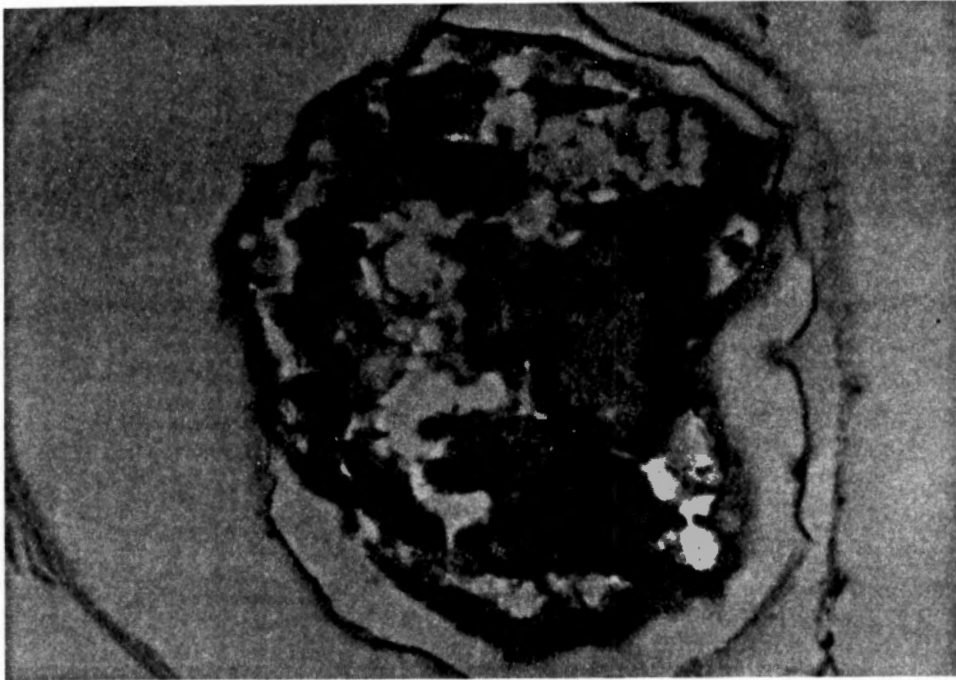


Figure 28. Fat Body of Euonymous Scale. This tissue is made up of adipose tissue of fat body (clear areas) (600 X).

CHAPTER 4

CONCLUSION

The results indicate that the digestive system of the Mexican mealybug is the basic form from which the others could have diverged off. These include reduction of the midgut and loss of the filter chamber, retention of adipose tissue as the predominant cell type in the fat body, and shortening of the hindgut which exits the body from a dorsal aspect in the diaspidid scale, *U. euonymi*. In the Tuliptree scale, there was a general enlargement of organ systems compared with those of the Mexican mealybug and the Euonymous scale (Table 1). However, there was a significant difference between the Mexican mealybug and the Tuliptree scale when length of the midgut was compared (Table 2). There was also significant differences between the Euonymous scale and the Tuliptree scale when foregut length and hindgut width were compared, and between the Mexican mealybug and the Euonymous scale with respect to hindgut length (Table 2). The Tuliptree scale and the Mexican mealybug only differed significantly when midgut lengths were compared (Table 2). In the Mexican mealybug there was a more complex filter chamber without the degree of intimacy with hindgut tissue. In the Euonymous scale the Malpighian tubule system was similar to that in the Mexican mealybug.

Weber (1930) contended that the digestive system seen in the pseudococcids was similar to those in cercopids and

Table 1

MEAN LENGTHS AND WIDTHS OF SPECIES						
ORGAN	<u>P. gossypii</u>		<u>U. euonymi</u>		<u>T. liriodendri</u>	
	L	W	L	W	L	W
FOREGUT	53.7	46.2	59.7	31.7	236.0	101.3
MIDGUT	161.3	51.7	-----		944.1	98.0
FILTER C.	178.3	106.2	-----		324.3	253.2
HINDGUT	401.3	205.3	92.3	14.3	879.0	591.2

Table 1. Mean Lengths and Widths for Digestive Tract.

Table 2

CHARACTERS LENGTH	SPECIES	T-TEST VALUE
FOREGUT	<u>P. gossypii</u>	0.03
	<u>U. euonymi</u>	
	<u>T. liriiodendri</u>	3.69 a
	<u>U. euonymi</u>	
	<u>P. gossypii</u>	2.83 a
	<u>T. liriiodendri</u>	
FILTER C.	<u>P. gossypii</u>	1.07
	<u>T. liriiodendri</u>	
MIDGUT	<u>P. gossypii</u>	4.16 a
	<u>T. liriiodendri</u>	
HINDGUT	<u>P. gossypii</u>	1.20
	<u>T. liriiodendri</u>	
	<u>P. gossypii</u>	3.44 a
	<u>U. euonymi</u>	
	<u>T. liriiodendri</u>	2.47
	<u>U. euonymi</u>	
WIDTH	<u>T. liriiodendri</u>	2.69 a
	<u>U. euonymi</u>	

a= Significant at $P < 0.01$.

Table 2. Variation of Level Significance for Means. Data is From Table 1.

aphidids. Thus, this system may represent the basic format for which other variants seen in the Neococcoidea may have evolved. The extent to which the digestive system diverges from the pseudococcid pattern reflects modification in the

form and function in scale insect evolution. From the system seen in the mealybugs, there is an enlargement of organ systems within the coccids with modification of the fat body tissue away from adipose cell types. This may reflect a difference in the type of wax secreted by these insects and loss in ability to produce honeydew (Miller and Kozstarab 1979). In the diaspidid scale insects, there is complete loss of midgut tissue and the filter chamber mentioned earlier. This too reflects a change in physiological needs.

Knowledge of the affinities and differences that exist internally between the three families is incomplete. This information may help in understanding the spectrum of physiological and morphological differences that pervade the Coccoidea. This will undoubtedly contribute to more studies in the future.

**PART II: COMPARATIVE ANATOMY OF THE CEPHALIC REGION
FOR TWO SPECIES OF MALE SCALE INSECTS (HOMOPTERA:
COCCOIDEA)**

CHAPTER 1

INTRODUCTION

The amount of literature pertaining to morphology of male scale insects is sparse when compared to the degree of representation of information on morphology for the females (Miller and Kosztarab 1979). This stems from lack of access to male specimens either because of their ephemeral nature or the lack of heterosexual species within the superfamily (Miller and Kosztarab 1979). Most research on males has dealt with taxonomy based on external morphology to coincide with that of the females (Boratynski 1953; Ghauri 1962; Giliomee 1961, 1967a, 1967b, 1968; Afifi 1968). Recently, work with males has revealed the unique nature of reproduction in scale insects that has generated interest in the morphology and ultrastructure of sperm (Nur 1970). The eyes have been implicated as important organs for identification of females, along with chemical identification through pheromones (Duelli 1979). This information has lead to research that would supplement current knowledge (Miller and Kosztarab 1979).

Two species of male scale insects, Phenacoccus gossypii Townsend and Cockerell, and Unaspis euonymi (Comstock), were selected as candidates for research. The two species occupy the relatively dichotomous positions phylogenetically.

CHAPTER 2

MATERIALS AND METHODS

Males of the Mexican mealybug and the Euonymous scale were obtained from colonies established on host plants (Coleus sp. and Euonymous japonicus Linnaeus, respectively), grown in a greenhouse on the University of Tennessee Agriculture campus, Knoxville, TN. Specimens of the Mexican mealybug were removed from their host plants by uncovering the test from around the insects with a microdissection spatula, picking them up and placing them into a phosphate-buffered 2% glutaraldehyde/ 2.5% formaldehyde, pH 7.2, fixative in vials. Specimens of the euonymous scale were obtained from plants brought into the laboratory and examined under a stereomicroscope, and were removed and processed as with the Mexican mealybug. The vials containing specimens and the fixative were transferred to a vacuum bell fitted to an aspirator fixture in a sink. Specimens were processed at room temperature for 4 hours. After extraction of the fixative with a Pasteur pipette, specimens were then rinsed three times in a buffer and dehydrated through a graduated series of ethanol concentrations from 30%-absolute in 15 minute intervals. The last series involved placing them into a solution of 50% tertiary butanol/ 50% ethanol, allowing the fluid to cover the specimens, followed by placing the vials

into an oven set at 40°C. Paraplast pellets were placed into the vials and left in the oven overnight to allow for infiltration of the paraffin into the specimens. After 24 hours, specimens were removed from the vials with an applicator stick, placed into a base mold, oriented to proper position, and then submerged with liquid paraffin. The specimens were then placed onto a cold plate and the paraffin allowed to solidify. After solidifying, the base molds were removed, the blocks trimmed, and placed onto a rotary microtome for sectioning. Serial sections at a 10 μ thickness were placed onto standard microscope slides, allowed to dry, and processed for staining. Three standard staining techniques were evaluated for use in the study. Gill's hematoxylin and eosin Y protocol was used following a modified technique from Humason (1979). A method using the metachromatic stain, toluidine blue, was also used, as well as a histochemical stain, Feulgens, for DNA (Humason 1979). Although the technique utilizing toluidine blue was simpler, the results were variable. The Feulgen staining method produced contrast between nuclear and cytoplasmic material but the technique was complex. The hematoxylin and eosin procedure produced good tissue results and was selected as the standard protocol.

Measurements were taken of widths and lengths for both longitudinal and cross-sectional sections of the cephalic region, antennae, the diameter of the ocelli and retinula cell

and retinula cell pigment length, and the cephalic ganglion for both species from an ocular micrometer on a microscope at high power under oil immersion. Measurements were taken in micrometers, means obtained and subjected to a non-pooled Student's T-test for significance.

CHAPTER 3

RESULTS

Sections of the head region between males of these species differed mainly length and width as noted by comparison of mean Table 1). The Mexican mealybug was 3-4 times larger than the euonymous scale. However, when these means were tested in a non-pooled Students T-test no significance could be determined ($P = >.05$).

Table 1

CHARACTER	MEAN			
	<u>P. gossypii</u>		<u>U. euonymi</u>	
	L	W	L	W
Cephalic Region	137.5	149.3	49.7	56.4
Antennae	83.3	34.1	84.2	10.7
Ganglion	61.0	69.5	87.0	73.7
Ocelli (Diameter)	28.4D	38.1V	17.7D	18.2V
Retinula Cell (Length)	6.4D	11.0V	4.6D	10.0V
Ret. Cell Pigment	20.4D	23.8V	8.8D	8.1V

D (Dorsal), V (Ventral)

Table 1. Mean Lengths and Widths of Cephalic Region.

Both possessed heads that were oval in shape, had two pairs of ocelli, dorsal and ventral, with antennae that arose from the anterior aspect between the dorsal and ventral pair of simple eyes. The antennae were filamentous and composed of 11 segments. Neither species possessed mouthparts. Comparison of mean lengths and widths of the cephalic exterior and ganglion morphology reveal that the Mexican mealybug is twice as large as that of the Euonymous scale (Table 1). The cephalic ganglion for the Mexican mealybug was organized within a more spacious head region than that of the Euonymous scale (Figures 1-2). There were areas where adipose tissue was deposited proximal to the ganglion in the Mexican mealybug not found in the Euonymous scale (Figures 1-2). Innervation

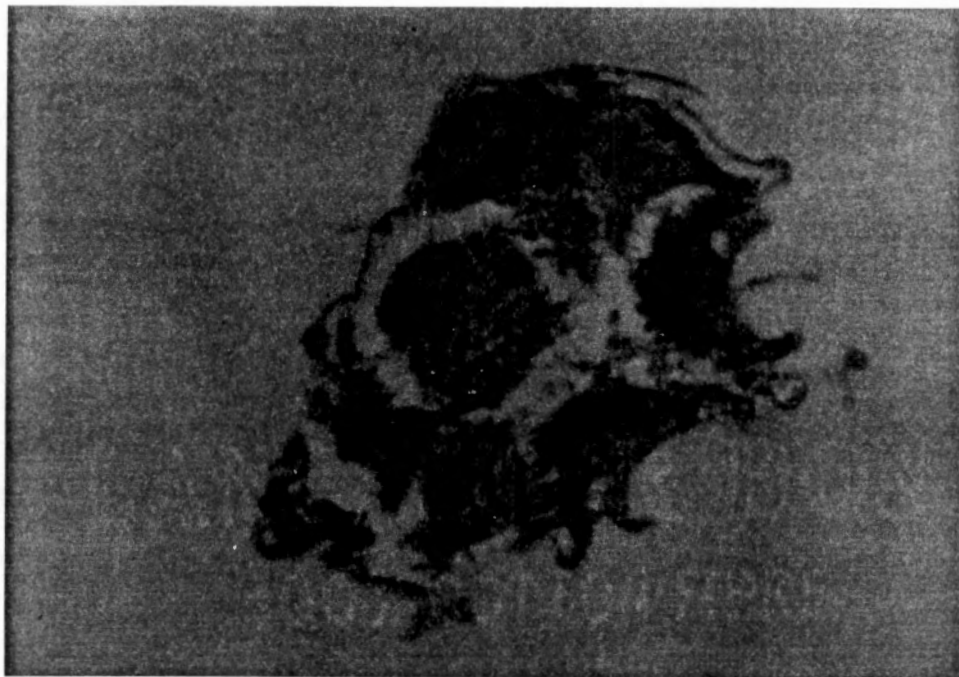


Figure 1. Cephalic Region of Mexican Mealybug. Crossection showing central ganglion and four ocelli (100 x).

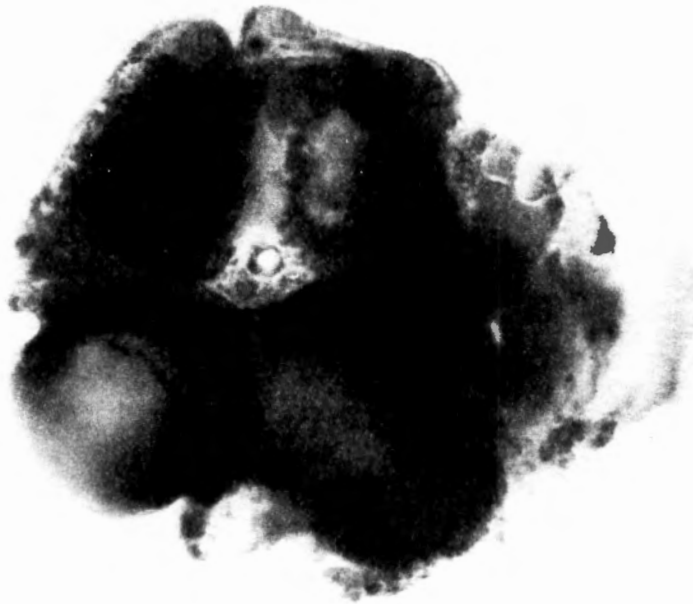


Figure 2. Cephalic Region Euonymus Scale. Part of the central ganglion (lower right) and ocellus (lower left) can be seen (200 x).

of the ganglia to the ocelli was more pronounced in the Mexican mealybug than the Euonymus scale (Figures 3-4, 7-8). The posterior aspect of the cephalic ganglion of the eonymous scale projected into the thorax (Figure 8) while the processes to the subesophageal ganglion did so in the Mexican mealybug. Delineation between the cell bodies, represented in the micrograph by the dense, dark staining nuclei around the cell processes, represented by the light region in the micrograph, was more pronounced in the Mexican mealybug (Figures 3-4).

Sections of the antennae indicated that the length was closely similar for both species (Table 1). However, the width by was three times larger for the Mexican mealybug than

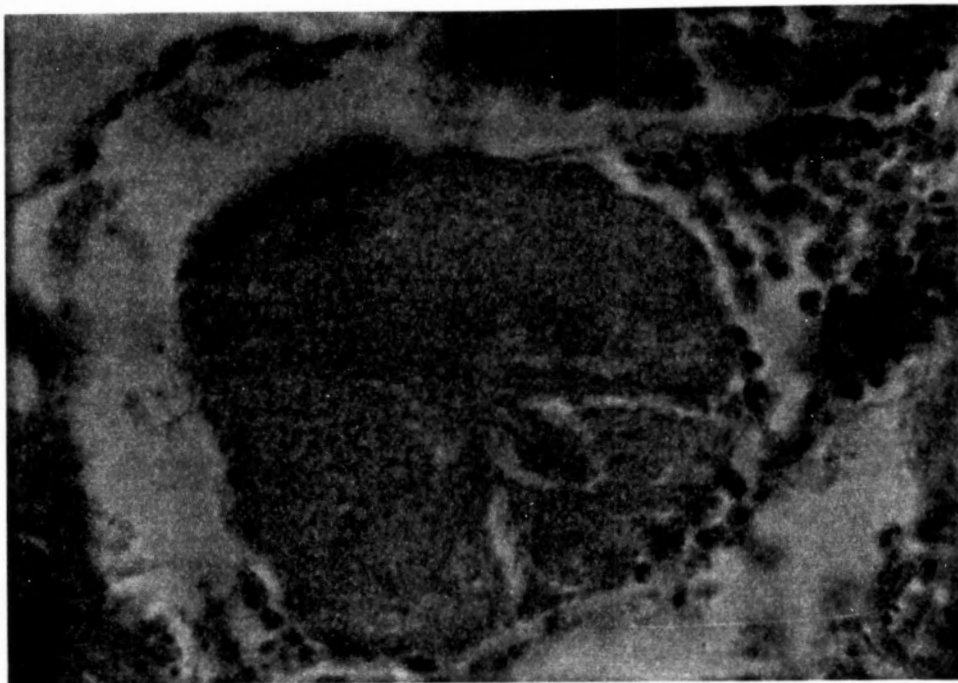


Figure 3. Cephalic Ganglion of Mexican Mealybug. Note light central mass, with darker staining region proximally (cell bodies) (200 x).



Figure 4. (3) Cephalic Ganglion of Euonymous Scale. Light micrograph (400 x).

the Euonymous scale (Table 1). Internally, the Mexican mealybug possessed a larger luminal space for muscle tissue and interneurons that transit through the antennae (Figure 5). The Euonymous scale antennae interspacing was smaller and denser with traversing tissue (Figure 6). There appeared was little difference between the two in the amount of tissue within the luminal space.

The diameter of the cornea of the dorsal ocelli for the Mexican mealybug was seven times greater than the Euonymous scale (Table 1). The ventral ocelli in the Mexican mealybug was three times that for the Euonymous scale (Table 1). The ocellar retinula cells in the Mexican mealybug were seven times those of the Euonymous scale in length (Table 1). The pigmentation of the retinula cells of dorsal and ventral pairs



Figure 5. Antennae of Mexican Mealybug. Light micrograph (200 x).



Figure 6. Antennae of Euonymous Scale. Light micrograph (400 x).

had little variation between the two species (Table 1). Thus, while the diameter of the ocelli and retinula cell lengths varied between the two species the amount of pigmentation did not. The Mexican mealybug also possessed an area of well defined corneagen cells between the cornea and the retinula cells (Figure 7) that was not as apparent in the Euonymous scale (Figure 8). The innervation of the ocelli from the cephalic ganglion was more pronounced compared with that of the euonymous scale (Figures 7-8).

Although the mean dimensions of the parameters between the species differed, there was little significant variation. When the means of the antennae were compared, the Mexican mealybug had dimensions that were 2 factors larger than in the

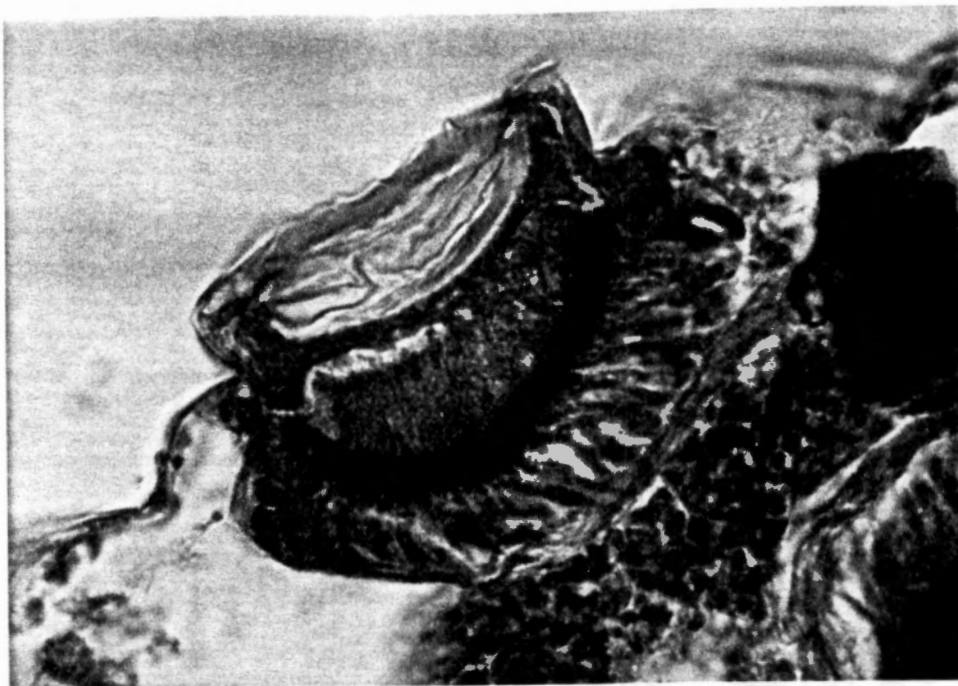


Figure 7. Ocelli of Mexican Mealybug. Ocellus showing innervation, pigment and the lens (200 x).



Figure 8. Ocelli of Euonymous Scale. Light micrograph (400 x).

euonymous scale (Table 1). Other dimensions of the cephalic region reflected more of a factor between 2-4 (Table 1). There was little difference between mean lengths in the pigmentation of the retinula cells of the ocelli suggesting that the function of this organ may be just as efficient in the euonymous scale, with smaller diameter cornea and retinula cell lengths, as in the Mexican mealybug (Table 1). The organization of the cephalic region and ganglion between the two species indicates a trend from a spacious, loosely and more randomly organized region in the Mexican mealybug to a more compact, less spacious organization in the Euonymous scale. These differences may reflect an increased dependence of the Euonymous scale male to specialize in chemosensory stimulus cuing as a mechanism for mate identification and location rather than the more generalized mechanisms possibly associated with the Mexican mealybug.

The lack of differences reflect a conservation of those characters in the males that determine sexual partners with the least amount of energy expended by the sexes of either species. Thus, the males have lost the use of effective mouthparts, shorter lifespans of only a few hours to a few days, retained wings for better motility and sexual partner location, and possessed larger antennae and ocelli. The females have retained a neotenic form with many of the adult characters repressed so that feeding and sexual efficiency are increased (Miller and Kosztarab 1979). This strategy means

that the males are only present as adults long enough to locate a mate, copulate and die while the females feed throughout their lifespan, producing hundreds of progeny in the process. The need for the male to locate mates during the short period of adult life places stresses on the species for greater efficiency of these tasks. The trend in the scale insects may be in greater efficient use of female sexual pheromone location with the male cuing from chemosensory stimuli.

The evidence, though inconclusive, shows that there is a difference in the morphology internally that could be used phylogenetically, but more research is needed throughout the taxa to determine how extensive these are.

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**PART III: HEMOCYTE TYPES OCCURRING IN THE MEXICAN
MEALYBUG, PHENACOCCLUS GOSSYPHII TOWNSEND AND COCKERELL**

CHAPTER 1

INTRODUCTION

Hemocytes of invertebrates like insects are not involved in the exchange of gases as erythrocytes, but are more analogous to the "white blood" cells of vertebrates implicated in immune response (Arnold 1974; Gupta 1986). In insects several cell types exist that include prohemocytes, plasmatocytes, granulocytes, spherulocytes, adipohemocytes, and oenocytoids (Wigglesworth 1950; Gupta 1979a, 1979b, 1985a; Brehelin and Zachary 1986). The type of cells found in the hemolymph in insects depends on several factors (Arnold 1974; Lackie 1981; Ratcliffe, Leonard and Rowley 1984). Important in this regard is the stage of development (Pathak 1986) and the state of health of the insect (Ratcliffe, Leonard and Rowley 1984). Although there has been substantial research on the forms and functions of these cells in insects there only a few have involved scale insects (Steinhaus 1949). In a study on the circulatory system of Icerya purchasi Maskell, Poisson and Pesson (1938) reported on the existence of many cellular elements. In 1967 Yadava presented data on studies of the circulatory system in female Saccharicoccus sacchari (Cockerell) with hemocyte descriptions.

The study of the internal anatomy in scale insects presented several opportunities for examination of various

tissues, including the hemocyte types. The objective of this study was to examine and determine the hemocyte types.

CHAPTER 2

MATERIALS AND METHODS

A population of the Mexican mealybug was maintained in a greenhouse on Coleus sp. plants raised on a 50% peat/ 50% soil mixture in pots on flats on the University of Tennessee Agriculture campus, Knoxville, TN. Twenty five specimens were obtained for treatment by prodding insects off the host plant with a microdissection spatula, transferred to a Petri dish. In the laboratory, each specimen was processed by piercing the ventral surface with a sterilized no. 1 insect pin between the meso- and metathoracic legs under a stereomicroscope. The venter offered the site where the least amount of contamination of the sample from the dorsal ostioles could occur. The resulting droplet of hemolymph was obtained by a Thoma pipette, diluted with an isotonic solution 1:100 (Gupta 1979d), and the contents of the pipette emptied onto a standard microscope slide. The droplet was lightly spread into a circular film 2 cm in diameter and allowed to air dry (Humason 1979). A droplet of Wright's blood stain was applied to this film and allowed to dry. The excess unreacted stain was gently washed from the slide by rinsing with distilled water from a squeeze jar. The slide was allowed to air-dry again, a droplet of Canada balsam or Permount applied and the slide cover-slipped. For examination under the phase-contrast

microscope the specimens were processed as above except the hemolymph droplet was diluted with an isotonic solution, applied to a microscope slide, and coverslipped. Both Wright's stain and phase-contrast specimens were examined under oil immersion at 100 X. Criteria for hemocyte identification followed were those outlined by Gupta (1979c).

CHAPTER 3

RESULTS

Four major types of hemocytes were found in samples of hemolymph extracted from the Mexican mealybug. These were the prohemocyte, plasmatocyte, granulocyte, and spherulocyte. All cell types were consistently found in each sample. The granulocytes and spherulocytes each made up the largest proportions of hemocytes in the samples, with 45% and 30%, respectively. The prohemocytes made up 15% of the total, while the plasmatocytes 10%. The prohemocytes were characterized by relatively small size, 5-7 μ diameter, a dense basophilic cytoplasm, and large nucleus (Figure 1). Plasmatocytes were characterized by an elongated spindle-like

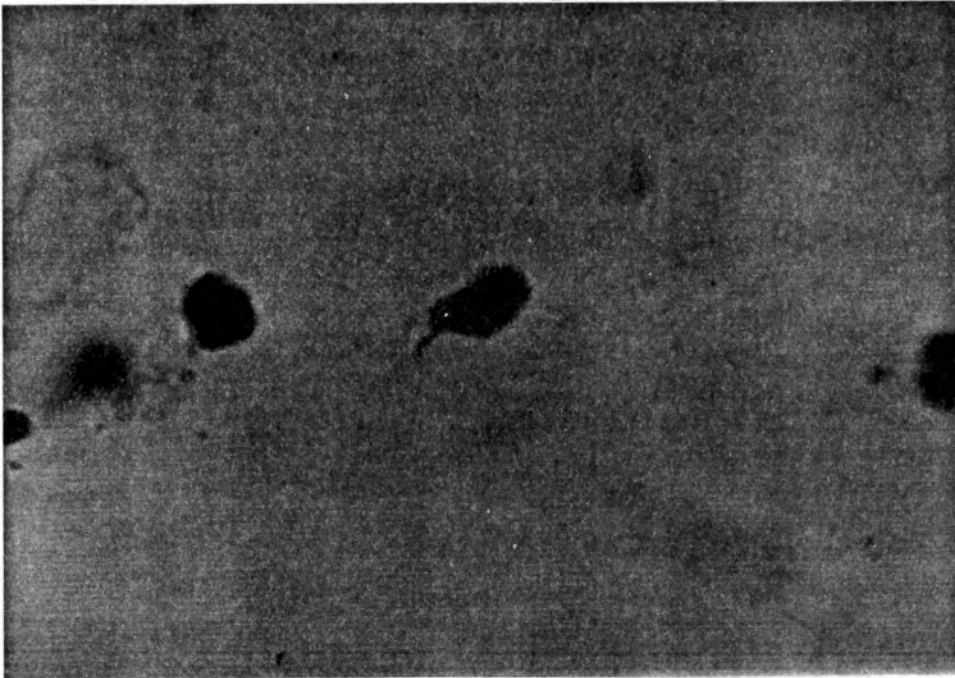


Figure 1. Prohemocyte of Mexican Mealybug. Light micrograph of Prohemocyte (center), Wright's stain (400 x).

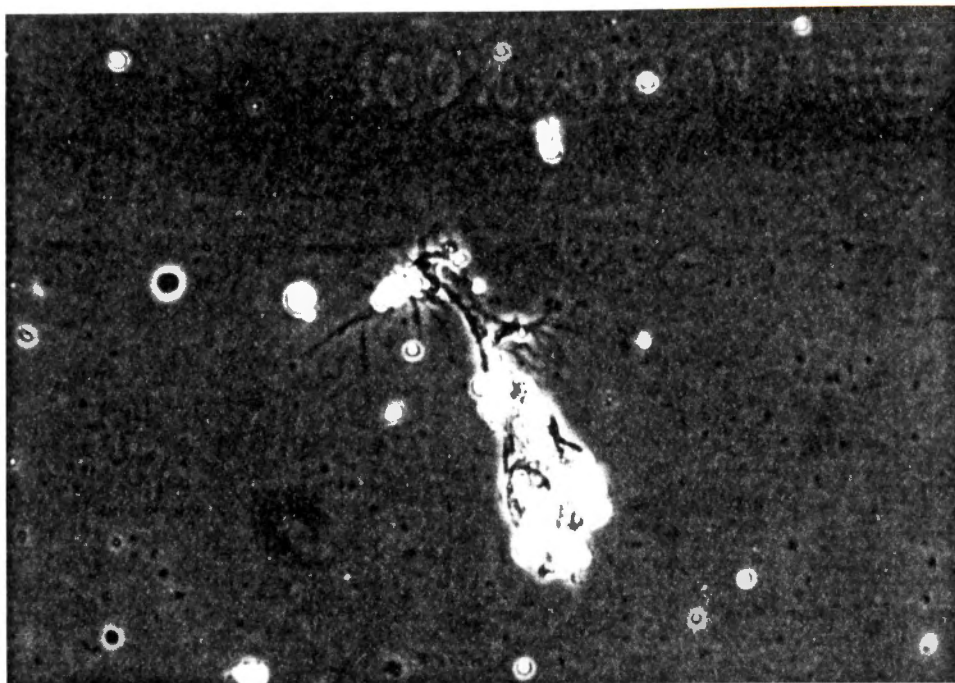


Figure 2. Plasmatocyte of Mexican Mealybug. Phase-contrast micrograph (200 x).

form, many pseudopodia, a centrally to eccentrically located nucleus, and size ranges between 10-15 μ in diameter (Figure 2). The cytoplasm in this hemocyte type was weakly basophilic and possessed a few granules. The granulocytes were between 10-15 μ in diameter, spherical, with a centrally located nucleus and many dark-staining granules in the cytoplasm (Figure 3). Spherulocytes were large, between 15-25 μ in diameter, possessed a large centrally located nucleus, and many large eosinophilic droplets in the cytoplasm (Figure 4).

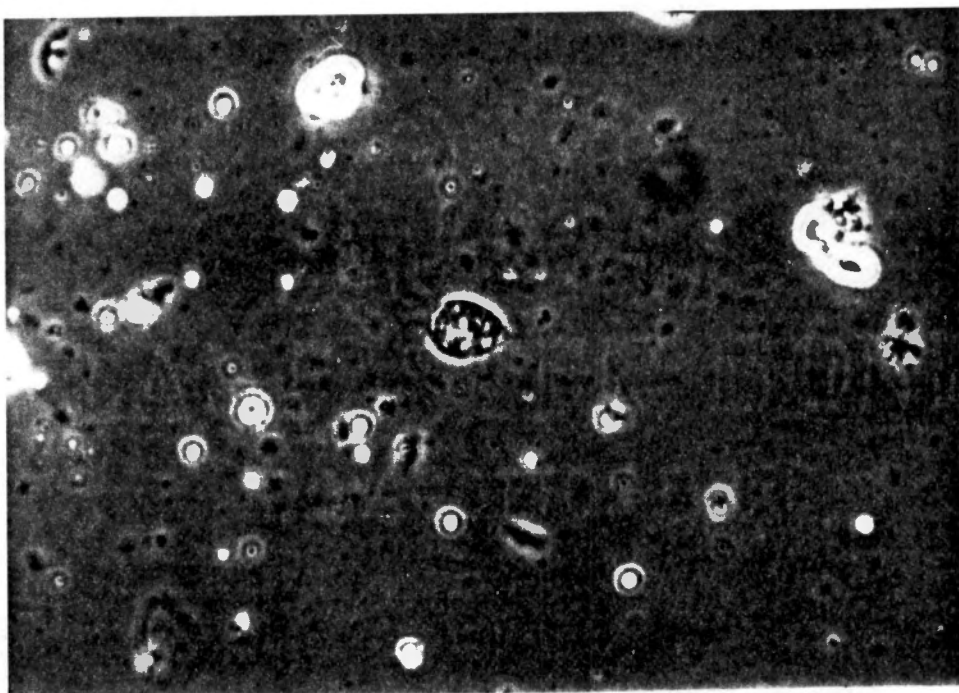


Figure 3. Granulocyte of Mexican Mealybug. Phase-contrast micrograph (200 x).

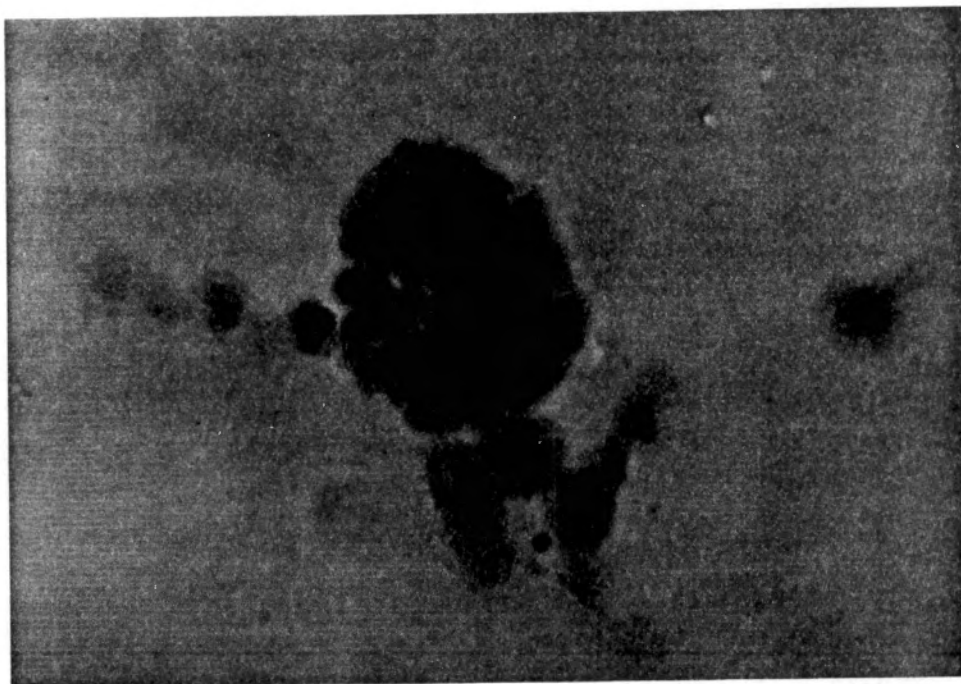


Figure 4. Spherulocyte of Mexican Mealybug. Light micrograph, Wright's stain (200 x).

CHAPTER 4

CONCLUSION

The prohemocytes are considered stem cells for other hemocyte types, and are found in the hemolymph in most all insect orders (Gupta 1979a, 1985a). They have been observed to transform into other hemocytes in culture (Sohi 1971).

Plasmatocytes have been implicated as the main phagocytic cell type within invertebrate taxa (Maramorosch and Shope 1975). Gupta (1979a) reported that this cell type may be an intermediate between the prohemocyte and other hemocyte types.

Granulocytes are the one cell type in which true specialization has been implicated (Gotz and Boman 1985, Gupta 1985a). These cells are able to phagocytize foreign material and attach themselves to larger particles in which granule release and melanization has been observed.

Spherulocytes are considered a specialized cell within the granulocyte complex (Arnold 1974; Brehelin and Zachary 1986). These cells are implicated as important for blood "clotting".

The results obtained agree with those results from other studies of the Homoptera (Gupta 1979a). Gupta lists the adipohemocyte as another hemocyte found within the Homoptera, however, there is much disagreement between insect hematologists as to the status of the adipohemocyte as a true hemocyte type (Gupta 1985b, Brehelin and Zachary 1986).

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VITA

Robert Carleton Brown was born in Chattanooga, Tennessee on September 27, 1960. He attended first grade at Bess T. Sheperd Elementary School in Chattanooga in 1966-67. He moved with his family to Morristown, Tennessee during the summer of 1967. He attended the Hamblen County Public School system and graduated high school August 1979. He entered the United States Navy that summer (August) and completed a four year tour of duty during which time he traveled throughout Europe and the Mediterranean area. He was honorably discharged October 1984 (from the reserves). He attended Walters State Community College from September 1983 through March 1986. In September 1986 he entered the University of Tennessee, Knoxville. He graduated on March 15, 1988 with a Bachelor of Arts degree in Microbiology with minors in Zoology and Biology. In July he entered the Entomology and Plant Pathology program at the University of Tennessee and received a Master of Science degree in Entomology May 1991.

#57
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