DEVELOPMENT OF AN ARTIFICIAL DIET FOR THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

This work is directed at the development of an artificial diet and a diet-based rearing system for *Homalodisca coagulata*. The approach taken to this work is to analyze the feeding mechanism and feeding dynamics of *H. coagulata* in order to use this information as a guide to the feeding needs of this insect. The assumptions behind this work are that the profile of components in xylem sap used by *H. coagulata* will be an optimal or at least suitable diet for this species, and details of the feeding biology of this insect (such as knowledge of specific feeding strategies and digestive enzymes) will help identify its dietary needs (Cohen 2003). Towards this end, studies were undertaken to pinpoint the details of feeding biology of this species, including how the insect may impact changes in the plant's sap profile.

OBJECTIVES

1. Develop an artificial diet for *H. coagulata.*
2. Develop an understanding of the morphology of the feeding system of this insect.
3. Develop an understanding of the digestive processes used by *H. coagulata* in handling its food.
4. Develop an understanding of the interactions between *H. coagulata* and its host plants to determine whether or not the insect is a passive feeder that simply ingests whatever the plant offers or an active feeder that manipulates or affects the plant's xylem sap composition.

RESULTS AND CONCLUSIONS

A series of diets has been formulated, and tests of these diets are underway. It has been demonstrated that the insects will feed on artificial diet presented through a membrane. Currently, feeding stimulants and profiles are being refined to maximize feeding rates. Mixtures that contain combinations of free amino acids and peptides are being tested now in light of the findings on the digestive physiology and biochemistry of *H. coagulata*.

The gross anatomy, fine anatomy, and utrastructure of the feeding system of *Homalodisca coagulata* (Say) (Homoptera Cicadéllidae) were studied with light (Figures 1-a, 1-b) (bright field, differential interference, fluorescence) and electron microscopy (cryofracture-based scanning and transmission). The mouthparts of *H. coagulata* (including the labium, labrum, and stylets) are relatively short in comparison with those of other Homoptera in relation to the ratio of these structures and the insect's body length. The bristles herein referred to as stylets, contain lateral, paired mandibular stylets, which have a dentition consistent with plant penetrating function. Typical of the Homoptera, *H. coagulata* produces a salivary sheath that extends from the exterior of the plant surface into the stem tissues terminating in the xylem elements. The sheath substance is produced by the paired salivary glands, which lie ventrally between the head and the prothorax. The sheath material fluoresces (Figure 2-a) when excited by various visible and UV wavelengths and can be localized within the plant tissues easily with fluorescence microscopy. Examination of 100 salivary sheaths by light and electron microscopy revealed that these structures are characteristically straight leading directly from the plant surface to the xylem bundles with no evidence of meandering or branching as is seen in aphids and whiteflies. The conspicuous clypeus lies on the anterior and ventral part of the head and marks the region of attachment of the powerful cibarial (sucking) pump muscles, which permit the ingestion of remarkable amounts of xylem sap (which is under negative pressure in the plant's vascular system).

Once xylem sap is ingested, it passes through the food meatus, mouth, and esophagus and empties into the anterior portion of the midgut (mg1). After the sap enters the midgut, it passes through the filter chamber (fc), where it is confined to a tubule that is proximate to a series of four Malpighian tubules and a length of the posterior midgut (MG2). The filter chamber is

extremely active in peristaltic movements that evidently increase the efficiency of concentration of the sap and removal of water to the Malpighian tubules, which remove the water and carry it directly into the hindgut where the water is stored in a bladder-like expansion of the hindgut until it can be discharged. The concentrated sap is processed by the midgut where the final nutrient products are absorbed by microvilli that are on the surface of a highly convoluted series of tubles.

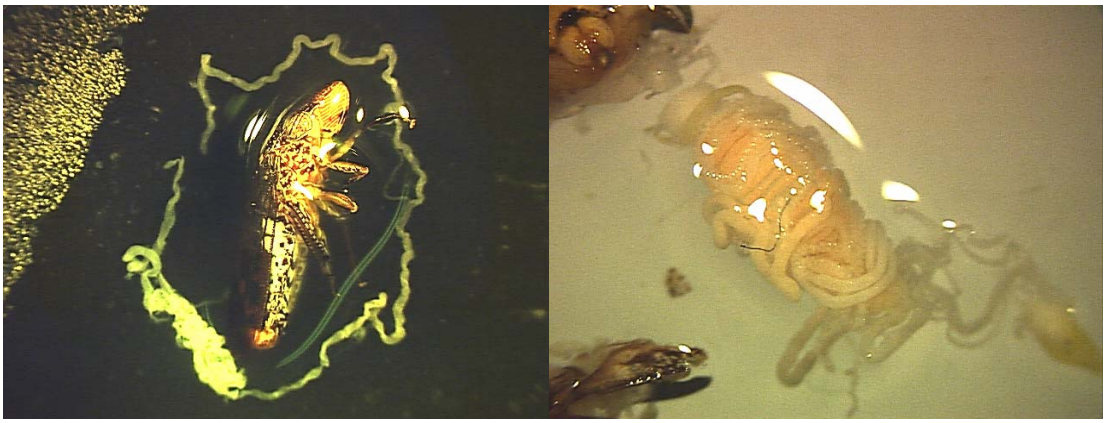
Digestive processes:
We tested for activities of aminopeptidase and general peptidase in the salivary glands (Figure 2-b), filter chamber (Figures 3-a, 3-b), anterior midgut, posterior midgut, and Malpighian tubules of the glassy winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera Cicadéllidae), and of the salivary glands anterior midgut, posterior midgut, and Malpighian tubules of the western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae). Both of these are fluid-ingesting species; however, *H. coagulata* is strictly a xylem sap feeder, and *L. hesperus* feeds on slurries of plant materials extracted from protein-rich tissues after pre-digesting plant tissues using extra-oral digestion. As a xylem sap feeder, *H. coagulata* was expected to lack ability to digest peptides because xylem sap is not known to contain substantial amounts of peptides or proteins. However, we found very high activities of aminopeptidase and general peptidase in the midgut of *H. coagulata*. In fact, the aminopeptidase activity from *H. coagulata* exceeded the activity of that enzyme from comparable regions of *L. hesperus* by several fold (Figures 4-a, 4-b). Given the fact that *L. hesperus* is known to ingest protein-rich foods, this finding provides a basis for re-examining our understanding of the *H. coagulata*-plant interaction.

Interactions between H. coagulata and host plants:
The profiles of free amino acids in the xylem sap in infested and uninfested sweet potato plants reflects an increase in the concentrations of most amino acids in the xylem sap from infested plants. Also, the concentration of ninhydrin positive substances was significantly higher in the sap from infested plants than it was for the uninfested counterpart. Most interestingly, when the xylem sap samples were filtered through molecular weight filters of 3 kDa and 30 kDa, there were higher concentrations of ninhydrin positive substances in the samples that ranged from 3-30 kDa in saps from infested plants than from uninfested plants. This supported the hypothesis that the feeding of *H. coagulata* impacted an increase in the available nitrogenous substances. This finding is in accord with the demonstration of an extremely active aminopeptidase in the midgut of this insect.

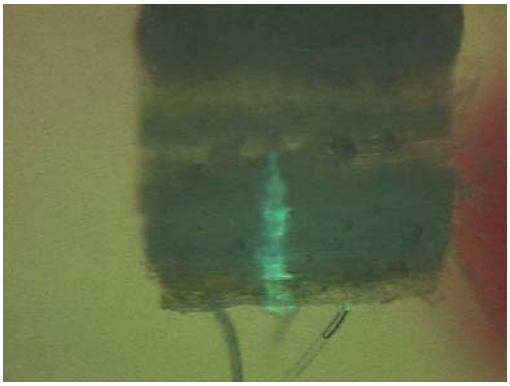
Artificial Diet: Preliminary Trials:
Based on the above findings about complex peptides as part of the feeding profile, a diet was devised to reflect the ability of the H. coaglulata to use such peptides. The following diet is currently being tested and is stimulating feeding response:

Proteose peptone 1.0 g	Cholesterol 0.0012* (*soluble at 0.0002 g/100 mL water)
Asparagine 0.25 g	β-sitosterol 0.0004
Glucose 0.010 g	Water 200 mL
Fructose 0.025 g	Stir until all components are dissolved.
Citric acid (anhydrous) 0.050 g	Filter through 0.22 µm filter
L-ascorbic acid 0.020 g	Final pH is 4.65.
Wesson salts 0.020 g	

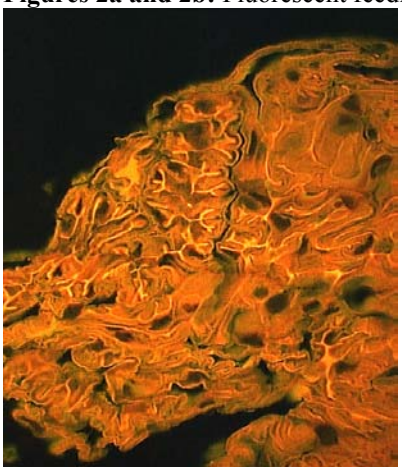
The diet is being presented in small Petri dishes covered with stretched Parafilm, and only a fraction of the nymphs are attempting to feed on the diet in the current presentation format. The mortality is still over 90% of the 1st instar nymphs placed on this diet, but those nymphs that feed last for at least one week and undergo a molt during that time.

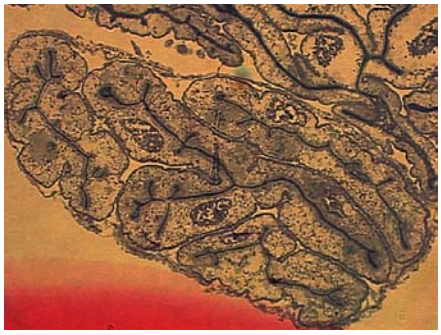


Figures 1-a. GWSS surrounded by dissected gut, **1 b** complete gut, intact.

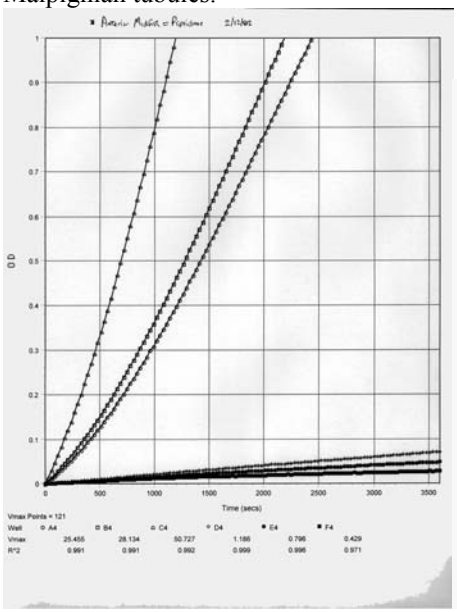


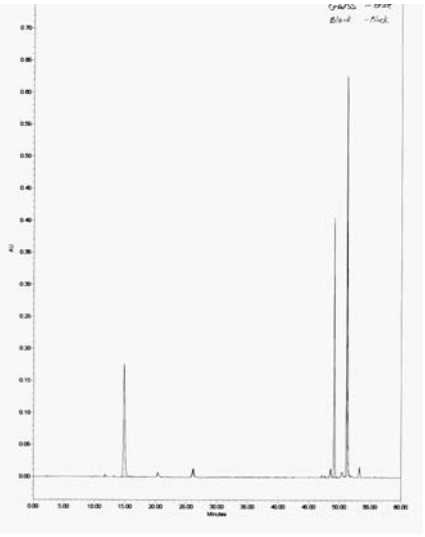
Figures 2a and 2b: Fluorescent feeding sheath in soy petiole and pair of salivary glands.





Figures 3a and 3b: Fluorescent image of filter chamber (100x) and close-up of filter chamber showing arrangement of Malpighian tubules.





Figures 4a and 4b: Kinetics of amino peptidase activity from GWSS posterior mid-gut (upper three lines) compared to activity from posterior midgut of tarnished plant bug (lower three lines), and chromatogram showing destruction of leucyl-glycine by GWSS posterior midgut extract.

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