DEVELOPMENT OF AN ARTIFICIAL DIET FOR THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT

The intent of this project was to advance the development of an artificial rearing system for the glassy-winged sharpshooter, GWSS (*Homalodisca coagulate*), the primary vector of Pierce's disease, PD (*Xylella fastidiosa; Xf*). In order to accomplish this, the simultaneous development of an artificial diet and diet delivery system suitable for insect feeding are needed. Diet formulations based, in part, on previous studies using GWSS (unpublished data), as well as on artificial diets developed for other Hemiptera (Mitsuhashi, 1979; Fu *et al.,* 2001; Coudron *et al*., 2002) and on the xylem chemistry of GWSS host plants (Andersen, *et al*., 1992; Gollan *et al*., 1992) were tested. Diets were evaluated based on their effects on life history analyses. Nitrogen may represent a nutrient limitation for xylem feeders. Therefore, three potential sources of nitrogen, i.e. proteins, peptides and amino acids, were evaluated via artificial diets. The ability of salivary and midgut proteolytic enzymes to digest proteins/peptides (Brandt, *et* al., 2004; Wright, *et al.,* 2006) is another important aspect of this project that would determine whether less costly nitrogen sources could be substituted for those commonly found in plants.

INTRODUCTION

Presently, the rearing of GWSS is labor-intensive and costly because of its dependence on the production of appropriate host plants, with researchers often needing to grow several species of plants to enable them to rear GWSS under optimal conditions. The development of an artificial diet would likely be more cost effective and portable, decreasing the costs and time-constraints associated with maintaining the insect in culture, thereby increasing the availability of high quality insects for Pierce's disease research and enabling researchers to rear this insect on site. The increased availability of GWSS to researchers will likely lead to more rapid developments in novel control measures for this major vector of PD. Furthermore, the coupling of an artificial diet with a suitable delivery system could improve our understanding of the relationship between GWSS nutrition, movement and host range and how they affect PD (including GWSS' varying abilities to acquire/maintain/transmit infectious *Xf* under different circumstances, e.g., via artificial membranes vs. plants, Redak *et al*., 2004). In addition, the diet delivery system alone would have other potential uses, such as studying the interactions between GWSS, *Xf*, and the host plant, as well as in testing potential anti-GWSS and anti-*Xf* control agents. This could be accomplished by incorporating into the feeding system: 1) selected host plant-associated compounds; 2) media containing the causative agent of PD (*Xylella fastidiosa*, *Xf*); 3) control agents including anti-GWSS or -*Xf* compounds (such as proteins to be engineered into host plants to control either GWSS or *Xf*; Dandekar *et al*., 2003; Lin, 2003; Meredith and Dandekar, 2003; Reisch *et al*., 2003) or anti-GWSS microbials (Kaya, 2003; Mizell and Boucias, 2003). In summary, the development of an artificial diet and a corresponding delivery system for GWSS could lead to insights that can be used to generate improved methods for controlling GWSS and, therefore, Pierce's Disease.

OBJECTIVES

1. Evaluate artificial diet delivery systems for rearing the glassy-winged sharpshooter.
2. Formulate and evaluate artificial diets for the development and reproduction of glassy-winged sharpshooter.

RESULTS

Objective 1. Evaluate artificial diet delivery systems for rearing GWSS.

Adult and immature GWSS were presented with over 25 variations of a diet delivery system (modifying both the membrane and presentation) and survivorship was recorded. An effective delivery system was then selected for further adult studies in diet formulation (details summarized below). For the adult delivery system preparation, a 2.5 cm wide and 15 cm long piece of Parafilm was cut, folded along its length and sealed. Approximately 25 ml of each diet formulation was put into the tube. Each tube was hung inside a polycarbonate culture vessel (each single unit measuring 77mm x 77 mm x 77mm, Phyto Technology Laboratory, Shawnee Mission, KS) using a plastic closure (Figure 1). The bottom portion of the tube was folded to exert pressure on the liquid diet inside the tube. The diet tubes were replaced twice a week. Observations on the fate of the adults were made daily.

Immature GWSS preferred a horizontal diet presentation with the most effective delivery a stretched Parafilm pouch that was placed over a screen at the top of the rearing system (Figure 2). In addition, development from 1st through 5th instars was also achieved using a plant surface-based delivery system.

Studies, in collaboration with Jones and Setamou at ARS in Weslaco, have demonstrated continuous feeding by adult GWSS for up to 39 days on artificial diets (Figure 3) presented through the specialized feeding tube that is prepared from Parafilm. In addition, molting was observed with immature GWSS that also fed from this system.

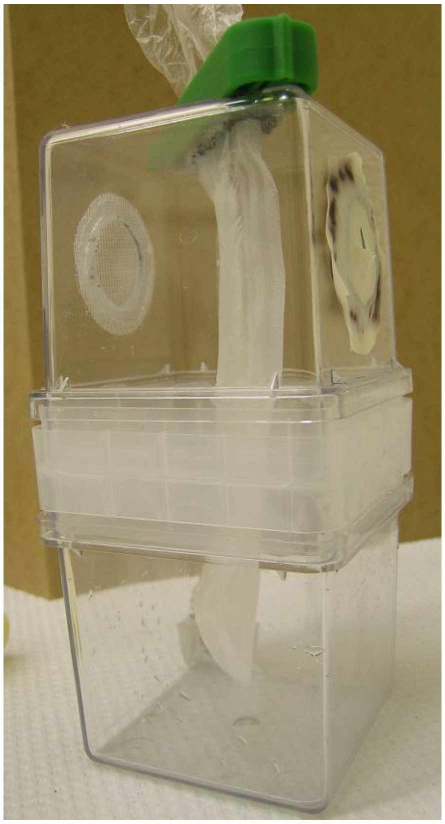

Figure 1. Double rearing system

Figure 2. Single rearing system

Objective 2. Formulate and evaluate artificial diets for the development and reproduction of GWSS
Numerous artificial diet formulations that contained differing combinations and concentrations of fructose, glucose, sucrose, asparagine, glutamine, lysine, cysteine, methionine, arginine, aspartic and glutamic acids, proteins and vitamins were evaluated. These treatment diets were compared to control diets that included plant-reared (cowpea) or artificial presentations that contained sucrose/fructose solutions or xylem extracted from cowpea or sunflower plants. An example of five dietary formulations evaluated for the development of adult GWSS depicts different survival responses by GWSS adults to changes in the carbohydrate and amino acid content of the diet (Figure 3).

[Control diet (5% sucrose) resulted in 50% survivorship of adult GWSS at 11 days. In comparison, 50% of adults feed Diet 5 were alive for 25 days. A significant increase in adult survival was noted with the addition of certain amino acids, such as glutamic acid and aspartic acid (Diet 5). The addition of 1% methionine resulted in a decrease in adult survival (Diet 4). Removal of fructose and glucose from the diet formulation did not reduce survivorship (Diet 3)].

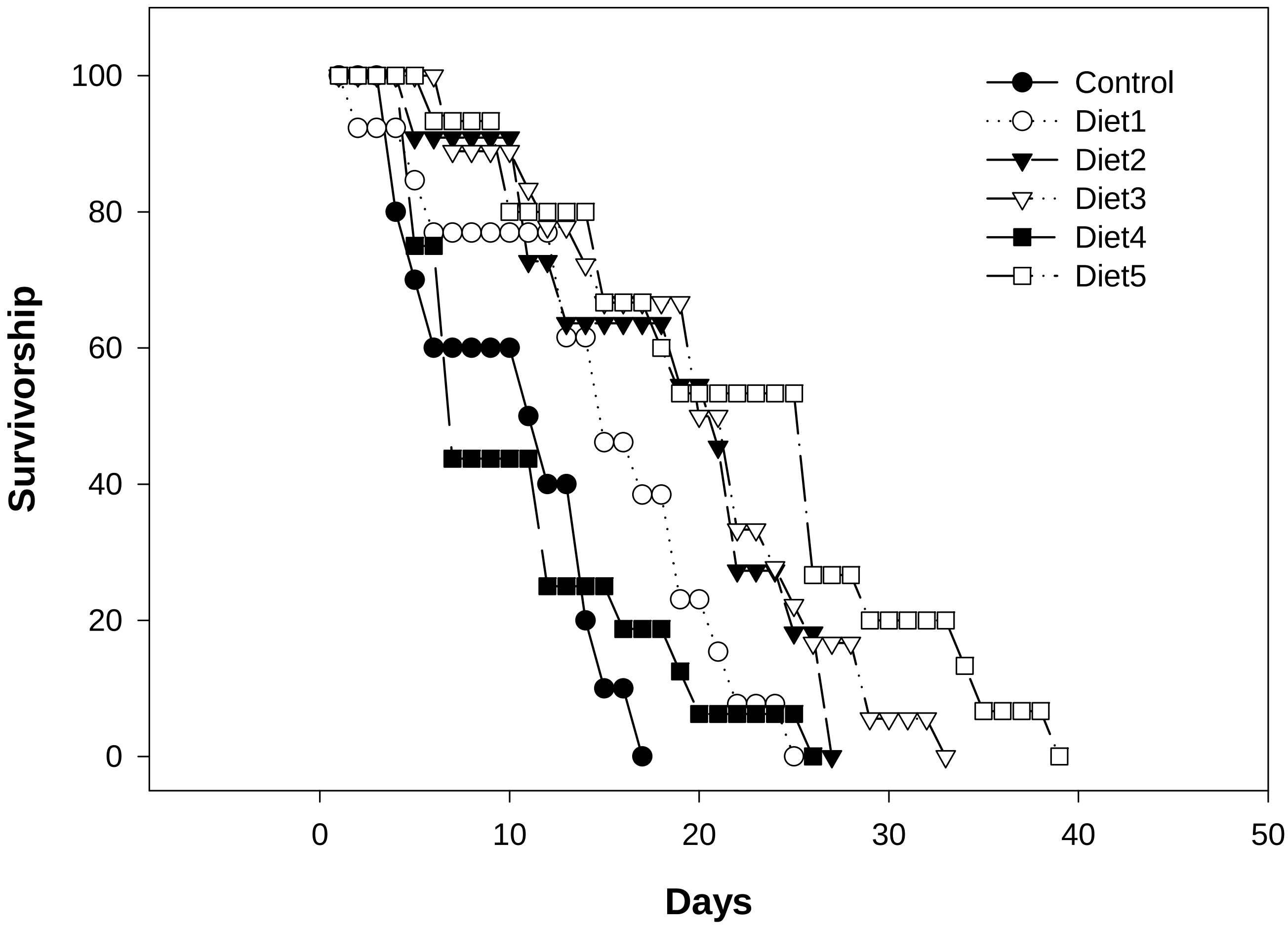

Figure 3. Survival of adult GWSS on artificial diets.

CONCLUSIONS

Our studies provide novel insights for advancing the development of an artificial rearing system: the best performance of GWSS reared on an artificial diet was accomplished through the simultaneous testing of different formulations and delivery system designs, i.e., testing of over 25 diet delivery systems in combination with over 10 diet formulations, and the best performance by nymphal and adult stages was not achieved with the same formulation or delivery system design. Our progress to date makes us confident that the development of an artificial rearing system for GWSS is achievable in the near future. As described in the original proposal, this achievement extends beyond the expectation of the project awarded.

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