DEVELOPMENT OF AN ARTIFICIAL DIET AND EVALUATION OF ARTIFICIAL OVIPOSITIONAL SUBSTRATES FOR THE IN VITRO REARING OF GONATOCERUS SPP. PARASITOIDS OF THE EGGS OF THE GLASSY-WINGED SHARPSHOOOTER

**Project Leader:**
Thomas A. Coudron
*USDA, ARS, BCIRL*
Columbia, MO 65203

**Researcher:**
Cynthia L. Goodman
*USDA, ARS, BCIRL*
Columbia, MO 65203

**Collaborators:**
Walker A. Jones
*USDA, ARS, KDLG Subtropical Agric. Res. Center*
Beneficial Insects Research Unit
Weslaco, TX 78596

Roger Leopold
*USDA, ARS, Red River Valley Agric. Res. Center*
Fargo, ND 58105

**Approximate Reporting Period:** Funding for the study was initiated in October, 2004 and the project is in the start-up phase at the time of this reporting.

**ABSTRACT**
The intent of this project is to develop an in vitro rearing system for one or more of the three mymarid species of *Gonatocerus* currently being reared and released in California to control GWSS. A complete in vitro rearing system will include both a growth-enhancing artificial diet for larval and pupal development as well as a suitable oviposition substrate, or “artificial egg”. Initial studies will formulate artificial diets based on those developed previously for hymenopteran parasitoids, with an emphasis being placed on diets for other egg parasitoids. To accomplish this, *Gonatocerus spp.* eggs and/or larvae will be dissected from host eggs and placed in cell culture plates containing selected diets. Comparisons will be made between the development of parasitoids on these artificial diets, and those developing on the natural host.

**INTRODUCTION**
Surveys of potential biological control agents in Texas (where GWSS is endemic and under natural control) and California revealed that *Gonatocerus* spp. parasitoids are the predominant natural enemy of GWSS in the field, parasitizing between 75-90% of GWSS egg masses (Phillips, 2000; Jones, 2002; Hoddle, 2003a). In California, over 90% of the eggs laid by the second generation of GWSS in late summer and early fall are parasitized by *Gonatocerus* spp., however, only 10 – 50% of the eggs laid by the first generation in the early spring are parasitized (Phillips et al., 2004; Hoddle, 2003b). This suggests that survival of overwintering adult parasitoids is low, or that the current cohort of species of *Gonatocerus* are not effective in parasitizing GWSS eggs early in the season (Hoddle, 2003b; Jones, pers. comm.). However, augmentation of *Gonatocerus* spp. populations in early spring may be able to significantly reduce the population of GWSS that vector the disease later in the season and could be used to reduce pesticide use thereby aiding in the development of a classical biological control program. The current list of species being considered for biocontrol of GWSS in CA include the solitary egg parasitoids *Gonatocerus ashmeadi* (which accounts for 80-95% observed GWSS egg parasitization in California) and *G. triguttatus* (the primary GWSS egg parasitoid in Texas), as well as the gregarious egg parasitoid *G. fasciatus* (which may have a greater host finding efficiency than the other two) (Hoddle, 2003a).

The implementation of current classical and augmentative biological control programs against GWSS has been complicated by a number of factors. Currently, no artificial diet exists for GWSS, and high costs are associated with rearing the sharpshooters in sufficient numbers to provide the necessary quantity of host eggs (Lauziere et al., 2002; Jones, pers. comm.). Long-term stockpiling of host eggs is not feasible at this time because host acceptance declines after refrigeration for 20 days at 13°C, and parasitized eggs only remain viable for 7 days at 2°C (Leopold, 2003). Consequently, augmentation of *Gonatocerus* spp. in many areas of California relies on the labor-intensive process of rearing the parasitoid on host eggs collected from the field (Jones, pers. comm.). Thus, the development of an artificial diet and ovipositional substrate as part of an in vitro mass rearing system for *Gonatocerus* spp. has a number of potential advantages over current rearing techniques. Additionally, in vitro rearing would also be more easily automated, reducing labor costs (Li-Ying, 1992; Qin, Beijing Univ., pers. comm.) and would provide an easier means for studying the reproductive and nutritional physiology of *Gonatocerus* spp.
Efforts to develop an artificial diet capable of supporting larval and pupal development will initially focus on testing established diets formulated for the in vitro rearing of other egg parasitoids, e.g., those used for rearing lepidopteran egg parasitoids including several *Trichogramma* spp. (Hoffman et al., 1975; Li-Ying 1992; Consoli and Parra, 1997; Xie et al., 1997; Grenier et al., 1998; Qin, Beijing Univ. pers. comm.), *Telenomus heliothidis* (Strand et al., 1988), and *Ooencyrtus* spp. (Masutti et al., 1994; Lee and Lee, 1994); a coleopteran egg parasitoid, *Edovum putteri* (Hu et al., 1999; Hu et al., 2001), and a pentatomid egg parasitoid, *Trissolcus basalis* (Volkoff et al., 1992). For studies on the development of an artificial ovipositional substrate, membranes that will be derived from a variety of sources will be tested, such as: oxygen-permeable films used for mass rearing *Trichogramma* spp. (Qin, Beijing University, pers. comm.), parafilm (Wittmeyer et al., 2001; Cooperband and Vinson, 2001), and polycarbonate, polyvinylchloride, polyethylene, and/or polypropylene membranes (Masutti et al., 1994; Morrison et al., 1983; Consoli and Parra 1999).

**OBJECTIVES**

1. Formulate an artificial diet capable of supporting the development and reproduction of *Gonatocerus* spp. parasitoids of the eggs of glassy-winged sharpshooter, *Homalodisca coagulata*.
2. Screen, modify, and evaluate existing materials for their suitability as ovipositional substrates for these egg parasitoids.
3. Develop and optimize an in vitro rearing unit, consisting of an artificial diet and ovipositional substrate, that can be utilized for *Gonatocerus* spp. oviposition, parasitoid development, and release.

**RESULTS AND CONCLUSIONS**

This project has just been funded. Preparation of quarantine facilities is complete and the identification of insect cultures to be used in our studies is underway. The process to hire an additional researcher has been initiated. Preliminary experiments have been conducted in collaboration with Leopold at ARS in Fargo that indicate cold-storage processes should offer suitable method(s) to preserve the natural host of the parasitoid for these studies.

**REFERENCES**


**FUNDING AGENCIES**

Funding for this project was provided by the CDFA Pierce’s Disease and Glassy-winged Sharpshooter Board.