EXPLORATION FOR BIOLOGICAL CONTROL AGENTS IN THE NATIVE RANGE OF THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
Surveys in the native range of the glassy-winged sharpshooter (GWSS) *Homalodisca vitripennis* are continuing to discover nymphal parasitoids and to determine the ecology and phenology of GWSS in undisturbed natural areas. Fifteen sites with stands of native *Vitis* spp. in southeastern Texas have been surveyed monthly from October 2005 to present. The focus is on big-headed flies (Pipunculidae), which are known to be nymphal parasitoids of sharpshooters. Several methods have been used to survey for the parasitic flies, including yellow sticky cards, malaise traps, sweeping, hand collection, and tethered nymphal sentinels. Larval pipuculids have been dissected from hand collected *Oncometopia orbona* feeding on mustang grapes. Numerous adult *Eudorylas* spp. have been collected by sticky traps, sweeping, and malaise traps that may be associated with GWSS. Peak populations of Pipunculidae, including *Eudorylas* and *Tomosvaryella* spp., occurred in February and October. Populations of GWSS began to increase in March and peaked in July. GWSS adults collected in March from survey locations were all positive for the presence of *Xylella fastidiosa* in their foreguts.

INTRODUCTION
The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis,* is native to Northeastern Mexico and the Southeastern U.S., and the origin of the invasive California populations is reported by de León et al. (2004) to be Texas. Most of the entomological and epidemiological information regarding this pest is derived from its status as a vector of Pierce's disease, *Xyllela fastidosa (Xf),* in cultivated hosts. Much less is known about the field ecology and phenology of GWSS and its natural enemies in its native habitat in the Southeastern U.S. Recent surveys in the native range and research on biological control agents has focused on egg parasitoids of GWSS (Mizzell and Andersen 2003, Hoddle and Tripitsyn 2004, Luck et al. 2004, Irwin and Hoddle 2005, Jones et al. unpublished data). *Gonatocerus* spp. egg parasitoids have been collected from the native range of Texas, Florida and Northeastern Mexico, and released in California where several species are now established (CDFA 2004). Nymphal parasitoids of GWSS, including Pipunculidae, have not been evaluated as biological control agents. Skevington and Marshall (1997) review the natural history and rearing of Pipinculidae. They indicate that many pipunculids are oligophagous and show specificity at the genus level. Five new pipunculid-sharpshooter host associations have been documented by Skevington et al. 2006 (submitted). The focus of our research is to discover, identify and evaluate the pipunculid parasitoids of GWSS and other sympatric sharpshooters. We will also use this survey of sharpshooters to determine the seasonal percentage of adults infected *Xf* in native habitats for comparison to agricultural settings in California where GWSS is invasive.

OBJECTIVES
1. Conduct monthly surveys in the native range of GWSS.
2. Determine the phenology and ecology of GWSS and other sharpshooters
3. Determine the species composition of GWSS natural enemies in their native habitat.
4. Develop methods for collection of parasitized GWSS nymphs and adult parasitoids.
5. Investigate the biology and biological control potential of GWSS nymphal parasitoid species.

RESULTS
Fifteen field sites have been established in southeastern Texas (Goolsby and Setamou 2005). The sites are located in eight different biogeographic zones. The transect starts at the southern tip of Texas in the Lower Rio Grande Valley in Weslaco, extending northwest to the Texas Hill Country near New Braunfels, northeast to the Piney Woods near Houston, and south along the coastal plain. Each site has natural stands of native *Vitis* spp. Five yellow sticky cards were placed monthly at each location starting in October 2005.

The mean number of GWSS and *Oncometopia orbona* (F.) adults in yellow sticky card traps for Giddings, TX are shown in Figure 1. The numbers of sharpshooters at this site are consistently high, which may be due to large stands of mustang grape, *Vitis mustangensis* and close proximity to Yegua Creek. *Oncometopia orbona* populations peak in early spring followed by GWSS. This phenology results in nymphal sharpshooter populations throughout the spring and summer which may be exploited by pipunculid parasitoids.

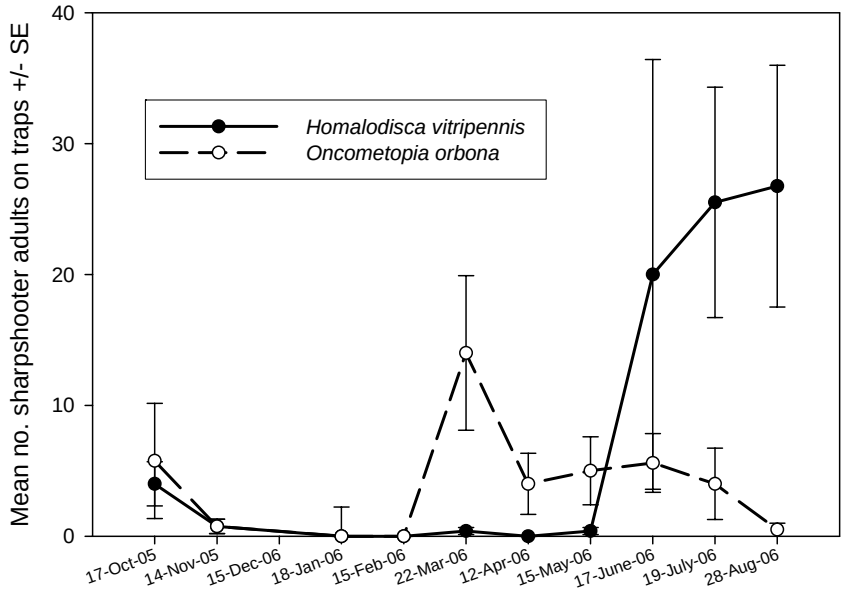

Figure 1. Yellow sticky trap catches of sharpshooter adults adjacent to mustang grape stands near Giddings, TX (Oct. 05- Aug 06).

Pipunculidae have been collected in yellow sticky traps from each survey location in Southeast Texas. Most individuals thus far have been recovered from October to February. Individuals collected in the yellow sticky traps fall into four different genera (Table 1). *Eudorylas* is the most likely parasitoid of GWSS based on its known biology and host associations. Further, an unknown species of *Eudorylas* was collected from *Oncometopia orbona* (F.) near Giddings, TX in October 2005.

Several methods have been investigated for recovery of Pipunculidae in addition to yellow sticky traps, including Malaise traps, hand collecting of adults for dissection and to hold for emergence of parasitic flies, sweeping, and tethering of nymphs. Malaise traps near Weslaco were placed next to naturalized grapes adjacent to a large natural area with diverse vegetation. Several *Eudorylas* sp. adults have been collected from the traps and held for identification. Once the species associated with GWSS has been identified, and the ideal time and location are determined, Malaise traps fitted with dry collection cones could be useful in collecting large numbers of adult pipunculids. Through dissections, pipunculid larvae have been recovered in *Oncometopia orbona* adults (Skevington et al. submitted). Several pipunculid flies have been recovered from sweeping but their host associations are not known. Adults in flight move in a characteristic slow hover, occasionally lighting on plants to feed on honeydew. Hundreds of GWSS adults have been hand collected from the survey locations and held in cages under greenhouse conditions, however without success in rearing out pipunculid adults. It is possible that the parasitic flies emerge from late instar nymphs (which are rare in the field), or the behavior of the parasitized individuals is altered, making them inaccessible for collection. Methods have been developed for tethering of sentinel nymphs to overcome the difficulties of collecting nymphs in the field. Current methods involve tethering of nymphs on silk threads glued to the thorax. The end of the tether can be placed on foliage in the areas frequented by the pipunculid adults. The optimal period for Pipunculidae appears to be late Fall through early Spring. Plans have been made to intensively survey for Pipunculidae during this time and to employ tethered nymphs in the most promising locations.

Table 1. Numbers of Pipunculidae adults collected from March to June 2006 in the yellow sticky traps.

Location	*Eudorylas*	*Tomosvaryella*	*Chalarus*	*Cephalops*
Weslaco	5	6		
George West	11	25		
Pleasanton	0	5	2	
New Braunfels	3	9		
Giddings	1	1		
Hempstead				1
Refugio				1
King Ranch	1			1

Sharpshooters collected in March 2006 were assayed for the presence of *Xf* using molecular techniques developed by Bextine et al. (2005). This date is significant because this is when 'red-winged' GWSS first appear in the field, which is indicative of a new generation of adults. High levels of *Xf* were detected in GWSS (13/13), *O. nigricans* (8/21), *O. orbona* (39/51). The assay of *Xf* in sharpshooter adults will continue for a full annual cycle.

CONCLUSIONS

Adult *Oncometopia* spp. reach a peak in late Spring, followed by GWSS which peaks in mid-Summer. Nymphal populations therefore must be common one to two months prior, which should correspond to peak pipunculid activity. Future efforts will be focused on collecting and evaluating pipunculids using sticky traps, Malaise traps, dissection of hand collected sharpshooters and tethered sentinel nymphs. Pipunculid immatures collected from GWSS and other sympatric sharpshooters will be sequenced and compared with sequence data from known adult populations. This molecular tool may help to determine the identity of the GWSS pipunculid and further refine the search areas and methods. Our goal for the next year is to evaluate these nymphal parasitoids of GWSS as biological control agents and export them to CDFA for mass rearing and release in California, to minimize the impact of GWSS on agricultural producers.

REFERENCES

Bextine, B., M. Blua, D. Harshman, and T.A. Miller. 2005. A SYBR green-based real-time polymerase chain reaction protocol and novel NDA extraction technique to detect *Xylella fastidiosa* in *Homalodisca coagulata*. Journal of Economic Entomology 98: 667-672.

CDFA 2004. Pierce's Disease Control Report to the Legislature: p. 20-24.

de León, J.E., W.A. Jones, and D.J.W. Morgan. 2004. Population genetic structure of *Homalodisca vitripennis* (Homoptera: Cicadellidae), the vector of the bacterium *Xylella fastidiosa* causing Pierce's disease in grapevines. Annals Entomological Society America. 97: 809-818.

Goolsby, J.A. and M. Setamou. 2005. Exploration for biological control agents in the native range of the glassy-winged sharpshooter, pp. 318-320. In Proceeding, Pierce's Disease Research Symposium, 5-7 December 2005, San Diego, CA. Complied by M. Athar Tariq, Peggy Blincoe, Melinda Mochal, Stacie Oswalt, and Tom Esser, Sacramento, CA

Hoddle, M.S. and S.V. Tripitsyn. 2004. Searching for and collecting egg parasitoids of the glassy-winged sharpshooter in the Central and Eastern USA. In: Pierce's Disease Program: Symposium Proceeding, Coronado, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, CA

Irwin, N.A. and M.S. Hoddle. 2005. Determination of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) egg ages suitable for oviposition by *Gonatocerus ashmeadi*, *Gonatocerus triguttatus*, and *Gonatocerus fasciatus* (Hymenoptera: Mymaridae). Biological Control 32: 391-400.

Luck, R.F., C.E. Coviella, and D. Morgan. 2004. Evaluation of olfactory responses by *Gonatocerus ashmeadi*, a parasitoid of glassy-winged sharpshooter egg masses on various host plants. In: Pierce's Disease Program: Symposium Proceeding, Coronado, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, CA

Mizell, R.F. and P.C. Andersen. 2003. Keys to management of the glassy-winged sharpshooter: interactions between host plants, malnutrition, and natural enemies. In: Pierce's Disease Program: Symposium Proceeding, Coronado, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Roger Spenser, Liz Houser, Amadou Ba and Tom Esser, Sacramento, CA

Skevington, J.H. and S.A. Marshall. 1997. First record of a big-headed fly, *Eudorylas alternatus* (Cresson) (Diptera: Pipunculidae), reared from the subfamily Cicadellinae (Homoptera: Cicadellidae), with an overview of pipunculid-host associations in the Nearctic region. Canadian Entomologist 129: 387-398.

Skevington, J.H., J.A. Goolsby and M. Setamou. New Records of Pipunculidae attacking Proconiine sharpshooters – the implications for parasitism of *Homalodisca coagulata* (Auchenorrhyncha: Cicadellidae: Proconiini). Canadian Entomologist (Submitted).

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AN IMMUNOLOGICAL APPROACH FOR QUANTIFYING PREDATION RATES ON THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT

The gut contents of 376 individual predators were assayed for glassy-winged sharpshooter (GWSS) remains using a multitude of ELISAs designed to detect predation on various GWSS life stages. We found that almost 10% of the predators examined contained GWSS remains in their guts. We recorded 10, 17, and 20 predation events on the GWSS egg, nymph, and adult stages, respectively. Of the predators examined in this study, *Collops vittatus* (20.6%) and *Hippodamia convergens* (16.7%) had the highest percentage of individuals positive for GWSS remains. Approximately 10% of five of the other predator species and none of the earwigs tested contained GWSS remains.

INTRODUCTION

Predaceous natural enemies can be important regulators of arthropod populations (Luff, 1983). However, accurately identifying key predators of most pests is very difficult because predators and their prey can often be small, elusive, and cryptic. Hence, visual field observations of predation are extraordinarily difficult to obtain. Perhaps the most frequently used experimental approach for evaluating predaceous natural enemies in the field are through studies conducted in field cages (Luck *et al.*, 1988). Such studies require manipulation of either the natural enemy or the targeted prey population(s) within the cage. Mortality of the pest can be estimated based on the presence or absence of the pest over time (Smith & De Bach, 1942; Luck *et al.*, 1988). Such studies have documented the qualitative impact of manipulated predator assemblages on many types of pests, but they do not provide quantitative information on predation rates or evidence of which predator in the assemblage is exerting the greatest biological control. Often the only direct evidence of arthropod predation can be found in the stomach contents of predators. Currently, the state-of-the-art predator stomach content assays include immunoassays (typically ELISA) for the detection of pest-specific proteins (Hagler & Naranjo, 1996) and PCR assays for the detection of pest-specific DNA (de León *et al.*, 2006).

ELISAs using pest-specific monoclonal antibodies (MAbs) have been widely used to identify key predators of certain pests, including the glassy-winged sharpshooter (GWSS) (Fournier *et al.*, 2006). The simplicity and low cost of ELISA lends itself to the efficient screening of hundreds of field-collected predators per day (Hagler & Naranjo, 2005). However, MAb development is technically difficult, costly, and time consuming for wide scale appeal (Greenstone, 1996). Moreover, pest-specific ELISAs share the same limitation as the other predator evaluation methods; the quantification of predation rates is impossible (see Hagler & Naranjo, 1996 for a review). PCR assays using pest-specific DNA probes might be less expensive to develop (Greenstone & Shufran, 2003), but PCR assays are also not quantifiable and they are more costly, technical, tedious, and time consuming than ELISAs (pers. obs.). These difficulties have resulted in a dearth of information on the quantitative impact that generalist predators have on suppressing pest populations.

The many shortcomings of each method of predator assessment described above were the impetus for us to develop a technique to more efficiently quantify predator activity. The technique combines our previous research using pest-specific MAb-based ELISAs to detect predation (Fournier *et al*., 2006) with protein marking ELISAs developed to study arthropod dispersal (Hagler *et al.*, 2002; Jones *et al*., 2006). In this study we erected 40, 1-m long field cages on selected citrus branches. We then placed (using a paper clip) a sentinel GWSS egg mass (ca. 6 to 12 eggs per mass) on the underside of a randomly selected leaf in each cage along with two individuals each of *Hippodamia convergens*, *Collops vittatus*, *Chrysoperla carnea*, *Labidura riparia*, *Geocoris punctipes*; and one individual each of *Sinea confusa* and *Zelus renardii*. One hour later, we released two GWSS adults and two nymphs into each cage. The four mobile GWSSs were each marked with a different protein before they were introduced into each cage. After 6 hours, each citrus branch was cut at its base, just below each cage, and immediately frozen on dry ice. Each predator was then analyzed by four protein-specific ELISAs to determine if they contained marked GWSSs in their guts. Additionally, the gut contents of each predator was examined by a GWSS egg-specific sandwich ELISA (Fournier *et al*., 2006) to determine the frequency of predation on GWSS eggs.