IDENTIFYING KEY PREDATORS OF THE GLASSY-WINGED SHARPSHOOTER IN A CITRUS ORCHARD

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Reporting Period: The results reported here are from work conducted October 1, 2005 to October 1, 2006.

ABSTRACT

Over 1,500 predators were screened for glassy-winged sharpshooter (GWSS) remains using a GWSS egg-specific monoclonal antibody (MAb) and several GWSS-specific genetic markers. Specimens were collected in 2002 and 2003 from a citrus orchard (Riverside, CA) harboring high densities of GWSS. We found that 6.2% of all specimens examined tested positive for GWSS remains. The most frequent predators to test positive included the assassin bug, *Zelus renardii* (Kolenati) (Hemiptera: Reduviidae) and the spiders *Trachelas pacificus* Chamberlin and Ivie (Araneae: Corinnidae) and *Olios* sp. (Araneae: Sparassidae) with 41, 22, and 19% of the specimens testing positive with either ELISA and/or PCR, respectively.

INTRODUCTION

Effective control of GWSS will require an areawide integrated pest management approach (AW-IPM). A major component of AW-IPM is the exploitation of the pest's natural enemies, which, when utilized to their greatest potential, can increase the effectiveness of other control tactics. Very little information exists on GWSS's predaceous natural enemies. Identifying the impact of predators can be challenging as they are usually small, elusive, nocturnal or cryptic. Direct visual field observations of predation are rare and often difficult to obtain. While predation studies using enclosures can provide some indication of predator impact, it fails to recreate natural conditions and can result in an overestimation of predation. A more valid method to qualitatively identify predators of key pests in nature is by the molecular analysis of predator gut contents for pest remains (reviewed in Sheppard and Harwood 2005). The state-of-the-art predator stomach content analyses include both MAb-based enzyme-linked immunosorbant assays (ELISA), which detect prey-specific proteins (Hagler et al. 1994ab, Schenk and Bacher 2004), and polymerase chain reaction (PCR)-based assays, which detect prey-specific DNA (Zaidi et al. 1999, Agustí et al. 2003). While DNA-based approaches reveal the prey identity at the species-level, they are unable to indicate which prey life stage is consumed. In contrast, pest-specific and life stage-specific MAbs can target a particular life stage of a given species, providing a higher level of precision to document predation (Hagler and Naranjo 1996). Combining both assays can provide a powerful tool to study predation on the GWSS.

To this end, genetic markers were designed using the cytochrome oxidase gene subunit I (COI) to detect and amplify a GWSS-specific fragment (de León et al. 2006), and a GWSS-egg specific MAb was developed to detect GWSS egg-specific protein (Hagler et al. 2002, Fournier et al. 2006).

OBJECTIVE

The main objective of this research is to identify the key predators of the different life stages of GWSS. More specifically, our aim is to determine the proportion of predators feeding on the various GWSS life stages in a citrus orchard. Using GWSS-specific ELISA and PCR assays, we examined the guts of 1,507 field-collected generalist predators. Results obtained from this research will aid in evaluating the efficacy of generalist predators for conservation biological control program.

RESULTS

Generalist arthropod predators were collected during 2002 and 2003 from a citrus orchard located at the Agricultural Operations Farm at the University of California, Riverside, CA. Collections were performed by beating the foliage or fogging the citrus trees with pyrethrum insecticide. Densities of GWSS were recorded as well (Blua and Akey, unpublished data). For each group of predators, we conducted lab trials to generate negative controls (i.e. individuals with no GWSS remains in their guts) and positive controls (i.e. individuals fed GWSS). Predators were frozen, sorted and then screened for GWSS remains with GWSS egg-specific sandwich ELISA and GWSS-specific PCR assays. Materials and methods employed were similar to the ones described in Fournier et al. (2006) and de León et al. (2006). Predators were scored positive for prey remains if the 197-bp specific GWSS DNA fragment was successfully amplified. With ELISA, specimens

were scored positive if they yielded an optical density response five standard deviations above that of their respective negative control mean (Sutula et al. 1986).

Table 1 reports the PCR and ELISA results for all the predator specimens collected from the citrus orchard (N=1,507). Our study showed that 6.2% of all specimens were found positive for GWSS remains. True bugs and spiders were the two groups with the highest percentages of positives, with respectively 28 and 18% of the specimens testing positive with ELISA and/or PCR. Among these groups, *Zelus renardii* (Hemiptera: Reduviidae), *Trachelas pacificus*, (Araneae: Corinnidae), and *Olios* sp. (Araneae: Sparassidae) were the most common predators of GWSS, with respectively 41, 22, and 19% of the specimens testing positive. Earwigs (N=661) and beetles (N=465), the two groups comprising the greatest number of specimens collected, only yielded 5.0 and 2.6% positive reactions, respectively.

Table 1. Results from predator gut content analyses using GWSS-specific PCR and ELISA. Predators were collected from GWSS-infested citrus trees in Riverside, CA.

Predator Group	N	% ELISA positive (a)	% PCR positive (b)	% overall positive (c)
True bugs (Hemiptera)	25	20%	23%	28%
Ants (Hymenoptera)	121	2.5%	1%	3.3%
Spiders (Araneae)	198	12%	12%	18%
Beetles (Coleoptera)	465	1%	2%	2.6%
Earwigs (Dermaptera)	661	2%	3.5%	5%
Others (various orders)	37	2.7%	0%	2.7%
Total	**1,507**	**3.2%**	**3.8%**	**6.2%**

(a) an individual was determined "positive" if GWSS egg-specific MAb detected egg protein in its gut.
(b) an individual was determined "positive" if GWSS-specific fragment was successfully amplified from its gut.
(c) % of specimens that tested positive for GWSS remains with either one, or both types of gut assay.

CONCLUSIONS

In contrast to our previous study (Fournier et al. 2005; Fournier et al., in preparation), in which predators (N=1,235) were collected from various ornamental plants in the urban areas of Bakersfield, CA and assayed with identical ELISA and PCR probes presented here, the current analyses with citrus-collected predators revealed a much lower percentage of overall specimens yielding positive response for GWSS remains (6.2% compared to 14.8%). Among other things, this observation is likely to be due to the differences in GWSS populations and predator complexes between the two systems. For instance, the host plants from which specimens were collected in the urban settings harbored higher abundance of spiders from the families Clubionidae, Salticidae and Agelenidae, which commonly prey upon GWSS. Similarly, lacewings and praying mantis were much more abundant in the urban settings than in the citrus orchard and commonly tested positive for GWSS remains.

Here we successfully implemented a GWSS-specific ELISA and PCR assay to analyze the guts of field-collected predators. Once the key predators of the various life stages of GWSS are identified, this information can be used to develop more ecologically-based management programs to control GWSS in California.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, the University of California Pierce's Disease Grant Program, and the USDA Agricultural Research Service.

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

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Reporting Period: The results reported here are from work conducted January 1, 2006 to September 30, 2006

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.