

IDENTIFYING KEY PREDATORS OF THE VARIOUS GLASSY-WINGED SHARPSHOOTER LIFESTAGES

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ABSTRACT

Using glassy-winged sharpshooter (GWSS) egg-specific monoclonal antibody (MAb) and GWSS-specific genetic markers that we previously developed and optimized, the guts of field-collected predators were screened for the presence of GWSS remains. We have examined the guts of over 700 generalist predators and our analyses revealed that frequent predators of the GWSS include spiders, assassin bugs, lacewings and praying mantis.

INTRODUCTION

Effective control of GWSS will require an area-wide integrated pest management approach (AW-IPM). A major component of Area-wide-Integrated Pest Management 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. 2004, de León et al. submitted), and a GWSS-egg specific MAb was developed to detect GWSS egg-specific protein (Hagler et al. 2002, Fournier et al. 2004, Fournier et al. submitted).

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 nature. Using GWSS-specific ELISA and PCR assays, we examined the guts of several hundred field-collected generalist predators. Results obtained from this research will aid in evaluating the efficacy of generalist predators for a conservation or an inundative biological control program.

RESULTS

From 2002 to 2004, generalist arthropod predators were collected from various species of shrubs and ornamental trees located in 20 sites in urban areas of Bakersfield, California. For each group of predators, lab trials were conducted to generate negative controls (i.e. individuals with no GWSS remains in their guts) and positive controls (i.e. individuals fed GWSS). Frozen specimens were shipped to USDA-ARS, Phoenix and screened by a GWSS egg-specific ELISA and a GWSS-specific PCR assay. All individuals were first homogenized in phosphate buffered saline and then aliquoted into two Eppendorf tubes in order to perform the two different assays (ELISA and PCR). For PCR assays, DNA was extracted using DNeasy tissue kit (Qiagen, protocol for insects). DNA samples were then subjected to the primer set HcCOI (forward 5'-

GGGCCGTAAATTTTACC-3' and reverse 5'-ACCACCTGAGGGGTCAAAA-3'; GenBank accession number AY959334) which amplifies a 197-bp GWSS fragment (de León et al. submitted). A sandwich ELISA was conducted on each predator using the modified protocol described by Hagler (1998). Predators were scored positive for prey remains if they yielded an ELISA response five standard deviations above that of their respective negative control mean (Sutula et al., 1986).

Table 1 reports the results of both PCR and ELISA tests for a sub-sample of field-collected predator specimens (N=795). We found that: 1) spiders, true bugs and praying mantis are common predators of motile GWSS life stages, and 2) lacewing is a common predator of the egg stage. Figure 1 shows the PCR results for the assassin bugs (*Zelus renardii* Kolenati) assayed. The analysis revealed that 2 of the 27 individuals contained sharpshooter DNA in their guts. Figure 2 shows the ELISA results for the field-collected lacewings (*Chrysoperla carnea* Stephens) assayed. The ELISA revealed that 8 of the 98 individuals tested contained sharpshooter egg antigen in their guts. The relatively high frequency of positive ELISA reactions suggests that lacewing may be a potential biological control candidate for GWSS eggs. Further ELISA and PCR assays are underway testing thousands of predators representing many additional species (e.g. beetles, ants, earwigs, other groups of spiders, etc), as well as specimens collected from different GWSS-infested crops (e.g., citrus).

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

Predator Group	N	PCR positive ^a (%)	ELISA positive ^b (%)
Arachnids (Spiders)	588	40 (7%)	66 (11%)
Hemipterans (True bugs)	61	13 (21%)	8 (12%)
Lacewings	98	8 (8%)	8 (8%)
Praying mantis	48	5 (10%)	2 (4%)
Total	795	66 (8%)	84 (10.5%)

^a an individual was determined "positive" if GWSS-specific fragment was successfully amplified from its gut.

^b an individual was determined "positive" if GWSS egg-specific MAb detected egg protein in its gut.

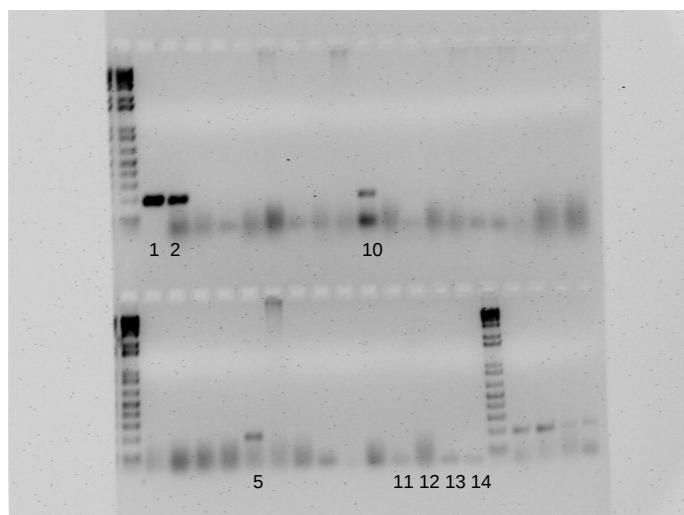


Figure 1. This gel presents the results of a PCR assay designed to detect GWSS remains in the gut of field-collected assassin bugs (N=27) using a GWSS-specific COI primer. The gel shows that GWSS DNA fragment (197 bp) was amplified from the following samples: positive control #1 (GWSS; the 1st sample of the upper gel), positive control #2 (*Z. renardii* that ate GWSS; the 2nd sample of the upper gel); two field-collected specimens (10th and 5th sample of the upper and lower gel, respectively). No amplification occurred for any of the negative controls (individuals that did not consume any GWSS: the 11th and 12th sample of the lower gel; and controls in which DNA extract was replaced by water: the 13th and 14th sample of the lower gel). Beyond the 14th sample, the lower gel reports results for a different species of predator.

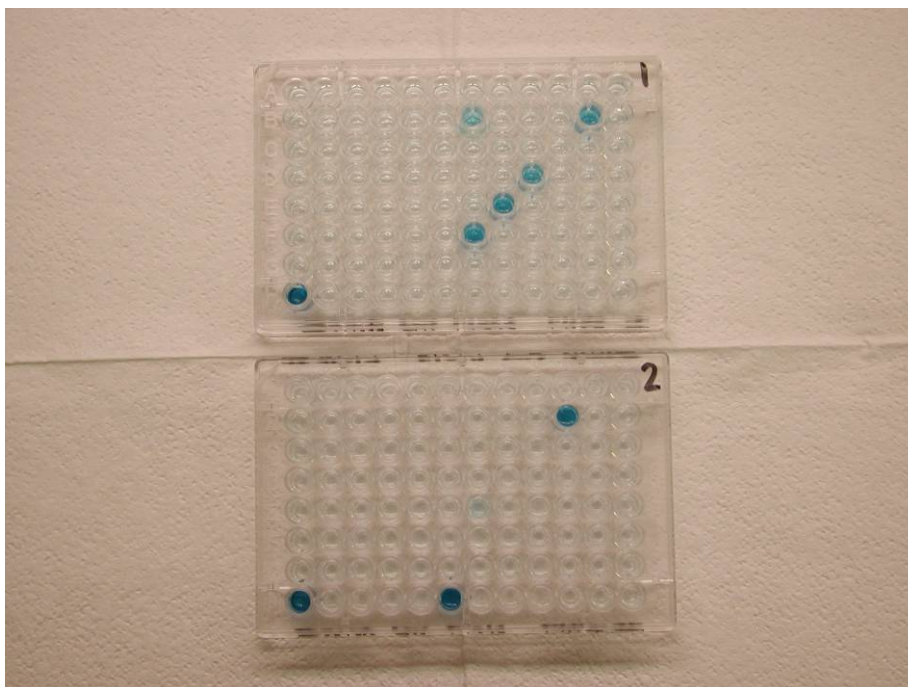


Figure 2. These ELISA plates present the results of an immunoassay designed to detect GWSS remains in the gut of field-collected lacewings (N=98) using a GWSS egg-specific MAb. Each plate included 1 positive control (=GWSS egg located in the lower left corner of each plate) and 24 negative controls (=C. carnea individuals that did not consume any GWSS egg located in columns 2, 3, and 4 of each plate). Blue coloration indicates that GWSS egg protein was detected in the sample (=positive reaction). The immunoassay revealed that 8 of the field-collected individuals examined here contained GWSS egg antigen in their gut. No positive reactions were recorded for the negative controls.

CONCLUSIONS

There has been increasing awareness over the past decade of the importance of generalist predators for biological control of insect pests (reviewed in Symondson et al. 2002) and predator gut content analyses offer a unique means for studying trophic interactions between predators and prey. 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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