IDENTIFYING KEY PREDATORS OF THE VARIOUS GLASSY-WINGED SHARPSHOOTER LIFESTAGES

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ABSTRACT
Glassy-winged sharpshooter (GWSS) egg-specific monoclonal antibody (MAb) and GWSS-specific genetic markers have been developed for use as diagnostic tools for predator gut content analysis. Feeding trials were conducted to determine how long a MAb-based ELISA can detect GWSS remains in the guts of *Chrysoperla carnea* and *Harmonia axyridis*. We found that *C. carnea* can yield positive ELISA reaction for the presence of GWSS egg antigen for up to 24 hours after eating an egg. Further results showed that the detection period of GWSS egg antigen in *H. axyridis* is less than 6 hours. Using mitochondrial COII primers specific to GWSS, we obtained successful amplification of GWSS DNA fragments from *H. axyridi* that consumed six GWSS eggs. Optimization tests are underway to increase the efficacy of GWSS-specific genetic primers to detect pest DNA in predator guts. Feeding trials with additional predators (*Zelus renardii*, *Sinea diadema*, and several spider species) are currently being performed.

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. Unfortunately, very little information exists on GWSS's predaceous natural enemies. Evidence of predation of GWSS eggs and adults has been observed in the field (JH pers. obs.); however, the composition of the predator complex, and the relative impact of each predator on GWSS mortality is unknown. A major obstacle is the difficulty of studying predators in their natural environment. Unlike parasitoids, predators rarely leave evidence of attack. Laboratory experiments can be used to evaluate the suitability of particular prey and the rates of predation. However, lab studies seldom translate to field situations. Direct field observations are sometimes used to identify predators of key pests, but the small size and cryptic nature of predators and GWSS make direct observations difficult and laborious. Predator gut content analysis represents a valid approach to investigate predation. Currently, the state-of-the-art predator stomach content assays include enzyme-linked immunosorbant assays (ELISA) for the detection of prey-specific proteins (Hagler 1998; Hagler & Naranjo 1994ab) and polymerase chain reaction (PCR) assays for the detection of prey-specific DNA (Symondson 2002). To this end, we have developed GWSS egg-specific MAbs (Hagler et al. 2002; Fournier et al. submitted) and GWSS-specific primers (de León & Jones 2004). Both assays provide an avenue to qualitatively assess the impact of predator species on GWSS populations.

OBJECTIVES
Our main objective is to identify the composition of the GWSS predator complex using pest-specific ELISA and PCR assays. However, several optimization studies are needed (e.g. detectability half-life) before these assays can be used to examine field-collected predators. Here we report results of laboratory tests on detection periods of GWSS egg antigen in the guts of two generalist predators, the green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) using a GWSS egg-specific ELISA. We also present preliminary results on predator gut content analysis using PCR.

RESULTS:
ELISA Response to Lacewing that Consumed GWSS Eggs
Predators were placed individually in Petri dishes and starved for 36 h. Lacewings were then fed one or two GWSS eggs (within a 30-min time frame) and isolated from food for 0, 6, 9, 12, 24, or 36h at 25°C, photoperiod of 16:8h (L:D), and then frozen (-80ºC). Negative controls were individuals that did not eat any GWSS eggs. Each lacewing was analyzed by indirect

ELISA for the presence of GWSS egg antigen (methods described in Hagler et al. 2002). Data indicate that the number of ELISA positive reactions decreased over time (Table 1). All negative controls yielded negative ELISA absorbance values. Significant differences between the mean absorbance of values of the lacewings fed GWSS eggs and their negative control counterparts was found in all post-feeding time intervals, except for time=24 and 36 h.

Table 1. ELISA results testing for the presence of GWSS egg antigen in the guts of *Chrysoperla carnea* (3rd instar larva).

Treatments [a]	Negative Control			Lacewing fed with GWSS eggs		
	Absorbance at 405 nm, mean ± SD	Critical value[b]	% positive reactions (N) [c]	Absorbance at 405 nm, mean ± SD	% positive reactions (N)	Signifi-cance[d]
0h	0.089±0.003	0.098	0 (15)	0.526±0.488	95 (19)	***
6h	0.072±0.006	0.090	0 (22)	0.176±0.142	62 (21)	***
9h	0.076±0.004	0.088	0 (19)	0.197±0.167	76 (21)	**
12h	0.074±0.007	0.095	0 (21)	0.147±0.149	43 (23)	*
24h	0.077±0.008	0.101	0 (14)	0.170±0.180	36 (22)	N.S.
36h	0.073±0.005	0.088	0 (22)	0.072±0.011	0 (22)	N.S.

[a] post-GWSS egg consumption intervals (hour).
[b] Mean + 3SD of the negative controls (Sutula et al. 1986).
[c] Based on the critical value of the negative control predators. N=total no. of individuals assayed for each treatment.
[d] Significant differences (*t* test) between negative control predators and their counterparts fed GWSS eggs: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; N.S., not significant.

ELISA Response to Multicolored Asian Lady Beetle that Consumed GWSS Eggs
Adult beetles were placed in individual Petri dishes and starved for 36 h. Each adult was fed six GWSS eggs (within a 60-min time frame) and isolated from food for 0 or 6h and then frozen (-80ºC). Negative controls were individuals that did not eat any GWSS eggs. We analyzed the dissected gut of each individual by indirect ELISA for the presence of GWSS egg antigen. All negative controls yielded negative ELISA absorbance values. We found that 65% of the individuals that ate GWSS eggs scored positive at time=0 h, and 8% at time=6h. A significant difference between the mean absorbance values of the beetles fed GWSS eggs and their negative control counterparts only occurred for the time=0h treatment.

Predator Gut Content Analysis Using PCR Assays
We are currently optimizing a PCR assay to detect GWSS DNA in the guts of various species of predators. Several pairs of primers were designed to amplify GWSS-specific fragments from: (1) randomly amplified polymorphic DNA (RAPD) based on sequence characterized amplified regions (SCAR); and (2) the mitochondrial cytochrome oxydase subunit I (COI) and subunit II (COII) genes (de León & Jones 2004). The size of amplified fragments of GWSS DNA varies from 166 to 302 bp. Adult *H. axyridis* fed six GWSS eggs were immediately frozen (-80ºC) after eating. Negative controls were beetles that did not eat any GWSS eggs. Each individual was homogenized in a lysis buffer solution, DNA was extracted using a DNeasy kit (Qiagen Inc., Valencia CA) and subjected to PCR using GWSS-specific COII primers. GWSS DNA was successfully amplified from *H. axyridis* extracts (Figure 1). Further tests are underway comparing the efficacy of different primer sets and determining the half-life detection interval of GWSS DNA in the guts of several predator species (*C. carnea*, *Z. renardii*, *S. diadema*, and several species of spiders).
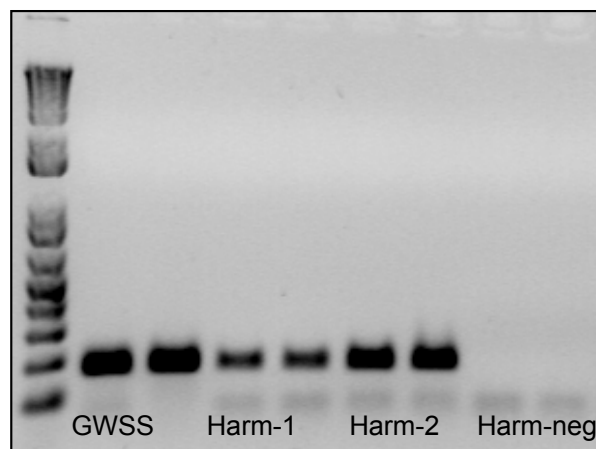
Figure 1. PCR assays were performed using GWSS-specific COII primers on *Harmonia axyridis*. This 2% agarose gel shows that GWSS DNA fragment (178bp) was amplified from the following samples (duplicates): positive control (GWSS), predators fed six GWSS eggs (Harm-1, Harm-2). No amplification occurred for the *H. axyridis* individual that did not consumed any GWSS eggs (Harm-neg).

CONCLUSIONS

We showed that molecular gut content assays can be used to detect GWSS remains in the guts of predators. Once optimization tests are complete we will assay extensive numbers of field-collected predators. We will be able to distinguish specimens that preyed upon immature and adult life stages of the GWSS via the PCR assay and those that consumed eggs via the ELISA assay. An understanding of the key natural enemies of GWSS will contribute to an areawide IPM approach for GWSS control. Once key predators are identified they can be better exploited for conservation and augmentative biological control programs.

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