

# PINPOINTING PREDATION EVENTS: A DIFFERENT MOLECULAR APPROACH

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## ABSTRACT.

A glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, protein marking system has been developed as a diagnostic tool for quantifying predation rates via gut content analysis. A field study was conducted to quantify predation rates on each of the GWSS lifestages. Specifically, two GWSS nymphs and two adults were marked with either rabbit IgG, chicken IgG, milk protein, or soy protein and then released into field cages containing a known assemblage of predators. Additionally, a sentinel GWSS egg mass was placed in each cage. In turn, the stomach contents of every predator in each cage was examined by four different protein-specific ELISAs and a GWSS egg-specific ELISA to detect for the presence of the targeted prey items. Here we present the results obtained for two of the predators examined; the convergent lady beetle, *Hippodamia convergens* and the praying mantis, *Stagmomantis carolina*. ELISA results indicated that two of the nine praying mantids examined fed on a single GWSS adult while five and two of the 78 lady beetles fed on a single GWSS adult and nymph, respectively. There was no GWSS egg predation detected and none of the predators consumed multiple GWSS prey.

## INTRODUCTION.

Predators can be important regulators of arthropod populations (Luff 1983). However, accurately identifying key predators of most pests is difficult because predators and their prey are often 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 is the cage study (Luck *et al.* 1988). Such studies require manipulation of either the natural enemy or the targeted prey population(s) within the cage. Pest mortality 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), *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) (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 too 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 (reviewed by Hagler & Naranjo 1996). 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 (Fournier *et al.* 2008). 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 more efficient screening technique for predator activity. Our goal is to: **(1)** quantify predation rates on GWSS nymphs and adults and **(2)** qualify predation on GWSS eggs. Using a multiple prey marking technique (Hagler 2006) and a GWSS egg-specific MAb (Fournier *et al.* 2006) we simultaneously examined the gut contents of predators for the presence of five GWSS prey items (e.g., GWSS egg protein, two protein marked nymphs and two protein marked adults). Here, we provide a summary of the gut content analyses for two of the predator species examined.

## MATERIALS AND METHODS.

### Laboratory Study.

The first experiment was conducted to determine if protein markers can be substituted for pest-specific MAbs in the immunological detection of prey in predator guts. GWSSs were not used in this study because the feeding study was conducted in an area quarantined for the GWSS (Phoenix, Arizona). Instead, we selected predators and prey for the feeding trials that represent extreme-case scenarios for detecting predation using molecular gut content assays. The “easy-case” scenario was a striped earwig, *Labidura riparia* (Pallas) (Dermaptera: Labiduridae), a large chewing predator, feeding on a large protein marked pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) larva. The “tough-case” scenario was a minute pirate bug, *Orius tristicolor* (White) (Heteroptera: Anthocoridae), a small piercing and sucking predator, feeding on a very small protein marked parasitoid, *Eretmocerus* sp. (Hymenoptera: Aphelinidae). In a series of lab studies, we fed these predators prey items marked with rabbit immunoglobulin G (IgG). In turn, the gut contents of each predator was analyzed by a rabbit IgG-specific ELISA to detect for the presence of rabbit IgG in their gut. Details of the methods used are given in Hagler (2006).

### Field Study.

Field studies were initiated to quantify predation rates on GWSS nymphs and adults using multiple protein markers and qualify predation on GWSS eggs using a GWSS-specific sandwich ELISA. Details of the experimental design are provided by Hagler (2006) and Hagler *et al.* (submitted). Briefly, we erected 40, 1-m long field cages on selected citrus branches. We then placed (using a paper clip) a single sentinel GWSS egg mass containing 6 to 12 eggs per mass on the underside of a randomly selected leaf in each cage along with two individuals each of the convergent lady beetle, *Hippodamia convergens* Guérin Méneville (Coleoptera: Coccinellidae), *Collops vittatus* Say (Coleoptera: Melyridae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *L. riparia*, *Geocoris punctipes* Say (Heteroptera: Lygaeidae); and one individual each of *Sinea confusa* Caudell (Heteroptera: Reduviidae) and *Zelus renardii* Kolenati (Heteroptera: Reduviidae). Moreover, a single praying mantis, *Stagmomantis carolina* (Johannson) (Mantodea: Mantidae), was placed in nine of the cages. One hour later, we released two uniquely marked GWSS adults and two uniquely marked nymphs into each of the 40 cages. The GWSS nymphs were marked with either non-fat dry milk or chicken IgG protein and the adults were marked with either rabbit IgG or soy milk protein.

After 6 h, 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.

## RESULTS.

### Laboratory Study.

The feeding studies showed that, regardless of the predator species and the size of protein-marked prey consumed, that the prey marking ELISAs can easily detect the mark in the predator's stomach for at least 12 h after feeding (Table 1; Hagler 2006). These results suggest that this marking technique will be effective for identifying key predators of marked GWSSs.

### Field Study.

All nine of the praying mantids were recovered from the field cages after the 6 h exposure period. The multiple gut content ELISA results for these nine individuals are presented in Fig. 1A. The results showed a strong positive response by individual 4 and 8 to the rabbit IgG ELISA. These data indicate that these two individuals consumed the rabbit IgG marked GWSS adult released into their respective cages. There was no evidence of predation on the GWSS nymph or egg lifestages.

Eighty lady beetles were released into the 40 field cages containing the marked GWSS prey items (2 per cage). Of these, 78 were recovered after the 6 h exposure period. The fate of the two missing beetles is unknown, but is likely due to escape

from the cages, intraguild predation, or being overlooked in the sorting process. The multiple gut content ELISA results for the 78 individuals recaptured after the 6 h study interval are presented in Fig. 1B. The ELISA results indicate that individual 7, 15, 38, 51, and 68 fed on a single GWSS adult (indicated by a positive soy or rabbit IgG ELISA reaction) and individual 60 and 76 fed on a single GWSS nymph (indicated by a positive milk or chicken egg white ELISA reaction); respectively. There was no evidence that any of these individuals fed on more than one GWSS. Moreover, there was no evidence that the beetles fed on GWSS eggs.

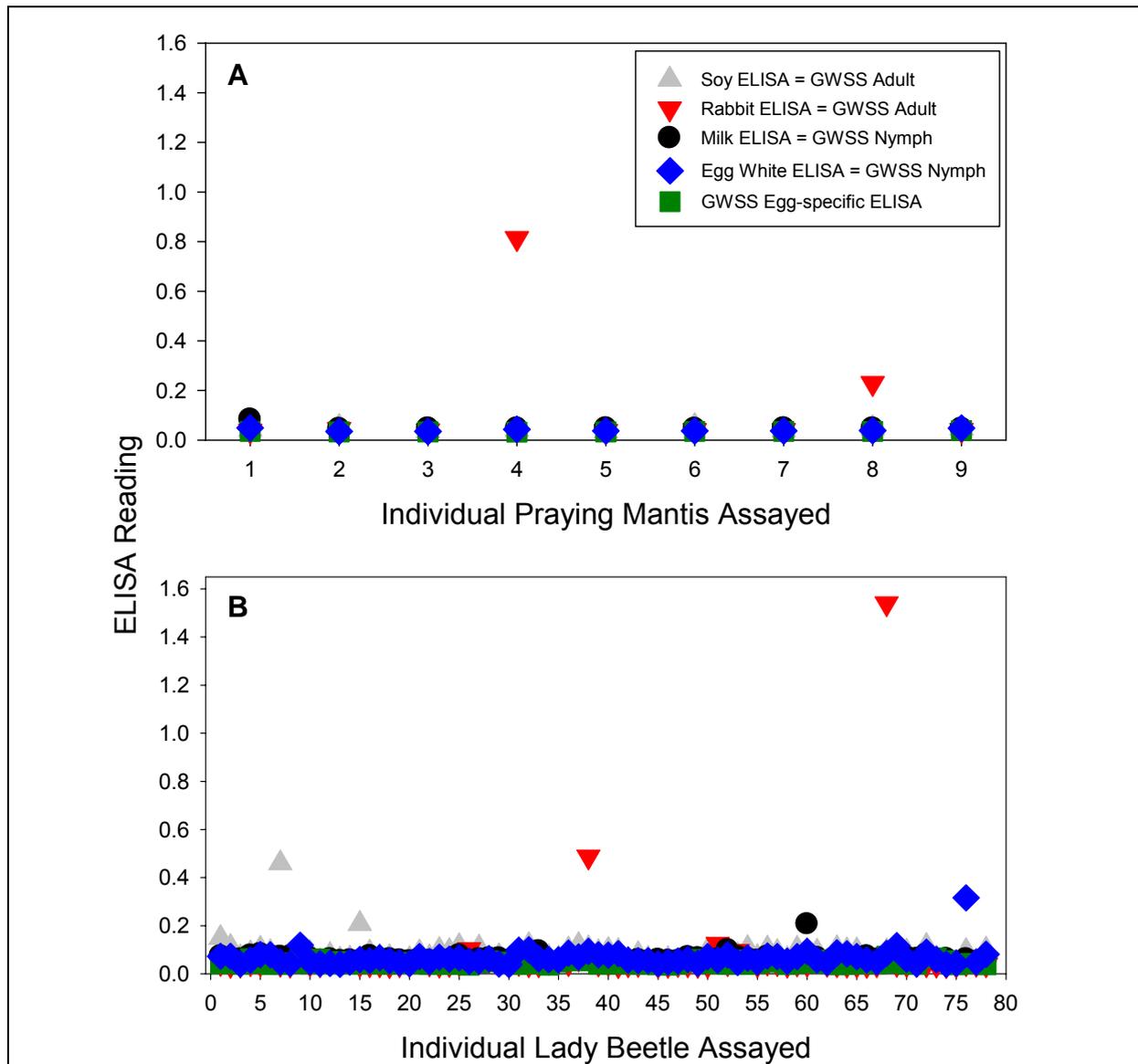
**Table 1.** Mean ( $\pm$ SD) ELISA readings for the retention of rabbit IgG in the gut of two types of predators that consumed either a single 2nd instar pink bollworm larva or an adult parasitoid (*Eretmocerus emiratus*) marked with 5.0 mg/ml of rabbit IgG (from Hagler 2006).

Predator	Hours After Feeding	n	Mean ( $\pm$ SD)	Percent Positive <sup>3</sup>
Earwig <sup>1</sup>	Negative Control	30	0.07 (0.02)	0
	0	30	1.29 (0.66)	100
	24	30	1.39 (0.58)	100
	48	30	1.16 (0.92)	100
Minute Pirate Bug <sup>2</sup>	Negative Control	32	0.04 (0.02)	0
	0	10	0.15 (0.10)	70.0
	3	10	0.07 (0.02)	70.0
	6	31	0.35 (0.31)	90.3
	9	10	0.17 (0.11)	80.0
	12	31	0.13 (0.13)	87.1

<sup>1/</sup> Earwig was fed a single rabbit-IgG marked pink bollworm larva.

<sup>2/</sup> Minute pirate bug was fed a single rabbit IgG-marked parasitoid.

<sup>3/</sup> Percentage of predators scoring positive by the rabbit IgG ELISA.



**Fig. 1.** ELISA results for every praying mantid (*Stagmomantis carolina*) and Convergent lady beetle (*Hippodamia convergens*) assayed for remains of differentially marked GWSSs.

DISCUSSION.

Although it is widely accepted that predators play a role in pest regulation, we still have an inadequate understanding of and ability to predict their impact in cropping systems. The impact that predators have on suppressing GWSS populations goes unrecognized due to the difficulties of assessing arthropod predation. The prey marking technique (Hagler 2006) combined with a GWSS egg-specific gut content ELISA (Fournier *et al.* 2006) circumvented many of the shortcomings of the current methods used to study predation. Here, we quantified predation on GWSS nymphs and adults by two predator species. Since these predation events were each detected with a specific protein ELISA, we are confident

that these results represent the first quantified results of predation using molecular gut content methods (e.g., immunological or DNA based).

#### ACKNOWLEDGEMENTS.

We thank Trina Brennan, Juanita Corza, Heather Costa, Marissa Gonzalez, George Green, Brian Hogg, Isabelle Lauziere, Theresa de la Torre, Dan Langhorst, Xiufeng Li, Scott Machtley, Tom Ortega, Christina Pflipsen, Erik Stone, and Heather Terry for excellent technical support. Funding for the project was provided by The University of California's Pierce's Disease Grant Program (USDA-CSREES) and the USDA-ARS.

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