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COMMUNITY ECOLOGY - METHODS PAPER

Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay

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Abstract A growing number of ecologists are using molecular gut content assays to qualitatively measure predation. The two most popular gut content assays are immunoassays employing pest-specific monoclonal antibodies (mAb) and polymerase chain reaction (PCR) assays employing pest-specific DNA. Here, we present results from the first study to simultaneously use both methods to identify predators of the glassy winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae). A total of 1,229 arthropod predators, representing 30 taxa, were collected from urban landscapes in central California and assayed first by means of enzyme-linked immunosorbent assay (ELISA) using a GWSS egg-specific mAb and then by PCR using a GWSS-specific DNA marker that amplifies a 197-base pair fragment of its cytochrome oxidase gene (subunit I). The gut content analyses revealed that GWSS remains were present in 15.5% of the predators examined, with 18% of the spiders and 11% of the insect predators testing positive. Common spider predators included members of the Salticidae, Clubionidae, Anyphaenidae, Miturgidae, and Corinnidae families. Common insect predators included lacewings (Neuroptera: Chrysopidae), praying mantis (Mantodea: Mantidae), ants (Hymenoptera: Formicidae), assassin bugs (Hemiptera: Reduviidae), and damsel bugs (Hemiptera: Nabidae). Comparison of the two assays indicated that they were not equally effective at detecting GWSS remains in predator guts. The advantages of combining the attributes of both types of assays to more precisely assess field predation and the pros and cons of each assay for mass-screening predators are discussed.

Keywords Conservation biological control · ELISA · Generalist predators · Gut content · PCR · Predator-prey interactions · Spiders

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Introduction

Ecologists increasingly use molecular gut content assays to study predator–prey interactions (see reviews by Symondson 2002; Sheppard and Harwood 2005; Gariepy et al. 2007). The major advantage of this over other approaches to study predation is that it allows rapid and precise assessment of predation with minimal disturbance to the study site, thus revealing predator's prey choice with little ambiguity (see Sunderland 1988; Luck et al. 1988 for reviews). For instance, Harwood et al. (2004, 2007a, 2007b) combined predator gut content assays with population monitoring to highlight non-random patterns of prey selection in different communities of generalist predators. Winder et al. (2005) used predator gut analysis to investigate population-level, spatial associations between carabid beetle predators and their prey. Sheppard et al. (2004) used stomach analysis as a framework to assess the threat of introduced biocontrol agents to Hawaii's endemic species, while Kasper et al. (2004) examined interspecific competition between native and invasive species of social wasps in southern Australia. Finally, the application of gut assays to soil organisms has recently contributed to a better understanding of below-ground food webs (Juen and Traugott 2005, 2007; Read et al. 2006).

Contemporary assays of stomach content include monoclonal antibody (mAb)-based, enzyme-linked immunosorbent assays (ELISA), which detect species-specific proteins (and sometimes life-stage-specific proteins) (Greenstone and Morgan 1989; Hagler et al. 1991, 1993, 1994; Symondson and Liddell 1996; Greenstone 1996; Fournier et al. 2006; Harwood et al. 2007a), and polymerase chain reaction (PCR)-based assays, which detect species-specific DNA (all life stages) (Agustí et al. 1999, 2003; Harper et al. 2005; de León et al. 2006; Harwood et al. 2007b).

Combining both a species- and life-stage-specific ELISA with a species-specific PCR assay can be a powerful tool for unveiling which prey life stage(s) is (are) most vulnerable to predation. Moreover, it can increase the accuracy and reliability of data obtained by serving as a double check (dual diagnostic assays) of the data obtained. Ours was the first investigation to: (1) mass screen a high number of field-collected arthropod predators using PCR, (2) combine the attributes of both ELISA and PCR assay to assess predation, and (3) directly compare the pros and cons of both assay types. Until now, field studies employing PCR-based gut content analyses have had small sample sizes, ranging from only 16 (Harper et al. 2006) to 353 (Juen and Traugott 2007) field-collected individuals. We studied 1,229 specimens from 30 taxa, which is the most comprehensive study to date using PCR-based gut content analysis.

The major goal of our research was to identify key predators of the glassy winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germer) (Hemiptera: Cicadellidae), formally known as *H. coagulata* (Say) (Takiya et al. 2006). GWSS is a polyphagous herbivore, native to the southeastern and southern coastal plains of the United States. It was first reported in California in 1989 (Sorenson and Gill 1996) and has since spread throughout southern California (Blua et al. 2001). While feeding on the plant's xylem fluid, it can acquire and transmit *Xylella fastidiosa* (Wells et al.), the bacterial pathogen responsible for several devastating plant diseases such as Pierce's disease in grape, almond leaf scorch, and oleander leaf scorch (Redak et al. 2004). Area-wide management strategies are currently implemented to suppress GWSS populations in California (National Research Council 2004). However, little effort has been expended to identify generalist predators of 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 protein (Fournier et al. 2006). Our results will provide a better understanding of the predators of GWSS and the foundation for their conservation in a biological control program for this pest.

Material and methods

Laboratory study

Predator feeding trials

Laboratory feeding trials were conducted (25°C and 40% RH) to determine how long GWSS DNA can be detected using PCR assay in a predator's gut following prey consumption. The test insects included third-instar green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae); adult multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae); and adult assassin bug, *Zelus renardii* (Kolenati) (Hemiptera: Reduviidae). These predators were selected because they are commonly found in California, represent an array of predators with different feeding habits (e.g., chewing and piercing and sucking mouth parts), and have been previously observed feeding on GWSS in both the laboratory and field (Fournier et al. 2006). *C. carnea* and *H. axyridis* were purchased from Rincon-Vitova Insectaries (Ventura, California, USA) and *Z. renardii* was taken from a laboratory colony (Riverside, California, USA). It should be noted that similar feeding trials for *C. carnea* and *H. axyridis* were previously conducted to determine how long GWSS egg-protein remained detectable in these predators' guts by the mAb-based ELISA (Fournier et al. 2006).

Prior to feeding trials, predators were individually placed in 4.0-cm-diameter Petri dishes containing only a wetted sponge for 1–4 days. *C. carnea* and *H. axyridis* were fed three and six GWSS eggs, respectively, and *Z. renardii* was fed one GWSS adult. Following prey consumption, predators were isolated from food (but not water) for 0, 3, 6, 9, 12, 18, or 24 h (n = 6 to 24 individuals per interval and 3 negative controls). For the 3-, 6-, 9-, and 12-h post-feeding intervals, individuals were held under constant light at 25°C. For the 18- and 24-h intervals, the predators were maintained under a 16-h:8-h (light:dark) photoperiod at 25°C. After each post-feeding interval, predators were frozen at −80°C and assayed using the PCR technique described below.

Statistical analyses

Half-life ($t_{1/2}$) of GWSS DNA detection was calculated for each of the three predator species used in the laboratory feeding trials based on the coefficients estimated using Logit regressions (STATA 2003).

Field study

Field samples

Arthropod predators were collected at various times from September 2002 to September 2005 at seven different urban landscape sites in Bakersfield, California, USA (all sites within 10 km of latitude: 35°21′26′N; longitude: 119°1′54′W). GWSS host plants from which predators were collected included *Agapanthus africanus* (lily of the Nile), *Buxus* sp. (boxwood), *Citrus* sp. (Valencia orange), *Eucalyptus cinerea* (silver dollar tree), *Euonymus japonica* (silver queen), *Gardenia jasminoides* ("mystery" gardenia), *Hedera helix* (English ivy), *Hibiscus* sp. ("Mrs. J.E. Hendrey" hibiscus), *Jasminum multiflorum* (star jasmine), *Lagerstroemia indica* (crape myrtle), *Lantana* sp. (lantana), *Ligustrum japonicum* ("Texanum" wax leaf privet), *Nerium oleander* (oleander), *Photinia* sp. (red tip photinia), *Punicia granatum* (pomegranate), *Pyrus calleryana* (ornamental pear), *Ulmus chinensis* (Chinese elm), *Vitis vinifera* (Thompson Seedless *cv* grape), *Xylosma* sp. (Xylosma). None of the sample sites were treated with insecticides during the study. Predators were collected from the foliage and branches of 3–12 individual plants for each plant species during each sampling period. The number of plants sampled depended on their availability at each collection site. To collect predators and mobile immature and adult sharpshooters, a 0.5- to 1.0-m section of each plant was beaten with a wooden dowel (ca. 80-cm long × 2.5-cm diameter) for 1 min to dislodge arthropods into a 62-cm-diameter cloth sweep net placed beneath the beaten foliage. All collections were made between 0830 hours and 1400 hours. Field-collected predators were immediately placed in a cooler containing dry ice and then stored at −80°C less than 2 h after collection.

From May 2003 to November 2004, GWSS egg, nymph, and adult densities were recorded once a month at each site. Nymph and adult densities were estimated by beating the foliage into a sweep net for 1 min as described above. GWSS egg density was estimated for each plant by counting 100 randomly selected leaves on 3–12 plants per site. This sampling protocol had been previously tested for efficacy by Daane et al. (unpublished data). The data presented are averaged across all sample sites and host plants.

Predator controls for field samples

Negative and positive control predators were obtained for each taxonomic group collected from our field survey. These predators were field-collected, purchased from insectaries (Rincon-Vitova, Ventura, California, USA), or taken from our laboratory colonies. Negative control predators were obtained by starving insect and spider predators for 2 and 14 days, respectively, in individual 4.0-cm-diameter Petri dishes that only contained a sponge saturated with water. Spiders were starved for a longer period because they can retain prey in their gut longer than insects due to a slower digestion rate (Greenstone 1983; Harwood et al. 2004). After each holding interval, arthropods were frozen at −80°C. Positive control predators were obtained by holding individual predators without food in individual Petri dishes for 48 h and then feeding them a GWSS egg, nymph, or adult. The positive control predators were frozen at −80°C immediately after they were observed feeding on GWSS. The negative and positive control predators were assayed using the ELISA and PCR assay described below.

Molecular gut content assays

Laboratory and field-collected predators were screened using a GWSS-specific PCR assay to determine whether they preyed on the various GWSS life stages. ELISA was also used to screen the field-collected predators to determine whether they preyed on GWSS eggs or adult females. Laboratory feeding studies for the ELISA had previously been conducted on *C. carnea* and *H. axyridis* (Fournier et al. 2006).

PCR assay

DNA extraction

Arthropods were weighed, placed individually in 1.5-ml microtubes, and homogenized using a sterile pestle in

180 µl of phosphate buffered saline (PBS, pH 7.4). A maximum of 50 mg of tissue was individually processed; specimens that weighed over 50 mg were sliced with a clean razor blade and homogenized in separate tubes. The homogenates were then centrifuged (8,000 rpm) at room temperature for 2 min. A 20-µl aliquot of supernatant from each sample was pipetted into a clean 1.5-ml microtube and stored at −80°C for subsequent ELISA (see below). The remaining 160 µl of the homogenized sample underwent DNA extraction using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, California, USA; protocol for insects). As described by Blankenship and Yayanos (2005), we included a blank extraction, which consisted of ultra-pure PCR grade H_2O substituted for gut content tissue, and assayed it simultaneously with sample extraction to control for contamination. Total DNA was eluted twice in the AE buffer provided by the manufacturer. The volume of buffer used was determined according to the predator weight (≤50 mg)—specifically, 30 µl if less than 5 mg, 50 µl if 5–12 mg, 60 µl if 12–20 mg, 75 µl if 20–25 mg, 100 µl if 25–40 mg, and 120 µl if 40–50 mg. The DNA extracts were stored at 4°C.

PCR amplification

All DNA extracts were subjected to a nested PCR-based format. The first amplification was performed with the primer set 28S (forward 5′-CCCTGTTGAGCTTGAC TCTAGTCTGGC-3′, reverse 5′-AAGAGCCGACATCGA AGGATC-3′; 580-bp; Burke et al. 1993; Werren et al. 1995), which targets a portion of a conserved gene in arthropods. We used the 28S-primer set for two purposes: (1) to determine the presence of suitable DNA, and (2) to control for the presence of PCR inhibitors (de León et al. 2006). DNA amplifications were performed in a 20-µl reaction volume containing 2 µl of DNA extract, 0.5 µl of 10 mM deoxynucleotide triphosphates (New England BioLabs Inc., Ipswich, Massachusetts, USA), 1 µl of each primer (5 µM), 2 U of *Taq* DNA Polymerase (New England BioLabs Inc., Ipswich, Massachusetts, USA), and 2 µl of 10× PCR buffer using a final $MgCl_2$ concentration of 2.0 mM. Samples were amplified in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, California, USA) for 44 cycles at 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s. A first cycle of denaturation was carried out at 94°C for 5 min.

We designed a new primer set (Ch) toward the 28S rDNA fragment of the arachnid *Cheiracanthium* sp. which was used in cases where the DNA was extracted from an arachnid and no amplification occurred with the 28S-primer set described above. The primers were designed from the accession number AY297294 reported in GenBank (forward 5′-GAAATCCTGTGGCGAGAGGAT-3′; reverse 5′-ACCCGGATCTGACGATCGATT-3′; 753-bp). This sequence was submitted to GenBank by W. P Maddison and M. C. Hedin from San Diego State University, San Diego, California, USA. Amplifications were performed as described for the 28S-primer set described above with the following modifications: reactions were performed for a total of 40 cycles at 94°C for 20 s, 53°C for 30 s, and 72°C for 50 s.

DNA samples were then subjected to the primer set HcCOI (forward 5′-GGGCCGTAAATTTTACC-3′ and reverse 5′-ACCACCTGAGGGGTCAAAA-3′; GenBank accession number AY959334) developed by de León et al. (2006) to detect and amplify a fragment (197-bp) of COI specific to *H. vitripennis*. Samples were amplified twice, at different times, to ensure better control. Amplifications were performed as described above with the following modifications: 3 µl of DNA, 1.4 mM $MgCl_2$ (or 2.5 mM for salticid spiders), samples were amplified for 31 cycles, and Tm was 60°C (62°C for praying mantis). Assays were performed to verify that the modified conditions carried out for salticid and praying mantis specimens did not trigger cross-reactions with other prey species present in the system. For each PCR conducted, one positive GWSS control (i.e., GWSS DNA extract), one blank negative control (i.e., ultra-pure PCR water), one positive predator control (i.e., an individual fed a GWSS), and one negative predator control (i.e., an individual not fed GWSS) were included in the amplification (see section below for a more detailed description). Samples from the blank extraction were also included in all amplifications (see section "DNA extraction" for details).

PCR products were separated by electrophoresis in 2% agarose gels (100 V, 60 min). Each gel was stained with ethidium bromide, and the bands on the gel were visualized using Quantity One Software (Bio-Rad Laboratories, Hercules, California, USA). Samples were scored positive when a band appeared on the 197-bp region of the gel.

GWSS egg-specific ELISA

Field-collected predators were screened using a GWSS egg-specific sandwich ELISA to determine whether they preyed on a GWSS egg(s) and/or a gravid female(s). This technique was described in detail by Fournier et al. (2006). Briefly, the wells of a 96-well Costar microplate (#9017; Corning Inc.; Corning, New York, USA) were coated with 80 µl of the primary antibody—mAb 6D5-2H1 (Fournier et al. 2006) diluted 1:1,000 in TBS. After 60 min at 27°C, the primary antibody was discarded and 400 µl of 1.0% non-fat dry milk (NFDM) was added to each well for 30 min. The NFDM was then discarded, and each well was coated with a 10-µl aliquot of a predator sample mixed with 70 µl of TBS (for total volume of 80 µl). After 60 min at

room temperature, the samples were discarded and wells were rinsed three times with TBS-Tween 20 (0.05%) and twice with TBS. Aliquots (80 µl) of the GWSS-specific HRP-conjugated secondary mAb (1D4-1D8) (Fournier et al. 2006), diluted 1:500 in 1% NFDM, were added to each well and incubated for 60 min at room temperature. Plates were then rinsed as above, and 80 µl of TMB One Component HRP Substrate (BioFX Laboratories, Owings Mills, Maryland, USA) was added to each well. The absorbance of each well was measured after 60 min using a SpectraMax 250 microplate reader (Molecular Devices Corp., Sunnyvale, California, USA) set at a wavelength of 650 nm.

Each 96-well ELISA plate included the following controls: (1) 7 PBS blanks, (2) a positive control (i.e., an 80-µl aliquot of one GWSS egg homogenized in 500 µl PBS), (3) 8–20 individual negative predator controls (i.e., predators not fed GWSS); and (4) a positive predator control (i.e., a predator fed a GWSS egg or gravid female). Some predator species do not readily eat eggs because they seek mobile prey. In such cases, these predator species' were fed a gravid GWSS female. Predators were scored positive for prey remains if they yielded an ELISA response five-standard deviations above that of their respective negative control mean. The five-standard deviation ELISA threshold value is higher than the conventional threshold value of three standard deviations (Sutula et al. 1986; Hagler et al. 1992). We selected a higher, more conservative threshold value for this study to reduce the risk of falsely scoring true negative ELISA reactions as positive for prey remains.

Results

Laboratory study

Predator feeding trials

The PCR assay was 100% effective at detecting GWSS DNA in all three types of predators immediately after the predators fed (Fig. 1). The fastest decline in percentage of individuals containing detectable quantities of GWSS DNA in their gut was observed in *C. carnea*, with only 10% testing positive after 24 h. The prey detection half-life for *C. carnea* was estimated at 11.0 h (Pseudo $R^2 = 0.34$, $\chi^2 = 0.000$; Fig. 1a). In contrast, 33% of *H. axyridis* (Fig. 1b) and 70% of *Z. renardii* tested positive for the presence of GWSS in their guts 24 h after feeding (Fig. 1c). Logit analyses could not accurately predict the prey detection half-life of GWSS DNA in the gut of *H. axyridis* due to lack of variation across time, nor could it predict half-life for *Z. renardii* due to the lack of time intervals tested beyond 24 h (Pseudo $R^2 = 0.03$, $\chi^2 = 0.189$). However, if we assume a linear relationship exists between percent positive for GWSS over time, then a crude estimated prey detection half-life would be 17.5 h and 51.0 h for *H. axyridis* and *Z. renardii*, respectively.

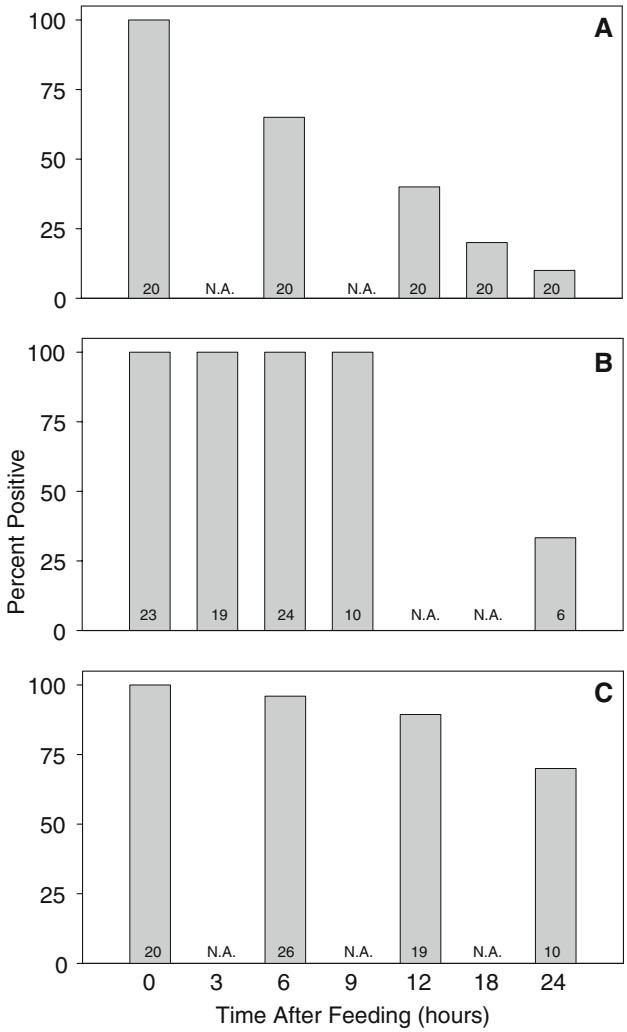

Fig. 1 Percentage of (**a**) *Chrysoperla carnea*, (**b**) *Harmonia axyridis*, and (**c**) *Zelus renardii* individuals testing positive for GWSS DNA 0–24 h after prey ingestion. The *numbers* inside the *vertical bars* are the sample size for each treatment. The estimated detection half lives were 11, 17.5, and 51 h, respectively. *N.A.* data not available

Field study

Field samples

A total of 1,229 field-collected predators representing 30 taxa were examined for GWSS prey remains by both GWSS-ELISA and PCR assay. Spiders were the most common predators encountered, accounting for 69% ($n = 850$) of the arthropod predator population. Of these, the Salticidae ($n = 233$), Clubionidae ($n = 210$), and Anyphaenidae ($n = 184$) were the most dominant taxa (Fig. 2). The

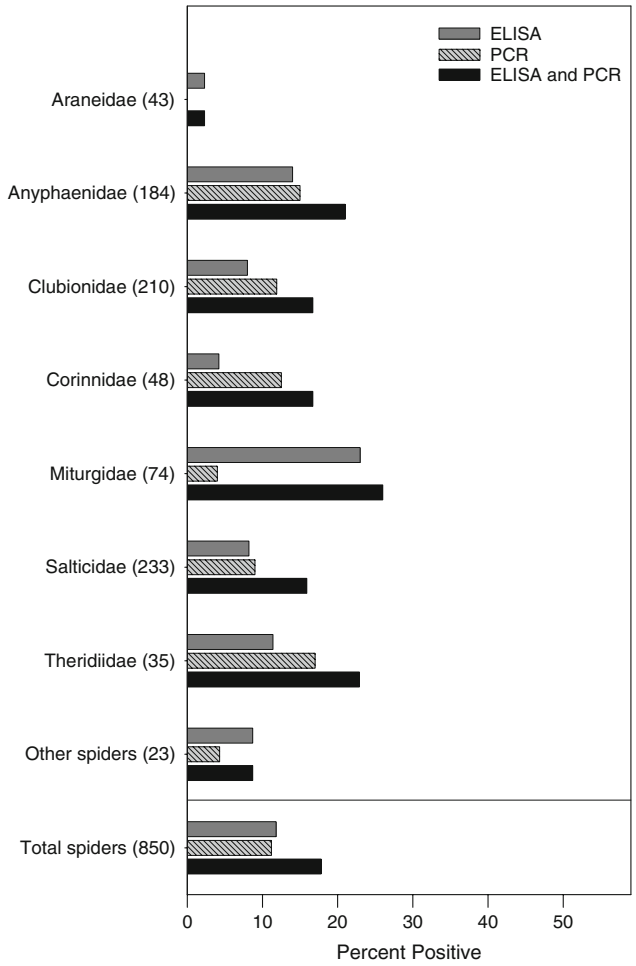

Fig. 2 Percentage of spiders testing positive for GWSS remains, tested using ELISA, PCR, and both techniques combined (= total specimens that scored positive using ELISA, PCR, or both). The *numbers in parenthesis* are the sample size for each taxa

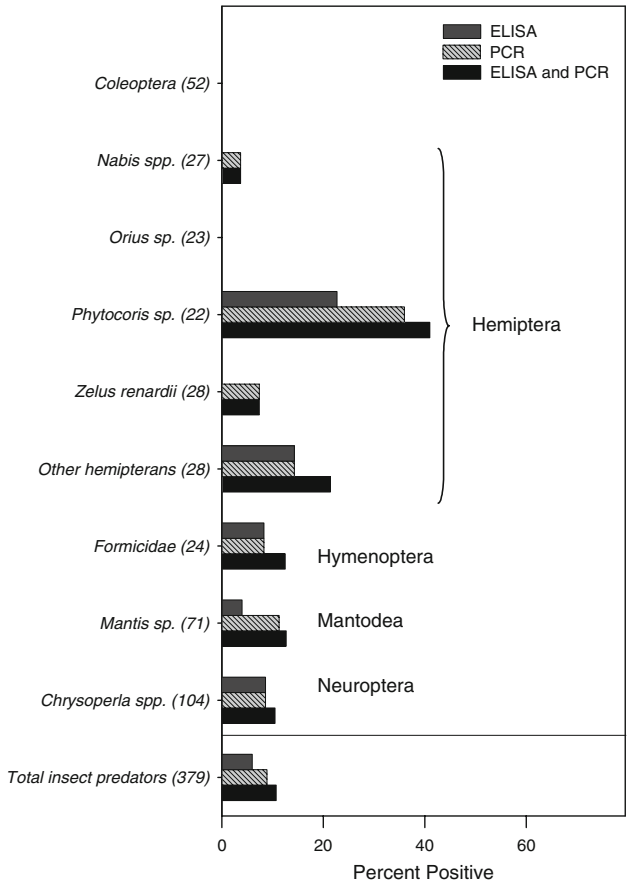

Fig. 3 Percentage of insects testing positive for GWSS remains, tested using ELISA, PCR, and both techniques combined (= total specimens that scored positive using ELISA, PCR, or both). The *numbers in parenthesis* are the sample size for each taxa

dominant insect predator taxa (n = 379) included 128 hemipterans (anthocorids, lygaeids, mirids, and reduviids), 104 lacewings (*Chrysoperla* spp., Neuroptera: Chrysopidae), 71 praying mantis (*Mantis* spp., Mantodea: Mantidae), 52 coleopterans (coccinellids and curculionids), and 24 ants (Hymenoptera: Formicidae) (Fig. 3). Overall, 15.5% (n = 192) of the 1,229 predators scored positive for GWSS remains by means of ELISA and PCR assay combined. Of these, 18% of the spiders contained GWSS remains in their guts (Fig. 2). Moreover, the five most abundant Araneae taxons—Salticidae (jumping spiders), Clubionidae (club spiders), Anyphaenidae (sac spiders), Miturgidae (long-legged sac spiders), and Corinnidae (corrinid sac spiders)—yielded a total of 16, 17, 21, 26, and 17% positive reactions for GWSS, respectively (Fig. 2). Species/genera that commonly tested positive included *Cheiracanthium* sp. (Miturgidae), *Hibana incursa* (Chamberlin) (Anyphaenida), *Trachelas pacificus* Chamberlin and Ivie (Corinnidae), *Theridion* spp. (Theridiidae), *Thiodina hespera* Richman and Vetter, *Phidippus audax* (Hentz), *P. johnsoni* (Peckham and Peckham), and *Metaphidippus vitis* (Cockerell) (Salticidae). Specimens testing positive by means of ELISA are more likely to have preyed upon gravid GWSS female than GWSS eggs because spiders usually prefer to attack mobile rather than immobile prey.

The study revealed that 11% (n = 41) of the insect predators tested positive for GWSS remains (Fig. 3). True bugs (Hemiptera), praying mantis (Orthoptera), ants (Formicidae), and lacewings (Neuroptera) accounted for 14, 13, 12, and 10% of overall positive reactions, respectively. No beetles (Coleoptera), most notably the lady beetles (*Hippodamia convergens* Guérin-Méneville and *H. axyridis*), were positive for GWSS remains.

Equal or higher proportions of positive reactions are expected when using the PCR assay because it detects predation on all GWSS life stages (de León et al. 2006). The GWSS egg-specific ELISA, however, only detects predation on the GWSS egg stage and adult female (gravid) stage (Fournier et al. 2006). Therefore, caution is advised when comparing the sensitivity of the two methods, since different

Table 1 Comparison of ELISA and PCR gut content assay results obtained for various spider and insect taxa

Taxon	No. assayed	Total positive by ELISA	Total positive by ELISA and PCR[a]	Total positive by ELISA and negative by PCR
Spiders				
Araneidae	43	1	0 (0.00)	1
Anyphaenidae	184	30	12 (0.40)	18
Clubionidae	210	25	10 (0.40)	15
Corinnidae	48	2	0 (0.00)	2
Miturgidae	74	17	7 (0.41)	10
Salticidae	233	19	6 (0.32)	13
Theridiidae	35	4	2 (0.50)	2
Other spiders	23	2	1 (0.50)	1
Total	850	100	38 (0.38)	62
Insect predators				
Chrysoperla spp.	104	9	6 (0.67)	3
Formicidae	24	2	1 (0.50)	1
Mantis sp.	71	3	2 (0.67)	1
Phytocoris sp.	22	5	4 (0.80)	1
Other hemipterans	28	4	4 (1.00)	0
Total	249	23	17 (0.74)	6

[a] The numbers in parentheses are obtained by dividing the total number of positive ELISA and PCR assays by the total number of positives by ELISA only. Values close to 1.00 indicate that the assays are equally sensitive; values close to 0.00 indicate that the assays are not equally sensitive

GWSS tissues were targeted. A method to compare the sensitivity of the two assay types is to first identify each individual sample testing positive when using ELISA and then verify whether it also tested positive using the PCR assay. For each predator taxon, we examined the positive results and found a huge discrepancy in sensitivity when using each assay, suggesting a high proportion of ELISA false-positive reactions and/or false-negative PCR reactions (Table 1). For example, we assayed a total of 184 anyphanenid spiders (Table 1). Of these, 30 (16.0%) scored positive by ELISA and only 12 (7.0%) by both ELISA and PCR, thus yielding an efficiency of 0.40 (12/30). In other words, 18 of the 30 scoring positive by ELISA scored negative by PCR. Ideally, the efficiency values yielded should be 1.0 or higher. In general, it appears that the discrepancy in sensitivity tends to be greater for spiders than insects (Table 1).

The population dynamics of GWSS from May 2003 to September 2005 and the proportions of positive predators for the 23 collection dates across this period of time are given in Fig. 4. Simple regressions did not reveal any specific pattern between the proportion of positive predators and prey density (linear regression: $F_{1, 22} = 0.16$; $P = 0.70$; quadratic regression: $F_{2, 22} = 0.09$; $P = 0.91$).

Controls

All negative and positive controls used in this study yielded the expected response: all predators deprived of GWSS foodstuff yielded negative gut content assay reactions and all predators fed GWSS foodstuff yielded positive gut content assay reactions (data not shown).

Discussion

Predator feeding trials

The *C. carnea* gut content PCR assay used in this study and the ELISA gut content assay used previously (Fournier et al. 2006) yielded similar results with GWSS egg detection half lives of 11.0 h and 11.8 h, respectively (Fournier et al. 2006). In contrast, the *H. axyridis* PCR assay detected GWSS egg remains for longer than those observed using ELISA—prey detection half lives of 17.5 h and 2.2 h, respectively. The PCR prey detection half-life of GWSS adults in the gut of *Z. renardii* was estimated at 51 h. These results illustrate how the prey detection interval can vary between both the predator species being examined and the type of assay used.

To our knowledge, this is the first time that the efficiency of an ELISA and PCR gut assay has been directly compared for efficacy. Our results presented here and those by Fournier et al. (2006) suggest that, for at least two predator species tested to date (*H. axyridis* and *C. carnea*), the PCR GWSS-specific assay can detect prey remains in the gut for a longer period than can the GWSS (egg)-specific ELISA. However, we caution that it is unlikely that the PCR assay will consistently detect prey for longer than ELISA for all potential predators. Variable prey retention intervals

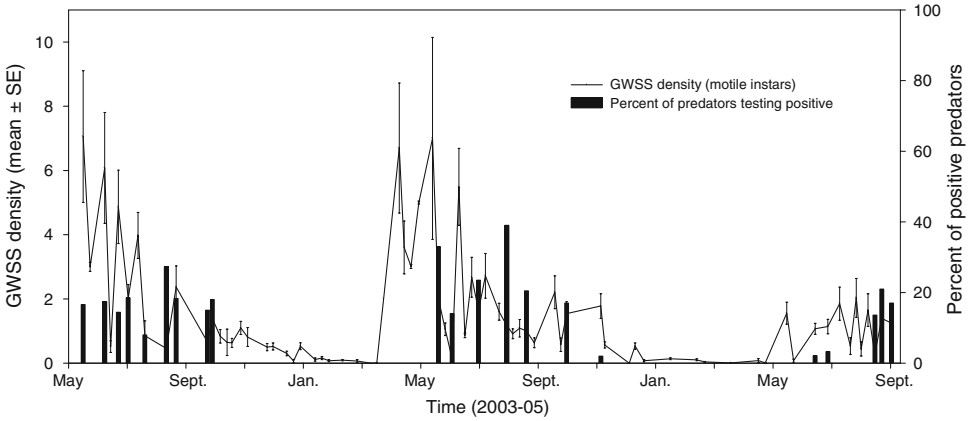

Fig. 4 Population dynamics of glassy winged sharpshooter from arthropod collections made once a month from May 2003 to September 2005 and proportions of positive predators across the same time interval. The line plot (*Y1 axis*) represents the density of GWSS motile life stages (mean number of nymphs and adults ± SE per sweep net collection) for all collection sites combined; vertical bars (*Y2 axis*) illustrate the proportion of predator specimens (spiders and insects combined) that tested positive for GWSS remains using PCR and ELISA. Of the 23 collection dates, 2 (8 October 2003 and 20 April 2004) failed to yield any positive gut assay reactions

between predator species have been reported in numerous studies using both ELISA and PCR gut assays (Hagler and Naranjo 1997; Zaidi et al. 1999; Chen et al. 2000; Harper et al. 2005).

Some researchers have implied that lengthy prey retention intervals are advantageous over short retention intervals (Agustí et al. 2003; Harper et al. 2005). Although such a long interval increases the chance of obtaining a positive assay reaction from a field-collected predator, it confounds the interpretation of the assay result because it cannot distinguish between an old and a recent predation event (Hagler and Naranjo 1996; Naranjo and Hagler 1998; Holland et al. 1999; Read et al. 2006). For instance, suppose that an ELISA or PCR assay can detect a targeted prey item in a given predator's gut for 100 h (Harwood et al. 2004, 2005; Harper et al. 2005) and that the predator feeds on the target prey every few hours throughout the day. It seems logical then that every field-collected predator in this scenario would yield a positive assay reaction due to the presence of multiple prey items in its gut. Conversely, if a prey item could only be detected for an hour in a predator's gut, then a more intense sampling schedule could be employed (e.g., every 1–4 h) to more accurately estimate (albeit still a qualitative estimation) its per capita predation rate in relation to host density and circadian feeding activity (Hagler 2006). In short, we contend that shorter prey retention intervals are more desirable in most instances for more precise estimates (e.g., semi-quantitative) of predation.

Field study: identification of key predators

Frequent predators of GWSS identified in this study using both a GWSS egg-specific ELISA and a GWSS species-specific PCR assay included a variety of arthropods including spiders (families: Salticidae, Clubionidae, Anyphaenidae, Miturgidae, and Corinnidae), green lacewings, praying mantis, ants, and several predaceous bugs (families: Lygaeidae, Miridae, Nabidae, and Reduviidae). The gut assay results yielded by the GWSS egg-specific ELISA further revealed that green lacewings, ants, big-eyed bugs, and the mired *Phytocoris* sp. are frequent egg predators.

This study and others demonstrate that spiders are the most abundant arthropod predators found in many natural and managed California ecosystems (Riechert and Lockley 1984; Riechert and Bishop 1990; Young and Edwards 1990). For instance, Costello and Daane (1999) reported that spiders constituted 98% of the total predator fauna found in California vineyards. In the present study, 69% of the predator fauna recovered from the urban landscapes we sampled consisted of a wide variety of spiders.

The gut content assay results revealed that 18% of the spiders and 11% of the insect predators captured contained GWSS remains in their guts, suggesting that spiders may prey upon GWSS more frequently than predaceous insects. However, this should be interpreted with caution (e.g., see above for an explanation of the disadvantage of long prey retention intervals) as spiders often exhibit longer retention times translating into greater detection periods in comparison with insects (Greenstone 1983; Greenstone and Shufran 2003; Harwood et al. 2001, 2004) due to their diverticula; organs that store partially digested food for extended periods of time.

None of the field-collected lady beetles contained GWSS remains in their gut, suggesting that they do not prey on any of the GWSS life stages. It is unlikely that these relatively slow moving and "stalking" predators can catch fast-moving and agile GWSS nymphs or adults. It is also unlikely that they feed on the GWSS egg stage because

their chewing mouthparts are not adapted to reach GWSS eggs which are protected in nature because they are oviposited underneath the a thin layer of the leaf epidermis. For instance, in the laboratory feeding trials, the beetles were reticent to feed on the eggs until we "teased" the embedded eggs away from the leaf tissue.

Comparing ELISA and PCR assays

To our knowledge, this is the first study to simultaneously use a pest-specific ELISA and PCR assay for predator gut content analyses. This provided us with a unique opportunity to directly compare the sensitivity, cost efficiency, and other attributes of each assay. It is important to remember two main differences between the two types of gut assays used. First, the ELISA only detects predation on the GWSS egg and adult (gravid) female life stages, and the PCR assay detects predation on all the GWSS life stages (Fournier et al. 2006; de León et al. 2006). Second, when compared with ELISA, the PCR detected prey remains for a longer period in the guts of the two predators (*C. carnea* and *H. axyridis*) that we have examined thus far (Fournier et al. 2006). If we assume that the PCR assay is as or more sensitive than ELISA for all the potential GWSS predators, then every predator scoring positive by means of ELISA should also be positive using PCR (but not vice versa). Our results showed that this assumption was only met for one of the taxa examined (Table 1, the "other" hemipterans). This finding suggests that either the ELISA is yielding some false-positive or the PCR assay is yielding some false-negative reactions. For instance, in exhaustive cross reactivity tests, the GWSS-specific ELISA also reacted to the egg stage (and gravid female stage) of the smoke tree sharpshooter (STSS), *H. liturata* (Fournier et al. 2006). Both the GWSS and STSS are known to co-exist in California (Blua et al. 2001; Park et al. 2006). Thus, predators that tested positive using ELISA could have ingested a STSS egg or gravid female instead of a GWSS egg or gravid female. However, this is unlikely because we did not collect any STSSs from any of the seven urban landscape collections sites (Daane, unpublished data). Further results from our study showed a large discrepancy in sensitivity between the two gut content assays between spiders and insects. Specifically, only 38% of spiders were concurrently positive when using ELISA and PCR compared with 74% of the insects (Table 1). The difference in gut assay response between the two taxa is an area for future investigation.

It is important to note that we do not contend that one assay is superior to another, but rather that there is a high degree of variability between the two assay formats. Clearly, more rigorous studies are needed to compare the efficacy of the two types of gut content assay formats among various predator species (Hagler 1998).

Application of pest-specific ELISA and PCR assays for mass-screening field-collected predators

There has been a sharp increase over the past 7 years in the number of PCR gut assays developed to study various aspects of arthropod predation (see Sheppard and Harwood 2005; Gariepy et al. 2007 for recent reviews). Advocates of the PCR gut assay approach have touted pest-specific PCR assays as the next significant step toward evaluating field-collected predators because, in theory, they are easier, faster, and less expensive to develop than mAb-based ELISAs (Chen et al. 2000; Symondson 2002). As a result, a growing number of ecologists are choosing PCR assays over mAb-based ELISAs to study predation. However, as Sheppard and Harwood (2005) accurately pointed out, most PCR gut content studies conducted to date have focused more on the development of the assays rather than on the application of the assays. Thus, only a few studies, each of which contains a relatively small sample size of field-collected predators, have been used to screen field-collected predators using the PCR approach (Hoogendoorn and Heimpel 2002; Agustí et al. 2003; Dodd 2004; Kasper et al. (2004); Harper et al. 2005; Foltan et al. 2005; Harwood et al. 2007b).

We believe that the gap in field data generated by PCR is inherent to the limitations we encountered in the present study. Specifically, we found that the PCR gut content analyses were extremely costly, time consuming, and tedious. For example, two highly trained technicians could only assay approximately 80 predators per day at of cost of about US $7.50 per predator. Conversely, two technicians could assay about 1,200 predators per day by ELISA for approximately $0.50 per predator. It should be noted that our estimated cost difference is similar to that estimated by two other independent researchers from other countries (Martin Erlandson, present communication). In short, we believe that once the burden of developing a pest-specific mAb is overcome (note: we contracted out the work to develop the mAb which cost US $12,000 and took a year to develop), the cost efficiency and simplicity of conducting an ELISA is more conducive for mass-screening predators than PCR (Sheppard and Harwood 2005). An example of a study that exploits the power of using a pest-specific ELISA to screen field-collected predators is given by Hagler and Naranjo (2005). In that study, over 32,000 predators, representing nine different taxa, were screened at minimal assay and labor costs to identify predators of the silverleaf whitefly, *Bemisia tabaci* (Gennadius). It should be noted that Chen et al. (2000) estimated the cost of conducting ELISA and PCR predator gut assays to be only US $0.21 and $0.28, respectively. The huge discrepancies in costs of our ELISAs (≈2.5-fold) and PCR assays (≈27-fold) merit further investigation.

Limitations of molecular gut content assays

Potential limitations are inherent in both the ELISA and PCR gut content analysis approach. First, false positive due to third trophic level interactions (secondary predation) can lead to the misidentification of the actual predator that consumed the targeted prey item (Harwood et al. 2001, 2004). Detection of secondary predation (e.g., a predator that consumes a lower order predator that previously consumed the targeted pest) via gut content analyses has been well documented using both mAb- and DNA-based assays (Harwood et al. 2001, 2004; Sheppard et al. 2005). Second, scavenging predators feeding on dead prey items could have a profound effect on the interpretation of ELISA and PCR assay results (Sunderland 1996; Calder et al. 2005; Juen and Traugott 2005; Foltan et al. 2005). In the present study, the vast majority of predator species that were collected are known to only feed on living prey. Third, some predator species may exhibit diel patterns of predation. That is, they might feed primarily during the day or night (Pfannenstiel and Yeargan 2002; Hagler 2006). Thus, the time of day that the predator is collected from the field may influence the results yielded from any given gut content analysis. For example, if the calculated detection half-life of target prey is 2 h for a strict nocturnal predator, then, collecting this predator during the day (2 h after light) will underestimate predation rates. In our study, we may have missed the nocturnal predators because all of the predator collections were made from between 0800 hours and 1400 hours (but see Costello and Daane 2005). Finally, predators' gut content ELISAs and PCR assays are not quantifiable. The numerous factors preventing the quantification of predation by pest-specific ELISA and PCR assay have been reviewed by Hagler and Naranjo (1996) and Naranjo and Hagler (1998).

The data presented here and elsewhere (Hagler 1998; Greenstone and Shufran 2003; Chen et al. 2000) suggest that there is a huge discrepancy in the sensitivity of gut content assays. Our aim was not to endorse one technique over the other, but rather to provide an assessment of the pros and cons of each procedure. While both types of gut content assay offer good methods to qualitatively estimate predation, they alone cannot provide researchers with quantitative estimates of predation. Therefore, the challenge remains to develop such a method. Until then, gut content assays must be used in concert with other predator evaluation techniques (Sunderland 1988; Luck et al. 1988; Naranjo and Hagler 1998) to assess predation.

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