BioControl (2013) 58:341–349
DOI 10.1007/s10526-012-9489-4

Gut content examination of the citrus predator assemblage for the presence of *Homalodisca vitripennis* remains

James R. Hagler · Felisa Blackmer · Rodrigo Krugner · Russell L. Groves · J. G. Morse · Marshall W. Johnson

Received: 15 May 2012 / Accepted: 24 September 2012 / Published online: 5 October 2012

Abstract A two-year study was conducted in a citrus orchard, *Citrus sinensis* L., to determine frequency of predation on glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar). A total of 1,578 arthropod predators, representing 18 taxa, were collected and assayed for the presence of GWSS egg protein by an enzyme-linked immunosorbent assay using a *Homalodisca*-species and egg-specific monoclonal antibody and then by polymerase chain reaction using a *H. vitripennis*-specific DNA marker. The gut content analyses revealed the presence of GWSS remains in the gut of 2.28 % of the total arthropod predator population, with 3.09 % of the spiders and 0.59 % of the insect predators testing positive. Moreover, a comparison of the two assays indicated that they were not equally effective at detecting GWSS remains in predator guts. Low frequencies of GWSS detection in the gut of predators indicated that GWSS are not a primary prey and that predators may contribute little to suppression of this pest in citrus.

Keywords Glassy-winged sharpshooter · Conservation biological control · ELISA · Generalist predators · Molecular gut content analysis · PCR · Predator–prey interactions · Spiders

Handling Editor: Patrick De Clercq

J. R. Hagler (✉) · F. Blackmer
United States Department of Agriculture (USDA)-Agricultural Research Service (ARS), Arid-land Agricultural Research Center, Maricopa, AZ, USA
e-mail: James.Hagler@ars.usda.gov

R. Krugner
USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA, USA

R. L. Groves
Department of Entomology, University of Wisconsin, Madison, WI, USA

J. G. Morse · M. W. Johnson
Department of Entomology, University of California, Riverside, CA, USA

Introduction

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is a serious cosmopolitan pest that was first detected in California, USA in the late 1980s (Sorensen and Gill 1996). GWSS transmits the bacterium, *Xylella fastidiosa* Wells et al., into a wide variety of economically important plants including citrus (Damsteegt et al. 2006) and grapevines (Purcell and Saunders 1999; Almeida and Purcell 2003), where it causes citrus variegated chlorosis in Central and South America (Chang et al. 1993) and Pierce's disease (PD) in the southern United States (Davis et al. 1978), respectively. In California, there are ≈335,000 ha of vineyards distributed throughout the state, which have an estimated economic value of $4.1 billion per year (CDFA 2006) and are threatened by GWSS. Citrus is

considered to be a key overwintering and first generation reproductive host (Blua et al. 1999) for GWSS and plays an important role in PD incidence in nearby vineyards (Perring et al. 2001; Park et al. 2006). To date, the primary PD management tactic used has been implementation of an area-wide insecticide application program to reduce GWSS populations in citrus groves, urban areas, and vineyards (Wendel et al. 2002; Hix et al. 2003; Park et al. 2006). This management effort has been focused in regions of the state where both citrus and grape production are present and GWSS populations are most dense. However, over-reliance on insecticide applications to suppress GWSS populations in citrus orchards could potentially eliminate GWSS egg parasitoids and predators that feed on it and other citrus pests such as cottony cushion scale, *Icerya purchasi* (Williston) (Hemiptera: Margarodidae). In short, ecologically sustainable, integrated management tactics that are less reliant on area-wide insecticide applications are warranted for long term pest control (CDFA 2006).

One such environmentally benign control tactic that fits into an integrated pest management program is biological control. To date, a significant amount of research has been dedicated toward evaluating the efficacy of GWSS egg parasitoids (Triapitsyn et al. 1998, 2003; Vickerman et al. 2004; Irvin and Hoddle 2005a, b). However, very little research has been conducted on the evaluation of its naturally occurring predaceous natural enemies (Fournier et al. 2008, Krugner et al. 2009).

Recently we examined the population dynamics of GWSS (eggs, nymphs and adults) and its associated natural enemies over a two-year period in a large citrus orchard that contained three separate irrigation treatment regimes. The present study was conducted in conjuncture with that study. Specifically, we conducted post-mortem gut assays on the predators collected over the course of that irrigation study.

Modern gut content assays include either 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), or polymerase chain reaction (PCR)-based assays, which detect species-specific DNA fragments (all life stages) (Agustí et al. 1999; Harper et al. 2005; de León et al. 2006; Harwood et al. 2007b). Here we assayed every field-collected predator by a *Homalodisca* egg-specific (e.g., it reacts with *H. vitripennis* and *H. lacerta* Ball) ELISA (Fournier et al. 2006). In addition, we also assayed most of the predator specimens by a *H. vitripennis*-specific PCR assay (de León et al. 2006). Using a *Homalodisca* life-stage-specific ELISA with an *H. vitripennis*-specific PCR assay can help to unveil which prey life stage(s) is (are) most vulnerable to predation. For example, a predator yielding a positive PCR reaction just indicates that it fed on a GWSS. In other words, this assay cannot differentiate between a predation event on a GWSS egg, nymph or adult. However, if the predator also scores positive by the ELISA, it can be deduced that it fed on the egg or gravid adult (which is not likely) lifestage. Here we examined 1,578 field-collected specimens from 18 taxa by an egg-specific ELISA. In addition, 1,148 of these predators were also examined by a *H. vitripennis*-specific PCR assay. Our major goal was to identify key predators of GWSS in a citrus agroecosystem.

Materials and methods

Experimental site

The study was conducted in conjunction with the work of Krugner et al. (2009). The goal of that study was to determine if different deficient irrigation regimes had an impact on GWSS and natural enemy population dynamics. Thus, the reader is referred to that study for thorough details regarding the experimental site and design. Briefly, the study was conducted at Agricultural Operations on the campus of the University of California, Riverside, USA from April 2005 to June 2007 in a 5.4 ha citrus orchard (*Citrus sinensis* L. Osbeck, cultivar Valencia) containing nine, 0.6 ha plots. Each plot contained 120 mature trees that were planted at a spacing of 6.8 by 5.9 m. The trees were irrigated with a micro-sprinkler irrigation system.

Insect sampling procedure

Populations of GWSS nymphs and adults, and potential natural enemies within experimental plots were sampled on three trees per plot using a beat net technique. Insects were collected between 06:00 and 07:30 (dawn to sunrise) each week from April to

December in 2005 and from February to December in 2006. One beat net sample unit consisted of arthropods collected from ten branches beaten four times. Collected arthropods were placed into paper bags and immediately frozen on dry ice. The samples were placed in a −80 °C freezer at the laboratory for later inspection under a dissecting scope. The numbers of GWSS nymphs, adults, and predators were recorded. The gut content of each predator was analyzed for the presence of sharpshooter egg protein by a *Homalodisca*-spp. egg-specific ELISA. In addition, the gut contents of the majority of these predators were also analyzed for the presence of GWSS DNA fragments by an *H. vitripennis*-specific PCR assay.

Molecular gut content assays

The *Homalodisca* species and egg-specific ELISA was used to screen all arthropod predators collected in the beat nets to determine whether they fed specifically on eggs or gravid adult females. In addition, all of the spiders and many of the insect predators were also screened using an *H. vitripennis*-specific PCR assay to determine if they preyed on the various GWSS life stages (e.g., this assay is not life stage specific). It should be noted that we only assayed 10 % of the 289 and 189 field-collected Chrysopidae and Coccinellidae, respectively, using the PCR assay. Our rationale for not assaying all of these individuals was threefold. First, we felt that it was not very likely that they would be capable of capturing and consuming an agile GWSS nymph or adult. Second, it is not very likely that chewing type predators are capable of feeding on a GWSS egg because they are deposited underneath the leaf epidermis (Fournier et al. 2008). Third, the PCR assay is very time consuming, labor intensive, and costly (Fournier et al. 2008; Aebi et al. 2011).

Homalodisca species and egg-specific sandwich ELISA

Field-collected predators were screened by a *Homalodisca* egg-specific sandwich ELISA to determine if they preyed on an egg(s) and/or a gravid female(s). The egg-specific ELISA is described in detail by Fournier et al. (2006). Briefly, the wells of a 96-well Costar microplate (#9017; Corning Inc., Corning, NY, USA) were coated with 40 µl of the primary antibody, MAb 6D5-2H1 (Fournier et al. 2006) diluted 1:500 in tris buffered saline (TBS). After 60 min at 27 °C, the primary antibody was discarded and 260 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 5 µl aliquot of a predator sample mixed with 35 µl of TBS (for total volume of 40 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 (40 µl) of the *Homalodisca* species-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 40 µl of TMB One Component HRP Substrate (BioFX Laboratories, Owings Mills, MD, 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, CA, USA) set at a wavelength of 650 nm.

PCR assay

DNA extraction

Arthropod specimens were weighed, placed individually in sterile 2.0 ml microtubes, and homogenized with in a TissueLyser (QIAGEN Inc., Valencia, CA, USA) for 1 min at 30 Hz in 180 µl of phosphate buffered saline (PBS, pH 7.2) using sterile 5 mm stainless steel beads. A maximum of 50 mg of tissue was individually processed. If a specimen weighed over 50 mg, it was homogenized in 360 µl of PBS. The homogenates were then centrifuged at 8,000 rpm (at 4 °C) for 4 min. The DNA was then extracted from the samples using the DNeasy Blood and Tissue Kit (QIAGEN Inc). Samples that were homogenized in 360 µl of PBS were split between two DNeasy mini spin columns. Total DNA was eluted twice in 30 µl of AE buffer provided by the manufacturer. The DNA extracts were stored at −80 °C. A 10-µl aliquot of supernatant from each sample was pipetted into a clean 1.5 ml microtube and stored at −80 °C for subsequent ELISA. The remaining 170 µl of the homogenized sample underwent DNA extraction using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA, USA; protocol for insects).

DNA quantification and normalization

DNA extracts were quantified and normalized prior to PCR amplification to control for PCR amplification variation and quenching. A 1.5 µl aliquot from each DNA sample was taken for quantification with Thermo Scientific's Nanodrop 3300 (West Palm Beach, FL, USA). Each quantified sample was then normalized to a concentration of 25 ng μl^{-1} using sterile TE Buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0).

PCR amplification

DNA samples were amplified using 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 only to *H. vitripennis*. The PCR amplifications were performed in 10 µl reaction volume containing: 3 µl of 25 ng μl^{-1} DNA extract, 0.25 µl of 10 mM deoxynucleotide triphosphates (New England BioLabs Inc., Ipswich, MA, USA), 0.5 µl of primers (5 µM), 0.2 µl HotStarTaq DNA Polymerase (QIAGEN Inc.), 1 µl of QIAGEN 10× PCR buffer, 0.55 µl of 25 mM $MgCl_2$ and 4 µl of RNase-free water. Samples were amplified in a gradient thermal cycler (Eppendorf Mastercycler® gradient, Eppendorf, Westbury, NY, USA) beginning with an initial denaturing step of 94 °C for 5 min followed by 50 cycles of 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 30 s. The PCR reaction was finished with a 10 min extension at 72 °C.

Predator controls for field samples

Negative control predators were obtained for each predator taxon collected from cotton fields located at the USDA-ARS in Maricopa, AZ, USA, purchased from insectaries, or taken from our laboratory colonies. The field-collected control predators were collected from areas not infested by GWSS. Live negative control insect and spider predators were starved for two 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. The negative control predators were assayed by the ELISA and PCR assays described above.

Scoring of the predator specimens

PCR products were separated by electrophoresis in 2 % agarose gels. Each gel was stained with ethidium bromide and a band on the gel indicating the presence of the GWSS DNA fragment was visualized using Quantity One Software™ (Bio-Rad Laboratories, Hercules, CA, USA).

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 1,000 µl PBS), and (3) eight individual negative predator controls (i.e., predators not fed GWSS). Field-collected predators were scored positive for prey remains if they yielded an ELISA response six standard deviations above that of their respective negative control mean (Hagler 2011).

Statistical analysis

Predator samples collected over the course of the two-year study were pooled by their respective taxon (family). Simple descriptive statistics showing the number of predators collected for each taxon and the proportion of their population testing positive for GWSS remains by ELISA and PCR assay are presented.

Results

The seasonal population dynamics of *H. vitripennis* and its associated predators in the citrus orchard over the two year period of the study are given in Krugner et al. (2009). A total of 1,578 field-collected predators, representing 18 taxa, were collected in the beat net samples over the duration of the study and then examined for *Homalodisca* species egg protein remains by an egg-specific ELISA (Table 1). In addition, 1,148 of these predators (e.g., all the spiders and some of the insects) were also examined for presence of prey DNA fragments by a *H. vitripennis*-specific PCR assay. Spiders were encountered more frequently than insect predators, accounting for 67.6 % ($n = 1{,}067$) of the arthropod predator

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

Specimens assayed			Total[b] (ELISA or PCR)		ELISA only[c]		PCR only[d]		ELISA and PCR[e]		Sensitivity[f] index
Class	Family	n[a]	# Pos	% Pos	# Pos	% Pos	# Pos	% Pos	# Pos	% Pos	
Insecta	Reduviidae	13	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Labiduridae	8	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Chrysopidae	289	1	0.35	1	0.35	1	3.45	1	0.35	1.00
	Coccinellidae	189	1	0.53	0	0.00	1	5.26	0	0.00	NA
	Nabidae	12	1	8.33	1	8.33	1	8.33	1	8.33	1.00
	Insect total	511	3	0.59	2	0.39	3	3.70	2	0.39	1.00
Arachnida	Agelenidae	5	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Anyphaenidae	330	9	2.73	6	1.82	5	1.52	2	0.61	0.33
	Araneidae	93	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Corinnidae	95	4	4.21	4	4.21	1	1.05	1	1.05	0.25
	Gnaphosidae	4	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Linyphiidae	2	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Mimetidae	1	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Miturgidae	428	16	3.74	9	2.10	12	2.80	5	1.17	0.56
	Oxyopidae	3	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Salticidae	92	4	4.35	1	1.09	3	3.26	0	0.00	0.00
	Tetragnathidae	2	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Theridiidae	3	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Thomisidae	9	0	0.00	0	0.00	0	0.00	0	0.00	NA
	Spider total	1,067	33	3.09	20	1.87	21	1.97	8	0.75	0.40
	Grand total	1,578	36	2.28	22	1.39	24	2.09	10	0.63	0.45

[a] The total number of predators collected from the citrus orchard. Note that all the predators were assayed by ELISA, but only 29 and 19 of the Chrysopidae and Coccinellidae were assayed by PCR, respectively

[b] Total number positive by ELISA, PCR, or both

[c] Total number positive by only the ELISA

[d] Total number positive by only the PCR assay

[e] Total number positive by both the ELISA and PCR assay (double confirmation of a feeding event)

[f] Sensitivity indices 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

population. Of these, the Miturgidae ($n = 428$) and Anyphaenidae ($n = 330$) were the most dominant spider taxa collected (Table 1). The two dominant insect predator taxa included 289 Chrysopidae (*Chrysoperla* spp.) and 189 Coccinellidae (*Hippodamia convergens* Guérin-Méneville).

Overall, 2.28 % (n = 36) of the arthropods yielded a positive gut reaction for the targeted prey remains by the ELISA or PCR assay. Only 1.39 % (22 out of 1,578 specimens) and 2.09 % (24 out of 1,148) of these predators scored positive for prey remains by means of only the ELISA or only the PCR assay, respectively (Table 1). Of the 36 predators that scored positive by ELISA or PCR, only ten specimens yielded a positive response to both assay types. The frequencies of predation ranged from 0 % for many of the taxa to 8.33 % for Nabidae (note that this represents only one positive gut assay reaction out of 12 specimens collected).

Equal (if they are feeding solely on GWSS eggs or gravid females) or a higher number of positive reactions are expected from the PCR assay because it detects predation on both GWSS genders and all life stages (de León et al. 2006), whereas the *Homalodisca*-specific ELISA only detects an egg or adult

female (gravid) predation event (Fournier et al. 2006). A simple method to compare the effectiveness of the two assay types is to sum the number of individuals testing positive for GWSS remains by both ELISA and PCR, and then divide this number by the number of positive reactions yielded by only the ELISA. If both assays are similar in sensitivity, the net value obtained should be $\geq$1.0. In other words, the PCR assay should always yield a positive reaction if the ELISA is also positive (e.g., an egg predation event), plus it should yield a positive reaction if it consumed only a GWSS nymph or adult male. We consistently found discrepancies in sensitivity between the assays. For example, nine Anyphanenidae yielded a positive reaction by ELISA or PCR (Table 1). However, only two yielded positive reactions by both ELISA and PCR. Furthermore six of these individuals scored positive in only the ELISA. The net result was a gut assay efficiency index of 0.33. This suggests that there was a high proportion of ELISA false positive reactions and/or false-negative PCR reactions. However, we believe we reduced to likelihood of obtaining ELISA false positive reactions by using a very conservative criterion for scoring the ELISA results. Specifically, we used the predator negative control mean ELISA OD value + 6SD to score a positive reaction instead of the conventional mean + 3SD method employed by most other researchers.

Discussion

This study showed that spiders were the most abundant arthropod predators collected in the beat nets. Other studies have also shown that spiders are more common in many natural and managed California ecosystems (Riechert and Lockley 1984; Riechert and Bishop 1990; Young and Edwards 1990). For instance, spiders constituted 98 % and 69 % of the total predator fauna found in California vineyards and urban areas, respectively (Costello and Daane 1999; Fournier et al. 2008). Moreover, a gut analysis of the urban predator population showed that 18 % of the spiders and 11 % of the insect predators contained GWSS remains, suggesting that spiders may prey upon GWSS more frequently than predaceous insects (Fournier et al. 2008). In the present study, we only detected GWSS prey remains in 0.59 % (3 of the 511 insect predators; note that only 81 of the insect predators were assayed by PCR) of the insect predator population, whereas 3.09 % (33 of the 1,067) of the spider population encountered tested positive for GWSS remains by the *Homalodisca*-specific ELISA or *H. vitripennis*-specific PCR assay.

Previous cross reactivity tests revealed that the ELISA used in this study also reacts to the egg stage (and gravid female stage) of the smoke-tree sharpshooter (STSS), *H. liturata* Ball (Fournier 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, we contend that this is very unlikely because STSS only comprised 1.1 % of the sharpshooter population in the citrus orchard (RK, pers. obs.). As such, the main difference between the two types of gut assays is that the ELISA only detects predation on the sharpshooter egg and adult (gravid) female life stages, whereas the PCR assay detects predation on both male and female GWSS life stages including eggs (de León et al. 2006). Therefore, if both assays were equally effective at detecting GWSS prey remains, then every predator scoring positive by ELISA should also be positive using PCR, but not vice versa. In other words, the number of positive PCR assay reactions should be equal (e.g., if it only fed on an egg) or greater (e.g., if it fed on any life stage) than the number of reactions yielded by the ELISA for any given taxon. The results from this study and that of Fournier et al. (2008) showed that this was clearly not the case. This can probably be attributed to highly variable efficiency between the two assay types and among the various predator species examined. For example, Fournier et al. (2006, 2008) and many others have shown that the prey detection half-lives exhibited by various predator species can be highly variable (Hagler and Naranjo 1997; Zaidi et al. 1999; Chen et al. 2000; Harper et al. 2005). For instance, for *Chrysoperla carnea* Stephens, the GWSS PCR assay and the ELISA yielded similar results with GWSS egg detection half-lives of 11.0 and 11.8 h, respectively (Fournier et al. 2006, 2008). In contrast, for *Harmonia axyridis* (Pallas), the 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. These results and those of others illustrate how the prey detection interval can vary between both the predator species being examined and the type of assay used (e.g., PCR assay, indirect ELISA, sandwich ELISA).

The lack of predation events recorded in this study was surprising, given that the field was heavily infested with GWSS eggs, nymphs and adults at various times throughout the year (see Krugner et al. 2009). The gut content assay results from this study and by Fournier et al. (2008) revealed that spiders may prey upon GWSS more frequently than predaceous insects. However, this should be interpreted with caution 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). The lack of positive reactions yielded by the predators for the presence of GWSS prey remains is likely due to two factors. First, the predator complex might be feeding on more desirable prey items or prey items that are easier to capture. For example, it is unlikely that a relatively slow moving and "stalking" predator can catch a fast moving and agile GWSS nymph or adult. An exception to this might be if a GWSS nymph is emerging from an egg, which is about a 2 h process (RK, pers. obs.). It is also unlikely that predators with chewing type mouthparts can feed on the GWSS egg stage because their mouthparts are not well adapted to reach GWSS eggs, which are protected in nature because they are deposited underneath a thin layer of the leaf epidermis. In laboratory feeding trials, Fournier et al. (2006, 2008) showed that insect predators were reticent to feed on GWSS eggs until they "teased" the embedded eggs away from the leaf tissue. Second, the low frequencies of recorded predation events may be an artifact of our sampling scheme coupled with short prey detection intervals. Specifically, the time of day that we collected the predators (e.g., dawn to sunrise) may have had an impact on the gut assay results. For example, some of the spider families (e.g., Anyphaenidae and Miturgidae) are nocturnal feeders. Therefore, they might yield a higher frequency of positive gut assay reactions due to the time of day in which they were collected in the field. This would be especially true for those predators that do not retain the prey very long. For example, as mentioned above, the prey detection half-life of a GWSS egg is only 2.2 h for *H. axyridis*. If GWSS can only be detected for 2.2 h, then a more intense sampling schedule should 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, shorter prey retention intervals are more desirable in most instances for more precise estimates (e.g., semi-quantitative) of predation, but a greater (more frequent) sampling effort is required.

In summary, our results show that there was very little predation detected on GWSS in the citrus orchard, which indicates that GWSS life stages were not the primary prey for the generalist predators examined. In all likelihood, these predators were feeding on more vulnerable prey items because GWSS nymphs and adults are very agile and the eggs are somewhat protected from predators in nature because they are deposited underneath the leaf epidermis (Fournier et al. 2008). Future studies are warranted on the prey retention period in more predator species and to determine if greater frequencies of predation can be detected if the predators are collected at different times of the day and night.

Acknowledgments We would like to thank Scott Machtley and Arnel P. Flores for their excellent technical support. This work was funded by the California Department of Food and Agriculture, the University of California's Pierce's Disease Grant Program, and the USDA-ARS.

References

Aebi A, Brown PMJ, De Clercq P, Hautier L, Howe A, Ingels B, Ravn HP, Sloggett JJ, Zindel R, Thomas A (2011) Detecting arthropod intraguild predation in the field. BioControl 56:429–440

Agustí N, de Vincente MC, Gabarra R (1999) Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: a new polymerase chain reaction-based technique for predator gut analysis. Mol Ecol 8:1467–1474

Almeida RPP, Purcell AH (2003) Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). J Econ Entomol 96:264–271

Blua MJ, Phillips PA, Redak RA (1999) A new sharpshooter threatens both crops and ornamentals. Calif Agric 53:22–25

California Department of Food and Agriculture (CDFA) (2006) County agricultural commissioner's data, calendar year 2005. California Department of Food and Agriculture, Sacramento, USA

Chang CJ, Garnier M, Zreik L, Rossetti V, Bové JM (1993) Culture and serological detection of *Xylella fastidiosa*, the xylem-limited bacterium associated with citrus variegated chlorosis disease. In: Moreno LWTP, da Graça JV (eds) Proceedings of the 12th conference of the international organization of citrus virologists, New Delhi. University of California, Riverside, USA, pp 294–300

Chen Y, Giles KL, Payton ME, Greenstone MH (2000) Identifying key cereal aphid predators by molecular gut analysis. Mol Ecol 9:1887–1898

Costello MJ, Daane KM (1999) Abundance of spiders and insect predators on grapes in central California. J Arachnol 27: 531–538

Damsteegt VD, Brlansky RH, Phillips PA, Roy A (2006) Transmission of *Xylella fastidiosa,* causal agent of citrus variegated chlorosis, by the glassy-winged sharpshooter, *Homalodisca coagulata*. Plant Dis 90:567–570

Davis MJ, Purcell AH, Thompson SV (1978) Pierce's disease of grapevines: isolation of the causal bacterium. Science 199:75–77

de León JH, Fournier V, Hagler JR, Daane KM (2006) Development of molecular diagnostic markers for the glassy-winged sharpshooter *Homalodisca coagulata* (Homoptera: Cicadellidae) for use in predator gut content examinations. Entomol Exp Appl 119:109–119

Fournier V, Hagler JR, Daane KM, de León JH, Groves RL, Costa HS, Henneberry TJ (2006) Development and application of glassywinged and smoke-tree sharpshooter egg-specific predator gut content ELISA. Biol Control 37:108–118

Fournier V, Hagler JR, Daane KM, de León JH, Groves RL (2008) Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay. Oecologia 157:629–640

Greenstone MH (1983) Site-specificity and site tenacity in a wolf-spider: a serological dietary analysis. Oecologia 56:79–83

Greenstone MH (1996) Serological analysis of arthropod predation: past, present, and future. In: Symondson WOC, Liddell JE (eds) The ecology of agricultural pests. Chapman and Hall, New York, USA, pp 383–399

Greenstone MH, Morgan CE (1989) Predation on *Heliothis zea* (Lepidoptera: Noctuididae): an instar specific ELISA assay for stomach analysis. Ann Entomol Soc Am 82:45–49

Greenstone MH, Shufran KA (2003) Spider predation: species-specific identification of gut contents by polymerase chain reaction. J Arachnol 31:131–134

Hagler JR (2006) Development of an immunological technique for identifying multiple predator-prey interactions in a complex arthropod assemblage. Ann Appl Biol 149:153–165

Hagler JR (2011) An immunological approach to quantify consumption of protein-tagged *Lygus hesperus* by the entire cotton predator assemblage. Biol Control 58:337–345

Hagler JR, Naranjo SE (1997) Measuring the sensitivity of an indirect predator gut content ELISA: detectability of prey remains in relation to predator species, temperature, time, and meal size. Biol Control 9:112–119

Hagler JR, Cohen AC, Enriquez FJ, Bradley-Dunlop D (1991) An egg-specific monoclonal antibody to *Lygus hesperus*. Biol Control 1:75–80

Hagler JR, Brower AG, Tu Z, Byrne DN, Bradley-Dunlop D, Enriquez FJ (1993) Development of a monoclonal antibody to detect predation of the sweetpotato whitefly, *Bemisia tabaci*. Entomol Exp Appl 68:231–236

Hagler JR, Naranjo SE, Bradley-Dunlop D, Enriquez FJ, Henneberry TJ (1994) A monoclonal antibody to pink bollworm (Lepidoptera: Gelechiidae) egg antigen: a tool for predator gut analysis. Ann Entomol Soc Am 87:85–90

Harper GL, King RA, Dodd C, Harwood JD, Glen DM, Bruford MW, Symondson WOC (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. Mol Ecol 14:819–827

Harwood JD, Phillips SW, Sunderland KD, Symondson WOC (2001) Secondary predation: quantification of food chain errors in an aphid–spider–carabid system using monoclonal antibodies. Mol Ecol 10:2049–2057

Harwood JD, Sunderland KD, Symondson WOC (2004) Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. Mol Ecol 13:3549–3560

Harwood JD, Bostrom MR, Hladilek EE, Wise DH, Obrycki JJ (2007a) An order-specific monoclonal antibody to Diptera reveals the impact of alternative prey on spider feeding behavior in a complex food web. Biol Control 41:397–407

Harwood JD, Desneux N, Yoo HJS, Rowley DL, Greenstone MH, Obrycki JJ, O'Neil RJ (2007b) Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. Mol Ecol 16:4390–4400

Hix R, Toscano N, Gispert C (2003) Area-wide management of the glassy-winged sharpshooter in the Temecula and Coachella Valleys. In: Tariq MA, Oswalt S, Blincoe P, Spencer R, Houser L, Ba A, Esser T (eds) Proceedings, 2003 Pierce's disease research symposium. California Department of Food and Agriculture, Coronado, CA, 8–11 December 2003. Copeland Printing, Sacramento, USA, pp 292–294

Irvin NA, Hoddle MS (2005a) The competitive ability of three mymarid egg parasitoids (*Gonatocerus* spp.) for glassy-winged sharpshooter (*Homalodisca coagulata*) eggs. Biol Control 34:204–214

Irvin NA, Hoddle MS (2005b) Determination of *Homalodisca coagulata* (Hemiptera: Cicadellidae) egg ages suitable for oviposition by *Gonatocerus ashmeadi*, *Gonatocerus triguttatus*, and *Gonatocerus fasciatus* (Hymenoptera: Mymaridae). Biol Control 32:391–400

Krugner R, Groves RL, Johnson MW, Flores AP, Hagler JR, Morse JG (2009) Seasonal population dynamics of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) in sweet orange trees maintained under continuous deficit irrigation. J Econ Entomol 102:960–973

Park YL, Perring TM, Farrar CA, Gispert C (2006) Spatial and temporal distribution of two sympatric *Homalodisca* spp. (Hemiptera: Cicadellidae): implications for areawide pest management. Agric Ecosyst Environ 113:168–174

Perring TM, Farrar CA, Blua MJ (2001) Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. Calif Agric 55:13–18

Purcell AH, Saunders SR (1999) Glassy-winged sharpshooter expected to increase plant disease. Calif Agric 53:26–27

Riechert SE, Bishop L (1990) Prey control by an assemblage of generalist predators: spiders in garden test systems. Ecology 71:1441–1450

Riechert SE, Lockley T (1984) Spiders as biological control agents. Annu Rev Entomol 29:299–320

Sorensen JT, Gill RJ (1996) A range extension of *Homalodisca coagulata* (Say) (Hemiptera: Clypeorrhyncha: Cicadellidae) to southern California. Pan-Pac Entomol 72:160–161

Symondson WOC, Liddell JE (1996) A species-specific monoclonal antibody system for detecting the remains of

field slugs, *Deroceras reticulatum* (Mueller) (Mollusca: Pulmonata), in carabid beetles. Biocontrol Sci Technol 6:91–99

Triapitsyn SV, Mizell RF, Bossart JL, Carlton CE (1998) Egg parasitoids of *Homalodisca coagulata* (Homoptera: Cicadellidae). Fla Entomol 81:241–243

Triapitsyn SV, Morgan DJ, Hoddle MS, Berezovskiy VV (2003) Observations on the biology of *Gonatocerus fasciatus* Girault (Hymenoptera: Mymaridae), egg parasitoid of *Homalodisca coagulata* (Say) and *Oncometopia orbona* (Fabricius) (Hemiptera: Clypeorrhyncha: Cicadellidae). Pan-Pac Entomol 79:62–63

Vickerman DB, Hoddle MS, Triapitsyn SV, Stouthamer R (2004) Species identity of geographically distinct populations of the glassy-winged sharpshooter parasitoid *Gonatocerus ashmeadi*: morphology, DNA sequences and reproductive compatibility. Biol Control 31:338–345

Wendel L, Ciomperlik M, Bartels D, Luvisi D, Elms D (2002) The area-wide pest management of glassy winged sharpshooter in Kern County. In: Tariq MA, Oswalt S, Blincoe P, Esser T (eds) Proceedings, 2002 Pierce's disease research symposium. California Department of Food and Agriculture, Coronado, CA, 15–18 December 2002. Copeland Printing, Sacramento, USA, pp 302–307

Young OP, Edwards GB (1990) Spiders in United States field crops and their potential effects on crop pests. J Arachnol 18:1–27

Zaidi RH, Jaal Z, Hawkes NJ, Hemingway J, Symondson WOC (1999) Can multiple copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? Mol Ecol 8:2081–2087

Author Biographies

James R. Hagler is a Research Entomologist at the USDA-ARS, Arid-Land Agricultural Research Center, Maricopa, Arizona, USA. His research concentrates on biological control, predator gut content analysis, and insect dispersal.

Felisa Blackmer is a Biological Science Technician at the USDA-ARS, Arid-Land Agricultural Research Center, Maricopa, Arizona, USA. Her research concentrates on designing and optimizing PCR protocols for predator gut content analysis.

Rodrigo Krugner is a Research Entomologist working on the biology, ecology, and biological control of insect vectors of plant pathogens.

Russell L. Groves is an Associate Professor at the Department of Entomology, University of Wisconsin, USA. His research concentrates on vegetable IPM and applied insect ecology with an emphasis on insect vector-borne disease epidemiology.

J. G. Morse is a Professor of Entomology at the University of California, Riverside, USA. His research concentrates on arthropod pest management on citrus and avocados in California with a focus on invasive species, biological control, and contaminants of import and export commodities.

Marshall W. Johnson conducts IPM research and extension on agricultural pests in orchard crops, recently focusing on innovative methods to extend information via videography.