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Evidence of intraguild predation on a key member of the cotton predator complex☆

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It is well known that most arthropod predators are generalist feeders and consume a wide range of prey species. As such, intraguild predation (IGP) is prevalent in virtually every agroecosystem. A polymerase chain reaction (PCR) assay was developed to examine the gut contents of predators that inhabit Arizona cotton for the presence of the DNA of green lacewing, *Chrysoperla carnea*, a common lower-tiered member of the predator community. A total of 1440 predators were captured using both a sweep net and whole plant sampling method. The gut content analyses revealed that over 10% of the predator population contained *C. carnea* DNA in their gut. Of these, IGP on *C. carnea* was detected almost twice as frequently in predaceous insects as spiders. The assassin bugs frequently encountered in cotton, *Zelus renardii* and *Sinea confusa* and orb-weaving spiders (Araneidae) were frequently identified as apex predators of *C. carnea*. These data provide a better understanding of predator–predator interactions in cotton and highlight areas for future research. That is, more thorough studies are needed to quantify prey selection of apex predators. Apex predators that frequently engage in IGP could be potentially antagonistic to the biological services rendered by the entire predator community.

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1. Introduction

An ideal predaceous biological control agent would be one that feeds exclusively on the herbivore pest(s). However, most arthropod predators are opportunists and do not discriminate between lower tiered herbivores, omnivores, or carnivores (Rosenheim et al., 1993, 1995; Snyder and Wise, 1999; Takizawa and Snyder, 2011). Clearly more studies are needed to identify the feeding proclivity of predators in their natural habitat. If any given predator species frequently engages in intraguild predation (IGP) it could reduce the biological control services provided by the entire predator community by preferentially feeding on those predators that are actually providing the biological control services (i.e., feeding on herbivore pests). As such, it is critical that we investigate the entire diet breadth of generalist predators instead of just focusing on predator–herbivore interactions.

Investigating predator activity is difficult due to the cryptic behavior exhibited by most arthropods. Direct visual observations of predation in situ are rare and can interrupt predator foraging behavior. Post-mortem, or indirect examination of predator gut contents, is a common method used to assess predation. Specifically, the prey-specific polymerase chain reaction (PCR) assay method has been widely used to examine trophic level interactions (Harwood et al., 2007b; Juan and Juen and Traugott, 2007; Moreno-Ripoll et al., 2012; Günther et al., 2014). These studies and many others (see Symondson and Harwood, 2014) have used molecular gut examinations to identify various aspects of predator feeding (e.g., predator–prey, scavenging, IGP, etc.) in natural, agricultural, and aquatic ecosystems. Recently we developed four prey-specific PCR assays to detect DNA in the guts of various members of the cotton predator community (Hagler and Blackmer, 2013). The targeted prey included two major cotton pests, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and *Lygus* spp. (Heteroptera: Miridae), and two of their natural enemies; *Collops vittatus* (Say) (Coleoptera: Melyridae) and *Geocoris* spp. (Heteroptera: Geocoridae). We used the gut assays to qualitatively evaluate the frequency of predation on these taxa. That study revealed substantial interguild predation occurring on the strict herbivore, *B. tabaci* (a pest); moderate intraguild predation on the omnivores, *Lygus* spp. (pests) and *Geocoris* spp. (beneficials) and little intraguild predation on *C. vittatus* (a beneficial). The assays also showed that pest remains were found more frequently in predaceous insects than in spiders; whereas there were no differences between the predatory insects and spiders for the targeted beneficials. In addition, there were very few green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) larvae collected in the cotton field. This seemed counterintuitive because we were readily capturing adults in our sweep net and whole plant samples, and frequently observing eggs on the undersides of cotton leaves (pers. obs.). We hypothesized that certain members of the cotton predator

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community were preferentially feeding on the immature *C. carnea*. Hence, we developed a *C. carnea*-specific PCR assay and then use it to re-analyze the predators collected and preserved by Hagler and Blackmer (2013) for the presence of *C. carnea* DNA. If our study supports our hypothesis, then more studies are needed to better elucidate prey preferences of those top-tiered predators that frequently engage in IGP. Such research will determine if top-tiered predators are a benefit or a detriment to the biological control services provided the predator assemblage inhabiting cotton.

2. Methods

2.1. *Chrysoperla carnea*-specific PCR assay

2.1.1. DNA extraction

Individual field-collected arthropod predator specimens were put in sterile 2.0-mL microtubes and homogenized in 180 μL of phosphate buffered saline (PBS, pH 7.2) using sterile 5-mm stainless steel beads and QIAGEN's TissueLyser (1 min at 30 Hz). A maximum of 50 mg of tissue was processed; specimens that weighed >50 mg were homogenized in 360 μL of PBS. The homogenates were centrifuged at 3935 *g* (4 °C) for 4 min. Each sample then underwent DNA extraction using the DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, CA, USA). Samples that were homogenized in 360 μL of PBS were split between two DNeasy mini columns. Total DNA was eluted 2× in 30 μL AE buffer that was provided by the manufacturer. The stock DNA extracts were frozen at −80 °C.

2.1.2. DNA quantification and normalization

DNA extracts were quantified and normalized prior to PCR amplification to control for amplification variation and quenching. A 1.5-μL aliquot of each DNA sample was used for quantification using Thermo Scientific's Nanodrop 1000. Each quantified sample was then normalized to a concentration of 40 ng/μL using sterile TE Buffer (10 mM Tris–HCL, 0.1 mM EDTA, pH 8.0).

2.1.3. PCR amplification

A primer set was designed for the *C. carnea* cytochrome oxidase subunit I gene (5′-CCTATTGTAATTGGAGGTTTTGG, 5′-TCCAGCATGAGC AATTCTTG, GenBank Accession Number AY743792) using Primer3 software (Untergrasser et al., 2012). The PCR amplifications were performed in a 10-μL reaction volume containing: 3 μL of 40 ng/μL DNA extract, 1 μL of both primers (2.5 μM), and 5 μL of HotStarTaq Master Mix (QIAGEN Inc.). Samples were amplified in an Eppendorf Mastercycler gradient thermal cycler (Eppendorf, Westbury, NY, USA) beginning with an initial denaturing step of 95 °C for 15 min followed by 10 cycles of touchdown PCR at 94 °C for 30 s, 69 °C for 30 s, and 72 °C for 30 s, −1 °C/cycle. Touchdown PCR was followed by 50 cycles at 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s. The PCR reaction was completed after a 10 min cycle at 72 °C. For each PCR assay, one *C. carnea* control, and one sterile TE negative control was included in the amplification.

PCR products were separated by electrophoresis (120 V, 25 min) in 2% agarose gels. Gels were stained with ethidium bromide and the bands on the gel were visualized using Quantity One Software™ (Bio-Rad Laboratories, Hercules, CA, USA). Predator specimens were scored positive by PCR if a band appeared at 207 bp on the gel.

2.2. PCR cross reactivity screening tests

Tests were conducted to confirm that the *C. carnea* primer set did cross react with the DNA of over 30 species (from 8 eight insect and 9 spider families) commonly encountered in cotton. A list of the arthropods screened for cross reactivity to the PCR assay is given in Hagler and Blackmer (2013). These specimens were collected from cotton and alfalfa fields located at the University of Arizona Maricopa Agricultural Center, Maricopa, Arizona, USA. Prior to DNA extraction, each predator and potential prey item was placed into an individual Petri dish that only contained a water saturated sponge. Each arthropod was left in the dish for >24 h to allow it to purge its gut of *C. carnea* DNA that they may have obtained prior to collection. Then, each individual was assayed by the *C. carnea*-specific PCR assay described above.

2.3. Field study

2.3.1. Predators

Except for exclusion of *Lygus* spp. and some of less common predators encountered, the same predator specimens examined by Hagler and Blackmer (2013) for the presence of *B. tabaci*, *Lygus* spp., *Geocoris* spp. and *C. vittatus* DNA were re-examined for the presence of *C. carnea* DNA using the PCR assay described above.

2.3.2. Study site

A thorough description of the study site and sampling procedures are described in Hagler and Blackmer (2013). Briefly, the predators were collected from a 1.5-ha cotton field (*Gossypium hirsutum* L.) located at the Maricopa Agricultural Center (GPS coordinates: 33° 04′ 37″ N, 111° 58′ 26″ W). The cotton (CV '*DP5415 RR*') was grown using standard agronomic practices. Predators were collected by a whole plant sampling procedure on August 8, 2007 and September 4, 2008 ($n = 100$ plants each year) and by sweep net sampling on August 6, 2007 and September 2, 2008 ($n = 100$ samples each year [10 sweeps per sample]).

2.3.3. Data analysis of predator gut content assays

The *C. carnea* (DNA)-specific PCR was performed on the field-collected predators to determine the proportion of individuals from each taxon that contained *C. carnea* DNA. Significant differences in feeding activity on *C. carnea* exhibited by: (1) arthropod class (insects vs. spiders), (2) sampling method (sweep net vs. whole plant), and (3) year (2007 vs. 2008) were determined by the proportions *z*-test with Yates correction for continuity (SigmaPlot ver. 11.0; Glantz, 1997).

3. Results

3.1. PCR cross reactivity tests

The list of arthropod taxa examined for cross reactivity to the *C. carnea*-specific PCR assay are listed in Table 2 of Hagler and Blackmer (2013). Assay results were positive only for the various lifestages of *C. carnea* that were analyzed (data not shown).

3.2. Field study

3.2.1. Arthropod predator abundance

The sweep net and whole plant sampling schemes only yielded 37 *C. carnea* larvae in 2007 and 2008. Of these, 22 were collected in the whole plant samples and 15 in the sweep samples. Moreover, 33 of the 37 were collected in 2008. A thorough description of the population dynamics of the predator community encountered in the cotton during the study is given in Hagler and Blackmer (2013). To summarize: (1) insect predators ($n = 832$) were captured more often than spiders ($n = 608$), (2) the sweep net sampling scheme collected more predators ($n = 1053$) than the whole plant sampling scheme ($n = 387$), and (3) the cotton field contained more predators in 2008 ($n = 841$) than in 2007 ($n = 599$) (Table 1; Fig. 1).

3.2.2. PCR gut assay results

As expected, all 37 of the field-collected *C. carnea* yielded a positive PCR reaction for *C. carnea* DNA (positive controls). Overall, 1440 field-collected arthropod predators, representing 15 taxa were analyzed for *C. carnea* remains. The frequencies of *C. carnea* predation recorded for

Table 1
The number (N) of insect and spider predators collected, the number positive (n) and the percent positive (%) for the presence of *Chrysoperla carnea* remains in their stomachs. The predators were collected by sweep net or whole plant sampling schemes.

Insects		2007 sweeps			2007 whole plants			2008 sweeps			2008 whole plants			Grand totals		
Family	Predominate taxon	N	n	%	N	n	%	N	n	%	N	n	%	N	n	%
Geocoridae	*Geocoris* spp.	211	30	14.2	3	0	0.0	161	5	3.1	19	2	10.5	394	37	9.4
Nabidae	*Nabis alternatus*	26	4	15.4	2	0	0.0	2	0	0.0	1	0	0.0	31	4	12.9
Reduviidae	*Sinea confusa*	16	6	37.5	1	0	0.0	1	0	0.0	2	0	0.0	20	6	30.0
	Zelus renardii	87	30	34.5	9	3	33.3	134	14	10.4	67	5	7.5	297	52	17.5
Melyridae	*Collops vittatus*	17	5	29.4	5	1	20.0	31	1	3.2	18	1	5.6	71	8	11.3
Forficulidae	*Forficula auricularia*	0			0			0			19	0	0.0	19	0	0.0
Insect totals		357	75	21.0	20	4	20.0	329	20	6.1	126	8	6.3	832	107	12.9
Spiders		2007 sweeps			2007 whole plants			2008 sweeps			2008 whole plants			Grand totals		
Family	Genus	N	n	%	N	n	%	N	n	%	N	n	%	N	n	%
Araneidae	Various	16	5	31.3	1	0	0.0	7	0	0.0	3	1	33.3	27	6	22.2
Clubionidae	*Clubiona* spp.	0			1	1	100.0	19	1	5.3	6	0	0.0	26	2	7.7
Corinnidae	*Trachelas* spp.	0			14	1	7.1	3	0	0.0	3	0	0.0	20	1	5.0
Dictynidae	*Dictyna reticulata*	31	3	9.7	13	0	0.0	23	0	0.0	41	2	4.9	108	5	4.6
Gnaphosidae	Various	0			0			2	0	0.0	11	0	0.0	13	0	0.0
Lycosidae	*Hogna* spp.	0			7	0	0.0	9	0	0.0	79	2	2.5	95	2	2.1
Miturgidae	*Cheiracanthium inclusum*	0			0			7	0	0.0	23	0	0.0	30	0	0.0
Salticidae	Various	14	5	35.7	5	1	20.0	41	1	2.4	15	0	0.0	75	7	9.3
Thomisidae	*Misumenops celer*	111	20	18.0	9	1	11.1	84	2	2.4	10	0	0.0	214	23	10.7
Spider totals		172	33	19.2	50	4	8.0	195	4	2.1	191	5	2.6	608	46	7.6
Grand totals		529	108	20.4	70	8	11.4	524	24	4.6	317	13	4.1	1440	153	10.6

each of the arthropod taxa examined are given in Table 1. Of the insect predators, the two reduviids, *Sinea confusa* (30%, $n = 20$) and *Zelus renardii* (17.5%, $n = 297$), had the highest proportion individuals testing positive for the presence of *C. carnea* DNA (Table 1). Other insect predators yielding relatively high frequencies of positive reactions included Nabidae (*Nabis alternatus*, 12.9%, $n = 31$), Melyridae (*C. vittatus*, 11.3%, $n = 71$), and Geocoridae (*Geocoris punctipes* (Say) and *Geocoris pallens* Stäl, 9.4%, $n = 394$). Overall, 7.6% of the spiders contained *C. carnea* in their gut. For spiders, Araneidae (several genera, 22.2%, $n = 27$), Thomisidae (*Misumenops celer* Hentz, 10.7%, $n = 214$), Salticidae (several genera, 9.3%, $n = 75$), and Clubionidae (*Clubiona* spp., 7.7%, $n = 26$) frequently contained *C. carnea* DNA in their guts (Table 1).

The gut content data were pooled to identify trends in feeding activity on *C. carnea* with respect to: (1) arthropod class, (2) sampling method, and (3) year of the study. The insect predators had a significantly higher frequency of positive PCR reactions for *C. carnea* than the spiders (Fig. 1a). Overall, 12.9% ($n = 107$ of 832) of the insects and 7.6% ($n = 46$ of 608) of the spiders contained *C. carnea* DNA in their gut. A total of 1053 and 387 predators were captured over the course of the study using the sweep net and whole plant sampling procedures, respectively (Fig. 1b). The predator gut assays revealed a significantly higher frequency of predator feeding events for the predators collected in sweep nets. Finally, the percentage of predation events recorded for *C. carnea* was significantly higher in 2007 (19.4%, $n = 599$) than in 2008 (4.4%, $n = 841$) (Fig. 1c).

4. Discussion

It is necessary to know the impact that generalist predators have on arthropod communities that occupy various trophic levels if we are going to identify the most promising candidates for conservation biological pest control (Hagler, 2006; Harwood et al., 2007a,b; Gagnon et al., 2011). Most predator gut assay examinations conducted to date have focused solely on identifying predators of a single herbivore pest (interguild predation; see Sheppard and Harwood, 2005). However, most predators are opportunists and will feed on weaker prey regardless if it is an herbivore or a lower-tiered predator. Therefore more studies are required to precisely pinpoint the proclivity of a given predator species to engage in interguild (effective biological control) and intraguild (ineffective or interference biological control) predation. Over two decades ago, Rosenheim et al. (1993) used field cage methodology to assess the degree of interguild and IGP occurring in a simple cotton arthropod community that was limited to either *Aphis gossypii* (Glover) and *C. carnea* together, or to a more complex community containing *A. gossypii*, *C. carnea* plus *Z. renardii*, *G. punctipes*, and *Nabis* spp. That study showed that *C. carnea* alone was effective at suppressing *A. gossypii*. However, *C. carnea* populations declined and *A. gossypii* populations surged once higher tiered predators were selectively introduced into the population. The conclusion from that study and a subsequent study (Cisneros and Rosenheim, 1997) was that higher tiered predators preferred *C. carnea*, thus hindering the biological services provided by the entire arthropod predator complex. Rosenheim et al. (1993) appropriately caution that the field cage methodology they used could have affected the outcome of their study because cage methods cannot precisely identify which member(s) of the predator assemblage were attacking *C. carnea*, and the experimental design required manipulation of the insect populations. The PCR gut assay approach used in this study complements field cage research by pinpointing which predators in the assemblage are feeding on *C. carnea* under natural field conditions.

The primary objective of this study was to examine field-collected predators for the presence of *C. carnea* remains. Prior to conducting this study it was essential that the PCR gut assay be optimized so that detectability was similar between the various predator species. As part of a separate study, such tests were conducted for four members of the cotton predator assemblage: *Hippodamia convergens*, *C. vittatus*, *G. punctipes*, and *Z. renardii*. In general, we were able to achieve reasonable standardization of the assay as *C. carnea* was detectible in these predators for 3 to 6 h after a meal (unpubl. data). These rather short detectability limits are similar to those reported in previous gut assay examinations (Hagler and Naranjo, 1997; Zaidi et al., 1999; Chen et al., 2000; Greenstone and Shufran, 2003; Harper et al., 2005; Fournier et al., 2008; Hagler and Blackmer, 2013).

The predator gut assay results were pooled to compare frequencies of predation events on *C. carnea* between arthropod classes (insects and spiders), sampling methods (sweeping and whole plant), and years (2007 and 2008). *Geocoris* spp. and *Z. renardii* were the most frequently encountered insect predator taxa. The gut content assays revealed that the two assassin bugs found in cotton, *S. confusa*

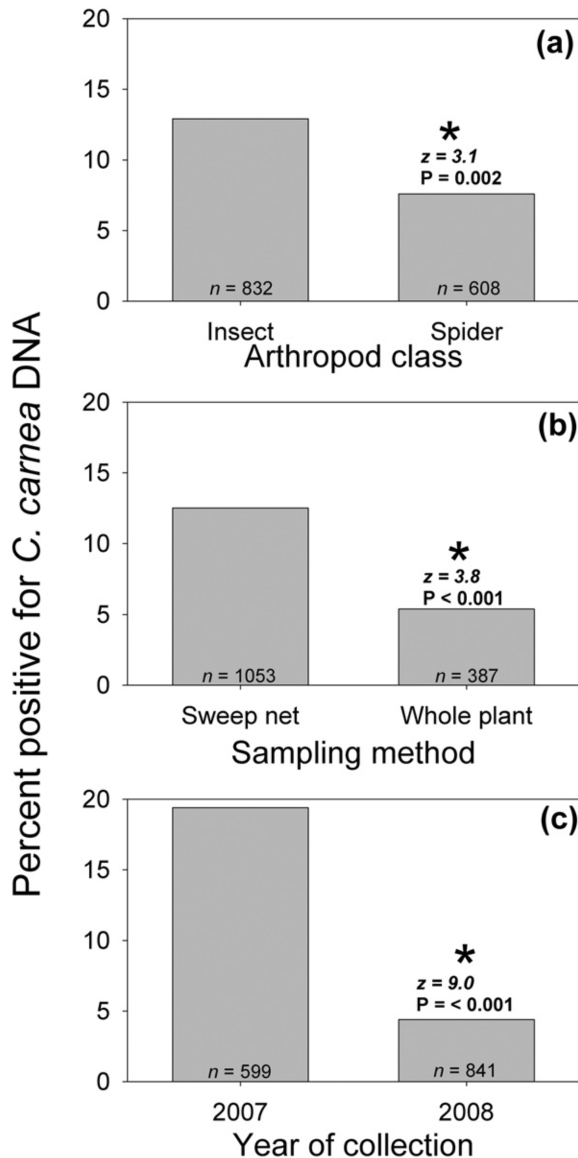

Fig. 1. A comparison of the frequencies of predation events recorded on *Chrysoperla carnea* between: (a) insect predators and spiders, (b) sweep net and whole plant sampling procedures, and (c) samples collected in 2007 and 2008. The numbers inside each bar above the x-axes are the sample sizes for each comparison and the numbers above the right vertical bar in each plot are the *z*-test statistic value and *P*-value. An asterisk denotes a significant difference between the paired treatment comparisons.

Caudell (Heteroptera: Reduviidae) and *Z. renardii* frequently preyed on *C. carnea*. Although these assassin bugs have been reported to feed on bollworm (*Heliothis* spp.) eggs when confined in a Petri dish (Lingren et al., 1968; Ewing and Ivy, 1943), they are mainly known to attack live and mobile prey (Ables, 1978; Cisneros and Rosenheim, 1997). Moreover, they typically employ a sit-and-wait (ambush) hunting strategy (Ables, 1978; Hagler, 2000). As such, they are probably not feeding on *C. carnea* eggs. Most likely they are attacking the larvae of *C. carnea* which are less mobile than the adult stage (Cisneros and Rosenheim, 1997). A high proportion of many of the other insect predator taxa examined (e.g., *N. alternatus*, *C. vittatus*, and *Geocoris* spp.) also contained *C. carnea* in their guts. All of these species have been shown to attack all the lifestages of *C. carnea* and other insects (Knowlton, 1944; Orphanides et al., 1971; Hussain, 1975; Hagler et al., 2004; Ramirez and Patterson, 2011; Zilnik and Hagler, 2013). However, it is unlikely that these predators are preying on *C. carnea* eggs because they are deposited on the tip of hair-like stalk that is about 1–2 cm in length (Canard et al., 1984). As such, an egg is somewhat protected from predators (Rùžička, 1997; Hayashi and Nomura, 2014). Again, it is most likely that these predators are attacking the less mobile larvae of *C. carnea*. Of the spiders encountered in the cotton, the various members of Araneidae complex (orb weavers) had the highest proportion of individuals (22.2%) containing *C. carnea* DNA in their guts. These spiders build flat, intricate, circular webs that are best designed to capture flying insects. As such, the adult life stage of *C. carnea* is probably the most vulnerable to attack by this taxa. The crab spider, *M. celer* was by far the most frequently encountered spider in the samples from cotton. Moreover, it contained the second highest proportion of individuals (10.7%) with *C. carnea* DNA in their guts. These relatively small sit-and-wait spiders are most likely ambushing early instar *C. carnea*. Our results and those of Hagler and Blackmer (2013) suggest the abundance of spiders in cotton and their proclivity to engage in both interguild and intraguild predation justifies that they be examined for biological control services as thoroughly as insect predators.

Another goal of this study was to determine if the method of arthropod collection could impact the gut analyses. The sampling scheme chosen for any given study could be a source of error for predator gut analyses research due to the possibility of predator-to-prey contact during the sweet net and post sweep net (e.g., sample bag) handling processes (King et al., 2008). Also, the predator could yield a positive PCR reaction for prey remains as a consequence of post collection consumption. Two studies reported no differences between the frequencies of predators testing positive for prey items when collected by hand picking vs. sweep netting or by hand picking vs. suction vacuuming (Harwood, 2008; Chapman et al., 2010). More recent research showed that harsh collection methods (e.g., sweep net and beat cloth) yielded higher proportions of positive gut assay reactions than non-harsh (e.g., hand picking and whole plant sampling) methods (Greenstone et al., 2011; Hagler and Blackmer, 2013). The sweep net method used here was less labor intensive, less time consuming, and collected almost three times more predators than the whole plant sampling method. Overall, 1053 insect predators and 387 spiders were collected in the sweep nets and whole plant samples, respectively. The gut analyses of these predators revealed a significantly higher proportion of positive PCR assay reactions yielded by the predators collected in the sweep nets. Whether this significant difference is an artifact of the sampling method is uncertain. Obviously the predators had a greater chance of direct contact (surface level contamination) with or feeding on the prey while confined in the sweep net and sweep net sample bag compared with the larger whole plant sampling bag. Also, differences yielded by the two sampling schemes could be an artifact of the two methodologies. For example, the sweep net scheme consisted of just collecting the predators from the top third of the cotton plant canopy whereas the whole plant scheme collected the predators throughout the plant canopy. Perhaps *C. carnea* populations are spatially distributed on the top portion of the plant as a response to prey availability. For example, *C. carnea* has been reported to be a voracious predator of various whitefly species (Gerling, 1986; Legaspi et al., 1996a,b). It is also well documented that all the whitefly lifestages are most commonly aggregated toward the upper half of the cotton canopy (Horowitz, 1986; Naranjo and Flint, 1994, 1995). Hence, it seems reasonable that a higher proportion of the predator population would contain *C. carnea* remains if the predators were only collected from the upper portion of the plant canopy. In short, choice of sampling scheme used for any given predator gut content analysis study warrants serious consideration.

Only 37 *C. carnea* larvae were collected during this study. Of these, only four were collected in 2007. However, the frequency of predation events recorded on *C. carnea* was four times higher in 2007 than in 2008. Interestingly, *B. tabaci* egg, nymph and adult populations were >10, 7 and 5 times higher, respectively, in 2008 compared with 2007 (Hagler and Blackmer, 2013) This raises several questions regarding the population estimates of *C. carnea* larvae in relation to the results yielded from the gut content analyses. First, were lower densities of

C. carnea larvae collected in 2007 due to natural year-to-year population variation? We previously documented large year-to-year fluctuations in *C. carnea* and other predator populations in the cotton agroecosystem (Naranjo et al., 2003, 2004). Second, were the lower densities due to greater predator pressure because there were less alternate prey available (e.g., *B. tabaci*) in 2007? Finally, were the lower densities in 2007 due to seasonal variation in the population? For example, the 2007 samples were collected in early August and the 2008 samples were collected in early September. Perhaps the one month difference in the crop phenology affected the encounter rate of *C. carnea*. In all likelihood, the year-to-year variation in population density and gut assay response are due to a combination of these factors.

Early gut content analyses focused exclusively on relatively simple qualitative evaluations of interguild feeding activity of the predator assemblage on one or two cotton pests (Hagler and Naranjo, 1994a,b). The present study along with that of Hagler and Blackmer (2013) have now simultaneously identified key predators of five prey species, two major herbivore pest and three lower-tiered predator species, in the cotton agroecosystem. Specifically, our studies identified predator species that frequently engaged in interguild predation on *B. tabaci* and *Lygus* spp. and IGP on *C. carnea*, *G. punctipes* and *C. vittatus*. However, these data are still only qualitative in nature because prey-specific PCR assays cannot precisely pinpoint how many prey items an individual predator consumed. A better understanding of food web population dynamics will require a more eclectic research approach that employs the more precise prey immunomarking gut analysis methodology (Hagler, 2006, 2011; Mansfield et al., 2008; Zilnik and Hagler, 2013) in combination with prey-specific gut analysis and inclusion/exclusion field cage methodologies.

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