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Use of a gut content ELISA to detect whitefly predator feeding activity after field exposure to different insecticide treatments[1]

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Abstract

A 2-year commercial-scale study was conducted to qualitatively evaluate the effect of different insecticide treatment regimes on the predator complex attacking *Bemisia tabaci* (Gennadius) in cotton. In 1996 three insecticide regimes were compared: a rotation of conventional broad-spectrum insecticides or one of two different regimes based on the initial use of the insect growth regulators (IGRs), buprofezin and pyriproxyfen. In 1997 the same three regimes plus an untreated control were compared; split-plots were sprayed once for *Lygus hesperus* Knight control using a broad-spectrum insecticide. Relative feeding activity for each predator species was compared between treatment regimes by analyzing the gut contents of predators for the presence of whitefly remains using a whitefly-specific enzyme-linked immunosorbent assay (ELISA). The ELISA results were combined with predator density data to obtain a qualitative pesticide impact index for each predator group. In total, we analyzed the gut contents of 32 262 field-collected predators, representing nine different taxa. Of these, *Pseudatomocelis seriatus* (Reuter), *Spanagonicus albofasciatus* (Reuter), and spiders consisting primarily of *Misumenops celer* (Hentz) are shown to be whitefly predators for the first time. Predator populations were usually reduced in plots that received applications of broad-spectrum insecticides for *B. tabaci* and *L. hesperus* control, but there were few treatment differences in the proportions of predators containing whitefly remains in their guts. However, the feeding activity of certain predator species in fields sprayed with broad spectrum insecticides was significantly reduced compared with those in IGR-based and control treatments. Overall, insecticide regimes using IGRs were less lethal to the whitefly predator complex than regimes consisting of only conventional, broad-spectrum insecticides, but differences in predator feeding activity on whitefly between the various insecticide treatment regimes were minimal.

Keywords: *Insect growth regulators, predator gut content examination, ELISA, conservation biological control,* Bemisia tabaci, Bemisia argentifolii

Introduction

Since the early 1990s, the silverleaf whitefly, *B. tabaci* (Gennadius), strain B [= *Bemisia argentifolii* Bellows & Perring] has threatened cotton production in the

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southwestern United States. In the last three decades whitefly control in cotton worldwide has heavily depended on broad-spectrum pyrethroids, carbamates, and organophosphates (Dennehy & Williams 1997). However, the whitefly has developed resistance to many of these insecticides (Horowitz & Ishaaya 1992; Ishaaya & Horowitz 1995; Dennehy & Williams 1997). In order to slow whitefly resistance to insecticides, a two-stage insecticide rotation program was established in Arizona in the early 1990s (Dennehy et al. 1995a,b). This threshold-based program consisted of initially applying carbamates, organophosphates, and cyclodienes followed by pyrethroids and non-pyrethroids later in the season. By the mid 1990s this 'conventional' insecticide resistance management proved ineffective for whitefly control due to resistance (Dennehy & Williams 1997). Furthermore, the broad spectrum insecticides being used were shown to be harmful to several whitefly natural enemies (Elzen 2001; Naranjo et al. 2002).

In an effort to control whitefly in Arizona, the United States Environmental Protection Agency granted an emergency use permit for application of the insect growth regulators (IGRs) buprofezin and pyriproxyfen for whitefly control in cotton in 1996 (Dennehy & Williams 1997). Buprofezin is a chitin inhibitor that mainly affects young nymphs (Ishaaya et al. 1988; Horowitz 1993). Pyriproxyfen is a juvenile hormone mimic that disrupts embryogenesis and prevents adult emergence (Ishaaya & Horowitz 1992). These two IGRs are effective for suppressing whitefly on cotton (Horowitz et al. 1994; Horowitz & Ishaaya 1994; Ishaaya & Horowitz 1995; Toscano et al. 2001) and are less harmful to some whitefly natural enemies than conventional insecticides (Gerling & Sinai 1994; Jones et al. 1995, 1998). Therefore, strategic rotations of these two IGRs with conventional insecticides in an integrated pest management (IPM) program for whiteflies should prolong the efficacy of both IGRs and conventional insecticides and conserve whitefly natural enemies (Dennehy & Williams 1997; Ellsworth & Martinez-Carrillo 2001).

In 1996 and 1997, large-scale experiments were conducted in Arizona to compare management regimes for whitefly in cotton. The major objectives were to compare the efficacy and cost effectiveness of different insecticide regimes for whitefly control, determine the proper action thresholds for deploying the IGRs, compare different application methods (e.g., air and ground), and evaluate resistance management strategies. These experiments also provided us with an invaluable opportunity to determine the impact that each whitefly insecticide regime had on the whitefly predator complex. First, we evaluated the lethal impact that the various management strategies had on 20 arthropod predator taxa found in cotton. Those results are presented in detail by Naranjo et al. (2003, 2004). Here, we present an assessment of the impact that each management regime had on predator feeding activity. We analyzed the gut contents of several key whitefly predators in cotton plots exposed to various insecticide regimes over two growing seasons using a whitefly specific enzyme-linked immunosorbent assay (ELISA) (Hagler et al. 1993). The ELISA provides a quick and accurate indirect method (i.e., a non-visual method) to assess predation (see Luck et al. 1988). We then calculated a qualitative pesticide impact index for each predator species. The index weights the proportion of each predator group ELISA-positive for whitefly in their guts by the density of each group exposed to the various insecticide regimes. To our knowledge this is the first study using a gut content assay to evaluate the impact of pesticide exposure on predator feeding activity.

Materials and methods

Study sites and experimental design

1996 Experiment. Studies were conducted on the demonstration farm of The University of Arizona's Maricopa Agricultural Center located near Maricopa, Arizona. Plots were planted in mid April with 'NuCOTN 33B' cotton and grown using standard agronomic practices. The experiment consisted of 14 treatments that formed an incomplete factorial design involving three main effects: insecticide regime, application method, and action threshold level. The experiment was arranged in a randomized complete block design with three replicates in 1.2–2.0-ha plots. The blocks consisted of three separate fields (total area ca. 72 ha). Use of the commercial demonstration farm precluded untreated control plots. Instead, a treated control representing a commercial standard control consisting of rotated conventional insecticides (see below) was established for comparison to regimes based on the newly available IGRs. The large plot design simulated commercial cotton production, minimized inter-plot interference due to arthropod movement, and accommodated the aerial application of insecticides (see below). The three insecticide regimes were designated as insecticide resistance management strategy-1995 (IRM-95), insect growth regulator strategy-1 (IGR-1), and insect growth regulator strategy-2 (IGR-2). IRM-95 plots were treated with a rotation of conventional insecticides using the insecticide resistance management strategy developed by Dennehy et al. (1995b); IGR-1 plots were treated first with buprofezin, followed by pyriproxyfen as needed, and IGR-2 plots were treated first with pyriproxyfen, followed by buprofezin as needed. If additional suppression was needed in either of the IGR regimes, a uniform rotation of conventional insecticides was used (Ellsworth & Watson 1996). These insecticide regimes are similar to those currently used by cotton farmers in the western US (Ellsworth & Martinez-Carrillo 2001). Insecticide sprays began in all plots between 3 and 8 July, 1996. The second main effect consisted of insecticide applied aerially (47 L/h) or by ground (140 L/h). Finally, the use of three different whitefly action threshold levels for applying the IGRs represented the third main effect. These levels were 0.5, 1.0, or 1.5 large whitefly nymphs (third or fourth instar) per leaf disk plus three to five adult whiteflies per leaf (Naranjo & Flint 1994, 1995). These threshold levels were chosen based on our experience that the IGRs would be most effectively deployed near the inflection point of pest population increase. A single threshold of five adults per leaf (Ellsworth et al. 1995) was used to schedule applications of insecticides in the conventional IRM-95 regime accounting for the incomplete nature for the third main effect. A threshold of five adults per leaf was also used to determine the need for additional conventional insecticide applications in the IGR regimes. Analyses of the impact of various treatments on predator populations revealed that there were no significant differences in predator populations among action threshold levels and mode of application. Thus only the main effect of insecticide regime was examined here (see Naranjo et al. 2003 for details). The entire study site was sprayed once with oxamyl for *Lygus hesperus* Knight control on 1 August, 1996. Seasonal usage of insecticides and rates applied in 1996 are summarized in Table I.

Predators were collected in each plot weekly from July 15 to September 9, 1996 using 38-cm diameter sweep nets. Twenty-five sweeps were taken in each of four random locations within each plot for a total of 100 sweeps per plot. The contents

Table I. Insecticide application history, Maricopa Agricultural Center, Maricopa, AZ, 1996.

	Treatment					
	Ground application			Aerial application		
	IGR-1	IGR-2	IRM-95	IGR-1	IGR-2	IRM-95
3–8 July	Buprofezin (392 g/ha)	Pyriproxyfen (60 g/ha)	Endosulfan (843 g/ha) + amitraz (280 g/ha)	Buprofezin (392 g/ha)	Pyriproxyfen (60 g/ha)	Endosulfan (843 g/ha) + amitraz (280 g/ha)
12–16 July			Oxamyl (561 g/ha) + profenophos (841 g/ha)			Oxamyl (561 g/ha) + profenophos (841 g/ha)
17–22 July	Pyriproxyfen (60 g/ha)		Fenpropathrin (224 g/ha) + acephate (561 g/ha)	Pyriproxyfen (60 g/ha)		
24 July		Buprofezin[b] (392 g/ha)				
1 August	Oxamyl[a] (843 g/ha)	Oxamyl[a] (843 g/ha)	Oxamyl[a] (843 g/ha)	Oxamyl[a] (843 g/ha)	Oxamyl[a] (843 g/ha)	Oxamyl[a] (843 g/ha)
5 August					Buprofezin[b] (392 g/ha)	
30 August		Buprofezin[c] (392 g/ha)				
4–8 September			Endosulfan (843 g/ha) + bifenthrin (90 g/ha)			Fenpropathrin (224 g/ha) + acephate (561 g/ha)

All rates given in grams of active ingredient per hectare.
[a]Applied for control of *Lygus hesperus* in all plots.
[b]Low threshold level only.
[c]Middle threshold level; high threshold level treatment for IGR-2 did not require an application of buprofezin within the natural enemy sampling interval.

from each sweep net sample were put into a plastic bag and immediately frozen on dry ice and later stored at −70°C. Predator samples were removed from the freezer, sorted and counted, and assayed for the presence of whitefly remains in their gut using the whitefly-specific ELISA described below. The predators we analyzed for whitefly prey remains were generally the most abundant taxa captured each year. The heteropteran predators assayed included both nymphs and adults (primarily adults) whereas only adults of the other taxa were examined.

1997 Experiment. Studies were conducted on the research farm of the Maricopa Agricultural Center, using the same cotton variety and agronomic practices, but the application method and action threshold treatments were eliminated from the experimental design. We used a randomized complete block, split-plot design with four main treatments replicated four times. The main treatments represented pesticide regimes designated as IGR-1, IGR-2, IRM-95 and UNTREATED. IRM-95 main plots were 0.11 ha in size and treated with a rotation of conventional insecticides; IGR-1 main plots were 0.12 ha in size and treated first with buprofezin, followed by pyriproxyfen, and then a rotation of conventional insecticides; IGR-2 main plots were similar in size, but treated first with pyriproxyfen, followed by buprofezin, and then a rotation of conventional insecticides (see Table II for details). UNTREATED main plots were 0.11 ha in size and were not sprayed with any whitefly insecticides. All insecticide applications were made by ground using a standard tractor sprayer. All IRM-95 applications were made at five adults per leaf (Ellsworth et al. 1995). The action threshold for IGR-1 and IGR-2 was one large whitefly nymph per leaf disk plus three to five adults per leaf (Ellsworth et al. 1996). On 25 July, 0.06 ha split plots of the IGR-1, IGR-2, and the UNTREATED main plots and all of the IRM-95 plots were sprayed with oxamyl at a rate of 0.45 kg per 0.40 ha to control a severe *L. hesperus* infestation which threatened the overall goals of the experiment. These sub-plots were designated as IGR-1_{Lygus}, IGR-2_{Lygus}, IRM-95_{Lygus} and UNTREATED$_{Lygus}$, respectively.

Table II. Insecticide application history, Maricopa Agricultural Center, Maricopa, AZ, 1997 for those plots that did not receive an additional application of broad spectrum insecticide for *Lygus hesperus* control.

	Main plot treatment[a]		
	IGR-1	IGR-2	IRM-95
29 July	Buprofezin (392 g/ha)	Pyriproxyfen (60 g/ha)	Endosulfan (841 g/ha) + amitraz (280 g/ha)
5 August			Oxamyl (561 g/ha) + profenophos (841 g/ha)
13 August	Pyriproxyfen (60 g/ha)		
20 August		Buprofezin (392 g/ha)	Fenpropathrin (224 g/ha) + acephate (561 g/ha)
4 September	Endosulfan (841 g/ha) + amitraz (280 g/ha)	Endosulfan (841 g/ha) + amitraz (280 g/ha)	Endosulfan (841 g/ha) + amitraz (280 g/ha)
12 September	Oxamyl (561 g/ha) + profenophos (841 g/ha)	Oxamyl (561 g/ha) + profenophos (841 g/ha)	Fenpropathrin (224 g/ha) + oxamyl (561 g/ha)

All rates given in grams of active ingredient per hectare.
[a]On 25 July, the entire IRM-95 main plot and split plots of IGR-1, IGR-2, and UNTREATED were sprayed with oxamyl at 1121 g/ha for *Lygus hesperus* control.

Predators were collected weekly from July 31 to September 16, 1997 in each plot using 38-cm diameter sweep nets. Twenty-five sweeps were taken in each of two randomized locations within each sub-plot for a total of 50 sweeps. The contents from each sweep net sample were processed as described above.

Whitefly-specific ELISA

Field-collected predators. We determined the proportion of predators that had recently fed on whitefly using an established whitefly-specific ELISA (Hagler et al. 1993). Each predator was macerated in 500-µl of Tris-buffered saline (TBS) (pH 7.4). A 100-µl aliquot of the macerated predator was placed into an individual well of a 96-well assay plate (Falcon Pro-Bind 3915, Becton-Dickinson & Company, Franklin Lakes, NJ). Each plate was incubated at 4°C overnight. Following incubation, the insect macerates were discarded from each plate and a 360-µl aliquot of 1.0% non-fat dry milk in distilled water was added to each well for 30 min at 27°C to block any unoccupied antigenic sites in the wells. The non-fat milk was emptied from each plate and a 50-µl aliquot of anti-whitefly monoclonal antibody was added to each well of the ELISA plate (Hagler et al. 1993). The ELISA plates were then incubated for 1 h at 27°C. The contents from each plate were discarded and the plates were briefly rinsed three times with PBS-Tween 20 (0.05%) and twice with PBS. Goat anti-mouse IgG/IgM conjugated to alkaline phosphatase (No. AMI 0705, BioSource International, Camarillo, CA) diluted (1:500) in 1.0% nonfat milk was added to each well (50-µl) of the plates for 1 h at 27°C. Plate contents were discarded and rinsed as described above. A 50-µl aliquot of substrate was added to each well using the ingredients supplied in a Bio-Rad (Hercules, CA) substrate kit (No. 172-1063). After 2 h the absorbance of each well was measured with a Molecular Devices Spectra Max 250 (Sunnyvale, CA) microplate reader set at 405 nm.

Negative control predators. Predators serving as negative controls were placed live into Petri dishes containing only water for 3 days. This time frame ensures that any whitefly prey remains present at the time of collection were excreted prior to their analyses by the ELISA (Hagler & Naranjo 1997). Negative control predators ($n = 8–16$ per ELISA microplate) were assayed by ELISA for each predator species alongside their field-collected counterparts. A field collected predator was scored positive for whitefly prey remains if its ELISA absorbance value exceeded the mean negative predator control reading by three standard deviations (Hagler & Naranjo 1994a,b).

Statistical analyses

The average ($\pm$SEM) number of predators collected in each sweep net sample was calculated for each predator taxa. A thorough statistical analysis of the week-to-week and seasonal predator population is provided by Naranjo et al. (2003, 2004). The proportion of individuals of each predator taxa scoring positive by ELISA for the presence of whitefly remains was determined for each sampling date. Differences in apparent predator feeding activity exhibited by each taxon exposed to the various whitefly treatment regimes over the entire season was determined using a proportions z-test calculation with Yates correction for continuity (Glantz 1992; SigmaStat Ver. 2.02, San Rafael, CA). Because the split-plot design was incomplete in 1997 two

analyses were completed. First we examined the effects of whitefly insecticides pooled over both *L. hesperus* insecticide split-plots after eliminating the IRM-95 treatment. We then examined all whitefly insecticide treatments in the split-plots receiving *L. hesperus* insecticide control sprays.

Qualitative analysis of pesticide impact

The relative impact of each whitefly treatment regime was measured for each predator species using a modification of the predator efficiency index of Ragsdale et al. (1981). This index was originally used to compare the efficiency of many different predator species on the pest *Nezara viridula* (L.) in relation to predator abundance and the proportion of individuals positive by ELISA for pest remains. We modified the use of the index to compare the impact of the different whitefly management regimes in relation to predator density (N) and proportion of each predator population (i) positive for whitefly remains (P). The resulting value for each regime was compared proportionally to the sum totaled value for the other treatment regimes ($\Sigma(NP)$).

$$\text{Qualitative Pesticide Impact Index} = [(N_i)(P_i)] \Big/ \left[\sum\nolimits_{i=1}^{j} (N_i P_i)\right] \times 100 \text{ (from Ragsdale et al. 1981)}$$

Results

Lethal effects

Detailed analyses of the effect of the whitefly management regimes on predator abundance are presented by Naranjo et al. (2003, 2004). In 1996, we found higher populations of all the predators examined here in the IGR treatment regimes compared with the conventional IRM-95 regime (Table III). The only exception was a non-significant difference (Naranjo et al. 2003) for *Drapetis nr. divergens* where slightly fewer flies were collected per 100 sweeps from the IGR-1 treatment regime.

In 1997, based on the split-plot analyses of predator densities excluding the conventional IRM-95 insecticide regime (see Materials and methods), seasonal average densities were almost always lower in the IGR regimes than the UNTREATED control (Table IV). A detailed statistical analysis revealed that three of the eight predator taxa were significantly reduced (Naranjo et al. 2004). However, it should be noted that our analysis of temporal effects suggest that reduction in predator populations in IGR plots appeared to be more closely related to reduction in whitefly density than to direct toxic effects of the insecticides. The use of insecticides for *L. hesperus* control reduced the densities of most of the predator taxa examined (Table V). In general, predator populations were reduced by $>30\%$ in those fields treated additionally for *L. hesperus* control (Table V). In 1997, based only on the analyses of split-plots receiving additional treatment for *L. hesperus* control, predator populations were almost always higher in the $\text{UNTREATED}_{\text{Lygus}}$ plots and lower in the IRM-95_{Lygus} plots than in the IGR-1_{Lygus} and IGR-2_{Lygus} plots (Table VI).

In short, the impact of the treatment regimes on predator density showed that: (1) the application of whitefly insecticides caused a net decrease in predator populations when compared to the UNTREATED plots in 1997, (2) the use of the IRM-95

Table III. The mean number (±SEM) of predators collected per 100 sweeps and the frequency of predators scoring positive for the presence of whitefly egg antigen in their gut. Predators were collected each week from fields exposed to various whitefly insecticide regimes from 15 July through 9 September, 1996. Percentages across each row followed by the same letter are not significantly different ($P < 0.01$, z-test for proportions).

	IRM-95			IGR-1			IGR-2		
Species	No. Collected (Mean±SEM)	Total Assayed	Percent Positive	No. Collected (Mean±SEM)	Total Assayed	Percent Positive	No. Collected (Mean±SEM)	Total Assayed	Percent Positive
D. nr. divergens	13.5 (2.7)	534	23.8 a	13.1 (1.3)	1906	24.6 a	17.0 (1.9)	1783	21.5 a
L. hesperus	13.0 (1.4)	625	41.0 a	20.7 (1.4)	3390	48.1 b	19.5 (1.1)	2710	51.4 b
O. tristicolor	11.8 (1.3)	557	14.5 a	17.5 (1.5)	2477	21.2 b	18.3 (1.3)	2355	22.7 b
Geocoris spp.	0.7 (0.2)	34	64.7 a	3.7 (0.4)	691	33.7 b	4.0 (0.5)	578	43.4 c
P. seriatus	1.1 (0.4)	54	55.6 a	5.0 (0.8)	827	30.0 b	4.7 (0.7)	687	42.8 a
Spiders	0.7 (0.2)	36	25.0 a	3.1 (0.3)	569	31.1 a	3.7 (0.4)	567	29.6 a
S. albofasciatus	4.3 (1.4)	179	16.2 a	5.1 (0.7)	856	31.5 b	4.9 (0.7)	680	32.5 b

Table IV. The mean number (±SEM) of predators collected per 50 sweeps and the frequency of predators scoring positive for the presence of whitefly egg antigen in their gut. Predators were collected each week from fields exposed to various whitefly insecticide regimes from 31 July through 16 September, 1997. Percentages across each row followed by the same letter are not significantly different ($P < 0.01$, z-test for proportions).

	UNTREATED			IGR-1			IGR-2		
Species	No. collected (Mean±SEM)	Total assayed	Percent positive	No. collected (Mean±SEM)	Total assayed	Percent positive	No. collected (Mean±SEM)	Total assayed	Percent positive
D. nr. divergens	35.2 (5.0)	2167	44.4 a	25.3 (3.4)	1563	50.7 b	23.4 (2.7)	1476	48.6 b
L. hesperus	15.6 (1.6)	985	35.3 a	10.8 (1.3)	668	34.6 a	9.7 (1.6)	615	31.9 a
O. tristicolor	6.8 (0.8)	406	54.4 a	5.9 (0.6)	333	46.2 b	6.4 (0.8)	364	53.3 a
Geocoris spp.	2.3 (0.3)	151	33.1 a	1.0 (0.2)	61	31.1 a	1.0 (0.2)	64	35.9 a
P. seriatus	1.6 (0.3)	67	46.3 a	1.0 (0.2)	59	40.7 a	1.4 (0.4)	82	35.4 a
Spiders	1.7 (0.2)	91	30.8 a	1.3 (0.2)	79	25.3 a	1.8 (0.3)	116	33.6 a
Z. renardii	0.6 (0.1)	36	52.8 a	0.4 (0.1)	23	21.7 b	0.4 (0.1)	24	33.3 ab
C. vittatus	0.3 (0.1)	19	15.7 a	0.2 (0.1)	15	13.3 a	0.3 (0.1)	18	27.8 a

Table V. The mean number (±SEM) of predators collected per 50 sweeps and the frequency of predators scoring positive for the presence of whitefly egg antigen in their gut. Predators were collected each week from fields that were either treated or not treated for *Lygus hesperus* control from 31 July through 16 September, 1997. Percentages across each row followed by the same letter are not significantly different ($P < 0.01$, z-test for proportions).

	Treated additionally for *Lygus hesperus* control					
	No			Yes		
Species	No. collected (Mean±SEM)	Total assayed	Percent positive	No. collected (Mean±SEM)	Total assayed	Percent positive
D. nr. divergens	33.6 (3.7)	3175	46.1 a	22.3 (2.3)	2031	49.6 b
L. hesperus	14.9 (1.5)	1424	34.6 a	9.2 (0.7)	844	33.5 a
O. tristicolor	6.6 (0.6)	338	56.2 a	6.2 (0.6)	502	46.0 b
Geocoris spp.	2.1 (0.2)	202	31.2 a	0.7 (0.1)	74	39.2 a
P. seriatus	1.9 (0.3)	152	38.8 a	0.8 (0.2)	56	44.6 a
Spiders	2.2 (0.2)	200	31.0 a	1.1 (0.1)	86	29.1 a
Z. renardii	0.5 (0.1)	50	42.0 a	0.4 (0.1)	33	33.3 a
C. vittatus	0.3 (0.1)	34	17.6 a	0.2 (0.1)	18	22.2 a

regime for whitefly control caused a net decrease in the predator population when compared to the two IGR regimes in 1996 and 1997, and (3) the single application of broad-spectrum insecticide for lygus control consistently caused a net decrease in the predator populations when compared to the non-lygus treated plots in 1997 (Table V & VI).

Feeding activity

The major focus of this study was to assess the seasonal impact that each treatment regime had on the feeding activity of several whitefly predator taxa. In 1996 a total of 22 095 predators, representing seven arthropod predator taxa were analyzed by ELISA for the presence of whitefly remains in their guts (Table III). The seasonal impact of the various treatment regimes on predator feeding activity varied between taxa. The proportion of *Drapetis nr. divergens* & spiders (86% of the spider population consisted of *Misumenops celer* (Hentz)) containing whitefly remains was not significantly different among the insecticide regimes (Table III). A significantly lower frequency of positive responses for whitefly was observed from the *L. hesperus*, *Orius tristicolor* (White) and *Spanagonicus albofasciatus* (Reuter) populations collected from the IRM-95 plots compared with the IGR regimes and a lower frequency of *P. seriatus* collected from the IGR-1 plots compared with the IRM-95 and IGR-2 regimes (Table III). Interestingly, a significantly higher proportion of positive responses for whitefly was observed from the *Geocoris* spp. (*G. punctipes* (Say) and *G. pallens* Stål) collected in the IRM-95 plots. The pesticide impact index clearly shows that predators collected from the IRM-95 regime accounted for the smallest proportion of predation (Figure 1).

A total of 9482 predators representing eight arthropod predator taxa were analyzed by ELISA in 1997 from the UNTREATED, IGR-1 and IGR-2 plots (Table IV). The proportion of the populations containing whitefly remains were not significantly different between the various treatment regimes for *L. hesperus*, *Geocoris* spp., *P. seriatus*, spiders (54, 13 and 12% of the spiders were *M.* celer, *Dictyna reticulata*

Table VI. The mean number (±SEM) of predators collected per 50 sweeps and the frequency of predators scoring positive for the presence of whitefly egg antigen in their gut. Predators were collected each week from fields exposed to various whitefly insecticide regimes from 7 July through 16 September, 1997 and an additional treatment of insecticide for *Lygus hesperus* control on 25 July. Percentages across each row followed by the same letter are not significantly different ($P < 0.01$, z-test for proportions).

	UNTREATED$_{Lygus}$			IRM-95$_{Lygus}$			IGR-1$_{Lygus}$			IGR-2$_{Lygus}$		
Species	No. collected (Mean±SEM)	Total assayed	Percent positive	No. collected (Mean±SEM)	Total assayed	Percent positive	No. collected (Mean±SEM)	Total assayed	Percent positive	No. collected (Mean±SEM)	Total assayed	Percent positive
D. nr. divergens	25.1 (4.3)	752	48.7 b	15.8 (3.7)	454	34.6 c	19.6 (4.0)	589	54.3 a	22.1 (3.8)	690	46.5 b
L. hesperus	12.6 (1.2)	393	33.9 a	4.1 (0.7)	131	25.9 a	8.1 (1.2)	236	34.3 a	6.8 (1.0)	215	32.1 a
O. tristicolor	6.7 (1.1)	190	45.8 a	2.6 (0.7)	78	17.9 b	5.9 (0.8)	154	46.1 a	6.0 (1.0)	158	46.2 a
Geocoris spp.	1.3 (0.2)	41	43.9 a	0.3 (0.1)	9	33.3 a	0.3 (0.1)	10	20.0 a	0.6 (0.2)	23	39.1 a
P. seriatus	1.2 (0.4)	27	51.9 a	0.2 (0.1)	4	25.0 a	0.5 (0.2)	13	53.8 a	0.8 (0.3)	16	25.0 a
Spiders	1.1 (0.2)	29	34.5 a	0.1 (0.1)	5	40.0 a	0.7 (0.2)	21	28.6 a	1.3 (0.3)	36	25.0 a
Z. renardii	0.6 (0.1)	16	37.5 a	0.1 (0.1)	2	0.0 a	0.3 (0.1)	8	25.0 a	0.3 (0.1)	9	33.3 a
C. vittatus	0.3 (0.1)	6	33.3 a	0.1 (0.1)	2	50.0 a	0.2 (0.1)	6	16.7 a	0.2 (0.1)	6	16.7 a

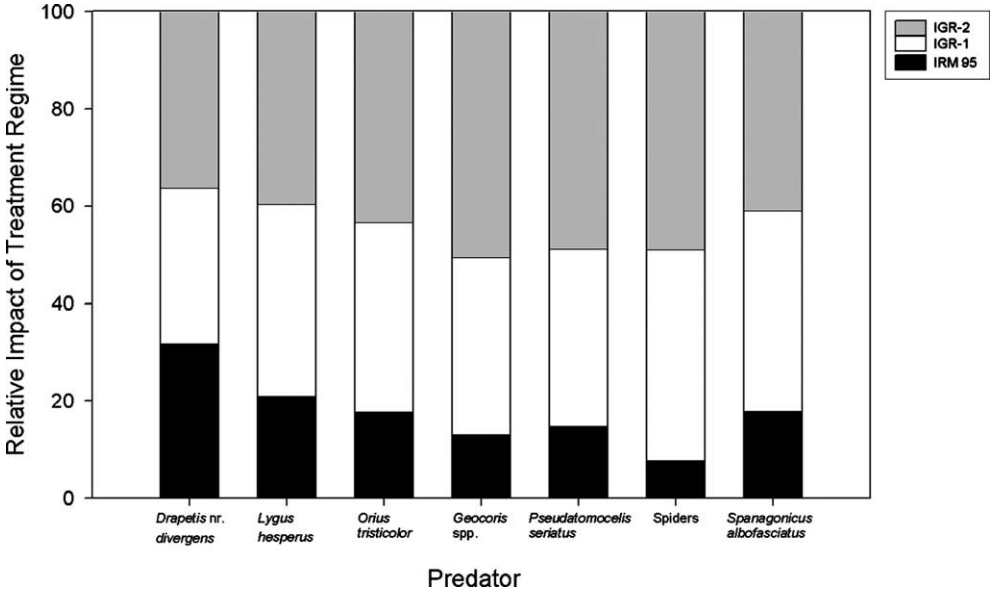

Figure 1. Relative impact of three treatment regimes on predation by seven predator groups during 1996. Contribution of each treatment was calculated using the predation index modified from Ragsdale et al. (1981).

Gertsch and Ivie, and *Thanatus vulgaris* Simon, respectively), and *Collops vittatus* (Say). However, a lower frequency of positive responses for whitefly remains were observed for the *O. tristicolor* and *Zelus renardii* Kolenati populations collected from the IGR-1 plots (note: the statistical differences for *Z. renardii* might be an artifact of small sample sizes). More than 1.5 times as many *D. nr. divergens* were collected from the UNTREATED plots than from either the IGR-1 and IGR-2 plots, but a significantly smaller proportion of the UNTREATED population contained whitefly in their gut. The pesticide impact index shows that the predators collected from the UNTREATED plots accounted for the majority of predation for most of the species examined ($\approx$40% for the most abundant predator species) (Figure 2A).

Results from the ELISA analysis between the split plots either receiving or not receiving *L. hesperus* control in 1997 showed a significantly lower frequency of positive responses for whitefly from the *D. nr. divergens* and *O. tristicolor* populations collected from the plots treated additionally for lygus (Table V). No significant differences in feeding activity between treatments were observed for the other six taxa examined. The pesticide impact index shows that the predators collected from the plots not treated additionally for lygus accounted for $\approx$60–65% of the predation (Figure 2B).

A total of 4329 predators were collected in 1997 from the four split plots that were treated for lygus control (Table VI). The proportion of the populations containing whitefly remains were not significantly different between the various treatment regimes for *L. hesperus*, *Geocoris* spp. (primarily *G. punctipes*), *P. seriatus*, spiders, *Z. renardii*, and *C. vittatus*. The most obvious decline in feeding activity was again detected for *O. tristicolor*. Only 17.9% of the *O. tristicolor* collected from the IRM-95$_{Lygus}$ regime contained whitefly remains while over 45.0% collected from the UNTREATED$_{Lygus}$, IGR-1$_{Lygus}$ and IGR-2$_{Lygus}$ contained whitefly remains. A significantly lower frequency of predation was also detected for the *D. nr. divergens*

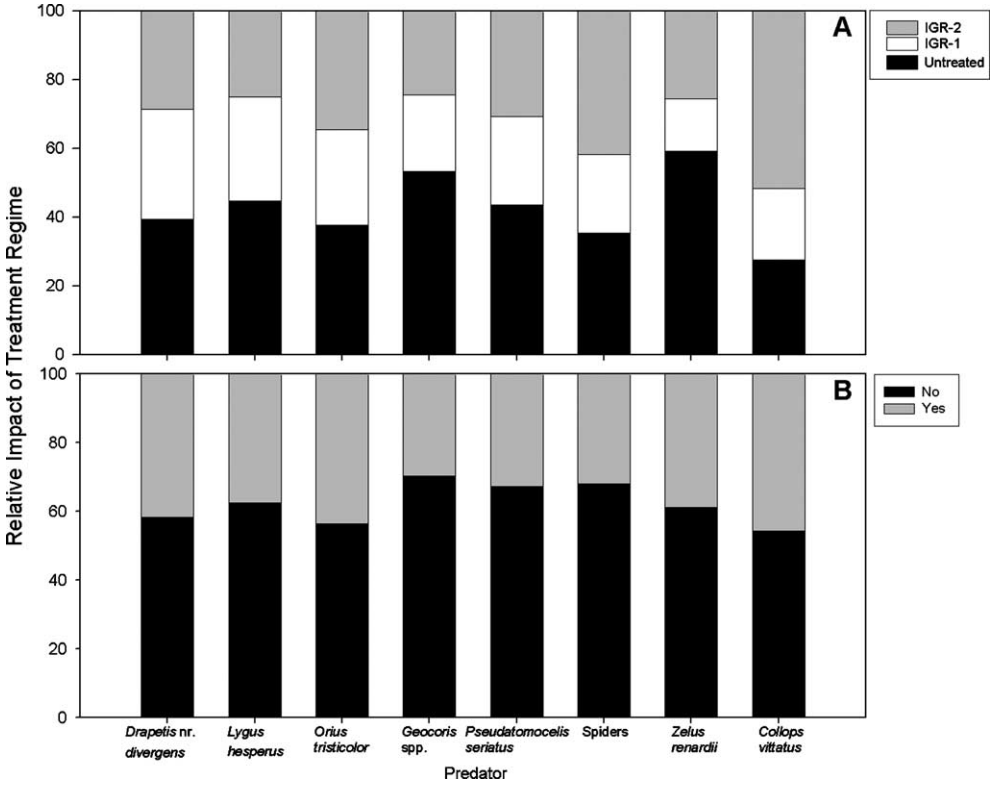

Figure 2. (A) Relative impact of three treatment regimes, pooled over both *Lygus hesperus* split plots, on predation by eight predator groups during 1997. (B) Relative impact of an additional application of insecticide for *Lygus hesperus* control on predation by eight predator groups during 1997. Contribution of each treatment was calculated using the predation index modified from Ragsdale et al. (1981).

collected in the IRM-95$_{Lygus}$ treatment regime compared with the IGR-1$_{Lygus}$, IGR-2$_{Lygus}$, and UNTREATED$_{Lygus}$ regimes (Table VI). The pesticide impact index shows that predators collected from the IRM-95$_{Lygus}$ regime accounted for only $\approx$10% of the total predator impact; whereas the UNTREATED$_{Lygus}$ predators accounted for 30–60% of the impact (Figure 3).

Discussion

This investigation was part of a large-scale, multidisciplinary study focused on examining and optimizing whitefly management strategies within a whole cotton production system. The goals were to control whitefly (Ellsworth et al. 1998; Ellsworth & Naranjo 1999), conserve natural enemies, limit insecticide use, and diversify insecticides used for whitefly control to minimize resistance. Our contribution to the study was to identify the lethal impact of several management regimes on the whitefly predator complex and to determine if there were any differences in feeding activity on whitefly by those predators that survived the insecticide exposure. To this end, we surveyed the whitefly predator complex in cotton on a weekly basis during two seasons and compared the effect of the various management regimes on the abundance and feeding activity of several whitefly predators.

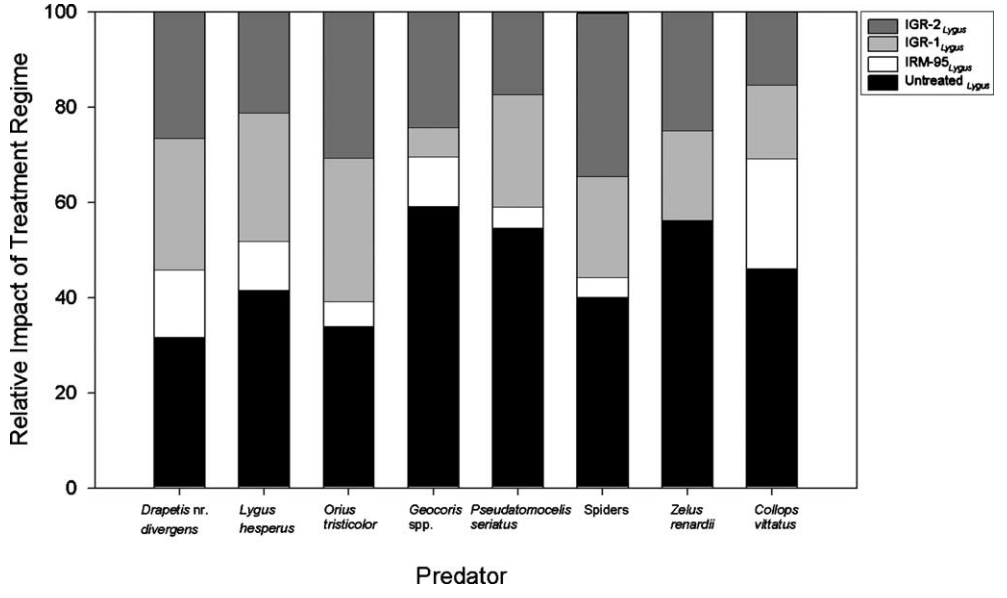

Figure 3. Relative impact of four treatment regimes receiving an additional treatment of insecticide for *Lygus hesperus* control on predation by eight predator groups during 1997. Contribution of each treatment was calculated using the predation index modified from Ragsdale et al. (1981).

Many laboratory studies have evaluated the impact of various classes of pesticides on predators (e.g., Wilkinson et al. 1979; Yu 1988; Hough-Goldstein & Keil 1991; De Clercq et al. 1995; Hamilton & Lashomb 1997; Elzen & Elzen 1999; Magagula & Samways 2000). The majority of the studies focused on predator survival after exposure to various types of pesticides. Fewer studies have examined the sub-lethal impacts of pesticide exposure on predators. Of those, the majority of the studies focused on predator fecundity, longevity, and various pesticide-associated abnormalities (e.g., Schmutterer 1990; Hough-Goldstein & Keil 1991; De Clercq et al. 1995; Magagula & Samways 2000; Elzen 2001). Relatively few studies have examined predator feeding activity after pesticide exposure. In a greenhouse, Hamilton and Lashomb (1997) showed decreased feeding activity by *Coleomegilla maculata* DeGeer and *Chrysoperla carnea* (Stephens) after eating *Leptinotarsa decemlineata* (Say) eggs exposed to various pesticides. Elzen and Elzen (1999) showed in a laboratory foliar insecticide residue bioassay that most of the chemicals they tested had no effect on egg consumption by *G. punctipes*. A few laboratory studies have evaluated the effects of IGRs on predators. De Clercq et al. (1995) found that pyriproxyfen was toxic to *Podisus maculiventris* (Say) when exposed by direct contact, residual contact, or ingestion. They also cited a study that reported that pyriproxyfen was toxic to *Coccinella septempunctata* L. and *C. carnea* (Yakti & Poehling 1988). Liu and Chen (2000) reported that low concentrations of buprofezin were harmless to *Chrysoperla rufilabris* (Burmeister), but higher concentrations reduced their probability of survival and increased larval developmental time in some instances. In contrast, Nagai (1990) observed no negative effects on *Orius* spp. after exposure to pyriproxyfen. Our study is unique because we evaluated the relative feeding activity of predators exposed to conventional insecticides and IGRs over extended periods of time under realistic field conditions using a whitefly-specific ELISA. Previously, this ELISA was used to

identify key native predators of whitefly (Hagler & Naranjo 1994a,b) and to compare the feeding activity between native and inundative released *H. convergens* (Hagler & Naranjo 2004). Generally, each predator population had a similar proportion of individuals positive for whitefly remains after exposure to the various insecticide treatments. However, in some instances, the broad-spectrum IRM-95 regime did cause notable decreases in feeding activity in some of the predator taxa examined, most notably, *O. tristicolor.* Populations of this predator consistently contained a smaller proportion of individuals with whitefly in their guts in the IRM-95 plots. Other notable instances where there was a significant decline in the proportion of a population containing whitefly remains were for *L. hesperus* and *S. albofasciatus* in 1996 and for *D. nr. divergens* in 1997 in the IRM-95 regime and for *P. seriatus* in 1996 in the IGR-1 regime.

This study also revealed that the two most common predators found in the cotton agroecosystem are species that are not generally regarded as major whitefly predators. The most abundant predator was *D. nr. divergens*, a predatory fly that feeds exclusively on adult whiteflies in the laboratory (Hagler 2002). Since *D. nr. divergens* feeds only on adults, and since the whitefly egg-specific ELISA can't differentiate between an egg and an egg-carrying (i.e., gravid) female meal (Hagler et al. 1993) a positive ELISA reaction can be attributed to predation on adult females. With the exception of perhaps *Z. renardii*, the other whitefly predators examined in this study readily prey on both whitefly eggs and adults (Hagler et al. 2004). As a consequence, we cannot be certain whether a positive ELISA reaction from most field-collected predator taxa is due to predation on whitefly eggs, adult females, or both lifestages (Hagler & Naranjo 1994a,b). The second most abundant predator residing in our cotton agroecosystem was the omnivore *L. hesperus*, despite the fact that insecticide was applied specifically to control it. Even though *L. hesperus* is a major cotton pest, it is also well documented as a predator of several other pests (Lindquist & Sorensen 1970; Bryan et al. 1976; Wheeler 1976; Bisabri-Ershadi & Ehler 1981; Cleveland 1987) including all whitefly lifestages (Hagler et al. 2004). Our results showed that the overall proportion of *L. hesperus* containing whitefly remains, regardless of the insecticide regime they were exposed to, ranged from 25 to 50%. In view of our results and the work of others, perhaps we need to evaluate this important pest because it might be beneficial in some situations (Wheeler 1976, 2000). Currently, studies are underway at our laboratory investigating the omnivorous feeding characteristics of nymphal and adult *L. hesperus* (JRH, in prep.).

Over half of the predators examined in this study have previously been identified as whitefly predators. For example, field-collected *D. nr. divergens*, *L. hesperus*, *O. tristicolor*, *G. punctipes*, *Z. renardii*, and *C. vittatus* have tested positive by ELISA for the presence of whitefly remains in their gut (Hagler & Naranjo 1994a,b; Hagler 2002). Moreover, predation by *D. nr. divergens*, *L. hesperus*, *O. tristicolor*, *G. punctipes*, and *C. vittatus* on whitefly has been quantified in laboratory studies (Hagler 2002; Hagler et al. 2004). This study reveals that 30–50% of the omnivorous *P. seriatus* and *S. albofasciatus* and the carnivorous spiders tested positive for whitefly remains. Given the propensity of these species to feed on whitefly and their abundance in cotton fields (e.g., *P. seriatus* and *S. albofasciatus* were among the most abundant arthropods in cotton fields from May through July [data not shown]) further studies on their contribution to biological control of whitefly are warranted.

While this whitefly-specific gut content ELISA proved useful for studying the feeding activity of predators exposed to various insecticide regimes, we would be remiss if we did not discuss a limitation of this technique. In this study, we assumed that a positive ELISA reaction was due to a predator feeding on at least one viable whitefly egg or adult female. A potential pitfall common with gut content ELISAs and other indirect methods of assessing predation (i.e., radiolabelling, electrophoresis, PCR, etc.) includes the possibility of obtaining false positive ELISA reactions due to third trophic level interactions (e.g., intraguild predation) or scavenger feeding. We have conducted laboratory studies that have shown that false positives ELISA reactions due to intraguild predation and scavenger feeding rarely occur (JRH, pers. obs.).

In conclusion, the use of pest-specific IGRs is generally regarded as a positive step towards conserving natural enemy populations. Clearly, strategic use of IGRs during the cotton growing season helped conserve the whitefly predator complex when compared to the conventional IRM-95 regime (Naranjo et al. 2003, 2004). The whitefly-specific ELISA was a useful tool for comparing the proportion of predators of various taxa containing whitefly remains after exposure to various insecticide regimes. In general, there were few differences in the feeding activity within each taxon. The most significant decrease in feeding activity was seen in the *O. tristicolor* populations exposed to the IRM-95 regime. The results presented here and by Naranjo et al. (2003, 2004) suggest that whitefly growth regulators play a significant role in conservation of whitefly predators, but the effect on feeding activity after exposure to the various classes of insecticides is minimal.

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References

Bisabri-Ershadi B, Ehler L. 1981. Natural biological control of the western yellow-striped armyworm, *Spodoptera praefica*, in hay in Northern California. Hilgardia 49:1–23.

Bryan DE, Jackson CG, Carranza RL, Neemann EG. 1976. *Lygus hesperus*: production and development in the laboratory. Journal of Economic Entomology 69:127–129.

Cleveland TC. 1987. Predation by tarnished plant bugs (Heteroptera: Miridae) of *Heliothis* (Lepidoptera: Noctuidae) eggs and larvae. Environmental Entomology 16:37–40.

De Clerq P, De Cock A, Tirry L, Vinuela E, Degheele D. 1995. Toxicity of diflubenzuron and pyriproxyfen to the predatory bug *Podisus maculiventris*. Entomologia Experimentalis et Applicata 74:17–22.

Dennehy TJ, Williams III, L. 1997. Management of resistance in *Bemisia* in Arizona cotton. Pesticide Science 51:398–406.

Dennehy TJ, Ellsworth PC, Nichols RL. 1995a. Whitefly management in Arizona cotton 1995. IPM Series 3. 1995. Tucson, AZ: University of Arizona.

Dennehy TJ, Ellsworth PC, Watson T. 1995b. Whiteflies in Arizona: pocket guide. No. 195009. Tucson, AZ: The University of Arizona, College of Agriculture.

Ellsworth PC, Martinez-Carrillo JL. 2001. IPM for *Bemisia tabaci*: a case study from North America. Crop Protection 20:853–869.

Ellsworth PC, Naranjo SE. 1999. Whitefly management with insect growth regulators and the influence of *Lygus* controls. In: Cotton, a college of agriculture report, series P-116. Tucson, AZ: University of Arizona. pp 339–354.

Ellsworth PC, Watson TF. 1996. Whiteflies in Arizona (No. 7): pocket guide '96. *Publication #196005*. Tucson, AZ: The University of Arizona, Cooperative Extension.

Ellsworth PC, Diehl J, Dennehy T, Naranjo S. 1995. Sampling sweetpotato whiteflies in cotton. *IPM Series No. 2*. Tucson, AZ: The University of Arizona, Cooperative Extension. p 2.

Ellsworth PC, Diehl J, Naranjo SE. 1996. Sampling sweetpotato whitefly nymphs in cotton. *IPM Series No. 6*. Tucson, AZ: The University of Arizona, Cooperative Extension.

Ellsworth PC, Naranjo SE, Castle SE, Hagler JR, Henneberry TJ. 1998. Whitefly management in Arizona: looking at whole systems. In: Cotton, a college of agriculture report, Series P112. Tucson, AZ: University of Arizona. pp 311–318.

Elzen GW. 2001. Lethal and sublethal effects of insecticide residues on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). Journal of Economic Entomology 94:55–59.

Elzen GW, Elzen PJ. 1999. Lethal and sublethal effects of selected insecticide on *Geocoris punctipes*. Southwestern Entomologist 24:199–205.

Gerling D, Sinai P. 1994. Buprofezin effects on two parasitoid species of whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology 84:842–846.

Glantz SA. 1997. Primer of biostatistics. New York, NY: McGraw-Hill. p 473.

Hagler JR. 2002. Foraging behavior, host stage selection and gut content analysis of field collected *Drapetis nr. divergens*: a predatory fly of *Bemisia argentifolii*. Southwestern Entomologist 27:241–249.

Hagler JR, Naranjo SE. 1994a. Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. Entomologia Experimentalis et Applicata 72: 59–66.

Hagler JR, Naranjo SE. 1994b. Qualitative survey of two coleopteran predators of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) using a multiple prey gut content ELISA. Environmental Entomology 23:193–197.

Hagler JR, Naranjo SE. 1996. Using gut content immunoassays to evaluate predaceous biological control agents: a case study. In: Symondson WOC, Liddell JE, editors. The ecology of agricultural pests: Biochemical approaches. New York, NY: Chapman & Hall. pp 383–399.

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. Biological Control 9:112–119.

Hagler JR, Naranjo SE. 2004. A multiple ELISA system for simultaneously monitoring intercrop movement and feeding activity of mass-produced predators. International Journal of Pest Management 50:199–207.

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*. Entomologia Experimentalis et Applicata 68:231–236.

Hagler JR, Jackson CG, Isaacs R, Machtley SA. 2004. Foraging behavior and prey interactions by a guild of predators on various lifestages of *Bemisia tabaci*. Journal of Insect Science, insectscience.org/4.1, 1–13.

Hamilton GC, Lashomb JH. 1997. Effect of insecticides on two predators of the Colorado potato beetle (Coleoptera: Chrysomelidae). Florida Entomologist 80:10–23.

Horowitz AR. 1993. Control strategy for the sweetpotato whitefly, *Bemisia tabaci*, late in the cotton-growing season. Phytoparasitica 21:281–291.

Horowitz AR, Ishaaya I. 1992. Susceptibility of the sweetpotato whitefly (Homoptera: Aleyrodidae) to buprofezin during the cotton season. Journal of Economic Entomology 85:318–324.

Horowitz AR, Ishaaya I. 1994. Managing resistance to insect growth regulators in the sweetpotato whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology 87:866–871.

Horowitz AR, Forer G, Ishaaya I. 1994. Managing resistance in *Bemisia tabaci* in Israel with emphasis on cotton. Pesticide Science 42:113–122.

Hough-Goldstein J, Keil CB. 1991. Prospects for integrated control of the Colorado potato beetle (Coleoptera: Chrysomelidae) using *Perillus bioculatus* (Hemiptera: Pentatomidae) and various pesticides. Journal of Economic Entomology 84:1645–1651.

Ishaaya I, Horowitz AR. 1992. Novel phenoxy juvenile hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweetpotato whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology 85:2113–2117.

Ishaaya I, Horowitz AR. 1995. Pyriproxyfen, a novel insect growth regulator for controlling whiteflies: mechanisms and resistance management. Pesticide Science 43:227–232.

Ishaaya I, Mendelson Z, Melamed-Madjar V. 1988. Effect of buprofezin on embryogenesis and progeny formation of sweetpotato whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology 81:781–784.

Jones WA, Ciomperlik MA, Wolfenbarger DA. 1998. Lethal and sublethal effects of insecticides on two parasitoids attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae). Biological Control 11:70–76.

Jones WA, Wolfenbarger DA, Ciomperlik M. 1995. Insecticide effects on immatures of native and imported *Eretmocerus* spp. In: Silverleaf whitefly: 1995 supplement to the 5-year national research and action plan. USDA-ARS 1995-2.

Lindquist RK, Sorensen EL. 1970. Interrelationships among aphids, tarnished plant bugs, and alfalfas. Journal of Economic Entomology 63:192–195.

Liu T-X, Chen TY. 2000. Effects of the chitin synthesis inhibitor buprofezin on survival and development of immatures of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). Journal of Economic Entomology 93:234–239.

Luck RF, Shepard BM, Kenmore PE. 1988. Experimental methods for evaluating arthropod natural enemies. Annual Review of Entomology 33:367–391.

Magagula CN, Samways MJ. 2000. Effects of insect growth regulators on *Chilocorus nigritus* (Fabricius) (Coleoptera: Coccinellidae), a non-target natural enemy of citrus red scale, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae), in southern Africa: evidence from laboratory and field trials. African Entomology 8:47–56.

Nagai K. 1990. Effects of a juvenile hormone mimic material, 4-phenoxyphenyl (RS)-2(2-pyridyloxy) propyl ether, on *Thrips palmi* (Thysanoptera: Thripidae) and its predator *Orius* sp. (Hemiptera: Anthocoridae). Applied Entomology & Zoology 25:199–204.

Naranjo SE, Flint HM. 1994. Spatial distribution of preimaginal *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development of fixed-precision sequential sampling plans. Environmental Entomology 23:254–266.

Naranjo SE, Flint HM. 1995. Spatial distribution of adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development and validation of fixed-precision sampling plans for estimating population density. Environmental Entomology 24:261–270.

Naranjo SE, Ellsworth PC, Chu CC, Henneberry TJ. 2002. Conservation of predatory arthropods in cotton: role of action thresholds for *Bemisia tabaci* (Homoptera: Aleyrodidae). Journal of Economic Entomology 95:682–691.

Naranjo SE, Hagler JR, Ellsworth PC. 2003. Improved conservation of natural enemies with selective management systems for *Bemisia tabaci* in cotton. Biocontrol Science & Technology 13:571–587.

Naranjo SE, Ellsworth PC, Hagler JR. 2004. Conservation of natural enemies in cotton: role of insect growth regulators for management of *Bemisia tabaci*. Biological Control 30:52–72.

Ragsdale DW, Larson AD, Newsom LD. 1981. Quantitative assessment of the predators of *Nezara virdula* eggs and nymphs within a soybean agroecosystem using ELISA. Environmental Entomology 10:402–405.

Schmutterer H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology 35:271–297.

Toscano NC, Prabhaker N, Castle SJ, Henneberry TJ. 2001. Inter-regional differences in baseline toxicity of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to the two insect growth regulators, buprofezin and pyriproxyfen. Journal of Economic Entomology 94:1538–1546.

Wheeler Jr, AG. 1976. Lygus bugs as facultative predators. In: Scott DR, O'Keefe LE, editors. Lygus bug: host plant interactions. Proceeding of a workshop at the XV International Congress of Entomology, 19 August 1976, Washington, DC. Moscow, ID: University Press of Idaho. pp 28–35.

Wheeler Jr AG. 2000. Predacious plant bugs (Miridae). In: Schafer CW, Panizzi AW, editors. Heteroptera of economic importance. New York, NY: CRC Press. pp 657–693.

Wilkinson JD, Biever KD, Ignoffo CM. 1979. Synthetic pyrethroid and organophosphate insecticides against the parasitoid *Apanteles marginiventris* and the predators *Geocoris punctipes*, *Hippodamia convergens*, and *Podisus maculiventris*. Journal of Economic Entomology 72:473–475.

Yakti R, Poehling HM. 1988. Zum Einfluss eines Insektenwachstumsregulators (DSC 24 300 I) auf die Entwicklung von *Aphis fabae* an *Vicia faba* unter besonderer Berücksichtigung von Nebenwirkungen auf Blattlausprädatoren. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 53:1033–1043.

Yu SJ. 1988. Selectivity of insecticides to the spined soldier bug (*Podisus maculiventris*) and its lepidopterous prey. Journal of Economic Entomology 81:119–122.