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Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs

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Abstract

The gut contents of field-collected, predaceous Heteroptera were assayed for the presence of eggs of the sweetpotato whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) and the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) using multiple enzyme-linked immunosorbent assays (ELISAs). Of seven species examined, *Geocoris* species and *Orius tristicolor* (Say) were the most frequent predators of sweetpotato whitefly with 32–39% of the individuals tested over the whole season scoring positive for whitefly antigens. With the exception of *Lygus hesperus* Knight, a major insect pest as well as a predator, the frequency of predation on pink bollworm eggs was much lower (0.7–14.3% positive over the season). Relatively few predators tested positive for both antigens (0.3–12.5%).

Introduction

Arthropod predator/prey interactions are difficult ecological processes to examine. Historically, the study of predation has relied mainly on inexact or indirect techniques, mainly as a direct consequence of the nature of predation, which unlike parasitism, rarely leave evidence of an attack. Many predators and their prey are small and either remain hidden, or are active at night which makes direct field observations of predation difficult.

Laboratory experiments can be used to evaluate the acceptability of particular prey and the rates of predation (e.g., Orphanides *et al.*, 1971; Henneberry & Clayton, 1985; Hagler & Cohen, 1991); however, these types of studies seldom translate to actual field situations where the requirements of predator search are more demanding, a variety of potential prey species are present, and both predator and prey are subject to changing environmental conditions. More direct techniques such as the microscopic analysis of predator gut contents have been used (James, 1961), but this process is highly labor intensive, can be inexact, and is not suitable for predator species that liquefy prey contents for consumption (Hengeveld, 1980). Indirect techniques of gut analysis, including the use of radioactive markers for tagging potential prey (Baldwin *et al.*, 1955; Jenkins, 1963; McDaniel & Sterling, 1979; McCarty *et al.*, 1980; Breene & Sterling, 1988) and electrophoresis (Murray & Solomon, 1978) have also been used, but such techniques can pose dangers to users and the environment, are often time-consuming, or do not possess the necessary specificity and sensitivity for particular species of prey. These difficulties have resulted in a lack of information on the impact that predators have on suppressing key insect pest populations.

One of the most promising techniques for studying predation is use of immunologically-based tests employing pest-specific monoclonal antibodies (MAbs) (Greenstone & Morgan, 1989; Whitten & Oakshott, 1990). Pest-specific MAbs used in an enzyme-linked immunosorbent assay (ELISA) give researchers a quick, sensitive, and cost-effective method to qualitatively examine arthropod predation without disrupting the normal feeding behavior of predators in the field (Hagler *et al.*, 1992).

Currently, sweetpotato whitefly (*Bemisia tabaci* Gennadius) and pink bollworm (*Pectinophora gossyp-*

iella (Saunders)) are the two most destructive pests of cotton in the southwestern United States. Annual economic losses incurred by pink bollworm averaged a quarter of the cotton crop value from 1966 to 1980 in the California Imperial Valley. Since 1980, cotton production there has decreased from over 100,000 acres to less than 15,000 acres, principally as a result of pink bollworm infestations (Henneberry, 1986; Natwick, 1987). Sweetpotato whitefly has been a pest in the Southwest since the early 1980's (Butler & Henneberry, 1984; Natwick & Zalom, 1984), and with the discovery of a new biotype, it has become a major pest of cotton and other field and vegetable crops. It has been estimated that whitefly outbreaks were responsible for over $100 million of total crop loss in California and Arizona in 1991 (USDA, 1992).

The crop loss caused by these two pests are exacerbated by the increased incidence of pesticide resistance and by secondary pest outbreaks subsequent to the destruction of the natural enemy complex by non-selective pesticides. These problems, coupled with increasing environmental awareness and pesticide costs are forcing growers to seek more environmentally safe and cost-effective pest control strategies. One such strategy may include a better conservation or even an augmentation of predaceous natural enemies as part of an integrated pest management program (Stern *et al.*, 1959). It is likely that many predaceous arthropods feed on sweetpotato whitefly and pink bollworm, yet the potential of these predators has not been fully evaluated.

We have developed pest-specific MAbs to sweetpotato whitefly and pink bollworm egg antigens that can detect a single prey item, i.e. one egg, within the gut of a predator up to 24 h after ingestion (Hagler *et al.*, 1993, 1994). We used these MAbs in multiple ELISAs to test simultaneously for the presence of sweetpotato whitefly and pink bollworm egg antigens in the guts of individual predators (Hagler & Naranjo, 1994). In this study, we focused on estimating the frequency of predation on these pests by seven species of predaceous Heteroptera commonly found in the cotton ecosystem in Arizona.

Materials and methods

Predaceous heteropterans were collected throughout the 1992 growing season from two, 2-Ha cotton fields located at the University of Arizona's Maricopa Agricultural Research Center, Maricopa, Arizona and one, 0.5-Ha field located at the Western Cotton Research Laboratory, Phoenix, Arizona. From 7 June to 6 September samples were taken at weekly to bimonthly intervals from each field using a modified Insectivac (Ellington *et al.*, 1984). Four, randomly selected, continuous 30-m rows of cotton were vacuumed in each field. The contents from each vacuum sample were put in a waterproof container and placed on ice. Upon return to the laboratory, predators were stored in a freezer set at - 80°C. All vacuum samples from each site were combined on each sampling date and all adult predators were identified to species. In some cases, the nymphal stage was separated from the adult stage for data analysis; however, for some predator species few nymphs were collected and so adult and nymphal stages were pooled for analysis.

On several sampling dates, additional predators were collected and kept alive for use as negative controls. These predators were fed cabbage looper, *Trichoplusia ni* (Hübner) larvae and water *ad lib* for a minimum of 72 h to ensure that any potential whitefly or pink bollworm antigens were eliminated from their guts (Hagler & Cohen, 1990). These individuals were then macerated in 250 μl phosphate buffered saline (PBS) and assayed for the presence of sweetpotato whitefly and pink bollworm egg antigen in their gut by the ELISAs described below. In most cases, it was not practical to assay the negative controls along with their field-collected counterparts because of space limitations on the ELISA plates. To account for the day to day variability inherent to the ELISAs, we assayed the negative control predators on different plates over several days. These results were then pooled and the mean (±SD) absorbance values were then calculated for each species. The negative controls permit evaluation of any predator constituents that may react with our antibodies and provide estimates of any inherent background noise associated with the ELISAs.

We used the ELISAs described by Hagler & Naranjo (1994) to determine the percentage of individual predators with sweetpotato whitefly and/or pink bollworm egg antigen in their gut. Initially, we were concerned that the predators collected in our vacuum samples might become contaminated with whitefly antigen during the sampling process. To eliminate any possibility of this we cleaned each predator by removing externally attached whiteflies and then irrigated each predator with PBS. We determined in a pilot test that this procedure was effective for cleansing any extraneous whitefly debris (JRH unpub. data). Whole individual field-collected predators were then ground in

250-μl of PBS. A 50-μl aliquot of each macerated predator was placed in an individual well of a 96-well assay plate (Falcon Pro-Bind 3915). A second 50-μl aliquot was placed in an individual well of a second assay plate. Each plate was incubated at 4°C overnight. Following incubation, the insect macerates were discarded from each plate and a 350 μl aliquot of 1% non-fat dry milk in distilled water was added to each well for 30 min at 37°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 MAb acetic fluid diluted 1:1,000 in non-fat milk (Hagler *et al.*, 1993) was added to each well of the first ELISA plate and a 50-μl aliquot of anti-pink bollworm MAb acetic fluid diluted 1:100,000 (Hagler *et al.*, 1994) was added to each well of the second plate. Each plate was accompanied with a positive whitefly or pink bollworm egg control (ca. 5 μg egg protein/well) and a PBS negative control. Both plates were then incubated for 1 h at 37°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 (TAGO Inc., Burlingame, CA) diluted (1:500) in 1.0% nonfat milk was added to each well (50-μl) of both plates for 1 h at 37°C. Plate contents were discarded and rinsed as described above. A 50-μl aliquot of p-nitrophenyl phosphate (1.0 mg/ml) substrate (Sigma Chem. Co., St. Louis, MO) was added to each well. The substrate buffer consisted of 1 M diethanolamine and 0.5 mM $MgCl_2$ (pH 9.8). After 1 h the absorbance of each well was measured with a Cambridge Technology Model 750 (Waterton, MA) microplate reader set at 405 nm. Predators were scored positive for the presence of sweetpotato whitefly egg or pink bollworm egg antigen if the absorbance values exceeded the mean negative control reading by three standard deviations (Schoof *et al.*, 1986; Sutula *et al.*, 1986). The percentage positive for each predator was tallied for each sampling date and over the entire season. We have found that the antigens cannot be reliably detected 1 d after ingestion by the predator (unpubl. data). Thus, these percentages reflect predation within the past 24 h.

A z-test statistic was computed for each possible pairwise combination of predators to determine significant differences in the proportion of positive responses for each pest species. The Yates correction for continuity was applied to each z-test calculation (Glantz, 1992).

Results

Most of the negative predator controls used to test for the presence of whitefly egg antigen yielded negative mean absorbance values (Table 1), that is, values lower than the PBS blank that the microplate reader was zeroed in on. The one exception was *Nabis* spp. which had a low mean absorbance value of 0.018. This indicates that none of the predators we surveyed had proteins that cross reacted with our whitefly MAb. Similarly, most of the negative control predators we screened for cross reactivity to the pink bollworm MAb yielded negative mean absorbance values.

A total of 9,178 individual predators representing six different genera were tested throughout the 1992 growing season by ELISA for the presence of whitefly and pink bollworm egg antigen in their guts (Table 2). Although there was considerable variation among species, over a quarter (26.7%) of the tested individuals were positive for the presence of whitefly egg antigen. The proportion of individual species of predators scoring positive for whitefly egg antigen ranged from 4.0% for *Nabis* spp. (primarily *N. alternatus* Parshley) to 39.4% for nymphs of *Geocoris* spp.

In comparison, pink bollworm egg antigen was not detected in predator guts as frequently, with only 13.3% of the individuals we screened scoring positive (Table 2). Of the numerically-dominant genera surveyed (i.e., *Geocoris* spp., *Orius tristicolor* (White), *Nabis* spp., and *Lygus hesperus* Knight), three showed less than 10% of the individuals scoring positive (Table 2). A notable exception was *L. hesperus* with over 30% of adults and just under 20% of nymphs testing positive for pink bollworm egg antigen.

The percentage of predators scoring positive for the presence of both whitefly and pink bollworm prey ranged from 0.3% for *Nabis* spp. to 12.5% for adult *L. hesperus* (Table 2). Overall, a relatively small proportion of the predators we examined had preyed on both pest species.

There were few definitive patterns in the proportion of positive responses to either sweetpotato whitefly or pink bollworm antigens over the season (Fig. 1). Overall, at least some *Geocoris* spp., *O. tristicolor*, and *L. hesperus* tested positive for sweetpotato whitefly antigen on almost every sample date over the growing season. For *Nabis* spp., *Zelus* spp. (primarily *Z. renardii* Kolenati), and *Sinea confusa* Caudell there were two to three dates when no individuals were found positive for whitefly antigens. Pink bollworm egg antigens were not detected in these predators on many sample

Table 1. ELISA results for negative control predators tested for the presence of sweetpotato whitefly (SPW) and pink bollworm (PBW) egg antigen

Predator	Stage	n	SPW Mean absorbance\[a] (±s.d.)	SPW A_{crit}\[b]	PBW Mean absorbance (±s.d.)	PBW A_{crit}
Geocoris punctipes\[c]	Adult	41	−0.017(0.012)	0.019	−0.030(0.030)	0.060
G. punctipes	Nymph	72	−0.046(0.019)	0.011	0.027(0.023)	0.096
Lygus hesperus\[d]	Adult	88	−0.003(0.013)	0.036	−0.039(0.017)	0.012
Nabis alternatus	Adult	81	0.018(0.037)	0.129	0.001(0.037)	0.112
Orius tristicolor	Adult	12	−0.026(0.013)	0.013	−0.003(0.020)	0.057
Sinea confusa	Adult	59	−0.018(0.017)	0.034	−0.077(0.016)	−0.029
S. confusa	Nymph	28	−0.004(0.028)	0.080	−0.057(0.033)	0.042
Zelus renardii	Adult	12	0.008(0.020)	0.068	−0.008(0.012)	0.027
Z. renardii	Nymph	39	−0.014(0.022)	0.052	0.023(0.016)	0.071

[a/] Absorbance was measured at 405nm.

[b/] A_{crit}= critical absorbance value based on mean + 3 s.d. of negative controls.

[c/] The critical value for *G. punctipes* was also used to calculate the number of field-collected *G. pallens* scoring positive for whitefly and pink bollworm.

[d/] The critical value for *L. hesperus* adults was also used to calculate the number of field-collected *L. hesperus* nymphs scoring positive for whitefly and pink bollworm.

Table 2. Frequency of predators scoring positive for the presence of sweetpotato whitefly, pink bollworm, and both pest egg antigens in their gut. Predators were collected 7 June through 6 September, 1992, Maricopa County, AZ

Insect	Stage	Number Assayed	Percentage of predators scoring positive for: Sweetpotato Whitefly\[a]	Pink Bollworm	Both
Geocoris spp.	Nymphal	315	39.4 a	4.1 c	3.8 c
Orius tristicolor	Adult/Nymphal	1,402	38.4 a	8.0 c	3.3 c
G. pallens	Adult	2,349	33.7 b	10.2 c	8.0 b
G. punctipes	Adult	615	31.7 b	3.6 c	2.4 c
Lygus hesperus	Nymphal	1,342	27.7 c	19.8 b	12.4 a
L. hesperus	Adult	1,709	20.1 d	30.1 a	12.5 a
Sinea confusa	Adult/Nymphal	42	14.3 d	14.3 bc	7.1 bc
Zelus spp.	Adult/Nymphal	303	12.2 d	11.9 c	4.3 c
Nabis spp.	Adult/Nymphal	1,101	4.0 e	0.7 d	0.3 d
Grand total		9,178	26.7	13.3	7.2

[a/] Percentages within columns followed by the same letter are not significantly different ($P<0.01$, z-test for proportions).

dates throughout the season and there were a number of dates when very few if any *Geocoris* spp. or *O. tristicolor* tested positive (Fig. 1). In contrast, *L. hesperus* nymphs and adults scored positive for pink bollworm antigens on every date that these predators were collected.

There was considerable variation in the percentage of specific predators found positive for whitefly and pink bollworm antigens on particular sample dates over

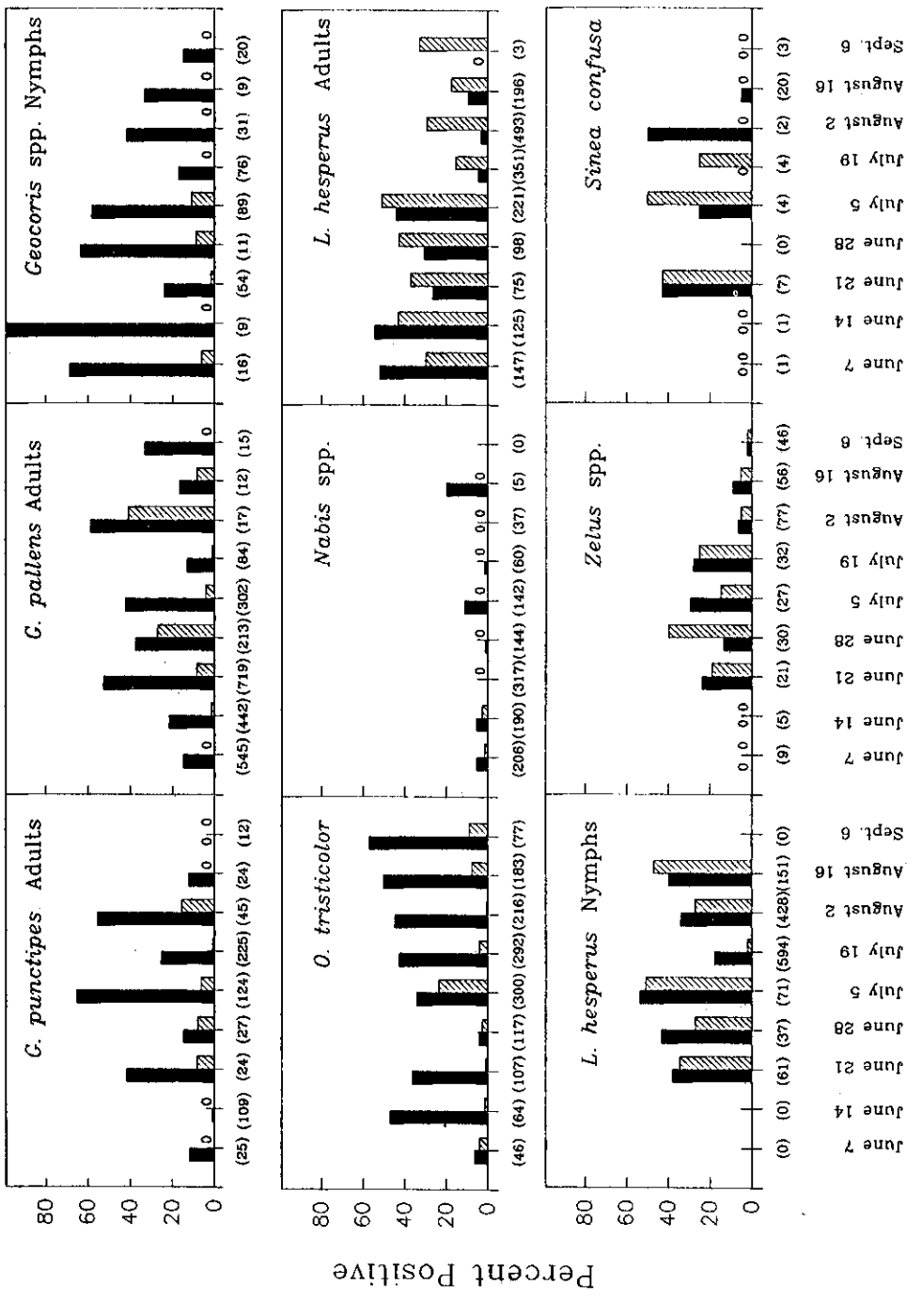

Fig. 1. Frequency of predators scoring positive for the presence of sweetpotato whitefly (solid bars) and pink bollworm (diagonal bars) egg antigen on nine different sampling dates throughout the 1992 growing season, Maricopa County, Arizona, USA. Predators examined are listed in the individual graphs and the numbers in parenthesis below the bars represent the number of insects assayed by ELISA.

the season (Fig. 1). More *Geocoris* spp. nymphs and *L. hesperus* adults were found positive for whitefly in the first half of the season, while *G. punctipes* (Say) adults appeared to feed on whitefly more in the latter half of the season. The percentage of *G. pallens* Stål adults, *O. tristicolor* adults and nymphs, *L. hesperus* nymphs, and *Zelus* spp. and *S. confusa* adults and nymphs testing positive for whitefly remained relatively constant over the entire season or showed no discernable pattern.

With the exception of *L. hesperus*, very few predators were found positive for pink bollworm antigens in the early portion of the season and the percentage rarely exceeded 20% over the remainder of the season for *Geocoris* spp. and *O. tristicolor* (Fig. 1). The percentage of *L. hesperus* adults testing positive for pink bollworm was higher the first half of the season, but remained steady for *L. hesperus* nymphs over the whole season. The greatest percentage of reduviids testing positive was found in mid-season. However, *Zelus* spp. were not commonly encountered in early June and *S. confusa* were rarely collected throughout the entire sampling period.

Discussion

The two MAbs used in this study are among the most specific and sensitive MAbs ever developed for predator-prey studies. This is exemplified by the low absorbance values of the negative controls (Table 1) and exhaustive tests for cross reactivity with other insect species (Hagler *et al.*, 1993, 1994). Using these two MAbs we were able to monitor simultaneously the frequency with which field-collected predators contain whitefly and pink bollworm egg remains in their bodies. Because these MAbs target for egg antigens, a positive response can also occur if a gravid adult female has been consumed (Hagler *et al.*, 1993, 1994). Adult pink bollworms are probably too large and elusive for most small predators to capture. However, adult whiteflies can be preyed on by all of the species we surveyed. This uncontrollable variable must be considered when interpreting our results.

As a group, the predaceous Heteroptera are among the most abundant species of predators in cotton and they are generally considered important predators of many pest insects (e.g., Whitcomb & Bell, 1964; Ehler, 1977). Compared with four other species, *Geocoris* spp. and *O. tristicolor* were the most frequent predators of sweetpotato whitefly. Combined with their abundance in our fields (Naranjo & Hagler, unpubl. data) these species may have a beneficial impact on whitefly populations, particularly when pest populations are low.

Very few *Nabis* spp. scored positive for whitefly or pink bollworm. The predominate species we encountered in our vacuum samples was *N. alternatus*, which has been identified as an effective predator on many insect pests in Arizona (Perkins & Watson, 1972; Stoner *et al.*, 1975). However, the minimal response of *N. alternatus* for whitefly and pink bollworm eggs indicates that they may be feeding preferentially on other insect species or other life stages of the two insects we studied. Alternatively, they may fail to locate and exploit these potential prey items.

The two reduviids we surveyed, *S. confusa* and *Z. renardii*, showed some evidence of feeding on whitefly and pink bollworm. While these predators have been recorded feeding on insect eggs (Ewing & Ivy, 1943; Lingren *et al.*, 1968) they seem to prefer live, mobile prey (Ables, 1978; pers. obs.). Therefore a positive response for either pest is likely due to feeding on adult females. Because these two reduviid species are not abundant, have slow development times, low reproductive rates, and long prey handling times they probably have little effect on whitefly or pink bollworm populations (Swadener & Yonke 1973a, b; Ali & Watson, 1978).

Although *L. hesperus* is considered a major insect pest, its predatory activity is well known (Lindquist & Sorenson, 1970; Bryan *et al.*, 1976; Cleveland, 1987). Our results demonstrated that pink bollworm eggs were readily preyed upon by both adults and nymphs of *L. hesperus*. The correspondence of *Lygus* feeding behavior and pink bollworm oviposition behavior may help explain why pink bollworm eggs are vulnerable to *L. hesperus*. Typically, *L. hesperus* feed on meristematic and reproductive tissues of the cotton plant. Prior to the presence of cotton bolls, pink bollworms lay their eggs on vegetative plant parts and show a preference for terminal growing points (Brazzel & Martin 1957; Henneberry & Clayton, 1982). Once fruiting structures are abundant by mid-season the majority of eggs are found on green bolls, often below the calyx which tightly surrounds the base of the fruit. These oviposition sites may increase the incidence of discovery of pink bollworm eggs by foraging *L. hesperus*. In view of our results and the work of others (Lindquist & Sorenson, 1970; Bryan *et al.*, 1976; Cleveland, 1987), perhaps we need to assess carefully the beneficial impact of *L. hesperus* in the cotton agroecosystem.

There were few trends in the frequencies of predators scoring positive for whitefly or pink bollworm over time (Fig. 1). Initially, we hypothesized that the frequencies would increase in time due to increasing pest populations. However, many of the predators sampled early in the season had approximately the same proportion of individuals scoring positive for either pest as those sampled later in the season. This indicates that predators can find these pests even when densities are very low in the early portion of the season. Another plausible explanation for the relatively high percentage of predators testing positive for whitefly in the early season may be due to the movement of predators from cantaloupe (*Cucumis melo* L.) patches adjacent to our field sites. In the southwestern United States these spring melons harbor huge populations of whiteflies that later infest cotton. Many of the predators scoring positive for whitefly in our cotton samples from June may have originated from these whitefly infested melons.

The use of multiple pest-specific MAbs in concert with gut content immunoassays helped us to gain a better understanding of predator feeding behavior under natural conditions. The methods applied here circumvent many of the difficulties typically encountered when studying predator/prey interactions. The ELISA technique is precise, rapid, economical, sensitive, and does not interfere with a predator's normal feeding behavior in the field. Potential pitfalls common with immunological assays and other indirect methods of assessing predation (i.e., radiolabelling and electrophoresis) include the possibility of obtaining false positive reactions due to third trophic level interactions or scavenger feeding. Either of these interactions would lead to an over-estimation of a predators efficacy (Breene & Sterling, 1988). We are currently investigating the movement of whitefly and pink bollworm egg antigen through the food chain. Another limitation with immunological assays is that the results are not readily quantifiable. Factors leading to spurious results in quantifying predation include temperature changes, digestive rate, prior metabolic status (such as degree of satiation), and prey size (McIver, 1981; Greenstone & Hunt, 1993; Symondson & Liddell, 1993). These factors must be addressed and compensated for before a precise quantitative estimate of predation can be made.

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