Qualitative Survey of Two Coleopteran Predators of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) Using a Multiple Prey Gut Content ELISA

JAMES R. HAGLER AND STEVEN E. NARANJO

Western Cotton Research Laboratory, USDA–ARS, 4135 East Broadway Road, Phoenix, AZ 85040

Environ. Entomol. 23(1): 193–197 (1994)

ABSTRACT A multiple serodiagnostic enzyme-linked immunosorbent assay (MS ELISA) is described that facilitated the simultaneous examination of insect predation on two economically important cotton pests, *Bemisia tabaci* (Gennadius) and *Pectinophora gossypiella* (Saunders). Using this MS ELISA in combination with specific monoclonal antibodies (MAbs) to both *B. tabaci* and *P. gossypiella*, we assayed 663 collops beetles, *Collops vittatus* (Say), and 613 convergent ladybird beetles, *Hippodamia convergens* Guérin-Méneville, for the presence of prey remains in the gut. A large proportion of each beetle population tested positive, suggesting that collops and ladybird beetles are active predators on both *B. tabaci* and *P. gossypiella*. The advantages and limitations of our multiple prey gut content assay (MS ELISA) are discussed.

KEY WORDS *Collops vittatus*, monoclonal antibodies, predation

COLLOPS BEETLES, *Collops vittatus* (Say) and convergent ladybird beetles, *Hippodamia convergens* Guérin-Méneville are generalist insect predators found on cultivated plants and weeds in Arizona. Some studies report that *Collops* spp. are efficient insect predators under highly artificial conditions in the laboratory (Dahms & Kagen 1938, Knowlton 1944, Walker 1957, Nielson & Henderson 1959) or in the greenhouse (Orphanides et al. 1971, Hussain 1975). However, little or no information exists on *C. vittatus* predation under field conditions. There is more literature on ladybird beetle predation, but most of these studies were also conducted under artificial conditions in the laboratory, greenhouse, or field cages (Martinez & Pienkowski 1982, Kring et al. 1985, Rice & Wilde 1988, Andow 1990, Evans 1991). The lack of field data regarding predator feeding behavior is caused in part by the difficulty of studying insect predation. Typically, predator/prey interactions are difficult to observe in nature because of the small sizes, cryptic behaviors, and nocturnal activities of both predator and prey. Moreover, because most predators preorally dissolve or chew their prey into unrecognizable fragments, direct gut observations are impossible (Miles 1972, Cohen 1989). In short, it is very difficult to determine what is eating what.

To date, the most promising method for measuring predation uses immunologically-based tests such as an enzyme-linked immunosorbent assay (ELISA) (Boreham & Ohiagu 1978, Ragsdale et al. 1981, Sunderland et al. 1987), which employ pest-specific monoclonal antibodies (MAbs) (Greenstone & Morgan 1989). Immunological methods facilitate examination of predation under natural, unmanipulated conditions (Hagler et al. 1992). We have developed species- and stage-specific MAbs to sweetpotato whitefly, *Bemisia tabaci* (Gennadius), and pink bollworm, *Pectinophora gossypiella* (Saunders) (Hagler et al. 1993, 1994). Using these MAbs, we can simultaneously examine predator gut contents for the presence of both pest-specific antigens.

In this study, we describe and apply a multiple serodiagnostic (MS) ELISA (i.e., multiple prey gut content assay) to evaluate the frequency that field-collected adult collops and adult ladybird beetles preyed on sweetpotato whitefly and pink bollworm at two different cotton fields in Arizona. This is the first study that we know of that employs multiple MAbs for the examination of the gut contents of an individual predator.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the USDA.

Materials & Methods

Adult collops and ladybird beetles were collected from two, 2.0-ha sites at the University of

Table 1. ELISA results for phosphate buffered saline (PBS), *Collops vittatus*, and *Hippodamia convergens* negative controls

Treatment	Pest MAb tested	n	No. positive reactions	Absorbance[a] $\bar{x} \pm$ SD	Critical value $\bar{x} \pm 3$ SD
PBS blank	anti-SPW	33	0	0.001 ± 0.024	—
PBS blank	anti-PBW	33	0	−0.004 ± 0.024	—
C. vittatus	anti-SPW	44	0	−0.013 ± 0.014	0.029
C. vittatus	anti-PBW	44	1	−0.020 ± 0.026	0.058
H. convergens	anti-SPW	97	3	−0.025 ± 0.018	0.029
H. convergens	anti-PBW	97	1	0.004 ± 0.022	0.069

Analysis was for the presence of sweetpotato whitefly (SPW) and pink bollworm (PBW) egg antigen in the guts of individuals using an anti-SPW and anti-PBW egg-specific monoclonal antibody (MAb).

[a] Absorbance was measured at 405 nm.

Arizona Maricopa Agricultural Research Center, Maricopa, AZ and one 0.5-ha site adjacent to the Western Cotton Research Laboratory, Phoenix, AZ. Each Maricopa site contained four quadrants consisting of 12, 120-m rows of cotton surrounded by four border rows. The Phoenix site contained four quadrants of six, 70-m rows surrounded by two border rows.

Vacuum samples were taken every 1–2 wk from each quadrant with a modified Insectivac (Ellington et al. 1984). Four randomly selected, continuous 30-m rows of cotton were vacuumed in each quadrant. Because of the low catches of collops and ladybird beetles at Maricopa, the samples from the two sites were combined for each date. Samples were only collected through 23 July 1992 at Maricopa because the cotton predator complex was destroyed by pesticide drift from nearby fields.

The contents from each vacuum collection were put in a 3.8-liter plastic carton and placed immediately on ice. Upon return to the laboratory, predators were stored at −80°C. Voucher specimens of *C. vittatus* and *H. convergens* used in this study were deposited at the Carl Hayden Bee Research Laboratory, USDA–ARS, Tucson, AZ.

Negative Predator Controls. Beetles serving as negative controls were collected from the field and provided with only water ad libitum for a minimum of 72 h. This allows the insects to egest any egg antigen they may have had in their gut at the time of collection (Hagler & Cohen 1990). Individual insects were placed in 250-μl phosphate buffered saline (PBS), macerated, and frozen (−80°C) until assay. Negative control beetles were assayed for the presence of egg antigens by the MS ELISA described below. Mean (± SD) absorbance values were calculated for each species.

Multiple Prey Gut Content Assay—MS ELISA. The indirect ELISA procedure described originally by Voller et al. (1976) was modified by Hagler et al. (1992). Individual field-collected collops or ladybird beetle adults were ground (whole body) in 250-μl of PBS. A 50-μl aliquot of each of the macerated beetles was drawn by pipet into individual wells of two uncoated 96-well ELISA assay plates. Each plate was incubated at 4°C overnight. Following incubation, the insect samples were discarded from each well and a 330-μl aliquot of 1% nonfat dry milk in distilled water was added to each well for 30 min at 37°C. The nonfat milk was discarded and a 50-μl aliquot of anti-*B. tabaci* MAb (Hagler et al. 1993) was added to the first ELISA plate and a 50-μl aliquot of anti-*P. gossypiella* MAb (Hagler et al. 1994) was added to the second plate. Each plate was accompanied with a positive *B. tabaci* or *P. gossypiella* egg control (≈5 μg egg protein/well) and a PBS negative control. Plates were incubated for 1 h at 37°C, then the MAbs were discarded, and the wells were briefly washed three times with PBS-Tween 20 (0.05%) and twice with PBS. Aliquots (50-μl) of goat antimouse IgG/IgM conjugated to alkaline phosphatase (TAGO, Burlingame, CA) diluted to 1:500 in 1% nonfat milk were added to each well of both plates. Plates were incubated for 1 h, then the conjugated antibody was discarded, and the wells were washed as noted above. A 50-μl aliquot of p-nitrophenyl phosphate (pNPP) substrate (Sigma, St. Louis, MO) was added to each well for 1 h, then the absorbance of each well was measured with a Cambridge Technology Model 750 (Cambridge Technology, Watertown, MA) microplate reader set at 405 nm. Field-collected beetles were scored positive for sweetpotato whitefly egg or pink bollworm egg antigen if the absorbance values exceeded the mean negative predator control reading by three standard deviations (Schoof et al. 1986, Sutula et al. 1986).

Results

Negative Predator Controls. All but one of the 44 collops negative controls we tested for the presence of whitefly and pink bollworm egg antigen had a negative response to the targeted antigens (Table 1). Similarly, four out of 97 of the ladybird negative controls scored positive for either pest antigen. Of the 97 ladybird beetles, three were positive for whitefly egg antigen and one for pink bollworm egg antigen. The remain-

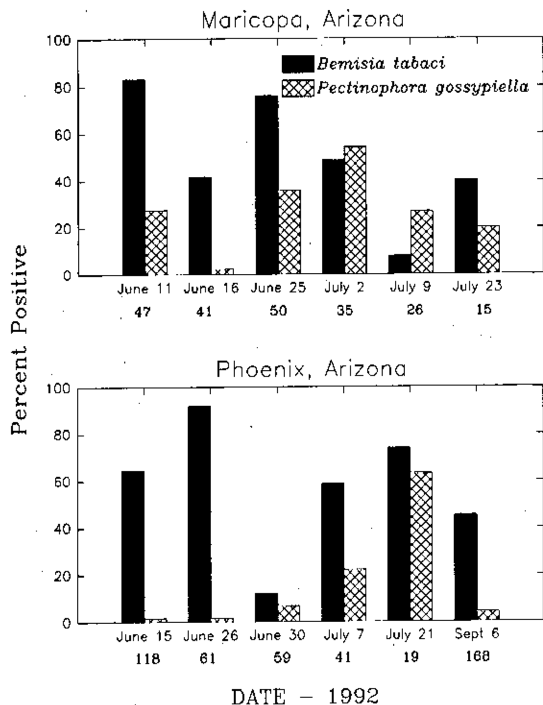

Fig. 1. Percentage of adult *Collops vittatus* scoring positive for the presence of *Bemisia tabaci* and *Pectinophora gossypiella* egg prey remains in their guts, 1992. Numbers below the sampling dates are the number of individuals examined. Note: there were no *C. vittatus* collected in our vacuum samples at Phoenix on the 2 d we sampled in August.

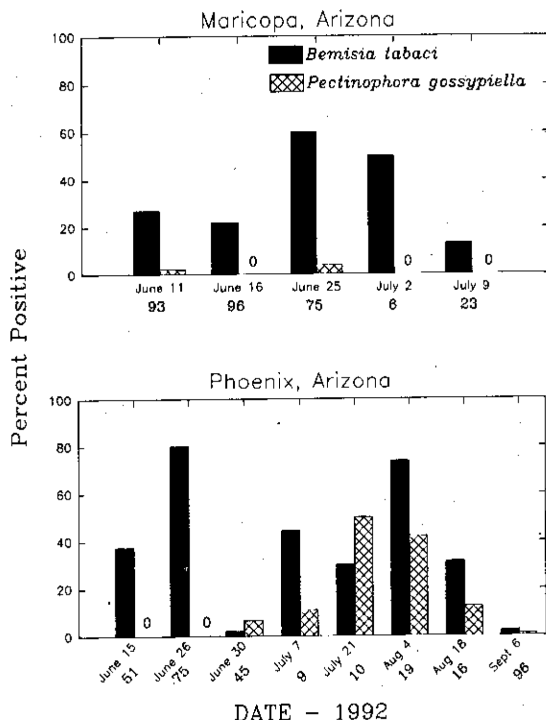

Fig. 2. Percentage of adult ***Hippodamia convergens*** scoring positive for the presence of *Bemisia tabaci* and *Pectinophora gossypiella* egg prey remains in their guts, 1992. Numbers below the sampling dates are the number of individuals examined.

der of the negative collops and ladybird beetle controls yielded absorbance values similar to the PBS blanks (Table 1).

Multiple Prey Gut Content Assay—MS ELISA. Adult collops beetles collected at Maricopa tested positive for whitefly egg (prey) remains from 8.3% (9 July) to 83.0% (11 June) of the time (Fig. 1). Overall, we detected whitefly egg antigen in the guts of 57.9% of the individuals we examined. The percentage of collops testing positive for pink bollworm egg antigen ranged from 2.4% (16 June) to 54.3% (2 July). Of the total collops population examined at Maricopa, 28.4% scored positive for pink bollworm egg antigen.

Whitefly egg antigen was detectable in collops collected at the Phoenix site 11.9% (30 June) to 93.4% (26 June) of the time (Fig. 1). When the samples from each date were combined, we detected whitefly egg antigen in 52.8% of the collops. The frequency of positive responses to pink bollworm eggs ranged from 1.6% (26 June) to 68.4% (21 July). Overall, 7.3% of the collops we sampled had pink bollworm egg remains in their guts.

Adult lady beetles collected at Maricopa tested positive for whitefly egg antigen from 13.0% (9 July) to 60.0% (25 June) of the time (Fig. 2). Overall, we detected whitefly egg antigen in the guts of 38.0% of the adult ladybird beetles we examined. The percentage of lady beetles testing positive for pink bollworm egg predation ranged from 0% (16 June, 2 July, and 9 July) to 4.0% (25 June). Only 1.0% of the ladybird beetles examined at Maricopa had pink bollworm egg antigen in their guts.

Whitefly egg antigen was present in 2.0% (30 June and 6 September) to 79.0% (26 June) of the adult ladybird beetles collected at the Phoenix site (Fig. 2). Overall, 33.0% of the ladybird beetles had whitefly egg antigen in their gut. The percentage of ladybird beetles scoring positive for pink bollworm egg antigen was higher at Phoenix than at Maricopa. Daily positive responses for lady beetle predation on pink bollworm eggs ranged from 0% (15 June and 26 June) to 50.0% (21 July) with 6.0% of the total population surveyed scoring positive for the presence of egg prey remains in their gut.

Discussion

Only a few of the negative predator controls scored positive for the presence of whitefly or pink bollworm egg antigen in their gut. These

data indicate that the starved beetles lacked whitefly or pink bollworm egg antigen in their systems and that there were no inherent antigens present in these beetles that cross reacted with our MAbs. These results are similar to previous studies where these two MAbs were tested for cross reactivity against other insect species (Hagler et al. 1993, 1994). The few positive scores in the negative controls probably can be attributed to human error or insufficient holding time for digestion of pest antigen present in the gut at the time of collection (Greenstone 1983).

A limitation of our MS ELISA is that these egg-specific and pest-specific MAbs cannot differentiate between an egg and an egg-carrying (i.e., gravid) female meal. However, we believe this limitation is only applicable to sweetpotato whitefly predation. For example, these two beetle species are relatively large and can easily capture, kill, and consume either an adult egg-carrying female whitefly or a whitefly egg. Therefore, a positive response could be attributed to feeding on either whitefly stage. Conversely, it is unlikely that these predators could capture, kill, and consume the large, cryptic, and mobile adult pink bollworm. Therefore, any positive scores in our MS ELISA for pink bollworm egg antigen could be attributed solely to egg predation.

Pink bollworm predation was not as common as sweetpotato whitefly predation. The lower proportion of beetles feeding on pink bollworm eggs could be attributed to several factors including the following: (a) the positive responses were probably associated with egg predation only; (b) pink bollworm egg populations were much lower than whitefly populations, thus decreasing the chance of a predator/prey encounter; and (c) the cryptic oviposition behavior (i.e., beneath the calyx) exhibited by pink bollworm moths makes their eggs less vulnerable to predators.

There were few patterns in the frequencies of beetles scoring positive for whitefly or pink bollworm ingestion over time (Figs. 1 and 2). Initially, we thought that the frequency of positive responses would increase in time because of the ever increasing pest populations. However, many of the predators collected 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 these two beetle species were able to find and feed on pink bollworms and whiteflies even when the pest densities were very low in the early portion of the season.

This is the first study that uses an ELISA in concert with multiple pest-specific MAbs to survey predator feeding patterns under natural, unmanipulated field conditions. The MS ELISA is ideal for a precise, rapid, and economical identification of insect predators (Hagler et al. 1992). We have shown in preliminary laboratory studies that the MS ELISA is sensitive enough to detect a single prey item in a predator's gut. Moreover, we can assay over 1,000 individuals per day at a cost of less than $0.10 per individual.

Results from this study are intended to provide only a qualitative estimate of adult collops and ladybird predation on whitefly and pink bollworm. Unfortunately, because of inherent constraints common with serological assays, quantification of the number of prey consumed by each predator is not yet possible. Abiotic factors, variable digestive rates, and variable meal sizes make it impossible to quantify predation at present (McIver 1981, Fichter & Stephen 1981, Sopp & Sunderland 1989).

Currently, we are using this sensitive MS ELISA to qualitatively evaluate other potential predators of sweetpotato whitefly and pink bollworm. The information gained from MS ELISA evaluations, combined with laboratory trials and thorough population dynamics studies can provide a better understanding of the role that predaceous natural enemies have on suppressing key agronomic pests.

Acknowledgments

We thank Christine Durand (Undergraduate Biology Research Program, University of Arizona), Pat Figuli, Scott Machtley, Jeanette Martin, Gary Owens, Jim Fields, Jennifer Katcher, Lisa Luddington, and Chris Webster (USDA, Phoenix) for their excellent technical assistance. Special thanks to Heather Costa (University of Hawaii), Gloria DeGrandi-Hoffman (USDA, Tucson), Hollis Flint (USDA, Phoenix)), Debbie Hagler (University of Arizona), Jay Rosenheim (University of California, Davis), and two anonymous reviewers for providing helpful suggestions to an earlier version of the manuscript. We would like to acknowledge Javier Enriquez and Debbie Bradley-Dunlop of the Hybridoma Core Support Facility at the University of Arizona for their expert assistance in developing the monoclonal antibodies used in this experiment. We especially thank Eric Erickson (USDA, Tucson) and the staff at the Carl Hayden Bee Laboratory for providing the laboratory facilities used in this experiment.

References Cited

Andow, D. A. 1990. Characterization of predation of egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 83: 482–486.

Boreham, P.F.L. & C. E. Ohiagu. 1978. The use of serology in evaluating invertebrate predator-prey relationships: a review. Bull. Entomol. Res. 68: 171–194.

Cohen, A. C. 1989. Ingestion efficiency and protein consumption by a heteropteran predator. Ann. Entomol. Soc. Am. 82: 495–499.

Dahms, R. G. & M. Kagen. 1938. Egg predation of the chinch bug. J. Econ. Entomol. 31: 779–780.

Ellington, J., K. Kiser, M. Cardenas, J. Duttle & Y. Lopez. 1984. The insectavac: A high-clearance, high-volume arthropod vacuuming platform for ag-

ricultural ecosystems. Environ. Entomol. 13: 259–265.

Evans, E. W. 1991. Intra versus interspecific interactions of ladybeetles (Coleoptera: Coccinellidae) attacking aphids. Oecologia (Berl.). 87: 401–408.

Fichter, B. L. & W. P. Stephen. 1981. Time related decay in prey antigens ingested by the predator *Podisus maculiventris* (Hemiptera: Pentatomidae) as detected by ELISA. Oecologia (Berl.). 51: 404–407.

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

Greenstone, M. H. & C. E. Morgan. 1989. Predation on *Heliothis zea* (Lepidoptera: Noctuidae): an instar-specific ELISA assay for stomach analysis. Ann. Entomol. Soc. Am. 82: 45–49.

Hagler, J. R. & A. C. Cohen. 1990. Effects of time and temperature on digestion of purified antigen by *Geocoris punctipes* (Hemiptera: Lygaeidae) reared on artificial diet. Ann. Entomol. Soc. Am. 83: 1177–1180.

Hagler, J. R., A. C. Cohen, D. Bradley-Dunlop & F. J. Enriquez. 1992. Field evaluation of predation on *Lygus hesperus* using a species- and stage-specific monoclonal antibody. Environ. Entomol. 21: 896–900.

Hagler, J. R., A. G. Brower, Z. Tu, D. N. Byrne, D. Bradley-Dunlop & F. J. Enriquez. 1993. Development of a monoclonal antibody to detect predation of the sweetpotato whitefly, *Bemisia tabaci*. Entomol. Exp. Appl. 68: 231–236.

Hagler, J. R., S. E. Naranjo, D. Bradley-Dunlop, F. J. Enriquez & T. J. Henneberry. 1994. A monoclonal antibody to pink bollworm egg antigen: A tool for predator gut analysis. Ann. Entomol. Soc. Am. (in press).

Hussain, M. 1975. Predators of the alfalfa weevil, *Hypera postica* in Western Nevada—a greenhouse study. N. Y. Entomol. Soc. 83: 226–228.

Kring, T. J., F. E. Gilstrap & J. Michels, Jr. 1985. Role of indigenous coccinellids in regulating greenbugs (Homoptera: Aphididae) on Texas grain sorghum. J. Econ. Entomol. 78: 269–273.

Knowlton, G. F. 1944. Collops feeding. J. Econ. Entomol. 37: 443.

Martinez, D. G. & R. L. Pienkowski. 1982. Laboratory studies on insect predators of potato leafhopper eggs, nymphs, and adults. Environ. Entomol. 11: 361–362.

McIver, J. 1981. An examination of the utility of the precipitin test for evaluation of arthropod predator-prey relationships. Can. Entomol. 113: 213–222.

Miles, P. W. 1972. The saliva of Hemiptera. Adv. Insect Physiol. 9: 183–256.

Nielson, M. W. & J. A. Henderson. 1959. Biology of *Collops vittatus* (Say) in Arizona, and feeding habits of seven predators of the spotted alfalfa aphid. J. Econ. Entomol. 52: 159–162.

Orphanides, G. M., D. Gonzalez & B. R. Bartlett. 1971. Identification and evaluation of pink bollworm predators in Southern California. J. Econ. Entomol. 64: 421–424.

Ragsdale, D. W., A. P. Larson & L. D. Newsom. 1981. Quantatative assessment of the predators of *Nezara viridula* eggs and nymphs within a soybean agroecosystem using an ELISA. Environ. Entomol. 10: 402–405.

Rice, M. E. & G. E. Wilde. 1988. Experimental evaluation of predators and parasitoids in suppressing greenbugs (Homoptera: Aphididae) in sorghum and wheat. Environ. Entomol. 17: 836–841.

Schoof, D. D., S. Palchick & C. H. Tempelis. 1986. Evaluation of predator-prey relationships using an enzyme immunoassay. Ann. Entomol. Soc. Am. 79: 91–95.

Sopp, P. I. & K. D. Sunderland. 1989. Some factors affecting the detection period of aphid remains in predators using ELISA. Entomol. Exp. Appl. 51: 11–20.

Sunderland, K. D., N. E. Crook, D. L. Stacey & B. J. Fuller. 1987. A study of feeding by polyphagous predators on cereal aphids using ELISA and gut dissection. J. Appl. Ecol. 24: 907–933.

Sutula, C. L., J. M. Gillett, S. M. Morrissey & D. C. Ramsdell. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70: 722–726.

Voller, A., D. Bidwell & A. Bartlett. 1976. Microplate enzyme immunoassays for the immunodiagnosis of virus infections, pp. 506–512. *In* N. R. Rose & H. Friedman [eds.], Manual of clinical immunology. American Society of Microbiology, Washington, DC.

Walker, J. K. 1957. A biological study of *Collops balteatus* Lec. and *Collops vittatus* (Say). J. Econ. Entomol. 50: 395–399.

Received for publication 8 June 1993; accepted 8 October 1993.