PREDATORS OF BUDWORM/BOLLWORM EGGS IN COTTON: AN IMMUNOLOGICAL STUDY

John R. Ruberson
Department of Entomology, Univ. of Georgia
Tifton, GA
Matthew H. Greenstone
USDA-ARS
Stillwater, OK

Abstract

Various predators were sampled in a 30-acre cotton field during the 1996 growing season and assayed for the presence of heliothine egg protein. A modified ELISA was used to assay the predators. A total of 3355 predators was collected and assayed. Of these, 6.49% proved to have egg antigen in them. Of the species assayed, big-eyed bugs (*Geocoris punctipes*), red imported fire ants (*Solenopsis invicta*), *Scymnus* lady beetles, and winter spiders (*Chiracanthium inclusum*) yielded the majority of positive responses. The frequency of positive responses correlated to some extent with the relative abundance of heliothine eggs in the field. However, abundance of cotton aphids relative to heliothine eggs during aphid outbreaks may have negative consequences for the efficacy of natural enemies against heliothine eggs, as all of the predators examined have catholic feeding habits.

Introduction

The importance of natural enemies for managing insect pests of cotton has become increasingly apparent in the southeastern United States, as the Boll Weevil Eradication Program (BWEP) and the deployment of transgenic Bt cotton have created a less toxic environment for arthropods in cotton fields. The beneficial fauna now present in many cotton fields has become quite diverse; however, the roles of the various species in suppressing pest populations are largely unknown. Identification of key natural enemy species, and quantification of their impact so that we can anticipate the suppressive capacity of particular beneficial species in the field, will increase our abilities to develop scouting procedures and thresholds that incorporate natural enemies. Such information is also essential for developing conservation efforts directed toward important natural enemy species.

Numerous natural enemies have been observed in cotton fields (Whitcomb and Bell 1964, van den Bosch and Hagen 1967, Lopez et al. 1996) and there is a considerable body of evidence suggesting that this complex of natural enemies can have a substantial impact on pest populations (e.g., Ruberson et al. 1994). Despite the lengthy natural enemy lists and the long history of research in this area (although this has been conducted by comparatively few researchers), little specific information is available on which natural enemies might be considered key species in cotton. Indeed, the diversity of the natural enemy complex has made efforts to determine key species very difficult.

Determination of key parasitoids of insect pests usually can be performed by collecting live specimens of the target species from the field, then holding them in the laboratory for parasitoid emergence. In this manner one can quantify and identify key parasitoids of target pests. Predators, however, present a serious challenge for those seeking to quantify their impact. Their activity is transient, and they rarely leave any sign of their activity. It is a difficult task to determine the complex of species feeding on a particular pest. Direct observation is one method that works well (e.g., Bell and Whitcomb 1964), but is excessively time consuming and costly for obtaining a small number of observations. Other techniques, such as the use of immunoassays, provide direct evidence of predation by a particular predator, but are often difficult to use for quantifying the predator's overall impact (Greenstone 1996, Sunderland 1996). Nevertheless, an important first step in understanding ecological processes in the field is discovering which natural enemies are feeding on key pests, and the frequency with which the natural enemy populations do so. Surveys and the use of immunotechniques are important tools in recognizing those players, and immunoassays provide a means of evaluating the frequency of predation events in a natural enemy population, preparatory to detailed quantitative studies (e.g., Hagler and Naranjo 1996). Once we establish which species are important, we can then begin to devise measures for scouting and conserving them.

The research results presented here are one step toward understanding and identifying key natural enemies. The objective of the research presented here was to identify predators of eggs of the tobacco budworm, *Heliothis virescens*, and the cotton bollworm, *Helicoverpa zea*, in cotton. The ultimate objective is to develop decision thresholds that incorporate both pests and natural enemies, and to develop scouting procedures relevant to evaluating populations of key natural enemy species.

Materials and Methods

We collected all predaceous arthropods obtained in sweep, shake, and whole-plant samples from two commercial cotton fields. One of the fields, however, had very poor weed management (more weeds than cotton, which influenced the makeup and activity of predators), thus the results from this field are not presented here. Samples were taken twice weekly at both locations. In addition to predators, heliothine eggs and cotton aphids were counted on 240 plants (8 centrally-located points in each of 30 plots in the field). Cotton aphids were counted on only two leaves of each examined plant -- one leaf in the upper third

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1095-1098 (1998)
National Cotton Council, Memphis TN

and one in the middle third of the plant. The predator samples consisted of a wide variety of species, including big-eyed bugs, pirate bugs, lady beetles (chiefly *Scymnus* species), hooded beetles, fire ants, and various spiders (see Table 1). All specimens were transported in coolers and transferred to a conventional freezer after being brought to the laboratory. Specimens were held in the freezer until processing for the ELISA bioassay.

Enzyme-linked immunosorbent assay (ELISA) was used to determine whether collected predators had fed on eggs. The monoclonal antibody used for the assays was provided by Dr. Matthew Greenstone and was cloned to produce sufficient material for the studies. Predators for assays were crushed and ground in borate saline solution and then run through a series of steps modified from Harlow and Lane (1988) for the two-antibody sandwich assay. Predators to be bioassayed were homogenized in microcentrifuge tubes, using teflon-coated micropestles, in the presence of 250 μl of buffer solution. 50-μl aliquots of the homogenized arthropod were applied to each of 3 wells in a 96-well microplate. The wells in the plate had been previously coated overnight with the monoclonal antibody (obtained from mouse; diluted 1:10,000 in buffer solution). Aliquots of positive (a single heliothine egg processed as the other samples) and negative controls (pre-immune mouse blood, beet armyworm eggs, and *Geocoris punctipes* or *Orius insidiosus* adults that had not fed on heliothine eggs) were also placed in three wells each. The arthropod aliquots were left in the wells overnight, then the plates were washed three times, using a buffered wash solution, in an automated plate washer. A blocking solution was added to each well, incubated for 2 hours at room temperature, then removed and each plate was washed 3 times as above. Each well was filled with monoclonal antibody solution, as above, and incubated at room temperature for 2 hours, after which they were washed 3 times. A rabbit-anti-mouse (RAM) polyclonal antibody solution was then added to each well and incubated for 2 hours at room temperature. The plates were again washed 3 times, and a commercially-produced enzyme-conjugated antibody (obtained from Sigma-Aldrich, St. Louis MO) was introduced to each well and allowed to incubate for 1 hour. The plate was again washed, and the substrate solution for the conjugated enzyme (alkaline phosphatase) was added to each well and incubated for 30 min. The reaction was stopped with 3N NaOH, and the absorbance values of the wells in the plates were immediately read with a microplate reader at a wavelength of 405 nm. A predator was considered to be positive for egg consumption if the mean absorbance value of the three aliquots representing that predator fell within two standard deviations of the positive control and exceeded the negative controls by at least two standard deviations. This rather subjective cutoff for positive responses probably errs on the conservative side -- we probably underestimated predation, possibly by a considerable margin. Nevertheless, by establishing this cutoff, we also minimize problems that may result from secondary predation, and other confounding events (see below).

Results and Discussion

The results of the assays for predators collected in 1996 are presented in Table 1. The predators shown were sampled between 10 July and 16 August, and a total of 3355 predators was assayed. During this time budworm and bollworm egg populations were variable (Table 2), never exceeding 60 eggs per 100 plants and averaging 4.6 eggs per 100 plants when the counts from all sampling dates are pooled. Aphid populations increased considerably during July, and then declined rapidly in early August (Table 2). The numbers of aphids (peaking at ~60 per leaf) relative to the availability of heliothine eggs (peaking at 0.43 per plant) during the period of aphid infestation may have had a diluting effect on egg predation. This remains to be examined in more detail, but given the catholic diets of the predators prey switching in the presence of overwhelming prey abundance seems likely.

Despite the low egg populations through most of the season, 6.49% of all predators collected were positive for the presence of egg proteins (Table 1), and the proportion of positive reactions tended to follow the abundance of eggs in the field (Table 2). Given the low numbers of eggs and the moderate levels of predators, this suggests that a high level of egg predation was occurring during this period. It must be pointed out, however, that false positives can occur when using immunological techniques to study predation (Sunderland 1996). It is possible that a predator which consumed another predator that fed on the target egg will test positive for the presence of the egg protein. Although this can happen, such results are highly unlikely, given the dilution and degradation of the protein likely to occur in such transfers, and the rather high absorbance value which we used to denote a positive result. We conclude that most, if not all, of the positive reactions recorded are signs of primary predation on eggs. Another potential pitfall is not knowing how many eggs a predator consumed -- a positive reaction does not have a strong quantitative component. Thus, when a positive reaction is obtained, it indicates at least one egg was consumed, but may actually be the result of multiple eggs being consumed. Again, as noted above, we tended to err on the conservative side.

Several species accounted for the bulk of the positive responses (Table 1): red imported fire ants, the big-eyed bugs *Geocoris* spp. (especially *G. punctipes*), *Scymnus* spp. lady beetles, and the winter spider *Chiracanthium inclusum* (Table 3). All of these species tended to be quite abundant.

Egg predation by *Geocoris punctipes* has been observed often in the field, but the overall impact of this species on egg populations is not clear (see Bell and Whitcomb 1964). Nevertheless, from our results it is apparent that this species

is an active egg predator and may be a key natural enemy of lepidopteran eggs. The high frequency of positive reactions among *Scymnus* spp. specimens suggests that these coccinellids may be quite important as egg predators (Table 1). These tiny coccinellids are well-known aphid predators, and can reach high population densities when aphids are abundant. The highest frequency of positive responses in *Scymnus* spp. was observed during the period when aphid populations were highest. *Scymnus* spp. can be found, however, at low levels even when aphids are rare in the field (J. Ruberson, pers. observation). Winter spiders previously have been reported to feed on heliothine eggs (Bohmfalk et al. 1982), but our data indicate that they may play an important role in managing heliothine pests. Winter spiders can be quite abundant in cotton (J. Ruberson, pers. observation.).

Several species known to feed on budworm and bollworm eggs tested positive for egg protein, but not at the levels that might have been expected. For example, the frequency of positive responses was surprisingly low for the insidious flower bug, *Orius insidiosus* (Table 1). This species is a noted predator of lepidopteran eggs (Winburn and Painter 1932, Barber 1936, Dicke and Jarvis 1962, Bell and Whitcomb 1964). Two factors may have contributed to the low frequency of positive reactions in *O. insidiosus*. First, in evaluating the decay rate of another antibody in *O. insidiosus*, Dr. James W. Smith (Texas A&M University) found that the half-life is very short -- less than 12 hours. Thus, if the bugs were not captured shortly after consuming an egg, they would yield negative results (J. W. Smith, pers. comm.). It is likely that this same rapid decay rate occurs with the antigen we are using. Second, with the high absorbance value cutoff used in our tests, we would have missed all but those that had eaten eggs within 6-8 hours of consuming an egg, based on Smith's decay rate. It is highly probable, then, that we seriously underestimated predation frequency in this species.

Adults and larvae of the convergent lady beetle (*Hippodamia convergens*) were found to have consumed eggs, as did green lynx spiders, *Peucetia viridans* (Table 1). Surprising positive results were obtained for several species, including the cotton aphid. Further testing is needed to substantiate these results, but some pest species, such as the cotton fleahopper and *Lygus hesperus*, are also known to act as predators on occasion (e.g., Hagler and Naranjo 1994). These results point to the complex interactions occurring among arthropods in cotton and reinforce the need to further examine these relationships to understand their effects on cotton production.

One particularly useful finding in this study was the relatively high positive response detected in big-eyed bugs, especially the more common species, *Geocoris punctipes*. This species is emerging from this and other studies (studies of beet armyworm natural enemies) as a key predator in cotton. It is also a fairly visible species, particularly in the adult stage, making it a good candidate for scouts to locate and count. Future studies will address this predator more closely, evaluating predation rates of various prey in the laboratory and field. It may hold considerable promise as an indicator species for efficacy of biological control -- one that could be counted and one that might lend itself to development of natural enemy thresholds.

Although our results provide insights into the complex of natural enemies that consume heliothine eggs, future studies will permit us to obtain more specific quantitative information. First, we must examine decay rates of the antigen in several of the key predators identified in this study. Knowing decay rates is vital for accurate interpretation of ELISA results -- understanding the decay curve allows the cutoff reaction to be set less arbritrarily. The appropriate experiments need to be conducted to address antigen decay rates (e.g., Greenstone and Hunt 1993). Second, more broad-based sampling of natural enemies will be necessary to fully characterize the natural enemy complex. The sampling conducted in the study reported here was all conducted during the day (between 0800 and 1200 EDT) and only on plants. The full spectrum of predators, therefore, was not represented in the samples. For example, ground-dwelling species and nocturnal species were excluded from sampling. These species also should be collected and assayed, although collection of such species is often challenging.

Acknowledgments

This research was supported by a grant from Cotton Incorporated and the Georgia Cotton Commission. Additional support was provided by USDA-NRICGP Grant No. 9503262. We also appreciate the assistance of Peggy Goodman in processing the samples and running numerous ELISA's. Our thanks to Drs. Gary A. Herzog (Univ. of Georgia), Phillip Roberts (Univ. of Georgia), and P. Glynn Tillman (USDA-ARS) for reviewing the manuscript. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

References

Barber, G. W. 1936. *Orius insidiosus* (Say), an important natural enemy of the corn ear worm. USDA Tech. Bull. 504.

Bell, K., and W. H. Whitcomb. 1964. Field studies on egg predators of the bollworm, *Heliothis zea* (Boddie). Florida Entomol. 47: 171-180.

Bohmfalk, G. T., R. E. Frisbie, W. L. Sterling, R. B. Metzer and A. E. Knutson. 1982. Identification, biology and sampling of cotton insects. Texas Agric. Exp. Sta. Bull. B-933.

Dicke, E. F., and J. L. Jarvis. 1962. The habits and seasonal abundance *Orius insidiosus* (Say) (Hemiptera-Heteroptera: Anthocoridae) on corn. J. Kansas Entomol. Soc. 35: 339-344.

Greenstone, M. H. 1996. Serological analysis of arthropod predation: past, present and future. In: W. O. C. Symondson and J. E. Liddell (eds.), The Ecology of Agricultural Pests: Biochemical Approaches, pp. 265-300. Chapman and Hall, London.

Greenstone, M. H., and J. H. Hunt. 1993. Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay. Entomol. Exp. Appl. 68: 1-7.

Hagler, J. R., and S. E. Naranjo. 1994. Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. Entomol. Exp. Appl. 72: 59-66.

Hagler, J. R., and S. E. Naranjo. 1996. Using gut content immunoassays to evaluate predaceous biological control agents: a case study. In: W. O. C. Symondson and J. E. Liddell (eds.), The Ecology of Agricultural Pests: Biochemical Approaches, pp. 383-399. Chapman and Hall, London.

Harlow, E., and D. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor NY.

Lopez, J. D., Jr., W. L. Sterling, D. A. Dean and D. A. Nordlund. 1996. Biology and ecology of important predators and parasites attacking arthropod pests. In: E. G. King, J. R. Phillips and R. J. Coleman (eds.), Cotton Insects and Mites: Characterization and Management, pp. 87-142. The Cotton Foundation, Memphis TN.

Ruberson, J. R., G. A. Herzog, W. R. Lambert and W.J. Lewis. 1994. Management of the beet armyworm in cotton: role of natural enemies. Florida Entomol. 77: 440-453.

Sunderland, K. D. 1996. Progress in quantifying predation using antibody techniques. In: W. O. C. Symondson and J. E. Liddell (eds.), The Ecology of Agricultural Pests: Biochemical Approaches, pp. 419-455. Chapman and Hall, London.

van den Bosch, R., and K. S. Hagen. 1966. Predaceous and parasitic arthropods in California cotton fields. Calif. Agr. Exp. Sta. Bull. 820.

Whitcomb, W. H., and K. Bell. 1964. Predaceous insects, spiders, and mites of Arkansas cotton fields. Ark. Agr. Exp. Sta. Bull. 690.

Winburn, T. F., and R. H. Painter. 1932. Insect enemies of the corn earworm (*Heliothis obsoleta* Fabr.). J. Kansas Entomol. Soc. 5: 1-28.

Table 1. Number of sampled arthropods testing positive or negative for presence of budworm/bollworm egg yolk protein.

Arthropod taxon	Total assayed	No. (%) Positive
Solenopsis invicta (Red Imported Fire Ant)	1171	47 (4.0%)
Geocoris punctipes (Big-eyed bug) nymph	170	14 (8.19%)
Geocoris punctipes (Big-eyed bug) adult	129	22 (17.05%)
Geocoris uliginosus (Big-eyed bug) nymph	5	1 (20.0%)
Geocoris uliginosus (Big-eyed bug) adult	24	1 (4.17%)
Orius insidiosus (Insidious flower bug) nymph	191	4 (2.09%)
Orius insidiosus (Insidious flower bug) adult	105	0 (0%)
Chrysoperla spp. (Green lacewing) larva	30	0 (0%)
Hippodamia convergens (Convergent lady beetle) larva	119	8 (6.72%)
Hippodamia convergens (Convergent lady beetle) adult	71	5 (7.04%)
Coccinella septempunctata (7-spotted lady beetle) larva	48	2 (4.17%)
Coccinella septempunctata (7-spotted lady beetle) adult	10	3 (30.0%)
Harmonia axyridis (Asian multi-colored lady beetle) larva	3	0 (0%)
Harmonia axyridis (Asian multi-colored lady beetle) adult	2	0 (0%)
Scymnus lady beetle larva	259	37 (14.29%)
Scymnus lady beetle adult	126	14 (11.11%)
Notoxus monodon (Hooded beetle)	531	3 (0.56%)
Podisus maculiventris (Spined soldier bug) nymph	2	0 (0%)
Podisus maculiventris (Spined soldier bug) adult	2	0 (0%)
Sinea sp. (Spined assassin bug) nymph	3	0 (0%)
Sinea sp. (Spined assassin bug) adult	2	0 (0%)
Zelus (Leafhopper assassin bug) nymph	9	0 (0%)
Nabis sp. (Damsel bug) adult	2	1 (50.0%)
Peucetia viridans (Green lynx spider)	61	2 (3.28%)
Chiracanthium inclusum (Winter spider)	203	51 (25.12%)
Pseudatomoscelis seriatus (Cotton fleahopper)	4	2 (50.0%)
Aphis gossypii (Cotton aphid)	73	1 (1.37%)
TOTALS	3355	218 (6.49%)

Table 2. Relative abundance of heliothine eggs and cotton aphids, and frequency of positive among predators on sample dates (1996, Tifton GA).

Sample date	No. eggs/100 plants	No. aphids/leaf	% positive predators
1 July	2.5	0.82	NA
8 July	3.3	13.7	NA
11 July	8.0	15.8	11.8
15 July	1.3	19.4	NA
18 July	44.2	58.7	NA
22 July	26.7	16.4	NA
25 July	14.6	18.7	10.4
29 July	1.3	24.6	6.5
1 Aug	4.6	38.9	0
5 Aug	40.4	17.6	2.2
8 Aug	5.4	2.8	5.0
13 Aug	55.8	2.8	3.2
16 Aug	43.8	4.2	8.3
20 Aug	5.8	6.3	NA
23 Aug	13.8	6.4	NA
27 Aug	28.8	4.7	NA
3 Sep	29.2	5.0	NA

Table 3. Relative contributions of selected predaceous species to total positive responses (percentages of all positive responses).

Sample date	RIFA[1]	G.p.[1]	*Scymnus* spp.	Winter spider	All others
11 July	29.0	19.4	38.7	6.5	6.4
25 July	15.6	15.6	42.3	20.3	6.2
29 July	16.6	8.3	8.3	25.0	41.8
5 Aug	40.0	20.0	0	20.0	20.0
8 Aug	31.3	16.1	3.2	32.3	17.1
13 Aug	0	34.4	0	65.6	0
16 Aug	30.7	11.6	0	26.9	30.8

[1]RIFA = red imported fire ant, *Solenopsis invicta*; G.p. = *Geocoris punctipes*