Characterizing and Estimating the Effect of Heteropteran Predation

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Heteropterans often are the numerically dominant species in the predator complexes of many agricultural systems, yet we have only a rudimentary knowledge of how they function in pest control. Our inability to predict the effect of these and other arthropod predators on pest population dynamics remains the most significant barrier to using predators as components of pest management systems. This problem stems in large part from the difficulty of measuring the activity of predators under field conditions. We briefly examine techniques for studying predation and evaluate them relative to measuring predation quantitatively. We then detail serological techniques, discuss their strengths and weaknesses, and exemplify use of a serological approach for studying the effect of heteropteran predation in the cotton ecosystem. Serology has a long history in the study of insect predation and it is one of the few methods that requires only minimal disruption of the system under examination. We used monoclonal antibodies developed to recognize egg antigens of pink bollworm, Pectinophora gossypiella (Saunders), and sweetpotato whitefly, Bemisia tabaci (Gennadius) strain B (= B. argentifolii Bellows and Perring), to study the native predators of these pests in cotton. Using a multiple-gut ELISA (enzyme-linked immunosorbent assay) we tested more than 22,000 individuals of 7 species of predaceous Heteroptera over 2 field seasons in central Arizona. Based on the frequency of positive ELISA responses and population densities, Orius tristicolor (White) and Lygus hesperus Knight, a recognized pest species, were found to be the dominant predators of pink bollworm and sweetpotato

whitefly eggs. Geocoris pallens (Stål), G. punctipes (Say), Nabis alternatus Parshley, Sinea confusa Caudell, and Zelus renardii Kolenati appear to be minor predators of eggs of these pests. We propose a new predation model that integrates the results of ELISA, predator population densities, and functional response behaviors. Preliminary analysis of pink bollworm egg predation suggests that the heteropteran predator complex was responsible for removing ≈20% of all pink bollworm eggs over the entire season. This effect was achieved at extremely low (and atypical) densities of this pest.

Since the 1940s, agricultural production has relied on insecticides as the primary method of controlling insect pests. Before this time, it was estimated that 7% of the world's crops were destroyed by insects. Some authorities now estimate losses at 13% (Wilson 1990). This increase in crop destruction over the last half century is caused, in part, by increased incidence of pesticide resistance, secondary pest outbreaks, and natural enemy destruction. Coupled with ever-increasing concerns about groundwater contamination, pesticide residues in foods, and the mounting cost of pesticides, producers are seeking environmentally safe and cost-effective methods for controlling insect pests. The maximization of natural control in crop production systems is a fundamental tenet of integrated pest management, and predacious arthropods are a key component of this natural control. Although it is widely accepted that predators play a role in pest regulation (Whitcomb 1980, Luff 1983), we still have an inadequate understanding of, and ability to predict, their effect in cropping systems.

Predacious heteropterans are a conspicuous component of the fauna of many agricultural ecosystems (e.g., Dicke and Jarvis 1962, Whitcomb and Bell 1964, Tamaki and Weeks 1972, Barry 1973, Pimentel and Wheeler 1973, Shepard et al. 1974, Benedict and Cothran 1975, Ehler 1977, Henneberry et al. 1977, Stoltz and Stern 1978, Morrison et al. 1979, Irwin and Shepard 1980, Wilson and Gutierrez 1980, Bechinski and Pedigo 1981, Trichilo and Leigh 1986, Bugg and Wilson 1989, Braman and Yeargan 1990, Coll and Bottrell 1991, Reid 1991, Yeargan 1998). As with other predator groups, we still know little about how these generalist predators affect economically important pest insects (e.g., Whitcomb and Bell 1964, Barry et al. 1973, Ehler et al. 1973, Elvin et al. 1983, O'Neil and Stimac 1988). Our relatively poor understanding of the function of predators stems directly from the fact that predation remains one of the most intractable ecological processes to study. Because of factors such as small size, nocturnal activity, cryptic behavior, preoral digestion, and often the lack of physical evidence, predators are extremely difficult to observe and study under natural conditions. Most of the widely used experimental methods for studying predation impose restrictions that limit their utility or bias the measurement of predation rates in the field.

Scope of Review

We begin by discussing briefly some of the various experimental methods that have been used for either quantifying or qualitatively describing the influence of predators on populations of prey insects. We highlight some of the advantages and disadvantages of using serological approaches and discuss some of the quantitative indices that have been used for determining the efficacy of arthropod predators through serology. Our major emphasis is to demonstrate how we used monoclonal antibodies to characterize and estimate the effect of native predators on 2 key pests of cotton in the southwestern deserts of the United States—pink bollworm, *Pectinophora gossypiella* (Saunders), and sweetpotato whitefly, *Bemisia tabaci* (Gennadius) strain B (=*B. argentifolii* Bellows and Perring) (Butler and Henneberry 1984, Natwick and Zalom 1984, Henneberry 1986). Specifically, we present an example of how we qualitatively characterized the contribution of various heteropteran predators to mortality of eggs of pink bollworm and sweetpotato whitefly. We then emphasize our recent efforts to quantify the effect of the heteropteran predator complex on pink bollworm eggs in cotton using a new predation index that integrates the results of ELISA (enzyme-linked immunosorbent assay), predator population densities, and functional response behaviors based on prey density in the field.

Experimental Methods for Studying Predation

Several authors have presented detailed discussions of various methods for studying predation (Kiritani and Dempster 1973, Boreham and Ohiaga 1978, Southwood 1978, Grant and Shepard 1985, Luck et al. 1988, Sunderland 1988). Our intention is not to present a detailed review of this material, but to summarize some of the more common methods and highlight their relative strengths and weaknesses in relation to quantifying predation in the field (Table 1). Several authors have categorized methods as either direct or indirect, or as qualitative or quantitative; we chose not to delineate methods in this fashion for several reasons. First, very few methods fit neatly into categories. Second, no single method will fully quantify predation in the field for any particular system. Thus, several techniques in tandem will be necessary.

Highly artificial laboratory experiments can be used to evaluate the suitability of particular prey, compare rates of predation between species, and examine predator behavior (e.g., Orphanides et al. 1971, Henneberry and Clayton 1985, Hagler and Cohen 1991). Unfortunately, such studies cannot be used to quantify predation in the field. Considerable information can be gathered on prey selection, handling times, searching behavior, and predator interactions through direct observation in the field (e.g., Whitcomb and Bell

Table 1. Summary of methods for studying predation

Method	Technical level	Field-level quantification	Major limitations	Major advantages
Correlation studies	Low	No	No cause–effect relationship	Association of predator(s) and prey
Lab feeding studies	Low	No	Prey densities artificially high, search requirements artificially low	ID potential prey of a predator
Direct observation	Low	Partial	Labor- and time-intensive, disruptive, biases toward easily observed predators	Directly reveals diet of predator, can measure periodicity of predation
Predator exclusion cage studies	Low	Partial	Prey cannot disperse, physical environment altered	Cage mesh size could be varied to exclude certain species or groups
Insecticidal check			Selection of proper material, sublethal effects, measures predator complex only	Prey can disperse, physical environmental mostly unaltered, large plot size possible
Predator–prey inclusion cage studies	Low	Partial	Prey cannot disperse, physical environment altered, alternate prey limited	Realistic predator and prey densities can be used, possible to measure impact of single species
Open Field Studies			Predators may disperse from release area, indigenous predators may complicate analyses	Same as above

Table 1. Continued

Method	Technical level	Field-level quantification	Major limitations	Major advantages
Gut dissection	Low	No	Labor-intensive, impossible for predators that liquefy prey	Characterize predator diet under unmanipulated field conditions
Prey marking	High	Partial	Potential danger to user and environment, (radioactive markers) sophisticated detection methods, manipulation of prey populations, secondary predation	No manipulation of predator populations, capability of mass screening of predators
Electrophoresis	Moderate	Partial	Problems in prey-specificity and band-pattern interpretation, difficult to quantify prey consumption from assay, secondary predation, time-consuming	No manipulation of predator or prey populations, best suited to characterizing predator diet breadth
Serology				
Polyclonal antibodies	Moderate	Partial	Antibody may not be species- or stage-specific, difficult to quantify prey consumption from immunoassay response, secondary predation	No manipulation of predator or prey populations, capability of mass screening of predators, sensitive
Monoclonal antibodies	High	Partial	Difficult to quantify prey consumption from immunoassay response, secondary predation	Same as above, species and stage-specificity possible

1964, Crocker and Whitcomb 1980, Nyffeler et al. 1992). However, it is difficult to observe nocturnal activity and to accumulate enough data to estimate even crude rates of predation. Further, disruption of the system during observation remains a constant concern. The microscopic analysis of predator gut contents (e.g., James 1961) can be used, but the process is labor intensive and inaccurate in many instances, and is ineffective for predators such as heteropterans that pierce and suck their prey (Hengeveld 1980). Studies that use cages or other techniques to either exclude predators from an area, or establish or enhance their presence, can provide useful information about the effect of a specific predator complex or species and can be used to estimate rates of predation (e.g., Barry et al. 1973, Ehler et al. 1973, Elvin et al. 1983). Care must be taken in interpreting results, however, because cages can significantly influence the microclimate and they prevent the free movement of both prey and predator. Despite their artificiality, cages can be used effectively to study predator–prey interactions at field densities of prey and predator (e.g., O'Neil and Stimac 1988, Wiedenmann and O'Neil 1992). Electrophoretic analysis of predator gut contents has been used successfully (e.g., Murray and Solomon 1978, Giller 1982), but this technique is time consuming and may not possess the necessary specificity and sensitivity. Radioactive markers (Baldwin et al. 1955, Jenkins 1963, McDaniel and Sterling 1979, McCarty et al. 1980) for tagging potential prey also have been used, but such techniques pose potential dangers to either users or the environment, and they require manipulation of the prey (i.e., marking).

Given the limitations and difficulties of the methods discussed above, we used a combination of techniques to characterize the predator complex of 2 cotton pests and to quantify predation rates in the field. Specifically, we employed immunologically based tests using pest-specific monoclonal antibodies, predator–prey inclusion techniques, and field estimates of predator and prey density.

Serological Approaches. The use of serology in the study of predation has a long history in entomology (Boreham and Ohiagu 1978, Southwood 1978, Sunderland 1988). Of all the methods summarized above (with the exception of electrophoresis), it is the one method that allows the examination and measurement of predation under natural, largely unmanipulated conditions (Brooke and Proske 1946, West 1950, Dempster 1960, Miller 1979). The only disruption involves the removal of predators from the field for laboratory assay. The basic technique relies on insect antigens stimulating an immunological response in a vertebrate host, which culminates in the production of serum polyclonal antibodies to constituents of that insect. These antibodies can be harvested and used as a diagnostic tool to probe the gut of a predator for the presence of prey remains. Unfortunately, polyclonal antibodies, although sensitive to extremely small amounts of prey antigens, tend to cross-react with related insect species (Frank 1967, Lund and Turpin 1977, Gardner et al. 1981, Miller 1981, Doane et al. 1985). However, with advances in hybri-

doma technology over the past 2 decades, investigators can now isolate individual antibody-producing cells grown in vitro and harvest antibodies with single-species specificity (Kohler and Milstein 1975). This is accomplished through cloning by limiting dilution until only 1 antibody is recognized in the cell culture (i.e., monoclonal). The result is an antibody that offers specificity and sensitivity unachievable with conventional polyclonal antiserum.

The use of serology shares several limitations common to other feeding trace methods, including problems of secondary predation that allow prey antigens to migrate up the food chain, predators feeding as scavengers, and predators that ingest sufficient antigen to respond in an assay but fail to kill the prey. An additional limitation that specifically affects the use of monoclonal antibodies is the high degree of technical knowledge and specialized facilities needed to produce useful antibodies.

Estimating Predation. The most important limitation of serological approaches stems from problems in quantifying predation. Antigen detection methods, such as ELISA, provide quantitative results—strength of response is directly proportional to the concentration of antigen (Sunderland 1988). But, translating these quantitative readings into an estimate of the number of prey consumed is complicated by several uncontrollable factors, including temperature, predator digestive rates, prior metabolic status (such as degree of satiation), and prey size (Pickavance 1970, McIver 1981, Fichter and Stephen 1981, 1984, Lovei et al. 1985, Sunderland et al. 1987, Sopp and Sunderland 1989, Hagler and Cohen 1990).

One of the most significant problems in associating a quantitative value of prey consumed with the strength of an assay's response is the confounding effects of meal size and the subsequent rate of digestion of that meal by the predator (Fig. 1). For instance, without additional information regarding the size of the meal or the time elapsed since ingestion, a response of 0.5 could translate to a small meal (e.g., 1 prey item) eaten <1 h ago or a relatively large meal (e.g., 3 prey items) eaten »7–8 h ago. An even more probable circumstance, particularly for predators consuming small prey such as insect eggs, is illustrated in Fig. 2. Here a predator continually consumes additional prey following an initial feeding bout. In this example, the same assay response (0.5) has 4 possible interpretations depending on when the predator was assayed relative to its first meal. Another important consideration is the effect of ambient temperature on the rate of digestion (Fig. 3). Once again a single assay response could have many interpretations. In this instance, one could estimate the temperature profile over the previous 24 h and possibly improve the accuracy of interpretation. A final consideration is the effect of prey age. We have found that the strength of the assay's response changes with age of the egg or adult female being consumed (unpublished data).

Given the problems highlighted above, few researchers have attempted to use the strength of the assay response for quantifying the rate of predation
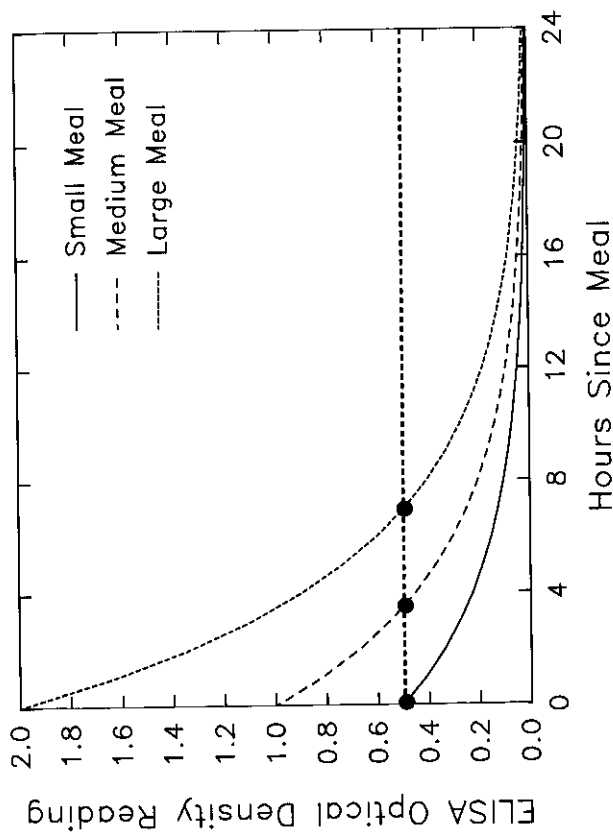
Fig. 1. Hypothetical decay in the quantitative response of an immunoassay to prey antigen in the gut of a predator in relation to size of initial meal. Dashed horizontal line highlights problems associated with the quantitative interpretation of a given strength of response.

(Sopp et al. 1992, see also Sunderland 1988). Sopp et al. (1992) attempted to use the quantitative relationship between an ELISA response and amount of prey biomass consumed by assuming that, on average, the predator's meal was half digested at the time of assay. Alternatively, several quantitative indices have been proposed that simply use the proportion of positive responses coupled with other data to infer rates of predation in the field and to facilitate comparisons across predator species (Table 2). Most indices account for the period over which the antigen remains detectable in the predator's gut. As shown in Fig. 3, this detection interval is temperature dependent and also may differ between predator species (e.g., Sunderland et al. 1987). Recently it has been suggested that a detectability half-life be substituted for the detection interval (Greenstone and Hunt 1993). Two of the indices (Table 2) assume that a positive ELISA response is the result of a single prey consumed. In some cases this assumption may be well founded. In an elegant study of predators of the broom beetle, *Gonioctena olivacea* (Forster), Dempster (1960) used information on relative prey abundance, predator searching behavior, and independent life table data to validate this assumption. The assumption of a single prey consumed per predator may be reasonable when prey are large relative to the predator or prey are relatively
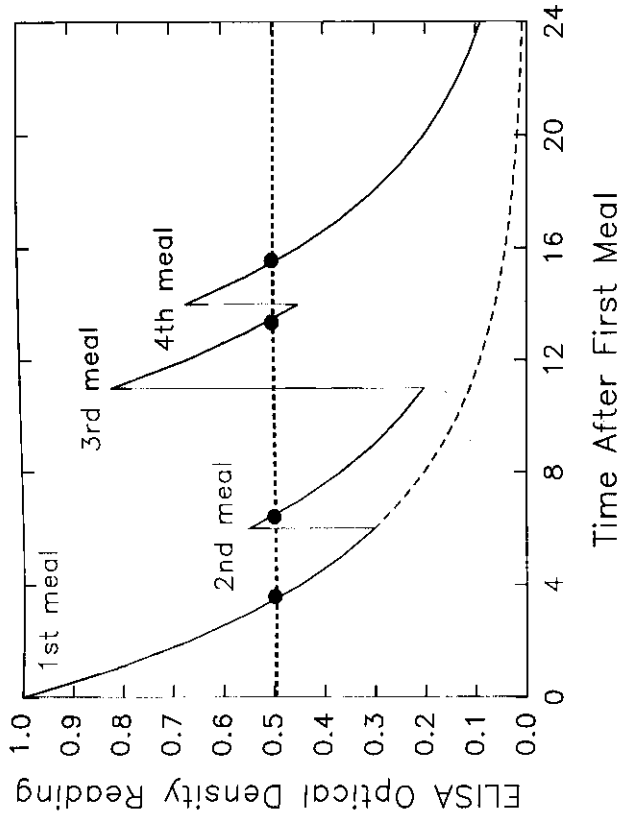
Fig. 2. Hypothetical pattern of decay in the quantitative response of an immunoassay to prey antigen in gut of a predator that fed repeatedly during a 24-h period. Dashed horizontal line highlights difficulty of translating strength of a response to number or biomass of prey consumed.

Table 2. Indices for quantifying predation using serological methods

Index[a]	Reference
1. pP/D	Dempster 1960
2. $Pp \underset{=}{<} (Pp)$	Ragsdale et al. 1981
3. PpT/D	Kuperstein 1979
4. PpTRothschild, 1966	
5. $Ln[1-p]P/D$	Nakamura and Nakamura 1977, Greenstone 1979
6. $Q_o P/fD$	Sopp et al. 1992
7. $pPT(M)/D$	New equation

[a]p, proportion of predators showing positive by ELISA; P, predator density; D, detection period ($\gg$ digestion rate[-1]; generally a function of temperature and predator species); T, predation rate measured in the laboratory or insectary; Q_o, prey biomass recovered; f, mean proportion of meal remaining; $T(M)$, predation rate as a function of prey density (M).
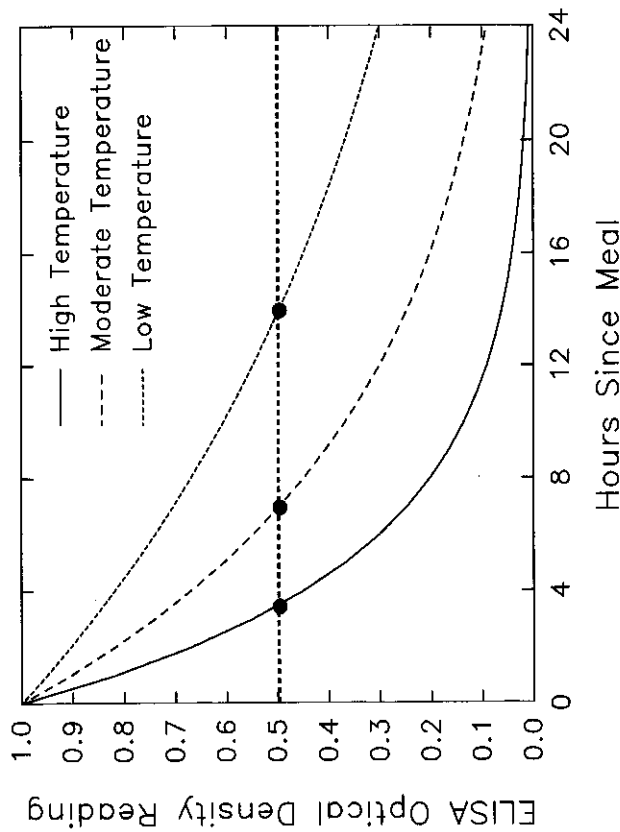
Fig. 3. Hypothetical decay in the quantitative response of an immunoassay to prey antigen in the gut of a predator after an initial meal in relation to temperature. Dashed horizontal line highlights problems associated with quantitative interpretation of a given strength of response.

scarce (or both), but this assumption cannot be universally held, particularly for predators feeding on small, abundant prey such as aphids and whiteflies. Other indices make some assumption about the number of prey consumed per positive predator. Methods for estimating the number of prey consumed vary from measurement of predator feeding rates in the laboratory or insectary (Rothschild 1966, Kuperstein 1979) to consumption rates based on the 0 term of the Poisson distribution (Nakamura and Nakamura 1977, Greenstone 1979). Interestingly, none of the proposed indices base prey consumption on the functional response—the fundamental relationship between prey density and rates of consumption. Therefore, we present an additional quantitative index (No. 7, Table 2) based on the predator's functional response measured under realistic conditions (see below).

Using Monoclonal Antibodies To Study Heteropteran Predation of Cotton Pests

The remainder of this paper presents some of our current research, which is focused on characterizing the heteropteran predator complex feeding on pink bollworm and sweetpotato whitefly and on quantifying the effect of

these native predators on the population dynamics of these insects in the desert southwestern cotton ecosystem. We discuss results from studies conducted during 1991 and 1992 in central Arizona.

Laboratory Components. *Antibody Library.* Researchers in several groups are beginning to amass a library of monoclonal antibodies for various insect pests of cotton. Lenz and Greenstone (1988) developed a monoclonal antibody that recognized 5th-instar *Heliocoverpa zea* (Boddie) for use in analyzing predation by *Podisus maculiventris* (Say) in the laboratory (Greenstone and Morgan 1989). Recently, monoclonal antibodies specific to the egg stage of the western tarnished plant bug, *Lygus hesperus* Knight, sweetpotato whitefly, and pink bollworm (Hagler et al. 1991, 1993, 1994) have been developed to study predation on eggs of these pests in the field (Hagler et al. 1992, Hagler and Naranjo 1994a, b). With this monoclonal antibody library we can simultaneously identify and assess the indigenous predators of these pests.

Antibody Specificity. The specificity of these antibodies was verified by screening them against numerous other insect species (Table 3). All the antibodies are highly species specific, and sensitivity is limited to 2 developmental stages—eggs and adult females. Relative to the performance of polyclonal antibodies, we consider this developmental stage cross-reactivity to be an insignificant limitation. The size difference between adults and eggs relative to the size of a given predator species and predator feeding behavior usually allows reasonable delineation of the stage of the prey attacked. For instance, it would be improbable for smaller predators such as *Geocoris* spp. and *Orius tristicolor* (White) to subdue and feed on an adult pink bollworm. Likewise, we have found that *Zelus renardii* Kolenati will not feed on lepidopteran eggs in the laboratory; thus, positive responses in this predator likely result from feeding on adult females. Predation on whiteflies is more problematic because the smaller heteropteran predators we have examined can probably attack both eggs and adults.

The strength of the immunoreaction with these antibodies declines with egg age; however, antigens can be detected in eggs up to the time of eclosion. Likewise, adult females of either species can be detected at any age, but antibody sensitivity changes with age and peaks around the time of maturity.

Multiple Gut Analysis. We use an indirect ELISA (Voller et al. 1976) to perform predator gut analyses. The sensitivity of the assay allows us to divide samples of a single predator among several microplates. The technique is rapid and lends itself easily to mass screening. Five hundred predators can be assayed for the presence of both pink bollworm and sweetpotato whitefly antigens in a single day. Negative controls (predators reared on diets that exclude pink bollworm or sweetpotato whitefly) are assayed with each predator species to estimate ELISA background noise. ELISA results are scored positive if optical density readings exceed the mean negative control reading by 3 standard deviations (Sutula et al. 1986). See Hagler and Naranjo (1994a, b) for further details of sample preparation and ELISA reagents and protocols.

Table 3. Specificity of monoclonal antibodies for sweetpotato whitefly, pink bollworm, and western tarnished plant bug

Species tested	Stage	Cross reactivity[a]		
		SPW	PBW	WTPB
Helicoverpa zea	Egg		—	—
Heliothis virescens	Egg		—	—
Pectinophora gossypiella	Egg	—	+	—
	Larva		—	
	Pupa		—	
	Adult		+[b]	
Spodoptera exigua	Egg	—	—	—
Trichoplusia ni	Egg	—	—	—
Chlorochroa uhleri	Egg		—	
Euschistus inflatus	Egg		—	—
Euthyrhynchus sp.	Egg		—	—
Geocoris punctipes	Egg	—	—	—
	Nymph	—		
	Adult	—		
Leptoglossus zonatus	Egg		—	
Lygus hesperus	Egg	—	—	+
	Nymph	—		—
	Adult	—		+[b]
Lygus lineolaris	Egg			—
	Nymph			—
	Adult			—
Orius insidiosus	Adult	—		
Sinea confusa	Egg			—
	Nymph			
Thyanta custator	Egg		—	
	Nymph			
Zelus renardii	Egg		—	
	Nymph			
Aphis gossypii	Nymph	—		
Bemisia tabaci (Strain A and B)	Egg	+		
	Nymph			
	Adult	+[a]		
Siphoninus phillyreae	Egg	—		
	Adult	—		
Trialeurodes vaporariorum	Egg	—		
	Nymph	—		
	Adult	+[a]		
Chrysoperla carnea	Egg	—		
	Larva	—		

[a] SPW, sweetpotato whitefly; PBW, pink bollworm; WTPB, western tarnished plant bug; + or −, the antibody did or did not react positively, respectively, in the ELISA to antigens from the indicated species or developmental stage.

[b] Positive reaction with adult female of tested species.

Field Components. Our study sites were located in Maricopa County, AZ, at the University of Arizona Maricopa Agricultural Center, and the Western Cotton Research Laboratory in Phoenix. Four and 3 cotton fields, 0.5-1.2 ha, were sampled during 1991 and 1992, respectively. These fields were maintained using standard agronomic practices for the area, except that no insecticides were applied.

Collection of Predators for Gut Analysis. A modified Insectavac (Ellington et al. 1984) was used to make mass collections of predatory arthropods in cotton at weekly to biweekly intervals during both years. On each date, 4 lengths (30 m) of row were vacuumed per field. Predators were immediately placed on ice for transport to the laboratory. Samples were sorted by species in the laboratory and were subsequently stored at −80°C until being assayed. We delineated 9 heteropteran predator species or groups as follows: adult *Geocoris pallens* Stål, adult *G. punctipes*, *Geocoris* nymphs (both species mixed), *Orius tristicolor* (adults and nymphs), *Nabis alternatus* Parshley (adults and nymphs), *Sinea confusa* Caudell (adults and nymphs), *Z. renardii* (adults and nymphs), *L. hesperus* adults, and *L. hesperus* nymphs. In total, 13,614 and 9178 predators were collected and assayed in 1991 and 1992, respectively. The number of predators assayed for any given sample date in 1992 is presented in Hagler and Naranjo (1994b). Patterns of abundance over the 1991 season were similar for most species.

Estimation of Predator Density. Concurrently with vacuum samples, we estimated absolute population densities of each predator species using whole-plant samples. We placed open-ended canvas bags (with drawstrings on each end) around the bases of 20 randomly selected cotton plants per field 24 h before sampling. The bottom drawstring was pulled tightly around the base of the plant and the top drawstring was left open around the top of the plant. The next day, the bags were pulled rapidly over the top of individual plants and the top drawstring was pulled closed to ensure that insects could not escape. Stems were cut at ground level, and bagged plants were taken to the laboratory where all predators were counted and recorded.

Densities of predator populations ranged from a low of 0.05 to a high of 4.0 per plant over both years depending on species. For the most part, few species displayed any definitive patterns of abundance over the season. *Geocoris* nymphs and *Z. renardii* both displayed late-season increases during both years whereas *O. tristicolor* displayed a late-season and a midseason peak of abundance in 1991 and 1992, respectively. Typically, most species fluctuated between 0.05 and 0.5 per plant from early June to late August.

Estimating Prey Density. Densities of pink bollworm eggs were estimated in both years by randomly collecting 100 green cotton bolls (10–20 d old) from each field per sample date and using a binocular microscope to count the number of eggs. In addition, 10–20 whole plants were collected on each date and examined for any eggs on vegetative structures. These counts, along
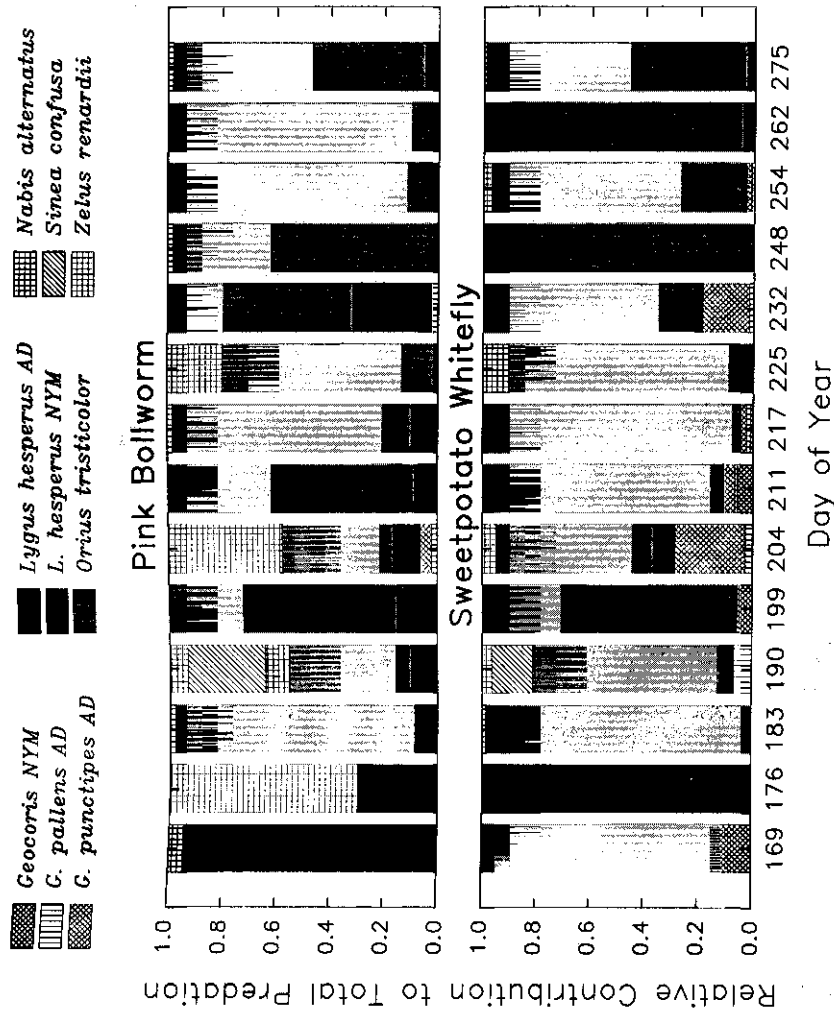
Fig. 4. Relative contribution of 7 species of heteropteran predators to consumption of pink bollworm and sweetpotato whitefly eggs and adult females over course of season in 1991. Contribution of each species or developmental stage calculated using Ragsdale's predation index (see Table 2). Density of predators and proportion of positive responses were derived from pooled estimates over 4 field sites in Maricopa County, AZ.

with estimates of bolls per plant, were used to estimate absolute pink bollworm egg densities. Relative densities of sweetpotato whitefly eggs were estimated by the method of Naranjo and Flint (1994) in 1992. Samples for this insect were not collected in 1991. Finally, 20 whole plants were collected from each field on each sample date to measure leaf area per plant.

Populations of pink bollworm were uncharacteristically low during both years of our study; densities of eggs per plant peaked at 12.2 and 1.8 in 1991 and 1992, respectively. Sweetpotato whitefly populations were not monitored in 1991, but in 1992 population densities were very low (<1 egg per leaf) from June through mid-July and increased dramatically beginning in late July. As many as 5000 eggs were found per leaf in late August. Despite the exponential increase in population density for sweetpotato whitefly and relatively higher populations of pink bollworm eggs in the latter part of the season, we found no relationship between increased frequency of predation by heteropterans and pest density (Hagler and Naranjo 1994b).

Synthesis

Characterizing Predation. Previously we have presented qualitative information on predation on pink bollworm and sweetpotato whitefly eggs by examining the percent of each heteropteran predator sample found positive for each antigen (Hagler and Naranjo 1994b). To gain further insight into the relative contribution of each predator species or group, we used the index (No. 2, Table 2) of Ragsdale et al. (1981). This index weights the proportion positive by the density of each species or group relative to the overall frequency of positive response and the density of all predators being examined. The index does not account for differing numbers of prey that might be consumed by different predators. We examined seasonal changes in the relative contribution of each predator group and the overall contribution of each group for the entire season.

The contribution of individual predator species and groups varied somewhat between years and over the season (Figs. 4 and 5), but 2 species dominated as predators of pink bollworm eggs and sweetpotato whitefly eggs and adult females. With very few exceptions, O. tristicolor and L. hesperus (adults and nymphs) accounted, either individually or in combination, for the majority of predation on these pest insects on all sample dates. On certain sample dates, a moderate fraction of total predation on pink bollworm was accounted for by Z. renardii and to a much lesser extent by S. confusa in both years; however, as discussed above, this is likely the result of predation on adult female moths. Geocoris spp. played a minor role in pink bollworm egg predation throughout 1991 (Fig. 4), and only G. pallens made any notable contributions on several dates in 1992 (Fig. 5). In comparison, the Geocoris complex were more active predators of sweetpotato whitefly eggs and adult

females in both years. Nevertheless, they were responsible for only a minor fraction of overall predation on these stages of this pest.

An integration of season-long results shows that O. tristicolor was by far the most significant predator of both pink bollworm eggs and sweetpotato whitefly eggs and adults in terms of frequency of positive responses and population density in 1991 (Fig. 6). O. tristicolor remained the dominant predator of sweetpotato whitefly eggs and adults in 1992, whereas nymphal L. hesperus was the most dominant predator of pink bollworm eggs. The remaining species of the heteropteran complex contributed <25% of all predation on eggs or adult females of either of these pest insects. These trends were not
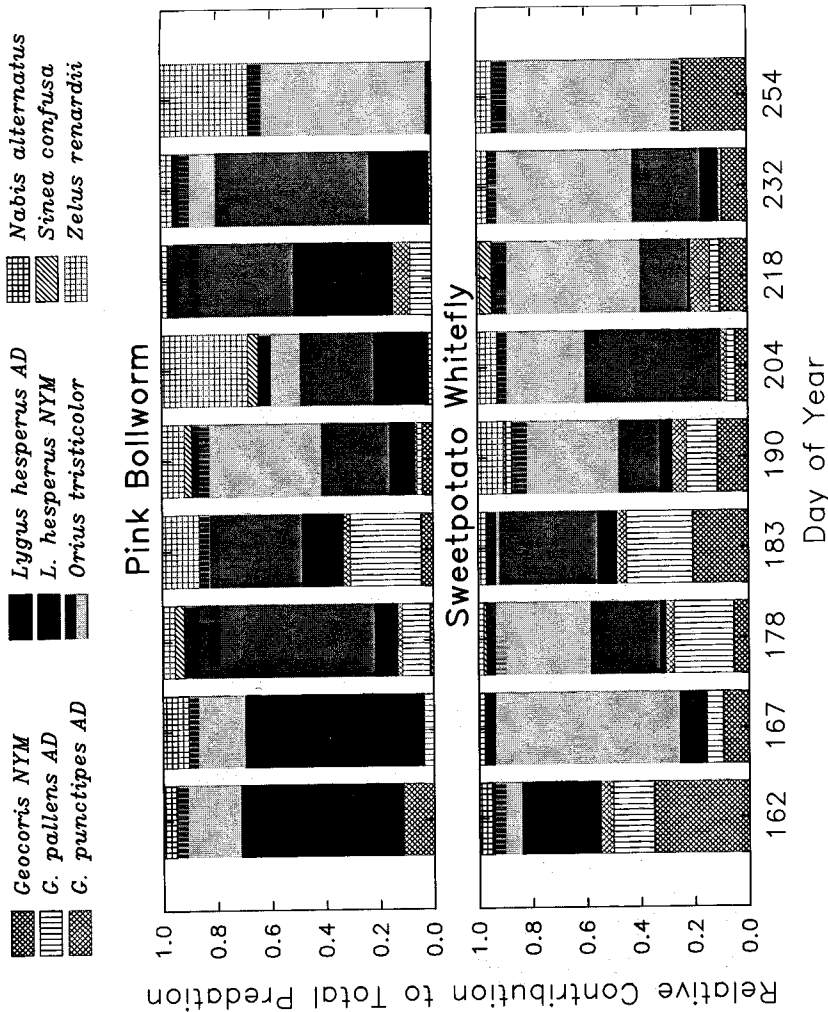
Total Predator–Days per Plant

Predator Complex — 1991 1992

Pink Bollworm Predation — 1991 / 1992

Sweetpotato Whitefly Predation — 1991 / 1992

Legend: Geocoris NYM · G. pallens AD · G. punctipes AD · Lygus hesperus AD · L. hesperus NYM · Orius tristicolor · Nabis alternatus · Sinea confusa · Zelus renardii

Fig. 6. Cumulative season-long abundance of 7 species of heteropteran predators (bar graph) and their contribution to consumption of pink bollworm and sweetpotato whitefly eggs and adult females in 1991 and 1992 (pie graphs). Data based on integration of results presented in Figs. 4 and 5.

whitefly in cotton (Hagler and Naranjo 1994b). It is well known that many heteropteran predators will feed phytophagously (Naranjo and Gibson 1996). It is also known that some pest heteropterans also may function as predators in some agricultural systems (McMullen and Jong 1970, Agnew et al. 1982, Cleveland 1987). The predatory nature of *L. hesperus* and other lygus bugs has been noted previously. For instance, *L. hesperus* was observed feeding on *G. punctipes* nymphs in the laboratory (Dumbar and Bacon 1972), and it is equally capable of using either plant or animal food to complete development (Bryan et al. 1976). Thus, it was not unexpected that *L. hesperus* was found to feed on eggs of pink bollworm and sweetpotato whitefly. Yet, the dominant contribution of this "predator" was surprising and may force us to reexamine the beneficial–negative role of this insect in the cotton ecosystem.

Quantifying Predation. The analyses we have presented so far enabled us to gauge the relative roles played by each member of the heteropteran

Pink Bollworm

Sweetpotato Whitefly

Axis: Relative Contribution to Total Predation

Day of Year — 162 167 178 183 190 204 218 232 254

Legend: Geocoris NYM · G. pallens AD · G. punctipes AD · Lygus hesperus AD · L. hesperus NYM · Orius tristicolor · Nabis alternatus · Sinea confusa · Zelus renardii

Fig. 5. Relative contribution of 7 species of heteropteran predators to consumption of pink bollworm and sweetpotato whitefly eggs and adult females over course of season in 1992. Contribution of each species or developmental stage calculated using Ragsdale's predation index (see Table 2). Density of predators and proportion of positive responses were derived from pooled estimates over 3 field sites in Maricopa County, AZ.

directly the result of seasonal predator population densities (Fig. 6, right). For example, in 1991 *O. tristicolor* and *L. hesperus* constituted ≈76% of all heteropteran predators examined but were responsible for >95 and 90% of the heteropteran predation on pink bollworm and sweetpotato whitefly, respectively. Thus, not only were these 2 predators most abundant, but they also were more frequent predators of the eggs or adult females (or both) of these 2 pest species. A similar, but more proportionate, pattern was true for results from 1992.

One of the more interesting results of our studies was the identification of *L. hesperus* as a significant predator of pink bollworm and sweetpotato
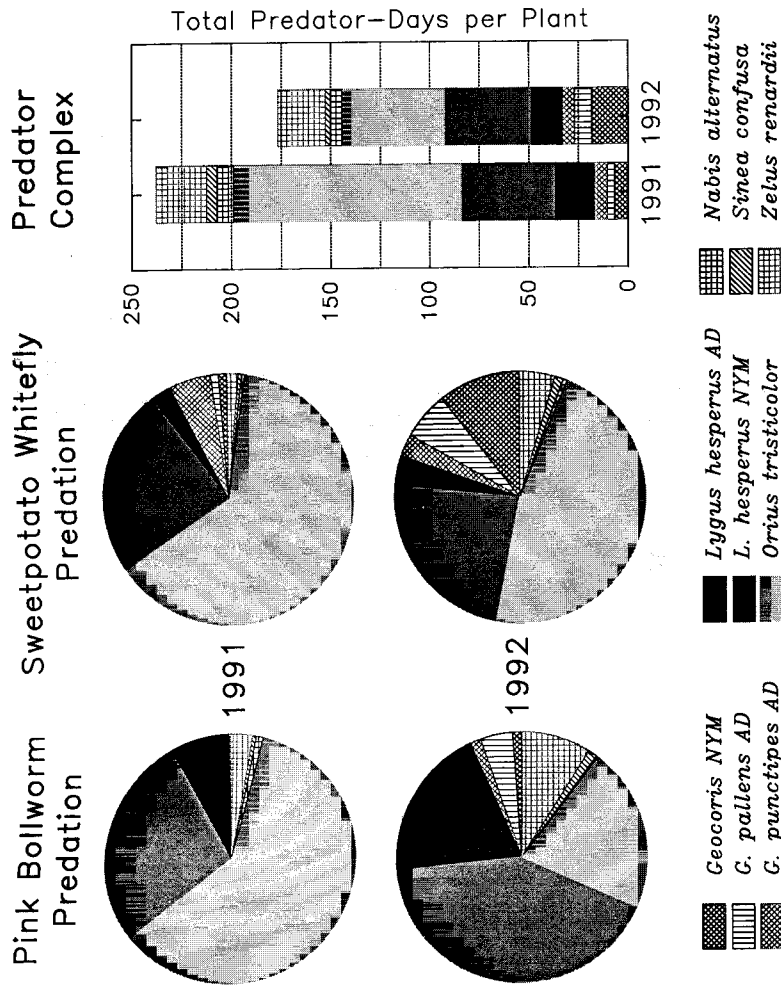
complex, but they fail to provide any quantitative measure of their effect. The quantification of predation requires an estimate of the number of prey eaten by each predator reacting positively in our ELISA. It is reasonable to assume that prey consumption is a function of prey density $T(M)$, where M is prey density and T is a mathematical expression representing the functional response. As a starting point we used the functional response model of O'Neil and Stimac (1988) given as

$$N_a = M[C_1 \exp(-C_2 M) + C_3], \quad (1)$$

where N_a is the per capita prey consumed, M is prey density (measured as number of prey per square centimeter of leaf area), C_1 is the maximum leaf area searched per predator above C_3; when $M = 0$, C_2 is the rate of decline in area searched as M increases, and C_3 is the minimum, asymptotic amount of leaf area searched at high prey density. This functional response has been used successfully to model predation by generalist predators of several soybean pests (O'Neil and Stimac 1988, Wiedenmann and O'Neil 1992). Incorporating equation 1 into our predation index (No. 7, Table 2) gives the daily rate of predation (r) by predator i as

$$r_i = (p_i P_i / D_i) M(C_1 \exp[-C_2 M] + C_3), \quad (2)$$

where p_i is the proportion of predators of species i showing positive by ELISA, P_i is the density of predator species i, and D_i is the period of time (in days) over which the antigen can be detected in the predator's gut after an initial feeding. We assumed D = 1 for the purpose of analyses presented here. Finally, equation 2 is constrained by the assumption that N_a must be at least 1 if a predator tests positive for prey antigen. Because of our experiences with the feeding behavior of *G. punctipes* and *O. tristicolor* in the laboratory we feel this is a reasonable assumption. These predators almost always consume the contents of 1 egg before moving to another, even if large numbers of eggs are provided.

We conducted a series of greenhouse experiments to estimate parameters of the functional response model (equation 1) for 2 representative heteropteran predators, *O. tristicolor* and *G. punctipes* preying on pink bollworm eggs (unpublished data). Briefly, individual predators were allowed to forage for 24 h on caged cotton plants of varying sizes containing variable numbers of pink bollworm eggs that were attached individually to the undersides of leaves (see Naranjo and Stimac 1987 for methods). The predators were obtained from laboratory cultures reared on a mixture of eggs of pink bollworm, cabbage looper, *Trichoplusia ni* (Hübner), and beet armyworm, *Spodoptera exigua* (Hübner), with a green bean added for moisture. We used adults 5–10 d old that had been starved for 24 h before testing. The number of eggs attacked and the amount of leaf area were recorded for each caged plant. Prey densities

(eggs per square centimeter of leaf area) varied from ≈0.001 to 0.15. The low end of this range was representative of densities of pink bollworm eggs estimated during 1991 and 1992.

We used estimates of predator and prey densities and ELISA results from 1992 to calculate the extent of predation on pink bollworm eggs. Because it was likely that positive responses for the assassin bugs *S. confusa* and *Z. renardii* resulted from feeding on adult female moths, we did not include these predators in the analysis. Data from our greenhouse studies indicated that the parameters of the functional response model (equation 1) differed between the 2 species that we examined, *G. punctipes* and *O. tristicolor* (unpublished data). However, when we used field estimates of prey density (pink bollworm eggs per square centimeter of leaf area), the functional response models for both species consistently predicted that <1 egg would be attacked per day. Thus, because we assumed that a positive ELISA response denoted consumption of at least 1 egg, our predation model collapsed to the simplified model of Dempster (1960) for the atypically low densities of pink bollworm eggs found in 1992 (and also in 1991). Based on the further assumption that the functional response behaviors exemplified by *G. punctipes* and *O. tristicolor* are representative of other heteropteran predators, we used this simplified model for the 5 predator species.

The effect of the heteropteran predator complex varied considerably between dates, and we estimated that the complex was responsible for attacking 18.9% of all pink bollworm eggs throughout the season (Fig. 7). Although the overall level of predation was relatively low, it was estimated to be relatively high on several individual dates. For instance, predators attacked ≈50% of the pink bollworm eggs present on 26 June (DOY = 178) and 8 July (DOY = 190). Pink bollworm populations did not reach economically significant levels at our field sites in 1992, but further analyses would be necessary to gauge the level of control provided by the heteropteran complex. Our results do suggest that these predators are capable of locating and attacking this prey at extremely low densities. Prey densities were typically <0.001 prey per square centimeter and never exceeded 0.005 in 1992. This former density is equivalent to a predator finding 1 egg in >1,000 square centimeters of leaf area. Further, these predators continued to attack pink bollworm eggs later in the season despite extremely high population densities of all stages of the sweetpotato whitefly.

Summary and Future Research

Predation remains one of the more difficult ecological processes to study, but one critical to understand if we are to use predators effectively in agricultural systems. No single method appears to provide accurate estimates of predation in the field. However, by combining several approaches, we may arrive at more realistic estimates of a predator's role. The serological analysis
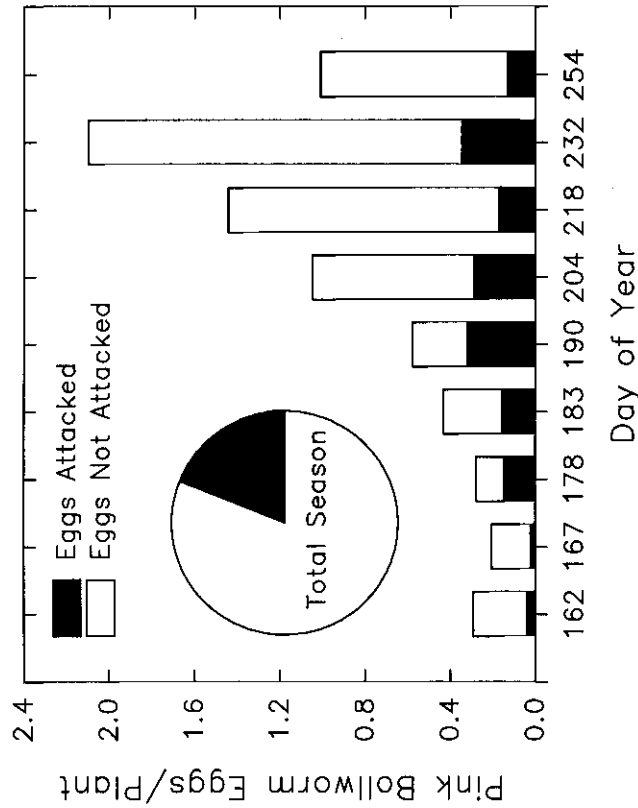
Fig. 7. Preliminary estimation of number of pink bollworm eggs attacked by the heteropteran predator complex in 1992. Results are based on synthesis of predator and prey densities, ELISA results, and predator behavior measured through functional response to prey density. Results did not include the 2 species of assassin bugs, *S. confusa* and *Z. renardii*, which likely fed only on adult female moths.

of predator gut contents is one of the few methods available that requires only minimal manipulation of the predator–prey system under study. Use of monoclonal antibodies further eliminates problems associated with species specificity characteristic of polyclonal antisera. Additional study, however, is necessary to assign a quantitative value to a predator found positive for prey antigens.

We have demonstrated an approach that couples serology, measurement of functional response behavior under realistic levels of prey density, and predator–prey population dynamics to estimate the effect of the heteropteran complex in cotton on eggs of the pink bollworm. None of the techniques alone would have yielded such information. The use of a functional response model alone likely would lead to overestimation of predation because each individual predator is assumed to behave similarly without regard to alternate prey or other factors that might dilute predation on the target prey. Indirectly, results from serological analysis integrate many of these complex factors in the field by identifying only those individuals in the population that actually fed on the target prey. For instance, if we had assumed that each predator

followed the functional response model without regard to the presence of pink bollworm antigen in their guts, our estimate of season-long impact would have been 46% of all eggs consumed rather than 19% (see Fig. 7). These 2 estimates represent alternative hypotheses that could be tested further through careful life table studies of pink bollworm population dynamics.

Many factors need additional study to refine estimates of predation in the field. These factors include more accurate definition of the time period during which prey antigen can be detected in the predator's gut in relation to temperature, subsequent meals of different prey, predator species, and the circadian rhythm of feeding activity. Also, the extent of false positives from predators feeding on other predators or feeding on dead prey needs to be defined. Finally, given the results of our functional response studies to date, it appears that 1 generic functional response model may be insufficient. Thus, the behavior of each predator needs to be examined separately. Many of these issues are currently under study in our laboratory.

Acknowledgments

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