Using gut content immunoassays to evaluate predaceous biological control agents: a case study

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ABSTRACT

Pest-specific monoclonal antibodies (MAbs) can be used to identify key predaceous biological control agents. A MAb library was used to (i) identify key indigenous predators of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) and pink bollworm, *Pectinophora gossypiella* (Saunders) and (ii) monitor the efficacy of an augmentative predator release targeted for these two pests.

The gut contents of over 10,000 individual predators indigenous to Arizona cotton fields were examined for the presence of whitefly and pink bollworm egg antigens by enzyme-linked immunosorbent assay (ELISA). Five predator species with frequent positive assays for whitefly proteins were (% positive): *Collops vittatus* (Say) (55%), *Geocoris* spp. nymphs (39%), *Orius tristicolor* (White) (38%) and *Hippodamia convergens* Guerin-Meneville (33%). The most frequent pink bollworm egg predator was *Lygus hesperus* Knight (adults 30%, nymphs 20%). *L. hesperus* was also the predator species with the highest percentage of individuals scoring positive for both pest egg antigens (12%).

In a separate experiment, we compared the efficacy of commercially reared *Orius insidiosus* (Say) against indigenous populations of *O. tristicolor*. The commercially reared predators were released into field cages, recaptured 36 hours later and examined by ELISA for the presence of whitefly and pink bollworm egg antigens. Some 4% of the released *O. insidiosus* and 26% of the indigenous *O. tristicolor* had whitefly egg antigen in their guts. Pink bollworm egg antigen
was found in the guts of 8% of the *O. insidiosus* and 5% of the *O. tristicolor*.

The immunologically based techniques described in this review can expedite the search for key indigenous predator species and also serve as a tool for monitoring the efficacy of augmentative biological control agents. The advantages and limitations of our techniques are discussed.

### 16.1 INTRODUCTION

Despite the heavy use of pesticides over the past half century, crop losses have almost doubled due to damage by insects (Wilson, 1990). With increasing concerns about the over-use of pesticides, growers are seeking more environmentally benign methods for controlling insect pests. One such method receiving renewed attention includes using predaceous natural enemies in an integrated pest management programme (Stern *et al.*, 1959). While it is accepted that predators are generally important in controlling pest populations, it is difficult to assess the ecological impact of specific predator species in cropping systems. The accurate identification of key entomophagous insects in any given system has been virtually impossible for several reasons: observers of predator–prey interactions disrupt the normal foraging process; predators, unlike parasites, rarely leave evidence of attack; and both predators and prey are often small, elusive and nocturnal.

Historically, many experimental methods have been employed for measuring and analysing predator–prey interactions. Laboratory and glasshouse experiments have been used to evaluate predator–prey interactions (e.g. Orphanides *et al.*, 1971; Henneberry and Clayton, 1985; Hagler and Cohen, 1991); however, these types of studies seldom translate to actual field situations. The microscopic examination of predator gut contents (James, 1961) is labour-intensive and unreliable, and unusable for predator species that liquefy prey contents before consumption (Hengeveld, 1980). Indirect gut analysis, including the use of radioactive markers (Baldwin *et al.*, 1955; Jenkins, 1963; McDaniel and Sterling, 1979; McCarty *et al.*, 1980) for tagging potential prey and electrophoresis (Murray and Solomon, 1978) have also been used, but such techniques either pose potential dangers to users and the environment, are too time-consuming, or do not possess the necessary specificity and sensitivity (Lister *et al.*, 1987; Sunderland, 1987; Luck *et al.*, 1988). These difficulties have resulted in a dearth of information on the impact that predators have on suppressing key insect pest populations.

Perhaps the most promising techniques for measuring predation are immunologically based tests employing pest-specific monoclonal antibodies (MAbs) (Whitten and Oakshott, 1990). Immunological methods of studying predation avoid many of the pitfalls inherent with the more traditional methods of study (Brooke and Proske, 1946; West, 1950; Boreham and Ohiagu, 1978; Miller, 1979). Insect pest antigens can stimulate an immunological response in a vertebrate which culminates in the production of serum polyclonal antibodies to the insect antigen. Once the antibodies have been produced in the vertebrate, they can be harvested and used serologically to probe predator gut contents. Unfortunately, most polyclonal antibodies tend to cross-react with other insect species (Davies, 1969; Lund and Turpin, 1977; Miller, 1981; Gardner *et al.*, 1981; Doane *et al.*, 1985). Cross-reactivity is unacceptable when working with an insect complex as diverse as that found in cotton. However, with advances in hybridoma technology, investigators now can isolate individual antibody-producing cells grown *in vitro* and harvest antibodies of single specificity (Köhler and Milstein, 1975). The results are monoclonal antibodies (MAbs) that offer specificity unachievable with conventional polyclonal antisera (Lenz and Greenstone, 1988). Furthermore, once the MAbs have been developed, the immunological assays employed are simple, rapid and economical.

Given all of the positive attributes of using MAb-based gut content immunoassays to investigate predator–prey interactions, it is somewhat surprising that very few investigators have actually adapted this technology to field research. This is due, in part, to the fact that the development of pest-specific MAbs is costly, time-consuming, and requires expertise in hybridoma technology. However, as more researchers from a wide variety of disciplines incorporate MAbs into their research, the techniques will probably become more refined and less expensive (Liddell and Symondson, 1996, this volume). Private industry has begun to develop MAbs on a contract basis. As the competition stiffens, the cost of developing pest-specific MAbs will decrease. Researchers interested in developing gut content immunoassays should be fully aware of the benefits and limitations of this qualitative technology (Sunderland, 1996, this volume).

The purpose of this chapter is to provide an example of how we have incorporated MAb technology into applied research aimed at identifying potential key predator species of the egg stage of the sweetpotato whitefly, *Bemisia tabaci* (*Gennadius*) (strain B) and pink bollworm, *Pectinophora gossypiella* (Saunders) in a cotton agroecosystem. The first part of the chapter summarizes the techniques that we have used to mass-screen indigenous predator species for the presence of whitefly and pink bollworm prey remains in the gut. Here, we summarize our research findings and discuss how these pest-specific MAbs have aided our efforts to identify key predators. The second part of the chapter presents preliminary data on how we have used our MAbs to assess
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the effectiveness of augmentative releases of the minute pirate bug, Orius insidiosus (Say) on these two pests.

16.2 SURVEY OF INDIGENOUS PREDATORS OF PINK BOLLWORM AND SWEETPOTATO WHITEFLY

16.2.1 Introduction

Dozens of insect species are common to cotton fields in the southwestern United States. Of these, many are potential biological control candidates. Due to the difficulties mentioned above, it is not feasible to investigate all of them by traditional methods (i.e. bioassays and behavioural experiments). However, with our pest-specific MAbs, we can quickly screen indigenous predators for the presence of prey remains in their guts. Our major emphasis here is to demonstrate how we utilized pest-specific MAbs to characterize the native predator complex on two economically important cotton pests, the pink bollworm and sweetpotato whitefly.

16.2.2 Methods

(a) Predator sampling

Indigenous predators were collected from 7 June to 6 September, 1992 from experimental cotton fields located on two, 2-ha sites at The University of Arizona’s Maricopa Agricultural Research Center, Maricopa, Arizona, and one, 0.5-ha site at The Western Cotton Research Laboratory, Phoenix, Arizona.

Predators were collected at weekly and bi-weekly intervals using a modified vacuum sampler described by Ellington et al. (1984). A randomly selected, continuous 30-m row of cotton was sampled in each of four quadrants per site. The contents from each vacuum sample were immediately placed on dry ice and transported to the laboratory where they were frozen at −80°C. Many of the predators collected in the vacuum samples were potentially contaminated with whitefly antigen (i.e. adult females) during the sampling process. To eliminate any possibility of external contamination each predator was cleaned by removing externally attached whiteflies and then irrigated with PBS. This procedure was effective in cleansing any extraneous whitefly debris (unpublished data).

On several sampling dates, additional predators were collected and kept alive for use as negative controls. The predators were fed cabbage looper, Tricoplusia ni (Hübner) larvae and water ad libitum for several days to ensure that any potential whitefly or pink bollworm antigens were eliminated from their guts (Hagler and Cohen, 1990). The negative controls were macerated in 250 μl phosphate-buffered saline (PBS) (pH 7.4) and assayed against pink bollworm and whitefly MAbs by the ELISAs described below. The mean (±SD) absorbance values were 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.

(b) ELISAs

The indirect ELISA procedure described originally by Voller et al. (1976) was modified by Hagler et al. (1992) and Hagler and Naranjo (1994a,b). Whole bodies of individual field-collected predators were ground in 250 μl of PBS. A 50 μl aliquot of each of the macerated insects was pipetted into individual wells of two uncoated 96-well ELISA plates (Figure 16.1). 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% non-fat dry milk in distilled water was added to each well for 30 min at 37°C. The non-fat dry 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 (Figure 16.1). Plates were incubated for 1 hour at 37°C, after which the MAbs were discarded and the wells were briefly rinsed three times with PBS-Tween 20 (0.05%) and twice with PBS. Aliquots (50 μl) of goat anti-mouse IgG/IgM conjugated to alkaline phosphatase (TAGO Inc., Burlingame, CA) diluted to 1:500 in 1% non-fat milk were added to each well of both plates. Plates were incubated for 1 hour after which the conjugated antibody was discarded, and the wells were rinsed as noted above. A 50-μl aliquot of p-nitrophenylphosphate (pNPP) substrate (Sigma, St Louis, MO) was added to each well for 1 hour and the absorbance at 405 nm of each well was measured with a Cambridge Technology Model 750 (Waterton, MA) microplate reader. Field-collected predators were scored positive for 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).

16.2.3 Results of the indigenous predator survey

The gut contents of over 10 000 individual predators representing nine of the most common predator species found in the cotton agroecosys-
tem for the presence of whitefly and pink bollworm egg antigen were screened (Table 16.1). Almost one-third (28.9%) of the individuals contained whitefly egg antigen, a high proportion which was not surprising. Whitefly egg densities ranged from <1 egg per leaf early in the season to >5000 eggs per leaf late in the season (Naranjo and Hagler, in press). The population of individual species of predators scoring positive for whitefly egg antigen ranged from 4.0% for *Nabis* spp. (primarily *N. alternatus* Parshley) to 55.0% for *Collops vittatus* (Say) (Hagler and Naranjo, 1994a,b).

Pink bollworm egg antigen was not detected in as many predators as whitefly egg antigen, with only 12.8% of the individuals screened scoring positive (Table 16.1). This relatively low proportion of individuals is probably due to low pink bollworm egg densities in our fields,

![Diagram](image-url)

**Figure 16.1** A diagrammatic representation of the steps in an enzyme-linked immunosorbent assay (ELISA): 1, Wells are coated with insect antigen; 2, wells are blocked with non-fat dry milk; 3, sweetpotato whitefly or pink bollworm egg-specific monoclonal antibody is added; 4, wells are washed and an enzyme-labelled secondary antibody is added; 5, wells are washed and an enzyme-specific substrate is added; and 6, absorbance is measured with an ELISA microplate reader. PBS, phosphate-buffered saline.
values ranging from 0.3 eggs per plant early in the season to only 1.8 eggs per plant late in the season (Naranjo and Hagler, in press). The proportion of individual species of predators scoring positive for pink bollworm egg antigen ranged from 0.7% for *Nabis* spp. (primarily *N. alternatus*) to 30.1% for adult *Lygus hesperus* Knight. Interestingly, although *L. hesperus* is considered a major pest, over one-quarter of the *L. hesperus* population surveyed scored positive for pink bollworm egg antigen. The correlation between *Lygus'* phytophagous feeding behaviour and their high predatory activity on pink bollworm eggs might explain why so many individuals scored positive for pink bollworm eggs, even when pest densities were low. *Lygus* feed on meristematic and reproductive tissues of the cotton plant, the same areas of the plant where pink bollworms oviposit (Brazzel and Martin, 1957; Henneberry and Clayton, 1982). These sites may increase the probability of discovery of pink bollworm eggs by foraging *Lygus* (Hagler and Naranjo, 1994b).

Overall, a relatively small proportion (7.1%) of the predators examined had preyed on both pest species. 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 16.1).

### 16.3 Examining the Efficacy of Augmentative Releases of *Orius insidiosus*

#### 16.3.1 Introduction

Despite the lack of predator efficacy data in the scientific literature, many producers of biological control agents promote predaceous insects as 'an environmental-friendly alternative to chemical control'. Currently, there are over 132 companies in North America alone selling beneficial organisms (i.e. predators and parasites) for augmentative biological control use (Hunter, 1994). Although probably environmentally safe (see Harris, 1990 for a review), these biological agents might be serving only as a placebo to the end-user. Hoy *et al.* (1991) suggests that action must be taken soon to evaluate these organisms before they are sold to consumers on a wide scale. Studies are needed to evaluate both the efficacy of augmentative releases and the quality of these 'domesticated' predators (Roush, 1990). If predators are improperly used or their effectiveness is misrepresented to the public, the biological control industry could suffer a loss of consumer confidence. Efficacy and quality issues need to be examined under realistic field conditions.

The effectiveness of two pirate bug species was assessed, indigenous *Orius tristicolor* (White) and mass-released *O. insidiosus*, using our pest-specific MAbs and multiple ELISAs. *O. insidiosus* was selected for this study because previous studies had indicated that a member of this genus frequently preyed on whitefly and pink bollworm (Table 16.1) and is readily available from producers of beneficials. Although the data set presented here is preliminary, it should give the reader an idea of how pest-specific MAbs can be incorporated into a programme designed to monitor the efficacy and quality of mass-produced biological control agents.

#### 16.3.2 Methods

**Predators**

Adult *O. insidiosus* were purchased from Arbico (Oracle, Arizona) and released into 1.83 x 1.83 m field cages located at The University of Arizona's Maricopa Research Station; 50, 100 or 250 *O. insidiosus* were released per cage. A fourth cage contained a no-release control (i.e. indigenous *O. tristicolor* only). These releases were made throughout the season on 28 June, 19 July and 26 July, 1993. Predators were released at approximately 8:00 p.m. on each date and allowed to forage within each cage for 36 hours, after which each cage was vacuumed as described above. Within minutes after collection the predators were frozen in dry ice and transported to the laboratory where they were assayed by ELISA for the presence of whitefly and pink bollworm egg antigens (Figure 16.1).

#### 16.3.3 Results from the augmentative release

Very few minute pirate bugs were collected in the vacuum sampler on each of the sampling dates. This was due, in part, to the relatively low release rates and the difficulty in finding these small predators. Because the recovery rates were so low for both the released and indigenous populations it was difficult to measure any treatment effects over time. To obtain an estimate of the proportion of individuals scoring positive for whitefly and pink bollworm throughout the entire season all of the vacuum samples were pooled. ELISA results from this experiment showed that 4.0% of the released *O. insidiosus* (*n* = 215) and 26.0% of the indigenous *O. tristicolor* (*n* = 133) had whitefly egg antigen in their guts. Pink bollworm egg antigen was found in the guts of 8.0% of the *O. insidiosus* (*n* = 215) and 5.0% of the *O. tristicolor* (*n* = 133). Overall, a relatively small proportion of the *Orius* examined had preyed on both pest species. Only 4.0% of the mass-released *O. insidiosus* (*n* = 215) and none (*n* = 133) of the indigenous *O. tristicolor* scored positive for the presence of both whitefly and pink bollworm egg antigens.
16.4 DISCUSSION

Pest-specific MAbs can be used to identify key indigenous predator species and to monitor the efficacy of introduced (augmented) biological control agents. *Collops vitatus*, *Hippodamia convergens* Guerin-Meneville, *O. tristicolor* and *Geocoris* spp. were the most active native predator species, particularly on whitefly. An interesting result from the indigenous predator survey was that *L. hesperus*, considered a major cotton pest, was also a significant predator of pink bollworm and whitefly (Hagler and Naranjo, 1994b). While the predatory nature of *L. hesperus* has been reported previously (Dunbar and Bacon, 1972), it was surprising that such a high percentage of these predators scored positive for both pests.

Once the key predators were identified, the research was focused on evaluating the efficacy of mass releases of *O. insidiosus*. Although *O. tristicolor* was ordered from the distributor of the beneficials, *O. insidiosus* was actually received. Consequently, the experiment was conducted using *O. insidiosus*.

Caged experiments revealed that the indigenous *O. tristicolor* populations were more frequent predators on whitefly eggs than the commercially reared *O. insidiosus*. However, the augmented predators had a slightly greater percentage of individuals scoring positive for pink bollworm than the indigenous predators. Unfortunately, because of the misidentification of these predators by the distributor it was impossible to separate differences due to a species effect (i.e. *O. insidiosus* versus *O. tristicolor*) from that due to an origin effect (i.e. mass-produced versus indigenous predators). The experiment still provides an example of how a pest-specific MAb library can be used to monitor (i.e. quality control) the efficacy of augmentative biological control agents. Clearly, a more thorough investigation is needed before any conclusions are made on the feasibility of using *Orius* spp. as augmentative biological control agents.

16.5 LIMITATIONS OF PREDATOR GUT CONTENT IMMUNOASSAYS

While gut content immunoassays are useful for studying predator–prey interactions it would be remiss if their limitations were not discussed. Potential pitfalls common with gut content immunoassays 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, scavenger feeding, or predators that ingest enough prey antigens to score positive in the immunoassay but fail to kill their prey. Any of these interactions would lead to an over-estimation of predation (Breene and Sterling, 1988). An additional limitation that especially hinders the widespread use of MAbs is the high degree of technical knowledge and specialized facilities needed to produce useful antibodies. Moreover, MAbs are targeted for pest egg antigens; therefore, 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 surveyed. This uncontrollable variable must be considered when interpreting results.

The most important limitation of using gut content immunoassays (and other indirect methods) to study predation is that the immunoassays are not quantitative. Antigen detection methods, such as the ELISA, dot-blot or radial immunodiffusion provide quantitative results in that strength of response is directly proportional to the concentration of antigen. However, translating these quantitative readings into an estimate of prey consumed is complicated by several interacting and uncontrollable factors (McIver, 1981; Naranjo and Hagler, in press; Greenstone, 1996, this volume; Sunderland, 1996, this volume). The interaction of the size of a predator's meal and the subsequent digestive rate are key factors to the strength of an immunoassay response. Thus, without additional knowledge of either the size of the meal or the elapsed time since ingestion, a given immunoassay response could translate to a small meal eaten recently or a large meal eaten several hours earlier. Moreover, if a predator continually consumes additional prey items after an initial meal the same assay response could have many interpretations depending on when the predator was assayed relative to its first meal. An additional issue is the effect of temperature on the rate of digestion (Fichter and Stephen, 1981, 1984; Sopp and Sunderland, 1989; Hagler and Cohen, 1990). Again, a single assay response could have many interpretations depending on the ambient temperature preceding the assay. Prey age (i.e. prey phenology) can also affect the quantitative outcome of gut content immunoassays. ELISAs are five times more responsive to freshly oviposited *L. hesperus* eggs than they are to 7-day-old eggs (Hagler et al., 1992).

A factor that can also affect the quantitative and qualitative response of gut content immunoassays is the variable digestive rates exhibited by different predator species. Symondson and Liddell (1993) have shown that two closely related carabid beetles have vastly different detection intervals after feeding on the same prey species in the laboratory. We have similar data suggesting that pink bollworm egg antigen can on average be detected for only 1 hour in *H. convergens* and for 48 hours in *O. insidiosus* (J.R. Hagler et al., in preparation).
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Given all of the drawbacks and limitations of gut content immunoassays, they remain valuable tools for conducting field research. The results from such assays when combined with field (predator and prey population densities) and laboratory (feeding experiments) studies can yield more accurate qualitative estimates of predation. Sopp et al. (1992) provide an excellent summary of some of the predator efficiency indices (PEIs) developed over the past 35 years. All of these PEIs are based on key assumptions that might be useful in certain situations. For instance, many such indices assume that a positive ELISA response is the result of a single prey consumed (Dempster, 1960; Boreham and Ohiagu, 1978; Ragsdale et al., 1981). These indices are more applicable if food resources are limited. Rothschild (1966) developed a PEI based on prey consumption under laboratory conditions, though this index is more applicable when prey populations are abundant and feeding is continuous. Another index has been suggested by Nakamura and Nakamura (1977) and Greenstone (1979) in which prey consumption is estimated as the zero term of a poisson distribution. Sopp et al. (1992) provide the only PEI that attempts to use the quantitative relationship between an ELISA response and the number of prey consumed in the laboratory. We have proposed a new PEI that is a modification of previously published PEIs, the major difference being that it incorporates prey consumption rates measured under more realistic conditions (i.e. greenhouse plant arenas rather than Petri dishes in the laboratory), takes into account the detection interval as a function of fluctuating field temperatures, and incorporates predator functional response behaviour (predator feeding as a function of prey density) (Naranjo and Hagler, in press). Surprisingly, none of the previously published PEIs has accounted for a predator's functional response.

16.6 SUMMARY

In summary, gut content immunoassays combined with field, laboratory and greenhouse studies can lead to better qualitative assessments of predation. However, uncontrollable variables inherent to gut content immunoassays of field-collected predators make precise quantitative assessments impossible. Biotic factors such as predator–prey phenology, number of prey eaten and prior metabolic status can all affect the outcome of an ELISA (McIver, 1981; Fichter and Stephen, 1981, 1984; Lovei et al., 1985, Sopp and Sunderland, 1989; Hagler et al., 1992). Furthermore, differential prey digestive rates of each predator species must be examined thoroughly in the laboratory before reliable qualitative assessments of predation can be made (Lovei et al., 1990; Symonds and Liddell, 1993; J.R. Hagler et al., in preparation).

In this review, an attempt has been made to present a realistic view of the benefits and drawbacks of using pest-specific MAbs to study predator feeding behaviour. Whether the advantages of using pest-specific MAbs to study insect predator–prey interactions outweigh the disadvantages is ultimately dependent on the researchers' needs. Data gathered using pest-specific MAbs can lead to a more effective utilization of predators in a biological control programme because it tests a predator's effect under natural and undisturbed field conditions. Pest-specific MAbs provide quick, efficient and cost-effective tools to identify qualitatively the effects that key predators have on key insect pests. Additionally, this technology can facilitate the stewardship of mass-produced predators into viable and trustworthy biological control programmes.

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REFERENCES


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