Measuring the Sensitivity of an Indirect Predator Gut Content ELISA: Detectability of Prey Remains in Relation to Predator Species, Temperature, Time, and Meal Size

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The gut contents of three species of insect predators that were fed either a variable or a fixed number of pink bollworm eggs but held at variable time and temperature regimes were assayed by an indirect enzyme-linked immunosorbent assay (ELISA). The sensitivity and efficacy of the monoclonal antibody-based ELISA was dependent on the predator species examined. Small predators were more immunoresponsive to the ELISA than large predators. Furthermore, the assay sensitivity was dependent on the number of prey consumed, elapsed time after feeding, and temperature at which the predators were held. The smaller predator species retained recognizable traces of prey remains for longer periods than larger predator species. The ELISA efficacy decreased with increasing ambient temperature. A series of regression equations have been developed to estimate the median detection interval of prey in a predator's gut that takes into account the predator species examined, the quantity of prey consumed, and ambient after-meal temperature. © 1997 Academic Press

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INTRODUCTION

One of the most important, yet least understood, aspects of insect biological control is the role that predators play in suppressing pest populations. Because many predators and their prey are too small and elusive to observe in nature, researchers have resorted to indirect methods for studying predation (Sunder-land, 1987). Indirect methods include microscopic (James, 1961), electrophoretic (Murray and Solomon, 1978), and immunological gut content analyses (Dempster, 1960; Boreham and Ohiagu, 1978). Microscopic gut content analyses are easy and affordable, but are ineffective for most predators because the prey is liquefied or chewed into tiny unrecognizable pieces (Miles, 1972; Pollard, 1990). Electrophoretic gut content analyses are sensitive but laborious and sometimes difficult to interpret or nonspecific (Giller, 1982a, 1982b, 1986; Fitzgerald *et al.,* 1986; Solomon *et al.,* 1996). Immunological gut content analyses used with a pest-specific monoclonal antibody (MAb) are both sensitive and specific. Unfortunately, pest-specific gut content immunoassays have not been readily adapted by researchers because the development of a pest-specific MAb is costly and time consuming and requires technical expertise (Greenstone, 1996). As a consequence, very little information exists on the interspecies sensitivity and detection period of gut content immunoassays using pest-specific MAbs.

Once the difficulties of development are overcome, MAbs can be incorporated into simple, rapid, inexpensive, and sensitive gut content immunoassays (Lenz and Greenstone, 1988; Greenstone and Morgan, 1989; Hagler *et al.,* 1992; Symondson and Liddell, 1993a). We have developed an indirect enzyme-linked immunosorbent assay (ELISA) which employs a species- and stage-specific MAb for examining predators of the pink bollworm, *Pectinophora gossypiella* (Saunders), eggs (Hagler *et al.,* 1994). This ELISA has been useful for identifying the frequency with which predator species feed on pink bollworm eggs in the field (Hagler and Naranjo, 1994a,b; 1996). However, preliminary studies in our laboratory, as well as previous studies (Sunderland *et al.,* 1987; Sopp and Sunderland, 1989; Symondson and Liddell, 1993b), suggest that gut content ELISAs vary in efficacy among predator species.

Differences in immunoreactivity can be attributed to

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a combination of confounding abiotic and biotic factors. Time elapsed after feeding, temperature, predator digestion rate, prey size, predator size, and the physiological state of a predator and prey can all affect the outcome of a gut content immunoassay (McIver, 1981; Fichter and Stephen, 1981; Lovei *et al.,* 1985; Hagler and Cohen, 1990; Hagler *et al.,* 1992; Sunderland, 1996). Before a precise estimate of predation can be made, these factors must be considered. One of the fundamental parameters for qualitatively or quantitatively estimating predation using immunoassays is the period of time that prey antigens remain detectable in a predator's gut. The detection interval is a key parameter in most indices that have been developed to assess predation using immunoassays (reviewed by Sopp *et al.,* 1992; Naranjo and Hagler, 1997) and is very important in comparative evaluations of different predator species feeding on the same prey.

In this study, we examined some of the variables that can affect the outcome of a gut content ELISA on the insidious pirate bug, *Orius insidiosus* (Say), big-eyed bug, *Geocoris punctipes* (Say), and convergent lady beetle, *Hippodamia convergens* Guerin-Meneville. We selected these three predator species because they are known to frequently prey on pink bollworm eggs (Hagler and Naranjo, 1994a,b) and they represent small (*O. insidiosus*), medium (*G. punctipes*), and large (*H. convergens*) predator species. In this paper, we examine what effect meal size, time, and temperature have on prey retention in the guts of three predator species using an indirect ELISA developed to detect pink bollworm egg antigen (prey) in a predator's gut.

MATERIALS AND METHODS

Predators. Adult *O. insidiosus* and *G. punctipes* originated from laboratory cultures maintained at our facility. Adult *H. convergens* were collected from a cotton field located near Phoenix, Arizona. Predators were maintained in an environmental chamber set at 27°C, 50% RH, and a photoperiod of 14:10 h (L:D). All predators were fed beet armyworm, *Spodoptera exigua* (Hübner), eggs, cabbage looper, *Trichoplusia ni* (Hübner), eggs, and green bean *ad lib.* for a minimum of 1 week prior to the feeding trials.

Effect of meal size on prey detection interval. Predators were deprived of prey for 48 h prior to testing. After starving, individual predators were put into a petri dish and allowed to consume 1, 3, 6, or 10 freshly deposited (<1 day old) pink bollworm eggs. Predators were then placed in an environmental chamber (25°C, 50% RH) on the rearing diet described above. In the first trial, we examined only *G. punctipes.* Predators were removed from the environmental chamber at 0, 12, 24, 48, or 72 h after feeding on the pink bollworm eggs and immediately frozen at −80°C. In the second

trial, we examined all three species. Because of the low ELISA responses for *G. punctipes* held more than 24 h in the first trial, we removed predators from the environmental chamber at 0, 12, or 24 h after feeding. Additionally, the largest meal for *O. insidiosus* was 6 eggs because this species was not capable of eating more during a single feeding bout. Each predator was homogenized in 500 µl of phosphate-buffered saline (PBS) and assayed for pink bollworm egg remains by the indirect ELISA described by Hagler *et al.* (1994). Each treatment included predators that were not fed pink bollworm eggs (negative controls). Individual predators were scored positive for the presence of pink bollworm egg remains if the ELISA absorbance value was three standard deviations above the value of the respective negative control mean (Sutula *et al.,* 1986; Schoof *et al.,* 1986).

Negative exponential or linear equations were fitted to describe the decay in the proportion of positive response with time after feeding for each predator species and meal size (SAS Institute, 1990). These models were solved in reverse to estimate the time interval associated with 75, 50, and 25% positive responses.

Effect of temperature on prey detection interval. Again, predators were starved for 48 h before testing. After starving, individuals were put in a petri dish and allowed to consume 5 freshly deposited (<1 day old) pink bollworm eggs. Individuals were then placed in environmental chambers set at 15, 20, 25, 30, or 35 ± 1°C and fed the rearing diet described above. In the first trial, we again examined only *G. punctipes.* Predators were removed from the environmental chambers at 24, 48, or 72 h after feeding on the pink bollworm eggs and immediately frozen at −80°C. In the second trial, we examined *O. insidiosus* and *G. punctipes.* Because *H. convergens* responded poorly in the meal size (prey size) experiment, they were omitted from this study. Predators were removed from the environmental chambers at 12, 24, 48, or 72 h after feeding. Each individual was homogenized in 500 µl of PBS and assayed for the pink bollworm egg antigen by the indirect ELISA described by Hagler *et al.* (1994).

We fitted negative exponential equations to describe the decay in the proportion of positive responses with time after feeding for each predator species and holding temperature (SAS Institute, 1990). These models were solved in reverse to estimate the time interval associated with 75, 50, and 25% positive responses.

RESULTS AND DISCUSSION

Effect of meal size on prey detection interval. The indirect gut content ELISA varied in efficacy for each of the predator species examined. All *O. insidiosus* assayed by ELISA immediately after eating (0 h) 1, 3, or 6

pink bollworm eggs contained easily recognizable traces of egg antigen in their guts (Fig. 1A). The strength of the ELISA response depended on the number of eggs consumed by *O. insidiosis.* The proportion of positive responses usually decreased as holding time after feeding increased. Regardless of the number of prey eaten, most of the *O. insidiosus* examined still contained recognizable quantities of prey remains in their gut 12 h after eating pink bollworm eggs, but not 24 h after feeding (Fig. 1A). The decline in the proportion of positive responses was relatively linear over a 24 h period for *O. insidiosus* feeding on 1 or 3 eggs (Fig. 1A). After feeding on 6 eggs, all the individuals tested remained positive after 12 h; however, there was a rapid decline in the response from 12 h to 24 h after feeding. The median detection interval for detection of prey (the time since feeding in which 50% of the individuals were scored positive by ELISA) was 13.5, 17.1, and 18.0 h for *O. insidiosus* that had eaten 1, 3, and 6 eggs, respectively (Table 1).

Most of the *G. punctipes* that were examined by ELISA immediately after eating pink bollworm eggs contained identifiable quantities of egg antigen in their guts. The ELISA absorbance value was positively correlated with the number of eggs ingested. Although only 57% of the individuals that ate a single egg reacted to the ELISA, almost all of those that ate 3 (89%) or 6 (97%) eggs scored positive, and all individuals eating 10 eggs scored positive at 0 h (Fig. 1B). The proportion of positive responses decreased as the holding time after feeding increased. None of the *G. punctipes* that ate a single egg had any detectable quantities of prey remains in their gut 12 h after eating (Fig. 1B). Only 70 to 75% of the *G. punctipes* that ate 6 or 10 eggs and assayed 12 h after feeding responded to the ELISA. The ELISA was ineffective at detecting pink bollworm egg remains in *G. punctipes* beyond the 12-h holding time, no matter how many prey were eaten (Fig. 1B). Pink bollworm egg antigen was detectable in very few of the *G. punctipes* examined 24 h after feeding (Fig. 1B). Data from both trials were pooled to examine the decay in positive responses over time. Because of the rapid decline in the proportion of positive responses, particularly with fewer than 3 eggs consumed, we used a double exponential model ($y = Ae^{-bx} + Ce^{-dx}$) to estimate the decay relationship over a 72-h period for
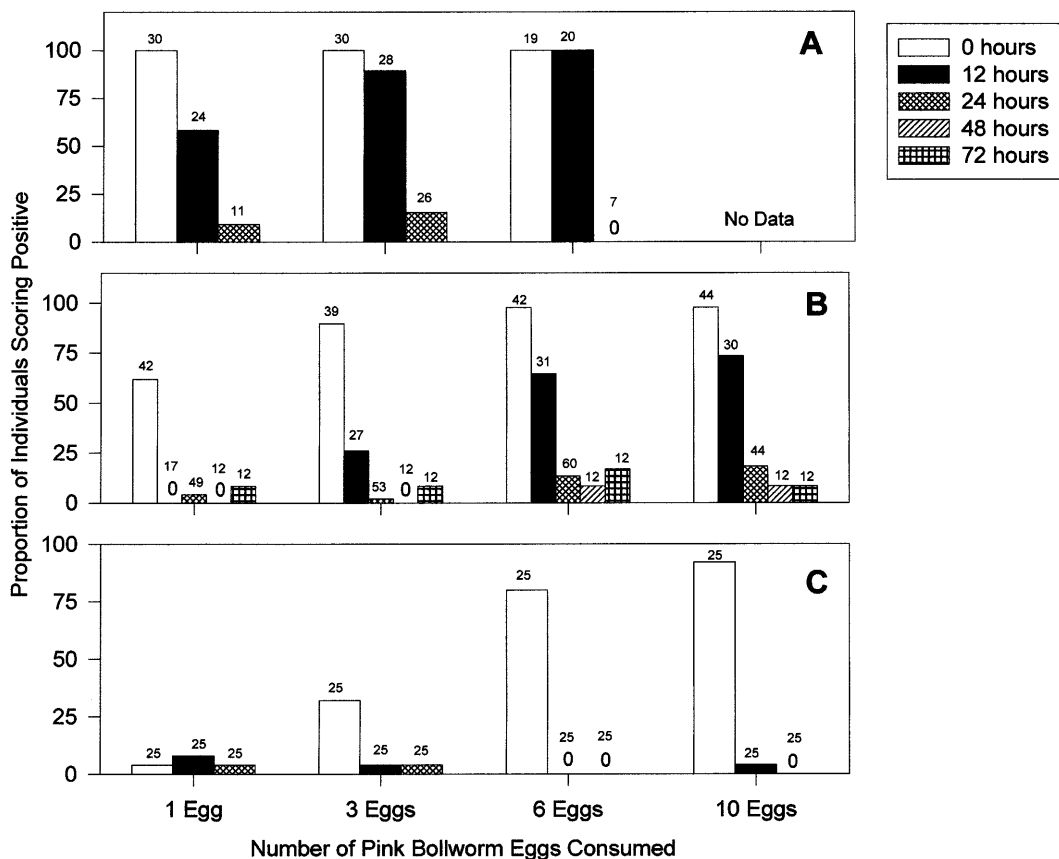

FIG. 1. Proportion of (A) *Orius insidiosus,* (B) *Geocoris punctipes,* and (C) *Hippodamia convergens* scoring positive for *Pectinophora gossypiella* egg remains 0, 12, 24, 48, or 72 h at 25°C after eating 1, 3, 6, or 10 eggs. The regression equation that predicts the prey detection intervals for each prey size is given in Table 1. The numbers above the bars represent the numbers of insects assayed by indirect ELISA for each treatment.

TABLE 1

Prey Detectibility (% Positive) over Time at 25°C in Three Different Predator Species after Consuming 1, 3, 6, or 10 Pink Bollworm Eggs

Species	Eggs eaten	Detection interval (hours)[a] at indicated % positive			Equation	r^2
		75%	50%	25%		
Orius insidiosus	1	6.9	13.5	20.1	$y = 1.012 - 0.038x$	0.99
	3	10.0	17.1	24.1	$y = 1.105 - 0.035x$	0.85
	6	15.0	18.0	21.0	$y = 1.000$[b] (for $x \leq 12$) $y = 2.000 - 0.083x$ (for $x > 12$)	—
	—	—	—	—	—	—
Geocoris punctipes	1	—	0.9	4.1	$y = 0.508 \exp^{(-0.287x)} + 0.114 \exp^{(-0.044x)}$	0.99
	3	1.5	5.5	12.4	$y = 0.858 \exp^{(-0.106x)} + 0.020 \exp^{(-0.0096x)}$	0.99
	6	3.9	9.8	20.9	$y = 0.900 \exp^{(-0.080x)} + 0.100 \exp^{(-0.010x)}$	0.94
	10	6.2	15.3	30.8	$y = 0.800 \exp^{(-0.051x)} + 0.198 \exp^{(-0.029x)}$	0.98
Hippodamia convergens	1	—	—	—	—	—
	3	—	—	2.6	$y = 0.32 \exp^{(-0.173x)}$[c]	—
	6	0.2	1.3	3.2	$y = 0.80 \exp^{(-0.365x)}$[c]	—
	10	0.8	2.3	5.0	$y = 0.92 \exp^{(-0.261x)}$[c]	—

[a] The length of time elapsed that prey can be detected in 75, 50, and 25% of the predators tested, respectively.

[b] Equation fit by assuming a piecewise linear function.

[c] Equation fit by assuming a simple exponential decay from 0 to 12 h.

G. punctipes (Fig. 2B). This relationship suggests that there is a rapid initial decay of detectable prey proteins within the first 24 h followed by a slower, more gradual decay up to 72 h after feeding. The median prey detection intervals were 0.9, 5.5, 9.8, and 15.3 h for *G. punctipes* that had eaten 1, 3, 6, and 10 eggs, respectively (Table 1).

The indirect ELISA was unreliable for detecting pink bollworm egg remains in the guts of *H. convergens*. Only those individuals examined immediately after eating 6 or 10 eggs responded with any consistency to the ELISA (Fig. 1C). Virtually all of the *H. convergens*

assayed 12 and 24 h after feeding yielded ELISA absorbance values similar to their negative control counterparts (Fig. 1C). *H. convergens* showed a rapid decline in the proportion of positive responses for all meals of three or more eggs; however, too few observations were available to fit a double exponential. Instead, we assumed a simple exponential decay between 0 and 12 h ($y = Ae^{-bx}$), set A = the proportion positive at 0 h, and estimated the parameter b as $(\ln P_0 - \ln P_{12})/12$, where P_0 and P_{12} are the proportion positive at 0 and 12 h, respectively (Fig. 2C). The median prey detection interval was only 1.3 and 2.3 h for *H. convergens* that had eaten 6 or 10 eggs, respectively (Table 1).

The results from this study showed that the sensitivity and the retention time of the indirect ELISA depended on the predator species, quantity of prey consumed, and length of time elapsed after feeding. The more eggs eaten by a given predator species the greater the probability of obtaining a positive ELISA reaction. Additionally, the median detection interval increased as the predator's meal size increased.

Effect of temperature on prey detectability interval. *O. insidiosus* held at relatively low temperature (≤25°C) after feeding on five pink bollworm eggs were more immunoreactive than individuals held at higher temperature (35°C) (Fig. 2A). Most *O. insidiosus* held at <25°C for 12 h after feeding had egg antigen detectable in their gut. Over half of the *O. insidiosus* held at low temperature (≤25°C) 24 h after feeding scored positive for pink bollworm prey remains. The detectability of prey remains decreased sharply for *O. insidiosus* held at ≥30°C after feeding. Of the *O. insidiosus* held for 12, 24, 48, and 72 h after feeding, almost all at 30°C and all at 35°C failed to respond to the ELISA. There was a rapid decline in the proportion of positive responses by this species at ≥30°C, but a more moderate decline between 15 and 25°C (Fig. 2B). We used a double exponential equation (see above) to model the decay relationship at all temperature regimes over a 72-h period. In general, the rate of decay increased with increasing temperature. The median prey detection intervals for *O. insidiosus* that consumed 5 eggs were 38.3, 26.2, 26.5, 4.5, and 1.8 h for those individuals held at 15, 20, 25, 30, and 35°C after feeding, respectively (Table 2).

Only about half of the *G. punctipes* exposed to low temperature (≤25°C) 12 h after feeding on five pink bollworm eggs responded to the ELISA (Fig. 2B). Prey remains were detectable in almost none of the *G. punctipes* held at 35°C 12 or more h after feeding. Again we used a double exponential decay equation to model the rapid decay in the proportion of positive response over a 72-h period (Fig. 2A). As with *O. insidiosus,* there was a general trend for a higher decay rate at higher temperature. The median prey detection intervals for *G. punctipes* that consumed five eggs were 5.5, 4.4, 4.4, 2.1, and
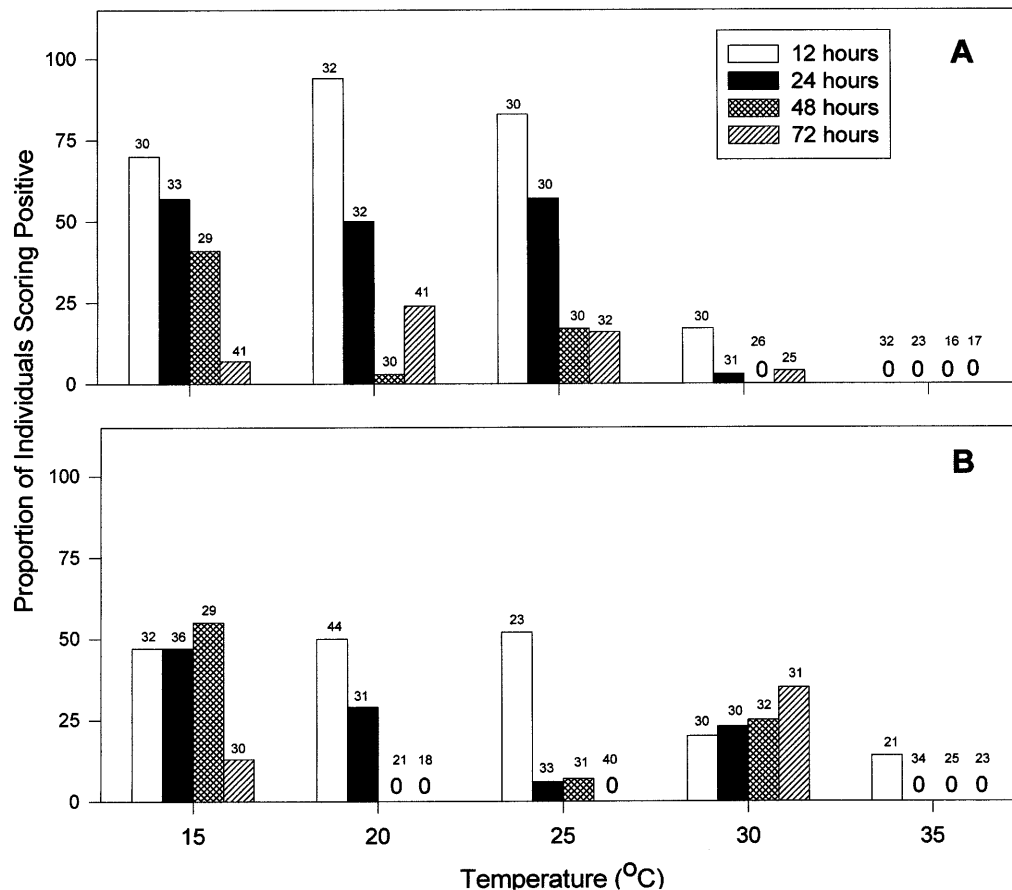
FIG. 2. Proportion of (A) *O. insidiosus* and (B) *G. punctipes* scoring positive for *P. gossypiella* egg remains after being held at variable postmeal temperatures 12, 24, 48, or 72 h after eating five eggs. The regression equation that predicts the prey detection interval for each temperature is given in Table 2. The numbers above the bars represent the numbers of insects assayed by indirect ELISA for each treatment.

1.3 h when held at 15, 20, 25, 30, and 35°C after feeding, respectively (Table 2).

The results from this study showed that the sensitivity and the retention time of the indirect ELISA depended on the predator species tested, the environmental temperature after feeding, and the time elapsed after feeding. The proportion of positive responses for prey remains and the median detection interval of prey decreased as temperature increased. Quantitative predator gut content ELISAs as a function of time and temperature have been documented before. Some of the studies which employed polyclonal antibodies also showed considerable species variation in predator gut content immunoassays (Sunderland *et al.,* 1987; Sopp and Sunderland, 1989). Most of the studies attributed interspecies differences in prey detection to variable metabolic rate as a function of time and temperature (e.g., Fichter and Stephen, 1984; Sopp *et al.,* 1992; Greenstone and Hunt, 1993). Sopp and Sunderland (1989) found that high temperature adversely affected the sensitivity of their predator gut content ELISA. Furthermore, prey retention time was less for staphyliniid (Insecta: Staphylinidae) predators than for carabid

(Insecta: Carabidae) or linyphiid (Arachnida: Linyphiidae) predators. Results from our study suggest that there is a very rapid decline in prey detection interval at a temperature above 30°C. In Arizona, a maximum daily field temperature above 40°C is common; however, the mean daily temperature rarely exceeds 33°C. Still, even at the more moderate temperature, egg remains may be detectable in predator guts for only a short period of time. Initially, we were concerned that a short prey detection interval would limit the application of gut content immunoassays in field situations. However, a relatively short detection interval may have some advantages. A long prey detection interval (e.g., 1 to 2 days) may improve the chances that a given predator will be found positive for prey antigen, but it also may decrease the chances of discerning patterns of predation. For example, a predator could have consumed a small meal immediately before collection, consumed a large meal many hours before collection, or consumed many small meals over time (Naranjo and Hagler, 1997). Furthermore, the probability of errors in estimating daily frequency or rate of predation increases because these values must be discounted over

TABLE 2

Prey Detectibility (% Positive) over Time at Variable Temperatures in Two Different Predator Species after Consuming Five Pink Bollworm Eggs

Species	Temperature (°C)	Detection interval (hours)[a] at indicated % positive			Equation	r^2
		75%	50%	25%		
Orius insidiosus	15	11.5	28.3	57.0	$y = 0.347 \exp^{(-0.024x)} + 0.644 \exp^{(-0.024x)}$	0.94
	20	12.6	26.2	49.6	$y = 0.290 \exp^{(-0.029x)} + 0.701 \exp^{(-0.029x)}$	0.92
	25	11.9	26.5	51.2	$y = 0.501 \exp^{(-0.028x)} + 0.498 \exp^{(-0.028x)}$	0.96
	30	1.9	4.5	9.2	$y = 0.977 \exp^{(-0.159x)} + 0.024 \exp^{(-0.003x)}$	0.99
	35	0.7	1.8	3.6	$y = 1.00 \exp^{(-0.384x)}$ [b]	—
Geocoris punctipes	15	5.5	17.5	61.9	$y = 0.466 \exp^{(-0.100x)} + 0.514 \exp^{(-0.012x)}$	0.92
	20	4.4	12.2	32.0	$y = 0.544 \exp^{(-0.099x)} + 0.425 \exp^{(-0.020x)}$	0.99
	25	4.4	10.8	21.2	$y = 1.899 \exp^{(-0.054x)} - 0.906 \exp^{(-0.043x)}$	0.98
	30	2.1	5.7	16.4	$y = 0.782 \exp^{(-0.167x)} + 0.199 \exp^{(-0.0001x)}$	0.99
	35	1.3	3.4	7.4	$y = 0.879 \exp^{(-0.230x)} + 0.101 \exp^{(-0.014x)}$	0.99

[a] The length of time that prey can be detected in 75, 50, and 25% of the predators tested, respectively.

[b] Equation fit by assuming a simple exponential decay from 0 to 12 h.

the detection interval. Thus, one could arrive at the same estimate of predation for a predator eating one meal every 2 to 3 days and one eating continually over a 2- to 3-day period. A shorter prey detection interval (<24 h) would be associated with fewer possible interpretations of predation patterns and may improve estimates of predation because it would be more likely that a positive response would be associated with a single feeding episode. This may be counterbalanced to some degree by increased chances of missing "positive" predators because of a short detection interval. However, even this limitation could be minimized by examining the daily feeding patterns of specific predator species combined with well-timed field collections. Overall, the resolution of measuring predation will depend on a better understanding of the biological characteris-

tics of the predator/prey systems under examination (Dempster, 1960).

We found considerable species variation in the detection interval among the three species examined in these studies. The frequency of positive ELISA reactions and the retention time was greater for smaller predators than for larger predators (i.e., *O. insidiosus* > *G. punctipes* > *H. convergens*). Results presented here showed that the indirect ELISA is more effective at detecting egg antigen in small predators than in large predators. This is probably due to the inherent insensitivity of the indirect ELISA format when assaying large, whole body predators. In an indirect ELISA, the microplate matrix is first coated with an aliquot of a homogenized predator. All proteins, whether they are extraneous predator proteins or the targeted prey in the predator's gut, have an equal chance to bind to a limited number of competitive binding sites on the ELISA matrix. Therefore, prey consumed by small predators have a greater chance of attaching to the ELISA microplate matrix than prey consumed by large, protein-rich predators. In effect, the extraneous, nontarget proteins associated with large predators "block" the targeted prey proteins from binding onto the ELISA matrix. The net result is a higher frequency of false-negative reactions with large predators.

The indirect ELISA's inability to detect prey remains in large, whole body homogenized predators might be resolved in several ways. First, predator guts or crops can be dissected and only the dissected portions assayed. This would decrease the amount of extraneous, nontarget predator proteins present in the sample. Gut and crop dissections have been used before, primarily with large predator species that are relatively easy to dissect (Dempster, 1960; Sunderland *et al.*, 1987; Hagler and Cohen, 1990; Symondson and Liddell, 1995). However, most pink bollworm egg predators are too small and soft bodied to be easily dissected (Hagler and Naranjo, 1994a,b). Second, a dot blot assay can be substituted for the indirect ELISA. Dot blots, although similar to indirect ELISAs, are more sensitive due to a greater number of competitive binding sites on a nitrocellulose membrane than on an ELISA microplate matrix. However, the major drawback with using a dot blot assay is that the immunoreaction is difficult to measure (Hagler *et al.*, 1995). Finally, a sandwich ELISA can be substituted for the indirect ELISA. A sandwich ELISA is designed to "pull out" a rare antigen (i.e., minute quantity of prey remains in whole body homogenized predators) from a complex mixture (Greenstone, 1996). While the sandwich ELISA is more sensitive than the indirect ELISA, it has some drawbacks. The major drawback is that a conjugated pest-specific secondary antibody must be developed. Unfortunately, conjugating a pest-specific secondary antibody is laborious, expensive, and requires technical expertise. Fur-

thermore, much of the precious primary monoclonal antibody must be sacrificed to develop a conjugated secondary antibody. However, once these drawbacks are overcome, a sandwich ELISA might be the best immunoassay format for assaying large, whole body predators that consume minute quantities of prey.

In summary, uncontrollable factors such as variable predator digestive rates (Symondson and Liddell, 1993b), predator prey sizes (Sopp and Sunderland, 1989; Symondson and Liddell, 1996), temperature (McIver, 1981), predator metabolic status (Lovei *et al.,* 1990), and the developmental stage of the prey (Hagler *et al.,* 1992) can all effect the quantitative outcome of gut content immunoassays (for an excellent review see Sunderland, 1996). The data presented here suggest that there is a huge discrepency in the sensitivity of a gut content immunoassay developed to detect pink bollworm egg remains in whole body homogenized predators. The predator species examined, a predator's exposure temperature, the quantity of prey consumed, and postmeal time all affected the qualitative and quantitative outcome of the indirect ELISA. These variables make the accurate quantification of predation very difficult using immunoassay procedures. While gut content immunoassays offer a good method of qualitatively estimating predation, they alone can't provide researchers with a reliable quantitative estimate of predation. Gut content immunoassays, with all of their advantages and limitations must be combined with the other predator evaluation techniques (for a review of these methods see Sunderland, 1987; Luck *et al.,* 1988; Naranjo and Hagler, 1997) to improve quantitative estimates of predation.

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