

Toward the Quantification of Predation with Predator Gut Immunoassays: A New Approach Integrating Functional Response Behavior¹

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Immunological methods have been widely used to identify key predator species and qualitatively evaluate predation of target prey. However, despite the quantitative nature of many immunoassays, the translation to number of prey attacked has been problematic because of the many factors that confound interpretation of the strength of the immunoassay response. We developed a new predation model that couples the proportion of predators positive for prey remains determined by enzyme-linked immunosorbent assay (ELISA), predator density, and predator functional response to prey density for estimating total prey attacked. We used single cotton plant arenas in the greenhouse to develop functional response models for two generalist predators, *Geocoris punctipes* (Say) and *Orius insidiosus* (Say), preying on *Pectinophora gossypiella* (Saunders) eggs. The model was validated and compared with other immunologically based predation models in multiple plant/multiple predator arenas. Our predation model was relatively accurate in predicting the total number of prey attacked by both predator species and was a significant improvement over previous models that rely on simple assumptions regarding predator attack rates. The model also improves the predictive capacity of the functional response model alone by correcting for the number of predators actually consuming prey. Sensitivity analyses indicated that model performance was most sensitive to accurate measurement of input variables such as temperature and the proportion of individuals positive for prey antigens by ELISA and less sensitive to changes in estimates of prey density. Accurate estimation of the functional response parameters is also important, especially for the behavioral parameter defining the decline in plant leaf area searched with increases in prey density. Limi-

tations of the model and application to the field are discussed.

Key Words: *Pectinophora gossypiella*; *Geocoris punctipes*; *Orius insidiosus*; monoclonal antibody; ELISA; functional response; gut content analysis; predation model.

INTRODUCTION

Arthropod predators are widely recognized as important contributors to the suppression of pest populations in many agricultural systems (Whitcomb, 1980; Luff, 1983). Progress in quantifying predation in agricultural systems has been hampered by the difficulty of studying predation in the field. Unlike parasitism, evidence of predation is seldom preserved in the field and researchers must generally rely on indirect and often less precise measures of activity (Kiritani and Dempster, 1973; Grant and Shepard, 1985; Luck *et al.*, 1988; Sunderland, 1988; Naranjo and Hagler, 1998). Many other factors, such as small size, nocturnal activity, cryptic behavior, and pre-oral digestion, contribute to the difficulty of observing and measuring predation under natural conditions.

Of the many methods used for studying predation, postmortem approaches are among the most direct and least likely to introduce bias through unintentional experimental disruption (Luck *et al.*, 1988; Sunderland, 1988). Postmortem methods include gut dissection and chromatographic, electrophoretic, PCR, and immunological analysis of predator gut contents. For over 50 years, immunoassay has been used for the study of predation in a number of agricultural and nonagricultural systems (Boreham and Ohiagu, 1978; Miller, 1979; Sunderland, 1988; Greenstone, 1996) and its application continues to grow. Depending on the type of antibody and assay system used, immunological methods can be species or stage specific, highly sensitive, and rapid enough to facilitate screening of thou-

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TABLE 1

Models for Quantifying Predation Using Immunological Methods

Index	Reference
A. pP/D	(Dempster, 1960)
B. PpR/D	(Kuperstein, 1979)
C. PpR	(Rothschild, 1966)
D. $-\ln[1 - p]P/D$	(Nakamura and Nakamura, 1977; Greenstone, 1979)
E. $Q_o P/fD$	(Sopp <i>et al.</i> , 1992)
F. $pPR(M)/D(\theta)$	(New equation)

Note. p , proportion of predators showing positive by immunoassay; P , predator density; D , detection period or detection half-life (function of temperature θ); Q_o , prey biomass recovered; f , mean proportion of meal remaining; R , per capita predation rate measured in the laboratory or insectary; $R(M)$, per capita predation rate as a function of prey density (M).

sands of predators in a short period (Sunderland, 1988; Hagler and Naranjo, 1996; Greenstone, 1996).

Despite these characteristics, immunoassays remain a qualitative method that provides direct evidence of predation by specific species but rarely provides quantitative estimates of the number of prey killed. Commonly used immunoassays, such as the enzyme-linked immunosorbent assay (ELISA), are quantitative in the sense that the strength of the reaction is proportional to the amount of antigen in the sample. However, many factors cloud the interpretation of the assay response in terms of how many prey that an assayed predator has eaten (Sunderland, 1988, 1996; Naranjo and Hagler, 1998). Assuming that the predator fed directly on live prey (i.e., did not scavenge or feed as a secondary predator), the strength of the assay response is a function of several uncontrollable factors, including the number of prey consumed, predator species, predator digestive rates, temperature since feeding, 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; Hagler *et al.*, 1997; Hagler and Naranjo, 1997). The problem is exacerbated for predators that may consume many prey over the course of a day, continually resetting the gut antigen concentration curve (Naranjo and Hagler, 1998).

A number of models have been developed for quantifying predation with immunoassays (Table 1). Sunderland (1996) delineates approaches that measure consumption rate (amount of prey biomass consumed) from those that measure predation rate (number of prey consumed). Given the problems highlighted above, few researchers have attempted to use the strength of the assay response for quantifying the rate of predation. Sopp *et al.* (1992) tested the use of predator density and the quantitative relationship between

an ELISA response and the amount of prey biomass consumed by assuming that, on average, the predator's meal was half-digested at the time of assay. This model predicts prey biomass consumed but could be translated to estimate number of prey consumed with additional knowledge of biomass per prey and prey-size preference. Sunderland (1988) proposed a similar approach several years earlier. All other models ignore the strength of the immunoassay response per se and depend simply on the proportion of predators positive for prey antigens. The basic form of these models is

$$N_a = pPR/D, \quad [1]$$

where N_a is the total number of prey attacked, p is the proportion of predators positive for prey antigens, P is predator density, R is per capita predation rate, and D is the antigen detection interval. Dempster (1960) assumed that a positive serological response resulted from a single prey being consumed, Kuperstein (1979) and Rothschild (1966) derived per capita attack rates from simple laboratory and insectary studies, and Nakamura and Nakamura (1977) and Greenstone (1979) assumed per capita prey consumption to be equal to the zero term (proportion of predators negative for prey antigens) of the Poisson distribution. The detection interval corrects the proportion positive for the length of time that prey antigens remain detectable in a predator's gut and is variously defined as the maximum detection interval, the mean interval, or the half-life of detection (e.g., Sunderland, 1988; Greenstone and Hunt, 1993). This parameter is especially important for quantifying predation and for directly comparing different predator species with different digestive rates. Relatively few studies have tested the validity of these models (Dempster, 1960; Sopp *et al.*, 1992), even though several have been used to estimate predator impacts from immunological and electrophoretic data (e.g., Ashby, 1974; Sunderland and Sutton, 1980; Doane *et al.*, 1985; Lister *et al.*, 1987; Hagley and Allen, 1990).

Various components of these models, including optimization of assay methods (Stuart and Greenstone, 1990; Greenstone and Trowell, 1994; Hagler *et al.*, 1995; Hagler, 1998), improvement of statistical methods for determination of proportion positive (Fenlon and Sopp, 1991), and the influence of various environmental and biotic factors on antigen detection intervals (Fichter and Stephen, 1981; Lovei *et al.*, 1985, 1990; Sopp and Sunderland, 1989; Hagler and Cohen, 1990; Symondson and Liddell, 1993; Hagler and Naranjo, 1997; Hagler *et al.*, 1997), have been investigated. However, surprisingly little attention has focused on improvement of the prey consumption parameter, R . Although it is widely recognized that predation rates measured in the laboratory or insectary under highly

artificial conditions would poorly predict rates of predation in the field (e.g., O'Neil, 1989), several of the models in Table 1 rely on just such data. The assumption of random rates of predation (e.g., Poisson) may be reasonable in some instances but is unlikely to be representative of all predator-prey systems. We propose a new predation model that attempts to improve and generalize prediction of the predation rate by replacing R with a functional response model, the fundamental relationship between prey density and rates of prey consumption. We developed functional response models for two representative generalist predators commonly found in field crops in the United States, *Geocoris punctipes* (Say) and *Orius insidiosus* (Say), preying on *Pectinophora gossypiella* (Saunders) eggs in greenhouse plant arenas. We then test predictions of prey attack from our new predation model (Table 1, Eq. [F]) with independent data and compare these with predictions from other proposed models. Finally, we evaluate the sensitivity of our new model to changes in inputs and model parameters and discuss the application and limitations of the approach.

MATERIALS AND METHODS

Insect sources. Adult *G. punctipes* and *O. insidiosus* were obtained from our laboratory cultures reared on lepidopteran eggs and green beans. Predators were reared in environmental chambers maintained at 27°C, 50% RH with a 14:10 h (L:D) photophase. Predators used in experiments described here were reared on mixtures of *Spodoptera exigua* (Hübner) and *Trichoplusia ni* (Hübner) eggs and green beans *ad lib.* for a minimum of 1 week prior to testing.

Plant sources. Cotton, *Gossypium hirsutum* L. (cv. Deltapine 50), plants were grown in greenhouses in commercial potting soil (Gardeners World, Phoenix, AZ) and fertilized weekly (Grow More, Gardena, CA). Plants were maintained in a herbivore-free environment; however, plants used in experiments described below were carefully searched and cleaned prior to use to ensure that they contained only those prey intentionally introduced.

Functional response studies. Greenhouse experiments were conducted to estimate rates of prey attack by *G. punctipes* and *O. insidiosus* as functions of prey density (prey per cm² leaf area), using *P. gossypiella* eggs as prey. The leaf area of cotton plants used ranged in size from 630 to 6700 cm². A total of 10 eggs (<1 day old) were attached, individually, to the undersides of leaves of a single plant with the aid of a fine camel's hair paintbrush and an adhesive, Plantgard (Polymetrics International, New York, NY), diluted 1:10 in water. Preliminary studies indicated that the adhesive did not alter the normal feeding behaviors of either predator species. Eggs were distributed evenly (single egg per leaf in most cases) over the available leaf area

of the plant in an attempt to simulate the oviposition behavior of female *P. gossypiella* on vegetative cotton (Brazzel and Martin, 1957; Henneberry and Clayton, 1982). This combination of prey number and plant size resulted in prey densities ranging from about 0.0015 to 0.016 prey per cm², which are representative of natural densities of this pest (Naranjo and Hagler, 1998). An individual plant with prey eggs was then placed into a wood-framed, screen cage (104 × 106 × 68 cm deep) and one 5- to 10-day-old adult predator was introduced following a 24-h period of starvation. Predators were randomly selected from a culture with a natural 1:1 sex ratio. The predator was allowed to forage for 24 h after which the total number of prey eggs attacked was counted and recorded. Eggs preyed upon by these predators could be distinguished by their characteristic sunken and hollow appearance. The total leaf area of the plant (upper and lower surface) was then measured with an LI-3100 area meter (LI-COR, Lincoln, NE; measurement error <1%). To the degree possible, each prey density was replicated three to four times. A total of 83 and 36 individual plant arenas were observed for *G. punctipes* and *O. insidiosus*, respectively. Eight additional arenas were examined without predators during this time period as controls; no egg predation was observed. Greenhouse temperatures were maintained between 17 and 36°C (average 26°C) and were recorded continuously with shielded StowAway data loggers (Intermountain Environmental, Logan, UT) over the course of these studies.

Functional response model. The behaviorally based model developed by O'Neil and Stimac (1988) was used to describe the functional response of each predator species to prey density. The per capita attack rate, R , is given by

$$R(M) = M(C_1 \exp[-C_2 M] + C_3), \quad [2]$$

where M is prey density measured as the number of prey per cm² of leaf area, C_3 is the minimum area searched, C_1 is the maximum area searched above C_3 when $M = 0$, and C_2 describes the exponential decline in area searched as M increases. The model assumes that predators forage at random and do not use host or plant cues to restrict search patterns. The model is parameterized by relating total leaf area searched by the predator, S , to prey density

$$S = C_1 \exp[-C_2 M] + C_3, \quad [3]$$

where, S is the product of total leaf area and the proportion of prey attacked. Nonlinear least-squares regression (Procedure NLIN, SAS Institute, 1989) was used to estimate model parameters (C_i) for each predator species using Marquardt's method. Lack-of-fit

analysis (Seber and Wild, 1989) was conducted to further test the adequacy of the models.

New predation model. The predation model predicts predation rate and not biomass consumption rate (Sunderland, 1996) and expands on models developed by Dempster (1960), Kuperstein (1979), and Nakamura and Nakamura (1977). The new model is given as

$$N_a = pPR(M)/D(\theta), \quad [4]$$

where the per capita rate of predation, R , is a function of prey density, M , and the detection half-life, D , is a function of temperature, θ . P and p are as defined above for Eq. [1]. Substituting the functional response model (Eq. [2]) for $R(M)$ and rearranging gives

$$N_a = (pP/D[\theta])M(C_1 \exp[-C_2M] + C_3). \quad [5]$$

Results from Hagler and Naranjo (1997) were used to develop temperature-dependent models for the detection interval half-life for each predator species based on a logistic equation given by $A/(1 + \exp[-(\theta - X)/B])$, where A , B , and X were fitted with nonlinear regression (SAS Institute, 1989). Predators in that study were randomly selected from a culture with a natural 1:1 sex ratio and starved for 48 h before being fed five newly laid *P. gossypiella* eggs. Because detection intervals could be <24 h, the quantity $p/D(\theta)$ was constrained to a maximum of 1 to guard against cases in which large numbers of predators in an arena were positive for prey antigens.

Immunological methods. Predators from the model validation studies (described below) were assayed for the presence of *P. gossypiella* egg antigen using an indirect ELISA. Individual predators were homogenized in 250 μ l of Tris-buffered saline (TBS). A 50- μ l aliquot of each macerated predator was placed in an individual well of a 96-well assay plate (Falcon Pro-Bind 3915). Each plate was incubated at 4°C overnight. Following incubation, the insect macerates were discarded from each plate and a 360- μ l aliquot of 1% nonfat dry milk in distilled water was added to each well for 30 min at 27°C to block any unoccupied protein-binding sites in the wells. The nonfat milk was emptied from each plate and a 50- μ l aliquot of anti-*P. gossypiella* MAb was added to each well of the ELISA plate (Hagler *et al.*, 1994). The ELISA plates were then incubated for 1 h at 27°C. The contents from each plate were discarded and the plates were briefly rinsed three times with TBS-Tween 20 (0.05%) and two times with TBS. Goat anti-mouse IgG/IgM conjugated to alkaline phosphatase (BioSource International, Camarillo, CA) diluted (1:500) in 1.0% nonfat milk was added to each well (50 μ l) of the plates for 1 h at 27°C. Plate contents were discarded and rinsed as described above. A 50- μ l aliquot of *p*-nitrophenyl phosphate (1.0 mg/ml) sub-

strate was added to each well using the ingredients supplied in a Bio-Rad (Hercules, CA) substrate kit (No. 172-1063). For the 24-h validation studies (see below), the absorbance of each well was measured with a Molecular Devices Spectra MAX 250 (Sunnyvale, CA) microplate reader set at 405 nm after 1 h. For the 6-h validation studies, the plates were allowed to sit overnight before measuring the absorbance. This additional incubation was needed due to some degradation in the anti-*P. gossypiella* Mab during storage. Preliminary studies indicated no background color development and excellent separation of positive and negative controls during this longer incubation period.

G. punctipes or *O. insidiosus* known to contain no *P. gossypiella* egg antigen were assayed by the indirect ELISA described above for use as negative controls. Test predators were scored positive for the presence of pink bollworm egg remains if the absorbance value exceeded the mean negative control ($n = 8-16$) value by three standard deviations (Sutula *et al.*, 1986). The proportion of predators scoring positive for prey antigens was tallied for each validation plant arena (described below).

Predation model validation. To test the ability of the new model (Eq. [5]) to predict total attack rates, we conducted a series of greenhouse studies similar to those described for functional response model development. Twelve adult predators (5-10 days old) were starved for 24 h and then placed in a screen-cage containing six cotton plants. As before, predators were randomly selected from a culture with a natural 1:1 sex ratio. Predators were allowed to forage for 24 h. Each plant contained 10 *P. gossypiella* eggs. The total number of prey eggs attacked on all plants was counted and recorded, and plant leaf area (upper and lower surface) was estimated as before with an LI-3100 area meter (LI-COR). A range of plant sizes similar to that used in model development was tested, resulting in prey densities ranging from 0.0014 to 0.017 prey per cm^2 . Predators were collected and frozen immediately at -80°C until they could be assayed by ELISA. A total of 80 and 40 arenas were tested for *G. punctipes* and *O. insidiosus*, respectively. To maintain a reasonable resolution for the estimation of proportion positive for prey antigens, we eliminated arenas in which <10 predators were recaptured after 24 h. Thus, a total 71 and 26 arenas were used in analyses for *G. punctipes* and *O. insidiosus*, respectively. Six additional arenas were examined without predators during this time period as controls; no egg predation was observed. Greenhouse temperatures were maintained between 17 and 36°C (average 26°C) and were recorded continuously with shielded StowAway data loggers (Intermountain Environmental) in all validation experiments.

Because of the relatively short prey antigen detection half-life for *G. punctipes* (≈ 10 h at 25°C; Hagler

and Naranjo, 1997) we conducted a second set of tests with this species to evaluate model performance without the correction for detection half-life ($D(\theta)$ in Eq. [5]). The arenas and conditions for these tests were identical to those described above. However, predators were allowed to forage for only 6 h (0800–1400 MST) before they were collected and frozen. A total of 40 arenas were tested but only 37 (recapture of ≥ 10 predators) were used in analyses. Prey densities ranged from 0.0018 to 0.018 prey per cm^2 . Because the models predict daily attack rates it was necessary to determine the periodicity of predation by *G. punctipes* so that 6-h results could be properly calibrated. Individual adult predators 5–10 days old were starved for 24 h and then provided an excess of *P. gossypiella* eggs and a green bean in 9-cm petri dishes. Experiments were initiated at 0800 h and individuals were allowed to feed for 6 h. The sheet containing eggs was then removed and replaced with a fresh sheet and the same individual was allowed to feed another 18 h. The number of eggs preyed on was recorded for each time interval. Predators were held in environmental chambers maintained at 27°C and a 14:10 h (L:D) photophase with lights on at 0600 h. Experiments were replicated with 25 individuals of each sex.

Predicted attack rates were compared to observed attack rates for the new model and for the models of Dempster (1960) and Nakamura and Nakamura (1977). When $p/D(\theta) = 1$, the per capita attack rate for the Nakamura model was set to $-\ln(0.01)$. The Kuperstein (1979) model was not tested because per capita predation rates from the laboratory were at least an order of magnitude greater than those measured in the greenhouse functional response arenas. The Rothschild (1966) model also was not tested because it doesn't correct for the detection interval. To evaluate model performance, we regressed observed attack rates on predicted rates and used a simultaneous F test to identify significant departures from slope and intercept values of 1 and 0, respectively (Procedure REG, SAS Institute, 1989). This method has been shown to be very powerful in rejecting invalid models, even when the regression reveals no underlying relationship (Mayer *et al.*, 1994). Sensitivity analyses were performed on model inputs and parameters to evaluate the relative importance of various factors in predicting rates of prey attack.

RESULTS

Functional response. Results from our single predator/single plant arenas (Table 2) fit the O'Neil and Stimac (1988) functional response model (Eq. [2]) well for both *G. punctipes* and *O. insidiosus*. As prey density (prey per cm^2) increased, the amount of plant leaf area searched by the predator declined exponentially (Fig.

TABLE 2

Parameters of the O'Neil and Stimac (1988) Functional Response Model and the Detection Interval Model for Two Predator Species

Parameter	<i>G. punctipes</i>	<i>O. insidiosus</i>
Functional response		
C_1	2198.16 (0.00)	2299.92 (0.00)
C_2	415.63 (21.30)	459.98 (42.18)
C_3	88.31 (25.99)	95.73 (37.39)
r^2	0.91	0.80
n	83	36
Lack of fit F test _{df} (P)	0.062 _{17,63} (0.99)	0.041 _{9,24} (0.99)
Detection interval		
A	25.09 (12.38)	27.29 (1.16)
B	-7.88 (3.49)	-0.99 (0.46)
X	21.04 (8.69)	28.38 (0.82)
r^2	0.94	0.98
n	5	5

Note. Numbers in parentheses following parameter values are asymptotic standard errors.

1). As a result, daily per capita attack rates remain relatively stable between one and three prey consumed over a wide range of prey densities for both predator species (Fig. 2). Lack-of-fit analysis provided further support that the models adequately describe the relationship between prey density and predator search (Table 2). Because a mixture of female and male predators were used in our studies, we assume that the functional response models predict the average per capita attack rate for natural populations of each species.

Detection interval. Hagler and Naranjo (1997) found a strong temperature dependence in the prey antigen detection half-lives for *G. punctipes* and *O. insidiosus* feeding on *P. gossypiella* eggs. However, relationships with temperature differed in the two species (Fig. 3). The antigen detection interval declined nearly linearly from 15 to 35°C for *G. punctipes*. We chose to fit the logistic model because a linear model would have led to unrealistic, negative retention values at high temperatures. A different pattern was observed for *O. insidiosus*, with little change in the detection half-life at temperatures between 15 and 25°C and a precipitous decline at temperatures above 25°C. At average greenhouse temperatures for our studies, detection half-lives averaged about 10 and 26 h for *G. punctipes* and *O. insidiosus*, respectively. Model parameters for the detection interval are presented in Table 2.

Predation model validation. The proportion of predators positive for prey antigens (after correction for the detection half-life) varied widely in our plant arenas and held no relationship to prey density for either predator species (Fig. 4). However, coupling this

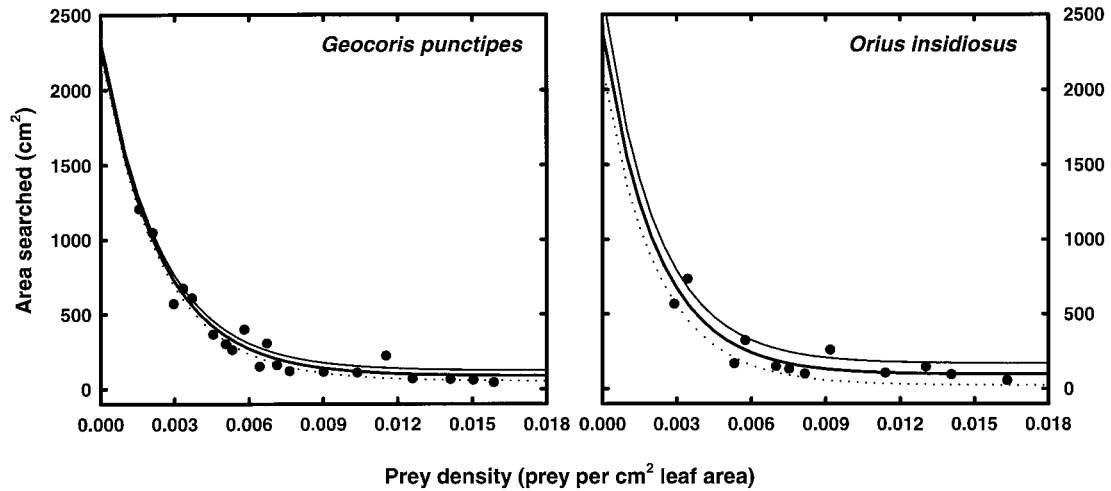


FIG. 1. Relationship between the amount of plant leaf area searched and prey density (prey per cm²) for two predator species preying on *P. gossypiella* eggs on cotton plants in the greenhouse. Each point represents the mean of three to four observations and the lines indicate the fit to the search model (Eq. [3]) and the 95% confidence intervals for mean prediction.

immunological component to the functional response model in the new predation model (Eq. [5]) resulted in accurate predictions of 24-h prey attack rates by *G. punctipes* (Fig. 5A). The simultaneous *F* test failed to reject the null hypothesis that the slope and intercept of the regression of observed on predicted attack rates are equal to 1 and 0, respectively (Table 3). The regression was highly significant and had a coefficient of determination of 0.74. The model was relatively poor at predicting small total rates of attack (≤ 4 total prey attacked). In many of these instances none of the predators scored positive for prey antigens despite the fact that some prey were attacked over the 24-h exposure period. These cases were not necessarily associated with low prey densities. For example, the average prey

density at which the model predicted 0 prey attacked was 0.0065 prey per cm² (see Fig. 4). Most likely, the few predators that attacked prey did so early in the 24-h exposure period, resulting in no detectable remains in their guts at the time of assay. The tendency of the model to underpredict predation at total attack rates greater than ≈ 20 arose primarily from underprediction by the functional response component of the model and not by the immunological component. That is, even when all predators were positive for prey antigens, the overall model underpredicted total predation. The immunological component is important because the simplifying assumption that all predators in an arena attacked prey at rates predicted by the functional response model leads to consistent overpredic-

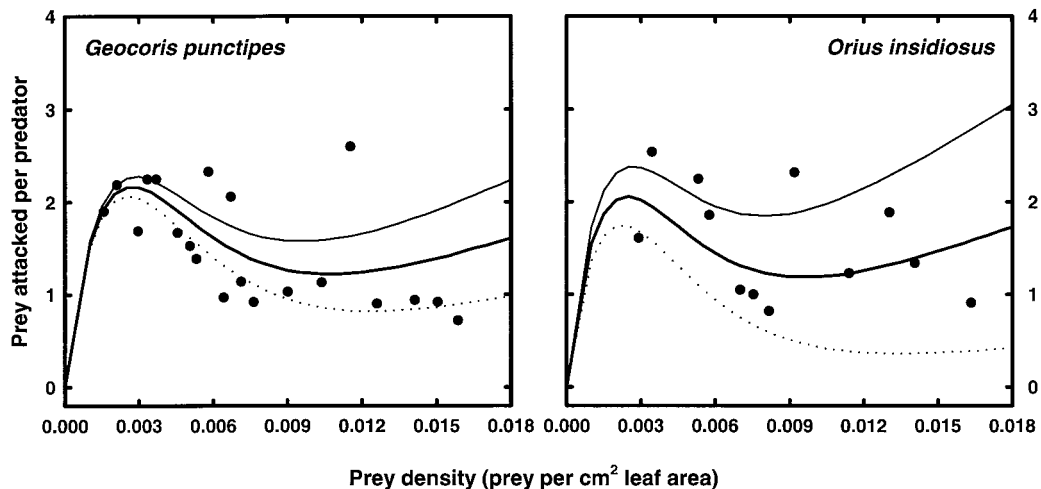


FIG. 2. Relationship between daily per capita attack rates and prey density (prey per cm²) for two predator species preying on *P. gossypiella* eggs on cotton plants in the greenhouse. Each point represents the mean of three to four observations and the lines indicate the functional response model (Eq. [2]) and the 95% confidence intervals for mean prediction derived from the data and model shown in Fig. 1.

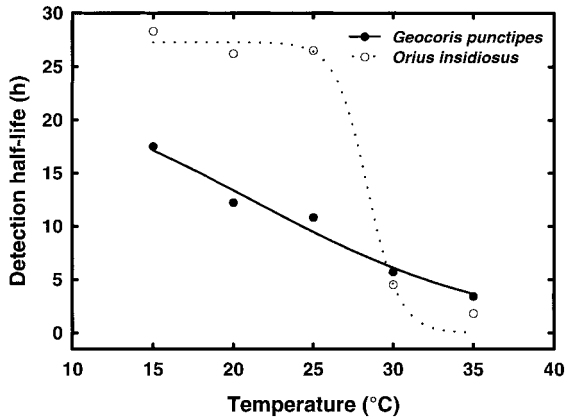


FIG. 3. Relationship between detection half-life of *P. gossypiella* egg antigens and temperature for two predator species. Data are from Hagler and Naranjo (1997) and the lines indicate fits to a logistic model.

tion of attack rates, especially at observed attack rates <20 (Fig. 5B, Table 3).

The Nakamura model greatly overpredicted prey attack rates, especially in instances when nearly all predators in an arena scored positive for prey antigens following correction for the detection interval (Fig. 5C). In contrast, the Dempster model consistently underpredicted total predation (Fig. 5D). This result was expected because the Dempster model assumes that immunologically positive predators attack one prey, whereas the functional response studies demonstrated that individual predators attacked ≈ 2 eggs per day over the range of prey densities observed here. Although the regressions for the Nakamura and Dempster models were highly significant, the simultaneous F tests clearly demonstrated that these models have poor predictive power (Table 3).

The new model was relatively accurate in predicting 24-h prey attack rates by *O. insidiosus* (Fig. 6A). Again, the simultaneous F test failed to reject the null hypothesis that the slope and intercept of the regression of observed on predicted attack rates are equal to 1 and 0, respectively (Table 3). The regression was highly significant and had a coefficient of determination of 0.62. As with *G. punctipes*, the model was relatively poor at predicting total rates of predation when prey attack rates were low (<3 total prey attacked) because of the failure of our assay to detect prey antigens in predators that had fed in many of these instances. Again, these cases were not necessarily associated with low prey densities. Instances of 0 attacks occurred at an average prey density of 0.0059 prey per cm^2 (see Fig. 4). The importance of the immunological component of the model was again demonstrated by the poor predictive capacity of the model without the immunological component (Fig. 6B, Table 3). In all but a few cases, total rates of prey attack were overpredicted when all predators in an arena were assumed to

attack prey at rates predicted by the functional response model alone.

The Nakamura model greatly overpredicted total attack rates by *O. insidiosus* in the several instances in which nearly all predators in an arena scored positive for prey antigens following correction for the detection interval (Fig. 6C). As a consequence it had poor predictive power overall (Table 3). The Dempster model consistently underpredicted total predation (Fig. 6D). As with *G. punctipes*, this result was consistent with findings in the functional response studies which indicated per capita attack rates of ≈ 2 eggs per day over a wide range of prey densities. Although the regressions for the Nakamura and Dempster models were highly significant, the simultaneous F tests again clearly demonstrated their poor predictive power (Table 3).

A final set of validation experiments was conducted with *G. punctipes* in which predators were allowed to forage for only 6 h rather than 24 h. The detection half-life was >6 h at the greenhouse temperatures used in these studies, allowing testing of the model without the correction for D , the detection interval, in Eq. [5]. Because all the predation models predict daily rates of prey attack, it was necessary to correct model output by the fraction of daily predation occurring in the 6-h period. In laboratory feeding studies with abundant prey we found that female and male *G. punctipes* would be expected to complete 68 and 74%, respectively, of total prey consumption per day in the first 6 h of exposure to prey (Table 4). Mixtures of females and males were used in plant arenas and so we discounted model output by multiplying predicted predation rates by an average value of 0.71 (assuming a 1:1 sex ratio). The new model was relatively accurate in predicting total prey attack rates (Fig. 7A). Again, the simultaneous F test failed to reject the null hypothesis that the slope and intercept of the regression of observed on

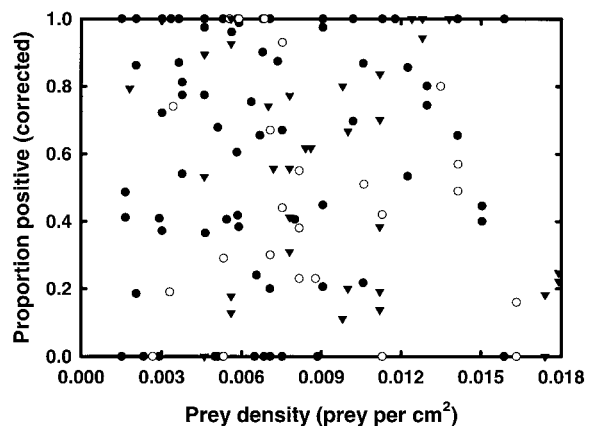


FIG. 4. Relationship between the proportion of predators positive by ELISA (corrected for the detection half-life) and the prey density. Closed circles, *G. punctipes* in 24-h arenas; open circles, *O. insidiosus* in 24-h arenas; closed triangles, *G. punctipes* in 6-h arenas.

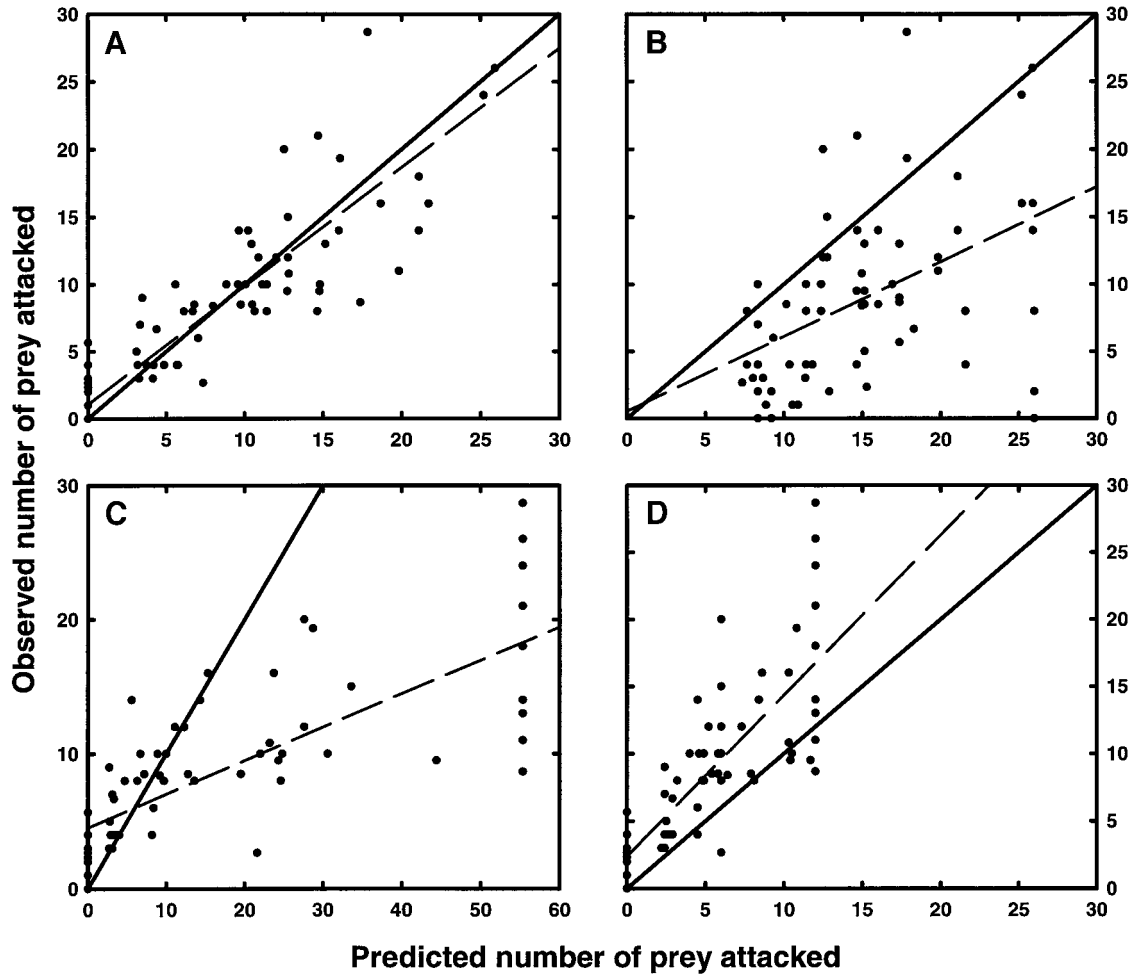


FIG. 5. Relationship between predicted and observed rates of predation for *G. punctipes* in 24-h arenas using (A) the new model (Eq. [5]), (B) the O'Neil functional response model (Eq. [2]) with the assumption that all predators in the arena are attacking prey, (C) the Nakamura model, and (D) the Dempster model. The solid lines represent $y = x$; the dashed lines are regressions fit to all the data.

predicted attack rates are equal to 1 and 0, respectively (Table 3). The regression was highly significant and had a coefficient of determination of 0.67. Once again the functional response model alone overpredicted predation in almost all instances (Fig. 7B, Table 3). Results from using the Nakamura and Dempster models were similar to previous evaluations (Figs. 7C and 7D, Table 3).

Overall, our results for the 6-h validations were insensitive to the assumption of a 1:1 sex ratio for the time correction. On average the intercept and slope values changed by 0.38 and 4.24%, respectively, if we assumed all females or all males in our arenas. Conclusions drawn from the simultaneous F tests remained unchanged.

Model sensitivity analysis. The sensitivity of the model to changes in three input variables was tested in a factorial design. Simulations were conducted for five levels of temperature (15, 20, 25, 30, and 35°C), proportion positive by ELISA (0.1, 0.3, 0.5, 0.7, and 0.9),

and prey density (0.001, 0.0021, 0.0045, 0.0094, and 0.02 prey per cm^2). Simulations were initiated with 100 predators and the evaluation criterion was the total number of prey attacked per day. For *G. punctipes*, ANOVA demonstrated that prey attack rates were most sensitive to changes in the proportion positive by ELISA, moderately sensitive to changes in prey density, and relatively insensitive to changes in temperature and interactions between the factors (Table 5). This pattern is plotted for a single prey density (0.01 per cm^2) in Fig. 8A and shows that, at high temperatures and with high proportion positive, the detection interval correction leads to a plateau in the response and a dampening of the effects of higher temperatures. In contrast, the high sensitivity to changes in temperature for *O. insidiosus* influenced the overall pattern of sensitivity to other inputs such as prey density (Table 5). The sensitivity to temperature is clearly related to the marked nonlinear nature of the detection half-life function for this species but also to the fact that longer

TABLE 3
Statistical Validation of Predicted Attack Rates for Various Predation Models

Test	Slope	Intercept	$F (P)^a$	r^2	n	$F_s (P)^b$
24 h (<i>G. punctipes</i>)						
New model	0.88	1.06	199.26 (<0.01)	0.74	71	2.32 (0.11)
Dempster	1.20	2.39	128.40 (<0.01)	0.65	71	30.86 (<0.01)
Nakamura	0.25	4.51	92.02 (<0.01)	0.57	71	553.40 (<0.01)
O'Neil	0.56	0.54	20.92 (<0.01)	0.23	71	46.34 (<0.01)
24 h (<i>O. insidiosus</i>)						
New model	0.86	1.41	39.28 (<0.01)	0.62	26	1.62 (0.22)
Dempster	0.95	3.25	46.58 (<0.01)	0.64	26	24.95 (<0.01)
Nakamura	0.18	5.25	12.08 (<0.01)	0.33	26	136.53 (<0.01)
O'Neil	0.23	3.83	2.44 (0.13)	0.09	26	49.41 (<0.01)
6 h (<i>G. punctipes</i>)						
New model	0.89	1.56	71.86 (<0.01)	0.67	38	1.42 (0.25)
Dempster	1.09	2.07	76.64 (<0.01)	0.67	38	14.25 (<0.01)
Nakamura	0.21	5.33	45.04 (<0.01)	0.54	38	438.54 (<0.01)
O'Neil	-0.20	12.64	0.63 (0.43)	0.02	38	51.20 (<0.01)

^a F test and P value for the regression of observed on predicted attack rates.

^b Simultaneous F test and P value for null hypothesis of slope = 1 and intercept = 0.

detection intervals at moderate and lower temperatures diminished the plateau that was so prominent for *G. punctipes* (Fig. 8B).

Parametric sensitivity analysis of the functional response model indicated that relatively small changes in the search behavior parameters could lead to relatively large changes in prey attack rates (Fig. 9). A 10% change in all three parameters simultaneously led to changes in prey attack rates as high as 22–29% at low to moderate prey densities (temperature and proportion positive were fixed at 25°C and 0.5, respectively). Prey attack rates were most sensitive to changes in C_2 , the parameter estimating the exponential decline in predator search with increasing prey density (Table 5), and this follows directly from the form of the equation.

DISCUSSION

Predation remains one of the most difficult to study ecological processes but one that is critical to understand if we are to use predators more effectively in agricultural pest control. Immunoassay of gut contents has been, and continues to be, the most widely used postmortem method for studying predation in the field. With careful recognition and consideration of the many pitfalls detailed by Sunderland (1988, 1996), immunoassay has significantly advanced our knowledge of the extent and function of arthropod predation in numerous managed and natural systems. Still, the promise of quantification has remained elusive.

We present an approach that expands on previous models emphasizing the estimation of per capita prey consumption rates from the proportion of positive responses in the immunoassay. We demonstrate that inclusion of a more realistic functional response model greatly improves the prediction of total prey attack

rates over previous models with simpler, but less realistic, estimates of per capita prey consumption rates that have gone untested in most applications. In an elegant study of predators of the broom beetle, *Gonioctena olivacea* (Forster), Dempster (1960) used independent life table data and predator searching behavior to support the assumption that each positive predator from his immunoassay was responsible for attacking a single prey. The consumption of a single prey may be reasonable when prey are large relative to the predator and when prey are scarce, but this assumption cannot be universally held and each system needs to be carefully examined to verify its validity. Our functional response studies for two generalist predators preying on moth eggs clearly indicate that the assumption of a single prey per predator would be valid only over a very narrow range of prey densities (see Fig. 2). As a result, the Dempster model consistently underpredicted prey attack rates in our system in which a wide range of realistic prey densities were examined.

The approach of Nakamura and Nakamura (1977) and Greenstone (1979) assumes that prey consumption is a random variate derived from the zero term of the Poisson distribution. The model predicts that per capita rates of predation increase exponentially as the proportion of positive responses increases. Indirectly, this is a functional response that implicitly infers a general positive relationship between prey abundance and the proportion of predators positive for prey antigens. That is, the model assumes that rates of predator encounters with prey increase with prey abundance, leading to more predators feeding on prey and higher per capita rates of predation. However, the Nakamura model consistently overpredicted prey attack rates in our plant arenas. The poor performance of this model

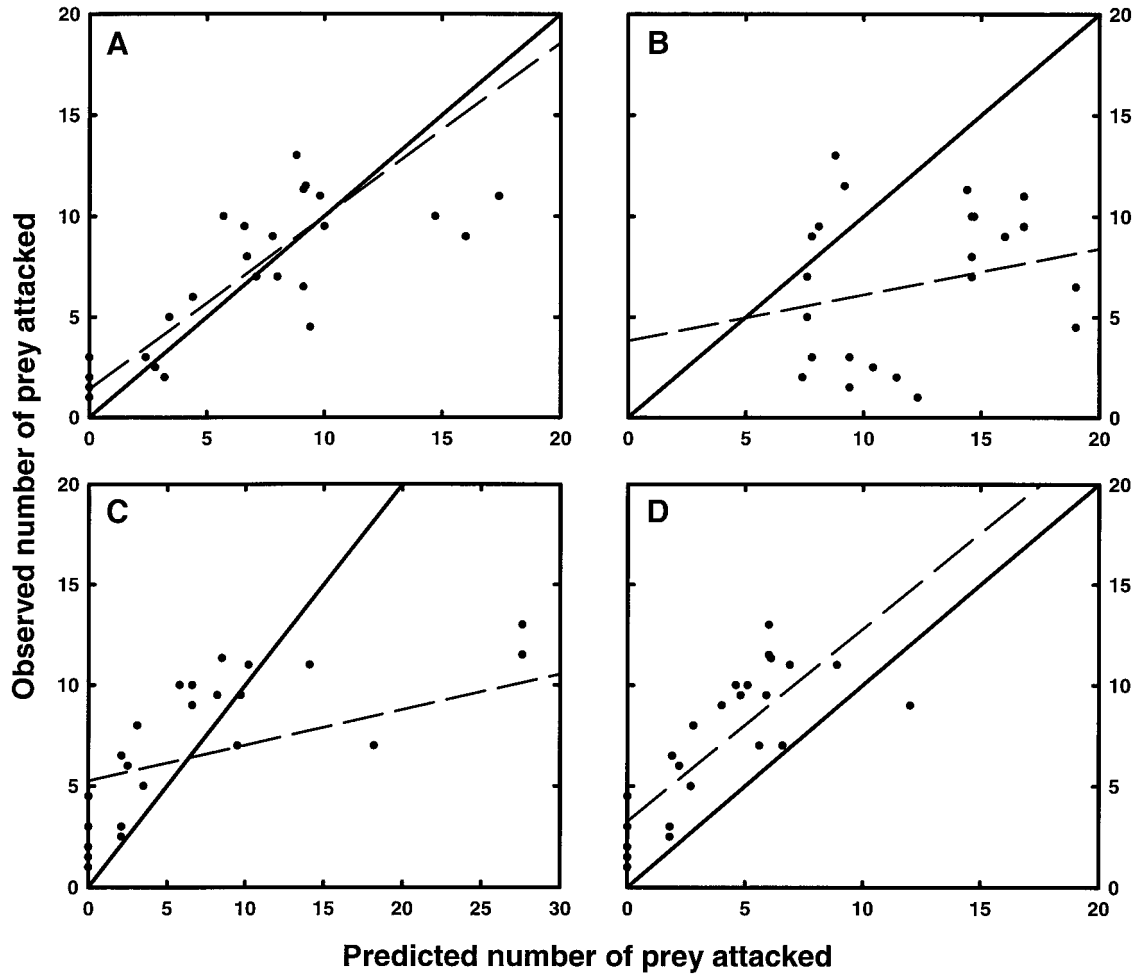


FIG. 6. Relationship between predicted and observed rates of predation for *O. insidiosus* in 24-h arenas using (A) the new model (Eq. [5]), (B) the O'Neil functional response model (Eq. [2]) with the assumption that all predators in the arena are attacking prey, (C) the Nakamura model, and (D) the Dempster model. The solid lines represent $y = x$; the dashed lines are regressions fit to all the data.

in our studies can be easily explained by the absence of any relationship between the proportion of positive responses and prey abundance, measured here as prey density (Fig. 4). We have reported a similar lack of correspondence between prey abundance and the proportion of positive predators in field studies involving generalist predators preying on both *P. gossypiella* and

Bemisia tabaci Gennadius (Hagler and Naranjo, 1994a,b).

The lack of such a relationship can be partially explained by the functional response behaviors observed in our study. Our data clearly demonstrate a decline in plant area searched by predators as prey density increases (see Fig. 1). Thus, prey encounters remain largely unchanged even as the number of prey per unit area increases and this is reflected by the relatively stable number of prey attacked over a wide range of prey densities (see Fig. 2). Interactions among the 12 predators in an arena may also contribute to this lack of pattern. The assumption that all predators are attacking prey in an arena was clearly invalidated by comparison of our results to predictions of the O'Neil and Stimac (1988) functional response model alone (see Figs. 5B, 6B, and 7B).

The models of Rothschild (1966) and Kuperstein (1979) propose the use of per capita prey consumption rates measured in the laboratory or insectary. Al-

TABLE 4

Mean (SE) Predation by *G. punctipes* on Eggs of *P. gossypiella* during Two Time Intervals over a 24-h Period

Sex	<i>n</i>	0800–1400 (6 h)	1400–1800 (18 h)	Proportion (6 h)
Female	25	57.9 (4.8)*	27.6 (3.1)*	0.68 (0.02)
Male	25	30.8 (2.7)	12.2 (1.8)	0.74 (0.02)
Total	50	44.4 (3.3)	19.9 (2.1)	0.71 (0.02)

Note. Asterisk indicates that egg consumption was significantly different between the sexes ($P < 0.001$).

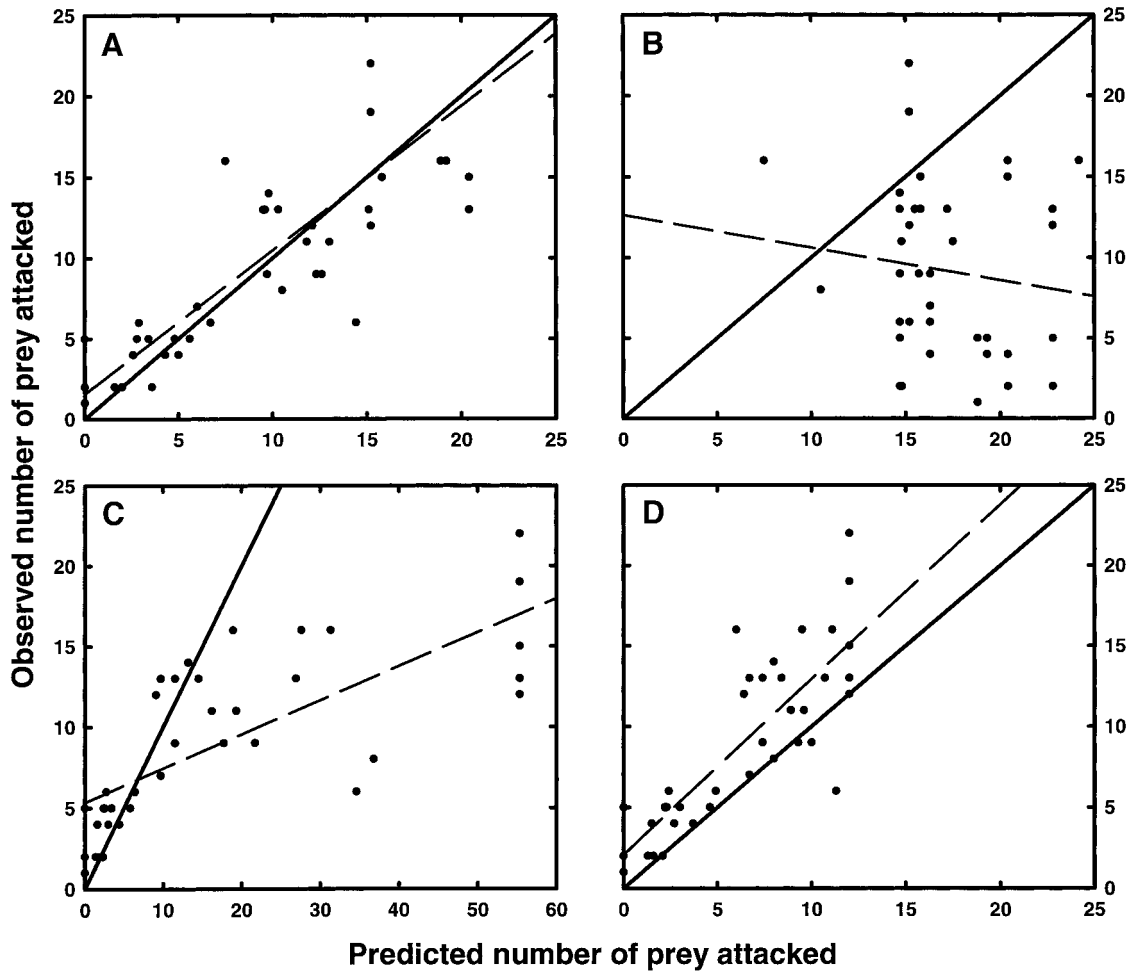


FIG. 7. Relationship between predicted and observed rates of predation for *G. punctipes* in 6-h arenas using (A) the new model (Eq. [5]), (B) the O'Neil functional response model (Eq. [2]) with the assumption that all predators in the arena are attacking prey, (C) the Nakamura model, and (D) the Dempster model. The solid lines represent $y = x$; the dashed lines are regressions fit to all the data.

though we did not test these models, it is clear that they would have grossly overpredicted rates of prey attack for the species examined here. Daily per capita rates of predation on *P. gossypiella* eggs in the laboratory were, on average, 20 times greater than the rates observed for *G. punctipes* forced to hunt for prey under realistic conditions. This result stresses the importance of the measurement of rates of predation under conditions more representative of the field (e.g., O'Neil, 1989).

We also did not test the alternative approach of estimating prey biomass consumption, which is theoretically more direct in terms of a predator's actual feeding history. That is, the concentration of prey antigens in the gut has some relationship to the amount of prey consumed. Sopp *et al.* (1992) demonstrated the utility of such an approach for estimating biomass consumption of predators feeding on the cereal aphid, *Sitobion avenae* (F.). The model performed better than the Rothschild, Kuperstein, and Dempster models and

may have application in systems in which immunore-sponse/antigen concentration curves are well defined and reasonable assumptions can be made about predator digestive rates, prey size, and prey-selection behavior of predators. Still, the many factors involved in explaining the amount of antigen in the gut that have been highlighted previously limit the general use of this approach in most systems.

The functional response is a fundamental component of predator-prey dynamics (Solomon, 1949) and various models have been proposed and used to quantify the relationship between prey density and predator attack rates (Hassell, 1978). Our predation model was tested using the functional response model of O'Neil and Stimac (1988), but other models could be easily substituted, depending on the particular system under investigation. Our choice of the O'Neil and Stimac (1988) model was based on its accuracy and generality in quantifying predation in several cropping systems and for a wide range of generalist predators preying on

TABLE 5

Factorial ANOVA of the Sensitivity of Rates of Prey Attack to Changes in Various Model Parameters

Factor	df	Prey attack rate (% variation explained)	
		<i>G. punctipes</i>	<i>O. insidiosus</i>
Model inputs ^a			
(A) Temperature	4	9.7	50.6
(B) Proportion positive	4	59.7	25.7
(C) Prey density	4	18.4	10.7
(A) × (B)	16	9.6	10.2
(A) × (C)	16	0.4	1.6
(B) × (C)	16	1.9	0.8
(A) × (B) × (C)	64	0.3	0.4
Total	124	100.0	100.0
Model parameters ^b			
C_1	2	13.6	11.2
C_2	2	82.8	83.6
C_3	2	3.1	4.7
$C_1 \times C_2$	4	0.5	0.5
$C_1 \times C_3$	4	0.0	0.0
$C_2 \times C_3$	4	0.0	0.0
$C_1 \times C_2 \times C_3$	8	0.0	0.0
Total	26	100.0	100.0

^a Temperature: 15, 20, 25, 30, 35°C; Proportion positive: 0.1, 0.3, 0.5, 0.7, 0.9; Prey density: 0.001, 0.0021, 0.0045, 0.0094, 0.02.

^b Each parameter altered $\pm 10\%$; temperature, proportion positive, and prey density fixed at 25°C, 0.5, and 0.01, respectively.

a wide range of target prey (Naranjo and Stimac, 1987; O'Neil, 1988, 1997; O'Neil and Stimac, 1988; Wiedenmann and O'Neil, 1992). This model emphasizes search behavior rather than the consumptive behaviors typically stressed by traditional functional response theory because the model is founded on a field-realistic definition of prey density.

Even though the O'Neil and Stimac (1988) model appears to have broad applicability for modeling the functional response of generalist predators, the influence of alternate prey in the field cannot be ignored. Cotton systems in the southwestern United States are inhabited by a large and diverse complex of arthropods (Van den Bosch and Hagen, 1966). Accordingly, we might expect alternate prey to be readily available and to be an important factor in affecting the functional response of generalist predators such as *G. punctipes* and *O. insidiosus* to the target prey studied here. However, because the functional response model embodies a predator search strategy that emphasizes leaf area searched, it may be possible to make adjustments to maintain accurate prediction of target prey even in the presence of alternate prey. That is, if predators are responding to total prey density, regardless of prey species, then the number of target prey attacked would depend simply on their density relative to other potential prey in the system. Even factors such as prey preference by predators and prey distribution (e.g., leaf

vs stem), which might further modify predator responses, could be tested and accommodated. Overall, these hypotheses need to be tested in the field, but if supported, could broaden the applicability of the O'Neil and Stimac (1988) model and the new model proposed here.

Regardless, the combination of immunological and functional response models proposed here has the potential to improve quantitative predictions of prey attack rates overall. We showed that predictions based solely on the functional response model were extremely poor because they were based on the assumption that all predators are attacking prey at the prescribed rate. Clearly, the proportions of predators attacking prey

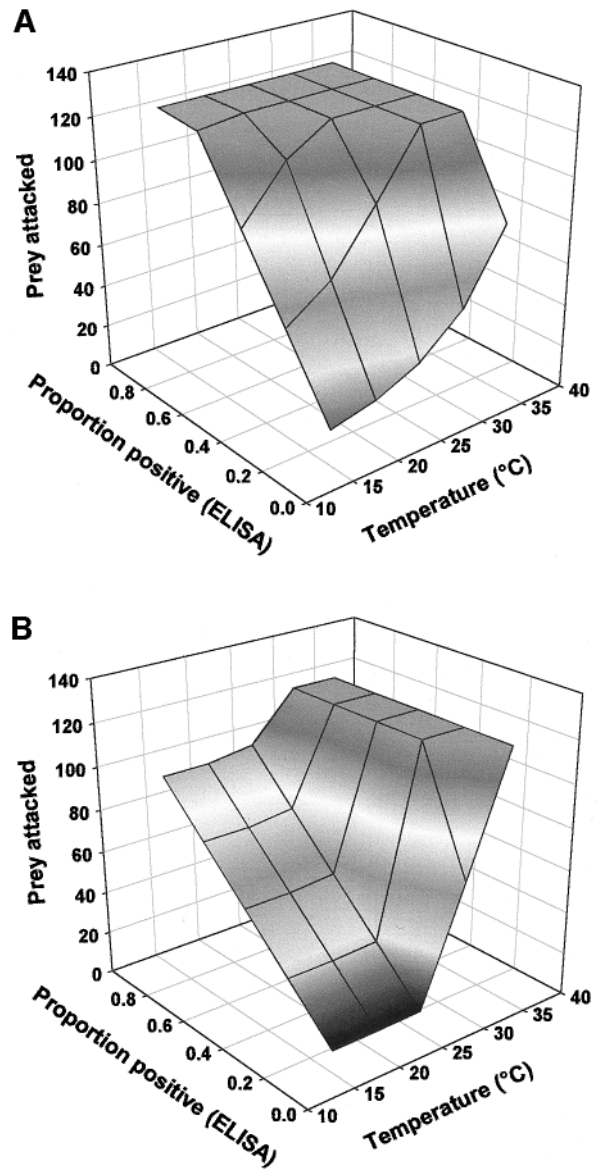


FIG. 8. Sensitivity of the predation model to temperature and proportion positive by ELISA for (A) *G. punctipes* and (B) *O. insidiosus*. Prey density was fixed at 0.01 prey per cm².

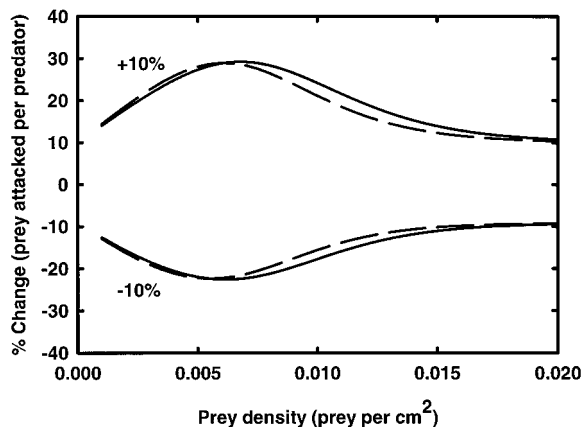


FIG. 9. Sensitivity of the predation model for *G. punctipes* (solid lines) and *O. insidiosus* (dashed lines) to simultaneous changes ($\pm 10\%$) in the three search parameters of the functional response model. Temperature and proportion positive were fixed at 25°C and 0.5, respectively.

can vary considerably and need not bear any relationship to prey density (see Fig. 4 and discussion above). In the field, the numbers of predators feeding on the target prey will be affected by additional factors, including the presence of alternate prey, predator life stage and reproductive status, interference from other predators, and a host of environmental variables. Although it would be difficult to account for all these factors, we suggest that an independent method of measuring predator feeding activity, such as an immunoassay, could improve predictions of predation models that are typically based solely on a mathematical representation of the functional response (Hassell, 1978).

The application and testing of our model in the field involves increased data requirements. First, additional data and effort will be needed to develop the functional response model and to monitor prey densities in the field. In our application, frequent measurement of plant leaf area and prey abundance would be needed. Estimation of prey antigen detection intervals for each predator species of interest would be required, however, such estimates would be needed for application of any of the immunological models discussed. Although our sensitivity analysis indicated that temperature was a very important factor, the average range of temperatures during the growing cycle of many crops would likely be considerably narrower. Thus, detection interval studies could be conducted over a smaller range or even at a single representative temperature. The divergent temperature-dependent detection interval curves for the two predator species studied here highlights the importance of separately examining each potential predator.

In addition to the issue of alternate prey as discussed above, several more assumptions also need consideration. First, the antigen detection intervals in the lab-

oratory are assumed to be applicable to the field, where environmental conditions are variable and predators may feed on other prey. Although predators in our detection interval studies (Hagler and Naranjo, 1997) were allowed to feed on alternate prey, the predators in our plant arenas were not (although they may have fed on plants directly), and this could affect the relationship between actual digestive rates and estimates of detection half-lives in the model. We further assume that immunological methods are selective and that problems such as scavenging and secondary predation are minimal. The antibody used here is species and stage specific (Hagler *et al.*, 1994) and preliminary studies indicate that there is a very minimal chance of detectable antigen migration up the food chain (unpublished data). We have not examined scavenging behavior but assume it would be minimal for predators feeding on eggs.

In summary, we proposed and tested a new model for quantitatively estimating predation using immunological methods. Our model performed better than previous models based on simpler but less realistic models of per capita prey consumption. The application of our model to evaluating predation in the cotton system will require further study to test the generality of the functional response component in the field and to measure detection intervals in a wider range of predator species. The general approach demonstrated here could have broader application to many systems and facilitate progress in quantifying predation through immunoassay without the problematic need to consider the strength of the immunoresponse.

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