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NOCTURNAL PREDATION OF LEPIDOPTERAN EGGS IN SOUTH TEXAS COTTON - 2002

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Abstract

Predation on eggs of the cotton bollworm, *Helicoverpa zea* Boddie, and the beet armyworm, *Spodoptera exigua* (Hübner), in south Texas cotton was measured using direct observation of sentinel eggs to quantify mortality, identify key predators and partition nocturnal and diurnal sources of mortality. Mortality (24 h) ranged from 56.8 to 89.1% of *H. zea* eggs and 57.7 to 84.2% of *S. exigua* eggs. There were 395 predation events observed in 2002. Predation was more commonly observed at night with 73.2% of predation events occurring at night. No differences in predation rates on eggs of either prey species were observed. The dominant predators were a complex of wandering spiders comprised primarily of *Hibana futilis* (Banks), *Hibana arunda* Platnick, and *Cheiracanthium inclusum* (Hentz) which were responsible for 28.4% of all nocturnal observations of predation (and 20.8% of all observations). Ants, dominated by *Solenopsis invicta* Buren, was the second most commonly observed predator (16.2% of all observations) and was the 4th most frequently observed nocturnal predator (14.5% of nocturnal observations). The 3rd and 4th most observed predators were the cotton fleahopper *Pseudatomoscelis seriatus* (Reuter) [13.4% of total and 17.0% of nocturnal observations (3rd)] and predatory mites [13.2% of total and 18% of nocturnal observations(2nd)]. Taxa that were primarily nocturnal represented the four arthropod groups most frequently observed feeding on lepidopteran eggs. Only the *Geocoris spp*. (*Geocoris lividipennis* Stål and *Geocoris punctipes* Say), which are exclusively day active, were among the five most frequently observed predators. Of the predators observed feeding nocturnally, only *Solenopsis invicta* has been studied as a predator of lepidopteran eggs, little or nothing is known about the predatory characteristics of the other taxa.

Introduction

Natural enemies are frequently cited as maintaining populations of lepidopteran pests of cotton such as the cotton bollworm, *Helicoverpa zea* Boddie, and the beet armyworm, *Spodoptera exigua* (Hübner), below damaging levels unless disturbed or decimated by pesticides (King & Coleman, 1989; Ruberson, et al., 1994). When detailed studies are conducted such as that of Stewart et al. (2001), predation is invariably implicated as the factor controlling pest densities. Despite this, our knowledge of the predators causing this mortality and their biology is extremely limited.

Identifying the predator species feeding on specific insect pests is an important step in determining the impact predation may have on pest populations. Studies have attempted to identify predators attacking lepidopteran pests in cotton (Whitcomb and Bell, 1964; Whitcomb, 1967; McDaniel and Sterling, 1979; 1982; Neussly and Sterling, 1994; Ruberson et al., 1994; Ruberson and Greenstone, 1998). However, those studies that used direct observation (Whitcomb and Bell, 1964; Ruberson et al. 1994 and to a lesser extent McDaniel & Sterling, 1982 and Nuessly & Sterling, 1994) inadequately addressed nocturnal predation, and studies using radioactive labeling may produce biased results due to intraguild predation (i.e., predation on predators). The use of immunological techniques (Ruberson and Greenstone 1998) may suffer from quick degradation of antigens within the predator (Sansone and Smith 2001). None of the studies that previously used direct observation to determine predation events in cotton partitioned diel patterns of predation.

It appears that the complex of predators which feed on lepidopteran eggs may vary temporally. In studies in soybean and corn, Pfannenstiel & Yeargan (2002) demonstrated that predators varied in their diel activity patterns and that predators active predominantly at night could cause the bulk of observable mortality in soybean. Careful observation of nocturnal predation in that study identified previously unknown predators In this study, we use direct observation to identify the important predators of lepidopteran eggs in cotton. There were two major goals to this study; first to determine the seasonal patterns of predation on eggs of lepidopteran pests in south Texas cotton and second, to identify the predators causing mortality, with a particular focus on noctural predators that might not have been identified in previous studies.

Materials and Methods

Predation on lepidopteran eggs was evaluated in two small cotton fields (Deltapine 54-15) planted on a 1.03 m row spacing with drip irrigation. Field one was planted in the first week of March in 2002 and was used to compare predation of *H. zea* and *S. exigua* eggs through the season. A second field was planted in late March and divided into twelve 16 row by 20 m plots and used for additional observation of predation events on *H. zea* eggs. There were 3.1-m bare ground buffers surrounding all plots and field edges. No pesticides were used during the course of this study.

Egg mortality and the predators responsible was quantified using the methods of Pfannenstiel & Yeargan (2002) but will be summarized here with appropriate changes noted. Field one was divided into quadrants and 30 sampling stations selected in each quadrant. In each quadrant there were 10 stations per row with 5 m between stations and 7 rows between sample rows. The 10 sites per row were alternately assigned to either *H. zea* or *S. exigua* using different colored flags so that there were 15 sites for each egg type in each quadrant. In the second field there were 9 stations per plot in 2002 with these stations at 3 m intervals with 4 rows between sample rows.

H. zea and *S. exigua* colonies were maintained in the laboratory by modified methods of Ignoffo (1965). Adults were placed in 3.8 l ice cream cartons lined with green florist paper for oviposition; a 10% sucrose solution was provided as a food source. Egg sheets were collected daily; paper on which eggs had been laid was cut into small (3 to 20 cm^2) sections containing either 10 *H. zea* eggs or one *S. exigua* egg mass each. Egg groups or masses were then placed in a refrigerator at 4°C to stop development until used or discarded after 4 d. Groups of 10 *H. zea* eggs (as opposed to 1 egg) were used to extend the amount of time that a predator feeds, thus increasing the probability of observing predation events. All eggs in each *S. exigua* egg mass (range 20 to ~160 eggs/mass) were counted and recorded before placement into the cotton field.

Eggs were transported to the field in an ice chest with ice and then attached to plants at 3 PM by stapling the eggs in the desired location. Afternoon was used for deployment of eggs for purely logistical purposes. *H. zea* eggs typically take 2.5 or more days to develop in the field in Texas and would be available to predators throughout this time (R. S. P. personal observation). Paper sections containing eggs were not used if any of the eggs were dislodged during transportation. Egg groups were attached to the top of a cotton leaf about 55 - 70% of plant height and this relative location was maintained as the cotton plants grew. Pests of field crops such as cotton often deposit their eggs on the foliage of the middle to upper parts of the plant (Terry et al. 1987, Sappington et al. 2001, R. S. P. personal observation) although often on the undersides of leaves. Placing the eggs on the top of leaves was done to facilitate observation. Neussly and Sterling (1994) found no differences in predation on *H. zea* eggs between the upper and lower leaf surfaces in cotton in central Texas.

Egg groups were observed at three-hour intervals (6 PM, 9 PM, 12 Midnight, 3 AM, 6 AM, 9 AM, 12 Noon, and 3 PM CDT) for the following 24 h. This distribution of sampling times results in 4 day (9 AM, 12 Noon, 3 PM and 6 PM) and 4 night samples (9 PM, 12 Midnight, 3 AM, and 6 AM CDT). Sunrise occurred as the 6 AM sample was being finished and sunset occurred just before the 9 PM sample was initiated, allowing for equal numbers of day and night samples despite a photophase lasting about 14 h. *H. zea* and *S. exigua* eggs take about 3 d to develop; due to their prompt refrigeration, eggs used during this study did not approach hatching at any time. At each observation period, predators observed feeding on the eggs were identified or collected for subsequent identification. All observations of predation could be assigned to day (9 AM, 12 Noon, 3 PM and 6 PM) or night (9 PM, 12 Midnight, 3 AM, and 6 AM). Eggs of each species were replaced when all eggs on the sheet had been consumed allowing accurate estimation of egg mortality (24 h). This experiment was conducted on seven dates in 2002. Sampling was initiated in early May and continued at two to four week intervals through late August continuing after the cotton would normally be harvested (late July - early August).

The temporal distribution of predation events was analyzed to determine the relative importance of nocturnal predation in south Texas cotton. The null hypothesis entering the study was that there would be no difference in the frequency of observed predation events between day and night or between types of prey. Although previous studies (Pfannenstiel & Yeargan 2002) have demonstrated significant differences in predation within crops due to photophase, without knowing *a priori* the important predators, it would be difficult to predict whether observed predation events would be higher during the day or at night. Observed predation events were divided into two groups for analysis occurring either during daylight (9 AM, 12 Noon, 3 PM and 6 PM) or at night (9 PM, 12 Midnight, 3 AM, and 6 AM). Deviation of the frequency of predation events from the expectation of a random distribution between these two periods was analyzed with the test of significance of a binomial proportion (Snedecor and Cochran 1989).

Results and Discussion

Impact of Predators on *H. zea* and *S. exigua* Eggs

Levels of predation varied through the season with no consistent differences between egg types (Fig. 1). Predation rates (24 h) were always > 50% and ranged from 56.8 to 89.1% on *H. zea* eggs and from 57.7 to 84.2% on *S. exigua* eggs (Fig. 1). Studies of egg predation in cotton have yielded consistently high estimated predation rates. McDaniel and Sterling (1982) observed an average of 77% daily predation rates of *Heliothis virescens* (Fabricious) eggs in cotton in central Texas. In a more detailed study, Neussley and Sterling (1994) demonstrated average total (~ 72 h) predation rates > 80% on *H. zea* eggs. Clearly, predation on lepidopteran eggs in cotton can vary but typically is quite high.

Diel Patterns of Predation on Lepidopteran Eggs

The predation events observed accounted for only a portion of the overall predation on eggs. Predation on eggs was observed 395 times during this study. Observations of nocturnal predation were considerably more common than during the day (Fig. 2) (χ^2=84.8, df=1, *P*<0.001) with 73.2% occurring at night. In the field where predation of *H. zea* and *S. exigua* was measured, there were no differences in observation of predation on eggs of either species during the day or night when assuming that the probability of observing predation on either egg type was equal (χ^2=0.1, df=1, *P*>>0.05 and χ^2=1.3, df=1, *P*>0.05, respectively) (Fig. 3). This is despite the larger size of the *S. exigua* egg masses which should take longer for predators to consume and might have led to a higher frequency of observed predation events in *S. exigua* eggs. The proportion of predation occurring during the night was high all season with the exception of the first week of June, the sampling date that more predation events were observed during the day.

Predator taxa observed feeding on eggs occasionally feed both during the day and night (most notably the formicids), however, the frequency of observed predation events for most taxa differed considerably between diel periods (Table 1). The dominant predator group at night was a complex of wandering spiders that were responsible for 28.4% of nocturnal observations (and 20.8% of all observations of predation). This group of spiders was composed primarily of *Hibana futilis* (Banks), *Hibana arunda* Platnick and *Cheiracanthium inclusum* (Hentz) which represented 58.5%, 15.9%, and 9.8%, respectively, of the 82 spiders observed feeding. Ants, dominated by *Solenopsis invicta* Buren, was the second most commonly observed predator (16.2%) and was the fourth most frequently observed nocturnal predator with 14.5% of nocturnal events. The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), was the 3rd most frequently observed feeding on eggs (13.4%) followed closely by predatory mites (13.2%). Both *P. seriatus* and the predatory mites were primarily nocturnal. It is notable that these four predator groups (or omnivores), all of which are primarily nocturnal, were the four most frequently observed predators overall and were responsible for 63.6% of all observations (Table 1).

The *Geocoris spp*. (*Geocoris lividipennis* Stål and *Geocoris punctipes* Say) were the most frequently observed predator during the day (26.4% of daytime observations) and the only day-active predator among the five most frequently observed (7.1% of all observations). Ants, predominantly *S. invicta*, and the coccinellid, *Hippodamia convergens* Guérin-Méneville, were tied as the second most frequent predators observed during the day (both with 20.8% of daytime observations). *H. convergens*, which was only observed feeding during the day, accounted for only 5.8% of total observations. A *Collops* sp. was the only other predator responsible for > 10% of daytime observations (12.3%) and accounted for only 3.3% of total observations.

A number of previous studies, including three in Texas (McDaniel & Sterling 1979, 1982, Nuessly & Sterling 1994) and two in Georgia (Ruberson et al., 1994; Ruberson & Greenstone 1998), documented the complexes of predators feeding on Heliothine eggs in cotton. The studies in Texas (McDaniel & Sterling,1979; 1982; Nuessly & Sterling, 1994), using radio-labeled eggs (^{32}P) all found the red imported fire ant (*S. invicta*) to be the dominant predator in central Texas cotton fields. Other predators were detected/observed in only very small numbers compared to *S. invicta* but included *Orius insidiosus* Say, *G. punctipes*, *P. seriatus* and spiders (dominated by *Phidippus audax* (Hentz). It is noteworthy though that the predator that they collected that had eaten the most labeled eggs per predator was *C. inclusum* although only a very small number of these were collected. *O. insidiosus* and *G. punctipes* have been observed to be primarily day active predators (Pfannenstiel & Yeargan 2002) and *P. seriatus* can be active both during the day and night (unpublished data). The only predator not active during the day that was observed by Nuessly and Sterling (1994) with any frequency was *C. inclusum* which made up only 0.32% of the observations (presumably all discovered due to feeding on radio-labeled eggs). A visual study of predation in Georgia conducted by Ruberson et al. (1994) in unsprayed cotton identified similar predators as the Texas studies with *O. insidiosus* most frequently observed followed by *S. invicta*, *G. punctipes*, and *Chrysoperla rufilabrus* Burmeister. However, the second study conducted in Georgia, (Ruberson & Greenstone,1998) yielded distinctly different results using an immunological assay to determine the important predators. This molecular technique avoids the danger of false positives due to intraguild predation and potentially addresses nocturnal predation. The two most frequently recovered predators testing positive for feeding on Heliothine eggs were the spider *C. inclusum* (23.4%) and *Scymnus* lady beetles (23.4%). This may be the first time that a spider or a *Scymnus* sp. has been implicated as a dominant predator of lepidopteran eggs. Although there is little data for *Scymnus* spp., they may be nocturnal (unpublished data). The two other predator groups that were frequently recovered positive in Ruberson & Greenstone's study were *S. invicta* (21.6%) and *G. punctipes* (16.5%) similar to the other studies. Three of the four taxa also had relatively high percentage of individuals that were positive for egg feeding [*C. inclusum* (25.1%), *G. punctipes* (12%), and *Scymnus* sp. (13.2%)], the proportion of *S. invicta* individuals testing positive was lower (4.0%). These studies paint a partial picture of the predator complexes causing mortality to eggs of lepidopteran pests but in all cases sampling was conducted during the day and they may have failed to identify important nocturnal predators. The results of the Ruberson and Greenstone (1998) study strongly suggest that other studies have inadequately identified important predators by not addressing nocturnal predation, this is also an important conclusion of this study.

These observations on the predator complex feeding on lepidopteran eggs documents that nocturnally active predators cause the bulk of mortality of *H. zea* and *S. exigua* eggs in south Texas cotton. Among the nocturnally active predators are several arthropod taxa not typically considered as important mortality agents of lepidopteran eggs in cotton such as the wandering spiders, mites and elaterids. The wandering spiders are of particular interest because they can consume large numbers of eggs in a single evening (>200 *H. zea* eggs/adult female/night for all three spiders mentioned here, R. S. P. unpublished data).

Diel Patterns of Predator Activity

The predators observed feeding on lepidopteran eggs varied in whether they were most often observed feeding during the day or at night (Table 1). Changes in frequency of observed predation probably reflect temporal changes in searching activity. The ants tended to be nocturnal, with 65.6% of the observed feeding events occurring between dusk and dawn. The wandering spiders, mites and elaterids were exclusively nocturnal. Cotton fleahoppers were most frequently observed at night (92.5%). The *Geocoris* sp. were observed exclusively during the day. Other, less frequently observed predators that were primarily day-active were *H. convergens* and a *Collops* sp.

Conclusions

The composition of the predator complex feeding on *H. zea* eggs at night was different than that feeding during the day. To accurately characterize the predators attacking particular prey, it is critical to investigate predation during nighttime as well as daytime hours. Studies using primarily diurnal visual observations (e.g. Ruberson et al., 1994) did not identify a significant proportion of the important predators due to inadequate observation of nocturnal predation.

Numerous arthropods feed on lepidopteran eggs in cotton. This predator complex causes significant mortality which is important in reducing populations of lepidopteran pests. While many arthropod species contributed to the overall high egg mortality rate, only a few were responsible for most of the observed mortality. Daylight observations would have correctly identified one of the two dominant predators in cotton (ants), however they would not have correctly identified the co-dominant predator group, the wandering spiders. Several other possibly important predator groups including the cotton fleahopper and mites would have also been missed. Increased consideration of nocturnal predation is likely to change our perception of the dominant predators in cotton and their impact on particular pests. The dominant predators identified in this study, particularly the wandering spiders, should be the focus of further studies to characterize their ecology and improve our understanding their contribution to the mortality of lepidopteran pests in cotton.

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Table 1. Frequency of observation of arthropods commonly feeding upon lepidopteran eggs in cotton. Observations of predation events are divided into the photophase observed and total observations for each taxa. The percent of observations attributable to each taxon within each diel period and for the total observed events are shown and the ranking of that predator for that diel period. Only predators that ranked among the top 5 for either diel period are shown.

Taxon	Day	%	Rank	Night	%	Rank	Total	%	Rank
Wandering Spiders	0	0.0	-	82	28.4	1	82	20.8	1
Formicidae	22	20.8	2	42	14.5	4	64	16.2	2
Cotton Fleahopper (*P. Seriatus*)	4	3.8	-	49	17.0	3	53	13.4	3
Mites	0	0.0	-	52	18.0	2	52	13.2	4
Geocoris spp. (*G. lividipennis* and *G. punctipes*)	28	26.4	1	0	0.0	-	28	7.1	5
Hippodamia convergens	22	20.8	3	1	0.3	-	23	5.8	-
Elaterids	0	0.0	-	14	4.8	5	14	3.5	-
Collops sp.	13	12.3	4	0	0.0	-	13	3.3	-
Chrysopidae	6	5.7	5	0	0.0	-	6	1.5	-
Totals (all observations)	106			289			395		

- Predators which did not make the top 5 ranking within the feeding period described in the column header.

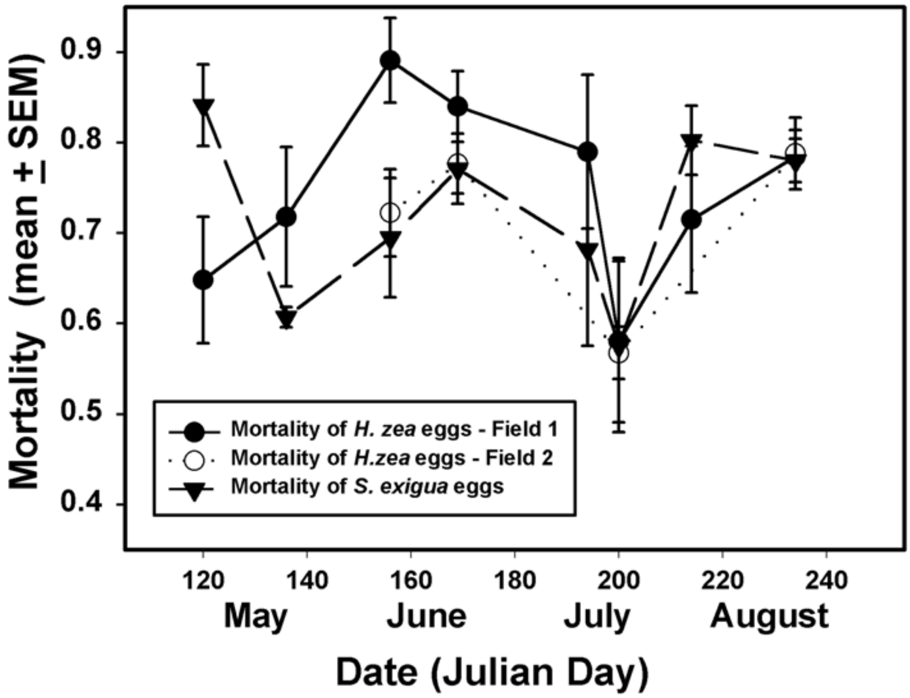

Figure 1. Mortality of H. zea and S. exigua eggs in cotton furing 2002 (Proportion fed upon or disappearing (24h) SEM).

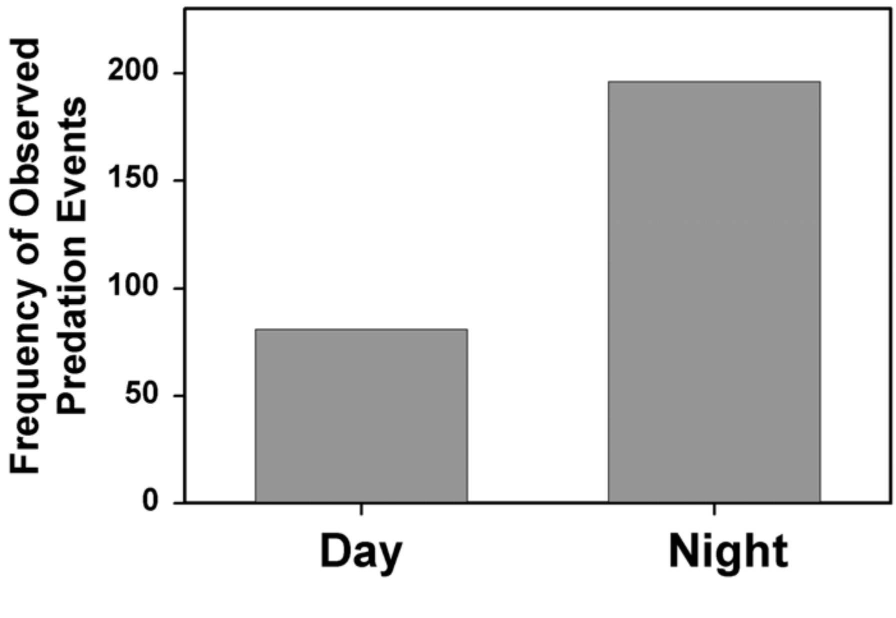

Figure 2. Diel observations of predation events during 2002.

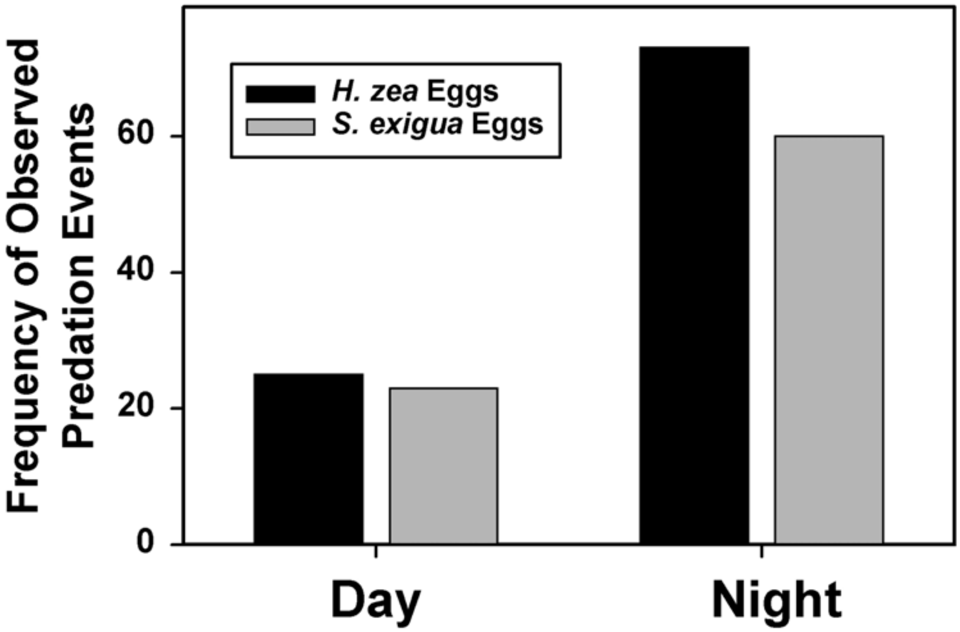

Figure 3. Frequency of predators observed feeding on H. zea and S. exigua eggs during the day and night during 2002.