Chapter Seven

Estimating Predation Rates with *H. armigera* Egg Cards: Preliminary Experiments.

Introduction

Estimation of parasitoid recruitment is often conducted using sentinel hosts placed in the field (Scholz 2000, Scholz *et al.* 2000). The same technique using sentinel prey can be applied to the estimation of predation and determining which predator species eat a particular type of prey (Pfannenstiel & Yeargan 2002). Stationary prey stages (eggs or pupae) are put in the field at natural densities and losses monitored (Whitcomb & Godfrey 1991, Mills 1997). It is assumed that these losses are a result of feeding by predators. In reality some losses may be due to abiotic mortality agents such as wind and rain. If the aim of the study is to obtain an accurate measure of predation rates in the field it is essential that the sentinel prey are equally susceptible to predation as the naturally occurring prey (Mills 1997). This assumption is rarely tested (Pfannenstiel & Yeargan 2002).

In Australia sentinel prey has been used to assess mortality by a number of predators of *Helicoverpa* spp. eggs and larvae in the field. Lytton-Hitchins (2000) used leaf discs with *H. armigera* eggs laid on them to assess foraging behaviour of ants in cotton. Titmarsh (1992) investigated mortality of *Helicoverpa* spp. eggs and larvae in artificially enhanced cohorts in cotton by a group of predators using life table studies. Scholz *et al.* (2000) used egg cards (20 *H. armigera* eggs per card) placed in a cotton field for 48 hours. The proportion of eggs that displayed feeding by ants and spiders was recorded after the exposure period. Egg cards have been used in sweet corn to assess spatial and temporal aspects of parasitism and predation by a range of species (Scholz 2000). Direct observation of the egg cards allowed the identification of an unrecorded predator of *Helicoverpa* spp., the mirid *Tytthus chinensis* (Stål). More mobile stages of prey have been used by some authors with mixed results. Whitcomb (1967) placed out second-instar *H. zea* larvae in a cotton field and constantly observed individuals. Despite some interesting records of the predator species preying on *H. zea* the author notes that the work was slower and more tedious than expected! This was primarily due to the difficulties involved in keeping track of uncaged mobile prey.

I used egg cards to investigate predation of *Helicoverpa* spp. in the field throughout this thesis (see Chapter eight and nine). Preliminary experiments were necessary to determine if the predation rate observed on egg cards is similar to that of naturally laid eggs. The results were used to develop a protocol for future egg card experiments. The following questions were addressed:

7.A What time of the day is the best time to place egg cards in the field?

7.B What effect does egg density on cards have on egg predation rate and on egg cards being found by predators?

7.C Does exposure time influence egg predation rate and the chance of egg cards being found?

7.D Is there a difference in predation rate of fresh eggs (less than 24 hours old) and old eggs that have been stored before use?

7.E Is there any difference between predation of eggs on egg cards and naturally laid eggs?

General materials and methods

Egg cards consisted of 20 *H. armigera* eggs laid onto paper towel in the laboratory. Adult male and female moths were placed into an oviposition cage immediately after eclosing from their pupal case. The walls of the cage were lined with paper towel and fresh honey solution was supplied every second day. The female moths after mating laid eggs onto the paper towel. Pupae were supplied from a culture at the Queensland Department of Primary Industries, Toowoomba. Scholz (2000, see appendix one) describes the culturing methods. Paper towel with eggs was attached to a six by two centimetre rectangle of white cardboard using a stapler. All cards were prepared less than 24 hr before use in the field, and all eggs had been laid less than 24 hr before the egg cards were made. In wheat fields cards were located on the upper side of the flag leaf. In lucerne and soybean cards were attached to the top third of the plant on the upper-side of a leaf. They were attached using a stapler half an hour before observations commenced. Cards were observed at half hourly intervals and the predators feeding on the eggs recorded. After the exposure time the egg cards were collected and placed in separate zip lock bags and transported to the laboratory. The eggs were each examined under a dissecting microscope and egg mortality based on characteristic damage was attributed to:

Sucking predators

Chewing predators

Missing (also counted as chewing predators, may be due to abiotic mortality agents)

Remaining (not consumed)

Scholz *et al.* (2000) (see fig. 1) provides photos of eggs damaged by different predators. The egg cards where stored in a temperature cabinet (14:10 light:dark; 24:20^{0}C; 65% relative humidity) until the remaining eggs hatched, or parasitism was observed. A data logger was placed out in the field during each experiment to record temperature and humidity.

Data analysis

The percentage of eggs eaten on each card was grouped into four categories: none eaten, less than or equal to 50 percent eaten, greater than 50 percent eaten and all eggs eaten. A Pearson's Chi-squared test was performed on the data matrix. The data was then grouped into egg cards found and those not found (no eggs consumed) for each treatment. A second Chi-squared test was performed. The statistical program S-plus was used for all analyses.

7.A Time of day effects

What time of the day is the best to place egg cards in the field?

Materials and methods

Egg cards were placed in the field during October of 2000 at different times of the day. Egg cards (n = 42) were placed in a wheat field (post-flowering stage) and lucerne field (three weeks post cutting) in a grid pattern with five metres between each card. All cards were observed for six hours, over three time periods: day, 10am to 4pm; dusk, 4pm to 10pm and dawn, 4am to 10am. After the exposure period egg cards were collected transported to the laboratory and treated as above.

The number of egg cards found by predators in each time period was standardised for the temperature conditions in each time period. A data logger was placed out in the field for the duration of the experiment and temperature recorded every 15 minutes. The temperature was graphed over all three time periods and the area under the curve estimated. The number of cards found by predators per hour was then divided by the area under the temperature curve at each site.

Results and discussion

The average percentage of eggs predated on the cards was greatest during the day in the wheat (table 1). However the greatest percentage of cards with predators observed on them was seen during the dusk period. The day results for the wheat experiment are confounded by the fact that these cards were left in the field for eight hours rather than the standard six. The percentage of cards found by predators per hour (fig. 1) was greatest for the dawn period in wheat. When the results were standardised for the temperature in each time period the dawn period in wheat showed the greatest activity (fig. 1). The dawn period in the lucerne had the highest average predation rate (table 1) and the dusk period had the greatest number of observed predators. There was a significant relationship between the percentage of eggs lost and the time periods (lucerne $\chi^2 = 20.33$, d.f. = 6, $P < 0.01$, wheat $\chi^2 = 44.75$, d.f. = 6, $P < 0.01$).

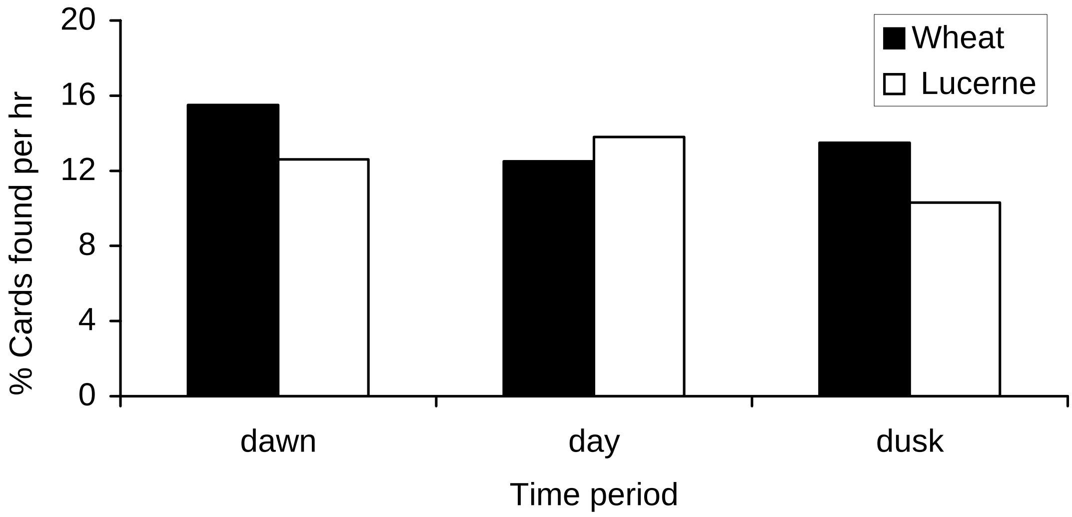
No common trends between the wheat and lucerne fields in the foraging pattern of predators across the time periods were observed (fig. 2). This may be partially due to the different predator groups that were observed foraging on the egg cards (Table 2 and 3). In lucerne Formicidae (ants) were the dominant predator group observed on the egg cards at dawn, day and dusk. Araneae were observed early morning and after sunset. The wheat had more diverse predator groups with Formicidae being rare. Scholz (2000) found Formicidae (*Iridomymex* spp.) to be significant predators of *Helicoverpa* spp. eggs on cards in a sweet corn field. Formicidae activity was restricted to the crop edges. This spatial pattern was attributed to cultivation practices that disrupt ant activity. Lucerne fields are not cultivated as frequently as conventional wheat fields and may support a more active and numerous ant fauna. Coccinellidae, adults and larvae, were observed throughout the day, and Clubionidae spiders in the morning and evening. Neuroptera (brown lacewing) adults and larvae were observed in the morning and during the cooler parts of the day. In lucerne, the greatest numbers of predators were observed after it became dark, and before it became too hot in the morning. The peak in activity at 9-10am in the wheat field was primarily due to foraging by lacewing larvae (fig. 2). No records were taken during the night period of 10pm to 4am. There are many species of nocturnal predators that may have been missed in this study.

Which time is best to put out egg cards will be influenced by the aim of the experiment. To observe which predators are feeding on *Helicoverpa* spp. eggs the dusk period would be best. This period had the highest numbers of predators seen on the cards feeding. Some predator groups that only forage at dawn may be missed. If you are interested in the numbers of eggs eaten then dawn or day time would be the most productive. For the purposes of this thesis the predator species observed on the cards and the predation rate of eggs per card are of importance. Therefore the dawn and dusk periods appear to be the best times for placing cards in the field. In these periods there is a high number of active predators (from a variety of groups) and temperature and humidity conditions are not extreme. Increased egg mortality may result from extreme weather conditions sometimes encountered in the field during the day (Titmarsh 1992). This may confound predation rates and reduce the number of eggs hatching per card.

Table 1. Summary of results of *H. armigera* egg cards exposed to predators at different times of the day (n = 42 cards per period). Note the underlined period (day in wheat) was two hours longer than the other experiments.

	Start time	End time	Exposure time	Mean % eggs predated/card (st.error)	% Cards with predators	Max. temp.	Min. temp.
Wheat							
Dawn	4am	10am	6hr	28.3 (4.58)	4.21	33	8
Day	8am	4pm	<u>8hr</u>	43.05 (3.93)	3.30	37	24
Dusk	4pm	10pm	6hr	38.45 (6.29)	4.4	27	14
Lucerne							
Dawn	4am	10am	6hr	30.71(6.29)	7.14	28	17
Day	10am	4pm	6hr	25.48 (4.80)	6.04	38	28
Dusk	4pm	10pm	6hr	16.9 (4.24)	7.88	31	17

A. Percentage of cards found per hour



B. Standardised data

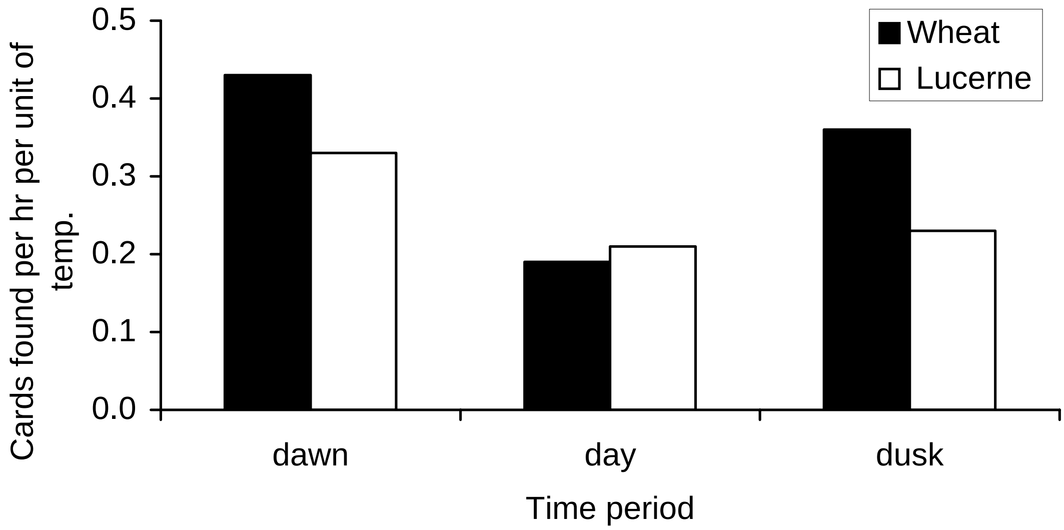
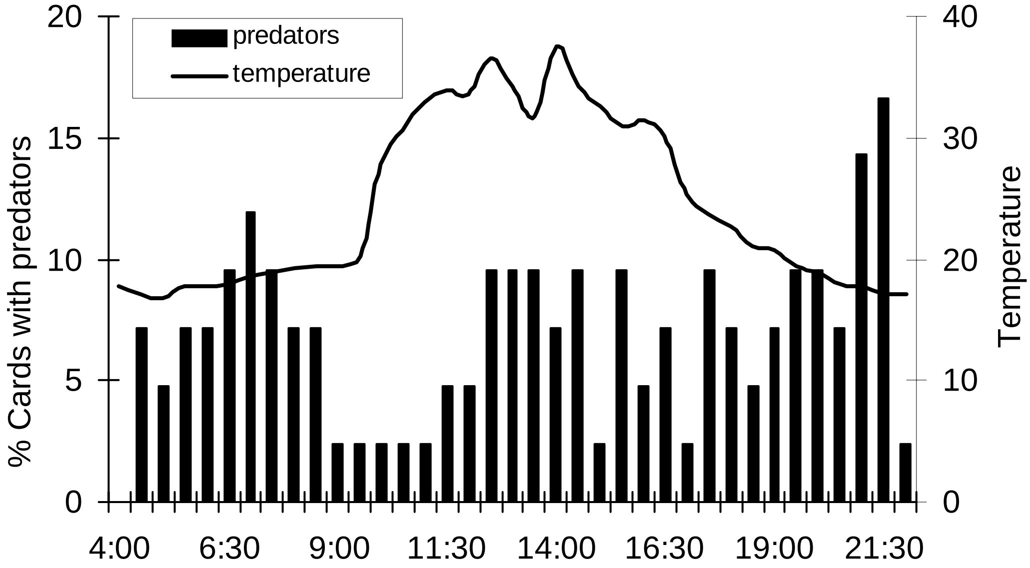


Figure 1. The percentage of *H. armigera* egg cards found by predators at different times of the day in a wheat and lucerne field. In **A.** the data is presented as the percentage of cards found per hour. In **B.** the data has been standardised for the temperature recorded for each time period.

A. Lucerne



B. Wheat

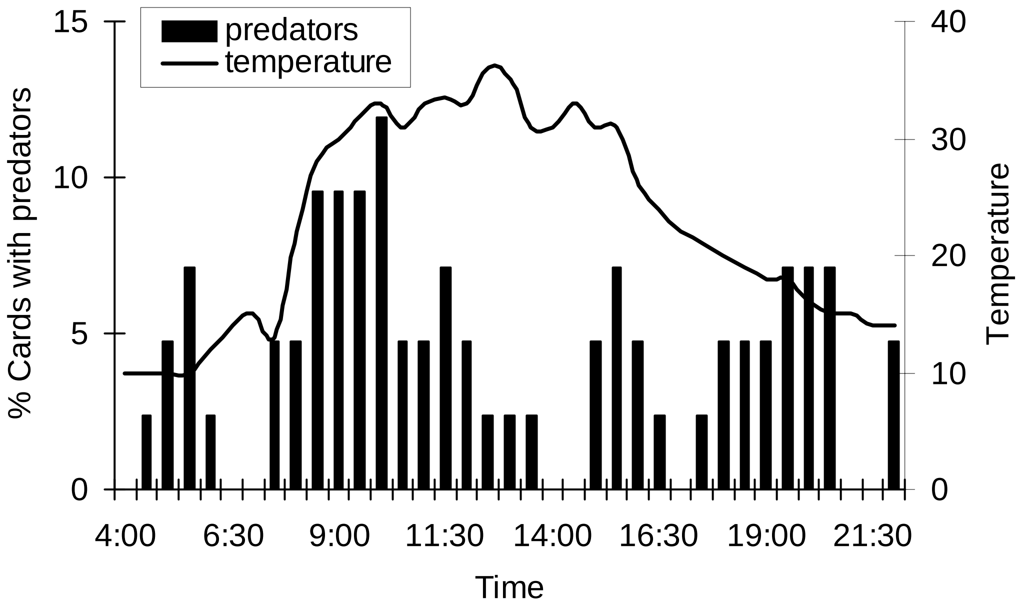


Figure 2. The foraging pattern of predators on *H. armigera* egg cards at different times of the day in **A.** lucerne and **B.** wheat fields. Observations were made on egg cards from 4:00am to 10:00am (dawn), 10:00am to 4:00pm (day) and 4:00pm to 10:00pm (dusk). Bars indicate the number of cards with predators seen on them during each observation period. The temperature (degree C) throughout the day, recorded on a data logger, is also shown.

Table 2. The foraging pattern of predator groups on *H. armigera* egg cards at different times of the day in a lucerne field. Stars indicate the presence of a predator on a single card, numbers indicate the number of cards on which the predator group was seen feeding at that time period (i.e. if they were seen on more than one card).

	T. pallidulus[1]	*M. frenata*[2]	small cricket	Clubionidae	Formicidae	unknown Araneae
4:00			*** 2**	*** 2**	*	
5:00			*** 2**		*** 3**	
6:00			*	*	*** 4**	
7:00		*			*** 4**	
8:00					*** 4**	
9:00					*** 2**	
10:00					*** 3**	
11:00					*** 3**	
12:00	*				*** 4**	
13:00					*** 5**	
14:00	*				*** 5**	
15:00					*** 5**	
16:00					*** 3**	
17:00					*** 4**	
18:00					*** 3**	*
19:00					*** 3**	*
20:00					*** 4**	*
21:00					*** 6**	*
22:00					*	

[1] *Taylorilygus pallidulus*, broken back bug.

[2] *Micraspis frenata*, striped ladybird.

Table 3. The foraging pattern of predator groups on *H. armigera* egg cards at different times of the day in a wheat field. Stars indicate the presence of a predator on a single card, numbers indicate the number of cards on which the predator group was seen feeding at that time period (i.e. if they were seen on more than one card).

	T. pallidulus[1]	*M. frenata*[2]	Ladybird larvae	*Micromus* spp.[3]	Lacewing larvae	small cricket	Clubionidae	Formicidae
4:00						*		
5:00				*		*	* 2	
6:00							*	
7:00			* 2			*		
8:00		*	* 2		*			
9:00		* 2	* 2		* 2			
10:00		*			*			*
11:00		* 2			*			
12:00		*						
13:00		*						
14:00								
15:00		* 3	*					
16:00	*	*	*					
17:00					*			
18:00					* 2		* 2	
19:00							* 4	
20:00							* 4	
21:00			*			*		
22:00			*			*		

[1] *Taylorilygus pallidulus*, broken back bug.

[2] *Micraspis frenata,* striped ladybird.

[3] *Micromus* spp., brown lacewing

7.B Egg density effects

What effect does egg density on cards have on egg predation and egg cards being found by predators?

Materials and methods

The effect of egg density on predation was investigated in a lucerne field in November, 2000 and a soybean field in March 2001. Three egg densities, five, 10 or 20 eggs per card, were used. Twenty replicate egg cards for each treatment were placed in a grid pattern in the lucerne field with five metres between each card. The treatments where assigned to each grid point randomly. In the lucerne field egg cards were observed at half-hourly intervals from 4pm to 10pm (six hours). At 10pm, 10 replicates of each treatment were collected and placed in separate zip lock bags and remaining egg cards were left in the field for a total of 24 hours. Half-hourly observations were not conducted on the egg cards placed in the soybean field.

<u>Data analysis</u>

Two Chi-squared tests (described previously) were performed on the data from the lucerne field after six hours exposure and 24 hours exposure, and the soybean field after 24 hours exposure. The exposure time was tested using a similar protocol and data from the egg cards with 20 eggs. A Fishers exact test was performed on the contingency tables.

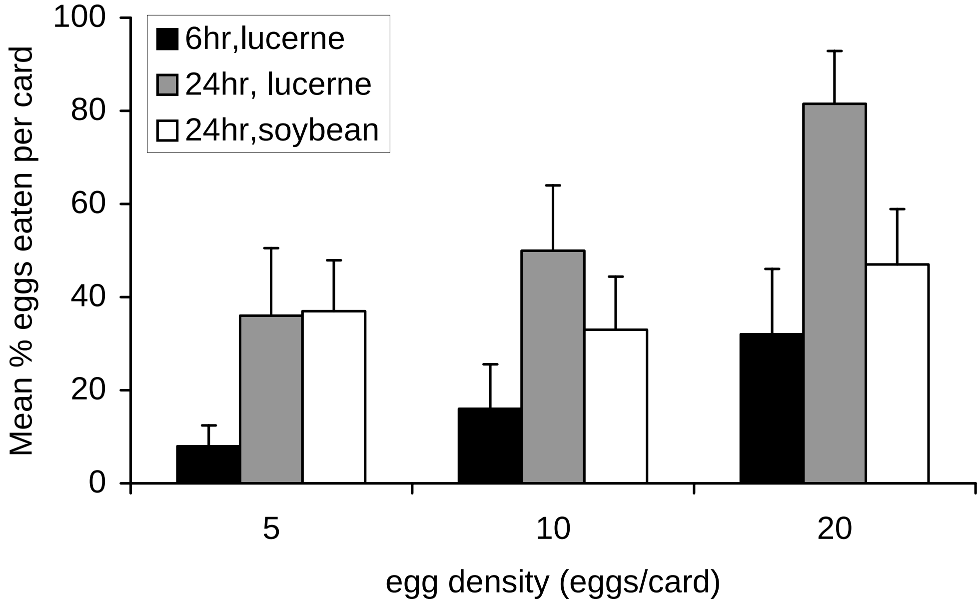
Results and discussion

Egg cards with greater numbers of eggs are easier for predators to find and proportionally more eggs are eaten than compared to lower density cards. The mean percentage of eggs lost rises as the egg density increases (fig. 3). The proportion of eggs eaten on each card was significantly related to egg density (lucerne 24 hours $\chi^2 = 128.98$, d.f. = 6, $P < 0.01$, lucerne six hours $\chi^2 = 62.75$, d.f. = 6, $P < 0.01$, soybean 24 hours $\chi^2 = 26.76$, d.f. = 6, $P < 0.01$). The likelihood of an egg card with small egg numbers being found by predators was lower than for egg cards with greater numbers of eggs (fig. 4).

Whether or not an egg card was found by predators was significantly related to egg density (lucerne 24 hours, $\chi^2 = 59.63$, d.f. = 2, $P < 0.01$; lucerne six hours, $\chi^2 = 18.75$, d.f. = 2, $P < 0.01$; soybean 24 hours, $\chi^2 = 22.42$, d.f. = 2, $P < 0.01$). This density dependent relationship is supported by Scholz (2000) who found that the parasitoid *Trichogramma pretiosum* Riley found high density (32 eggs per card) egg patches more quickly than low density patches (two eggs per card). However there was no significant difference in the levels of predation and parasitism for the egg densities studied. Predation rates calculated from egg cards containing 20 eggs may overestimate naturally occurring predation. This fact must be kept in mind when drawing conclusions about *Helicoverpa* spp. predation in the field from egg card experiments, given that *Helicoverpa* spp. generally lay only single eggs on plants. Many eggs may be laid per plant however a moth ovipositing 20 eggs on a single leaf is highly unlikely. Experiments using single eggs on a card and single eggs naturally laid, or painted onto leaves (i.e. no card see 7.E) would provide more information on the relationship between egg card predation and naturally occurring predation. The advantage of using 20 eggs per card is that information on predation can be obtained in the field in a relatively short time span. Experiments using single eggs on cards would require much longer exposures, and many cards, to ensure predators have time to find the prey item.

The egg cards placed in the lucerne field were exposed for either six hours or 24 hours. The predation rates obtained for the highest egg card density (20 eggs per card) showed a significant relationship between the percentage of eggs lost on the cards and exposure time ($\chi^2 = 53.33$, d.f. = 3, $P < 0.01$). After six hours 50 percent of the cards had been found by predators, compared to 90 percent of cards after 24 hours. The relationship between exposure time and egg cards being found by predators was significant ($P < 0.01$). For all egg densities the rate at which egg cards were found (cards found per hour) in the first six hours was higher than the rate at which they were found in the next 18 hours (fig. 4). Greater numbers of predators were recorded foraging on the cards within the lucerne at the end of the dusk time period (fig. 5, table 4). This peak in activity on the egg cards was mostly due to Formicidae (ants) finding more cards, however ants were present throughout the entire observation period (4pm to 10pm). Spiders increased in activity in the dusk period (table 4) in this experiment and in past experiments in lucerne (table 2) and wheat (table 3) fields. The parasitism rate in the soybean experiment was extremely high. Most (> 90%) of the eggs remaining on the cards were parasitised regardless of egg density. The wasps that hatched from the eggs were all *Trichogramma* spp. On the basis of these results a more detailed experiment was conducted on exposure time of egg cards in the field.

A. Eggs eaten per card



B. Cards found

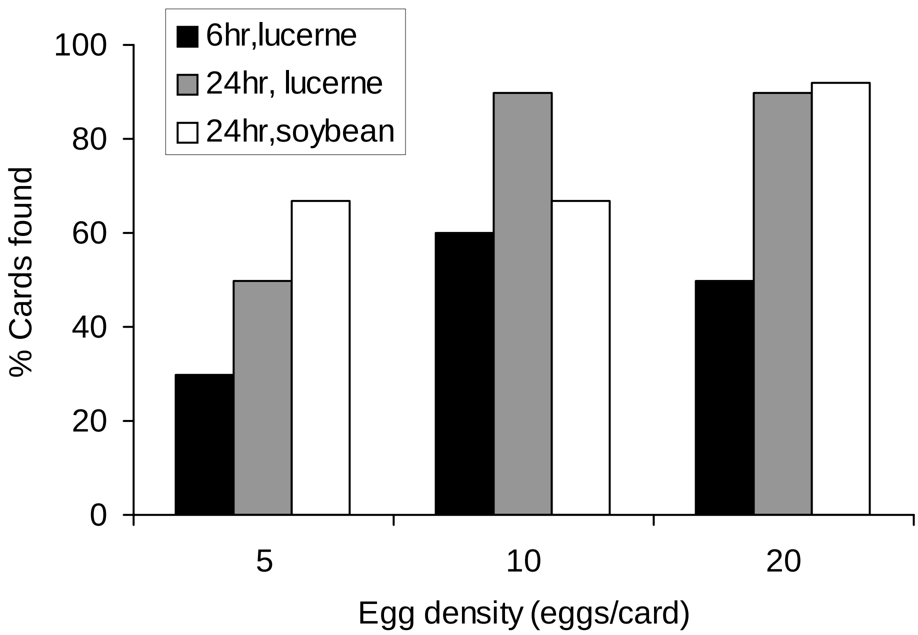


Figure 3. Predation rate of *H. armigera* eggs on cards with different densities of eggs. **A.** shows the predation rate in mean eggs eaten per card at different egg densities, **B.** shows the location rate of cards with different egg densities by predators. Cards were exposed for six hours and 24 hours in the lucerne and 24 hours in the soybean. Bars indicate standard error (n = 10 replicates per treatment).

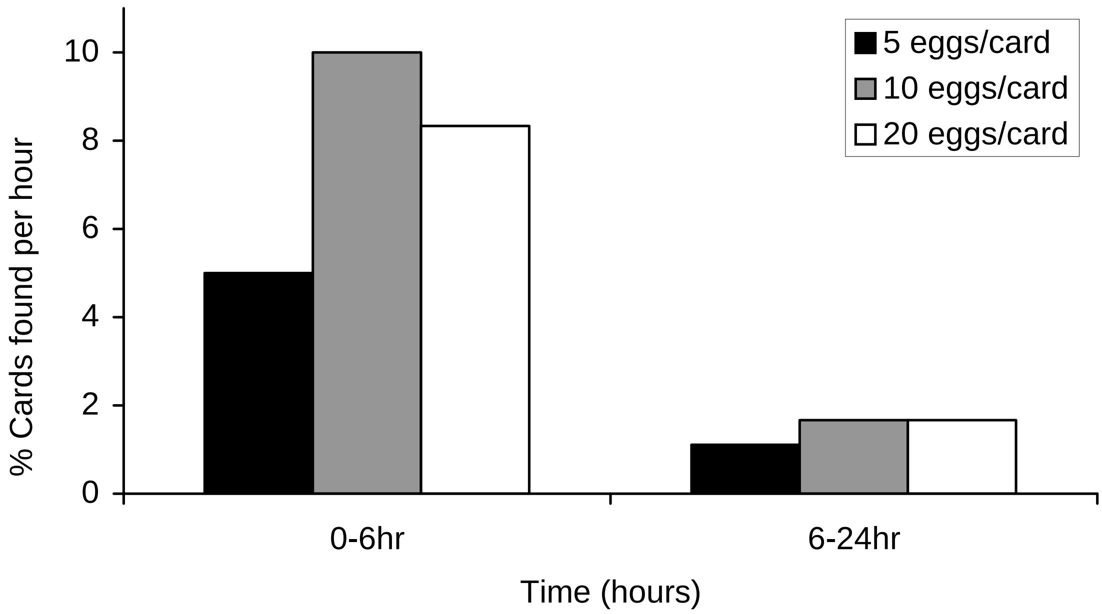


Figure 4. Location rate per hour of *H. armigera* eggs cards with different egg densities. Bars show the change in the rate at which cards were found during the first 6 hours (0-6) and the next 18 hours (6-24) in a lucerne field (n = 10 replicates per treatment).

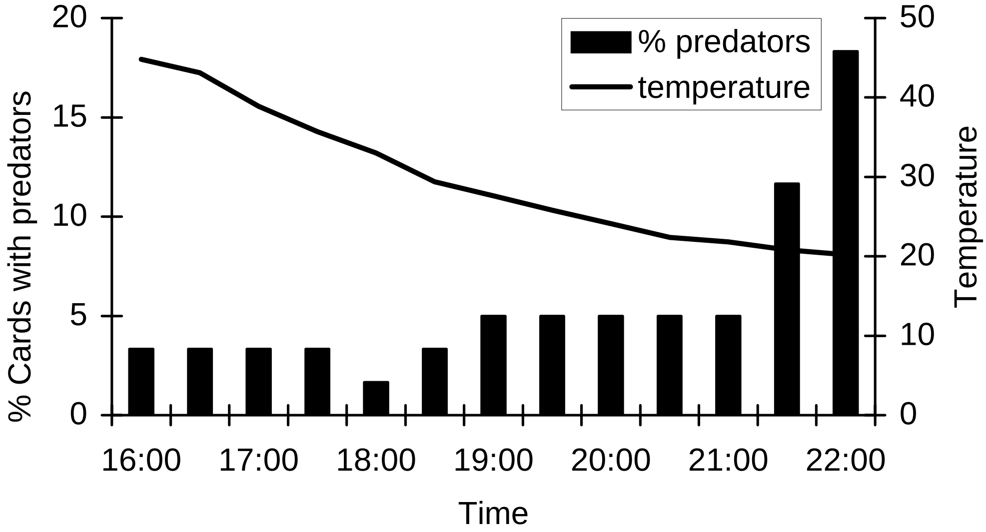


Figure 5. The foraging pattern of predators on *H. armigera* egg cards from 4pm to 10pm in a lucerne field. A total of 20 cards were exposed for six hours. Bars indicate the number of cards seen with predators during the observation period.

Table 4. The foraging pattern of predator groups on *H. armigera* egg cards at different times of the day in a lucerne field. Stars indicate the presence of a predator on a single card, numbers indicate the number of cards on which the predator group was seen feeding at that time period (i.e. if they were seen on more than one card).

	Oxyopidae	*M. frenata*[1]	small cricket	Clubionidae	Formicidae	*C. liebknechti*[2]
16:00					*** 3**	
17:00					*** 2**	
18:00			*		*	*
19:00		*	*		*** 3**	
20:00			*		*** 2**	
21:00	*** 2**			*	*** 5**	
22:00	*** 2**				*** 9**	

[1] *Micraspis frenata,* striped ladybird.

[2] *Campylomma liebknechti,* apple dimpling Bug

7.C and 7.D Effect of exposure time and egg condition

Does exposure time influence egg predation rate and the chance of egg cards being found?
Is there a difference in predation rate of fresh eggs (less than 24 hours old) and old eggs that have been stored before use?

Materials and methods

The predation rate of the eggs throughout 24 hours was examined further due to the results of the two time intervals trialed in the pervious preliminary experiment (7.B). Egg cards were prepared as described previously with 20 eggs per card. As an added treatment eggs that had been oviposited 24 hours earlier and stored in a cold temperature cabinet (13^0C, no light, 50% humidity) were used also. The treatments included egg cards exposed in the field for six, 12, 18, and 24 hours and old eggs exposed for 24 hours. In total 60 egg cards were placed in a grid pattern in lucerne with five metres between each card. Treatments were randomly allocated to each grid point. The egg cards where placed out at 4pm and the first collection occurred after six hours (10pm) and the final collection after 24 hours (4pm the next day). No observations were made on the egg cards whilst they were in the field.

Data analysis

The two Chi-squared tests (described previously) were performed on the data from egg cards in a lucerne field after six, 12, 18 and 24 hour exposure. The data comparing new eggs with old eggs was compared using the same protocol. A Fishers exact test was used to determine if egg predation was independent of treatment. The same test was used for egg cards found and not found by predators.

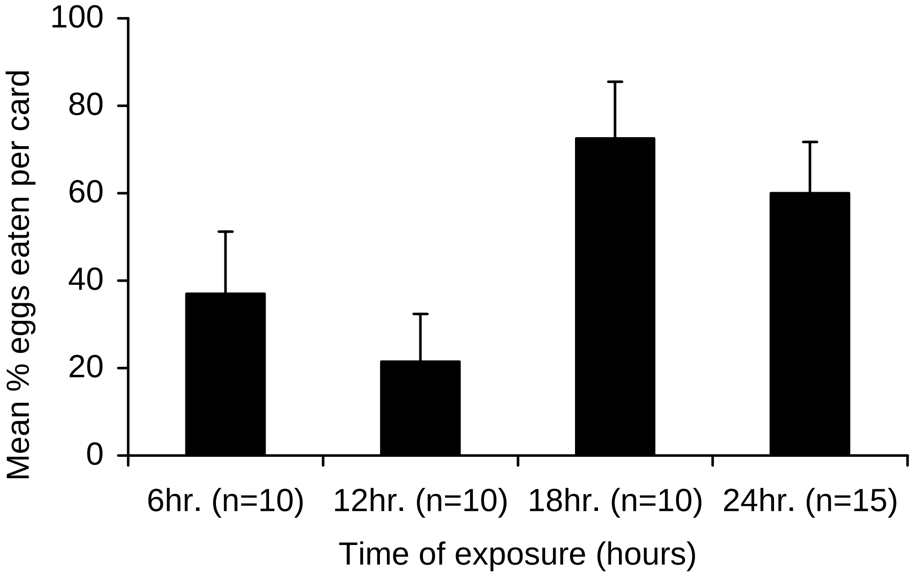
Results and discussion

The numbers of eggs lost per card and the number of cards found by predators increased as the exposure time in the field lengthened (fig. 6). There was a significant relationship between exposure time and the percentage of eggs lost on the cards (χ^2 = 122.69, d.f. = 9, $P < 0.01$). After 18 hours there was an average of 73% of eggs lost and after 24 hours 60%. The same trend was observed for egg cards found. After six hours 60 percent of cards were found, after 12 hours, 70 percent, after 18 hours, 100 percent and after 24 hours, 80 percent. This relationship was significant (χ^2 = 50.18, d.f. = 3, $P < 0.01$). There appeared to be little difference in predation rate between 24 and 18 hours. As no added predation was observed at

24 hours (all cards were found by predators after just 18 hours) it is preferable to leave cards out for the shorter time period.

There was no observable difference in predation rate of new eggs and old eggs that had been stored prior to use (fig. 7). There was no relationship between the percentage of eggs eaten on the cards and the treatments (P = 0.72). There was also no relationship between the numbers of egg cards found and the treatments (P = 0.60). Predators found 80 percent of the new egg cards compared to 93 percent of the old egg cards. This means that eggs that have been stored from the previous days egg lay can be used for making egg cards. This becomes essential when many egg cards are required in the field at the same time.

A. Eggs eaten per card



B. Number of cards found

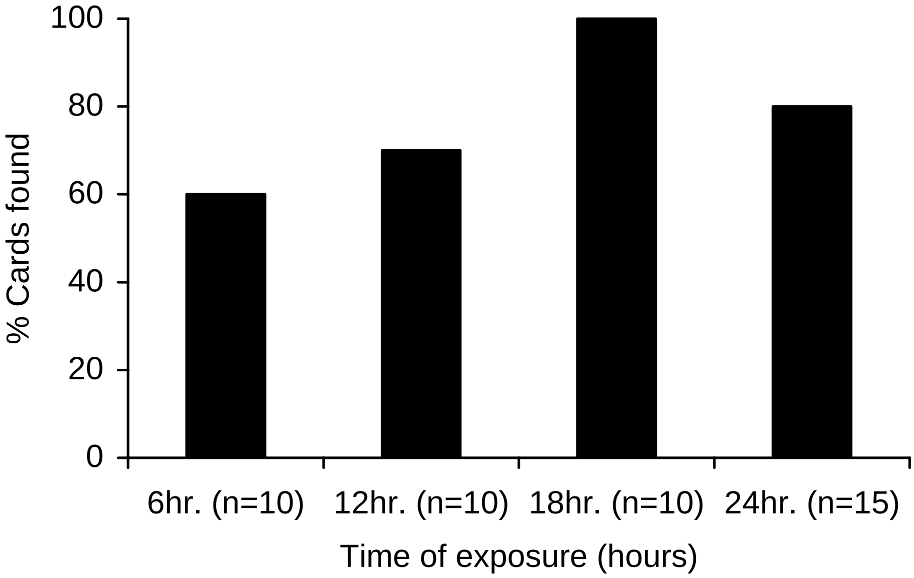
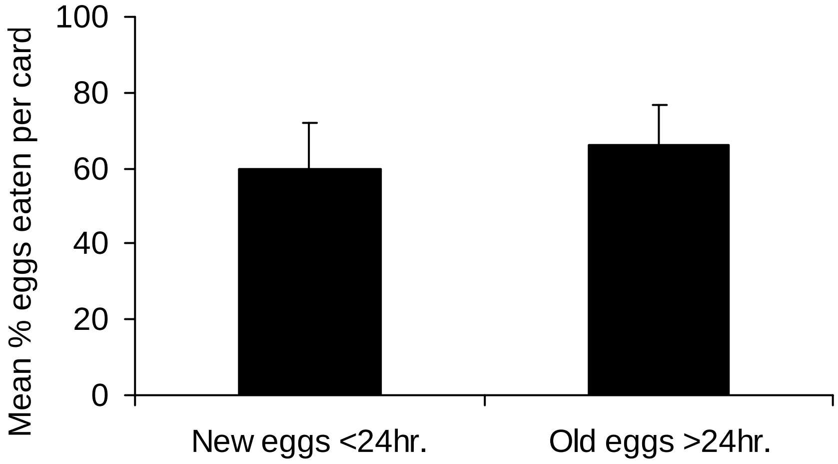


Figure 6. Predation rate **A.** of *H. armigera* eggs on cards exposed for different lengths of time and **B.** Location rate of cards exposed for different lengths of time in a lucerne field. Bars indicate standard error.

A. Eggs eaten per card



B. Percentage of eggs eaten

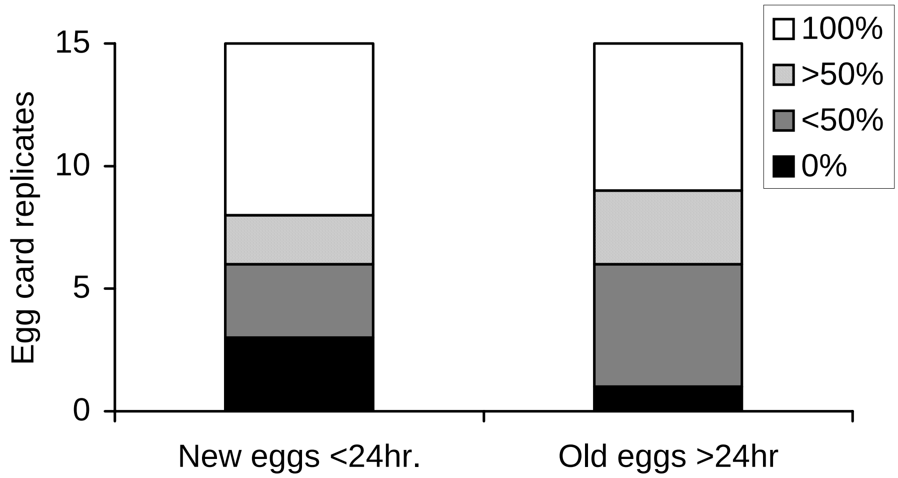


Figure 7. Eggs consumed on *H. armigera* egg cards made with fresh eggs (less than 24 hr old) or old (stored for greater than 24 hr) and exposed for 24 hr in a lucerne field. Mean predation rate **A.** of eggs per card and **B.** percentage of eggs consumed on each card. Bars indicate standard error (n = 15 cards per treatment).

7.E Effect of the card itself

Is there any difference between predation of eggs on egg cards and naturally laid eggs?

Materials and methods

This question was answered whilst conducting the spatial grid sampling (2000/01) and exclusion experiments using open and closed bags in soybean field Gilbert A (2001/02) (see Chapter eight). At 42 points within the grid egg cards were placed on a soybean plant or lucerne plant. At six points within the grid (one per grid row in the soybean) a control was set up on the plant next to the egg card. The control consisted of twenty naturally laid eggs on the upper surface of the soybean leaf. The previous day a mated female *H. armigera* moth was placed in a small upturned solo cup that was taped to the leaf. Overnight the moths oviposited onto the leaf surface. The moths and the solo cups were removed from the leaf just prior to the start of the exposure time and excess eggs were removed with a paintbrush. This was a difficult procedure that was not always successful because the moths often did not lay or died prior to oviposition. A more reliable second control was used were twenty eggs were placed on the upper side of a leaf using a paintbrush and some water. The egg cards and the controls were left exposed for 18 hours from 4pm to 10am the next day.

During the 2001/02 season a more concentrated effort was made to determine if there was a difference in predation observed on egg cards and naturally laid eggs. Due to the difficulties encountered with forcing moths to oviposit on leaves in the field only glued on eggs were used. Experiments were conducted which involved setting up sites within the soybean field at least 20 metres apart. At each site there was an egg card in a closed mesh bag, an egg card in an open bag, an exposed egg card and twenty eggs glued onto a leaf using a paintbrush and egg albimum. Each egg card consisted of 20 eggs and each of the treatments were one metre apart on the same soybean row. There was concern that the increased density of eggs within a small area would bias the predation rates observed. An extra treatment of a single egg on a card was placed at each of the sites. This was compared to a single egg on a card isolated from other treatments at 20 metres away from the site.

The experiment was conducted in Gilbert A soybean during January 2001. There were 10 replicates per treatment and 12 replicates for the single egg card that was isolated. The experiment was repeated during March 2001 with the same number of replicates. Treatments were exposed for 18 hours from 4pm to 10am the next day. The results of the exclusion experiment will be presented later (Chapter nine) and only those results relating to the exposed egg cards, single egg cards and the painted on eggs are relevant here.

Data analysis

The proportion mortality for each egg card was calculated by dividing the total numbers of eggs sucked, chewed and missing by 20. The average proportion mortality was compared between egg cards and painted on eggs for the grid data. The paired data (egg card and painted eggs at each site) from three grids in the 2000/01 season was used in the analysis. The data was not distributed normally so a Wilcoxon signed rank test was used to compare means. The number of egg cards found and not found (no eggs lost) for each treatment was calculated and compared. A contingency table was constructed with the percentage of egg cards found and not found for each treatment (egg cards and painted eggs). A Fishers exact test was used to assess if the number of cards found was independent of treatment.

The same tests were performed on the exclusion experiment data comparing exposed (no cage) egg cards and painted on eggs. The two experiments were analysed separately. An additional test was performed to determine if single eggs near the other treatments are more likely to be found by predators than those isolated from other treatments. A contingency table was constructed with the percentage of egg cards found and not found for each treatment (near treatments and isolated). A Fishers exact test was used to assess if the number of cards found was independent of treatment.

Results and discussion

Results of the exclusion studies in soybean (fig. 8) show that egg cards are easier for predators to find than eggs painted on leaves. In the first experiment on average 68 percent of the eggs on cards were lost compared to 40 percent for the painted eggs. In the second experiment the trend was the same with 56 percent of the eggs on cards lost compared to 45 percent for the painted eggs. Whilst more eggs were taken from the egg cards in both experiments this difference was not great enough to be significant (experiment one, $H = 1.89$, $N = 10$, $P = 0.06$, experiment two, $H = 0.46$, $N = 10$, $P = 0.65$). The results were different when looking at whether or not treatments were found by predators. For both experiments all egg cards were found (100%) however only half (50%) of the painted eggs were found. The percentage of egg cards found was significantly related to the treatment ($\chi^2 = 64.03$, d.f. = 1, $P < 0.01$).

The results from the grid data appear to be biased by the use of water rather than egg albimum to paint eggs onto leaves. The grid data showed that on average 70 percent of eggs that are painted on to the leaves are lost and 30 percent of those on egg cards (fig. 8). The difference between the means of the two treatments is significant (H = -3.55, N = 22, P < 0.01). The result is the same when looking at whether or not treatments were found by predators. All of the painted eggs were "found" (100 %) however only three quarters (73 %) of the egg cards were found. The percentage of egg cards found was significantly related to the treatment (P < 0.01). The egg albmium glued the eggs to the leaves much better than water so in the grid controls more eggs would have fallen off the leaves. These lost eggs were included in the mortality counts as being taken by chewing predators. The action of predators was inflated for the water painted eggs. This is supported by examination of the painted eggs in the exclusion experiments. Due to the strength of the egg albimum, chewing predators often left egg remnants on the leaf, however the leaf surface of the water controls was usually clean. The results of the exclusion experiments are a more accurate representation of the effect of egg cards and painted eggs on predation rate.

It could be argued that painted on eggs are not comparable to naturally laid eggs. It is unknown what prey-derived cues predators detect when looking for prey and at what spatial scale such cues operate. It has been shown that parasitoids can be attracted to eggs by volatiles produced by plants in response to oviposition (Hilker & Meiners 2002). A similar response has not yet been demonstrated for predators but predators can respond to plant volatiles released in response to larval feeding damage (Shimoda & Dicke 2000, Shimoda & Takabayashi 2001). When using egg cards plant responses to oviposition would be absent, however plant responses to the mechanical damage caused by stapling the card onto the leaf may have an effect. Painted on eggs do alleviate the need for the card itself. One possible reason why more eggs on cards were eaten is because the cards themselves are attractive, or just easier to find amongst a mass of green soybean plants. Painted on eggs provide an adequate control for the card itself.

The single eggs on cards were included in the exclusion experiments to test if the clumping of treatments together on the same row made it easier for predators to find cards and consume eggs. In the first experiment predators found 30 percent of the single egg cards within the other treatments and 42 percent of the single egg cards isolated from the other treatments. The percentage of egg cards found was independent of treatment (P = 0.10). Cards isolated from the other treatments were found just as easily as those within other treatments, suggesting no localised effect of increased egg density. In the second experiment 40 percent of single egg cards within other treatments were found by predators, however none of the isolated single egg cards were found. The percentage of egg cards found was significantly related to the treatment ($P < 0.01$). This weak clumping effect may be partially due to the small number of replicates for each treatment. If the two experiments are combined the percentage of egg cards found is not significantly related to the treatment ($P = 0.09$). When the two experiments are combined predators found 35 percent of the single egg cards within the other treatments and 23 percent of the isolated single egg cards.

The results suggest that having egg cards within one metre of each other on the same row does not greatly increase their likelihood of being found by a predator. This may not be the case for cards placed on the same plant or even adjacent plants. Further experiments would provide valuable information on the foraging behaviour of predators in the field. In particular whether after encountering prey items on one plant the predator then spends time searching on the same plant or adjacent plants for more prey items.

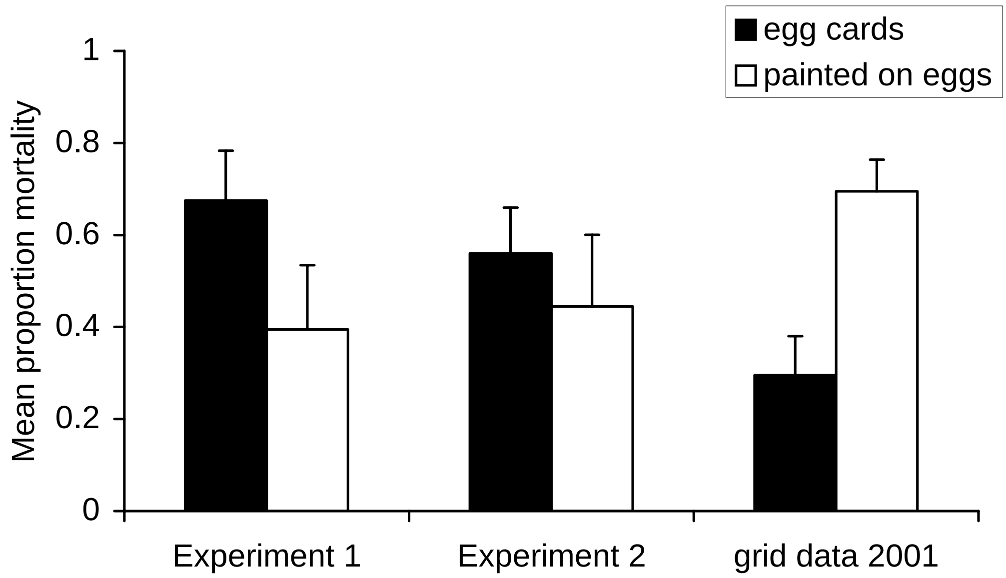


Figure 8. Predation of *H. armigera* eggs on egg cards or painted onto the leaf surface. The eggs were placed in a soybean field for 18 hours. Experiments 1 and 2 were conducted in the same field in the 2001/02 season and the grid data comes from three grids in the 2000/01 season (see Chapter eight). The painted on eggs in the grid data experiments were painted onto the leaf using water, in experiments one and two the eggs were painted on with egg albimum. Bars indicate standard error.

Chapter Summary

- The dawn and dusk periods appear to be the best times for placing cards in the field. In these periods there is a high number of active predators (from a variety of groups).
- Egg cards with greater numbers of eggs on them are easier for predators to find and proportionally more eggs are eaten than compared to lower density cards. Predation rates calculated from egg cards containing 20 eggs will overestimate predation rate. The advantage of using 20 eggs per card is that information on predation can be obtained in the field in a relatively short time span.
- The number of eggs on cards lost and the number of cards found by predators increased as the exposure time in the field lengthened. There appeared to be little difference in predation rate between 24 and 18 hours. It is preferable to leave cards out for the shorter time period (18 hours) to decrease effort required to obtain predation estimates.
- There was no observable difference in predation rate of new eggs and old eggs that had been stored prior to use. Eggs that have been stored from the previous days egg lay can be used for making egg cards.
- The predation rate of eggs on cards is higher than that for eggs painted on leaves. This difference was found to be not significant. The technique used to attach the eggs to the leaf (water or egg albimum) greatly affects the results obtained. Egg cards seem to overestimate true predation rate on naturally laid eggs in the field.
- Egg cards placed within one metre of each other on the same row does not increase their likely hood of being predated. This may not be the case for cards placed on the same plant or even adjacent plants.
- The following protocol for egg card experiments will be followed for the remainder of the thesis: Egg cards will be made in the morning from fresh eggs and older stored eggs if necessary. There will be twenty eggs per card. They will be placed out in the field at 4:00pm and exposed for 18 hours then collected at 10:00am the next day. Observations will be conducted from 4pm to 10pm and 4am to 10am if required.
- This protocol enables estimates of relative egg predation in the field (even if they are inflated) to be obtained in a reasonable time frame and is logistically acceptable. The results of egg card studies will provide a relative estimate of predation that can be used to investigate variation in within-field predator activity (see Chapter eight).

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