

## Chapter Nine

### ***H. armigera* Egg and Larval Mortality in the Field: How Much is Due to Predation?**

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## Introduction

*Helicoverpa* spp. like most Lepidopteran species experience very high rates of mortality at the first instar larval stage (Zalucki *et al.* 2002). Factors such as predation, competition, pathogens, locally induced plant changes and microclimate changes have all been suggested as causes of mortality (Zalucki *et al.* 1986, Kyi *et al.* 1991, Zalucki *et al.* 2002). The relative importance of each factor in the field is rarely determined. Life table studies have improved our knowledge of survival across stages but have rarely managed to clearly demonstrate the causative agents of mortality in each stage. Titmarsh (1992) conducted a thorough study on the mortality of immature *Helicoverpa* spp. in a variety of agricultural crops on the Darling Downs. Cohorts within exclusion cages were used to estimate stage-specific effects of predation. The results were confounded by the presence of the cage but predators appeared to be a relatively unimportant source of mortality of early stages of *Helicoverpa* spp. This result was attributed to insecticide applications in the study area that reduced predator numbers overall. The majority of *Helicoverpa* spp. mortality was explained by plant host effects, and to a lesser extent weather conditions. Another study in cotton (Stanley 1997) found similarly high levels of background mortality of *H. punctigera* neonates in cages. When predators were added to cages that had been seeded with *H. punctigera* larvae and left for six days, no discernible additional losses above the 70 percent recorded in the control cages was observed (Stanley 1997). Titmarsh (1992) reported poor larval survival on soybean but recorded no evidence of larval predation. Failure to detect predation of first instars was attributed to higher humidity within the cages for a number of days caused by rain or irrigation. It seems inappropriate to dismiss the impact of predators on *Helicoverpa* spp. larval survival in soybean until further studies have been conducted.

The literature surrounding exclusion experiments, utilising cages or insecticides, for estimating predator impact in the field will be reviewed in other chapters (see Chapter ten). Studies by Titmarsh (1992) illustrate many of the confounding effects associated with exclusion cages. The presence of the cage not only restricts movement of the predators and the pests but also changes the environmental conditions for both. Despite these difficulties exclusion cages are useful for investigating mortality of mobile prey stages such as *Helicoverpa* spp. first instar larvae. If results from cage experiments are to be relevant to the field situation, the conditions in the cage must be similar to that in the field (Seymour & Jones 1991). Generally, larger cages provide a high level of environmental complexity because lots of plants can be enclosed (Stanley 1997). However, it can be time consuming to search for introduced prey and/or predators in large cages and they are costly to produce. As a result

large cage studies often suffer from low replication and variable results. Smaller cages, enclosing a single plant, or parts of a single plant, are easier to search and less costly to produce in large numbers. However a high prey density within a small cage can lead to confounding effects resulting from food shortage and cannibalism.

Here I use small exclusion cages to estimate the proportion of mortality caused by naturally occurring predators of *H. armigera* first instar larvae in soybean fields. Some experiments were also conducted using *H. armigera* eggs. The relative importance of different predator groups was evaluated using different exclusion techniques and a predator addition treatment.

## **Materials and methods**

### Larval survival: Preliminary experiment (2000/01)

A preliminary experiment using exclusion cages was conducted in Mendel soybean field late in the first season (2000/01). *H. armigera* egg cards were made using 20 eggs laid two days earlier (brown ring stage) so that the larvae were near to hatching (see Chapter seven). Cards were transported to the field and stapled to the upper side of leaves in the top third of the plant. Target plants were isolated from surrounding plants by cutting out contiguous plants in the same row, and trimming leaves from plants in adjacent rows. There were three treatments; open cage, closed cage and no cage and ten replicate egg cards per treatment. Cages consisted of a vacuum sampler bag (20cm diameter opening, 40cm length), which had an opening at one end and was made out of a fine mesh (0.5mm) material. Arthropods were removed from plants by shaking them and carefully examining the leaves that were eventually enclosed in the cages. Egg cards were attached to a leaf then a cage was placed over a number of stems and sealed using string. For the open cage treatments the bag was not tied shut. For the no cage treatment the egg card was left exposed.

Cards were checked every two days for a total of eight days. The egg card leaf and surrounding leaves on the same plant were examined for larvae and their stage and location recorded. A waterproof pen was used to mark the location of the larvae on the leaf. On the final day each plant was destructively examined by firstly cutting off the egg card leaf then removing all leaves until the stem was exposed. All leaves and the plant stem were examined for larvae. Cages were gently removed and the insides of the bags examined for larvae.

Larval survival: Further experiments (2001/02 and 2002/03)

The preliminary experiment was repeated in a number of fields with minor modifications to the methodology in the next season (2001/02). Three exclusion experiments (early, mid and late season) were conducted in Gilbert A soybean and one experiment was conducted in Gilbert C soybean field late in the season. One experiment was conducted in Harvey mungbean field late in the season (2001/02). There were three treatments; open cage, closed cage and no cage with 10 to 12 replicates per treatment. Cages consisted of bags (30cm diameter opening, 40cm length) made from the same fine mesh (0.5mm) material as the vacuum sampler bags but they were open at both ends to allow easy access. Drawstrings at both ends were used to seal the bag. Experiments ran for six days and on the final day each plant was destructively examined and the surviving larvae counted.

In the final season (2002/03) larval exclusion experiments were conducted within a single soybean field (Gilbert A). The methods were the same as in previous seasons, however extra treatments were added. A ground cage treatment was used to exclude ground dwelling predators from the plant. The cage consisted of a rectangular plastic container (size) with the bottom removed. The container was placed over a group of plants and buried two centimetres into the soil. A thick line of Tangle-trap<sup>®</sup> (The Tanglefoot Company, Grand Rapids, United States) was painted around the outside edge to prevent arthropods climbing up the cage and onto the plants. Leaves touching the ground were removed and the plant was separated from adjacent plants in the same row.

A predator inclusion treatment involved a closed cage into which predators were added for the duration of the experiment (six days). In the first experiment two adult *Dicranolaius bellulus* (Guerin-Meneville) beetles (red and blue beetles) were introduced per cage, in the second experiment two adult *Harmonia octomaculata* (Fabricius) beetles (maculate ladybirds) were introduced per cage. Predators were collected from the soybean field and adjacent lucerne using a sweep net and held individually in solo cups (14:10 light:dark; 24:20<sup>0</sup>C; 65% relative humidity) for 24 hours without food prior to use.

Egg survival (2001/02)

The survival of *H. armigera* eggs in exclusion cages was briefly investigated as part of a preliminary experiment on egg cards (see Chapter three). Two experiments (mid and late season) were conducted in Gilbert A soybean field. Egg cards were made using 20 freshly

laid (less than 24 hours old) *H. armigera* eggs per card. There were three treatments; open cage, closed cage and no cage and ten replicate egg cards per treatment. Egg cards were left in the field for 18 hours, from 4:00pm to 10:00am the next day, and then returned to the laboratory and examined under a dissecting microscope. Egg mortality was attributed to sucking predators or chewing predators according to the condition of egg remains (see Chapter seven). Missing eggs were counted as chewing predators and the remaining eggs (not consumed) were recorded. Egg cards were stored in a temperature cabinet (14:10 light:dark; 24:20<sup>0</sup>C; 65% relative humidity) until the remaining eggs hatched, or parasitism was observed.

### Data analysis

The loss of larvae in the field was attributed to a number of factors. Plant effects include larval death due to host plant defences and poor nutrition. Whilst plant effects were not directly assessed in this study, previous research has shown that they can affect *Helicoverpa* spp. larval survival (Kyi *et al.* 1991). Plant effects were not assessed in egg experiments because eggs were laid on paper towel not directly onto the leaf surface. Physical effects such as rain, heat and humidity may directly cause the death of a larva or cause them to fall off the plant and then die. Predator effects included the actions of generalist predators as well as parasitoids, diseases, and viruses. Other effects included death due to operator error and larvae not counted on plant by researcher, or larva moved out of search area. Their effects were believed to be small in most experiments.

It was assumed that mortality factors operating in each of the treatments could be partitioned as follows:

Treatment	Mortality due to:			
No cage	Plant effects	Physical factors	All Predators	Other
Open cage	Plant effects		All Predators	Other
Closed cage	Plant effects			Other
Ground cage	Plant effects	Physical factors	Foliage-dwelling Predators	Other
Closed cage + Predators	Plant effects		Added Predators	Other

The partitioning of mortality factors in each treatment lead to three simple equations:

1. No cage - Closed cage = Mortality due to Predators + Physical factors
2. Open cage - Closed cage = Mortality due to Predators
3. No cage - Open cage = Mortality due to Physical factors

4. Closed cage + Pred. – Closed cage = Mortality due to added Predators

5. No cage – Ground cage = Mortality due to ground-dwelling predators

These equations are an oversimplification of what occurs in the field but they provided a estimate of mortality imposed by each factor. Maximum predation rate (equation one) and minimum predation rate (equation two) were expressed as a percentage. Equation two could not be calculated for the Harvey experiment in mungbean and the second egg mortality experiment in Gilbert A because the open cage treatment did not fall between closed cage and no cage results.

For statistical analysis results were expressed as mean proportion of eggs or larvae surviving per treatment at the end of the exposure period. Data was arc sin transformed for normality before analysis. Treatment means in the preliminary experiment were compared using a nonparametric one-way ANOVA (Kruskal Wallis rank sum test). In the next season (2001/02) the four soybean experiments were combined and analysed using a two-way ANOVA. The same analysis was repeated on the four soybean experiments in the final season (2002/03). The interaction between treatment and experiment was tested in each season. A nonparametric one-way ANOVA was used to compare the treatment means in the single mungbean experiment (2001/02). The same analysis was used for each exclusion experiment using *H. armigera* egg cards (2001/02). The two ground predator exclusion experiments (2002/03) were combined and analysed using a two-way ANOVA. The two predator inclusion experiments were both analysed separately using a nonparametric one-way ANOVA. All statistical analysis was conducted in the S-Plus program.

#### Environmental conditions in cages

An experiment was conducted to see if environmental conditions within the cage treatments (open and closed) differed from natural field conditions. Three data loggers were taped to the stem of a soybean plant in Gilbert A field. One data logger had a closed cage sealed over it, the second had an open cage, and the third was left exposed. Temperature and humidity were recorded every 15 minutes for 24 hours. This experiment was repeated in the following season.

#### Predator abundance

For some exclusion experiments an estimate of arthropod abundance was obtained using beat sheet samples. One-metre samples were taken at ten sites per experiment in the same area as the cage treatments. The beat sheet was laid on the ground with the tail of the sheet covering

the adjacent row and the target row knocked over the sheet four times using a stick. Arthropods falling onto the sheet were recorded. Numbers of predators was totalled for all ten beat sheets and the predator to pest ratio calculated so larval and egg mortality could be related to predator abundance.

#### No-choice feeding tests in laboratory

Feeding tests were performed on the two species of predator used in the predator inclusion experiments in season 2002/03. *H. octomaculata* and *D. bellulus* were collected from soybean (Gilbert A) and lucerne fields using a sweep net from February to March 2003. Predators were kept chilled whilst being transported back to the laboratory where they were individually placed in petri dishes with a moist dental wick. They were each starved for 24 hours in a constant temperature cabinet (14:10 light:dark; 24:20<sup>0</sup>C; 65% relative humidity) before being offered prey items. Each predator was used once.

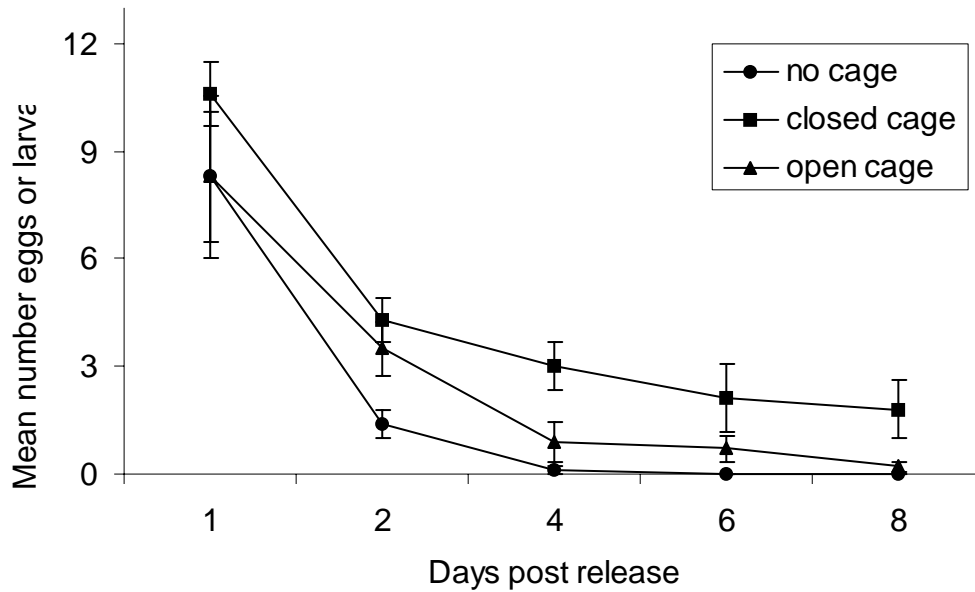
Fresh (less than 24 hours old) *H. armigera* eggs oviposited on paper towel were counted and cut into 20 egg sections. The 20 eggs were placed in each petri dish and the predators returned to the constant temperature cabinet for a further 24 hours. After the exposure time the numbers of eggs that had been consumed by each predator was recorded. For the larvae feeding experiments ten first instar *H. armigera* larvae were transferred to a petri dish using a fine brush. The dish was sealed around the edges with masking tape and left for 24 hours and the number of larvae eaten was recorded. Control Petri dishes consisting of ten larvae and no predators were left for 24 hours. Numbers of larvae that had disappeared during this period was recorded and used to correct the results. Individual *H. octomaculata* were offered eggs (n = 10) and first instar larvae (n = 12) but individual *D. bellulus* (n = 7) were only offered eggs.

### **Results**

#### Larval survival: Preliminary experiment

After eight days the proportion of larvae that survived in closed cage treatment (0.17) was significantly greater than in open cage (0.03) and no cage treatments (0.0) ( $H_{10} = 10.19$ ,  $P < 0.01$ ) (fig. 1). There was a very high rate of mortality in the closed cage treatments. Between 17 and 14 percent of the mortality could be attributed to the action of predators and three percent to physical factors. In all treatments none of the eggs hatched straight away and only half of original eggs survived to hatch into larva (fig. 1). In future experiments eggs were released just prior to the larva hatching (dark head stage), rather than at the brown ring

stage. Additionally a single larval count at the end of six days and a new cage that had drawstrings at both ends were used to reduce disturbance to plants and larvae.



**Figure 1.** Mean numbers of *H. armigera* eggs or larva surviving in a closed cage, open cage and no cage (exposed) treatments in a soybean field. There were ten replicates per treatment and the prey was exposed for eight days. On day 1 prey was at egg stage, days 2 to 6 very small larval stage and by day 8 small larvae stage. The bars indicate standard error.

Larval survival: Further experiments

In all experiments the greatest survival of larvae was observed in the closed cage treatments, which excluded predators (fig. 2). In most experiments survival in the open cage treatments was in between that of closed cage and no cage treatments. For all experiments in both seasons there was a significant difference between the proportions of larvae surviving in each of the treatments (2001/02  $F_{2,119} = 85.17$ ,  $P < 0.01$ , 2002/03  $F_{2,111} = 43.27$ ,  $P < 0.01$ ). In the second season there was a significant difference between experiments ( $F_{3,111} = 11.38$ ,  $P < 0.01$ ) within the season and a significant interaction between treatment and experiment ( $F_{6,111} = 3.22$ ,  $P < 0.01$ ). Overall larval survival was very low within soybean fields (mean proportion surviving =  $0.10 \pm 0.03$  standard error,  $n = 8$  experiments) when all mortality factors were combined (i.e. the no cage treatments).

In Gilbert A soybean field (season 2001/02) there was a decrease in larval survival in the closed cage treatment as the plants matured. Maximum percentage mortality due to predators decreased over the season: from 38% (early) to 25% (late), (table 1). The mortality attributed to physical factors ranged from three to 17 percent in Gilbert A field (table 1). Average predator mortality estimates across the whole season in Gilbert A field ranged from a maximum of 33 percent to a minimum of 23 percent, with 10 percent mortality due to physical factors. The lowest survival rate was seen in the mungbean field (Harvey) and the percentage mortality that could be attributed to the action of predators was very low (15%) (table 1). The mungbean field had fewer predators (total 24 per 10m of row) and the predator to pest ratio was low (Predator: Pest = 0.69) in comparison to soybean fields (Gilbert A late 24 predators per 10m of row, Predator: Pest = 1.70), (table 2). In the mungbean field there was still a significant difference between mean larval survival in each of the treatments ( $p$ -value  $< 0.01$ ). In the second season survival of larvae in the no cage treatments decreased as the season progressed (fig. 2). Abundance of predators increased in the soybean field as the season progressed (table 2). However the maximum mortality that could be attributed to the action of predators fluctuated between 14 (early1) and 53 (mid2) percent (table 1). In the final season (2002/03) mortality attributed to physical factors ranged from four to 25 percent.

Ground-dwelling predator exclusion

Survival of larvae in the ground cage treatments was usually in between that of the closed cage and no cage treatments (fig. 3). In one experiment survival was the same as in the no cage treatment. The mortality that could be attributed to the actions of ground-dwelling

predators ranged from none to nine percent (table 1). There was a significant difference between mean larval survival in each of the treatments ( $F_{2,84} = 27.30, P < 0.01$ ) and between each experiment ( $F_{2,84} = 29.77, P < 0.01$ ). The ground cages used did not exclude all ground-dwelling predators but did reduce the numbers gaining access to the plants and larvae.

#### Predator inclusion experiments

In the first predator inclusion experiment (using *D. bellulus*) mean larval survival in each of the treatments was significantly different ( $H_{11} = 13.07, P < 0.01$ ) (fig. 3). However there was very little difference between mean survival in the closed cage treatments, mean proportion survived 0.40 ( $\pm 0.03$ ) and the closed cage with predators added, 0.31 ( $\pm 0.06$ ). Mortality that could be attributed to the actions of the caged predators was only eight percent (table 1). In the second experiment (using *H. octomaculata*) survival in predator inclusion treatments was closer to that found in the no cage treatments. There was a significant difference between mean larval survival in each treatment ( $H_9 = 18.57, P < 0.01$ ). The mortality estimate was much higher at 50 percent (table 1).

In the laboratory no-choice feeding tests both *D. bellulus* and *H. octomaculata* individuals consumed nearly all of the *H. armigera* eggs offered to them over 24 hours. *D. bellulus* individuals consumed an average of 78 percent ( $\pm 8.6$ ) of eggs offered, equal to 16 eggs in 24 hours. *H. octomaculata* individuals consumed an average of 99 percent ( $\pm 0.8$ ) of eggs offered, equal to 20 eggs and 84 percent ( $\pm 2.9$ ) of larvae offered, equal to 8 first instar larvae in 24 hours.

#### Egg survival

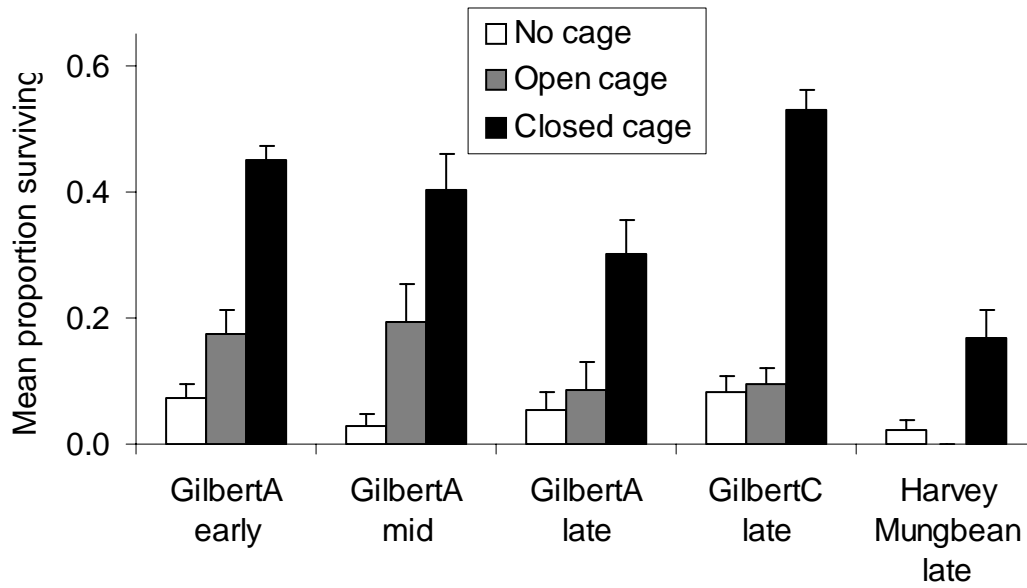
Egg mortality estimates were obtained after egg cards had been exposed in a soybean field for 18 hours. The greatest number of eggs survived in the closed cage treatments for the experiments early and late in the season (fig. 4). There was a significant difference between the mean proportions of eggs surviving in each of the treatments (first experiment  $H_{10} = 13.07, P < 0.01$ , second experiment  $H_{10} = 10.23, P < 0.01$ ). The proportion of mortality that could be attributed to the action of predators was higher for the early experiment (56%) than for the late season experiment (28%) (table 3).

#### Environmental conditions in cages

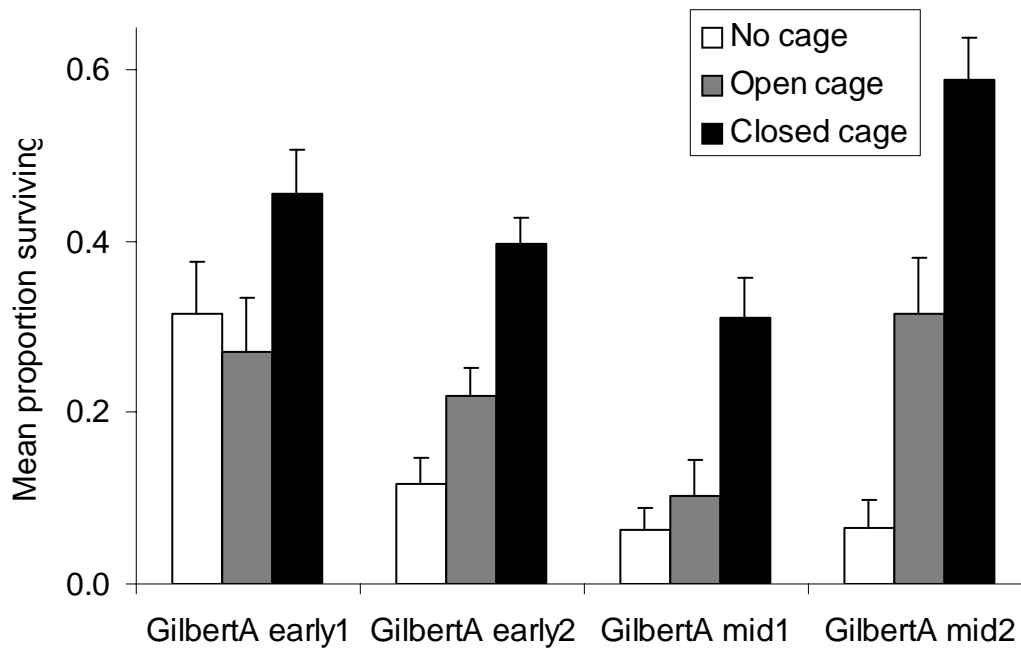
Temperature and humidity in a single closed cage, open cage and no cage was measured in soybean using data loggers (fig. 5). Overall mean humidity across 24 hours was lower in the

no cage treatment (mean  $65.5 \pm 1.8$ ) than compared to the open cage ( $69.1 \pm 2.2$ ), and the closed cage ( $66.5 \pm 2.1$ ). However, this difference between treatments was small. A similar trend was seen for temperature in each of the treatments: no cage ( $25.6 \pm 0.5$ ), open cage ( $26.3 \pm 0.6$ ) and closed cage ( $26.1 \pm 0.5$ ). Almost identical results were obtained in the following season so they have not been presented here.

A. Season 2001/02



B. Season 2002/03



**Figure 2.** Proportion of *H. armigera* larvae that survived in a closed cage, open cage and no cage (exposed) in the field over two seasons. Twenty larvae were used for each treatment (10-12 replicates per treatment) and each experiment lasted for six days. All experiments were conducted in soybean with the exception of a single experiment in a mungbean crop. The bars indicate standard error.

**Table 1.** Estimates of percentage mortality of *H. armigera* first instar larvae imposed by predators and physical factors in each exclusion experiment.

Field	Time during season	Days exposed	eqn 1. Max. predation	eqn 2. Min. predation	eqn 3. Physical factors	eqn 4. Added predators <sup>1</sup>	eqn 5. Ground predators <sup>2</sup>
Season 2001/02							
Gilbert A	early	5	37.7	27.4	10.3	-	-
Gilbert A	mid	6	37.3	20.7	16.6	-	-
Gilbert A	late	6	24.6	21.5	3.1	-	-
Gilbert C	late	6	44.9	43.6	1.3	-	-
Harvey <sup>3</sup>	late	6	14.6	NA	NA	-	-
Season 2002/03							
Gilbert A	early1	6	14.0	NA	NA	-	8.9
Gilbert A	early2	6	28.1	17.8	10.3	8.3	6.2
Gilbert A	mid1	6	24.9	20.9	4.0	-	0.0
Gilbert A	mid2	6	52.5	27.5	25.0	50.1	-

NA - Open cage results did not fall between Closed cage and No cage results

<sup>1</sup> In first experiment two *D. bellulus* per cage, in second experiment two *H. octomaculata* per cage

<sup>2</sup> A cage around the base of the plant was used to exclude ground-dwelling predators from the larvae

<sup>3</sup> Harvey experiment was in mungbean, all others in soybean

Dash indicates treatments not included in experiment

SB - Soybean, MB - Mungbean

**Table 2.** Results of beat sheet sampling of arthropod abundance during each exclusion experiment. Ten, one metre beat sheets were collected at each sampling time in soybean (SB) and a single mungbean (MB) field.

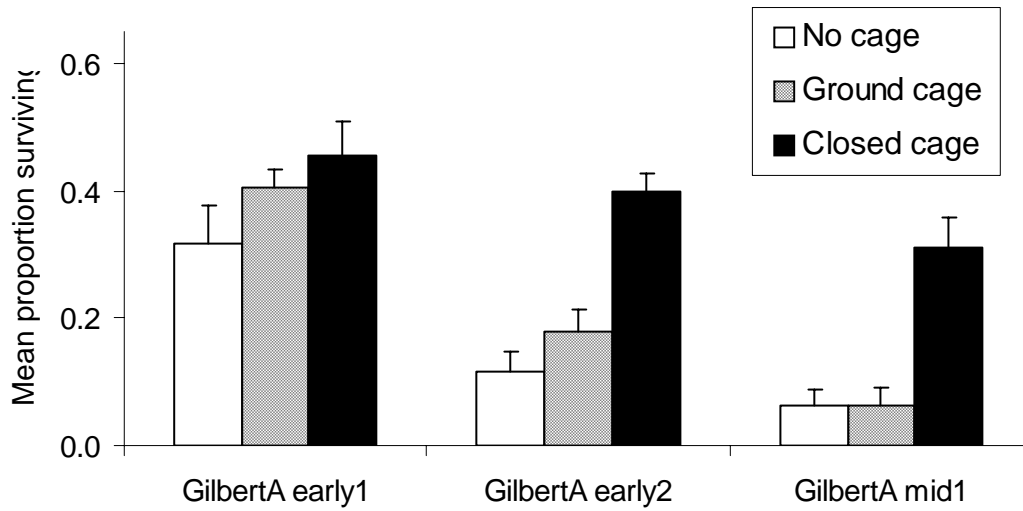
Field	Time during season	Crop	Predators/ 10m	Araneae/ 10m	Pred. to pest ratio	Mean plant height (cm) <sup>1</sup>
Season 2001/02						
Gilbert A	early	SB	-	-	-	-
Gilbert A	mid	SB	44	17	0.92	NA
Gilbert A	late	SB	46	15	1.70	NA
Gilbert C	late	SB	-	-	-	-
Harvey	late	MB	24	9	0.69	NA
Season 2002/03						
Gilbert A	early1	SB	2	1	0.40	15.50 (0.56)
Gilbert A	early2	SB	3	2	0.08	28.80 (2.11)
Gilbert A	mid1	SB	23	4	0.40	57.80 (2.04)
Gilbert A	mid2	SB	48	11	0.59	85.44 (2.64)

NA - measurement not assessed in the field

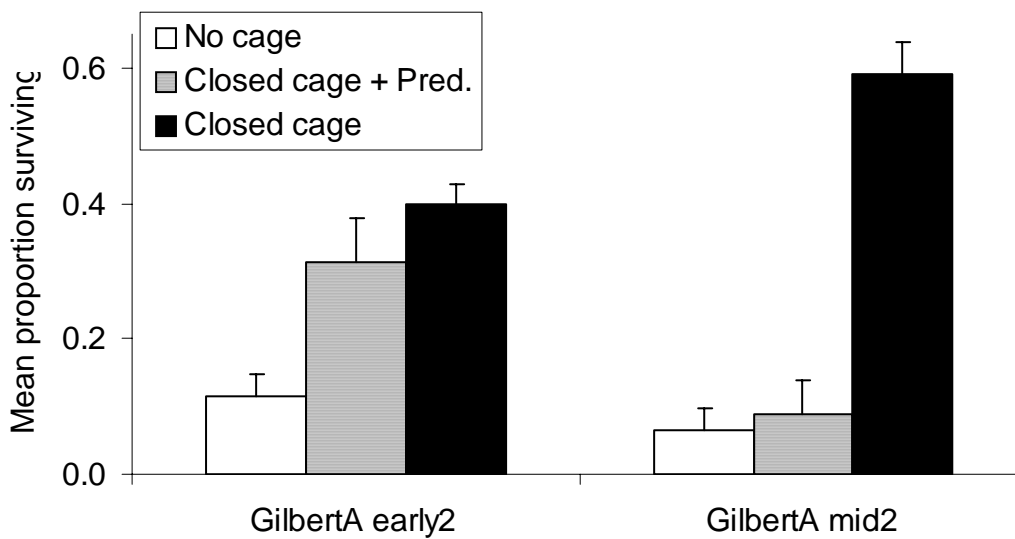
Dash indicates no measurements taken during experiment

<sup>1</sup>Mean of 10 samples, numbers in brackets indicates standard error

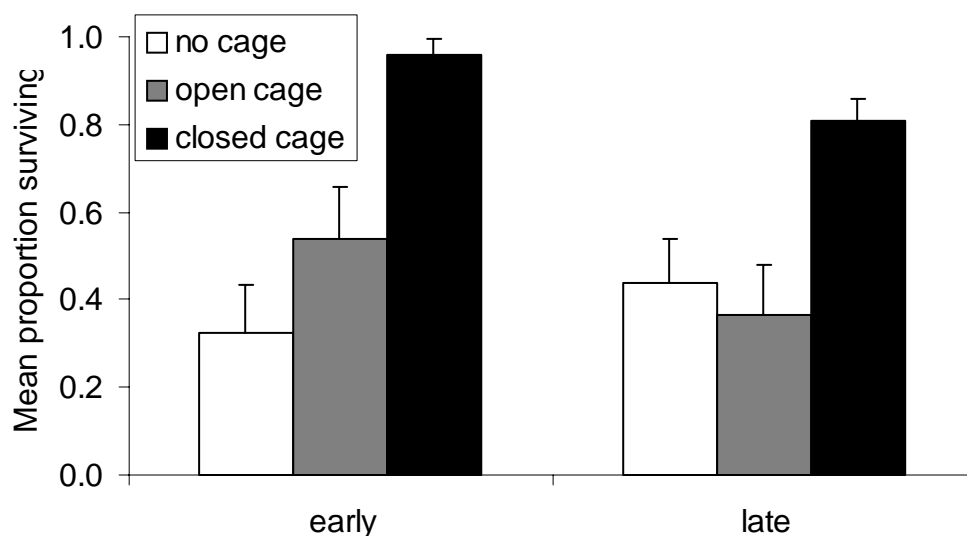
**A. Ground-dwelling predators excluded**



**B. Predator Inclusion**



**Figure 3.** The proportion of *H. armigera* larvae that survived during **A.** ground predator exclusion experiments and **B.** predator inclusion experiments in a soybean fields during season 2002/03. In the first experiment (Gilbert A early2) two *D. bellulus* were introduced per cage and in the second experiment (Gilbert A mid2) two *H. octomaculata* were introduced per cage. The bars indicate standard error.



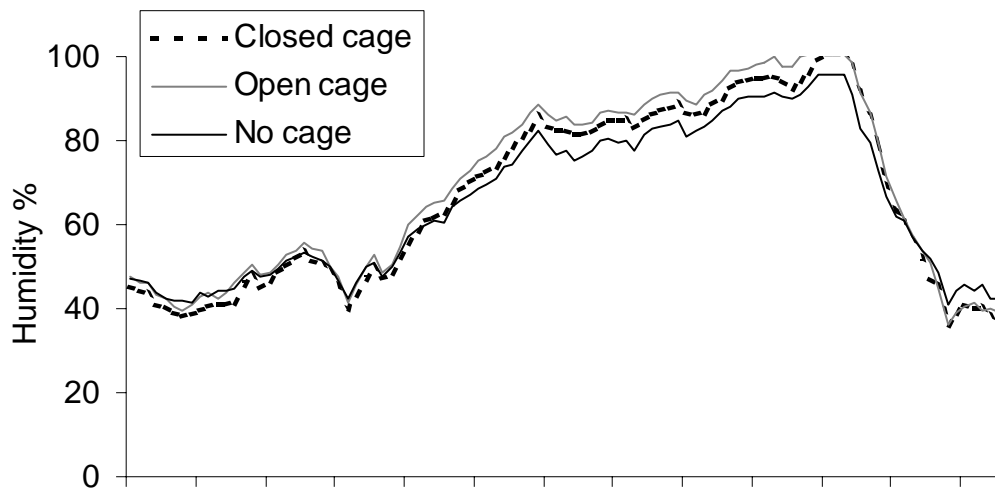
**Figure 4.** The proportion of *H. armigera* eggs on cards that survived in a closed cage, open cage and no cage (exposed) in a soybean field; 20 eggs per card were used for each treatment (10 replicates per treatment) and each experiment lasted for 18 hours. The bars indicate standard error.

**Table 3.** Estimates of the percentage mortality of *H. armigera* eggs imposed by predators in each exclusion experiment.

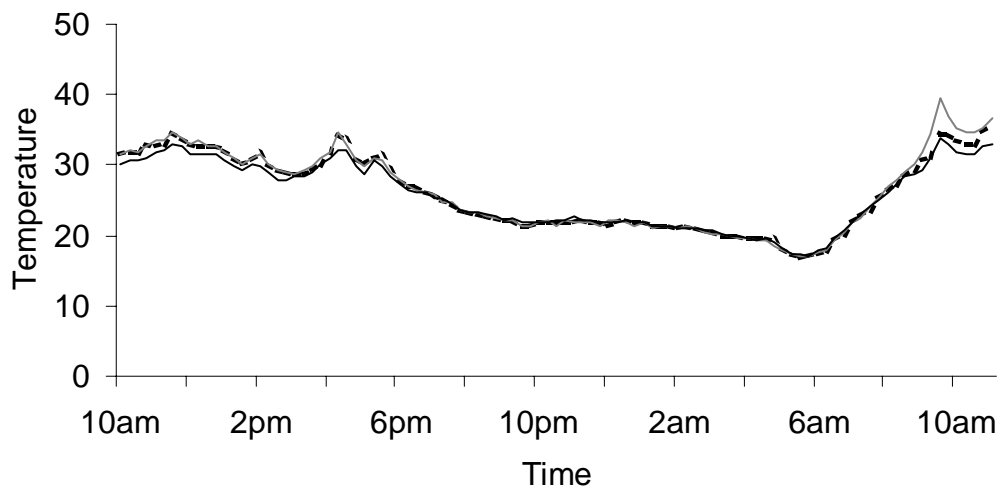
Field	Time during season	Crop	Hours exposed	eqn 1. Max. predation	eqn 2. Min. predation
Season 2001/02					
Gilbert A	early	Soybean	18	56.0	38.0
Gilbert A	late	Soybean	18	28.0	NA

NA - Open cage results did not fall between Closed cage and No cage results

**A. Humidity**



**B. Temperature**



**Figure 5.** Humidity (%) **A.** and temperature ( $^{\circ}\text{C}$ ) **B.** measured every 15 minutes in a closed cage, open cage and no cage treatments in a soybean field. Data loggers were tapped to the stem of soybean plants and left for 24 hours.

## **Discussion**

### Larval survival: Preliminary experiment

In the preliminary experiment there was a very high rate of mortality in the closed cage treatments. High mortality in the closed cages may be partially attributed to egg mortality immediately after release (before the larvae had time to hatch). In addition it was difficult to find the surviving larvae amongst the soybean foliage (even in the closed cage treatments). Opening and removing of cages every two days may have dislodged some larvae. These larvae would have been inappropriately classed as missing due to predation. Due to the experimental problems discussed, results from this experiment cannot be viewed as an accurate estimate of predator impact. The protocol for further exclusion experiments was changed to avoid some of these problems (see below).

### Larval survival: Further experiments

Significantly greater survival was observed when predators were excluded from the larvae using small cages. Using the open cage treatment I was able to partition out the proportion of survival that was a result of the altered conditions within the cage, to get a better estimate of mortality due to the action of predators. No attempt was made to find larvae that had moved off the plants, onto the ground, in the open cage and no cage treatments. These larvae may have been classed incorrectly as missing due to predation.

First instar *H. armigera* larval survivorship within soybean fields was generally very low (average of 10% survival) when all mortality factors were taken into consideration across both seasons. The average predator mortality estimates for Gilbert A soybean field was very similar across seasons, 23-33 (2001/02) and 21-30 percent (2002/03). Titmarsh (1992) found that larval mortality due to predation was 12 percent in soybean fields. He found higher levels of larval mortality due to predation in pigeonpea (31%) and vegetative cotton (29%). In Gilbert A soybean field there was a decrease in larval survival in closed cage treatment as the plants matured, suggesting that plant effects had a greater impact on larval survival as the season progressed. However the physical factor mortality estimates actually decreased as the season progressed (from 10 to 3%) (table 1). As the plants mature the canopy becomes enclosed and may ameliorate some of the more extreme environmental conditions that the larvae are exposed to. An alternative explanation is that as the plants matured the larvae were harder to find in the increased area of soybean foliage and so more were thought to have died due to predation.

The lowest survival rate was seen in the mungbean field. This experiment was conducted late in the season after pods had formed. At this time the plant was unsuitable for development of the larvae and high rates of mortality were observed in the closed cage treatment mainly due to plant effects. This conclusion is supported by the low predator mortality (14%) recorded and the low abundance of predators in the field (table 1 and 2).

Ground-dwelling predators, such as carabids, Lycosidae and ants, are highly abundant within unsprayed soybean fields (see Chapter eight). Their predaceous activities are difficult to quantify because they are often nocturnal or hard to observe (Lytton-Hitchins 1999, 2000). An attempt was made to estimate mortality imposed by ground-dwelling predators by excluding them from plants artificially infested with *H. armigera* larvae. Estimates of mortality imposed by ground-dwelling predators decreased from nine percent to zero as the season progressed. One possible explanation for this may be that the ability of the ground cages to effectively exclude predators decreases as the plants mature. As the plants matured it was harder to separate the ground caged plants from contiguous plants on the same row and plants in the adjacent rows. Soybean leaves often touched the ground and allowed access for both ground-dwelling and foliage-dwelling predators. Estimates obtained suggest that ground-dwelling predators have little impact on larval mortality however total predator mortality was also low during these experiments. For example, in the experiment in which ground dwelling predator mortality reached nine percent, total predator mortality (ground-dwelling and foliage-dwelling predators) only reached a maximum of 14 percent (table 1). Other studies that use more effective exclusion cages have shown that ground-dwelling predators limit herbivore abundance (Cicadellidae and Thysanoptera) (Lang *et al.* 1999).

#### Predator inclusion experiments

Both *D. bellulus* and *H. octomaculata* are capable of consuming large numbers of *H. armigera* eggs and first instar larvae in limited search arenas. Under similar no-choice laboratory conditions it has been shown that *D. bellulus* is capable of eating an average of  $34.4 \pm 3.59$  eggs per day, and the ladybeetle *C. transversalis* can consume an average of  $30.8 \pm 6.63$  per day when offered 100 eggs per day (Johnson 1999). Horne *et al.* (2000) found that *D. bellulus* consumed all ten eggs offered per day and approximately 2.5 first instar larvae per day. Prey consumption estimates calculated from no-choice feeding tests in limited search arenas should be considered the maximum amount of prey that the predator can consume. In a field situation with a more complex search arena and abundant alternative prey

estimates may be reduced (see Chapter ten). This was seen in the inclusion cage studies in soybean fields in which an average of 8 percent of larval mortality was attributed to introduced *D. bellulus* and 50 percent to introduced *H. octomaculata* (table 1). The large difference in results between the two species may be an artefact of caging. In the first experiment lots of soybean leaves were enclosed in the cage with the predators. Larvae within the cage were able to hide from predators amongst the tightly packed leaves so survival was high within these treatments. In the second experiment care was taken not to enclose too many leaves in the cage allowing the predators access to prey. This problem illustrates the many confounding factors inherent in cage studies and care must be taken when interpreting results.

### Egg survival

Maximum predator mortality imposed on *H. armigera* eggs on cards ranges from 28 percent (late experiment) to 56 percent (early experiment) in Gilbert A soybean field (table 3). These results are higher than those obtained by Titmarsh (1992) who found that egg mortality due to predation ranged from eight to 24 percent in soybean fields. Mortality estimates are much lower than that of the no cage treatments alone (No cage mortality; early 67%, late 56%) without comparison with the control treatments. Egg cards alone (no caged controls) will provide a quick estimate of predator mortality in the field, however they overestimate the true predation rate (see Chapter seven).

This chapter attempts to partition the mortality recorded in the field into discrete categories (predation, physical factors etc.) according to the survivorship in each treatment. In reality mortality factors all act in combination and contemporaneously to produce the observed pattern of larval and egg survivorship. Partitioning out the activity of predators alone is very difficult and results presented should be considered an estimate of the actual predation rate in soybean fields. Nonetheless, estimates of larval predation rate recorded throughout this study suggest that the naturally occurring arthropod predators within unsprayed soybean fields are capable of imposing high levels of mortality on *H. armigera* first instar larvae.

### Chapter Summary

- Small exclusion cages (no cage, open cage, closed cage) were used to estimate the proportion of mortality caused by predators of *H. armigera* eggs and first instar larvae in soybean fields.
- Larval survival was greatest in the closed cage treatments from which predators were excluded. In most experiments the survival in open cage treatments was intermediate between that of the closed cage and no cage treatments.
- Overall larval survival was very low within soybean fields; mean proportion surviving, 0.10 ( $\pm 0.03$  standard error,  $n = 8$  experiments) when all mortality factors were combined.
- Average predator mortality estimates for Gilbert A soybean was very similar across seasons, 23-33 percent (2001/02) and 21-30 percent (2002/03).
- Maximum predator mortality imposed on *H. armigera* eggs on cards range from 28 percent (late experiment) to 56 percent (early experiment) in GilbertA soybean field.
- Ground cages were used to exclude ground-dwelling predators (Lycosidae, ants, Carabidae) from larvae. Survival of larvae in ground cage treatments was usually in between that of the closed cage and no cage treatments. Mortality that could be attributed to the actions of ground-dwelling predators range from none to nine percent.
- Predator inclusion experiments involved a treatment that consisted of a closed cage with two predators added for the duration of the experiment. In the experiment using *D. bellulus* mortality estimates due to the added predators was only eight percent. In the experiment using *H. octomaculata* mortality estimates was much higher at 50 percent.
- *D. bellulus* individuals consumed an average of 16 *H. armigera* eggs in 24 hours ( $78\% \pm 8.6$ ) in no-choice feeding tests. *H. octomaculata* individuals consumed an average of 20 eggs ( $99\% \pm 0.8$ ), and an average of 9 first instar larvae ( $84\% \pm 2.9$ ) over 24 hours in the no-choice feeding tests.
- Estimates of larval predation rate recorded throughout this study suggest that the naturally occurring arthropod predators within unsprayed soybean fields are capable of imposing high levels of mortality on *H. armigera* first instar larvae.

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