

Australian Cotton CRC

FINAL REPORT

**Enhancing the Impact of Early Season Predation on
Helicoverpa spp.**

CRC17C

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REPORTS

Part 1 - Summary Details

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Part 3.3 – Final Reports

1. Project Background

Eggs and larvae of *Helicoverpa* spp. are preyed upon by a diversity of insect predators in cotton (Johnson et al. 2000). Predators can cause significant mortality to *Helicoverpa* spp., thereby reducing their abundance in the field (Room 1979, Bishop and Blood 1981, Scholz et al. 2000, Wade et al. 2002 and 2003). Techniques have been developed to incorporate the general contribution of predators into pest control decisions such as the predator to pest ratio (Mensah 2002a) and the use of lucerne refuges to provide an 'on-farm' source of predators (Mensah 2002b). The assassin bug has been identified as a potential predator of *Helicoverpa* spp. larvae, if assassin bug populations within cotton crops can be augmented artificially through mass rearing programmes (Grundy and Maelzer 2000). Anecdotal evidence suggests that selective removal of ants results in higher survival of *Helicoverpa* spp. However current understanding of the behaviour, ecology and impact of individual predator species is limited. This hinders our ability to conserve predators, by modifying some agricultural practices, and to build on the predator/pest ratio to actively incorporate particular predator species or groups into pest management strategies.

Agricultural practices can affect predator species diversity, and predator-prey dynamics. The use of insecticides is an obvious example (Lytton-Hitchins and Wilson 1999, Ma et al. 2000) but other common practices such as soil cultivation can also affect predators (Hulugalle et al. 1997). While an understanding of these effects is important, we also need more information on predator behaviour, particularly in relation to prey consumption in order to make predictions about the impact predators have on *Helicoverpa* spp. This requires a greater understanding of predator behaviour as well as the interaction between availability of alternative prey and predation on *Helicoverpa* spp. However predation is difficult to measure under natural conditions because predators are often cryptic, secretive or nocturnal. Unless observed in the act of feeding upon a prey item, predators leave little trace of their activity. Direct observation is a useful but time-consuming way to quantify predator behaviour. The development of serological techniques (ELISA) to detect prey in the gut contents of individual predators is a significant advance in the assessment of predation because these methods provide a direct link between predator and prey (Greenstone 1996). Initial investigations by Dr Lytton-Hitchins (project CSE69C) demonstrated the potential for ELISA tests to quantify predation on *Helicoverpa armigera*.

Efficient, accurate methods to monitor the abundance of beneficial arthropods are also important, if predators are to be fully incorporated into grower pest management strategies. Dr Brad Scholz and colleagues (QDPI) have pioneered the use of a beatsheet to measure the abundance of beneficial arthropods (Scholz et al. 2001). A greater understanding of predator ecology combined with improved monitoring methods and new technologies that help conserve predators (e.g. selective insecticides, Ingard cotton and stubble retention) could greatly enhance the contribution of predators to management of *Helicoverpa* spp. in cotton.

This project investigated how key insect predators contribute to the suppression of *Helicoverpa* spp. in cotton and explored the interactions between predator abundance, agronomic practices and predation of *Helicoverpa* eggs and larvae. The key beneficial groups investigated were predatory beetles, predatory bugs and ants. Several important collaborations were also developed which contributed additional information or resources to the project.

2. Project Objectives and Achievements

AIMS

1. To investigate the behaviour and ecology of key predators in relation to the suppression of *Helicoverpa* spp.
2. To explore the interaction between predator abundance, agronomic practices and predation of *Helicoverpa* eggs and larvae.
3. To determine whether a greater diversity of insect predators results in greater predation.

MILESTONES

2000-01

- a. Finalise development of ELISA technique for a range of coccinellid species and begin development for other species (follows from CSE69C).
- b. Set up initial experiments to quantify the effects of individual predator species or groups of species.

2001-02

- a. Undertake initial field experiments on the impact of agronomic practices on predator abundance and diversity and *Helicoverpa* spp. mortality due to predation.
- b. Finalise observations on predation on *Helicoverpa* spp. by ants and other early season predators (follows from CSE69C).
- c. Set up a second set of experiments to quantify the effects of individual predator species or groups of species.

2002-03

- a. Undertake further field experiments on the impact of agronomic practices on predator abundance and diversity and the level of predation on *Helicoverpa* spp.
- b. Set up a third set of experiments to quantify the effects of individual predator species or groups of species.
- c. Set up initial experiments to look at the effect of the availability of alternative prey on the level of predation on *Helicoverpa* spp.

2003-04 (to 2nd week in January 2004)

- a. Finalise experiments on the effects of agronomic practices.
- b. Complete second experiment to look at the effect of the availability of alternative prey on the level of predation on *Helicoverpa* spp.
- c. Develop recommendations for industry on farming systems to conserve or enhance the impact of early season predation on *Helicoverpa* spp. mortality.

Summary of project achievements including related collaborations

- ELISA protocols developed and calibrated to establish detection limits for 10 common predator species. This procedure uses a monoclonal antibody specific to *Helicoverpa armigera* to detect predation on eggs and larvae of this pest.
- Calibration of an ELISA protocol for the assassin bug, *Pristhesancus plagipennis*, a potential agent for augmentative release against cotton pests (in collaboration with Dr Paul Grundy, QDPI).
- Collections of insect predators for ELISA over two full cotton seasons (2001-02 and 2002-03) to quantify predation on *H. armigera* using the monoclonal antibody.
- Measurement of predation on *H. armigera* (using the monoclonal antibody) when pest numbers were augmented by placing *H. armigera* eggs into the cotton crop.
- Development and field trial of an alternative ELISA method that uses rabbit proteins as a marker for potential prey, then detects presence of the rabbit protein in predators that have consumed marked prey (in collaboration with Dr James Hagler, USDA). The detection rates of the two ELISA methods were compared using laboratory-fed and field collected predators.
- Measurement of predator diversity and abundance over two cotton seasons (2001-02 and 2002-03) under different agronomic conditions. This led to an assessment of the Beneficial Disruption Index as a measure of insecticide impacts on beneficial arthropods in cotton crops (in collaboration with Mr Martin Dillon and Dr Mary Whitehouse, CSIRO Entomology).
- A series of laboratory, glasshouse and field experiments to examine predation by native coccinellids on *H. armigera* eggs and the cotton aphid, *Aphis gossypii*.
- Development of rearing methods for the bigeyed bug (*Geocoris lubra*) and a laboratory investigation of predation by this species on *H. armigera* eggs and larvae. Results from this work will be published in collaboration with Drs Brad Scholz and Marie-Louise Johnson (QDPI).
- Investigation of ant behaviour and predation on *H. armigera* eggs under different agronomic conditions (a CRC Summer Scholarship project that followed on from project CSE69C). Findings from this project are summarised in the Summer Scholarship Final Report to the CRC and have been published in the Australian Journal of Entomology.
- Comparison of the beat sheet method with visual and suction sampling for the measurement of beneficial arthropod abundance (a collaborative CRC Summer Scholarship project with Mr Martin Dillon, CSIRO Entomology, and Ms Sandra Deutscher, CSIRO Plant Industry). Findings from this project have been summarised in the Summer Scholarship Final Report to the CRC and several industry publications.
- All publications arising from this project and related collaborations are listed in section 9.

3. Methods

a) Detection of predation on *H. armigera* using a monoclonal antibody

Most predators used to develop and calibrate the ELISA protocols were collected from crops at ACRI using a beat sheet or sweepnet. *P. plagipennis* were provided by Dr Paul Grundy (QDPI). The monoclonal antibody (Mab) used for the indirect ELISA assays was developed by Stephen Trowell (CSIRO Entomology) and collaborators (Trowell et al. 2000). The Mab responds specifically to *H. armigera* and not *H. punctigera*.

Individual predators were crushed in buffered saline solution (500µL, pH of 7.5) and centrifuged to separate out large debris. Each predator was assayed only once. Assay plates were incubated at 25°C for all predatory beetles and at 4°C for all predatory bugs throughout the procedure. A 100µL aliquot of each macerated predator was placed in a well on a 96-well assay plate and incubated overnight to allow antigens to bind to the assay plate. The next day, a 1% solution of non-fat dry milk in distilled water was added to each well for 1h to block unoccupied antigenic sites. Then 100µL of the primary Mab was added to each well for 2h, to bind with any *H. armigera* antigens present. The plate was washed with buffered saline solutions before the addition of the secondary antibody to each well for 1h (100µL, diluted 1:500 in 1% non-fat milk). This secondary antibody forms a link between the primary Mab and the colour reagent used to visualise positive reactions. The plate was washed a second time before the colour reagent (100µL of HRP substrate solution) was added to each well for 2h (1h for *N. kinbergii* and *P. plagipennis*). The absorbance of each well was read using a Benchmark Microplate reader set at 415 nm. Every plate included negative controls, positive controls and reagent blanks to provide confirmation that all reagents were working satisfactorily and to calibrate the plate reader.

To establish the expected absorbance from the target prey *H. armigera* eggs and neonate larvae were assayed using this ELISA protocol. A range of insects commonly found in Australian cotton crops was also subjected to ELISA to determine whether false positives might occur following consumption of prey other than *H. armigera*. To measure the decay period of the antigen, individual predators were held in a petri dish (50mm diameter) with a single *H. armigera* egg or neonate. The predators were checked approximately every 15 minutes and if the prey was missing then consumption was assumed. Predators were kept isolated prior to these tests and provided with aphids and water for 72 hours (*G. lubra* and *N. kinbergii*) or with water only for at least 24 hours (*D. bellulus*). Fed predators were frozen at intervals from 0-48h after consumption of *H. armigera* and kept at -80°C until assayed. A subsample of individuals from each species was frozen after the isolation period to act as controls. A predator was considered positive for *H. armigera* if its absorbance was greater than three standard deviations above the mean absorbance of all control specimens tested.

Predators were collected from cotton crops at ACRI and commercial farms in the 2001-02 and 2002-03 cotton seasons. The predators were collected directly from beat sheets, stored in liquid nitrogen at the field site then transferred to a -80°C freezer in the laboratory. Visual checks were also made to collect eggs and larvae of *Helicoverpa* spp. and determine the proportion of *H. armigera* present at the time of predator collection. In addition, *H. armigera* populations were augmented in two areas of unsprayed conventional cotton (Field A1 and Block 17) at ACRI during the 2002-03 season. In each area a 10 row x 10 m section was marked out and *H. armigera* eggs added at a density of 10 eggs/m row. The eggs were attached to paper towel (5 eggs/piece, 2 pieces/m) and stapled to the leaves of the plants. Six beat sheet samples were taken from each section and predators collected 24h after the eggs were set out. Control areas of Field A1 and Block 17 that did not receive additional *H. armigera* eggs were sampled simultaneously and predators collected (6 beat sheets/area). This procedure was repeated three times over the season in December, January and February.

b) Detection of predation on *H. armigera* using rabbit protein to mark the target prey

The following protocol is based on methodology developed by James Hagler (USDA) to measure predation on cotton pests in Arizona (Hagler and Durand 1994, Hagler and Miller 2002).

Rabbit protein marker (rabbit IgG) was diluted in deionised water and applied to *H. armigera* eggs at a rate of 5 mg/mL using a perfume atomiser. In the laboratory clusters of approximately 5-10 marked *H. armigera* eggs were fed to the predatory beetles *Dicranolaius bellulus* and *Hippodamia variegata*. The predators were held in individual petri dishes and observed until the eggs were consumed. Fed predators were frozen at -80°C at 0, 1, 2, 4 and 24h after eating. Each predator was subjected to two assays: the indirect ELISA described previously to detect *H. armigera* and a sandwich ELISA to detect the rabbit protein marker. The duplicate assays allow a direct comparison of detection rates for both ELISA methods.

Predators were prepared for this ELISA in a similar fashion to the indirect ELISA (i.e. crushed in buffered saline solution and centrifuged). The first stage of the sandwich ELISA to detect the rabbit marker was to coat the assay plate with anti-rabbit IgG (the primary antibody, 100 μL per well) and incubate the plate overnight at 4°C . The next day, a 1% solution of non-fat dry milk in distilled water was added to each well for 30 min at 27°C to block unoccupied sites. Then a 100 μL aliquot of each crushed predator was added to each well and incubated for 1 h at 27°C to allow antigens to bind to the primary antibody. The plate was washed with buffered saline solutions before the addition of the secondary antibody (rabbit IgG conjugated to HRP) to each well for 1h at 27°C . This second antibody forms a link between the rabbit protein (which is already bound to the primary antibody) and the colour reagent. The plate was washed a second time before the colour reagent (100 μL of HRP substrate solution) was added to each well for 2h at 27°C . The absorbance of each well was read using a Benchmark Microplate reader set at 415 nm. Every plate included negative controls, positive controls and reagent blanks to provide confirmation that all reagents were working satisfactorily and to calibrate the plate reader.

In November 2004 rabbit-marked *H. armigera* eggs attached to paper towel were added to a 10 row x 10 m section of conventional cotton at the QDPI research station in Biloela. Egg density was 10/m (similar to previous augmentation trial at ACRI with unmarked *H. armigera* eggs). Predators were collected by visual searching of 6 x 1m sections of cotton in the release plot at 12, 24, 36 and 48h after egg placement. All predators were subjected to an indirect and sandwich ELISA to compare the percentage of positive responses for each method.

c) Predator diversity and abundance under different agronomic conditions

The abundance and diversity of beneficial insects and spiders were monitored in 15 fields on seven farms in the Namoi, Gwydir and Macintyre valleys and one field at ACRI during 2001-02. These fields represented a range of cropping systems and varieties (conventional, Ingard, unsprayed, dryland, irrigated, retained stubble). Beat sheet samples and visual checks were taken at each site every 2-4 weeks from November to February (12 x 1 row metre beat and visual samples per field per sample date). All beneficial predators were counted and identified, usually to species. Insect predators were collected for subsequent ELISA analysis to test for consumption of *H. armigera*. All *Helicoverpa* spp. eggs and larvae were collected and reared through to determine parasitism rates and emerged parasitoids identified. Similar methods were used during the 2002-03 season when 14 fields at six farms were monitored (usually one conventional and one Ingard field per farm). Three fields at ACRI were also sampled over this season: Block 17 (conventional and Ingard cotton), Field A1 (conventional) and Field C1 (rotation trial managed by Dr Hulugalle, NSW Agriculture).

d) Predation of native coccinellids on *H. armigera* and the cotton aphid, *Aphis gossypii*

The effect of prey density and aphid availability on consumption of *H. armigera* eggs was tested under laboratory conditions for transverse, variable, three banded and striped ladybirds (*Coccinella transversalis*, *Coelophora inaequalis*, *Harmonia octomaculata* and *Micraspis frenata* respectively). Two species (*C. inaequalis* and *H. octomaculata*) were then used to examine the effect of predator and prey density on *H. armigera* egg consumption in glasshouse cages. Finally, *H. armigera* egg consumption by the same two predator species was tested in field cages under natural conditions.

Adult and larval ladybirds of all species were collected from crops and weeds around Narrabri, Wee Waa and Pilliga during the 2000-01 cotton season. The ladybirds were kept in a perspex cage in a glasshouse at $35 \pm 5^\circ\text{C}$ and aphid-infested cotton plants placed inside the cage as a food source. Water was supplied in specimen jars with cotton wicks through the lid. Additional ladybirds were added to the colony to maintain numbers as necessary over the season. All predators were used only once and were starved for 24h prior to an experiment. Cotton aphids (*Aphis gossypii*) were reared on cotton plants in the same glasshouse. *H. armigera* eggs were supplied from a laboratory colony maintained by CSIRO Entomology at ACRI. Eggs were stored at 10°C and used within 48h of collection from the moth colony.

For the laboratory tests single adult ladybirds were confined in a 100 x 70 x 50 mm plastic container with 3, 6 or 12 *H. armigera* eggs and a small piece of moistened sponge to provide water. After 24h the number of missing eggs was recorded. Each egg density level was replicated 15 times for the four ladybird species (*C. transversalis*, *C. inaequalis*, *H. octomaculata* and *M. frenata*). The entire experiment was then repeated for the four predators and three egg densities with the addition of approximately 15 - 20 aphids to each container.

Perspex cages (480 x 500 x 600 mm) were used for the glasshouse experiments. 1, 3 or 6 adult ladybirds were confined in each cage with a young cotton plant but no aphids. 3, 6 or 12 *H. armigera* eggs were individually glued to three leaves on the cotton plant with a water-based glue. Predators were left in the cages for 24h and the number of missing eggs was then counted. Each combination of predator and prey density was replicated 10 times for two ladybird species (*C. inaequalis* and *H. octomaculata*).

The field cages had a 1m^3 metal frame with a fine mesh cover and two zips on opposite sides. Tent pegs through two eyelets at each corner of the cover secured the cage to the soil. Frames were placed approximately 10 m apart over single rows of cotton in field 18 at ACRI from December 2001 to February 2002. The covers were only kept over the frames during the course of an experiment. Cages were assigned to one of four treatments: addition of five *H. octomaculata* ladybirds, addition of five *C. inaequalis* ladybirds, control (no predators added) or open cage control (no predators added, cage sides left open to allow free insect movement). Five sentinel egg cards, each with five *H. armigera* eggs, were stapled to leaves in the upper canopy of the cotton plants within the cage, left for 24h and the number of missing eggs recorded. Sentinel egg cards were used instead of directly gluing the eggs to leaves because it was too difficult to find the eggs again unless they were attached to visible cards. This experiment was replicated four times from December to February, with four cages per treatment in December and three cages per treatment for all other replicates. All insects (pest and beneficial) already present on plants inside the cages were left untouched.

e) Rearing methods for the bigeyed bug, *Geocoris lubra* and consumption of *H. armigera*

The effect of prey type and temperature on survival and development of *G. lubra* was examined under controlled conditions. Adult and juvenile *G. lubra* were collected with a beat sheet in March 2002 from unsprayed Ingard cotton at Cotton Seed Distributors, Wee Waa, to start cultures. Colonies of the prey species *A. gossypii* and *H. armigera* were kept at ACRI.

For the first rearing method, *G. lubra* were placed in 5L white plastic buckets covered with cloth gauze and containing scrunched paper towels to provide concealment. Water was provided on a moistened sponge and live aphids on cotton terminals as prey. Cotton dental wicks were added as an egg-laying substrate. These wicks were collected every two days then held in small plastic containers (100 x 70 x 50mm) until the eggs hatched. Juveniles were separated into individual containers (100 x 70 x 50mm) lined with paper towel and provided with a damp sponge and approximately 20 live aphids. Prey and water were replenished every two days. All containers and buckets were held in a constant temperature room at 25°C, 12L:12D and ambient humidity. Rearing conditions were similar for the second rearing method except that aphids were replaced with loose *H. armigera* eggs and the containers were moved to a constant temperature cabinet at 27°C, 16L:8D and 75% relative humidity.

f) Ant predation on *H. armigera* eggs

Behavioural observations of ants were made from December to early January in 2001-2002. The duration and type of behaviour was recorded on handheld Psion data loggers using Observer 3.0 software. Observations were made in irrigated, conventional cotton at ACRI (field 18, 31 observations), "Lowana", Pilliga (field 3, 7 observations) and "Milchengowrie", Boggabri (field W3, 8 observations), in irrigated, Ingard (Bt) cotton at Cotton Seed Distributors, Wee Waa (plots 17 and 18, 3 observations) and in dryland conventional cotton at "Glenwarrie", Edgeroi (Halls field, 3 observations). Continuous observations were taken for 30 minutes from each of three 1 m sections of cotton at each site on each sampling date. Before observations began in a 1 m section, 10 sentinel cards (10 x 15 mm) were stapled to leaves in the upper canopy of the observed section. Three *H. armigera* eggs were attached to each card with water based glue and the cards were refrigerated at 4°C overnight until placed in the field. Fresh eggs came from the *H. armigera* colony at ACRI.

4. Results

a) Detection of predation on *H. armigera* using a monoclonal antibody

The degree of variability in response to the Mab from the target species, *H. armigera*, indicates there is some risk of false negatives with this assay at both incubation temperatures (Figures 1 and 2). Absorbance for *H. armigera* eggs increased at 4°C with a longer incubation period during the final stage of the assay (Figure 2). Of the 175 insects tested for cross-reactivity to the Mab, mean absorbance was 0.172 ± 0.031 nm, suggesting that the risk of false positives from alternative prey species is minimal (Figure 1a).

Greater variability in the response to the Mab was apparent for control specimens of predatory beetles (all coccinellids and *D. bellulus*) compared with the cross-reactivity tests (Figure 1b). Further experimentation with the ELISA protocol suggested non-specific binding of the secondary antibody contributed to the variation. However it was not possible to reduce this background noise (by lowering the concentration of the secondary antibody) without also losing the ability to detect true positive results. This meant that critical thresholds for the predatory beetles were relatively high (Table 1). Such high thresholds do increase the likelihood of false negatives, particularly for neonate prey. Neither adult *N. kinbergii* nor adult and nymphal *G. lubra* showed significant response to the Mab (Figure 2). For both predators, less than 3% of the laboratory-reared *H. armigera* tested showed absorbance values lower than the critical thresholds for these two predators (Table 1). Given the low variability in absorbance for the control specimens tested, the chance of false positive or negative results seems quite low for *N. kinbergii* and *G. lubra*. Control specimens of assassin bugs of all instars showed greater response to the Mab than the smaller predatory bugs (Figure 2), although the critical thresholds for each instar were still relatively low, compared to the observed response from *H. armigera*.

When predators were known to have fed on *H. armigera*, antigen detection declined exponentially with increasing digestion time for *D. bellulus* ($F_{1,5} = 40.74$, $P = 0.001$, $R^2 = 0.891$) and adult *G. lubra* ($F_{1,5} = 189.45$, $P < 0.0001$, $R^2 = 0.974$). For *D. bellulus* detection of a single egg was only possible for approximately 2h after consumption and the chance of detecting consumption was below 30% within 1h (Figure 3a). The detection period for *G. lubra* that had fed on a single egg was also quite short (from > 70% immediately after consumption to < 10% at 4 h, Figure 3b) although the probability of detection was generally greater than for *D. bellulus*. The probability of detecting consumption of a single larva was low for *G. lubra* (below 25% immediately after consumption). Adult *N. kinbergii* that had fed on one *H. armigera* larva showed a noticeably different pattern of antigen detection over time that did not follow the expected exponential decay (Figure 3c). In fact, detection peaked at 29% when 24 h had elapsed. Although the detection period was more extended in this species than for *D. bellulus* or *G. lubra*, the probability of detection was low overall. Antigen detection in adult and nymphal assassin bugs (fed a 3rd instar *H. armigera* larva) also varied over time in an unexpected fashion, with peak detection usually occurring several hours after prey consumption (Figure 3d). However the probability of detection was generally fairly high (mostly > 50%) and the detection period was extended, with some positive results found 48h after consumption. Predation on a single *H. armigera* egg could not be detected in any of the coccinellid predators tested, although *Hippodamia variegata* that had fed on multiple eggs did test positive using this ELISA protocol (see results from the rabbit marker technique for more details). In general, these calibration limits for the different predators are conservative because they are based on the smallest meal size (one egg or larva). Antigen detection in these predatory beetles and bugs did increase when more than one *H. armigera* egg or larva had been consumed.

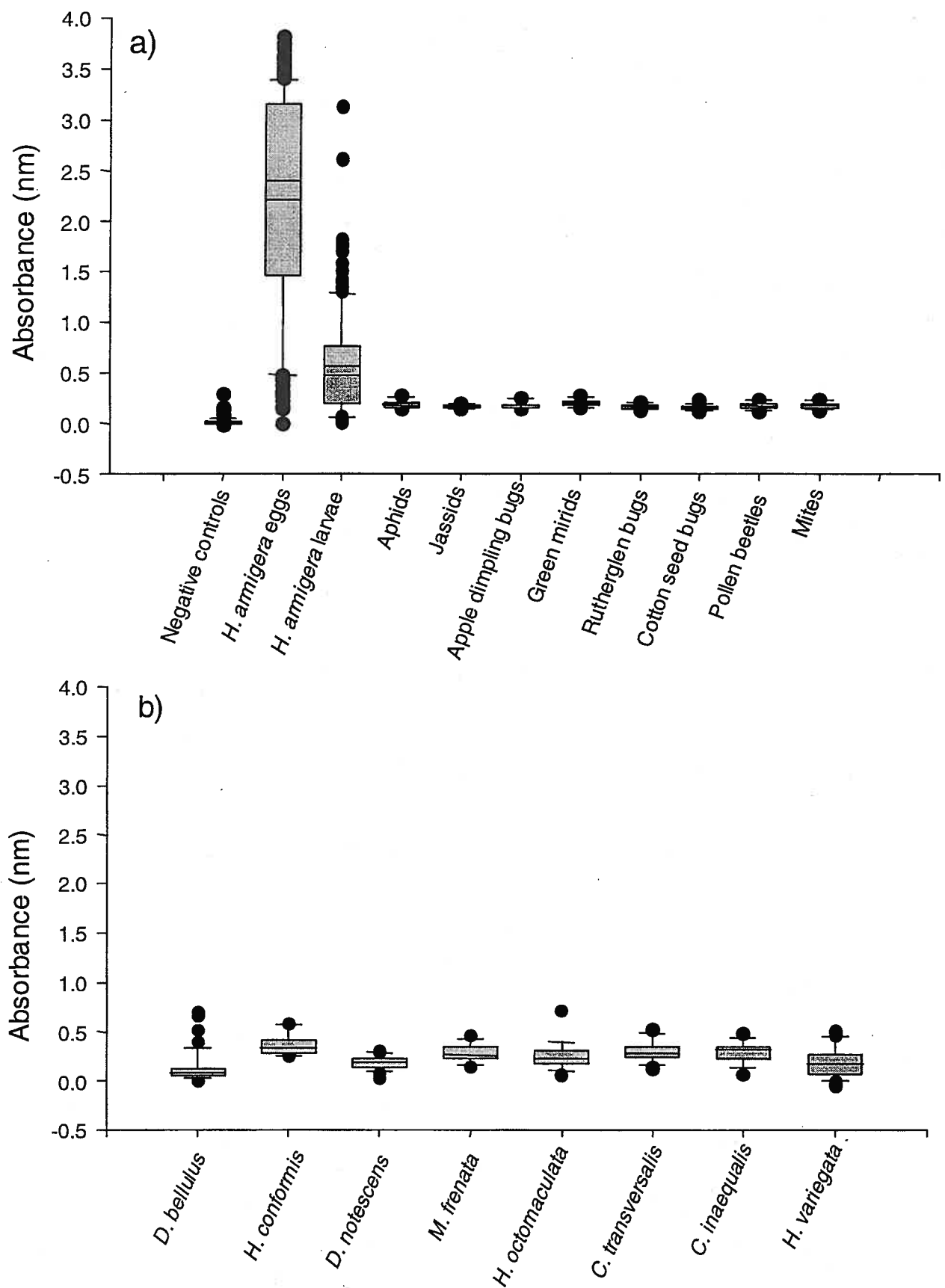


Figure 1. Absorbance of a) negative controls, *Helicoverpa armigera* eggs and larvae and a selection of insects commonly found on Australian cotton crops; and b) control specimens of predatory beetles, all subjected to ELISA using the Mab specific for *H. armigera*. All plates incubated at 25°C.

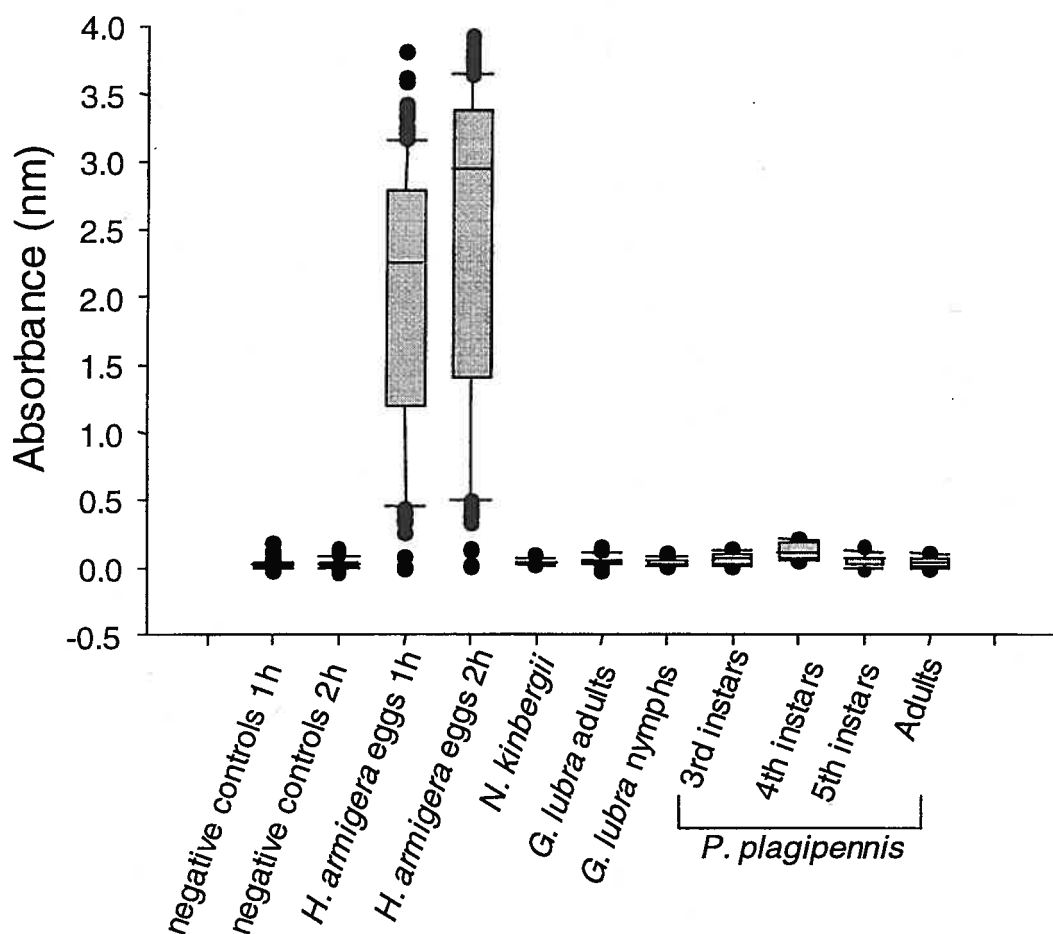


Figure 2. Absorbance of negative controls, *Helicoverpa armigera* eggs and control specimens of predatory bugs, all subjected to ELISA using the Mab specific for *H. armigera*. All plates incubated at 4°C.

Table 1. Critical absorbance thresholds for predatory beetles and bugs to determine positive reactions for recent feeding on *H. armigera* eggs or larvae.

Predator	Positive threshold (nm)
<i>Dicranolaiius bellulus</i> – red and blue beetle	0.576
<i>Harmonia conformis</i> – common spotted ladybird	0.663
<i>Diomus notescens</i> – minute two spotted ladybird	0.392
<i>Micraspis frenata</i> – striped ladybird	0.547
<i>Harmonia octomaculata</i> – threebanded ladybird	0.676
<i>Coccinella transversalis</i> – transverse ladybird	0.602
<i>Coelophora inaequalis</i> – variable ladybird	0.603
<i>Hippodamia variegata</i> – amber spotted ladybird	0.659
<i>Nabis kinbergii</i> – damsel bug	0.095
<i>Geocoris lubra</i> – bigeyed bug (adults)	0.160
<i>G. lubra</i> (nymphs, averaged across all five instars)	0.120
<i>Pristhesancus plagipennis</i> - assassin bugs (3 rd instar nymphs)	0.192
<i>P. plagipennis</i> (4 th instar nymphs)	0.307
<i>P. plagipennis</i> (5 th instar nymphs)	0.187
<i>P. plagipennis</i> (adults)	0.157

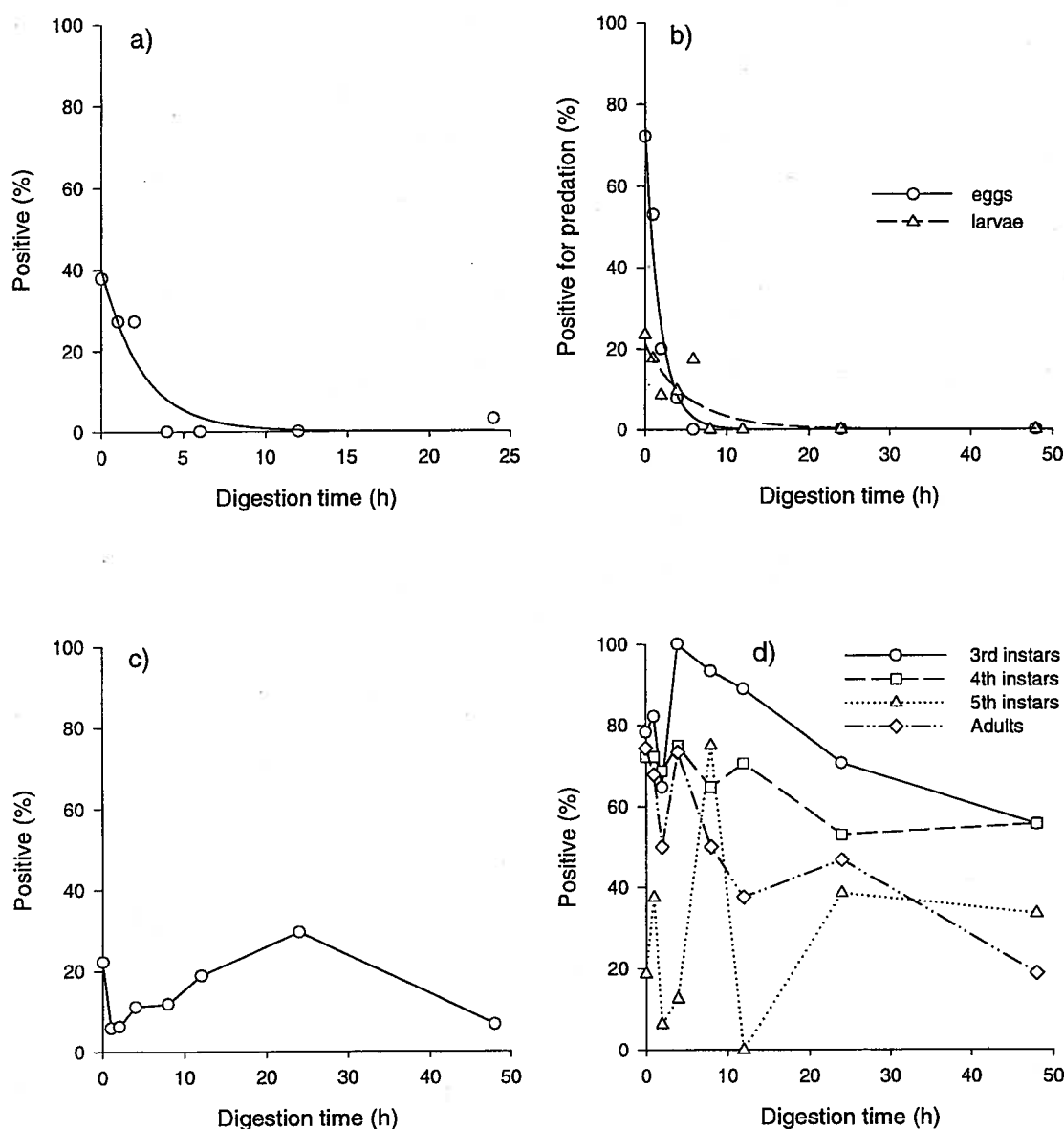


Figure 3. Percentage of positive ELISA detections with increasing time after prey consumption using the monoclonal antibody specific to *H. armigera* for a) *D. bellulus* fed one egg ($y = 40.32e^{-0.40x}$), b) *G. lubra* fed either one egg ($y = 75.21e^{-0.54x}$) or one neonate larva ($y = 21.29e^{-0.19x}$), c) *N. kinbergii* fed one neonate larva and d) 3rd, 4th, 5th instar and adult *P. plagipennis* fed one 3rd instar larva.

Of the field collected predators, *N. kinbergii* and *G. lubra* had the highest predation rates in both seasons although the percentage of positive individuals for both species declined slightly in 2002-03 compared with 2001-02 (Table 2). Despite the large number of individuals collected, only a small percentage of *D. bellulus* were positive for recent predation on *H. armigera* in both the 2001-02 and 2002-03 cotton seasons. Some coccinellids tested positive for recent predation in 2001-02 but none in 2002-03. No assassin bugs were collected from the field samples. *H. armigera* formed a greater percentage of the *Helicoverpa* spp. population in 2002-03 (39%) than 2001-02 (13%). In the *H. armigera* augmentation trial, almost twice as many predators collected from the adjacent control plots tested positive for recent predation, compared with predators from the plots with additional *H. armigera* eggs (3.7% compared with 1.5%, Table 3).

Table 2. Number of predators collected (N) and the percentage of positive detections (P %) for recent predation on *H. armigera* in each season.

Predator	2001-02		2002-03	
	N	P %	N	P %
<i>Nabis kinbergii</i>	95	28	111	22
<i>Geocoris lubra</i>	50	14	292	12
<i>Dicranolaius bellulus</i>	758	1	2221	1
<i>Hippodamia variegata</i>	16	0	116	2
<i>Harmonia octomaculata</i>	28	11	0	-
<i>Coccinella transversalis</i>	42	5	25	0
<i>Diomus notescens</i>	33	3	6	0
Other coccinellids	3	0	1	0
Total	1025	5	2772	3

Table 3. Number of predators collected (N) and number positive (P) for recent predation on *H. armigera* in plots with *H. armigera* eggs added (10 eggs/m) and control plots without additional eggs.

Predator	Egg addition plots		Control plots	
	N	P	N	P
<i>Nabis kinbergii</i>	17	3	23	11
<i>Geocoris lubra</i>	5	1	6	0
<i>Dicranolaius bellulus</i>	435	2	306	2
<i>Hippodamia variegata</i>	17	1	11	0
Other coccinellids	3	0	4	0
Total	477	7	305	13

b) Detection of predation on *H. armigera* using rabbit protein to mark the target prey

For *D. bellulus* and *H. variegata* that had fed on multiple *H. armigera* eggs, detection of the rabbit protein marker with the sandwich ELISA was extremely likely, even 24h after feeding (Table 4). There was no evidence of non-specific binding with the rabbit protein marker that could lead to false positives, unlike the Mab and the critical thresholds for the sandwich ELISA were much lower (0.099 nm for *D. bellulus* and 0.110 nm for *H. variegata*). Detection of *H. armigera* with the Mab and indirect ELISA was also higher in these predators that had consumed multiple eggs, compared with predators fed only a single egg. However detection of predation with the Mab was unlikely at 24h after consumption.

More than 120 predators were collected from the plot with marked *H. armigera* eggs attached to the foliage. The sandwich ELISA detected nearly 5x more predation than the indirect ELISA in these predators (Table 5). For those predators that did not have critical thresholds determined for the rabbit marker from laboratory tests (i.e. all predators except *D. bellulus* and *H. variegata*), a default threshold of 0.100 nm was used (mean + 4sd of negative controls). Despite the lack of established protocols for spiders with either ELISA method, several of these predators showed extremely strong positive responses from visual inspection of the assay plates.

Table 4. Number of individuals tested (N) and percentage of positive detections (P %) with increasing time after prey consumption using either the rabbit protein marker and sandwich ELISA or the Mab specific to *H. armigera* and indirect ELISA. Predators had fed on multiple *H. armigera* eggs marked with rabbit IgG.

Time after feeding (h)	<i>Dicranolaius bellulus</i>			<i>Hippodamia variegata</i>		
	N	Rabbit (P%)	Mab (P%)	N	Rabbit (P%)	Mab (P%)
0	20	100	100	18	100	83
1	18	100	94	18	100	67
2	18	100	94	16	100	13
4	17	100	71	16	100	0
24	15	33	0	15	100	7

Table 5. Number of predators collected (N) and the percentage of positive detections (P %) for recent predation on *H. armigera* using either the rabbit protein marker and sandwich ELISA or the Mab specific to *H. armigera* and indirect ELISA.

Predator	N	Rabbit (P %)	Mab (P %)
<i>Dicranolaius bellulus</i>	11	64	18
<i>Hippodamia variegata</i>	45	18	0
<i>Coccinella transversalis</i>	38	24	5
<i>Diomus notescens</i>	3	0	0
<i>Geocoris lubra</i>	9	0	0
<i>Cheiracanthium</i> spp.	12	33	8
Other spiders	3	33	33
Total	121	24	5

c) Predator diversity and abundance under different agronomic conditions

The most abundant beneficial predators in the sampled fields for the 2001-02 season were spiders (58%), particularly lynx and jumping spiders, and predatory beetles (28%) such as *D. bellulus* (Figure 4). Of the *Helicoverpa* eggs and larvae collected, 13% were identified as *H. armigera*, 56% as *H. punctigera* and 31% could not be identified to species (includes unhatched eggs, parasitism, accidental lab loss, etc). Five species of parasitoids were reared from the field collected *Helicoverpa* eggs (*Telenomus* spp. and *Trichogramma* spp.) and larvae (*Chaetophthalmus* sp., *Heteropelma* sp. and *Microplitis demolitor*). Parasitism rates were low (4% of eggs parasitised and 6% of larvae parasitised) and 12% of larvae were virus infected. Mean abundance of all beneficial insects and spiders increased over the season in fields with a Beneficial Disruption Index (defined by Hoque et al. 2000) of less than 8 at the end of the season (Figure 5). Fields with a higher BDI (ranging from 28-36 at the end of the season) had consistently low numbers of beneficial predators. Insecticide use was the major factor affecting beneficial insect and spider abundance in these fields and it was not possible to detect additional effects from different agronomic practices such as stubble retention or dryland cultivation for fields sampled in 2001-02.

Red and blue beetles, *D. bellulus*, dominated the insect fauna for the 2002-03 season (49% of 5669 predators counted, Figure 4). Spiders were the other major group (28%) with predatory bugs only 14% of all predators counted. Of the *Helicoverpa* eggs and larvae collected, 39% were identified as *H. armigera*, 19% as *H. punctigera* and 42% could not be identified to species. There was 4% mortality due to parasitism (mostly *Microplitis* spp. and *Trichogramma* spp.) and < 2% mortality from viral disease.

In irrigated cotton fields beneficial insects and spiders increased in abundance from November 2002 to February 2003 ($F_{3, 585} = 51.5$, $P < 0.001$ and $F_{3, 585} = 85.1$, $P < 0.001$ respectively, Figure 6). On average, spiders were more abundant in Ingard cotton than conventional ($F_{1, 585} = 7.7$, $P = 0.006$) whereas the abundance of beneficial insects did not differ significantly between Ingard and conventional cotton ($F_{1, 585} = 0.8$, $P = 0.39$). Mean abundance of beneficial arthropods for each field declined as BDI increased although the trend was only marginally significant (linear regression, $F_{1, 10} = 4.6$, $P = 0.06$, $R^2 = 0.32$).

One dryland site was sampled during the 2002-03 cotton season. Spiders were more abundant in both Ingard and conventional cotton at this site compared with other sites sampled (44% of all beneficial fauna). In the conventional cotton beneficial insect and spider abundance peaked in January (mean abundance of 11 insects/m and 6 spiders/m) then declined after a pyrethroid application to the crop. Insect predators were more abundant in Ingard cotton than conventional at this site (mean of 5 and 2 insects/m respectively).

The tillage and rotation trial managed by Dr Hulugalle (NSW Agriculture) in Field C1 at ACRI contains replicate blocks of three treatments: maximum tillage and continuous cotton, minimum tillage and continuous cotton, minimum tillage and cotton/wheat rotation. The same insecticide regime is applied to all blocks, which were all planted with conventional cotton. This trial has run continuously since the 2000-01 season. Beneficial insect and spider abundance peaked in February 2003 (mean of 21 insects/m and 3 spiders/m). *D. bellulus* were particularly abundant in Field C1, comprising 66% of all beneficial arthropods found. Although there was no significant difference in total abundance of beneficial arthropods between the three treatments ($F_{2, 132} = 0.52$, $P = 0.6$), ants were more abundant in the minimum tillage plus wheat rotation blocks, particularly in December, compared with the other two treatments ($F_{2, 132} = 8.59$, $P = 0.0003$, Figure 7).

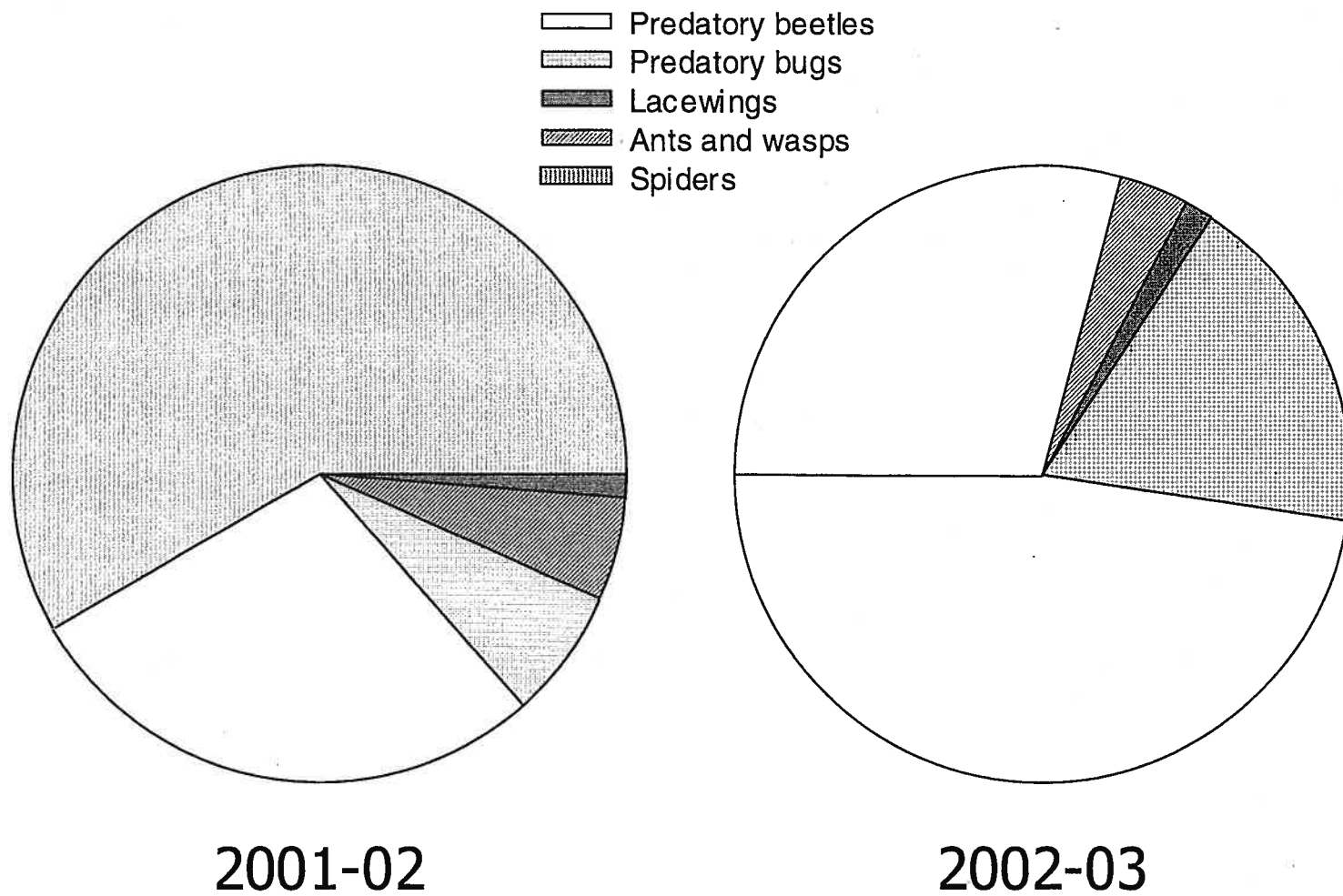


Figure 4. Abundance (%) of beneficial arthropods in all cotton crops sampled during the 2001-02 and 2002-03 cotton seasons.

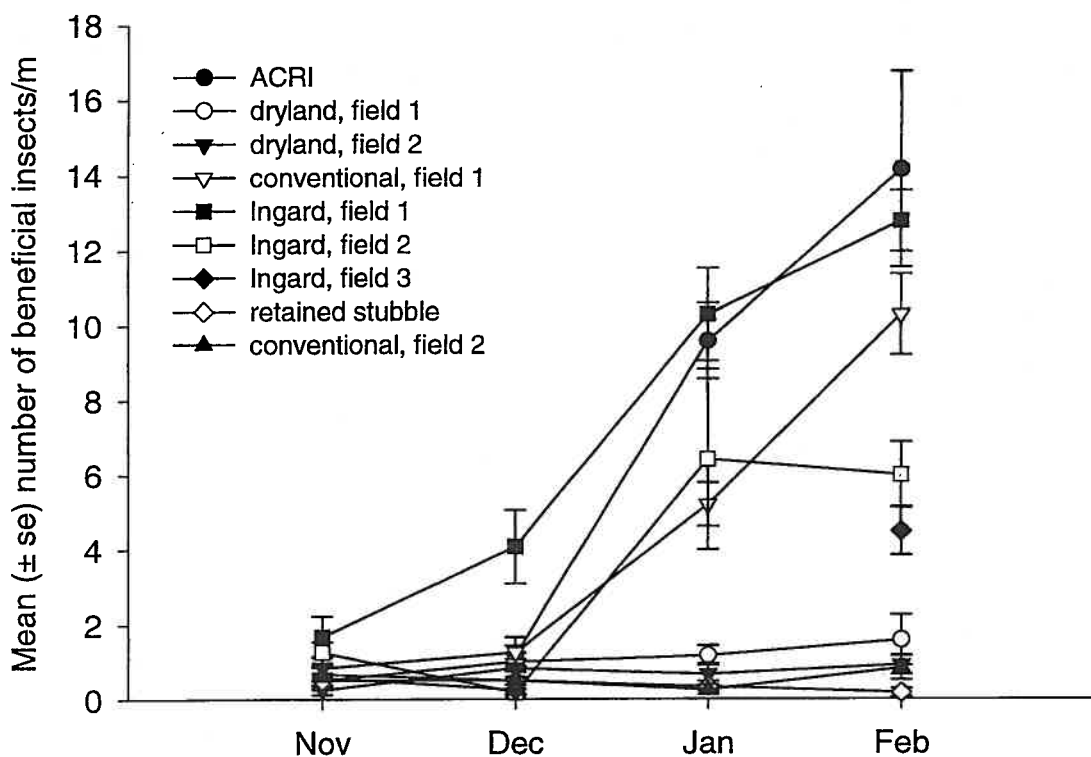


Figure 5. Mean ($\pm$ se) abundance per metre row of cotton for all beneficial arthropods collected in each field and each month. Very soft or no spray regimes were applied to conventional field 1, Ingard fields 1, 2 and 3 and at ACRI. Harder spray regimes were applied to dryland fields 1 and 2 and the retained stubble field.

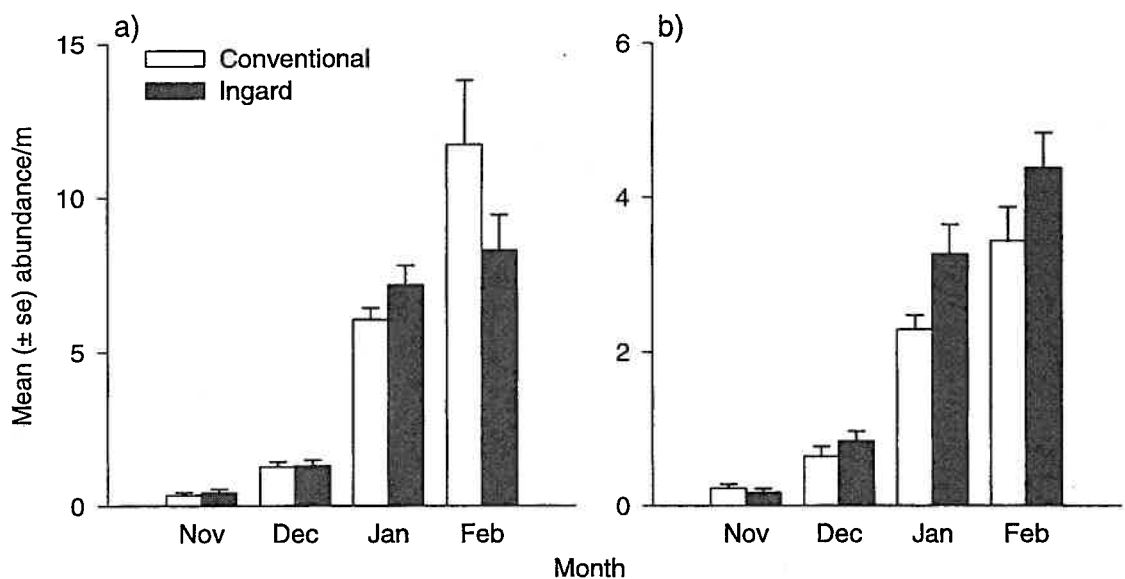


Figure 6. Average abundance of a) beneficial insects and b) spiders in conventional and Ingard cotton fields sampled from November 2002 to February 2003.

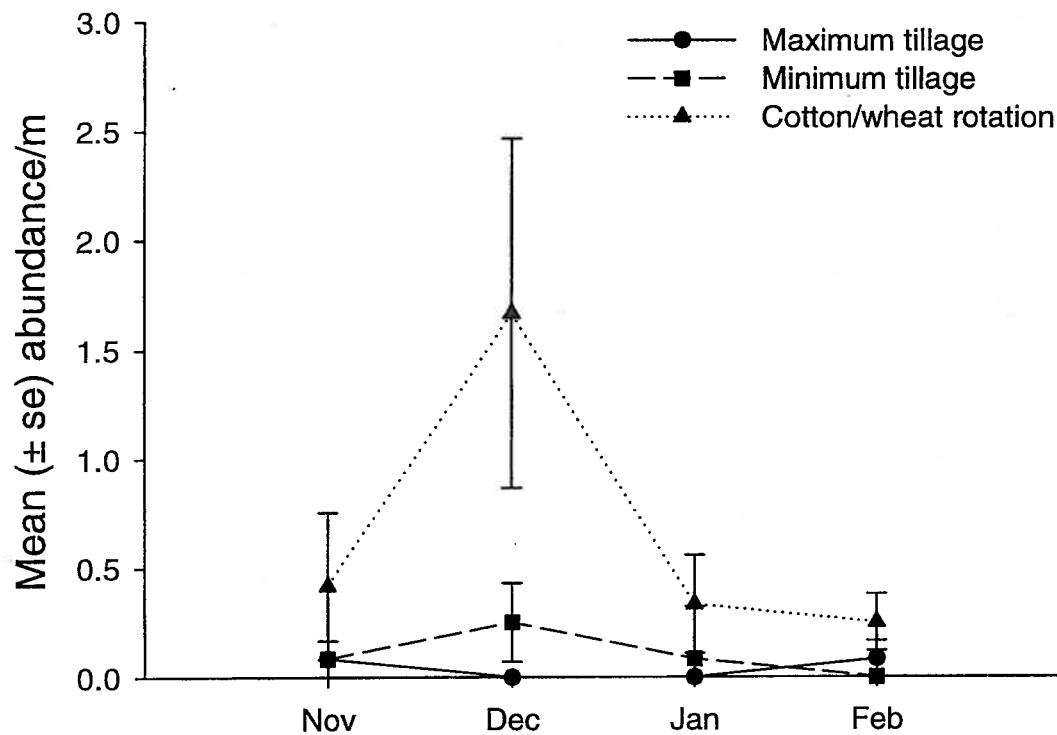


Figure 7. Average abundance of ants in field C1 at ACRI for the 2002-03 cotton season. This field is divided into replicate blocks of three treatments: maximum tillage and continuous cotton, minimum tillage and continuous cotton, minimum tillage and cotton/wheat rotation.

d) Predation of native coccinellids on *H. armigera* and the cotton aphid, *Aphis gossypii*

Under laboratory conditions, *H. armigera* egg consumption increased with higher prey density and differed between predator species (Table 6). Although alternate prey (aphids) did not affect egg consumption significantly overall, the interaction between species and aphids shows that the feeding patterns of some coccinellids changed when aphids were present. *C. transversalis* and *M. frenata* consumed more *H. armigera* eggs in the absence of aphids than when aphids were available (Figure 8a-b). *H. octomaculata* consumed the most eggs of the four ladybird species tested (Figure 8c) and *C. inaequalis* consumed the least (Figure 8d), with or without aphids available.

The two coccinellid species tested in the glasshouse cages consumed similar amounts of *H. armigera* eggs although their responses to prey and predator density differed (Table 7). Egg consumption by *H. octomaculata* increased as egg density increased but predator density did not affect egg consumption (Figure 9a). In contrast, egg consumption by *C. inaequalis* increased with both prey and predator density, particularly at the higher egg densities (Figure 9b).

Under field conditions, the number of eggs missing declined from December to February ($\chi^2 = 21.56$, $df = 3$, $P < 0.001$), although there was no consistent pattern in the amount of egg loss between the different cage treatments (Figure 10).

Table 6. Logistic analysis of predator species, prey density and alternate prey effects in the laboratory tests of *H. armigera* egg consumption. * $P < 0.0001$.

Factor	df	χ^2
Predator species	3	291.59*
Prey density	2	45.32*
Aphids	1	0.01
Species x prey	6	51.54*
Species x aphids	3	25.14*
Prey x aphids	2	70.23*
Species x prey x aphids	6	32.17*

Table 7. Logistic analysis of predator species, predator density and prey density effects in the glasshouse tests of *H. armigera* egg consumption. * $P < 0.001$, ** $P < 0.05$.

Factor	df	χ^2
Predator species	1	< 0.005
Predator density	2	54.92*
Prey density	2	5.32
Species x predator density	2	20.78*
Species x prey density	2	5.89
Predator density x prey density	4	22.75*
Species x predator density x prey density	4	12.89**

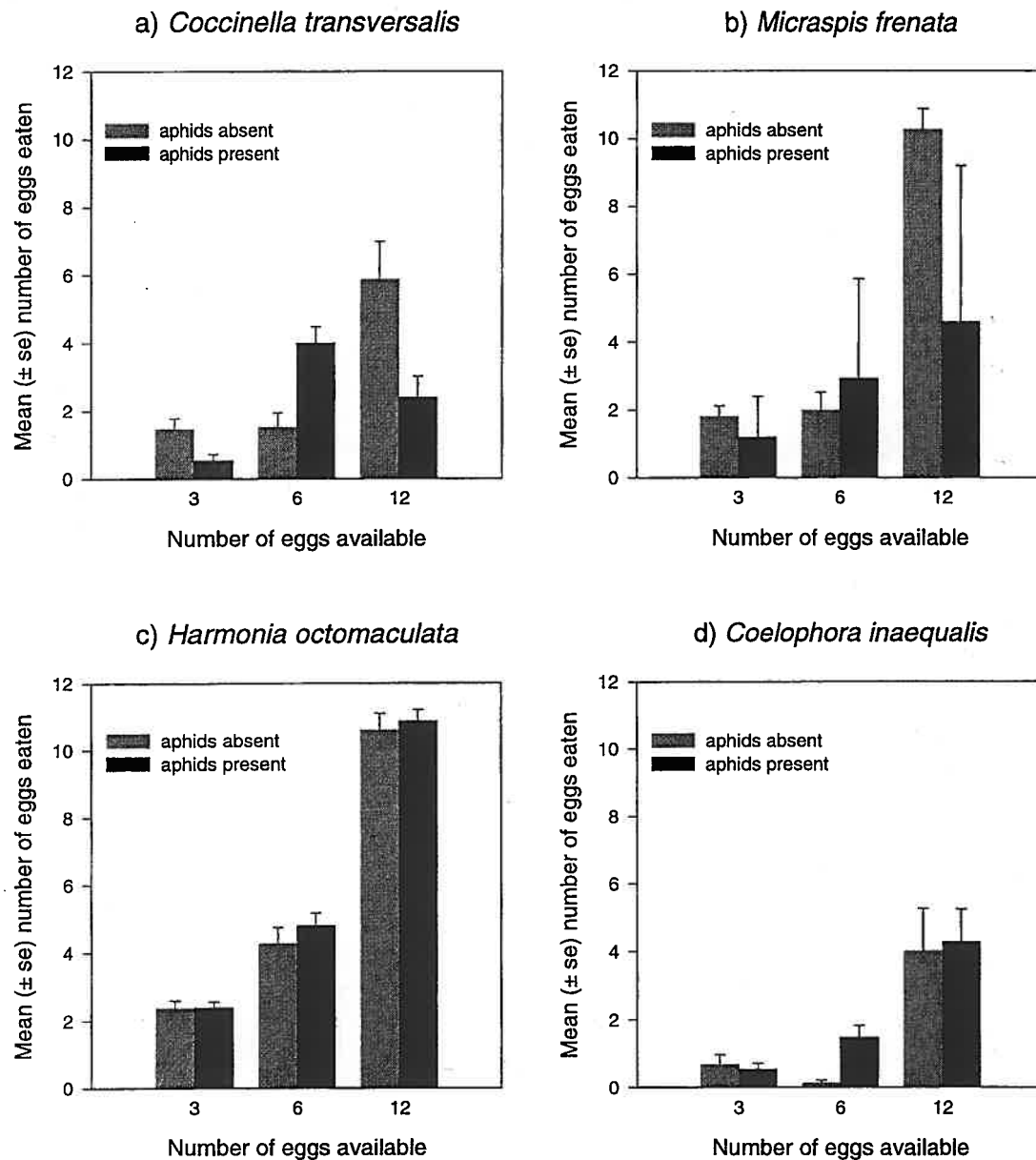


Figure 8. Mean ($\pm$ se) consumption of *Helicoverpa armigera* eggs at three densities by four coccinellid species under laboratory conditions, with and without aphids as alternate prey.

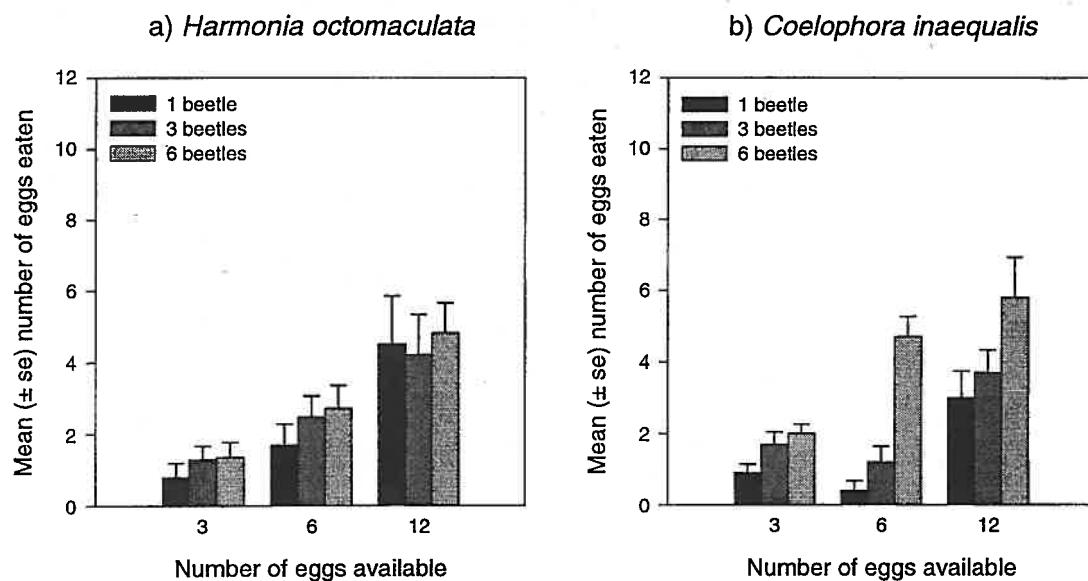


Figure 9. Mean ($\pm$ se) consumption of *Helicoverpa armigera* eggs by two ladybird species at different predator and prey densities in perspex cages under glasshouse conditions.

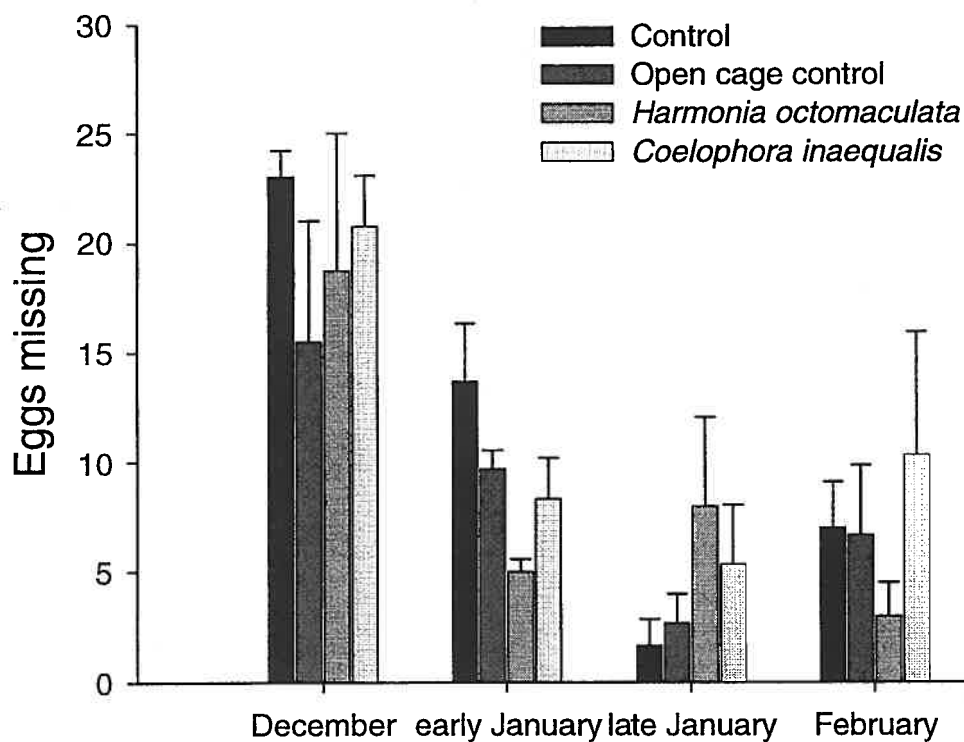


Figure 10. Mean ($\pm$ se) disappearance of *Helicoverpa armigera* eggs from field cages containing two coccinellid species and from control cages with no coccinellids added.

e) Rearing methods for the bigeyed bug, *Geocoris lubra* and consumption of *H. armigera*

G. lubra eggs hatched more quickly and total development time was shorter at 27°C than 25°C (Table 8). Very few nymphs reached adulthood on a diet of aphids alone ($n = 12$). Breeding success improved dramatically at 27°C ($n = 219$). More than 50 nymphs hatched after just three days at the higher temperature and survival to adulthood increased to 70%. *G. lubra* ate a higher proportion of *H. armigera* neonates (46% on average) than eggs (6% on average). Predation of *H. armigera* eggs and neonates increased with longer feeding times (Figure 11), however single *H. armigera* eggs were not eaten by *G. lubra* in this experiment. Predation also tended to increase when more prey items were offered for both eggs and neonates.

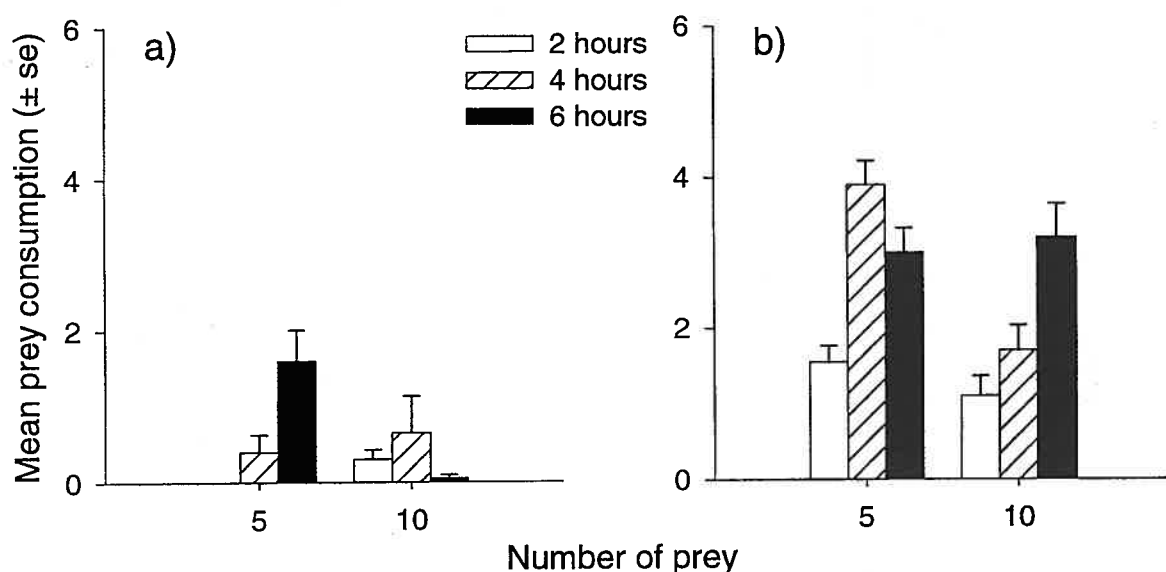


Figure 11. Mean ($\pm$ se) consumption by adult *Geocoris lubra* of a) *Helicoverpa armigera* eggs and b) neonate larvae after two hours, four hours and six hours exposure to either five or ten prey items.

Table 8. Mean ($\pm$ se) development time to egg hatch and adulthood and survival to adulthood (%) for *Geocoris lubra* reared at different temperatures and on different prey.

Temperature (°C)	Prey	Egg hatch (days)	Adulthood (days)	Survival (%)
25	Aphids	11.79 $\pm$ 0.12	39.92 $\pm$ 1.10	8.4
27	<i>H. armigera</i> eggs	8.35 $\pm$ 0.31	26.01 $\pm$ 0.39	70.7

f) Ant predation on *H. armigera* eggs

Iridomyrmex spp. were seen most frequently whereas *Paratrechina* spp., *Pheidole* spp. and *R. metallica* were less frequently observed in the cotton crops during the 2001-02 season. *Iridomyrmex* spp. spent most of the observed time on the ground (70%), very little time (<1%) on egg predation and were seen more frequently on the cotton plants in 2001-02 than any other ant taxa. Egg predation occurred twice at ACRI (two and three eggs taken respectively) and once at Lowana (four eggs taken). Hence the rate of egg predation by *Iridomyrmex* spp. was 0.03% eggs/hour in 2001-2002. No egg predation was recorded in 2001-02 for any other ant taxa.

5. Conclusions

Key predators of *H. armigera*: their ecology and the effects of agronomic practices

The predatory bugs *G. lubra* and *N. kinbergii* were significant predators of *H. armigera* in Australian cotton crops during seasons of low and high *H. armigera* abundance. It is difficult to compare the relative importance of these two predators for the control of *Helicoverpa* spp. because of the different antigen decay rates for *G. lubra* and *N. kinbergii*. The higher proportion of positive results from *N. kinbergii* may reflect the longer detection interval in this predator or a true difference in predation rates between the two species. *N. kinbergii* can reproduce and develop successfully on multiple prey, not just *Helicoverpa* spp. This has the advantage that this predator can maintain populations within the cotton system even when *Helicoverpa* spp. are less abundant. Development studies with *G. lubra* and other species of *Geocoris* have demonstrated the importance of protein-rich prey, such as *Helicoverpa* eggs for successful reproduction of these predators (Cohen and Debolt 1983, Lopez et al. 1987, Eubanks and Denno 1999 and 2000, Mansfield et al. submitted ms). The abundance of *G. lubra* within the crop system is also affected by prevailing weather conditions because this species requires warm temperatures for successful reproduction and development. Although growers have no control over the weather, they can take weather forecasts into account when assessing the potential for *G. lubra* to suppress *H. armigera* within their crops. Two other predatory bugs (the brown smudge bug, *Deraeocoris signatus* and minute pirate bugs, *Orius* spp.) often become abundant in late season cotton crops, however the impact of these predators on *H. armigera* was not addressed in this project.

The effect of predatory beetles on *H. armigera* populations is less certain due to difficulties with application of the ELISA technique to this group of predators. The red and blue beetle, *D. bellulus*, was the most abundant predator collected for ELISA tests yet only a small fraction of the individuals tested were positive for predation on *H. armigera*, using the indirect ELISA and Mab. A much higher percentage of these predators tested positive using the rabbit protein marker and sandwich ELISA, suggesting that predation rates were underestimated by the other method. However field testing of the rabbit marker protocol was very limited (a single event on small, early season cotton plants) and predation on the marked *H. armigera* eggs may have been greater than normal because very few other prey were available at the time of the field trial. Results from both ELISA protocols indicate that predatory coccinellids may have some impact on *H. armigera* populations, particularly when their preferred food source (usually aphids) is absent from the crop. While these predators can feed on other prey, the importance of their preferred food to coccinellid populations should not be underestimated. These predators cannot reproduce successfully in the absence of their preferred prey and adult beetles travel large distances in their search for suitable reproduction sites. Hence native coccinellids tend to be very transient within cotton crops during seasons of low aphid abundance. The impact of *H. variegata*, a recent arrival to Australia (Franzmann 2002), on native coccinellids and on crop pests is not yet certain. This species has spread rapidly within Australia and was the dominant coccinellid species in the 2002-03 season.

Four ant taxa are commonly found on cotton foliage: *Iridomyrmex* spp., *Paratrechina* spp., *Pheidole* spp. and *Rhytidoponera metallica*. Of these taxa, only *Iridomyrmex* and *Pheidole* spp. have been observed taking *H. armigera* eggs that had been deliberately placed in the crop during the 1999-2000 and 2001-02 seasons (Mansfield et al. 2003). *Pheidole* spp. had a higher predation rate than *Iridomyrmex* spp. (0.14% eggs/hour c.f. 0.03% eggs/hour) and spent more time on the cotton foliage than the other ant taxa, however the overall impact of such predation is unlikely to be significant for pest control. Ant populations are severely disrupted by cultivation, flood irrigation and insecticide use, particularly applications of

endosulfan and fipronil (Lytton-Hitchins and Wilson 1999, Wilson et al. 1999, Mansfield et al. 2003). Dryland cotton crops may support a greater diversity of ants in the absence of cultivation or insecticide effects (Elias and Mansfield 2002).

Spiders, such as *Cheiracanthium* spp., have been recorded previously as predators of *Helicoverpa* spp. (Scholz et al. 2000). However the lack of diagnostic keys to identify spider species prevented development of the indirect ELISA for any of these predators. Results from the rabbit marker field tests suggest this group should be the subject of future research, because it is likely that spiders do contribute to control of *Helicoverpa* spp.

Insecticide use remains the key factor affecting the abundance and diversity of beneficial arthropods in Australian cotton crops (Mansfield et al. submitted ms). When a soft insecticide regime is used, the abundance of beneficial arthropods increases dramatically over the course of the cotton season with the growth of the crop canopy. Ingard cotton crops usually support a greater abundance of beneficial arthropods, however it is not yet clear if this difference is solely attributable to the softer insecticide regime typically applied to these crops. Minimum tillage combined with a cotton/wheat rotation can increase ant abundance relative to other cultivation systems. Dryland cotton crops may support a greater diversity of ants and spiders than irrigated systems, although this needs to be confirmed by further research over a wider range of field sites.

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

This project has improved our understanding of insect predator ecology and has demonstrated the importance of predatory bugs as key predators of *H. armigera*. Difficulties with the methodology have limited the overall assessment of predatory beetles; it does appear that coccinellids may contribute to suppression of *H. armigera* in situations where their preferred prey is absent. Development of two effective ELISA methods for the assessment of predation has provided tools for further research into predator ecology, if such research is considered important to the industry. Collaborative projects with other researchers at ACRI have validated the Beneficial Disruption Index as a measure of insecticide impact on beneficial arthropods and demonstrated the relative efficiencies of different insect sampling techniques. The outcomes of this project and associated collaborations should assist growers to maximise the effects of beneficial insects within their cotton crops and reduce insecticide use. In consequence, production costs will decrease, making the cotton industry more sustainable. This will benefit rural communities that depend on the cotton industry for employment and income.

7. Summary of advances in methodology arising from the project

a) technical advances achieved

Not applicable

b) other information developed from research

ELISA as a method to measure predation

Prior to the development of serological methods such as ELISA, direct observation was the only way to link a predator with a particular prey item. ELISA tests allow the assessment of many individual predators for recent predation on the target prey whereas direct observations are usually limited to a small number of individuals, due to time and resource constraints. The indirect ELISA using the Mab specific to *H. armigera* proved highly effective for detecting predation in predatory bugs but was less effective in predatory beetles. Other studies have found similar limitations for indirect ELISA tests using monoclonal antibodies specific to the target prey (Hagler and Naranjo 1997, Hagler et al. 1997, Hagler 1998). The total protein content of the predator (higher in beetles than in bugs) can affect the ability of

these tests to detect true positive results although the exact mechanism is unknown (Hagler et al. 1997). In contrast, the sandwich ELISA is generally more effective on predatory beetles than predatory bugs, particularly when combined with rabbit protein as a prey marker (Hagler and Durand 1994, Hagler 1998). This difference is partly due to the feeding modes of the two predators. Chewing predators, e.g. beetles, consume all or most of the prey including the outer shell or exoskeleton that carries the protein marker. Sucking predators, e.g. bugs, consume the internal contents of the prey but leave most of the outer shell, reducing their contact and ingestion of the protein marker. Another factor that contributed to the higher detection rates of the sandwich ELISA for this study was that the rabbit protein marker persisted longer in the gut contents of predators than did the antigen detected by the *H. armigera* Mab.

Therefore, it appears that when an ELISA technique is chosen to assess predation, the user must consider the type of predators to be tested and the situation in which the tests will be used. The rate of antigen decay is an important limiting factor for all ELISA tests. The faster the antigen is digested the shorter the detection period. The observed rate of digestion depends on many factors, most outside the control of the operator (Hagler and Naranjo 1997). However when a Mab is to be developed specifically for this purpose, ideally the chosen antigen-Mab combination will have the optimum decay rate for the intended environment. If the target prey has a patchy distribution and variable levels of abundance, a slower decay rate may aid identification of key predators by increasing the likelihood of detection. On the other hand, if the target prey is usually widespread and highly abundant, a faster decay rate allows better quantification of predation over time. The final point for consideration is that a prey-specific antibody allows measurement of predation on natural prey populations with minimal interference in the predator-prey interaction. However the rabbit protein marker must be physically applied to prey and those marked prey then released into the environment. The mode of prey marking and release could affect predator behaviour, thereby altering the predation rate from the natural situation.

c) are changes to the Intellectual Property register required?

Not applicable

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

A research proposal has recently been approved by CRDC that will use the rabbit protein marker and sandwich ELISA to further investigate predation on *Helicoverpa* spp. and pest mirids.

(b) for the future presentation and dissemination of the project outcomes.

Project outcomes have been presented through scientific and industry publications. Dr Mansfield frequently gave presentations to scientific and industry audiences (e.g. the Ecological Society of Australia and the IPM Short Course for Cottongrowers). A full publication list is given in section 9, including scientific manuscripts in preparation. Results from this project will also be presented in August 2004 at the International Congress of Entomology in Brisbane.

(c) for future research.

Spiders are a major predatory group found in Australian cotton crops that have not been addressed in this project. A recent CRDC project has examined the biodiversity of spider populations in cotton crops around Australia and it is likely that the new research proposal mentioned above will further investigate spider ecology. While this project has demonstrated the impact of some predatory bugs on *H. armigera*, it has also suggested that the impact of

some predatory beetles is not equal to their apparent abundance. The effect of these findings on empirical management techniques such as the predator:pest ratio needs to be investigated. Another issue not specifically addressed by this project is whether predators discriminate between *H. armigera* and *H. punctigera*. Although such discrimination seems unlikely, given the ecological similarity of the two species within cotton crops, experimental tests of predator preferences for these two prey species are needed to confirm this.

9. List the publications arising from the research project and/or a publication plan.

PUBLICATIONS

Refereed Journals

Mansfield, S., Elias, N. & Lytton-Hitchins, J. A. (2003). Ants as egg predators of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) in cotton. *Australian Journal of Entomology* **42**: 349-351.

Conference papers

Mansfield, S. (2002). Consumption of *Helicoverpa armigera* eggs by the ladybirds *Harmonia octomaculata* and *Coelophora inaequalis*. Proceedings of the 11th Australian Cotton Conference, Brisbane, Qld. pp. 321-327.

Reviews and Book Chapters

Mansfield, S. (2004). Book review of "Insects and Pest Management in Australian Agriculture" for *Austral Ecology* **29**: 359-360.

Manuscripts in preparation

Mansfield, S., Scholz, B., Armitage, S. & Johnson, M.-L. Rearing methods, development time and prey consumption of the bigeyed bug, *Geocoris lubra* Kirkaldy (Hemiptera: Geocoridae). Submitted to Australian Journal of Entomology, 4 June 2004.

Mansfield, S., Dillon, M. L. & Whitehouse, M. E. A. Are communities really disrupted? An assessment of insecticide spray regimes (as measured by the Beneficial Disruption Index) on insect and spider communities in Australian cotton. Submitted to Agriculture, Ecosystems and Environment, 29 June 2004.

Mansfield, S. Development of an ELISA to detect predation on *Helicoverpa armigera* eggs and larvae in Australian cotton crops. (ms in prep.)

Mansfield, S. & Hagler, J. Comparison of the efficacy of two ELISA methods for assessing predation by predatory beetles on *Helicoverpa armigera*. (ms in prep.)

PRESENTATIONS AND PUBLIC RELATIONS

Conferences Presentations

Mansfield, S. (2004) The impact of predatory bugs on *Helicoverpa armigera* in Australian cotton crops. Paper, International Congress of Entomology, 16 August 2004, Brisbane, Qld.

Mansfield, S. (2003) Detection of predation on *Helicoverpa armigera* in Australian cotton crops using ELISA. Paper, Annual Meeting of the Ecological Society of Australia, 8-10 December, Armidale, NSW.

Mansfield, S. (2003) Key predators of *Helicoverpa armigera* in Australian cotton crops. Paper, XIII International Entomophagous Insects Workshop, 27-31 July, Tucson, Arizona.

Mansfield, S. (2003) Cotton predators exposed. Presentation, Annual review of the Australian Cotton CRC, 24-25 July, Armidale, NSW.

Mansfield, S. (2003) Key predators of *Helicoverpa* spp. in Australian cotton. Paper, Farming Systems IPM Forum, 25-26 June, Toowoomba, Qld.

Mansfield, S., Dillon, M. L. & Whitehouse, M. E. A. (2002). Do beneficials pay their way? Poster, Annual Meeting of the Ecological Society of Australia, 2-6 December, Cairns, Qld.

- Mansfield, S. (2002) Enhancing the impact of early season predation on *Helicoverpa* spp. Presentation, Farming Systems Evaluation and Planning Meeting, 19-20 June, Toowoomba, Qld.
- Mansfield, S. (2002) Insect predators in cotton. What can they do for you? Presentation, Annual Review of the Australian Cotton CRC, 17-18 June, Toowoomba, Qld.
- Mansfield, S. (2001) Enhancing the impact of early season predation on *Helicoverpa* spp. Presentation, Annual Review of the Australian Cotton CRC, 17-18 June, Narrabri, NSW.

Seminars, Workshops & Trade Shows

- Mansfield, S. Know your beneficials: why, who, what & how? Presentation to the IPM Short Course, 11 September 2003, ACRI, Narrabri, NSW.
- Mansfield, S. Research update presented at a meeting of the Two-River Areawide Management Group, 17 June 2003, Pilliga, NSW.
- Mansfield, S. Beneficial insects in cotton, Presentation at the Lower Namoi Field Day, 13 March 2003, Breeza Plains, NSW.
- Mansfield, S. Beneficial insect sampling and identification. Presentations to the Integrated Pest Management Short Course for cotton growers, 18 December 2002 and 31 January 2003, St. George, Qld.
- Mansfield, S. Research Q&A seminar, 12 September 2002, Certification Workshop for Cotton Consultants of Australia Inc., Wee Waa, NSW.
- Mansfield, S. Beneficial insect sampling and identification, Integrated Pest Management Short Course for cotton growers, 6 December 2001 and 31 January 2002, Trangie, NSW.
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- Deutscher, S., Dillon, M., McKinnon, C., Mansfield, S., Staines, T. and Lawrence, L. (2003) A Good Beating. *The Australian Cottongrower* **24** (3): 24-27.
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Media interviews

- Research summary included in a poster highlighting IPM in cotton for the 75th anniversary celebration of CSIRO.
- Media release from CSIRO Entomology,
<http://www.ento.csiro.au/publicity/pressrel/2001/01mar01.html>

GRANTS

- Dillon, M. L., Mansfield, S. and Deutscher, S. Comparison of established sampling methods with the new beat sheet technique for measurement of pest and predator abundance in cotton. Australian Cotton CRC Summer Scholarship Project awarded to Carla McKinnon, November 2002 to February 2003.

Mansfield, S. Ants as egg predators of *Helicoverpa* spp.: Measurement of abundance and the effect of agronomic practices. Australian Cotton CRC Summer Scholarship Project awarded to Natalie Elias, December 2001 to February 2002.

10. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

Results from this research project should increase grower confidence in the importance of beneficial arthropods, particularly predatory bugs, for the control of *Helicoverpa armigera*. The economic analysis of Hoque et al. (2000) has demonstrated that growers can achieve higher gross margins while using a softer insecticide regime intended to conserve beneficial arthropods. The findings of this project support this outcome by providing direct evidence of predation on *H. armigera*. However this project has also shown that not all beneficials have equal impact on *H. armigera*. This means that growers need to consider not only the total abundance of beneficial arthropods within their cotton crops, but also the species composition of the beneficial community. While such considerations will increase the complexity of crop management decisions, in the long term a deeper understanding of the beneficial community should improve pest control and increase the sustainability of the cotton industry.

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Part 4 – Final Report Executive Summary

This project investigated how key insect predators contribute to the suppression of the cotton bollworm, *Helicoverpa armigera*, in Australian cotton crops and explored the interaction between predator abundance and diversity, agronomic practices and predation of *H. armigera* eggs and larvae. Several strategies were used to determine the importance of different insect predators in cotton: manipulative experiments, direct monitoring of insect abundance in commercial cotton crops, observation of predator behaviour under natural conditions and development of diagnostic laboratory tests (ELISA) to detect recent consumption of *H. armigera* by predatory arthropods.

The predatory bigeyed bug (*Geocoris lubra*) and damsel bug (*Nabis kinbergii*) were significant predators of *H. armigera* in cotton crops during seasons of low and high *H. armigera* abundance. Over two consecutive cotton seasons (2001-02 and 2002-03), 12-14% of *G. lubra* and 22-28% of *N. kinbergii* tested positive for recent predation on *H. armigera* using ELISA. Juvenile *G. lubra* survive and develop better on a diet of *H. armigera* compared with a diet of aphids under laboratory conditions, further suggesting that *H. armigera* is an important food source for this predator. Warm temperatures (above 27°C) also favour breeding of this species.

Although the predatory red and blue beetle, *Dicranolaius bellulus*, was highly abundant in both the 2001-02 and 2002-03 seasons, only 1% tested positive for recent predation on *H. armigera*. However the diagnostic ELISA test is less effective with predatory beetles than predatory bugs, so these results may underestimate the rate of predation for this species. Most species of native ladybirds found in Australian cotton crops feed primarily on aphids but may feed on *H. armigera* in the absence of their preferred prey.

Early in the growing season, ants (*Pheidole* and *Iridomyrmex* spp.) were observed to prey upon *H. armigera* eggs in small quantities. Cultivation and flood irrigation severely disrupt ant populations in cotton crops and limit their impact as predators. Minimum tillage combined with a cotton/wheat rotation can increase ant abundance relative to other cultivation systems. Some spiders such as the yellow nightstalker (*Cheiracanthium* spp.) are likely to feed upon *Helicoverpa* spp., although their impact on *H. armigera* was not specifically addressed in this project.

Insecticide use remains the key factor affecting the abundance and diversity of beneficial arthropods in Australian cotton crops. When a soft insecticide regime is used in both conventional and Ingard cotton fields, the abundance of beneficial arthropods is likely to increase dramatically in response to crop growth over the course of the season.

This project has improved our understanding of insect predator ecology and has demonstrated the importance of predatory bugs as key predators of *H. armigera*. When making pest management decisions, growers need to consider not only the total abundance of beneficial arthropods within their cotton crops, but also the species composition of the beneficial community.