Part 3 – Final Report Guide (due within 3 months on completion of project)

(The points below are to be used as a guideline when completing your final report.)

Background

1. *Outline the background to the project.*

The advent of transgenic Bt cotton has had a major impact on the cotton industry by largely controlling Lepidopteran pests. This has lead to a drop in the number of insecticides used on cotton which has made cotton more amenable to Integrated Pest Management (IPM) Strategies. This has yielded major benefits to the grower in terms of costs saved, and to the community in general in terms of reduced insecticides in the environment.

Mirids threaten this process. The decline in insecticide usage allows them to survive and reproduce whereas before they were suppressed by insecticides applied to control *Helicoverpa* spp. Mirids are capable of causing major damage such as tipping out plants, shedding squares and deforming bolls, which can result in an economic loss in production. However there is only a limited range of broad-spectrum insecticides available to control mirids and these usually result in a dramatic decline in beneficial numbers, thereby disrupting IPM systems and increasing the risk of mite, aphid or whitefly outbreaks.

High numbers of mirids, however, do not necessarily indicate heavy crop damage. Pest managers perceive mirids as "unpredictable". They report observing large numbers of mirids causing little damage, and vice versa (Whitehouse, pers. obs.). The problem is made worse because damage not caused by mirids is sometimes attributed to mirids, such as damage from other sucking pests, and damage from physical factors (such as excessive heat which can cause parrot beaked bolls by inducing sterility; Hearn & Constable 1984). This adds to the problem of identifying mirid damage, and consequently mirid control.

Growers could be applying insecticides to control mirids under conditions when they are unlikely to be causing economic damage. As the sprays available to control mirids are disruptive to the beneficial community, they could have a strong affect on the predators of mirids, thereby exacerbating the problem. Excessive insecticide use could reduce the benefits offered by Bt cotton and thereby incur an unnecessary financial cost to the grower.

The concern about mirids has raised fears that they could trigger a rise in the amount of insecticides applied to cotton. To counteract this problem, mirid thresholds have been refined (Khan et al 2006) to reduce the number of unnecessary sprays for mirids, but this has complicated mirid management as the threshold now varies 6 fold throughout the country. In addition, pest managers also need to consider fruit retention when making a decision to spray. Given the perceived unpredictability of the pest and the variable nature of the thresholds, there is concern as to whether pest managers are following the recommendations.

The aim of this report is to present the results of three studies which aimed to identify current mirid management methods, identify potential predators which could assist with mirid management and test their effectiveness, and identify other interspecific interactions which could influence mirid damage to cotton.

Project structure and structure of this report

This final report covers four years of work, but in reality it encompasses three separately funded bodies of work: The first body of work was a project titled: "Impact of predation on emerging cotton pests" that ran for one year (2004-05). It looked at the feasibility of using rabbit protein to mark pests, and to identify the predations of mirids, aphids and *Helicoverpa* eggs in cotton.

The second body of work was a project titled: "Mirid Predation" that was funded for 2 years (2005-07). The aim of this project was to test if predators could reduce mirid numbers and mirid damage; if other factors, such as the sexual status of the mirids or the presence of alternative prey or competitors, could reduce mirid damage; and (if any reduction in mirid damage was detected) if any managerial practices could enhance this reduction.

The third body of work was an extension of the Mirid Predation project which ran for one year (2007-08). The aim of this work was to write up and make available the results from the mirid project.

Because of the overlap between these bodies of work, they will not be presented in the chronological order outlined above, but in four sections, each containing its own Methods, Results and Discussion.

The first section "**Mirid Spray Survey**" will report on the mirid survey conducted in 2005/06 and 2006/07. The aims of the survey were to identify: 1) the spray triggers (or the factors influencing the decision to spray for mirids); 2) the control options chosen (the insecticides used and the spray rates); and 3) the ramifications of the decisions (whether spraying above or below threshold resulted in higher or lower yields on commercial farms). The overall aim of this section was to see if the management recommendations for mirids were supported by commercial farm results. The second aim was to identify how to best support an IPM approach to the control of mirids and how to further develop IPM.

Mirids are seen to be very variable in the amount of crop damage they inflict. This anomaly could be due to 1) differences in the beneficial community (see below); 2) the presence of competitors and alternative food sources; or 3) the condition of the mirids themselves. For example, mirids are known to feed on animal as well as plant material. The presence of other pests in the cotton (such as *Helicoverpa* eggs, aphids or mites) may be an alternative food source to mirids, reducing the likelihood of mirids attacking cotton fruiting bodies. The effect of an alternative food source may also be dependant on the life stage of the mirid. Males and females are likely to have different requirements when they enter cotton. Males are more likely to need energy and so probably will feed on nectaries. Females will need protein for egg production, and so may be more likely to attack bolls and squares, especially once they are mated. Thus mirid damage could be an interaction between the reproductive state of the mirids, and the presence of other pests. The second section "**Modifiers of Mirid Damage**" will report on the effect of mirid sexual status on the amount of damage inflicted on cotton fruit. It will also report on the effect of competitors and possible alternative food items, such as *Helicoverpa* eggs, mites and aphids, on cotton fruit damage by mirids.

One of the aims of IPM is to used a number of different approaches to control pests. If pest managers are aware of the beneficials in their crop they will have more confidence in allowing them to control low numbers of pest without resorting to insecticide applications. Unfortunately, the predators of mirids and other emerging pests are not well known. The second section of this report, "**ELISA work**", presents the results of work designed to identify predators of mirids, aphids and *Helicoverpa* eggs in cotton by releasing marked pests into cotton, collecting predators and testing these predators for the mark. The aim was to modify a technique which involved applying exotic antigen markers (such as the rabbit IgG protein) to target prey and then testing for the presence of the marker in predators using enzyme-linked immunosorbent assays (ELISAs) with the sandwich technique. The first part of this work focused on establishing if mirids and aphids could be reliably marked. The second part tested whether the mark could be detected in predators which had eaten marked prey; and the third part used this information to identify predators of marked mirids, aphids and *Helicoverpa* eggs collected from cotton crops.

Once predators have been identified, the next step is to test the effectiveness of key predators at limiting mirid numbers and damage. The third section "**Predators**" focuses on animals identified from the ELIZA work as mirid predators. The aim of this section is to establish if any or all of these predators can control mirid numbers and mirid damage; both on their own in laboratory experiments, in the presence of other predators, and within cotton crops. Part of this section includes contributions from two summer scholarship students, Mark Barnett and Katie Broughton.

Statistics

Statistical analysis was conducted using the Genstat program (Payne 2000). Some samples lacked information, which meant that they could not be used in all statistical tests. Wherever possible I used parametric techniques (in particular ANOVA and REML) but when the data could not be normalised I used non-parametric techniques including the Friedman's Two-Way analysis of variance by rank, Chi-squared test or Fisher's exact test (in all cases the α value was 0.05, Siegel & Castellan 1988, unless otherwise stated). I used a direct canonical correspondence analysis (CCA) to examine the importance of different factors in mirid management decisions.

Throughout the report, results are presented as histograms. Unless otherwise stated, the same letter under a histogram column indicates no significant difference between these columns.

Objectives

2. List the project objectives and the extent to which these have been achieved.

Below are the objectives for the three bodies of work and comments on their success. Their achievements are discussed in greater detail in the sections indicated.

Objectives for the initial project: "Impact of predation on emerging cotton pests":

1. *Adapt the rabbit IgG protein marker method to assess predation on mirids and cotton aphids using laboratory and glasshouse studies.*

This work was completed, see **ELISA Work** section for more detail

2. *Use the rabbit IgG protein marker method to assess seasonal variation in predation of mirids, aphids and Helicoverpa amongst the most commonly occurring invertebrate predators in cotton fields, under varying management regimes.*

This work was completed, see **ELISA Work** section for more detail

3. *To determine if super-predation (predation of predators) can confound the outputs from rabbit IgG – based assessments, given the apparent sensitivity of the marking method.*

This objective was not fully explored; see **ELISA Work** section for more detail

Objectives for the second project "Mirid predation":

4. *Establish if key predators can reduce mirid numbers and mirid damage in cotton crops*

This work was completed, see **Predator** section for more detail.

5. *Gauge current response patterns to mirid pressure to establish if any changes in spraying patterns are due to increases in mirid numbers, or decreases in grower thresholds.*

This work was completed, see **Mirid Spray Survey** section for more detail

6. *Identify management techniques which maximise the opportunity for predators to control mirids.*

This work was not completed as I thought it was better to understand the effect of the predators on mirids before trying to manipulate the predators on farm. Insights into management methods in use that reduced the cost of mirid damage were identified by the mirid survey (see **Mirid Spray Survey** section for more detail).

7. *Establish if the presence of alternative food sources and competitors causes an increase or decrease in mirid damage in cotton.*

This work was completed; see **Modifiers of Mirid Damage** section for more detail.

8. *Establish if differences in the foraging demands of male and female mirids influences crop damage*

This work was completed, see **Modifiers of Mirid Damage** section for more detail

Additional objective with the addition of extra funds:

9. *Use ELISA techniques to identify if key mirid predators attack different pests as they mature*

This work was completed, see **ELISA Work** section for more detail

Objectives for the one year extension to "Mirid Predation":

10. *Understand whether predators, alternative food types and mated status of mirids influence mirid damage*

This work was completed, see **Predators** and **Modifiers of Mirid Damage** sections for more detail

11. *Make available information on the predators of Helicoverpa eggs, Aphids and mirids, as identified using ELISA techniques*

This work was achieved. I will be presenting the results of this work as a speaker at the 3rd International Symposium on Biological Control of Arthropods (Christchurch) in February 2009 and I have written a proceedings paper on this topic for this meeting (see attachments).

12. *Make available information on the mirid spray survey*

This work was achieved. The Mirid Spray Survey section has been submitted to internal review in CSIRO in preparation for submission to the scientific journal "Agricultural Systems". In addition, two articles on this work have been published in the Australian Cottongrower: one in 2006 on the first survey, and on this November on the final results. Another version of the results have been sent out to all the participants in survey, the results were also published in the proceedings of the 2008 14th Australian Cotton Conference.

13. *Make available information on the communities of Bt and conventional cotton*

This work is not directly related to mirid control, but it has been achieved. I will be presenting the results of this work as a speaker at the 3rd International Symposium on Biological Control of Arthropods (Christchurch) in February 2009 and I have written a proceedings paper on this topic for this meeting (see attachments).

1 Mirid spray survey

METHODS

Background

Mirid thresholds are based on both the number of mirids found in the field, and the retention of fruiton the plant. The retention threshold for mirids is 60% throughout Australia. If the plant is maintaining more than 60% retention of its fruit, then the plant can compensate for damage caused by low mirid numbers; but if plant retention drops below 60% then it is susceptible to damage as it will have less change of compensating for any further loss.

The mirid number threshold is more complicated than the retention threshold as it varies six fold with respect to the climatic region and the sampling method. Two main methods for searching for mirids are the visual search, which involves carefully searching by eye one metre of a row of cotton and counting any mirids found; and the beatsheet method, which is described elsewhere (eg Mansfield et al 2006) and involves placing a large plastic sheet under a row of cotton and knocking it with a one metre stick held horizontal to the ground, causing any insects in the cotton to fall out onto the sheet, where they are counted. The threshold (using a visual search) in the cool region (upper Namoi, Macquarie, Lachlan/Murrumbidgee) is 0.5 mirids/m, while in the warm region (Emerald, Theodore, Darling Downs, St George, McIntyre, Gwydir and lower Namoi) it is 1 mirid/m (Farrell 2006). The beatsheet method is more effective and shows less variance between users than visual searches (Detucher et al 2003, Wade et al 2006), thus the beatsheet threshold in both the cool and warm regions is three times that of the visual search (1.5 and 3 mirids/m respectively; Khan et al 2006).

The above thresholds are most pertinent to a period of about five weeks from “first flower” (which occurs at about 800 degree days), when mirids are their most destructive (Khan et al 2006). Before this period small squares that are lost are more readily replaced, and after this period bolls that are maturing are less susceptible to mirid damage, to the point that they are immune to mirids once they are 20 days old. In this report, I assumed that all mirid control was occurring during the vulnerable period, so I used the thresholds based on this period. Thus this is a conservative estimation of the mirid thresholds.

Survey

Growers, managers, agronomists and consultants who had agreed to participate in the survey were sent a questionnaire by email or fax every fortnight during the cotton growing season. In the first survey, participants were asked to respond if they had sprayed for mirids; in the second survey participants were asked to respond if they had found mirids in their target field whether or not they had sprayed. In the second survey, more effort was taken to follow particular fields, and only Bt cotton was included in the survey. The participants choose a target field (which was the first field in which they found mirids) and reported in the questionnaire their management of mirids in that field for the rest of the season. They only needed to fill out the survey if they had seen mirids in their “target field” during the previous fortnight. Some participants, particularly consultants, reported on more than one field.

The questionnaire consisted of four sections (**Appendix 1**): section one recorded the statistics of the field during that survey (such as crop stage, number of mirids, sampling method, fruit retention); section two was a subjective assessment of the importance of various factors on the decision to spray/not to spray for mirids (such as rating the importance of retention, mirid numbers, crop stage, the presence of beneficials); section three recorded details about the spray (if the field was sprayed for mirids) such as product used and spray rate; and section four recorded the reasons for the choice of spray used (such as, cost, availability, preservation of beneficials). In sections 2 and 4, the importance of the factors to the decision was rated along a scale of A to D (A= very important, B=important, C=slight influence, D= no influence).

Before the season started, prospective participants were asked to provide information about themselves, their experience in cotton, and the amount of cotton they expected to be managing or growing. At the end of the 2006/07 season participants were asked the number of bales/ha their target field had yielded; and (as 2006/07 was a drought affected year) if the field had been affected by water stress (which was ranked from 0=no stress to 3=very stressed). Care was taken to record all mirid spray applications applied to the target fields, and as much information as possible of the spray events.

Additional statistical information

Data independence. One of the problems with this survey, as with most surveys, is that different participants put in different amounts of effort, so that the results will be distorted by those who put in the most amount of effort. To try and manage this problem, the responses were divided up into areas, where participants with similar response patterns were grouped. This enhanced analyses across all cotton regions, but has made comparisons between areas problematic. As participants were only asked to respond if they had mirids in their fields, one of the biggest problems was that it was difficult to tease apart differences between regions caused by differences in “effort” or “mirid pressure”, as both of these factors would result in some areas providing more reports. Because of these complications, comparisons between areas were only made cautiously, and readers need to keep this caveat in mind.

RESULTS

Characterisitics of the data

In the 2005/06 season the pilot survey involved 14 participants (4 growers, 5 consultants, 3 agronomists and 2 managers) These participants reported on 38 spray events (that either targeted mirids, or mirids were the second target) in the Namoi (n=27), Gwydir (n=5), and McIntyre (n=6) valleys. Between 1-5 sprays were reported per respondent, although the respondents did not necessarily report the spray events from one field. When asked what factors influenced their decision to spray, all of the growers and managers but none of the agronomists and consultants reported that “associates advice” was very important. Most agronomists and consultants reported that “associates advice” had “no influence” on their decision to spray. These results reflect the influence that consultants have on field management. Because of this finding, the fields in the 2006/07 survey were grouped according to the consultant that managed the field.

The 2006/07 survey obtained information from of 57 participants (35 consultants, 9 agronomists, 9 growers and 4 managers) from all the valleys in Australia growing cotton that season (Emerald, Theodore, St George, Darling Downs, McIntyre, Gwydir, Namoi, Macquarie, and Lachlan/Murrumbidgee). Because the cotton growing valleys in the warm region are very diverse, these were further divided up into three areas: Tropical (Emerald and Theodore), Downs (Darling Downs), and Middle (St George, McIntyre, Gwydir and lower Namoi). The Cool region (upper Namoi, Macquarie, Lachlan/Murrumbidgee) was left as one area (Fig. 1.1).

The respondents provided information on 255 mirid records in 80 fields, including 107 spray events. In all, the survey followed fields managed by 51 consultants. Most consultants reported directly to the survey, some were represented only by their clients, while others were represented by both themselves and their clients. There was large variation in the number of fields managed by each consultant in the survey, and the number of reports per consultant (Fig. 1.1). The most comprehensively surveyed area was the tropical area, where consultants involved in the survey managed about 90% of all cotton grown in this region. Not only were most of the consultants from this area involved in the survey, but they each reported on many fields from different farms, and provided the greatest number of responses.

The area that was most poorly represented in the survey was the middle area, where consultants involved in the survey managed about 25% of all cotton grown in this one area. Participants mainly reported on one field (as requested) and gave fewer responses per field. The number of responses reported could reflect either the enthusiasm of the participant, or the mirid pressure. In total, about 137,000 ha of cotton was grown in Australia in the 2006/07 season, and the consultants of the fields in the survey managed about 37% of this crop.

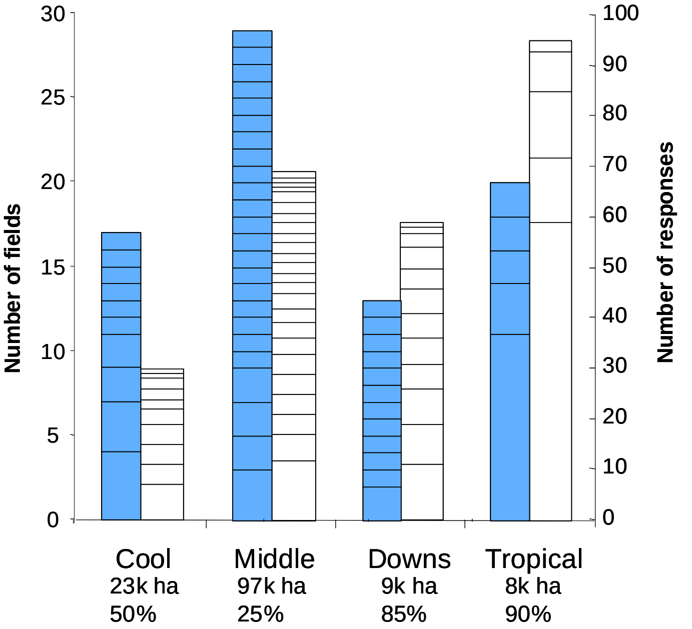


Fig. 1.1 The number of fields reported in the survey by each consultant (shaded histograms), and the total number of responses from each consultant (clear histograms) in the different areas in the 2006/07 survey. Each block in a histogram represents one consultant. The number under the histogram indicates the amount of cotton grown in that region (in thousands of hectares) and the percentage indicates the approximate proportion of that area managed by consultants in the survey.

The spray triggers

The mirid number threshold

When mirid thresholds are standardized to that for beatsheets in warm regions, many pest managers sprayed below threshold in both seasons (the shaded region in Fig. 1.2). Not surprisingly, nearly all the no-spray events were also below threshold (in the shaded area of Fig. 1.2c). So very few people didn't spray once the threshold had been reached. Some pest managers in the 2006/07 season used fruiting factor rather than % retention. In all of these 11 cases, spray events occurred only once mirid number threshold had been reached.

One reason for the high number of sprays occurring before threshold was management constraints ("insurance sprays" Fig. 1.2b). These were constraints identified by some pest managers to explain spray applications below threshold (eg: last opportunity to use a ground rig before irrigation, or spray plane going over anyway).

Adhesion to mirid threshold levels varied between areas (Chi sq =16.65, df=3, n=91) with the Downs area more likely to spray for mirids below threshold, and the Cool area more likely to sprays for mirids above threshold. However the differences in compliance to thresholds were due to changes in the threshold, not changes in pest managers responses to mirids. If the raw numbers of mirids which triggered sprays (as the first target) are compared between regions and sampling methods, there is no difference in the number of mirids triggering sprays (REML analysis, df=3, Wald/df= 1.07, P=0.36 NS, n=90). The only factor that had a significant effect on the number of mirids triggering sprays was the crop stage (as the crop got older, the number of mirids triggering a spray increased; linear regression: F=6.93, df=1,78, P=0.01, explaining 7 % of variance). Thus pest managers responded to mirid numbers in a similar way throughout Australia, irrespective of sampling method or climate.

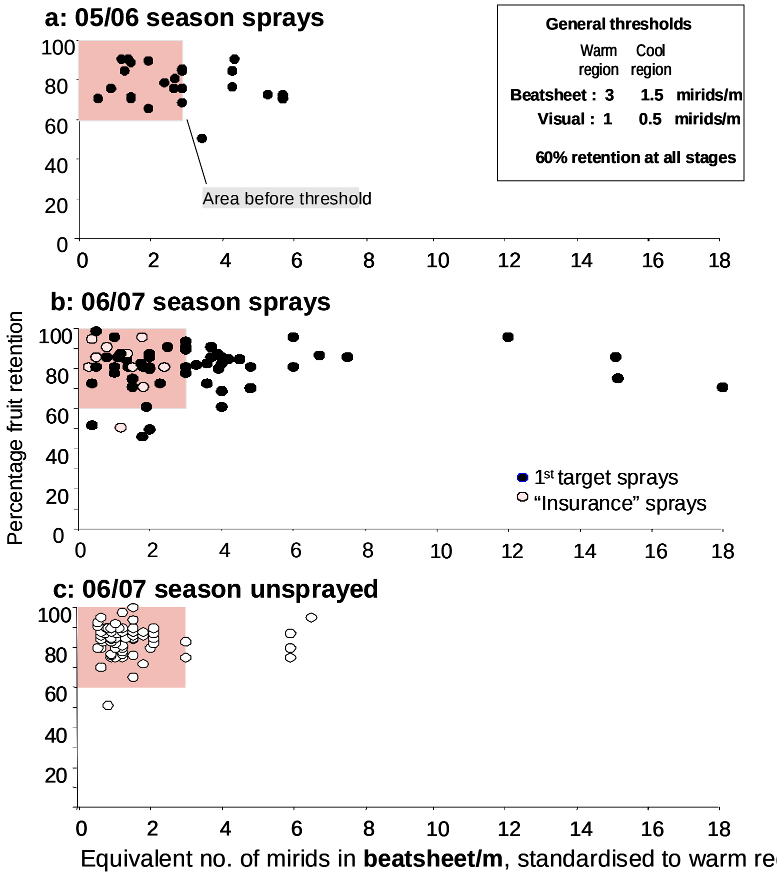


Fig. 1.2 The relationship between spraying for mirids, and both the retention and mirid number thresholds. All mirid numbers were standardized to the equivalent number of mirids in a beatsheet sample in the warm region. Only spray events targeting mirids first are included, and samples in which retention was measured as fruiting factor are not included. "Insurance sprays" occurred because of management constraints (see text).

This similarity is apparent when the mirid numbers which triggered sprays are examined (Fig. 1.3). In 3 of the 4 threshold categories, the actual threshold used by pest managers was between 1-1.5 mirids per metre. In the cool region, this meant that the visual sampling threshold was actually higher than that recommended. In the warm region, the pest managers using visual sampling applied the recommended threshold, but in neither the cool or the warm region did pest managers raise their threshold to that recommended for beatsheets. This indicates that pest managers are reluctant to accept the much higher thresholds recommended for beatsheets.

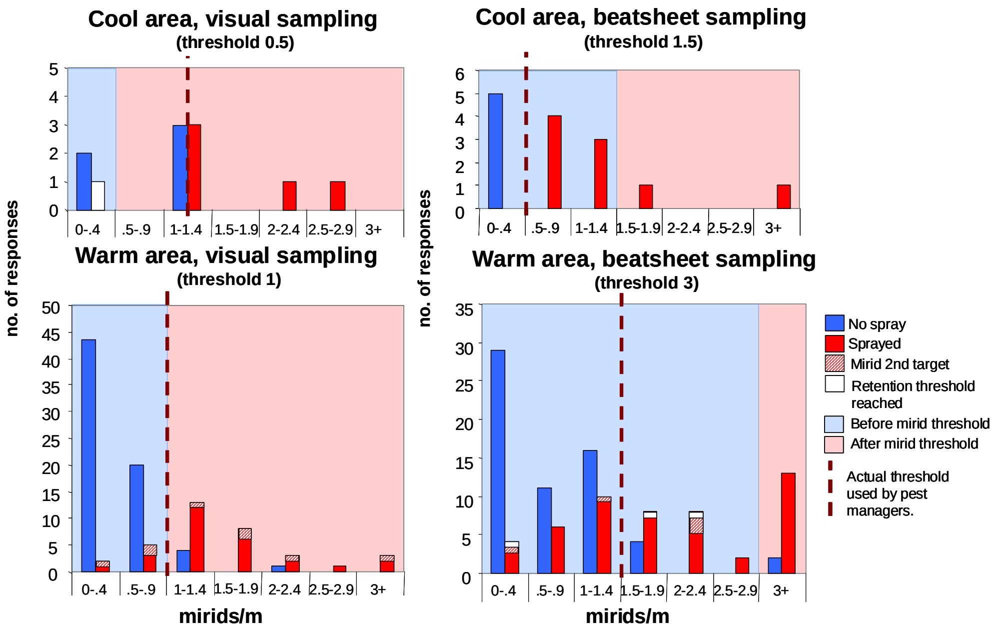


Fig. 1.3 Showing the relationship between the recommended thresholds (the line between the blue and pink shading) for both the different regions and sampling methods; and the thresholds used by the pest managers (dashed line). Pest managers using beatsheets did not raise their threshold to the beatsheet level.

The retention threshold

The percentage retention of the crop was significantly higher when pest managers didn't spray than when they did spray (REML, Wald/df=0.09, p=0.003, df=1,6; data grouped by number of mirids; Fig. 1.4). But the degree to which retention was important was difficult to gauge from this because there were very few reports (only 5) of retention less than the recommended threshold (60% retention). A large number of pest managers sprayed on very high retention, even when mirid numbers were below the industry threshold, or even below the "defacto" threshold calculated in Fig. 1.3 (Fig. 1.4).

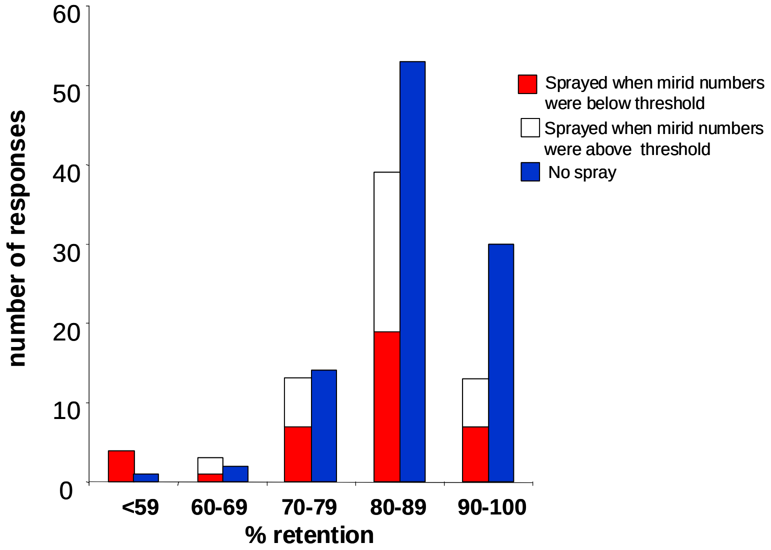


Fig. 1.4 The relationship between retention level and whether pest managers sprayed for mirids.

To understand why pest managers sprayed on high retention thresholds and low mirid numbers I used a Canoco Correspondence Analysis (CCA) to compare the importance of different factors in the decision to spray on responses with high retention (>60%) and mirid numbers which were 1) above industry threshold, 2) between the industry and the defacto threshold (identified in Fig. 3, or 3) below all thresholds (that is very low mirid numbers). The factors compared were both those in section 2 of the questionnaire (Appendix 1, Fig. 1.5) and whether the spray was an "insurance spray" or not (Fig. 1.2). I found that the factors in section 2 of the questionnaire had no particular association with any of the three spray groups. However, one factor that was strongly associated with spraying on high retention and mirid numbers below all thresholds was insurance sprays (Monte Carlo test of CCA (499 permutations): P=0.004 for first axis, variance explained by first axis =7.1% Fig. 1.6). Of the eight insurance sprays, all except 1 were on mirid numbers below all thresholds and high retention.

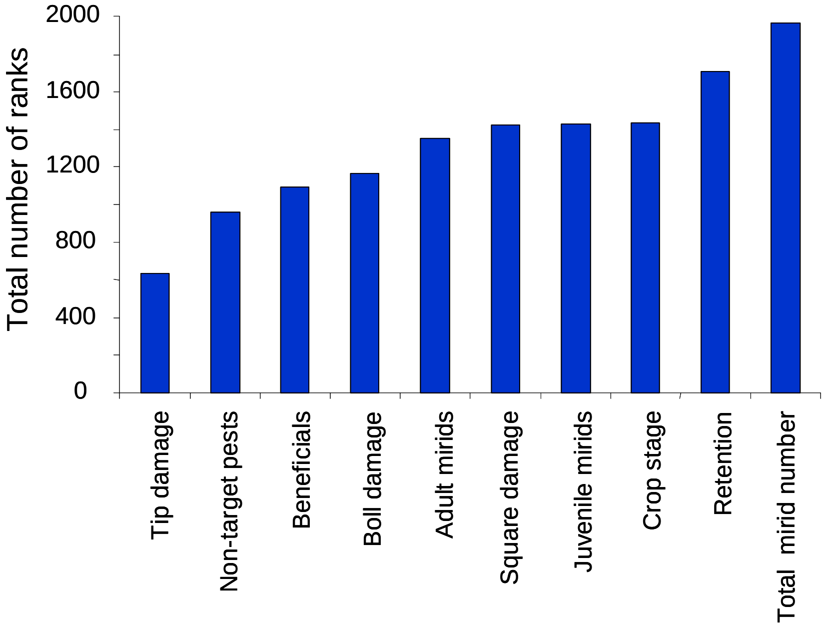


Fig. 1.5 The importance of different factors when deciding whether to spray for mirids in the 2006/07 season. There is a significant difference between the factors, with total mirid numbers the most important, and tip damage the least important. Friedmans test= 581, df=9, P=0.0001; significant difference between total ranks = 112.

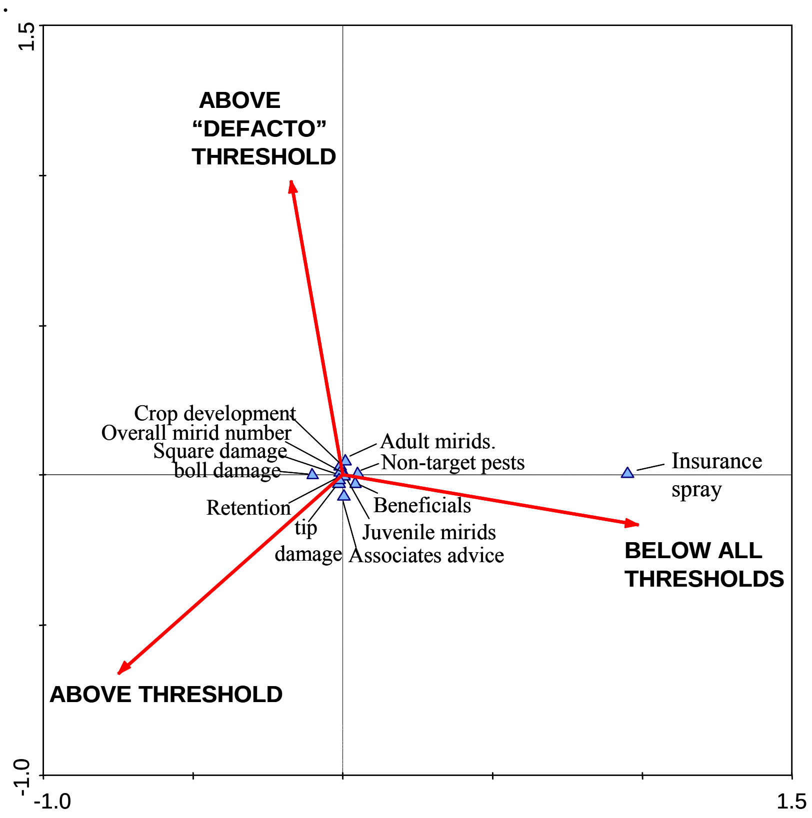


Fig. 1.6 A Canonical Correspondence Analysis comparing the importance of different factors in the decision to spray below thresholds. Only "insurance sprays" had a significant association with spraying below all thresholds.

The reports from respondents (including those with low retention) on the importance placed on retention provides information on how pest managers are using retention to manage mirids. **Fig. 1.7** shows the relationship between the importance placed on retention, and the percentage retention measured. There was a significant difference (even when different regions were taken into consideration) in the importance placed on retention depending on whether the pest manager sprayed above the mirid number threshold, below the threshold, or didn't spray for mirids. In particular, when pest managers sprayed below threshold, the relationship between importance and percentage retention varied: low percentage retentions were reported as important and high percentage retentions were reported as unimportant in making the decision to spray. But when mirid numbers were above threshold, or pest managers decided not to spray, then the importance of retention was not correlated with percentage retention. Thus pest managers significantly varied the importance they placed on retention only when they decided to spray mirids below the mirid number threshold.

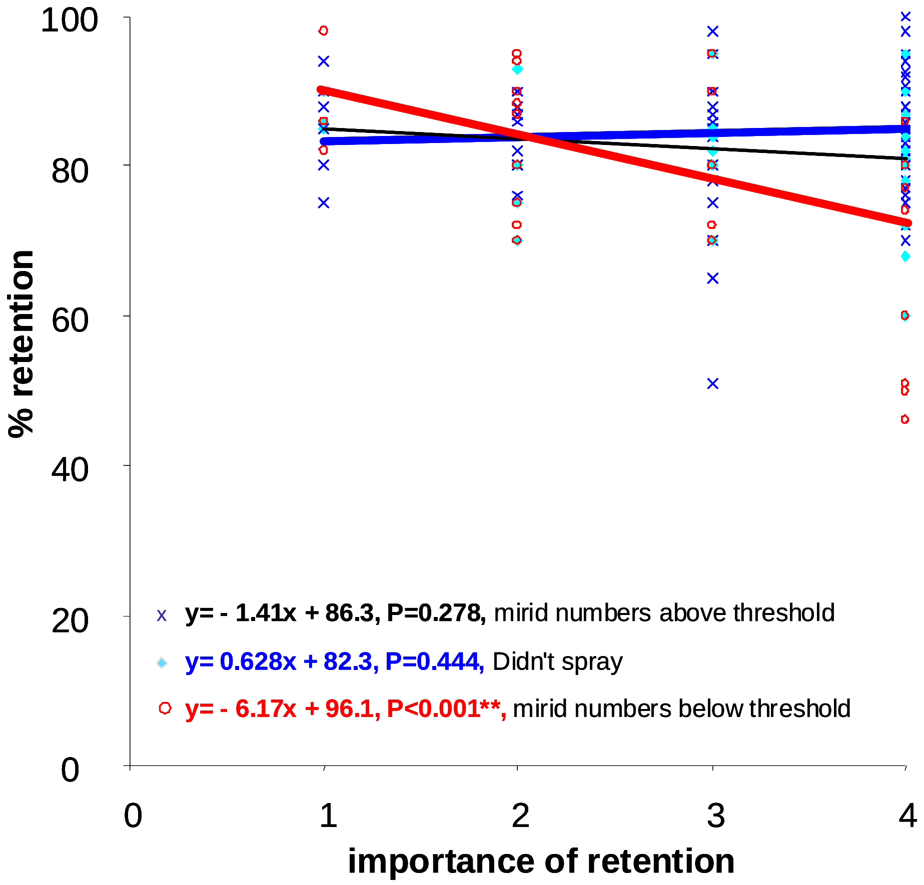


Fig. 1.7 The relationship between the importance placed on retention (x axis) and the percentage of retention measured (y axis). Pest managers varied the importance they placed on retention only when the decided to spray mirids below the mirid number threshold.

Factors influencing the decision to spray

The factor reported as most important when deciding to spray for mirids in the 05/06 survey was the overall number of mirids (rather than the number of adults or juveniles); fruit retention and square damage were also considered important; while tip damage had little influence on the decision to spray (See Whitehouse 2006).

In the 2006/07 survey, the total number of mirids was again the most important factor when deciding whether or not to spray for mirids, followed by retention. Crop stage, the presence of juvenile or adult mirids, and square damage were equally important; and tip damage was the least important factor when deciding whether to spray (Fig. 1.5, Friedman non-parametric ANOVA: df= 9,239; test statistic = 581, P<0.001).

The 2006/07 survey also showed that different areas valued different factors. Cool, Downs and even Middle areas viewed the importance of the factors in a similar manner, while those in the Tropical area responded quite differently, placing more importance on square damage and beneficial insects, and less on non-target pests or tip damage. Boll damage was also more important in the non-tropical areas. Retention was less important to pest managers in the cool and warm areas relative to the middle and tropical areas.

Control Options

Mirid control in the survey was dominated by an overwhelming dependence on fipronil (Fig. 1.8). In the 2005/06 survey 55% of the sprays were fipronil, in the 2006/07 survey, 63% were fipronil. Although this is a large increase, when only those valleys used in the 2005/06 survey from the 2006/07 survey are compared with the 2005/06 survey, the difference is not significant (chi square, df=1, chisq= 0.32, P=0.5). Spray rates of fipronil and dimethoate varied greatly. Fipronil rates were very low and were evenly spread between 30 and 60 ml/ha (125-62.5 ml/ha is the recommended rate). Dimethoate rates were evenly spread between 100 and 500ml/ha (340-500 ml/ha is the recommended rate). Additives to insecticides were prominent. Salt was used with both fipronil and dimenthoate, with a large increase in the amount used with fipronil in the 2006/07 survey, largely due to an increase in the tropical area. Oil was used in conjunction with fipronil, dimethoate, endosulfan and indoxacarbn (Fig.1.8).

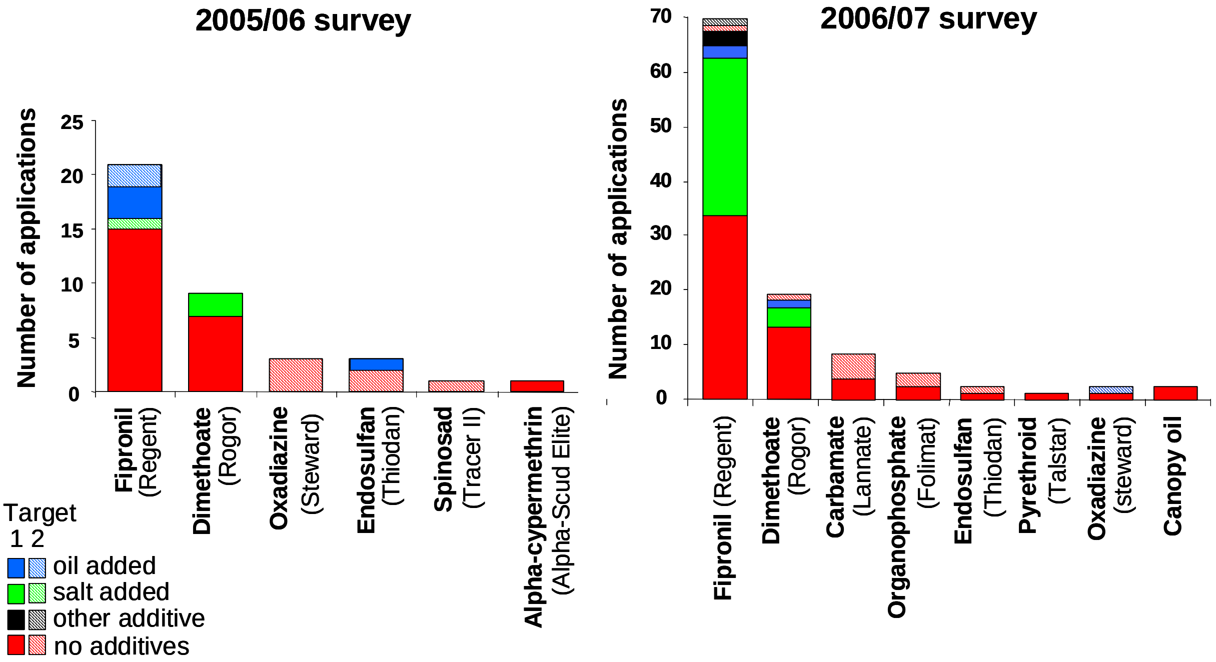
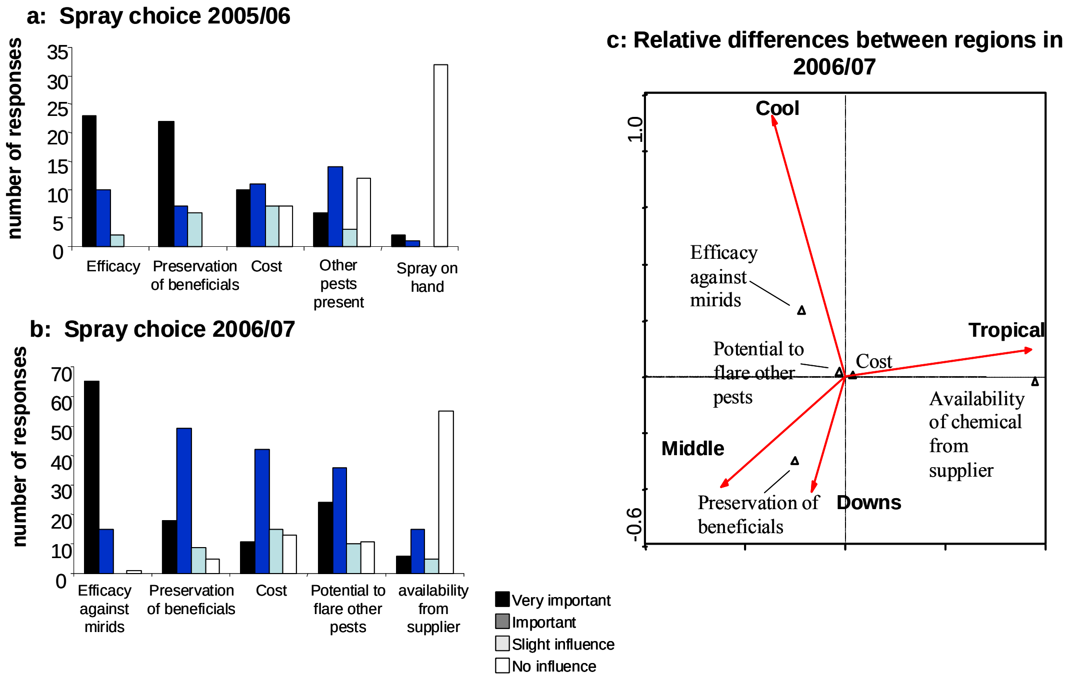


Fig. 1.8 Sprays used to control mirids when they were the primary or secondary targeted pest.

Mirid control was occasionally combined with the control of other pests. In the 2005/06 season, sprays to control mirids were also used to control *Helicoverpa* and *Nezara viridula* (Linnaeus) (Pentatomidae; see Whitehouse 2006). Of the 27 sprays targeting mirids in 2005/06, 10 had *Nezara viridula* as a secondary target, and 2 also targeted *H. armigera* eggs. When mirids were the secondary target, 5 sprays targeted *H. armigera* grubs, and 3 targeted *Nezara viridula*. Not surprisingly, when mirids were not the first target, the insecticide applied differed (Fig. 1.8) with both Indoxacarb and Spinosad only used when mirids were the secondary target (although in 2006/07, mirids were targeted with Spinosad). In the 2006/07 season, only Bollgard crops were monitored, so no sprays targeted *H. armigera*, but 5 targeted jassids (Cicadellidae), 5 *Nezara viridula*, 1 *Piezodorus hybneri* (Gmelin) (Pentatomidae), and 1 targeted whitefly (Aleyrodidae). Fewer mirid sprays also targeted other pests (6 targeted jassids as secondary pests; 2 *Nezara viridula*, 1 mites

(Tetranychidae), 1 *Campylomma liebknechti* (Girault) (Miridae) and 1 fleabeetles (Chrysomelidae)). The low amount of secondary targets may reflect the low pressure year.

The choice of insecticide in the 2005/06 and the 2006/07 survey were influenced by the same factors, In both surveys insecticide choice was most strongly influenced by efficacy, and then by the desire to preserve beneficials (Fig 1.9a,b). Within these preferences there were some differences between areas. An ordination analysis revealed that while all regions equally valued the preservation of beneficials and the cost of the sprays, Cool areas were more concerned about efficacy relative to other regions, and Downs and Middle areas about the preservation of beneficals (Fig. 1.9c). Tropical areas seemed to be particularly concerned about availability of the insecticide (monte carlo test of CCA in Fig. 1.9c, P=0.002, Fig. 9c explains 11.4 % of all variance).


a: Spray choice 2005/06

b: Spray choice 2006/07

c: Relative differences between regions in 2006/07

Fig 1.9a & b. The factors influencing the choice of spray used by pest managers to control mirids. Overall, efficacy and preservation of beneficals had the strongest influence. Between regions the importance of these factors varied. **Fig. 1.9c:** The Canonical Correspondence Analysis shows that middle and downs areas were relatively more concerned about the preservation of beneficials, and cool regions more concerned about efficacy.

Ramifications

Spray applications

Spray applications to 80 fields are included in this survey. Spray applications per field varied from 0 to 3 over the course of the season. This number was strongly influenced by area, with Cool and Middle areas peaking at 1 spray per field, and Downs and Tropical areas peaking at 2 sprays per field (one way ANOVA: df=3,77; F=9.46; P<0.001).

Re-spraying fields

One way to avoid costs is to avoid re-spraying a field for mirids. Management decisions which can reduce the likelihood of re-spraying are therefore advantageous. A simple chi-square pooling all areas was used to see if spraying above threshold in the first mirid spray applied to a field reduced the likelihood of re-spraying that field. The results showed that if a field was first sprayed when mirids were over threshold, then it was less likely to be re-sprayed for mirids (chi-sq, P=0.035, df=1, n=67).

However, the likelihood of re-spraying could have been affected by both the area in which the fields were located, and variability in the insecticide application rates between fields. These problems were addressed by comparing the application rates of fipronil (the most common insecticide used) by grouping rates into "below 40ml/ha" and "40ml/ha and above"; and by lumping the areas into "northern" (Tropical + Downs) and "southern" (Middle + Cool). Unfortunately, sparse data meant one analysis couldn't include the effects of spray rates, area, mirid thresholds and likelihood of respraying. So I did two log linear analyses: one focusing on mirid thresholds (a 2x2x2 log linear analysis comparing (i) spraying above or below the mirid threshold, (ii) spray rates, and (iii) re-spraying or not re-spraying the field); and the second one focusing on the effect of area (a 2 x2 x 2 log linear analysis comparing (i) the effect of the northern and southern areas, (ii) spray rates, and (iii) re-spraying or not re-spraying the field).

The overall model of the first analysis focusing on mirid thresholds was significant (log-linear; df=7, r=4, P<0.001) although the three way interaction between spray rates, threshold and re-spraying was not. There was also no link between spray rate and the likelihood of spraying above or below threshold. Nevertheless, within the model there was a significant interaction between threshold and re-spraying (r= 8.52, P=0.004); and between spray rate and re-spraying (r=9.66, P=0.002); indicating that re-spraying was more likely to occur when mirids were below threshold (supporting the chi-square results above); and that re-spraying was also associated with using application rates below 40 mls/ha.

The overall model of the second analysis focusing on area was also significant (log-linear; df=7, r=4, P<0.001, Fig. 10), although the 3-way interaction between areas, spray rates and respraying was not. It showed that area had a strong interaction with spray rate (r=15.75; P<0.001); and the likelihood of a second spray was linked to area (r=10.45; P=0.001). This means that fields in the northern area were both more likely to be re-sprayed, and received lower spray rates. In addition, spray rate directly influenced the likelihood of respraying (r=4.21, P=0.04), supporting the results of the first log linear analysis, in that fields whose first spray rates were below 40ml/ha were more likely to be re-sprayed (and vice versa).

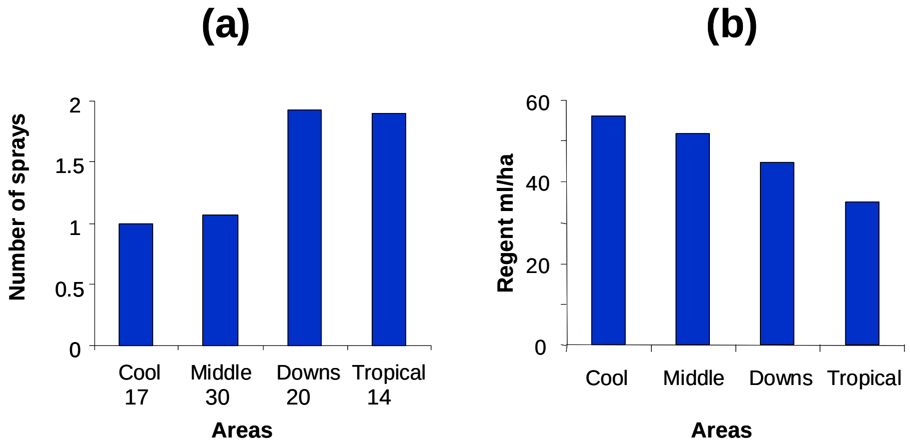


Fig. 1.10 Average number of sprays per field in each area (a) and the average regent spray rates per field in each area (b).The numbers under the histogram are the number of fields in that sample. Fields in the north were more likely to be resprayed, and received lower rates of regent per spray.

Mirid thresholds and yields

Given that mirids have the potential to reduce yield, is there a cost to only controlling mirids once they reach the recommended threshold? To answer this question I compared the yield of 71 fields located throughout the cotton growing valleys that had received any mirid spray when mirids were below threshold, with those that were only sprayed once mirids were over threshold (Fig. 1.11). The 2006/07 season was strongly affected by drought, so I took this into account by asking pest managers if their field had been drought affected (0=no stress, 1= slight stress, 2=stressed, 3=very stressed). I found that while water stress had a strong affect on yield, whether growers had sprayed above or below threshold had no effect (REML, df=60, deviance = 111; affect of water stress: P<0.001; affect of threshold: P=0.084; areas were treated as blocks). The number of sprays a field received had no additional affect on yield. If anything, there was a slight trend for fields that were only sprayed for mirids once threshold was reached to have slightly higher yields (although this wasn't significant: 0.084). Thus there was no yield loss if pest managers only sprayed mirids once they reached threshold.

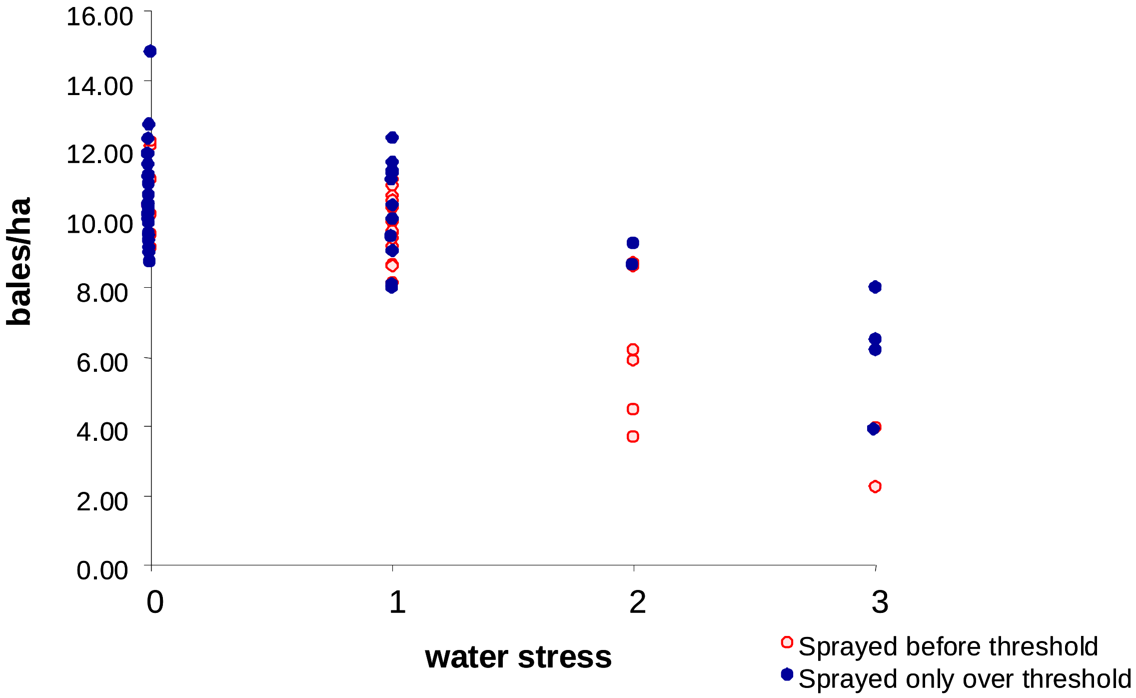


Fig. 1.11 The relationship between yield, water stress, and spraying mirids before or over threshold. Spraying mirids only over threshold had no effect on yield, if anything there was a trend towards a yield advantage (REML, P=0.084, df=1,60).

DISCUSSION

Support for mirid management recommendations

The results from the survey vindicate the recommended mirid thresholds, and indicate that there is no cost to yield if spraying is delayed until mirid numbers are above threshold. If anything, there was a trend for yields to be higher if fields were only sprayed above threshold. Spraying only once mirids were above threshold also reduced the need to re-spray a field, which has economic and resistance management advantages. Thus current mirid management recommendations are well supported by these results.

When synthetic pesticides were first introduced, yields increased as pesticide use increased (Kogan 1998). However, more recent work looking at 80 crop combinations, in 62 projects from 26 countries found that in over 60% of the projects, yields increased as pesticide use declined (Pretty 2008) probably reflecting a move to more targeted insecticide applications. Work which compared different spray regimes using the concept of "BDI" (Beneficial Disruption Index) also showed an economic advantage to less, more targeted insecticide applications (Hoque 2002). Results presented here also support the argument that a more targeted application of insecticides can reduce cost without reducing yield, thereby increasing profitability while increasing compliance with IPM.

Compliance with mirid management recommendations

Pest managers in this survey were following the guidelines recommended for mirid management. Both mirid numbers and retention were seen as important, and thresholds were used, but to varying degrees.

Pest managers accepted the mirid numbers threshold for visual surveys, but not for beatsheets. They seemed to be reluctant to accept that three times the number of mirids are found in beatsheets than in visual surveys and that thresholds should be adjusted accordingly. This may be leading to a number of unnecessary sprays and the associated costs. To solve this problem, the industry needs to either build up the pest managers confidence in the high beatsheet threshold, or encourage the use of visual surveys for mirids.

Very few responses reported retention levels below the threshold of 60% and of these, most reported spraying for mirids. Retention rate was used by pest managers when deciding to spray for mirids, but not used by pest managers when deciding not to spray for mirids. Greater confidence (that high retention rates and low mirid numbers indicate that you don’t need to spray for mirids) could reduce many unnecessary sprays and hopefully overcome managerial constraints or “insurance sprays” currently triggering many applications.

Economic thresholds are problematic. They are defined as the threshold at which the grower will suffer an economic cost, and thus are important to IPM because they encourage a move away from a total eradication approach to pest control. However, they are problematic because, by definition, they are partly driven by the market cost of the produce which is continually changing. Economic thresholds can also differ between individual growers as they base their threshold on what they perceive is a realistic yield (Orr 2001). Some yield perceptions, which were based on the early success of the chemical eradication approach to pests, can be unrealistic (Lewis et al 1997) and hinder the adoption of a more general threshold. Alternatively, a static threshold provides a simple number which growers can use to justify not spraying for a pest. Although such thresholds are an oversimplification of a complex problem, it is this simplicity that has made them endurable (Pedigo et al 1986). However, to be more realistic, thresholds need to be dynamic like the crop and the pests they are trying to manage (Wilson & Morton 1993). They need to vary throughout the season, or in respect to the presence of beneficials (Mensah 2002). Therefore an effective threshold is a simplification of an economic threshold which includes some variability.

Mirid thresholds in Australian cotton are dynamic in that they vary throughout the season, between different climatic regions, and in respect to the different sampling methods (Khan et al 2006). In the mirid survey, pest managers largely complied with the visual survey warm region recommendation of 1 mirid/metre during the most vulnerable part of the season, but there was little evidence that pest managers adjusted their threshold to match their climatic conditions and sampling methods. Many lacked confidence in this aspect of the threshold’s dynamic nature.

Retention levels were used to justify spraying for mirids, but not as a reason not to spray for mirids. Thus the retention threshold is not functioning well. The 60% retention threshold was originally developed on research in conventional cotton which has lower retention levels than Bt cotton because of greater susceptibility to insect pressure. There is a perception in the industry, not always supported by results, that higher retention means higher yields, which may be hindering compliance with this threshold.

There was a high reliance on fipronil to control mirids. This chemical accounted for 63% of the insecticides in the 2006/07 survey, and was often applied multiple times to fields at low rates. This is a concern from a resistance management perspective, especially as areas which were more likely to re-spray were also more likely to use the lower rates. Interestingly, spraying mirids above threshold reduced the likelihood of re-spraying, unless the rates were particularly low. Pest managers are probably spraying at low rates to help preserve their beneficials (which ranked high in influencing their choice of spray). Work needs to be done to see if very low rates of fipronil are still effective at controlling mirids.

The importance of beneficials in this survey was encouraging; especially as few predators of mirids had been formally identified (but see sections 2 and 3). It may also be indicative of a more systems based management of pests developing in Australian cotton.

One of the biggest challenges to maintaining and developing IPM is that researchers from different disciplines and pest managers need to work together effectively. This is difficult because the different groups operate out of different paradigms, so there is a danger that they will interpret the same results from totally different perspectives. Petrie (1976) identified that a barrier to people from different disciplines solving problems together is that their interpretive use of learning will differ; that is, what the two groups see as background information and foreground information will differ, resulting in the same facts being interpreted quite differently (Fig. 1.12).

Fig. 1.12 "Young Girl-Old Woman Illusion." From *MathWorld*--A Wolfram Web Resource. http://mathworld.wolfram.com/YoungGirl-OldWomanIllusion.html Depending on which lines are viewed as foreground or background, the viewer will see either an old woman (looking down) or a young girl (looking away to her right)

This may explain the problem encountered in the mirid survey where pest managers were reluctant to accept the higher threshold for beatsheets. Formal studies have shown that beatsheets are three times more effective than visual searches at finding mirids and that they are more consistent (Detucher et al 2003, Wade et al 2006) so researchers are happy to accept the higher beatsheet threshold. However bug checkers report seeing insects flying off the beatsheets before they are counted (they don't see the animals they miss while doing visual surveys). They therefore regard visual searches as more reliable than beatsheets. Researchers focus on the mirids caught, while pest managers focus on the possible mirids that they missed. This difference in interpretive learning between researchers and pest managers is probably the biggest barrier to threshold compliance.

To overcome the differences in beatsheet interpretation, two approaches could be used. First, researchers could encourage pest managers to use visual searchers rather than beatsheets. The negative side to this approach is that a lot of information about the beneficial community in the cotton would not be collected. Alternatively, more work could be done to give pest managers more confidence in beatsheets, such as involving them in demonstrations (at grower meetings on farms) that show that they can find more mirids using a beatsheet than a visual search.

In order for IPM to grow towards the ideal of a totally integrated system, researchers and growers will need to understand the "interpretive knowledge" (Petrie 1976) of each other's philosophies.

An aim of the work presented here was to enhance an IPM approach to the management of mirids in cotton. To do this, growers, consultants, agronomists and managers were heavily involved in the survey from the beginning to make sure that the survey was relevant to them, and to help encourage their ownership of the results. The results were also presented in a manner that has direct relevance to the growers, such as by demonstrating that following mirid thresholds could reduce costs without sacrificing yield. The study revealed that the interpretive knowledge of pest managers has not been taken into consideration with the use of beatsheets, which may explain the lower compliance to the thresholds of this sampling technique. Nevertheless, there are ways of overcoming this problem, and by involving pest managers in the survey, the survey will have a better chance of enhancing IPM development with respect to sucking pests in cotton.

2 Possible Modifiers of Mirid Damage

One of the reasons that pest managers do not keep to mirid thresholds is that mirid damage is perceived to be unpredictable – sometimes a few mirids can cause a lot of damage and vice versa. This section examines factors that could result in this variability, including the effect of the sexual status of the mirids, and the possibility that mirids, which are also predators, can switch between feeding on plant protein to feeding on animal protein.

METHODS

Table 2.1 gives an overview of the experiments carried out to test the possible modifiers of mirid damage, including lifehistory status of the mirids, or the presence of alternative food or competition.

Location	Title	Modifier	description	Date
Laboratory	**2.1 Reproductive status 1**	Life history/ aphids/mites	Tested the effect of the mirid's reproductive status on its damage to cotton. Light aphid & mite contamination.	Nov 2006
Laboratory	**2.1 Reproductive status 2**	Life history/ aphids	Tested the effect of the mirid's reproductive status on its damage to cotton. Heavy aphid contamination	Jan 2007
Laboratory	**2.1 Reproductive status 3**	Life history/ aphids	Tested the effect of the mirid's reproductive status on its damage to cotton. Light aphid contamination.	Nov 2007
Laboratory	**2.2 Effect of mites**	Mites	Tested the effect of mites on mirid damage to cotton	Nov 2007
Laboratory	**2.4 Effect of aphids & eggs**	Aphids & *Helicoverpa* eggs	Tested the effect of aphids or very heavy Helicoverpa lays on mirid damage to cotton	March 2006
Laboratory	**2.4 Effect of eggs on damage 1**	*Helicoverpa* eggs	Tested the effect of Helicoverpa eggs on mirid damage to cotton	June 2006
Laboratory	**2.4 Effect of eggs on damage 2**	*Helicoverpa* eggs	Tested the effect of Helicoverpa eggs on mirid damage to cotton	Oct 2006
Laboratory	**2.4 Efect of eggs on damage 3**	*Helicoverpa* eggs	Tested the effect of Helicoverpa eggs on mirid damage to cotton	Nov 2006

For all of the experiments outlined above, I set up glass house experiments using large pots (25cm diameter x 25cm high) containing four cotton plants at early boll stage which were caged using a light netting (Fig. 2.1). Into each cage were placed one of the different treatments. At the end of the experiment, all animals left in the cages were identified and counted, and plant data was recorded, such as plant height, number of nodes number of fruiting bodies, and any damage to fruiting bodies.

Fig.2.1 Glasshouse cages used in these experiments

2.1 Reproductive status

The aim of these experiments was to see if the life history stage of the mirids influenced the amount of damage they caused to fruiting bodies.

Three cage experiments (Fig. 2.1) were undertaken. In the first experiment (which had light aphid and mite contamination) 6 reps of 4 unmated males, 4 unmated females, 4 mated males and 4 mated females and a control of no mirids were set up in cages (total of 30 cages).The second experiment (which was heavily infested with aphids) was the same as the first, with 6 reps of 4 unmated male, 4 unmated females, 4 mated males and 4 mated females and a control of no mirids (total of 30 cages). The third experiment, which had light aphid contamination, had 5 reps of 4 unmated males, 4 unmated females, 4 mated males and 4 mated females and a control of no mirids in each of the the cages, and a 6th treatment (5 reps) of 4 4th instar mirids.

The cages were left for a week, after which time they were checked for surviving invertebrates and the plant statistics were recorded, including the number of damaged fruit.

2.2 The effect of mites

The aim of this experiment was to see if the presence of mites as an alternative food would reduce mirid damage to fruiting bodies.

This experiment (Nov. 2007) included 5 reps of 4 treatments. The treatments were: 6 4th instar mirids, 6 4th instar mirids+mites, mites only, and control (nothing added to cage). The cages had been seeded with mites about two weeks before the beginning of the experiment, so that mite colonies were established by the time the mirids were added.

The cages were left for a week, after which time three leaves at position three were taken from each cage to check for mites. The rest of the plants were checked for surviving invertebrates (including mites) and the plant statistics were recorded, including the number of damaged fruit.

2.3 Effect of eggs on mirid damage

The aim of these experiments was to see if the presence of an alternative food (*Helicoverpa* eggs; hereafter referred to as just “eggs”) would reduce the amount of damage to cotton fruiting bodies.

In the first experiment (March 2006), 6 reps of 4 treatments were set up in glasshouse cages decribed above (Fig.2.1). The treatments were: control (nothing added to cage), mirids only (2 male, 2 female mirids), aphids (2 male, 2 female mirids + 10 female aphids), eggs (2 male, 2 female mirids + about 100-230 *Helicovera* eggs, (laid over night by 5 moths). The cages were left set up for 2 weeks, after which time they were checked for surviving invertebrates and the plant statistics were recorded, including the number of damaged fruit.

The next three experiments were analysed together. Work was carried out on cotton variety Sicala 40B. In the first experiment (June 2006), 5 reps of 4 treatments were set up. These were: 5 juvenile mirids (3rd instar) only, 10 eggs + 5 juvenile mirids (3rd instar), 30 eggs +5 juvenile mirids (3rd instar), and control (nothing added to cage). Eggs were replaced on the second and 5th day to keep the egg pressure constant. In the second (Oct. 2006) and third (Nov. 2006) experiment, 6 reps of 5 treatments were set up (all mirids in these treatments were 3-4 instars). The treatments were: 5 mirids, 5 mirids+10 eggs, 5 mirids+30 eggs, 5 mirids+60 eggs, and control (nothing added). Eggs were replaced once on the 3rd day to keep egg pressure constant. In all three experiments the cages were left set up for 1 week, after which time they were checked for surviving invertebrates and the plant statistics were recorded, including the number of damaged fruit.

RESULTS

2.1 Reproductive status

In these experiments, which suffered from contamination from aphids and mites, there was little or no effect of mirid reproductive status on cotton fruit production. For example, there was no evidence that mated status influenced the number of undamaged fruit (ANOVA; covariate: plant height; df =5,62; P=0.5) and only a trend for its influence on the average boll weight (ANOVA; covariate: plant dry weight; df=5,62; P=0.053). But mated status did have an effect on total boll weight (ANOVA; covariate: plant dry weight; df=5,64, F=3.1; P=0.016; Fig. 2.2a) with male and unmated female treatments producing more fruit dry weight. What was also surprising is that there was a significant difference in plant height between treatments (ANOVA; covariate: boll dry weight; df=5,67; F=2.6; P=0.032; Fig. 2.2b) where the largest plants were found in the control and mated females. These results suggest that plants either put their energy into growth or fruit.

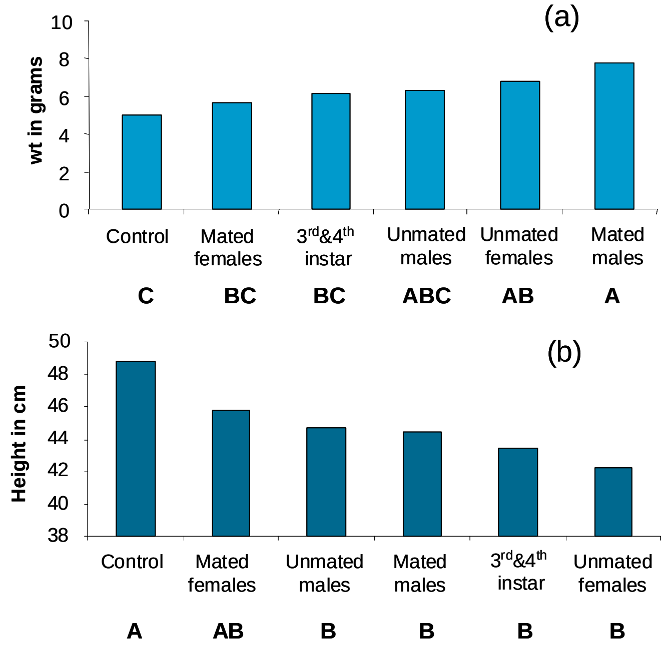


Fig. 2.2 The effect of mated status on (a) total dry fruit weight (P=0.016) and (b) plant height (P=0.032).

Although mites and aphids were positively correlated with both total dry fruit weight (regression analyses; df=1,88; P<0.001; variance explained = 24.3%; Fig 2.3) and log average dry fruit weight (ANOVA; df=1,83; P<0.001; variance explained = 32.5%), they were not correlated with the treatments (ANOVA: df=5,82; P=0.7). Thus the aphid/mite contamination may have influenced the pattern seen in Fig 2.2, but not caused it.

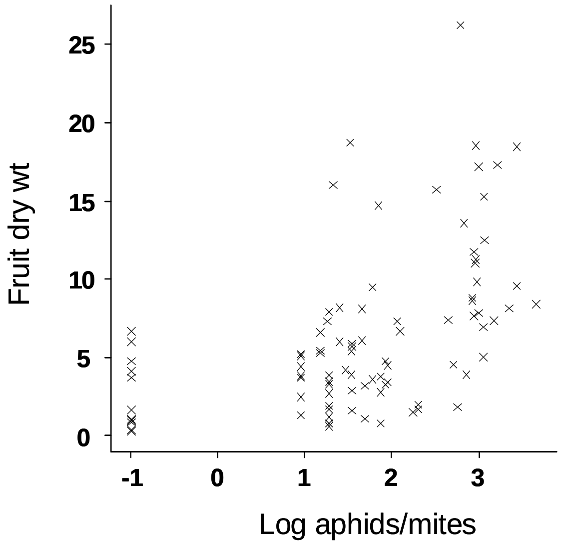


Fig. 2.3 Relationship between boll dry weight and the number of aphids/mites in the cage. A mite/aphid number increased, so too did fruit dry weight.

2.2 The effect of mites

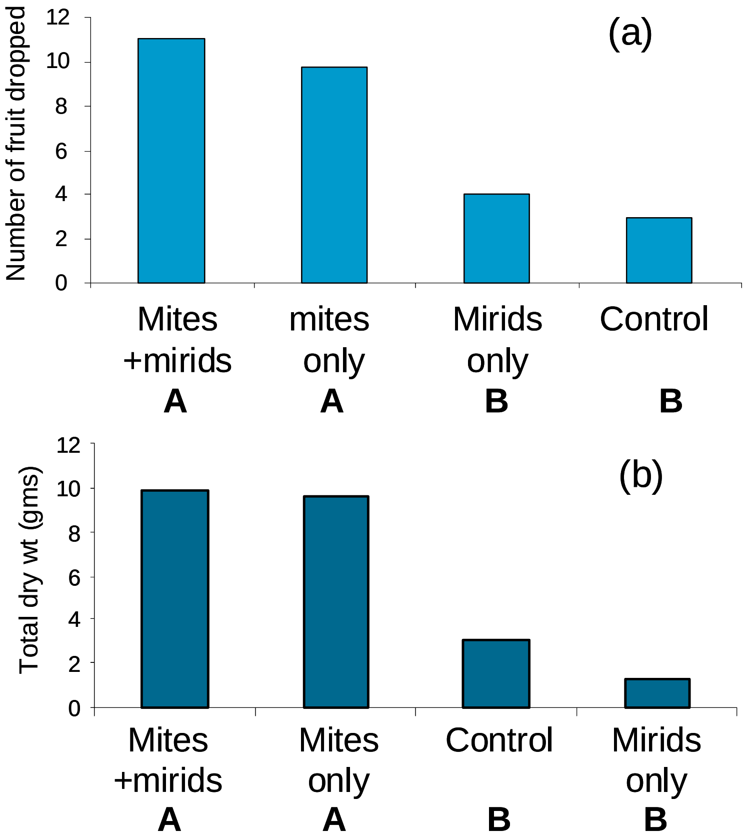


Fig.2.4 The effect of the presence of mites and aphids on the number of fruit the cotton plants shed (a) and the total dry fruit weight of the fruit left (b).

The results from the cage counts suggest that there may be a reduction in the number of mites in the presence of mirids (ANOVA; df=3,13; F=12; P<0.001; lsd=22.7; average number of mirids in: mites only treatment = 63; mites+mirids = 22; control = 15; and mirids only = 3). However, the results from the leaf samples indicate that although there were fewer mites in the mite+mirids treatment compared to the mites only treatment, this differences was not significant (ANOVA; df=3,13; F=204; P<0.001;lsd=0.35, average log number of mites in: mites only treatment = 2; mites+mirids = 1.8; control = -1; and mirids only = -1).

There was only a trend that treatments reduced the number of undamaged fruit (ANOVA; df=3,12; F=2.8; P=0.088); but the treatments did influence the number of fruit that dropped off the plants during the experiments: cages containing mites dropped significantly more fruit than those that didn't (ANOVA; df=3,14; F=11; P<0.001; Fig2.4a). However, the total dry fruit weight of the fruit left in the cages containing mites was also significantly heavier (ANOVA; df=3,13; F=8.8; P=0.002; Fig 2.4b).

2.3 Effect of Eggs on mirid damage

The first experiment found that the presence of aphids and eggs did not decrease the drop in retention caused by mirids (ANOVA; df=3,19; F=14.9; P<0.001; Fig. 2.5) However, this experiment ran for two weeks, during which time the caterpillars hatched from the very heavy egg-lays (equivalent to c.400/m) and began to cause damage.

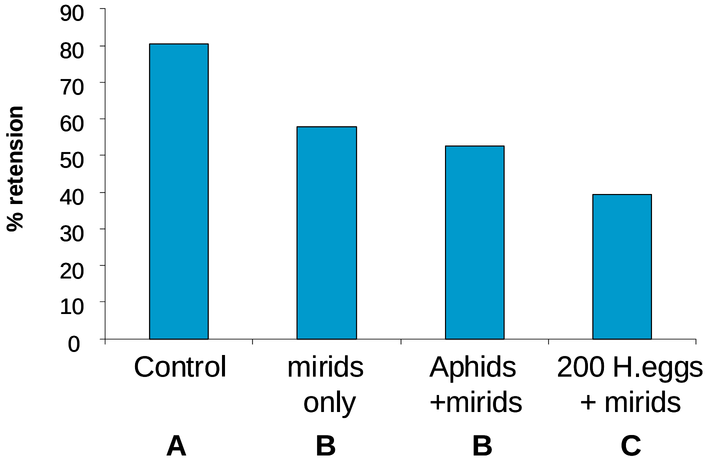


Fig. 2.5 The effect of mirids and potential prey on retention. The presence of aphids and large numbers of H.eggs did not increase retention.

The next set of experiments used lower, more realistic numbers of Eggs. These experiments found mirids in the presence of at least 60 eggs (about 120 eggs/m or 12 eggs/mirid) caused less damage to fruit than mirids on their own (ANOVA; df=4,62; F=7.5; P<0.001; Fig 2.6).

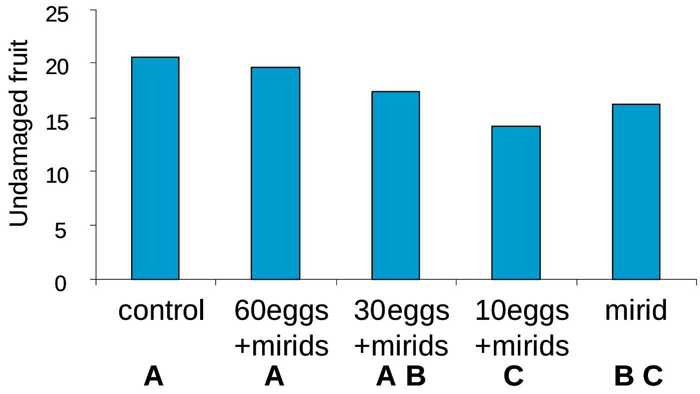


Fig. 2.6. The effect of mirids and potential prey (H.eggs) on yield. The presence of more than 30 H.eggs decreased the negative effect of mirids.

DISCUSSION

These results suggest that there is little or no effect of mating status of the mirids on the amount of damage they cause to cotton fruiting bodies. So the sexual status of mirids in the cotton is unlikely to have an effect on the amount of damage seen in the field.

In addition, mites and aphids as potential food items do not reduce mirid damage. Although mirids may slightly reduce, at least in the short term, mite numbers, any effect of mites on mirid damage to cotton is overridden by the effect of mites on cotton production. So when managing mirids in the presence of mites and aphids, the management of the mites and aphids should be the greatest concern.

In these studies there was some evidence of short term compensation by the plant to the presence of mites and aphids in the presence of mirids, in that the plant put more resources in the fruit it retained. This effect is likely to be very short lived under field conditions and unlikely to influence yield.

These results did suggest that the presence of *Helicoverpa* egg lays could influence mirid damage in cotton. Reasonably heavy egg lays (60 eggs per cage, or 12 eggs per mirid) but not extremely heavy lays (200+ eggs per cage) reduced fruit damage, suggesting that the mirids were feeding on *Helicoverpa* eggs in preference to cotton fruiting bodies. This has major ramifications to Bt crops. If this finding can be repeated under field conditions, then reasonably heavy *Helicoverpa* egg lays in Bt crops with mirid pressure could be a good thing as they could reduce mirid damage.

The work with Eggs needs to be followed up, and other potential prey of mirids, such as thrips, need to be tested. Mirids are viewed as predators in other crops, so if a cotton crop can maintain an alternative animal food source for the mirids during the critical 5 week period, mirid sprays could be avoided.

3 ELISA Work

One way to avoid the excessive use of insecticides is to ultilize the predators of pests in cotton. However, identifying predators of invertebrate pests is problematic, as these animals are often cryptic or nocturnal (Hagler & Cohen 1990) and leave little evidence of a predation event. The development of prey-specific immunological and DNA-based techniques to detect prey in the gut contents of individual predators has been a major advance to understanding predator/prey interactions (Greenstone 1996, Sheppard & Harwood 2005). The aim of this work was to develop the anti-rabbit IgG sandwich ELISA further by testing it on two emerging pests: the cotton aphid (*Aphis gossypii* Glover (Hemiptera: Aphididae), hereafter referred to as "aphids") and the green mirid (*C. dilutus*, hereafter referred to as "mirids") and comparing the results to those for *H. armigera* eggs (hereafter referred to as "eggs") and then using the technique in the field to identify potential predators of these pests. The first aim was to establish if mirids and aphids can be marked, how long the mark lasts. The second aim was to see if the mark could be detected in predators which had consumed marked prey. The third aim was to release marked prey in the field and identify predators of those prey.

METHODS

3.1 Testing marked prey.

The initial step in this work was to see if marking mirids and aphids with rabbit IgG protein was comparable to marking eggs in terms of the ease of marking, and its strength and permanence. Alive eggs, mirids and aphids were marked by dunking, spraying, or sucking. The concentration of the rabbit protein in all cases was 5 mg/ml.

Dunking - The animal was placed in a small plastic specimen vial (diameter 5cm, height 6cm) containing a small amount of rabbit protein. After it had contacted the protein it was removed from the vial and allowed to dry. This technique was most successful with eggs. Mirids and aphids were anesthetised with CO_2 before being dunked.

Spraying - The animals were sprayed with a Nebulizer which produces a very fine, fog-like mist (Hagler 1997). Aphids were sprayed for 2 min while standing on a leaf. Mirids were placed in a specimen via, anaesthetised with CO2, and then sprayed for 1 min, shaken slightly, and sprayed again for 1 min (making a tot al of 2 mins). Eggs were sprayed for 2 mins in a specimen vial.

Sucking – This method was only used with aphids and involves the aphids obtaining the mark by consuming it. Aphids were placed in film canisters (diameter 2cm, height 5cm) which were sealed on top with Parafilm®. A large drop of rabbit protein in a 10% sugar solution was placed on top of the Parafilm®, and was covered with another layer of Parafilm®. The aphids were left in the container for 24 hours. Only aphids found on the Parafilm® lid (and therefore may have been feeding) were used in this test.

All animals were kept at room temperature (23^{o}C) between marking and testing at 0, 6, 12, 24, 48 or 96 hours after marking. If an animal was not tested immediately, it was stored in the -80^{o}C Freezer. Some eggs were also tested after being marked, killed by freezing and then left at room temperature for 1 and 2 weeks. Between marking and testing, eggs were individually kept in eppendorf tubes, aphids were placed on cotton leaves in specimen vials, and mirids were placed in specimen vials containing a bean and some unmarked *Helicoverpa* eggs (their normal diet). Mirids were only tested 0, 24, 48, and 96 hours after marking. Some marked H.eggs were allowed to hatch. These were tested 0, 6, 24, 48, and 96 hours after hatching. The significance level for the absorbance readings was determined by taking the negative controls (8 unmarked prey/ plate) and calculating the mean +(3*SD). Absorbencies below this level indicated unmarked prey. The original absorbencies of the marked prey were compared.

3.2. Testing predators in the laboratory

Predators were placed in a small petri dish with either marked or unmarked prey and left for half an hour, after which time they were checked to see if they had eaten the prey. They were then removed from the petri dish and placed in a clean vial. Predators were tested using ELISA techniques to see if they had been marked 0,6, 24, 48, or 72 hours after being exposed to the prey. Predators which fed on unmarked prey were used as the negative control.

Predators will be referred to by their generic name, except for the jumping spiders which were not identified to species and were collectively referred to by family (Salticidae). The *Oxyopes* (Araneae: Oxyopidae) (lynx spiders) used in this work were one of three species found in cotton in the Namoi Valley (NSW Australia): *O. molarius* L. Koch, *O. amoenus* L. Koch, and *O. gracilipes* (White) (Whitehouse & Grimshaw 2007) of which *O. molarius* is the most common. The other predators used in this study were: yellow night stalkers: *Cheiracanthium* sp (Araneae: Clubionidae); red & blue beetles: *Dicranolaius bellulus*; white-collared ladybird: *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae); brown smudge bug: *Deraeocoris signatus* (Distant) (Hemiptera: Miridae); damsel bug: *Nabis kinbergii* Reuter (Hemiptera: Nabidae); and the big eyed bug *Geocoris lubra* Kirkaldy (Hemiptera: Geocoridae). Specimens of these animals have been deposited within the spider collection of ACRI at Narrabri.

3.3. Testing predators in the field

2004/05 season

Mirids: About one hundred 1-2 instar mirids were knocked out with CO2 and then sprayed for 2 minutes with rabbit protein using a nebulizer in order to mark them. Once the mark had dried 4 mirids were removed a positive controls, and the rest were divided into 2 groups to be released in the field that morning. The 2 release sites were randomly chosen. At each site the 50 mirids were gently brushed onto 5th to 3rd position leaves. The following morning the site of the release was sampled using 5 beatsheets (10 beatsheets per sampling date): one beatsheet over the release site, one on either side of the release point, and one on each of the adjacent rows. Any predators dislodged in the beatsheets were taken and tested for rabbit protein using the ELISA techniques. Ten releases were conducted during the season.

Aphids: About two hundred aphids were knocked out with CO2 and then sprayed for 2 minutes with rabbit protein using a nebulizer in order to mark them. Once the mark had dried 4 aphids were removed a positive controls, and the rest were divided up among 10 3rd position leaves to be released in the field in the morning. Each leaf, containing about 20 aphids, were stapled to a 4th position leaf at 10 random locations around the field in the morning. That afternoon (about 4 pm) the locations where the leaves were added were sampled using a beatsheet and all predators collected (10 beatsheets per sampling date). Three 3rd position leaves were checked at the beatsheet site to gauge the resident aphid population. If high numbers of aphids were found then we conducted aphid washes on the samples. Five releases were conducted during the season.

Helicoverpa eggs. Eggs collected from laboratory colony moths were marked by dunking and left to dry. Once the mark had dried 4 eggs were removed a positive controls, and the rest were divided up among 10 cards, 5 cm x 2 cm. At one end of the card was a small piece of double-sided Sellotape (0.5 cm x 1 cm) to which we stuck about 250-300 eggs. That morning the ten cards were stapled to a 3rd position leaf at 10 random locations around the field. The following morning the cards were collected and the location where the card was stabled was sampled using a beatsheet and all predators collected (10 beatsheets per sampling date).The top third of the plants at the sample site were checked for naturally laid *Helicoverpa* eggs.

2006/07 season

Mirids were prepared the same way as in the 2006/07 season, except that they were initially divided into two groups, those that were 1-2 instars, and those that were 3-4 instars. Two lots of 25 1-2 instar mirids were sprayed with chicken protein, while two lots of 25 3-4 instar mirids were sprayed with rabbit protein. At two random locations in a Bt field of cotton, one lot of 1-2 mirids and one lot of 3-4 mirids were released. The following day the the site of the release was sampled using 5 beatsheets (10 beatsheets per sampling date): one beatsheet over the release site, one on either side of the release point, and one on each of the adjacent rows. Any predators dislodged in the beatsheets were taken and tested for rabbit and chicken protein using the ELISA techniques. If they tested positive for rabbit protein, then it was assumed that they had eaten a large 3-4 instar mirid nymph, if they tested positive for chicken protein, then it was assumed that they had eaten a small 1-2 instar mirid nymph. five releases were conducted during the season.

Eggs and aphids were prepared the same way as in the 2005/06 season, except that Eggs were marked with chicken protein. Both marked Eggs and aphids were released at mid-day at two randomly chosen sites in alternate weeks to the mirid marking. The following morning the site of the release was sampled using 5 beatsheets (10 beatsheets per sampling date): one beatsheet over the release site, one on either side of the release point, and one on each of the adjacent rows. Any predators dislodged in the beatsheets were taken and tested for rabbit and chicken protein using the ELISA techniques. If they tested positive for rabbit protein, then it was assumed that they had eaten an aphid, if they tested positive for chicken protein, then it was assumed that they had eaten a Helicoverpa egg. Five releases were conducted during the season.

Analysis

The results of both seasons will be presented together.

Anti-rabbit IgG sandwich ELISA Protocol

To test if pests or predators had been marked by the rabbit (or chicken) protein, each animal to be tested was placed individually in an Eppendorf tube and stored in a -80 freezer until needed. The sample was then ground in 500µl of Tris-buffered saline (TBS, pH 7.5) in the Eppendorf tube, centrifuged for 3 mins at 1400rpm, and then stored in the -80 freezer overnight. The sandwich ELISA is described in detail by Hagler (2006). Each assay plate was coated with 100 µl per well of anti-rabbit IgG (product no. R2004, Sigma-Aldrich, Castle Hill, NSW 1765) or anti-chicken IgG (product no. C2288 Sigma-Aldrich) that had been diluted 1:500 in milli Q water and incubated overnight at 4°C. The next day the primary antibody was discarded and 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 antigenic sites. The milk solution was then discarded and a 100 µl aliquot of each crushed arthropod sample was added to each well and incubated for 1 h at 27°C. The sample was discarded and the plate washed three times with TBS-Tween 20 (0.05%) and twice with TBS. Anti-rabbit IgG peroxidase conjugate (product no. A6154, Sigma-Aldrich) or anti-chicken IgG peroxidise conjugate (product no. A9046, Sigma-Aldrich) diluted 1:1000 in 1% milk was added to each well (100 µl) and incubated for 1h at 27°C. This solution was then discarded and the plate washed as before. Finally, 100 µl of HRP substrate solution was added to each well and incubated for 2h at 27°C. The absorbance of each well was read at 415 nm. Each plate included negative controls, positive controls and reagent blanks.

3.1 Testing marked prey.

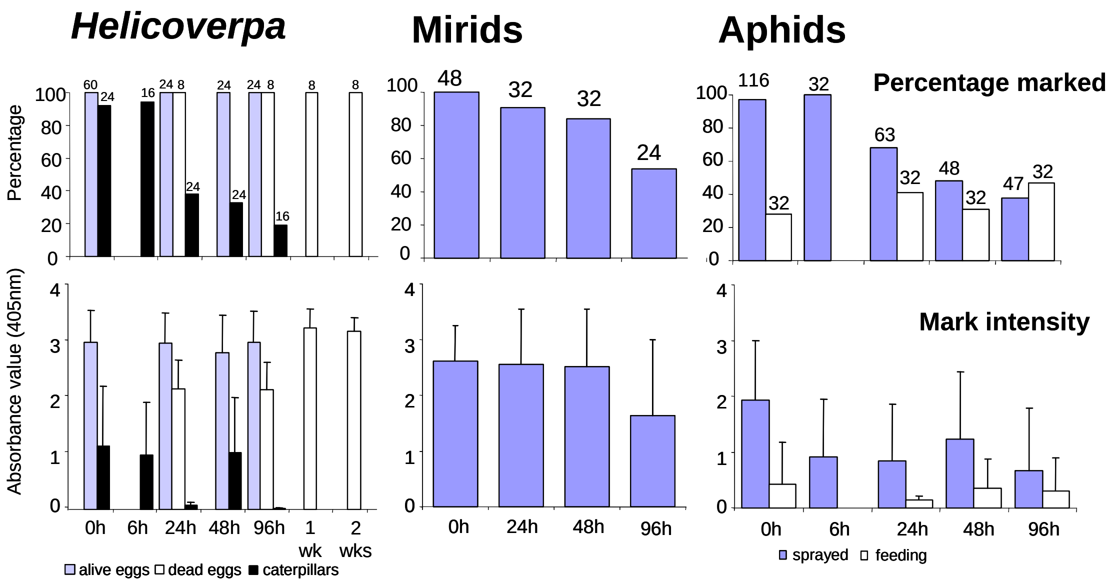


Fig. 3.1 Percentage of *Helicoverpa* eggs, mirids and aphids that remained marked, and the intensity of the mark, after various time intervals. All animals were marked with rabbit protein by spraying, except "feeding" aphids, which were marked with rabbit protein by allowing them to feed off a sugar solution containing the protein. The number above the percentage columns are the total number of animals tested. The time interval refers to the time since marking for the eggs, mirids and aphids (and the time since hatching for the caterpillars). The mark intensity was calculated using only samples that recorded a positive mark.

All eggs were easily marked and stayed marked indefinitely, with high absorbance values (Fig. 3.1) indicating the presence of the rabbit IgG protein marker. Even caterpillars hatching from their marked eggs retained a mark for 24 hours (although not at a high intensity) before the proportion of marked animals starts to fall. Mirids were easily marked, but the proportion marked and the mark intensity declined after a few days. Aphids were difficult to mark. External marking was the most effective for aphids, but the proportion of animals marked and the mark intensity dropped off after 6 hours. This loss of marking intensity is possibly due to the aphid moulting and their rapid life cycle, combined with their waxy cuticle. We tried to counteract the effect of the waxy cuticle by adding Maxx Organosilicone Surfactant to the protein solution, but as this had no effect on the results, we stopped using it. The percentage of aphids marked by feeding was very low (30%) and the mark intensity was also low.

3.2 Testing predators in the laboratory

The 151 negative controls in this experiment, which were spread among the absorbance plates, were predators that ate unmarked prey. Of these, three species groups were represented by only one or two samples (Iridomyrmex =2, *Deraeocoris* =1, *Geocoris* =1). The rest were represented by more than 20 samples, although not all of these six predators ate all three prey types as some predators showed no interest in some prey

The six predators with more than 20 samples were then tested for outliers using box-whisker plots in Genstat 11.1. This revealed that the distribution of absorbencies for the six species was not normal, with 9 "far" outliers (more than 3 times the interquartile range beyond the quartiles) spread among the predators. These extreme data points were excluded from further calculations. A REML analysis revealed that prey type had no effect on the absorbance readings (P=0.997, d.f.= 2,122.1; F=0) while the predator had a significant effect (P=0.009; d.f.= 5,121.3; F=3.25). There was also a significant difference between Orders (predicted means of Araneae, Coleoptera, and Hemiptera respectively = 0.034, 0.064, 0.023; P=0.007, d.f.=2, 133, F=5.1) but not within Orders (Araneae: P=0.139, d.f.= 2, 43.4, F=2.1; Coleoptera: P=0.59, d.f.=1, 47.7, F=0.3, Hemiptera were only represented by one species). Critical absorbance levels (mean+3*SD) were calculated for the three Orders across all plates, and for all predators combined (Araneae= 0.173, Hemiptera= 0.174, Coleoptera = 0.279, All = 0.222). The "All" critical absorbance was used with marked predators which did not belong to either Hemiptera, Coleoptera or Araneae.

Feeding in the laboratory

We tested 672 predators, belonging to seven taxa, for their likelihood to become marked after eating aphids, mirids or *Eggs*. This group included 40 Iridomermex (ants) and 12 *Deraeocoris* (Brown smudge bugs). In both of these groups only one individual caught a prey item, so they were not used in the majority of the analyses. The ants also behaved abnormally because they had been removed from their nest.

We found that the spiders (*Oxyopes*, *Cheiracanthium* and Salticidae) in particular were well marked, with over 70% of all species obtaining a mark from eating marked prey (Fig. 3.2). About 30% of the beetles which fed on marked prey obtained a mark, while 25% of Nabis became marked.

Many predators which didn't eat the prey also became marked. In particular, over 20% of spiders were marked when they didn't feed on the prey (Fig. 3.2).

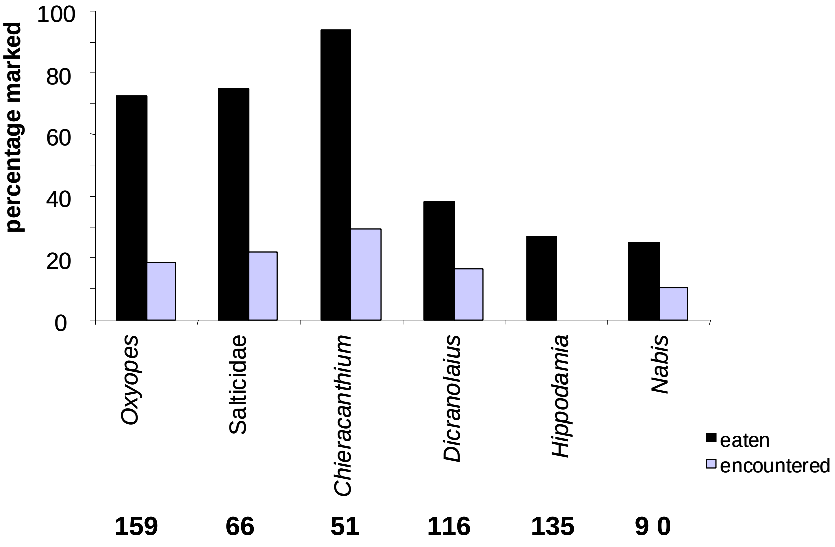


Fig. 3.2 Percentage of predators marked after feeding on, or just encountering marked prey (all prey species combined).

<u>Mark intensity:</u> The absorbance readings differed between predators, but unlike the tests on their baseline absorbance readings, the prey consumed influenced the absorbance readings (Fig 3.3; REML; effect of Predator: P<0.001, d.f.= 5,138, F=9.79; effect of prey type: P=0.002, d.f.=2,139.3,F=6.48, no interaction effect). Again the most strongly marked predators were spiders, with beetles and bugs showing similar absorbance readings. Predators which had eaten mirids were also more strongly marked, than those that had eaten *Helicoverpa* eggs or aphids.

Predators which were marked positive from encountering marked prey but not feeding did not show different absorbance levels either in respect to predator type (ANOVA, P=0.182, F=1.64, df=4,40) or prey encountered (ANOVA, P=0.767, F=0.27, df=2,42).

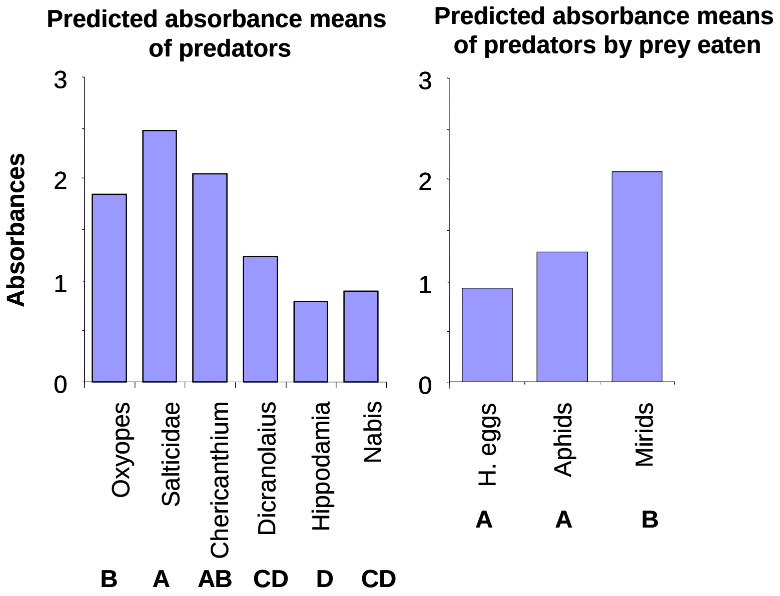


Fig. 3.3 Estimated absorbance means (using REML) for predators marked by feeding on marked prey.

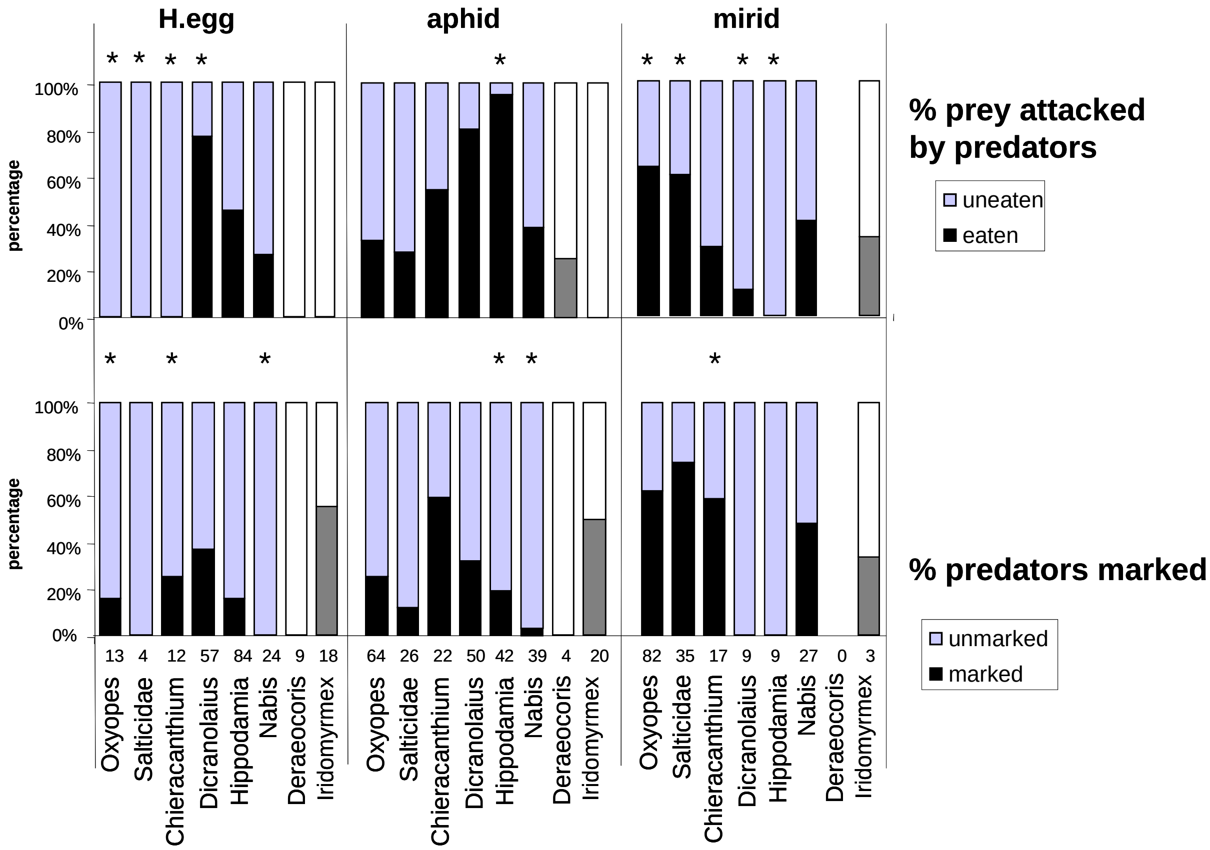


Fig. 3.4 Comparing the percentage of predators marked positive for feeding on marked prey with the actual number which ate marked prey. The * above the top graph indicate where predators significantly differed from the others in their tendency to attack that prey. The * between the graphs indicate instances where the number of marked predators significantly over or under estimated the number of actual feeding events. Ants and smudge bugs were not used in the analysis (see text).

Prey attack rates: We compared the number of prey eaten by the different predators to see if, under laboratory conditions, they varied in their likelihood to attack different prey. We excluded ants from the analysis, because they were behaving aberrantly, and smudge bugs because their numbers were too small. We found that predators varied in their likelihood to attack different prey ($\chi2 = 300$, df=10, $P<0.001$; Fig.3.4). The spiders (*Oxyopes*, Salticidae and *Cheiracanthium*) were significantly less likely to attack eggs than the other predators, and *Oxyopes* and Salticidae were more likely to attack mirids. *Hippodamia* were significantly more likely to attack aphids, and less likely to attack mirids. The beetle *Dicranolaius* was more likely to attack eggs, and less likely to attack mirids (Fig. 3.4).

We then tested to see if there was a significant difference between the percentage of predators which ate prey, and the percentage marked positive for feeding on prey. We found that there was an overall difference between the two groups ($\chi2 = 201$, df=17, $P<0.001$; Fig.3.4). In particular, *Oxyopes* were over-marked fore, Cheiracanthium were over-marked for eggs and mirids, *Hippodamia* were under-marked for aphids, and *Nabis* were undermarked for eggs and aphids.

3.3 Testing predators from the field

In the 2004/05 season we collected over 5500 predators of mirids, aphids and *Helicoverpa* eggs for testing using ELISA techniques. Through testing marked controls and recaptured mirids, we estimate that about half of all the aphids and mirids that predators encountered at the study site would be marked, while 99% of all eggs would be marked. Of the 2505 predators tested, 69 (2.5%) were marked positive for mirids; of the 1226 predators tested, 25 (2%) were marked positive for H.eggs; and of the 1368 predators tested, 54 (3.9%) were marked positive for aphids. So aphids were the most successful prey, even though they were the hardest to mark.

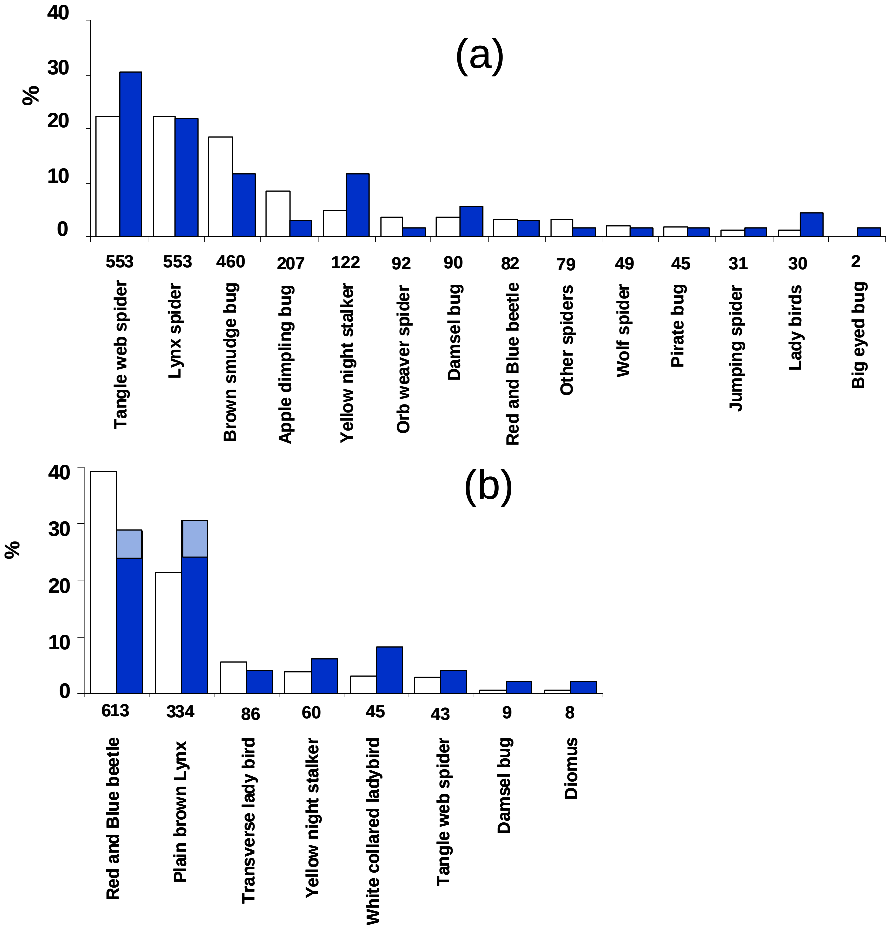


Fig. 3.5 The predators marked positive for mirid predation in the 2004/05 season (a) and the 2006/07 season (b). The number under the histogram is the number sampled of that species. The white bars represent the proportion of that species in relation to all animals captured; the blue bars indicate the proportion of that species in relation to all animals marked. In (b), the light blue indicates the proportion of that species marked positive for small mirids.

In the 2006/07 season, over 3500 predators were collected for testing using ELISA techniques for both rabbit and chicken markers (over 7000 tests). Although chicken protein was an effective maker, we had some problems under field conditions as mirids released in the field on the 25th of January marked with chicken protein had no detectable mark. This affected the mirid predator results for small mirids; of the 1557 predators tested, 42 (2.7%) were marked positive for large mirids, but only 7 (0.5%) were marked positive for small mirids, Fig 3.5). Thus the 2006/07 survey is biased towards the predators of large mirids, while the 2004/05 survey was sampling predators of small mirids.

Of the 2033 predators tested, 40 (2%) were marked positive for H.eggs; while of the 2033 predators tested, 102 (5%) were marked positive for aphids (Fig. 3.6).

The dominant predators of the different prey species was strongly affected by predator availability. The 2004/05 and 2006/07 seasons were quite different, in that the 06/07 season was a drought, with red& blue beetles dominating the beneficial community. Thus red and blue beetles were the most dominant predator for all prey species (excep mirids) in this season. Among the mirid predators, lynx spiders were consistently marked as dominant, although Tangle web spiders (which were very common in the first season) challenged them in the first season. YNS and Damsel bugs were also consistently marked above their abundance for mirids, as were ladybirds. YNS and ladybirds again were marked above their abundance for eggs along with a range of other predators, including mirids in the second season (Fig. 3.6). The aphid predators are more variable between season, and seemed to have been strongly influenced by the presence of Red and blue beetles. In 2004/05, when Red and blue beetles were uncommon, many predators were marked above abundance. In 2006/07 when nearly 45% of the predators marked were red and blue beetles, fewer predators were marked above abundance.

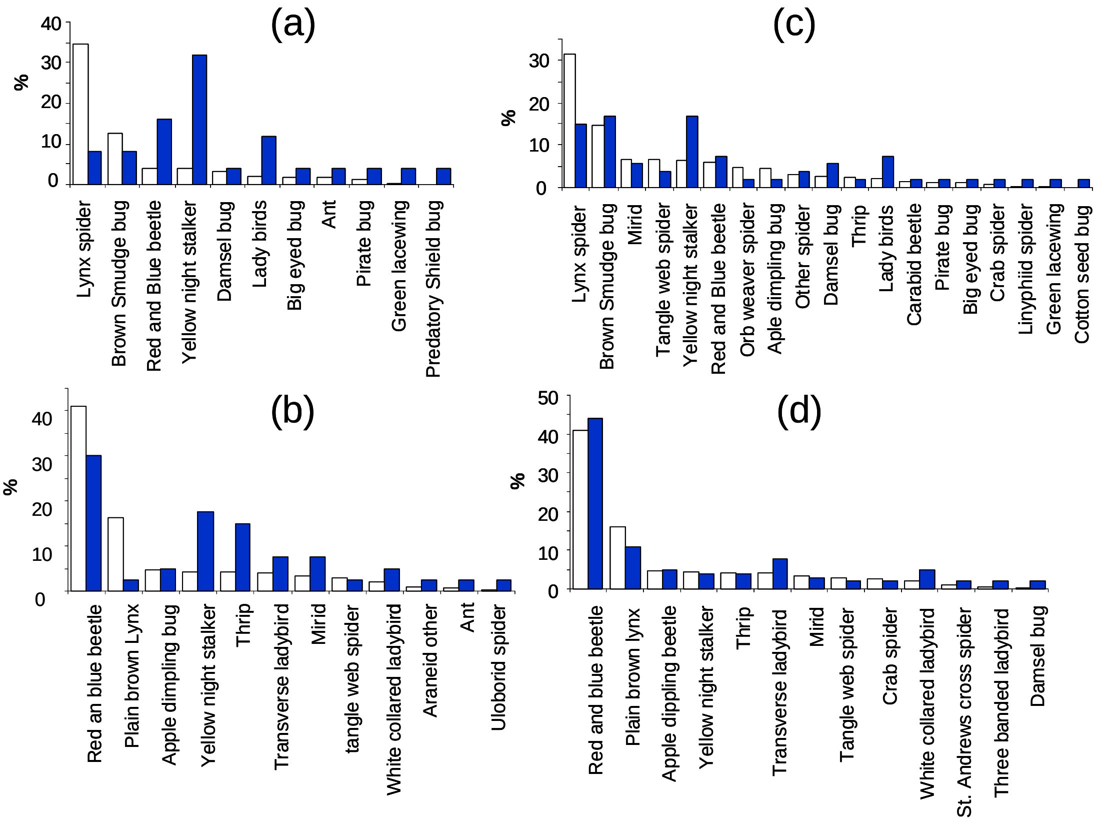


Fig. 3.6 The predators marked positive for both eggs in the 2004/05 season (a) and the 2006/07 season (b) and aphids in the 2004/05 season (c) and the 2006/07 season (d). Key the same as in Fig. 3.5.

Effect of predator size on prey type. In the first season, both Lynx spiders and YNS larger size classes caught proportionally more mirids (Fig. 3.7). In the second season there was no difference between the size classes, and a high proportion of very small lynx spiders were marked positive for catching large mirids.

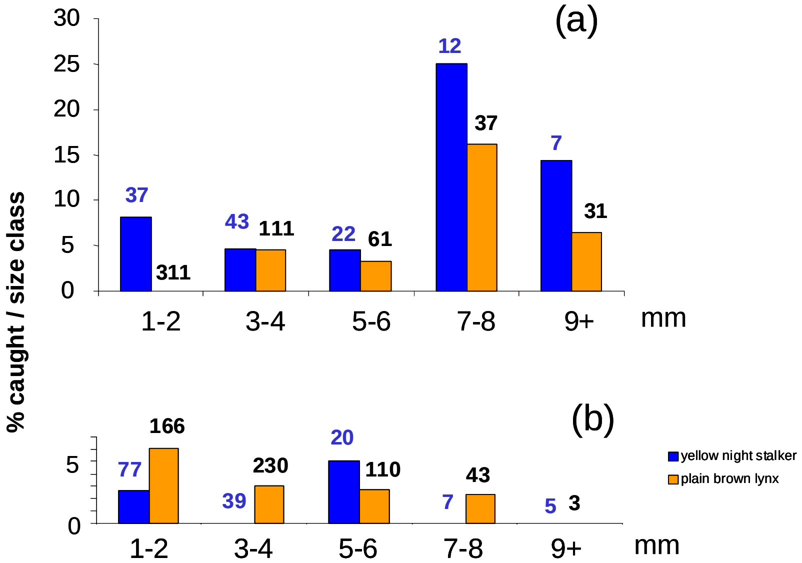


Fig. 3.7 The proportion of different size classes of Lynx spiders and Yellow night stalkers which caught mirids in the 2004/05 season (a) and the 2006/07 season (b). The numbers above the histograms are the number of predators caught in that size class for that species.

DISCUSSION

Marked prey

The results presented here show that mirids, and to a lesser extent aphids, can be effectively marked with rabbit protein and used to identify the predators of these animals. The work also showed that it was possible to mark eggs and then follow the activities of the neonates that emerged. (Presumably the neonates obtained the mark from eating their egg shells). Marked mirids in particular were effective to the extent that predators which consumed marked mirids had absorbance readings higher than those that consumed marked eggs. The rabbit protein marker on mirids was stable for 24 h, which would allow sufficient time for marked animals to be released in the field and consumed before sampling for predators needed to begin. The markings on aphids, however, deteriorated quickly, probably due to the rate at which these insects moult. Surveys for predators of aphids would need to be conducted about 6 hours after the marked aphids were released.

Testing predators in the laboratory

Predators differed significantly in their baseline absorbance (their absorbance readings before being exposed to a marker), and in their likelihood of obtaining the mark after eating or encountering marked prey. The differences in baseline absorbance could lead to biases in field collected predators, and could complicate work looking at predator prey interactions at the community level. However, the effect of the baseline absorbance readings probably has little effect on the final absorbance of the marked predator, especially when considering the degree to which predators differ in their ability to acquire the marker by consuming marked prey. Spiders, whose baseline absorbance readings were low, had significantly higher absorbance readings after consuming marked prey than beetles, whose baseline absorbance readings were high. This means that positive readings were clearer than in species whose absorbance readings are always low. Perhaps a more pragmatic approach would be to adopt a conservative critical absorbance level by calculating the mean baseline absorbance readings from all predators, add four times the standard deviation rather than the usual three times the standard deviation (Hagler & Naranjo 2004, Mansfield et al. 2008) and then use a simple multiplicative correction factor to accommodate differences between plates (Jones et al. 2006). This conservative approach would enable all predators in the community to be tested without the need to conduct prior tests to determine species-specific critical absorbances for every predator species that may be collected.

The difference between the predator groups in their tendency to acquire the protein marker could be caused by phylogenetic differences in their digestive processes, or due to differences in their feeding behaviour. The spiders in this study all lunge at and grab their prey before wrapping it up and feeding. Bugs such as *N. kinbergii* just stab their prey, while beetles such as *H. variegata* and *D. bellulus* move over their prey and start chewing. The amount of handling of prey by spiders prior to feeding may explain their higher absorbance values.

The difference between predators in their tendency to acquire the protein marker was reflected in the discrepancies between the percentage of predators marked and the percentage of predators that fed on different prey. In particular, *Cheiracanthium* were over represented and *N. kinbergii* were under represented when marked predators were directly compared to predators which actually ate prey. Overcoming such a bias is difficult, and would be common to all external marking techniques. Developing a quick and reliable way to mark prey internally with rabbit protein could assist with this problem.

The discrepancy between the marked and fed predators was enhanced by the high proportion of predators which became marked without feeding on the prey. Ants were a striking example of this as they didn't feed, but they did move around and made contact with the prey item in the petri dish. The artificial nature of the petri dishes may have enhanced the likelihood of marking through only encountering the prey in this study. Another explanation may be the sharing of food between ant workers (trophallaxis), which enables a single feeding event by one ant to produce more than one 'marked' individual (Hagler 2006, Morris *et al.* 1998). Nevertheless, if predators can obtain a mark in the field by contacting the prey rather than consuming the prey, then this highlights a potential problem when identifying predators from the field. The problem may be particularly relevant when using egg cards as prey items for predators. If the eggs are marked in situ on the cards, then both the eggs and the card will be marked. Therefore in field studies, care must be taken to only release marked prey (and not marked substrate such as cards), as predators could potentially obtain the marker from walking over the substrate rather than consuming the prey.

Testing predators in the field

These results show that the predators of mirds, aphids as well as eggs can be successfully identified in the field using these ELISA techniques. Although laboratory results revealed some biases in the system, comparisons can be made between seasons and within predator species. One of the striking observations is the versatility of generalist predator assemblanges. Because red and blue beetles were dominant in the second season and focused their foraging efforts on aphids, the consumption of aphids by the other predators adjusted accordingly.

The mirid predator assemblage was diverse throughout the season, with lynx spider consistently important predators. However, a surprise in the mirid predator assemblage were the high scores of ladybirds, red and blue beetles, and smudge bugs, none of which would eat mirids in the laboratory. This is particularly surprising in the second year when the marked mirids were large. It is unlikely that ladybirds and smudge bugs attacked and killed 4th instar mirids. What is more likely is that these animals scavenged either dead marked mirids, or the exoskeletons of marked mirids. Likewise, in the second season it is unlikely that 1-2 mm lynx spiders were attacking 4th instar mirids. In this season smudge bugs were also less common, so small lynx spiders may have taken their role in the second season and been marked accordingly.

The importance of YNS as predators of all prey groups needs to be accepted with some caution, as these animals in laboratory conditions were particularly easy to mark in comparison to other predators.

These results show that sandwich ELISAs that detect the presence of exotic protein markers are very sensitive and are an effective way of identifying potential predators of marked prey. However caution must be taken when comparing predators from different phylogenetic groups which use different foraging methods. Any field collected animals that are marked (indicating that they are predators of the marked prey) should be further checked to confirm that they are predators, and not just scavengers or incidentally marked. Nevertheless, the technique has the potential to reveal cryptic predator/ prey interactions and expose predators of target pests. As such it is a useful tool in the conservation biological control of pests.

4. Predators

The aim of this section is to test the ability of some of the mirid predators identified from the ELISA tests to attack mirids. In particular, yellow night stalkers, lynx spiders, and damsel bugs will be tested. The first experiment (4.1) looks at the ability of YNS, damsel bugs and lynx spiders to control mirid numbers and mirid damage. The second group of experiments (4.2, 4.3) takes a more detailed look at lynx predation on mirids. (Some of this work was presented as part of a summer scholarship by Mark Barnett; it is presented here for completeness.) The next group of experiments (4.4, 4.5, 4.6, 4.7) look at the response of mirids to lynx spider and damsel bugs, and the effect of lynx spiders and damsel bugs on each other (some of this work was presented as part of a summer scholarship by Mark Barnett and Katie Broughton). The final group of experiments (4.8, 4.9) look at the ability of these predators to control damage under field conditions.

METHODS

Table A gives an overview of the experiments carried out to test the ability of predators to control mirid numbers and mirid damage. Some of the work was conducted at part of summer scholarship work (indicated below) and is included here to give a complete picture of the ability of mirid predators.

Location	Title	description	Date	Summer Scholarship contribution
Laboratory	**4.1 Lynx, YNS & damsel bug effect on mirid damage**	Tested the ability of lynx spiders YNS, and damsel bugs to control mirid numbers and damage.	2007	-
Laboratory	**4.2 Lynx preferences**	Preferred mirid prey size of 2 species of Lynx spiders and two size classes	2005	Barnett
Laboratory	**4.3 Lynx consumption**	Tested how many mirids a lynx spider could eat	2005	Barnett
Laboratory	**4.4 Chemical detection**	Tested if mirids could detect the present of predators	2005, 2007	Barnett/ Broughton
Laboratory	**4.5 Lynx/damsel bug interactions**	Testing whether lynx spiders eat damsel bugs or vice versa	2005	Barnett
Laboratory	**4.6 Predator/mirid interactions**	Tested the behavioural response of mirids and predators to each other	2007	Broughton
Field	**4.7 Predator Cage Experiment**	Tested if lynx spiders and damsel bugs could control mirid numbers and damage in field cages	2005	Barnett
Field	**4.8 Spot innoculations**	Tested if locally high concentrations of a predator within a cotton field could increase yield or aid maturity at that point.	2006	-
Field	**4.9 Plot innoculations**	Tested if plots augmented with a predator could increase yield or aid maturity within the plot.	2006	-

Background

The damsel bugs, lynx spiders, and mirids used in these experiments were caught in the field using sweep nets or raised in laboratory cultures. Green mirids were distinguished from brown mirids using Malipatil & Cassis (1997) and *O. molarius* was distinguished from other common lynx spiders using Whitehouse & Grimshaw (2007). *N. kinbergii* is the only damsel bug in Australian cotton (eg Whitehouse et al. 2005). All lynx spiders and damsel bugs in the behaviour experiment were field caught. Laboratory cultures were continually stocked with field-caught animals, so that individuals used in these experiments would be no more than one or two generations removed from the field.

The cotton plants used in these experiments were: Bollgard® Sicala 40B®, Sicot 289B®, and Sicot 43B®. The experiments took place at the Australian Cotton Research Institute (30°11'S: 149°33'E) where squares develop about 40 days after sowing, with the first flowers appearing after c.70 days and develop into mature bolls after a further 70 days (Hearn & Constable 1984). The plants used in these experiments had bolls in the middle and lower parts of the canopy, and squares at the top. At this stage they are most vulnerable to mirid damage (this period usually lasts for about 5 weeks in December/January from first flower to early boll formation, Khan et al. 2006). Mirids damage the squares and the bolls (up to 20 days old) and can also damage the apical tip of the growing plant (Sadras & Fitt 1997). Unless otherwise stated, we used adult mirids or 4-5th instar mirids in these experiments. These cohorts are known to produce the greatest damage to cotton (Khan et al. 2006).

4.1 Lynx, YNS & damsel bug effect on mirid damage

The ELISA work indicated that lynx spiders, damsel bugs and yellow night stalkers may be predators of mirids. To compare the ability of yellow night stalkers against that of lynx spiders and damsel bugs to control mirids, the same experimental design as that used in section 2 was used (Fig. 2.1).

Three experiments were conducted. The first experiment consisted of three replicates of 5 treatments which were: control (nothing added to cage); mirids only (4 females + 1 male); mirids+lynx (4 females + 1 male mirid; 2 female lynx); mirids+YNS (4 females + 1 male mirid; 2 female Yellow Night Stalkers); mirids+damsel bugs (4 females + 1 male mirid; 2 damsel bugs). In the second and third experiments there were no damsel bugs available so only the first four treatments were tested (three replicates in both experiments).

A fourth experiment, using the same setup (4 cotton plants caged in a pot)was conducted with 5 replicates of 4 treatments: control (nothing added to cage); mirids only (12 juvenile mirids: 3rd -4th instar); mirids+lynx (12 juvenile mirids; 3 female lynx); mirids+YNS (12 juvenile mirids; 2 female +1 male Yellow Night Stalkers).

4.2 Lynx preferences

The aim of this experiment was to establish if plain brown lynx spiders (*Oxyopes molaris* L. Koch), stocking lynx spiders (*Oxyopes gracilipes* (White)) and damsel bugs attack mirids. If they did attack mirids, the next step was to see if they preferred a particular size class of mirids. Two experiments were conducted. In the first experiment, Ten female plain brown lynx spiders (average size 42 mg, 16.6 mm), ten juvenile plain brown lynx spiders (average size 4.6 mg, 4.3 mm) and ten damsel bugs (average size 5.8 mg, 10 mm) were tested to see if they were more likely to attack any of four mirid size classes (1st instar:; 3rd instar; 4/5th instar; and adult; average mass and length = 0.1mg 1.9mm; 1.4mg 3.1mm; 4.1mg 5.6mm; 7.4mg 7.2mm respectively). In the second experiment ten female and juvenile stocking lynx spiders were tested with the four mirid size classes. This experiment was run twice.

To control for satiation, predators were fed a *Helicoverpa* grub (about one half their body weight) two days before the experiment. To start the experiment, lynx spiders and damsel bugs were weighed and placed in individual cages made from plastic specimen jars (diameter: 4cm, height:5.5cm) where they remained for the four day duration of the experiment. Each day a mirid was randomly chosen from one of four size classes and added to each predator's cage. After 1 hour we checked if the mirid had been captured. If the mirid had not been captured, it was removed from the cage. After four days each predator had been exposed to one mirid from all four size classes.

4.3 Lynx consumption

Five adult female plain brown lynx spiders ranging in weight from 37.2mg to 53.8mg were placed in individual petri dishes and allowed to settle. Prior to the commencement of this experiment, the spiders were starved for 7 days in order to increase their appetite. An adult mirid (average weight 7.4mg) was placed into each dish and the time until it was attacked by the spider was recorded. Five minutes after the first mirid was attacked a second mirid was placed into the dish and the spider's actions observed. The spider was given 1 hour after finishing the first mirid to attack the second mirid. If the second mirid was attacked within the hour, the process was repeated. If the next mirid was not taken up within the hour, the spider was assumed to be satiated.

Once satiated, the spiders were tested after 3 and 24 hours to see if they had regained their appetites. To do this the spider, still in the initial petrii dish, was offered an adult mirid. If the spider did not attack the mirid within 15 minutes it was assumed to be still satiated. If the spider did consume the mirid it was assumed to have regained its appetite.

4.4 Chemical detection

Predators can reduce pest pressure without killing the pests by stopping them foraging. To see if damsel bugs or lynx spiders have non-predatory effects on mirids even when they are not present, individual mirids were tested in Petri dishes which had previously contained either lynx spider or damsel bug predators, another mirid, or nothing. Two sets of experiments were conducted, one during the day, and one during the insect's night using the reverse cycle room. Mirids can be active both diurnally and nocturnally (Broughton, Whitehouse & Mansfield, unpubl. data), so we tested them both during the day and night.

Diurnal test.

Petri dishes containing a filter paper and a square from a cotton plant were set up with either: (i) a lynx spider, (ii) a damsel bug, or (iii) were left empty (control). After 2-3 hours any occupants of the Petri dish were removed, and an adult female mirid was placed in the Petri dish and allowed 30 seconds to settle before it was tested, by observing it for 5 mins and noting its behaviour every 5 seconds (60 observations within the 5 min period). After 5 mins the mirid was removed and then retested after ½ hour in another Petri dish that had been empty (control), or had just had either a lynx spider or a damsel bug removed from it. After ½ hour the mirid was retested in a third Petri dish, so that each mirid had been tested once with each treatment. The order of the Petri dishes was randomly assigned. Ten mirids were tested.

Nocturnal test.

For 2-3 hours prior to the test, Petri dishes containing a filter paper were set up with either: (i) a lynx spider, (ii) a damsel bug, (iii) a lynx spider for one hour and then a damsel bug for the final hour, (iv) a mirid or (v) had been empty (control). After the occupant had been removed, an adult female mirid was placed in a Petri dish. The test mirid was allowed 30 seconds to settle before it was recorded for 5 minutes using an infra-red night vision camera (NESS 100-466 Digital b/w camera, low light Ultra IR OSD). The footage was recorded on a PowerPlex eDR400, 4 Channel Digital Video Server and later viewed and the mirid's behaviour was recorded every 5 seconds (60 observations per test). After 5 minutes the mirid was removed and retested every ½ hour four times more in a fresh Petri dish that had contained either (i), (ii), (iii), (iv) or (v) until each mirid had been tested with each treatment once. The order of the Petri dishes was randomly assigned. Five lots of five mirids were tested (25 mirids were tested in total).

Mirid behaviour was categorized as 'standing still' (no movement), 'fidgeting' (moving legs slightly in no discernable pattern) 'grooming' (moving legs through mouthparts), 'feeding' (mouthparts inserted into square), 'walking' (moving at normal gait), 'running' (moving at least three times normal gait).

4.5 Lynx/damsel bug interactions

As both lynx spiders and damsel bugs are generalist predators, either could attack the other. Therefore, the aim of the intraguild experiment was to test if lynx spiders or damsel bugs were more likely to attack the other, and whether this depended on their relative size. To alternate the size advantage between lynx spiders and damsel bugs, three size classes of lynx spiders (adult female, juvenile and small) were paired with adult damsel bugs. Due to a limited number of damsel bugs available, both male and female damsel bugs were used in this experiment. Male damsel bugs are similar in size to female damsel bugs, and a study which looked at their tendency to attack *Helicoverpa* eggs found no difference between the sexes (Wade et al. 2003).

Next, 10 adult female lynx spiders were paired with 10 adult damsel bugs (5 female, 5 male); 10 juvenile lynx spiders were paired with 10 adult damsel bugs (5 female, 5 male, average mass: 5.8 mg); and 10 small lynx spiders (average mass: 1.2 mg) were paired with 10 adult damsel bugs (3 female, 7 male). In the first 10 pairs, lynx spiders, which were 7x larger than damsel bugs, had the size advantage; in the second 10 pairs there was no size advantage; and in the final 10 pairs, damsel bugs, which were 5x larger, had a size advantage (Fig. 3.1).

Each pair was placed in a 9 cm diameter Petri dish. After one hour we recorded if either predator had killed the other.

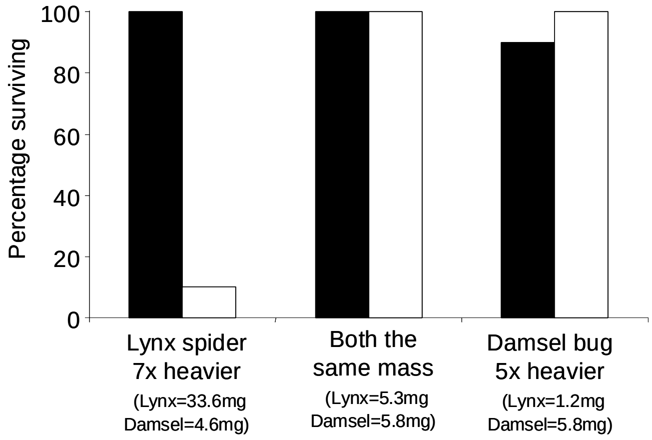


Fig. 4.1 The percentage of lynx spiders (black) and damsel bugs (white) surviving when paired in a Petri dish for one hour. In all three categories, 10 pairs were tested. In all cases the average mass is provided.

4.6 Predator/mirid interactions

The behavioural experiment was set up in the reverse-cycle Day/Night Room and ran for two hours after dark when mirids were most active. The temperature and humidity of the room remained at about 28°C and 50% respectively. Five potted cotton plants at about the 10-12th node stage (with a mixture of bolls, flowers and squares) were used in each experiment. On each plant, 4 4th instar mirids were tested in one of 5 treatments: (i) no predators (control); (ii) 1 lynx spider; (iii) 2 lynx spiders; (iv) 2 lynx spiders and 2 damsel bugs; and (v) 4 lynx spiders. Adult female and sub-adult lynx spiders and adult damsel bugs were used as predators. Due to a shortage of predators some animals were used more than once: 2 damsel bugs were used twice (but both were paired with first-time damsel bugs); and of the 24 lynx spiders used, 14 were used once, 2 were used twice, 5 were used 3 times, and 3 were used 4 times. Reused spiders were used in different treatments, and additional tests were done to make sure they did not bias results. A small drop of Celco™ correcting fluid was put on the wings of the damsel bugs to stop them flying away. Correcting fluid has been used in other experiments to mark ants (Whitehouse & Jaffe 1996) and spiders (Whitehouse & Jackson 1993) successfully without altering their behaviour. The damsel bugs did not seem affected by the correcting fluid as they did not try to remove it and no increase in grooming behaviour was observed. The experiment was repeated 5 times with the treatments rotated between the different plants to remove any potential bias caused by differences in plant structure (thus 100 mirids were used in this experiment). Plants were replaced if they lost too much fruit (retained less than two fruiting bodies) or became too old and unsuitable (10 plants were used in total).

Each plant in an experiment was set up in a large tray containing water (to prevent the mirids from escaping). Four 4th instar mirids were placed on each plant, followed by the predators. The animals were given 10 minutes before the first observations were made. Plants were checked every 30 mins to find the mirids and predators. Once an animal was found we noted: 1) its location in the plant (at the top, middle, or bottom of the plant, calculated by dividing the height of the plant evenly into 3 sections; 2) its position on the plant (on the stem, leaf or a fruit); and 3) its behaviour (standing still or feeding, walking, fidgeting or grooming). In the analysis, fidgeting and grooming were recorded as one behaviour, as were standing still and feeding because it was difficult to distinguish between these behaviours in the experiment.

We carefully searched for the animals on the plants using a red light from a HDL33A *Energizer* LED Headbeam (c. 20 lux). Preliminary experiments had found that this light did not disturb the mirids (Whitehouse, Broughton and Mansfield, unpubl. data). If a mirid was found in the water it was placed back on the plant. If a mirid was still missing after 10 minutes of searching, we moved on to the next plant and recorded that mirid as missing. Each plant was checked 5 times in the 2 hour period.

4.7 Predator cage experiment

Food web experiment

Five cages, which consisted of a cylindrical wire frame covered with white micromesh, each of 0.5m^3 volume, were set up over cotton plants in a 7 ha cotton field at the Australian Cotton Research Institute. Prior to enclosing the plants in the cages, the plants were vigorously shaken in an attempt to remove any large arthropods. The cages were then set in place, and 5 different combinations of mirids and predators were added. These included: (i) the ‘Mirids only’ treatment of 15 mirids only (5 small, 5 medium and 5 large); (ii) the ‘Mirids +Lynx’ treatment of 15 mirids and 4 lynx spiders; (iii) the ‘Mirids+Damsel bug’ treatment of 15 mirids and 4 damsel bugs; (iv) the ‘All’ treatment of 15 mirids, 4 lynx spiders, and 4 damsel bugs; and (v) the Control where nothing was added.

The cages were then sealed and left for one week before they and the plants therein, were removed from the field. At this point the contents of the cages (including plants) were sampled by fumigating with chloroform, and then collecting all animals present. The plants in each sample were then mapped and inspected for tip and fruit damage (squares were scored “damaged” if they were discoloured inside, bolls if they had dark puncture marks on the outside). All animals collected were identified and counted. In particular, we counted the remaining mirids, large lynx spiders and damsel bugs. The experiment was replicated four times over a 5 week period (20 separate cages) from early flower to early boll set, when the cotton was most susceptible to mirid damage.

4.8 Spot inoculations

The aim of this experiment was to see if a local increase in mirid predators could reduce mirid damage within a field of cotton. Six potential mirid predators were selected to be tested, based on the results of the ELISA experiments. Inoculation points 1m long were randomly located within a 2 ha field of Sicot 71 B RR (Bt) cotton. At each location one of 9 treatments were applied (3 replicates): 1) control (nothing done to location), 2) the location was beatsheeted but nothing was removed, 3) location was sprayed with propagite, 4) Damsel bugs were added, 5) tangle web spiders were added, 6) Jumping spiders were added, 7) plain brown lynx spiders were added, 8) Yellow night stalkers were added, 9) ladybirds were added. At each location 9 inoculations of one of these treatments were conducted over the season (sprayed location received applications of propagate). Between 2 and 4 predators were added at each inoculation (depending on availability).

The invertebrate community in the plots was sampled 9 times during the season using beatsheets placed adjacent to the inoculation location.

At the end of the season, maturity picks were conducted at the 1m inoculation point, and one metre on the same row on either side of the inoculation point. Boll damage was compared using a chart in which bolls were graded from not damaged (0) to very damaged (4). Boll number, boll damage, lint and seed weight were calculated from the cotton collected from the inoculation points.

4.9 Plot inoculations

Twenty-five plots 8 rows by 10m long were set up in a 5 x 5 matrix using Sicot 80 BRF cotton (bordered by 1 fallow row, two rows of sorghum, one fallow row; and across rows: 3 metres chipped). Each plot was inoculated with one of five treatments: control (no inoculation), mirids only, mirids+lynx, mirids+YNS, mirids+damsel bugs, and mirids+spray. Plots were inoculated once a week 11 times during the season, but every second time only the predators were added (6 inoculations with mirids+predators, 5 with predators only. The spray plots were sprayed 3 times during the season with regent (70mls/ha).

Each time plots were inoculated with mirids, 10 mirids (1male, 1 female and 8 juveniles) were added. However, the number of predators added to the plots was more variable due to the number available. Between 10 and 2 lynx spiders were added (usually *Oxyopes molaris*), and 1-8 Yellow night stalkers were added to each appropriate plot. These numbers were less than anticipated.

The community in the plots was sampled 5 times during the season using beatsheets placed adjacent to the inoculation location to see if the communities were altered by the inoculations.

At the end of the season, maturity picks were conducted at 2 1m locations in the centre of the plot. Boll damage was compared using a chart in which bolls were graded from not damaged (0) to very damaged (4). Boll weight, lint, and seed number? were calculated from the cotton collected from the inoculation points. Bulk picking of plots was also undertaken.

RESULTS

4.1 Lynx, YNS, and damsel bugs effect on mirid damage

The first three experiments were analysed together. These showed that there was no significant difference in the number of fruit in the different treatments (ANOVA (plant height covariate); df=3,29; P=0.45; F=0.92), but there was a significant difference in the number of mirids between treatments, with fewer mirids in the lynx spider and control treatments than the mirid, YNS and damsel bug treatments (ANOVA; df=4,32; P<0.001; F=16.3; lsd=1; mean number of mirids in the different treatments: damsel bug=4, mirid=2.7, YNS=2.3, lynx spider=1.1, control=0.1).These experiments were all contaminated with aphids, so the experiment was repeated again.

In the fourth experiment, the presence of both lynx spiders and YNS reduced the number of mirids in the cages (Fig. 4.2 (a), ANOVA, df=3,16, P<0.001, F=9.2) while there was a trend for there to be less fruit in the mirid treatment (Fig 4.2 (b), ANOVA, df=3,11, P=0.062, F=3.3).

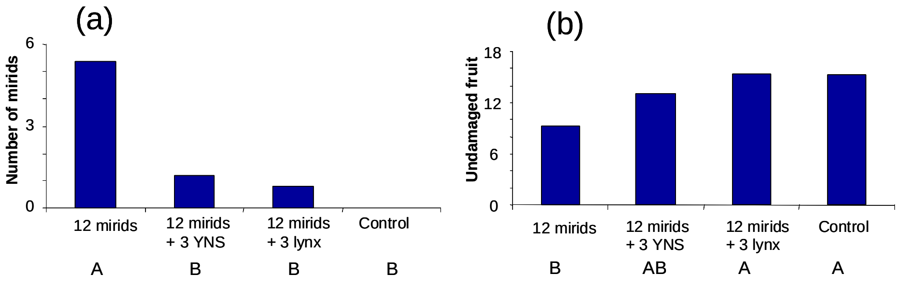


Fig 4.2 (a) There were significantly less mirids in the lynx spider and YNS treatments than the mirid treatment, while there was only a trend in the number of fruit between treatments (b).

4.2 Lynx and damsel bug preferences

Plain brown lynx spiders and damsel bugs.

Small juvenile lynx spiders were more likely to capture smaller mirids (χ^2=12.62, d.f.=3, P=0.006, Fig. 4.3). Adult female lynx spiders were more likely to capture larger mirids (χ^2=15.83, d.f.=3, P=0.001) and were more likely to take 4th instar to adult mirids than juvenile lynx spiders (Fisher's exact test; P<0.001). Juvenile lynx spiders showed a trend to take more 1st to 3rd instar mirids in comparison to adult female lynx spiders (Fisher's exact test; P=0.052). Only one damsel bug attacked a mirid, consuming a 4th instar (Fig. 4.3).

Stocking lynx spiders.

Stocking lynx spiders were not as effective at catching mirids as plain brown lynx spiders (df=1, chisq.= 10.6, P=0.001) and adult stocking lynx spiders showed no preference for any size class (Fig. 4.3).However, juvenile stocking lynx spiders also seemed to prefer smaller mirids.

4.3 Lynx consumption

The spiders consumed an average of 4 mirids (range 0 to 7) over 12 hours before becoming satiated (Fig. 4.4). The average time that it took for each spider to consume a mirid was 1:55 hrs (ranging from 1:30 hrs to 2:05 hrs). All satiated spiders ignored the mirid when tested after 3 hours but attacked and consumed the mirid after 24 hours. Spider 5 did not attack any mirids, not even after 3 or 24 hours.

Three of the 4 spiders which ate during the experiment consumed two mirids at the one time (spiders 1 and 2 both once and spider 3 twice). The reactions of the mirids when confronted with an adult female lynx spider was to either freeze immediately on touching the spider and then back away, or to move rapidly in the opposite direction to the spider.

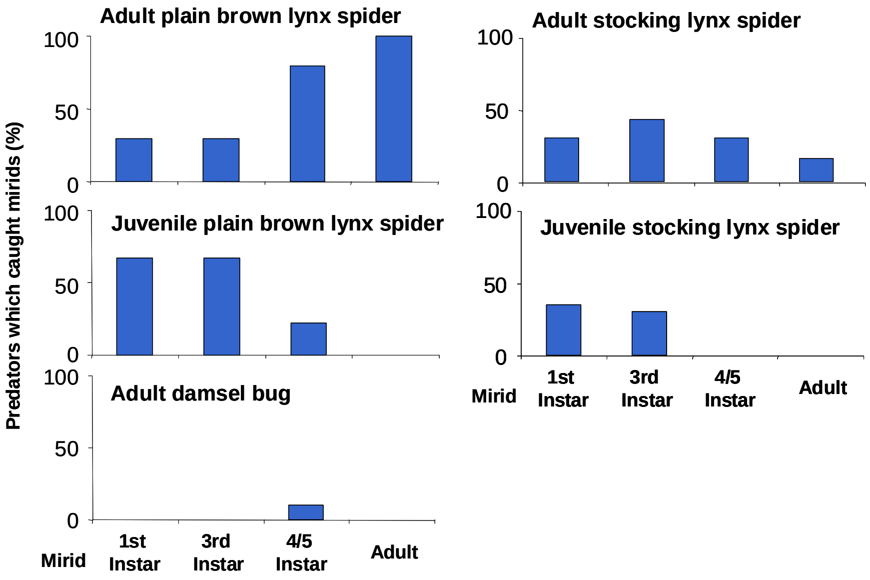


Fig. 4.3. The percentage of different mirid instars captured by adult plain brown lynx spiders (n=10), juvenile plain brown lynx spiders (n=9), adult damsel bugs (n=10), adult stocking lynx spiders (n=19) and juvenile stocking lynx spiders (n=20).

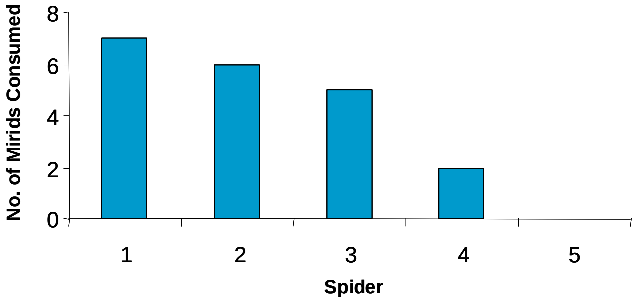


Fig 4.4 Number of mirids consumed by each spider during 12 hour test.

4.4 Chemical detection

Diurnal test.

Mirids tested in petri dishes which had contained either a damsel bug, a lynx spider or nothing, mainly stood still, fidgeted, or walked. These behaviours accounted for 91.2% of all the observations with mirids spending most of their time standing still (mean observations standing still: 42.8, walking: 6.4, fidgeting: 5.9). For each of the most common behaviours, the mirids responded differently to the treatments (standing still: Friedmann's statistic = 9.38, P=0.009, df = 2; fidgeting: Friedmann's statistic = 8.6, P=0.014, df = 2; walking: Friedmann's statistic = 7.8, P=0.02, df = 2). For all three behaviours there were no significant differences between mirid behaviour on lynx spider or damsel bug Petri dishes but there were between lynx spider and the control Petri dishes (critical difference for α= 0.05: 10.7; differences between lynx spider and damsel bug treatments: standing still = 6, fidgeting = 5, walking = 9; differences between control and lynx spider treatments for: standing still = 13.5, fidgeting = 13, walking = 12). Mirids were significantly more likely to stand still on control Petri dishes than lynx spider Petri dishes, and more likely to walk and fidget on lynx spider Petri dishes than control Petri dishes.

Other mirid behaviours that were observed but were too infrequent to analyse were feeding (n=2: 1=lynx, 1= lynx and control treatments), running (n=3: 1= lynx, 2= damsel bug treatments); and grooming (n=9: 2= lynx, 2= lynx and control, 2= damsel bug and lynx, 1= damsel bug treatments).

Nocturnal test.

There was no significant difference between the likelihood of mirids to walk, stand still or fidget in the different treatments (filter paper contaminated by: lynx; lynx followed by damsel bug; damsel bug; mirid; control) when tested at night on filter paper only (standing still: Friedmann's statistic =4.1, P=0.36, df = 4; fidgeting: Friedmann's statistic = 1.9, P=0.53, df = 4; walking: Friedmann's statistic = 6.9, P=0.106, df = 4).

4.5 Lynx/damsel bug interactions

Adult female lynx spiders, which are 7 times larger than adult damsel bugs, were likely to kill the damsel bugs, but adult damsel bugs which were 5 times larger than juvenile lynx spiders were not likely to kill the small lynx spiders ($\chi^2 = 7.31$, P=0.026, d.f.=2; Fig. 4.1).

4.6 Predator/mirid interactions

Mirids moved away from the middle of the plant and towards the top of the plant as predator pressure increased (Fig 4.5; treatment x location on plant: F= 13.2, d.f. =8, 321, P=0.001; LSD1%: mirids =0.57, Lynx spider =0.62, Damsel bug =0.25) and mirids were less likely to stand on stems as predator pressure increased (treatment x substrate on plant: F= 3.1, d.f.=8, 340, P=0.002). There was a sharp drop in the amount of time mirids spent standing still or feeding in the presence of any predators, while the likelihood of fidgeting increased (Fig 4.6; treatment x activity: F= 5.4, d.f.=8, 321, P<0.001).

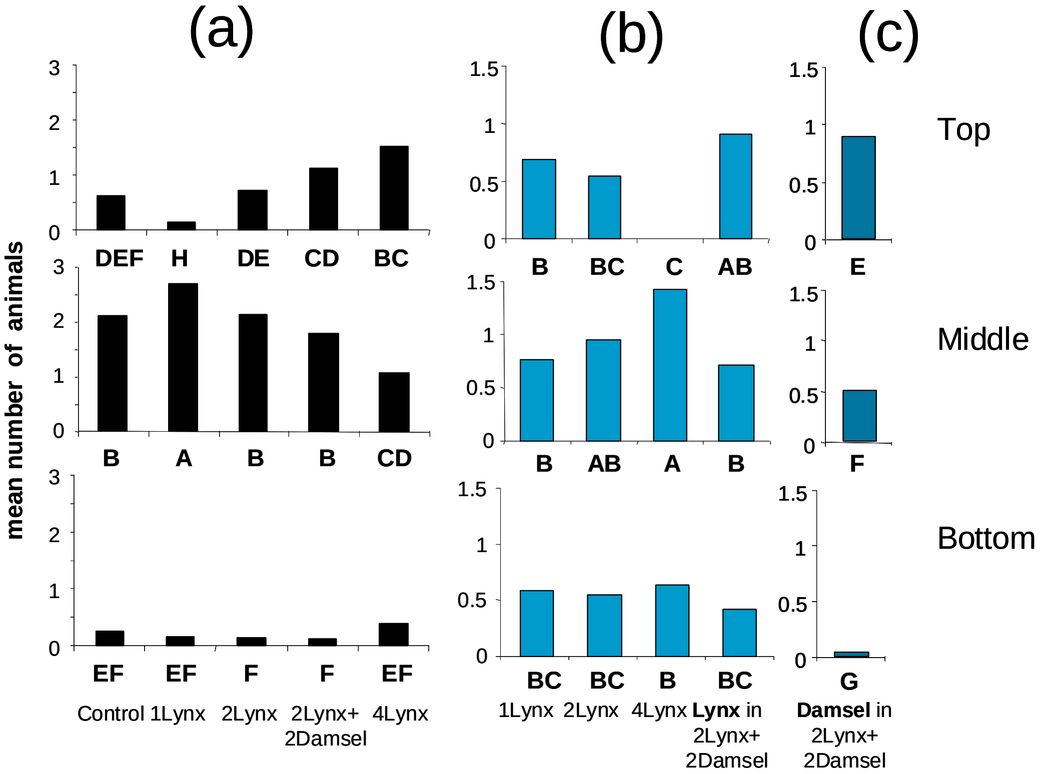


Fig. 4.5 (a) The effect of predator density on the number of (a) mirids (b) Lynx spiders or (c) Damsel bugs at the top, middle or bottom of the cotton plants (total mirids =covariate). See text for details.

All predators were more likely to be found on leaves than stems or fruit (lumped together because of sparse data on fruit; ANOVA: d.f. =1, 173; substrate: F= 13.75, P<0.001). When on the stems, lynx spiders often adopted a predatory stance, in which legs I and II were held forward and above the substrate, and the spider either faced downwards or towards the "trunk" of the cotton (to observe lynx spiders catching mirids on stems and leaves, please go to:

http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/SuckingPestPublications/Mirid_Predation_by_Lynx_Spiders.aspx)

More damsel bugs were found at the top of the plant than at the middle or bottom (Fig 4.5 (c); ANOVA: d.f. = 2,44; location: F = 24.25; P<0.001). Alternatively, there were fewer lynx spiders at the top of the plant as predator density increased, except in the presence of damsel bugs(Fig. 4.5 (b)); at the predator density of 4/plant, there were significantly more lynx spiders at the top of the plant when half the predators were damsel bugs than when all were lynx spiders (ANOVA: d.f. = 8,243; treatment x location: F=7.85, P<0.001).

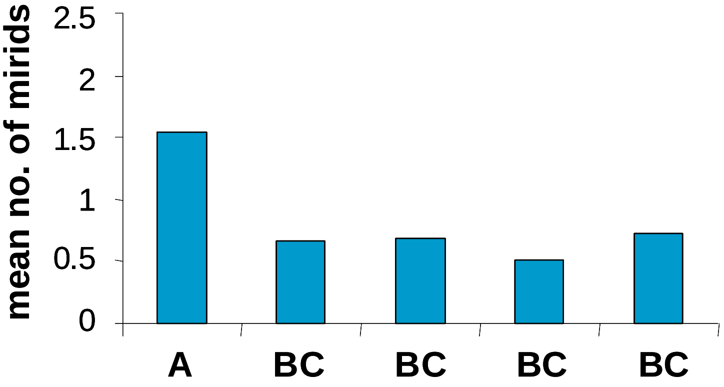


Fig. 4.6 The effect of predator density on the number of mirids standing still or feeding, (with total mirids on the plant as a covariate, LSD(1%)=0.63). In the presence of any predator mirids were less likely to stand still or feed.

Because some spiders were used more than once, we were concerned that specific spiders may be more likely to be at the top of the plant, and this may have influenced the results. To control for reusing spiders, we tested the eight spiders used more than three times to see if the treatment response of these spiders differed in their preference for the top of the plant compared with the other spiders. We found that spiders used more than three times followed a similar spatial distribution to the other spiders, i.e. more individuals moving to the top of the plant in the presence of damsel bugs (REML, W=36.9, d.f.=3, P<0.001; average proportion of re-used lynx spiders at the top of the plant for 1 lynx: 0.34, 2 Lynx: 0.18, 2 Lynx + 2 Damsel bug: 0.42, and 4 Lynx: 0.08). Therefore reusing spiders did not appear to bias our results.

Mirid deaths. Of the 100 mirids tested in this experiment, 9 were killed: one by a damsel bug and 8 by a lynx spider. There was no significant difference between the number of mirids killed and the amount of exposure to damsel bugs or lynx spiders (Fisher's exact test: NS; mid P value = 0.676). Two mirids were killed in the treatments "2 lynx spiders", and "2 lynx & 2 damsel bugs", while five were killed in the "4 lynx spider" treatment. Six of the mirids were caught in the middle of the plant, two at the bottom and one at the top. Seven were caught on leaves while two were caught on stems.

4.7 Predator cage experiment

There was a significant difference in the number of mirids surviving in the different treatments (Fig. 4.7 (a); ANOVA (square root transformation): F= 27; d.f.= 4,12; P<0.001). The Mirids+Damsel bug treatment contained the greatest number of surviving mirids (70.0% survival), which was not significantly different from the Mirids only treatment (65%); while cages containing lynx spiders had significantly lower numbers of mirids (Mirids+Lynx treatment: 21.7% survival; All treatment: 26.7% survival) The control cage had an average of 1.50 mirids at the end of the experiment indicating that although the plants were vigorously shaken prior to being enclosed, they still retained some invertebrates. An additional 21 lynx spiders were also collected from the cages over the four weeks, but of these only three were subadults, and 18 were spiderlings less than 4 mm in body size. Two additional very small damsel bug nymphs were found in the Mirids only treatment. There was no difference in the survivorship of damsel bugs or lynx spiders when they were the sole predator, or when they were caged with each other in the All treatment, (Fig. 4.7 (a), ANOVA, Damsel: F=0.27, d.f.=1, P=0.64; Lynx: F=0.18, d.f.=1, P=0.7).

During the 4 weeks of experiments, no tip damage occurred to any of the plants in any of the combinations. Damage was recorded to the squares and bolls of the plants with, in some cases, as much as 100% boll damage being inflicted in “Mirids only” cages. As the season progressed, the bolls accumulated external damage. The number of damaged squares was lower in the last release because by then it was late in the season and most of the squares had already flowered.

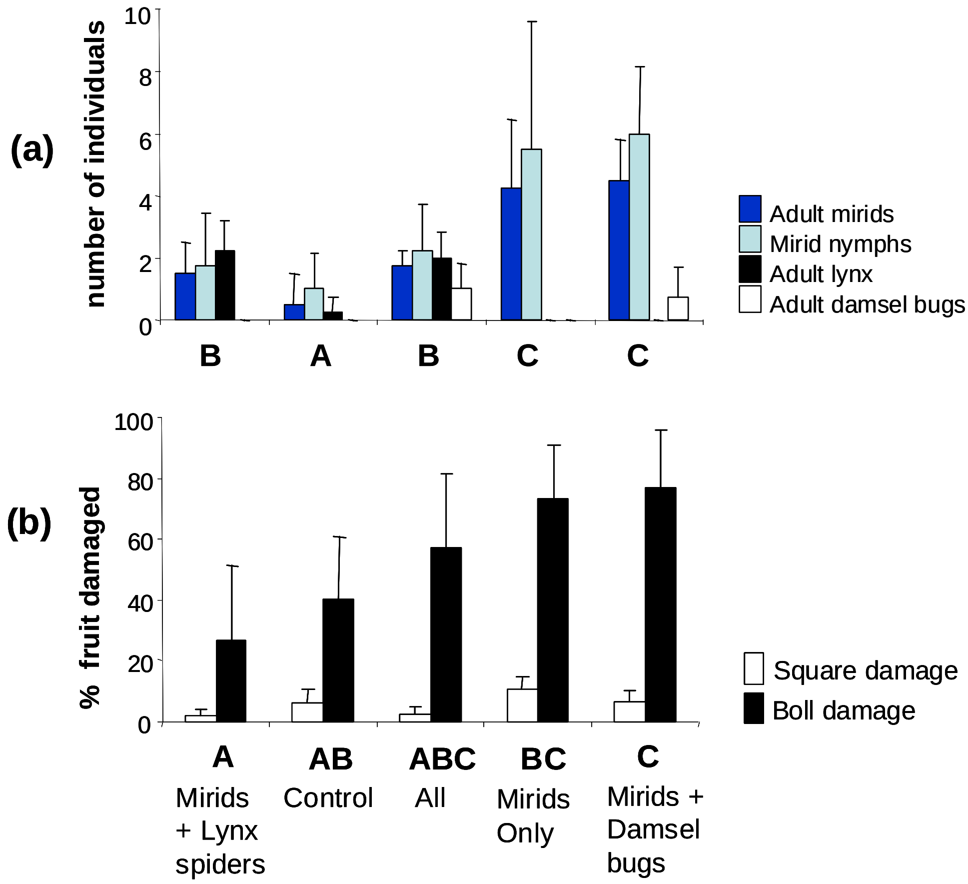


Fig. 4.7 The effect of the different treatments on: (a) the number of mirids surviving the different treatments; and **(**b) the proportion of fruit damaged.

Although there was no significant effect of treatments on square damage (ANOVA test: F=3.1, d.f.=4, 11, P=0.06) there was a trend with the Mirids only treatment receiving the greatest amount of damage to the cotton squares (mean=11%) while the least damage occurred in Mirids+Lynx treatment (mean=2%, Fig. 4.7 (b)).

There was a significant effect of treatment on boll damage (Fig. 4.7 (b); Friedmann’s statistic = 12, P=0.016, d.f. =4). The Lynx treatments fared the best with an average of only 27% damage, while the Mirid+Damsel bug treatments fared the worst with an average of 77% damage. The Mirids+Lynx treatment suffered significantly less boll damage than the two treatments without lynx spiders, while the Mirids+Damsel bug treatments suffered as much damage as the Mirid only treatment.

Other prominent animals found in the cages included jassids, thrips and brown smudge bugs. We found no significant difference between treatments in the number of thrips (average number per sample: 23; ANOVA: F=0.76, d.f.=4,12, P=0.571) nor jassids (average number per sample: 73; ANOVA: F=1.87, d.f.=4,12, P=0.181) but brown smudge bugs showed a trend to differ (average number per sample: 5; ANOVA: F=8.90, d.f.=4,12, P=0.055) with the highest numbers found in the All treatment and the lowest numbers found in the Mirids+Damsel bug treatment.

4.8 Spot inoculations

Mirids were more abundant in the Bt than the conventional crop, probably because the conventional crop was badly chewed out by *Helicoverpa* spp. There was no difference in the yield between the nine treatments in the spot inoculation experiments in the field but there was a difference in the maturity picks, with inoculation sites of YNS, ladybirds, tangle web spider, and plain brown spiders maturing first, and the control, the beatsheet location, and damsel bug location taking the longest to mature (ANOVA, df=8,40; F=5.2; P<0.001).

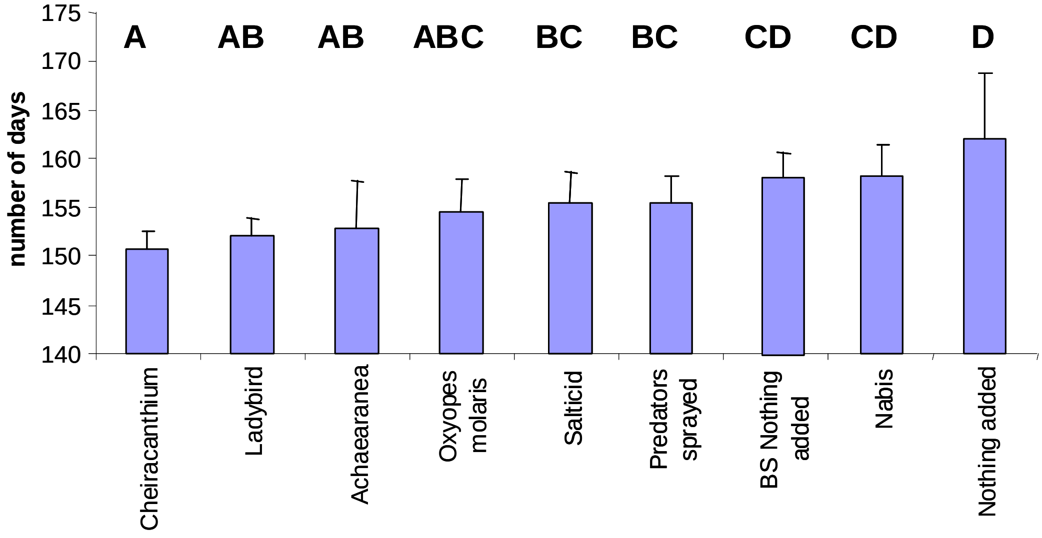


Fig. 4.8. The age of the crop at the inoculation site at 60% maturity for all treatments in the Bt crop. Three metres were sampled at each sight.

4.9 Plot innoculations

There was no effect of treatment on the days to 60% maturity (Fig. 4.9). This crop matured much earlier than that in the previous year. The plots were also heavily invested by mites, which appeared to have been triggered by the regent spray treatment, which was aimed at removing any mirids from the plot.

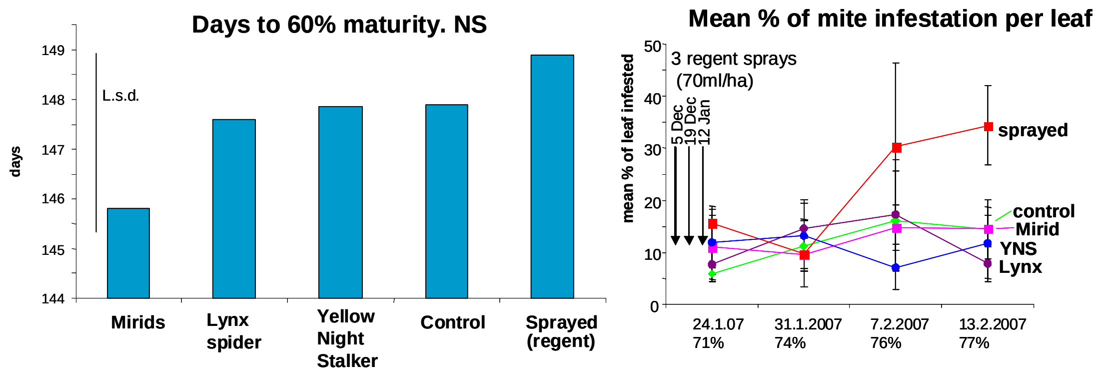


Fig. 4.9. The age of the crop in the inoculation plots at 60% maturity for all treatments. , and the mean number of mite infestations in the different crops.Three metres were sampled at each sight.

DISCUSSION

Potential mirid predators

The experiments in this section tested a number of potential mirid predators identified from the ELISA work. While damsel bugs did attack mirids, they did not seem to be very effective at controlling mirid numbers either in the laboratory (Exper. 4.1) or field cages (Exper. 4.7). This study suggests that damsel bugs would not be useful mirid control agents.

Yellow night stalkers (YNS) controlled mirid numbers in the glasshouse (Exper. 4.1) but they were not as effective at controlling mirid damage. Nevertheless, the spot inoculation experiment did indicate that they could be effective at reducing time to maturity under field conditions (Exper. 4.8)

Lynx spiders were found to be effective predators of mirids (Expers. 4.2, 4.3, 4.6, 4.7) although there were differences between species. There are three species of lynx spider in the Namoi Valley (Whitehouse & Grimshaw 2007) and we tested *Oxyopes molarius* and *gracilipes*. *O. gracilipes* (the stocking lynx) is a smaller animal than *O. molarius* (plain brown lynx) and not as common in cotton (Whitehouse & Grimshaw 2007). It was not as effective as a mirid predator as the plain brown lynx under laboratory conditions. Plain brown lynx spiders were particularly keen mirid predators, catching up to 7 adult mirids in 12 hours and capable of consuming two mirids at once (Exper. 4.3). Adult female plain brown lynx preferred larger adult mirids, while smaller, juvenile plain brown lynx preferred younger nymphs (Exper. 4.2). Plain brown lynx were also able to reduce mirid damage (Exper. 4.1, 4.7) making them potentially very effective mirid control agents. Although they did reduce time to maturity in the spot inoculation experiment (Exper 4.8) more work is still needed to assess their effectiveness within a crop.

Mirid response to predators

In the nocturnal part of Exper. 4.4, mirids did not respond to chemical cues. However, in the diurnal part of Exper. 3.3, mirids in Lynx spider treatments were significantly less likely to stand still, and more likely to walk than those in Control treatments. Thus mirids were responding to the presence of lynx spiders without encountering them. These results suggest that a response to chemical cues was context specific (although it was difficult to see "fidgeting" using the night vision cameras because of the resolution, and this could have contributed to the null result). Nevertheless, an increase in movement in the diurnal experiment could indicate a reduced likelihood to settle down to feed, which could explain why the Lynx treatment in Exper.4.7 had less damage with the same number of mirids. The same reduction in "fidgeting" in the presence of predators was also seen in Exper. 3.5. Thus predators in a cotton crop can reduce damage by reducing mirid numbers and also by changing mirid behaviour.

Foodweb interactions

Exper. 4.7 showed that cages containing female lynx spiders reduced the number of mirids in the cage, even in the presence of damsel bugs. However, cages containing only damsel bugs and mirids did not contain fewer mirids than cages with only mirids. These results suggest that lynx spiders were the main predators of mirids in the cages and that damsel bugs did not contribute to mirid mortality.

This conclusion is supported by Exper. 4.2, where lynx spiders were more effective predators of green mirids than damsel bugs; in particular, adult females focused on large nymphs or adults and juvenile lynx spiders focused on smaller instars. Lynx spiders usually attack prey between 50 and 80% of their body size (Nyffeler et al. 1992). As spiders probably gauge body size in respect to both the length and weight of their prey, adult mirids were still a bit small (43.4% length and 17.5% weight) in comparison to an adult lynx spider, but far too large (167.4% length and 160.9% weight) in comparison to the juvenile lynx spiders used in these experiments. Alternatively, third instar mirids were within the preferred attack range of juvenile lynx spiders (74.4% length, 30.4% weight). This difference is useful in terms of biological control. Most damage is inflicted by the larger sized mirids (Khan et al. 2006) so the presence of adult lynx spiders may reduce damage, and reduce influxes of mirids into crops, whereas the presence of smaller lynx spiders could reduce the numbers of mirids reaching a damaging size.

Damsel bugs are classified as generalist predators that will attack mirids (Farrell 2006; Whitehouse et al unpubl. data). But in Exper. 4.2 only one mirid was attacked. A low attack rate of damsel bugs on mirids was also reported in Exper. 4.1 and 4.7, where the presence of damsel bugs did not reduce mirid numbers in the field cages. Thus damsel bugs appear to be a minor predator of mirids, and may preferentially attack other known prey species such as lepidopteran larvae and eggs (Siddique & Chapman 1987; Wade et al. 2003). Nevertheless a damsel bug did attack and consume a mirid in Exper. 4.6, and so can have an impact of mirid density, but the extent of this impact seems to be low.

TheExper. 4.5 confirmed that lynx spiders are top predators in this food web, while damsel bugs are secondary predators. When lynx spiders have a 7% size advantage they readily attack damsel bugs; when both animals were the same size there was no predation; but when damsel bugs had a 5% size advantage they did not readily attack lynx spiders. Damsel bugs are also considered a prey of the striped lynx spider, *Oxyopes salticus*, in America (Young & Lockley 1985), and it seems that the Australian counterparts follow suit.

Nevertheless, there was no evidence that lynx spiders increased damsel bug mortality in the All treatment of the food web experiment. Although lynx spiders could attack damsel bugs, they did not do so to any significant degree in Exper. 4.7.

The 'lynx spider/damsel bug/mirid' food web, in terms of mirid number, seems to be a case where one predator causes significant mortality of the prey but the other predator has no significant impact, either on the prey or the other predator("trivial multiple predator effect", Sih et al. 1998). Such interactions are very common (eg Dinter 2002; Denno et al. 2004; Vance-Chalcraft & Soluk 2005) and indicate that the strength of the connections within a food web can have more of an effect on community dynamics than web connectivity (Vance-Chalcraft & Soluk 2005).

Although in the confined cages damsel bugs disrupted the effect of lynx spiders to control mirids, there were some positive results from the field experiments (Exper. 4.8) which showed that increasing the local abundance of lynx spiders date to maturity could be reduced. This result is very promising, as the treatment was exposed to other natural predators and pests, and it indicated that increasing a range of predators was beneficial. Unfortunately, the plot experiment (Exper. 4.8) was inconclusive: we were not able to supplement the plots with the predator (and mirid) numbers necessary to see a sustained change in the communities in the plots, the crop matured more quickly anyway, and the effect of mites overrode any effect of mirids. This experiment, though, did emphasize that the effect of mites overrides any effect of mirids.

The role of Behaviour

While density mediated interactions suggest that the 'lynx spider/damsel bug/mirid' food web is an example of "rivial multiple predator effects", it seems that this is not the case for the behaviourally mediated interactions in the four trophic level 'lynx spider/damsel bug/mirid/cotton' food web. In Exper 4.7, the All treatment containing lynx spiders, damsel bugs and mirids had significantly less mirids than the Mirids only treatment; and yet there was no significant difference in damage to the cotton bolls between the two treatments. In addition, the Mirids+Lynx treatment had the same number of mirids as the All treatment, but suffered significantly less damage to bolls.

The fact that the same mirid densities produced different amounts of boll damage has important ramifications to the management of mirids in cotton. It indicates that you can manage the pest without managing the damage. Often the effectiveness of a biological control method is measured by the extent to which it reduces the target pest population (Grundy 2004). Here the mirid numbers have been reduced, but the damage has not, indicating that management of this pest has not been achieved. To assist with the control of this pest it is important to understand how this pattern can occur.

As damsel bugs are not exerting an effect on prey abundance, the effect must be behavioural. Presumably the presence of damsel bugs has altered the mirids' behaviour so that they were more likely to attack bolls. However there is no evidence to support this in Exper. 4.6. Mirids responded to increasing predator densities, irrespective as to whether the predators were lynx spiders or damsel bugs. For example, mirids were more likely to "fidget" and less likely to stand still or feed in any treatments with predators; and the number of mirids at the top of the plant increased with predator density. Bolls are more common in the middle and bottom section of the plant, so the increase in predator pressure causing mirids to move to the top of the plant should have reduced boll damage.

Although mirids did not appear to behave differently in the presence of damsel bugs, lynx spiders did. In the behavioural experiments, damsel bugs were significantly more common at the top of the plant than elsewhere on the plant. This is a well known characteristic of damsel bugs (Wade et al. 2003). However, at the same predator density, there were more lynx spiders at the top of the plant in groups of lynx spiders and damsel bugs, than in groups of lynx spiders only.

The presence of lynx spiders at the top of the plant in the presence of damsel bugs could indicate why mirids in the All treatment did more damage than the same number in the Mirids+Lynx treatment. The presence of damsel bugs in the All treatment may have drawn lynx spiders to the top of the plant, leaving the bolls, which occur lower down on the plant, more exposed to mirid attack. Thus the same number of mirids in treatments with and without damsel bugs could do more damage to bolls in the treatment with damsel bugs because there were less lynx spiders near the bolls to suppress the mirids feeding.

However, while the results indicate that in mixed predator groups of lynx spiders and damsel bugs the predators would be concentrated at the top of the plant, more work is needed to confirm that dropping the densities of lynx spiders near the bolls in the middle of the plant would provided less feeding suppression by mirids. Fig.4.6 indicates that across the whole plant, feeding suppression occurs at all predator densities, so it is not clear that less lynx spiders in the middle of the plant would mean less feeding suppression.

Nevertheless these results suggest that lynx spiders are capable of controlling mirid damage by reducing mirid numbers and reducing mirid foraging.

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Outcomes

3. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

All the following outcomes were achieved, except the third one: identifying the most effective management techniques. Nevertheless, although we did not manipulate mirid management techniques on farms, we did identify the most effective techniques that were used by the growers on farms in our survey. These were: to only spray mirids once they were over threshold, and if mirids were to be controlled with Regent, to use no less than 40mls/ha.

In respect to the last outcome, "greater understanding of the effect of Bt on mites", the experiments and analysis have been achieved, but the paper is still in prep. As this work isn't directly related to this project and mirid predation, it was not presented in the previous section. The mite work has been presented as a talk I gave at the ASSAB conference in 2008. More information on the mite work is available on request.

Expected Science Outcomes	Expected Industry/Applied Outcomes: all achieved
Greater understanding of mirid predation	Establishing which predators can reduce mirid damage in cotton crops
Information on spray patterns	By knowing what triggers growers to spray for mirids, we can better tailor new mirid management techniques to the growers
Information on management techniques and mirid numbers	By identifying the most effective management techniques for controlling mirids using the beneficial community, we can help growers control this pest without resorting to hard insecticides
Insights into community ecology in general and foraging webs in particular.	By understanding the interactions between mirids and their potential prey items and competitors we will be better able to predict when mirids will cause damage.
Insights into nutrient requirements of male and female mirids	Identifying differences in the foraging behaviour of male and female mirids will improve predictions on when mirids will cause damage in cotton

Expected Science Outcomes (NB: A direct science outcome might not be applicable for all extension outputs.)	**Expected Industry/ Applied Outcomes** *Eg These growers gain knowledge and change practices in pesticide application.*
Greater understanding of predator/ prey interactions and the function of food webs.	Identification of predators and prey which can help control mirids in cotton.
Greater understanding about prey switching between hosts and the effect of relative prey and predator size on mirid predation.	Identification of predators which can help control mirids, aphids and *Helicoverpa* eggs in cotton.
Greater understanding on the dynamics controlling mirid sprays.	Information on the insecticides used, and whether different spray regimes are correlated with different yields.
Greater understanding of how Bt cotton influences the non-target arthropod community.	Greater understanding of the effect of Bt on mites.

4. Please describe any:-
    a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
    b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
    c) required changes to the Intellectual Property register.

N/A

Conclusion

5. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

This work has provided the industry with a number of positive findings with respect to mirid management in cotton. For example, the work has shown that growers can be frugal with their insecticide applications for mirid control without compromising yield.

However, it also showed that pest managers were less likely to follow the recommended thresholds when they used the beatsheet method to sample for mirids. This revealed a problem that occurs with any group of people from different disciplines working together; and it is one of the biggest challenges to maintaining and developing IPM: people from different disciplines are likely to interpret the same results differently because they differ in what they see as background and foreground information. The only way to overcome this problem is to keep asking the different stakeholders questions that probe their interpretation of results to ensure that everyone is aware of each other's perspective.

The results of this work also show that the presence of alternative food, in the form of prey such as *Helicoverpa* eggs, may reduce the impact of mirids on cotton crops.

A third finding of the work is that there are predators capable of reducing mirid numbers and mirid damage in cotton. In particular, the plain brown lynx spider (*Oxyopes molaris*) is a veracious mirid predator, especially once it is large. More work is needed to ascertain how to quantify their effectiveness in field crops. In addition, work is needed to ensure that these animals are abundant in crops during the critical period for mirids.

The final finding of the work is that other secondary pests, such as mites and aphids which may be flared by mirids sprays, cause more damage to crops than the mirids. Any effect of mirids was overridden in these experiments by the effect of mites and aphids.

The results of the mirid predation work has vindicated the mirid thresholds and identified weaknesses in compliance with mirid thresholds. It has identified key mirid predators and field conditions that could reduce the likelihood of mirid damage. It holds a lot of promise for the management of mirids and further development of IPM. Central to this development is the further integration of mirid control with the management of other pests and crop agronomy. The results indicate that it may be possible to avoid the use of insecticides for mirid control partly by managing the crop ecosystem to ensure large number of predators and alternative food for mirids are available at times when the crop is most vunerable to mirid damage. Achieving this goal would support the cotton industries BMP endevours.

Extension Opportunities

6. Detail a plan for the activities or other steps that may be taken:
    (a) to further develop or to exploit the project technology.
    (b) for the future presentation and dissemination of the project outcomes.
    (c) for future research.

The project has revealed a number of factors that should be followed up. For example, the project has highlighted an underlying problem of interdisciplinary work, which is that results are interpreted differently by individuals from different philosophies. It highlights the need to recognize when this is happening, and that it is important to ask questions about the interpretation of results to make sure that the different parties can see how each other are interpreting the "facts".

To encourage adherence to the recommended mirid thresholds, the industry needs to actively promote beatsheets as reliable so that pest managers increase their confidence in the higher beatsheet thresholds. Opportunities for extension work of this include demonstrations at farm walks which show that beatsheets reveal 3 times the number of mirids than visual searches.

Alternatively, some pest managers may be better off just using visual searches to sample for mirids, and they should be encouraged to do so. Very few people used sweepnets to sample for mirids in this survey. This method also uses the same threshold as that of beatsheets (three times that of visual surveys), so it may encounter resistance. If the industry wants to promote sweep nets as a method to measure mirids is would need to gauge if pest managers are prepared to use the higher threshold with sweep nets.

The project has also highlighted the need to integrate the management of *Helicoverpa* eggs, aphids and mites with that of mirids. For example, the presence of *Helicoverpa* eggs could delay the need to spray for mirids. Other potential food items of mirids, such as thrips, should be tested to see if they have a similar effect on mirid damage. The damage caused by mites and aphids overrode the effect of mirids. This information needs to be extended to the pest managers so that they don't manage mirids at the expense of mite control.

The project also showed the important role that predators, and in particular lynx spiders, play in controlling mirids. This needs further development. Spiders behave differently to insects in that they cannot respond quickly to outbreaks (like ladybirds do to outbreaks of aphids). Large spiders need to be grown up in the crops. More work is needed to understand how to most effectively enhance the spider community in cotton crops, and in particular ensure that there are good-sized spiders at critical times. We also need to check in field studies that lynx spiders are as effective in the field as they are in the laboratory.

A concerning finding from the project was the impression that regent rates lower than 40mls/ha were more likely to result in a respray for mirids. This needs to be tested formally. We need to know the effect of low rate regent on the beneficial community, and whether it is better (from an IPM perspective) to spray twice with a low rate, or once with a higher rate.

Publications

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

(NB: Where possible, please provide a copy of any publication/s)

Publications

Whitehouse M.E.A., Mansfield S., Hagler J., Nobilo J., Harden S. IN PRESS. The use of ELISA techniques to clarify predator/prey interactions in cotton. Proceedings of the **3rd International Symposium on Biological Control of Arthropods**, Christchurch, New Zealand (Feb 2009).

Whitehouse M.E.A., Wilson L.J., Fitt G.P., Constable G.A. IN PRESS. Integrated Pest Management and the effects of transgenic cotton on insect communities in Australia: lessons from the past and future directions. **Proceedings of the 3rd International Symposium on Biological Control of Arthropods**, Christchurch, New Zealand (Feb 2009).

Mansfield S., Hagler J. R. & Whitehouse M. E. A.2008 A comparative study of the efficiency of a pest-specific and prey-marking ELISA for detection of predation. **Entomologia Experimentalis et Applicata**.127:199-206.

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Whitehouse M.E.A.2008 Spraying for mirids and benchmarking the triggers: what's working and what needs more development. **Proceedings of the 14th Australian Cotton Conference.**

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Whitehouse M.E.A. 2006. Spraying for mirids – and benchmarking the triggers. **The Australian Cottongrower**, 27 (4): 17-19.

Whitehouse M.E.A. 2006. Benchmarking what triggers mirid sprays in cotton – the results from the pilot study. **The proceedings of the 13th Australian Cotton Conference.** pp 533-536.

Whitehouse M.E.A. and Barnett M. 2006. Mirid predation. **2006 Lower Namoi Field Day Book**. 49-50.

Reports

Whitehouse M.E.A. & T. Farrell. 2007. Mirid Management Benchmarking **Cotton Tales Gwydir Valley.**

Whitehouse M.E.A. & T. Farrell. 2007. Mirid Management Benchmarking **Cotton Tales Namoi Valley.**

Whitehouse M.E.A. 2006 The mirid spray survey. **In Field Yarns** Feb 2006.

Whitehouse M.E.A. 2006. Mirid predation. **Cotton Tales Namoi Valley** Jan 2006

Whitehouse M.E.A. 2005. Mirid predation. **Cotton Tales Gwirdyr Valley** Dec 2005.

Manuscripts submitted

Whitehouse M.E.A., Barnett M., Mansfield S. & Broughton K. Intraguild interactions and predator effects: the potential role of Lynx spiders and damsel bugs in the control of green mirid damage in cotton. In review with: **Ecologial Entomology**

Whitehouse M.E.A. The IPM of mirids in Australian cotton: understanding why and when pest managers spray for mirids. In internal CSIRO review, for submission to **Agricultural Systems**.

Manuscripts in prep

Whitehouse M. E. A. Nobilo J. The potential for mirid predators and alternative mirid food sources to reduce mirid damage in cotton. Target journal: **Biological Control**.

Whitehouse M.E.A., Manfield S., Nobilo J., Hagler J. Identifying predators of mirids, aphids and *Helicoverpa* eggs in cotton using ELISA techniques. Target Journal: **Entomologia Experimentalis et Applicata**.

Whitehouse M.E.A., Wilson L.S., Downes S., Cave L., Norman P. Does Transgenic Bt-cotton affect Spider Mite abundance? Target Journal: **Environmental Entomology**

Presentations at Conferences

2008. **14th Australian Cotton Conference** (Australia). Organising and presenting the hands-on workshop titled "Emerging insect pests.". Co-presenters: M. Khan, R. Sequiera, S. Maas, G. Rudd.

2007 **Australasian Society for the study of Animal Behaviour** (Australia). Does Transgenic Bt-cotton affect Spider Mite abundance? (Talk).

2007 **XVII International Congress of Arachnology** (Brasil). Spiders in Australian Cotton: Community structure and pest control in the transgenic era. (Talk).

2007 **Australasian Society for the study of Animal Behaviour** (Australia). Mirid foraging behaviour: understanding fickle mirid damage in cotton.(Talk).

2006. **11th Congress of the International Society for Behavioural Ecology** (France) Intraguild interactions and predator effects: the potential role of Lynx spiders and damsel bugs in the control of green mirid damage in cotton.(Poster, M. Barnett co-author).

2006. **Australasian Society for the study of Animal Behaviour's 2006 Conference** (Australia). Intraguild interactions and predator effects: the potential role of Lynx spiders and damsel bugs in the control of green mirid damage in cotton. (Talk, M. Barnett co-author).

2006. **13th Australian Cotton Conference** (Australia). Organising and presenting the hands-on workshop titled "Green mirids and green vegy bugs: sucking your profits all the way to the gin.". Co-presenters: R. Mensah, M. Khan, S. Deutscher, D. Murray

Lectures

2008 10 April **University of Sydney**. Lecture on Evidence of a latitudinal gradient in spider diversity in Australian cotton

2008 14 July **University of Technology**, Sydney . Lecture on Spiders in Australian cotton: community structure and pest control in the transgenic era.

2004, 2005, 2007, 2008 **CRC Cotton Production Course Residential Schools IPM short course** Guest lecturer (University of New England, Australia)

Presentations at industry and community gatherings

2008 27 Nov. **CRDC Final Report Presentation.** Talk titled: mirid predation: contributing to Integrated Pest Management in Cotton.

2008 13 Nov. **Division of Entomology CSIRO seminar.** Talked titled: The IPM of mirids in Australian transgenic cotton: understanding why & when pest managers spray for mirids.

2008 16 Oct.r **Cotton Catchment Communities CRC review.** Talk entitled: "Mirids: a challenge to Integrated Pest Management in cotton"

2008 25 June **Northern farming systems IPM researchers forum.** Talk entitled: Mirid management with predators and the results of the mirid spray survey.

2008 13 May **AGM of Cotton Consultants Association** (Narrabri). Talked entitled: Mirid management with predators and the results of the mirid spray survey

2008 15 April **Emerald East Area Wide meeting** (Emerald) Susan Maas organizer.Talked on mirids and gave an update of the mirid spray survey.

2008 2 April. **Upper Namoi Cotton Field Day** (Boggabri) Andrew Watson organizer. Talked on mirids and gave an update of the mirid spray survey.

2008 12 Mar.: **Lower Balonne Cotton Field Day** (St George) Dallas King organizer. Talked on mirids and gave an update of the mirid spray survey.

2007 6 Aug. **Deparamento de Agronomia-Entomologia, Universidade Federal Rural de Pernambuco; Recife, Brazil**. Talk titled:Spiders in Australian cotton: community structure and pest control in the transgenic era.

2007 24 July **Northern Farming systems IPM Forum** Mirid predators, prey and insecticide regime

2006 8 Dec. **Namoi Cotton Consultants Association** Talked on mirids

2006 15 Nov **Cotton Growers Association at Bullarah** Talked on mirids

2006 2 Nov **Cotton Consultants Association at Warren** Talked on mirids

2006 26 Oct **Cotton Growers Association at Moree** Talked on mirids

2006 24 Oct **Cotton Growers Association at Brookstead** Talked on mirids

2006 17 Oct **Cotton Growers Association at Goolgorie** Talked on mirids

2006 Sept. **Cotton Growers Association at Emerald** Talked on mirids

2006 10-12 Sept. **Silverleaf Whitefly Tour** Talked on mirid management in Toowoomba, Dalby, Goondiwindi, and St George.

2006 31 July **Division of Entomology CSIRO Montpelier** (France) Talk titled: From landscape ecology to community ecology: what spiders, mirids and cotton can tell us about diversity gradients and food webs.

2006 20 June **Silverleaf Whitefly & sucking Pest Management workshop** (Toowoomba). Talked on mirid management.

2006 24-25 May **Cotton Trade Show**. Presented current understanding of the effect of mirids as pests

2006 17 May **Lower Namoi Cotton Growers Association at Narrabri.** Talked on mirid survey results.

2006 16 May **Cotton Consultants Association at Narrabri.** Talked on mirid survey results.

2006 9 May **Farrer Memorial Agricultural High School for boys** (Year 11 & 12 Ag students). Talked on: IPM and the role of beneficials, with special reference to mirid management

2006 8 May **Calrossy Anglican School for Girls** Talked on 'IPM and the role of beneficials, with special reference to mirid management' Also talked about work/life balances.

2006 4 May **Division of Entomology CSIRO Canberra.** Talked titled: From landscape ecology to community ecology: what spiders, mirids and cotton can tell us about diversity gradients and food webs.

2006 May **Country Women's Association** meeting. Talk titled: IPM in a Bt landscape

2006 5 April **Postgraduate tour "Cotton – your industry, your future?**" Talked on mirid predation.

2006 2 March **Lower Namoi Cotton Growers Association Annual Field Day** Talked about Mirid predation

2005 13 Oct **Cotton Consultants Association** first meeting of the season: Talked on setting up a survey of mirid spray decisions.

2005 12 Oct **DPI Entomologists** (In Narrabri)Talked on spider communities in cotton, combining practical and theoretical approaches.

2005 21 Sept **Lower Namoi Cotton Growers Association Meeting.** Talked on setting up a survey of mirid spray decisions.

2005 Aug **Australian Cotton Cooperative Research Centre** Final Science Review. Predators of *Helicoverpa*, aphids and mirids.

2005 July **Northern Farming systems IPM Forum** Predators of Mirids, Aphids and *Helicoverpa* eggs

2005 May Short meeting with Senator Colbeck (Parliamentary secretary to the federal agricultural minister), the senior advisor to the agricultural minister, a team of senior executives from the **Dept. of Agriculture Fisheries and Forestry**, and NSWDP. Talked on: emerging pests and CRC collaborations

2005 May **Calrossy Anglican School for Girls** Talked on IPM, beneficials and Bt

2005 April **Biotech field day.** I had a stand on spiders and other beneficials in Bt cotton.

2005 April **ABC radio.**Interview: discussed spiders and other beneficials in Bt cotton.

2005 Feb **Contrasting cotton and sugar: Bundaberg Women in sugar visit.** Talked on: Beneficials in cotton

2004 Dec **Narromine farmwalk – IPM.** Talked on: beneficials and pests in cotton

B. Have you developed any online resources and what is the website address?

Yes, with the help of David Larsen I have set up display of mirids fighting on the web: http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/SuckingPestPublications/Mirid_Predation_by_Lynx_Spiders.aspx

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

With the advent of Bt cotton, mirids have become more of a pest in cotton and are attracting a number of insecticide applications. This has the potential to disrupt IPM in cotton and increase the risk of mite, aphid or whitefly outbreaks.

The aim of this project was to identify factors which could influence mirid damage to cotton. In particular, the project looked at current mirid management methods, testd the effectiveness of potential predators and identified other interspecific interactions which could reduce mirid damage to cotton.

We found that pest managers that only sprayed for mirids once the numbers had exceeded the recommended threshold suffered no yield loss, and if anything it was beneficial to the profitability of the field. Nevertheless, we did find that pest managers were less likely to use the beatsheet threshold than the visual survey threshold, indicating that more extension work is needed in this area.

The project was able to identify a number of predators that could reduce mirid numbers and affect mirid feeding behaviour. In particular the plain brown lynx spider, which is very common in cotton, was a very efficient predator of mirids. More work is needed to confirm their effectiveness under field conditions.

The project also showed that mirids may not attack cotton if alternative foods, such as *Helicoverpa* eggs, are available. Thus a *Helicoverpa* egg lay in a Bt crop could be advantageous if the field has a heavy mirid infestation because it could reduce the likelihood that the mirids present will attack the cotton. This finding needs to be confirmed under field conditions.

The reproductive status of the mirid had little effect on mirid damage, but damage caused by mites and aphids overrode any damage caused by mirids. This indicates that if pest managers have to choose between mirid and mite control, they should be more concerned about controlling the mites.

The results of this project indicate that insecticide applications to control mirids can be kept to a minimum; 54% of the sprays in the mirid survey were applied to mirid numbers below threshold. If these sprays were irradiated, there would be a huge saving in insecticides, no cost in yield, and a large advantage in terms of the development of IPM in cotton. The results of this project indicate that this should be the goal of mirid management in cotton.