Cotton Catchment Communities CRC

SUMMER SCHOLARSHIP REPORT - 2007/08 SEASON

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Diurnal Movement of Mirids in Response to Lynx Spiders

Abstract

With the introduction of Bt transgenic cotton to control Lepidoptera pests, there has been a decrease in insecticide applications. This has resulted in an increase in the numbers of secondary pests such as the Green mirid (*Creontiades dilutus*).

The aim of this project was to identify the times that mirids are most active, as well as to examine mird behaviour in response to chemical cues and the presence of their predators, Lynx spiders and damsel bugs. It was found that mirids are nocturnal, with peak activity at dawn. Mirids did not respond to chemical cues in the nocturnal experiment, however it was found that mirids do respond to the presence of predators through altering their location on the cotton plant.

These results have important implications for the improvement of Integrated Pest Management Programmes. Predators do not need to kill pests to reduce damage. Encouraging the presence of predators may reduce the need to spray to control mirid damage.

Introduction

Insects can cause major damage to cotton plants, seriously affecting yield. Integrated Pest Management (IPM) strategies are being implemented within the cotton industry in order to reduce insecticide use. In the past, *Helicoverpa* spp. were the main pests in cotton and insecticides were used to control them. Since the introduction of *Bt* cotton (a genetically modified (GM) variety that expresses a toxin within the plant tissue for the control of Lepidoptera) insecticide use has been reduced by 80% (Fitt et al In Press). However with the reduction in insecticide use, there has been an increase in damage caused by secondary pests. Green mirids (*Creontiades dilutus*) are one of the more significant secondary pests in GM cotton crops because they attack cotton squares and bolls.

A strategic aim of the CCC CRC Farm Program is to improve profitability and sustainability of cotton production by halving pesticide use through Integrated Pest Management for insects. However, currently about half of the insecticide sprays applied for mirids are below the recommended threshold (Whitehouse 2007), indicating that many of these applications may not be necessary. One reason for a lack of confidence in the threshold is that damage from mirids is not always well correlated with mirid density. To reduce the number of unnecessary insecticides targeting mirids, we need to better understand conditions under which mirids cause damage to cotton.

Mirids go through five nymphal instars before reaching the adult stage. It is known that feeding by fourth instar and adult mirids causes the most damage to cotton (Khan *et al* 2006). Mirids pierce the plant tissue with their stylet and release a pectinase which destroys the plant cells, causing death of the affected tissue (Pyke & Brown, 1996). Cotton plants are most vulnerable to mirid damage for about five 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).

In a previous Summer Scholarship (Barnett 2006) it was shown that lynx spiders (*Oxyopidae molarius*) on their own reduce both mirid numbers and mirid damage to cotton bolls, whereas lynx spiders combined with damsel bugs (*Nabis kinbergii*) reduce mirid numbers but cause no significant reduction in mirid damage. It was suspected that the presence of lynx spiders might change mirid behaviour, while damsel bugs may disrupt the interaction between mirids and lynx spiders. In order to understand the interactions between these animals we need to understand more about mirid behaviour; in particular, when mirids are most active, whether they use chemical cues, and how mirids behaviourally respond to the presence of lynx spider and mirid predators.

The aim of this research project was to investigate why lynx spiders could suppress mirid foraging, while the presence of damsel bugs removed the suppression. The first step was to find out when mirids were most active, so that experiments could be conducted when mirids were most likely to respond. To do this we used time budgets to identify the peak activity periods of mirids. The second step was to see if observations using light would disrupt the mirid’s behaviour. This was important if we were to successfully observe the mirids at night. The third step was to see if mirids were using chemical cues left on substrate to recognise that a predator in near. Chemical cues left by damsel bugs could potentially mask those left by lynx spiders if mirids are using this information. Finally, the fourth step was to see how mirids responded behaviourally to the presence of lynx spiders and damsel bugs. These results have been combined with those of Mark Barnett and written up as a scientific paper for Ecological Entomology (see attached manuscript) which is currently in review.

Methods

Insect cultures and host plants

The damsel bugs, lynx spiders, and mirids used in this study 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). *Nabis kinbergii* is the only damsel bug in Australian cotton (Whitehouse *et al* 2005). All lynx spiders and damsel bugs in the experiments 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 variety used in these experiments was Bollgard® Sicot 43B®.The plants used in this study had bolls in the middle and lower parts of the canopy, and squares at the top. Unless otherwise stated, we used adult mirids or 4-5th instar mirids in these experiments.

Experiment 1: Time Budgets

Mirid behaviour was recorded using four infra-red night vision cameras (NESS 100-466 Digital b/w camera, low light Ultra IR OSD) in a reverse-cycle Day/Night Room which was kept at about 28°C and 50% RH. The footage was recorded on a PowerPlex ℯDR400, 4 Channel Digital Video Server for later viewing. A circular section of cotton leaf (50mm diameter) and the centre of a cotton square were set up in a plastic Petri dish containing agar to keep the plant material fresh. The Petri dish was placed in a separate larger container surrounded by water to prevent the mirids from escaping. Four identical observation arenas were set up simultaneously, one under each camera and one fourth instar mirid was added to each Petri dish (Fig. 1). The cameras recorded the mirids' behaviour for 24 hours. This experiment was repeated four times (n = 16 mirids).

Fig. 1. The infa-red cameras and video apparatus in use in Experiment 1.

Experiment 2: Chemical Cues

Individual mirids were tested in Petri dishes which had previously contained either a lynx spider, damsel bug, another mirid, or nothing. In the Barnett summer scholarship work, a diurnal version of this experiment was conducted. In this Summer Scholarship, we tested the mirids during their night.

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 the same camera and video equipment used in Experiment 1. 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 categorised 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).

Experiment 3: The effect of the presence of predators on mirid behaviour

Experiments were carried out just after dusk when the mirids were most active (see results of experiment 1). Animals were located on the plant by carefully searching for them using a red light. To make sure that this would not disrupt the mirids we did a preliminary experiment to test the effect of light on the mirids.

3.1 The effect of lights on mirids

The aim of this experiment was to test if shining white or red light from a HDL33A *Energizer* LED Headbeam on mirids in the dark disturbed the mirids (white light: 200 lux, red light approximately 20 lux). This experiment was performed in the reverse-cycle Day/Night Room and carried out shortly after dark using the four night vision cameras used in Experiment 1. Eight Petri dishes were set up under the four cameras (two petri dishes/camera). The Petri dishes were placed in a larger container of water to prevent mirids escaping. One 4th instar mirid was placed on each Petri dish. The mirids were allowed 5 minutes to settle. Either a red or white light was shone on 4 of the mirids for 15 seconds. After 2 minutes the alternative light was shone on the other 4 mirids. This was repeated every 4 minutes until the mirids had been exposed to the same light four times before changing the colour of the light and repeating another four times. Thus each mirid experienced one light colour once every 4 minutes 4 times, before the light colour was changed and the pattern repeated. Behaviour was recorded 15 seconds before the light, 15 seconds during the light and 15 seconds after the light. This experiment was repeated 3 times (therefore testing a total of 24 mirids).

For the first replicate, the plastic Petri dish contained agar supporting part of a cotton leaf (a 50 mm diameter circle) and the centre of a cotton square. For replicates 2 and 3 the Petri dishes were made of glass and had been autoclaved. They contained filter paper only to make it easier to observe the mirids behaviour through the night vision cameras.

3.2 Predators on plants with mirids

This experiment was set up in the reverse-cycle Day/Night Room (28°C and 50% RH) and ran for two hours after dark. 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 with 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 randomly 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).

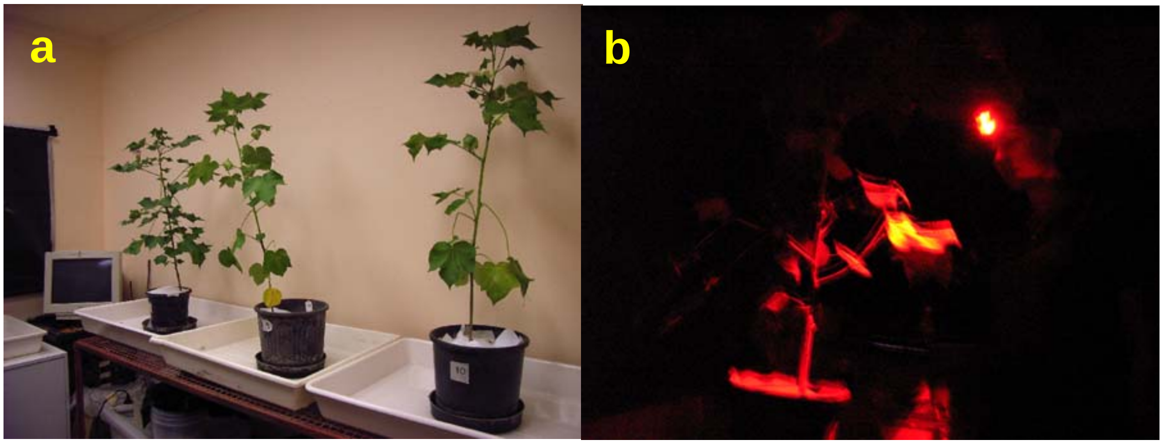

Fig. 2. a: Three of the five plants set up for Experiment 3.2. **b**: KB searching for mirids and predators during the experiment

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 its location on the plant (at the top, middle, or bottom of the plant, calculated by dividing the height of the plant evenly into 3 sections), position on the plant (on the stem, leaf or a fruit), and its behaviour (standing still or feeding, walking, fidgeting or grooming) was noted. 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 used a red light to carefully search for the animals on the plants. 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.

Results

Experiment 1: Time Budgets

In this experiment, the mirids either stood still, fidgeted, ran or walked. No animals were observed feeding. On many occasions it was impossible to locate the mirids. Therefore, to analyse the results, mirid behaviour was classified as “standing still”, or “active” (which included fidgeting, walking or running). The 24 hour day was divided into 8 3-hour time block (therefore each time block consisted of 10800 seconds) We then tailed up the number of seconds the mirids were seen either active or standing still during a time block. The number of seconds the mirid was visible during the time block was the co-variate. Two mirids were not recorded properly by the camera and were excluded from the analysis.

There was a significant difference in the amount of time mirids were active between the 8 time blocks (ANOVA, df=7,54; F=7; P<0.001), with most activity occurring at dawn, or during the night (Fig. 3).

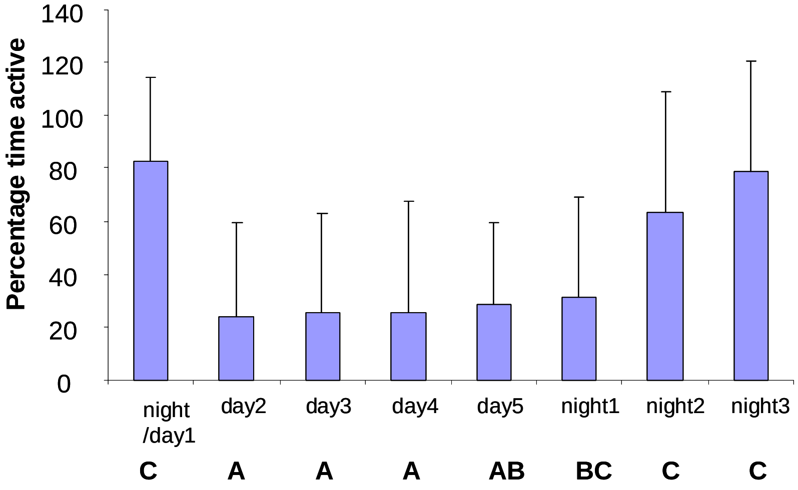

Fig. 3: The proportion of time when mirids were active. Mirids became more active during the night, with most activity occurring with dawn. The same letter under the graph indicates time blocks which show the same level of activity. The statistical analysis was conducted on actual time spent being active, not the proportions.

Experiment 2: Chemical Cues

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; Fig. 4).

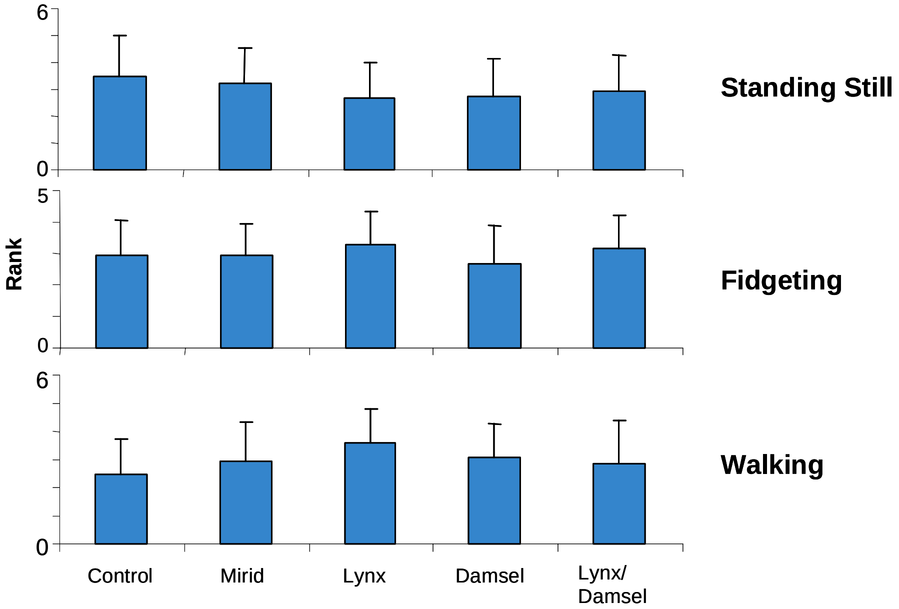

Fig. 4. The rank totals of mirids walking, fidgeting or standing still in Petri dishes exposed to either lynx spiders, damsel bugs or nothing (control). All 10 mirids tested were exposed to each type of Petri dish. The same letter above the histogram indicates responses not significantly different from each other for that behaviour.

Experiment 3: The effect of the presence of predators on mirid behaviour

3.1 The effect of lights on mirids

The presence of lights did not appear to disturb the mirids. The most frequently observed behaviour was standing still (average duration was > 13s of a 15s observation) across all treatments. Exposure to red or white light did not affect the duration of this behaviour significantly (F = 0.96, df = 2, 570, P = 0.38, Fig. 5) either when the light was on or after it was turned off compared with before the light was turned on (F = 2.28, df = 1, 570, P = 0.13). All other mirid behaviours (fidget, walk, run) occurred too infrequently to analyse (average duration < 1.5s). As we found no evidence of shining a torch influencing mirid behaviour, we were comfortable using this technique below.

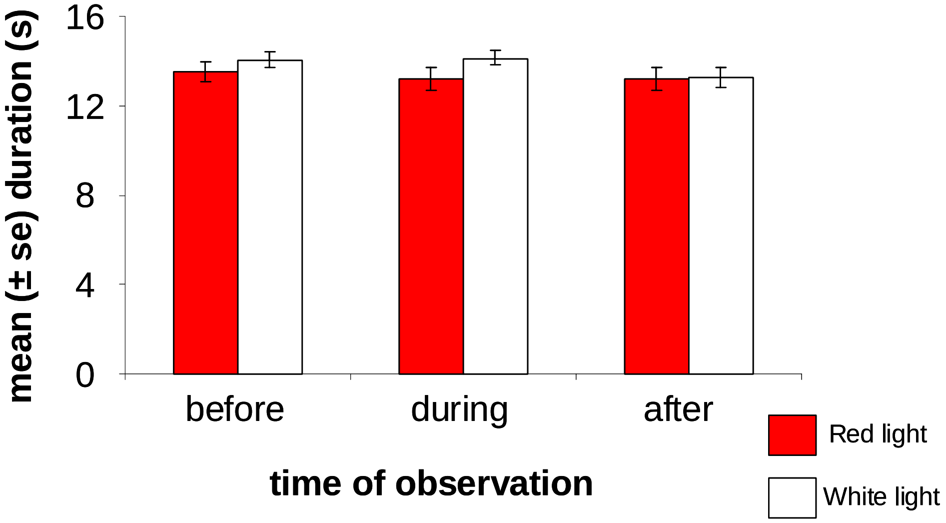

Fig. 5. Time spent Standing by mirids before, during and after shining red or white light.

3.2 Predators on plants with mirids

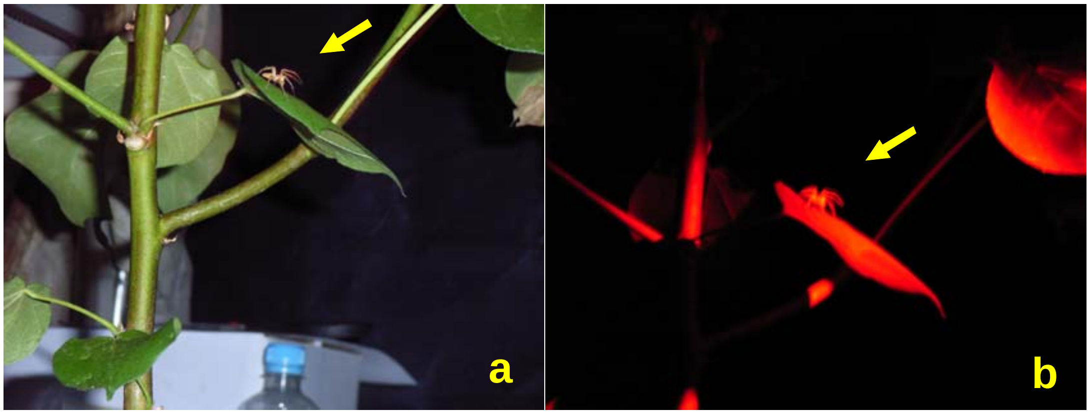

Fig. 6. A lynx spider (arrowed) in a predatory pose on a leaf under normal (a) and red (b) light.

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 (Fig. 6) while two were caught on stems.

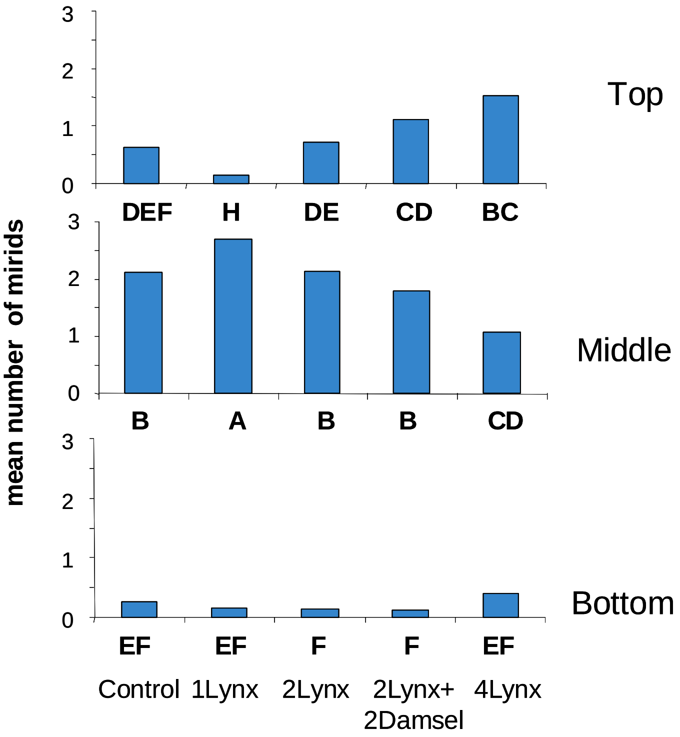

Fig. 7. The effect of predator density on the number of mirids at the top, middle or bottom of the cotton plants (with total mirids on the plant as a covariate). The same letter below the histogram indicates responses not significantly different from each other (l.s.d.=0.44). Mirids moved away from the central section of the plant with increasing predator pressure.

Mirids moved away from the middle of the plant and towards the top of the plant as predator pressure increased (Fig. 7. treatment x location on plant: F= 13.2, df =8, 321, P=0.001, covariate = total number of mirids found on a plant); and they were less likely to stand on stems and more likely to stand on leaves as predator pressure increased (Fig. 8; treatment x substrate on plant: F= 3.13, df=8, 340, P=0.002 covariate = total number of mirids found on a plant). 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. 9. treatment x activity: F= 5.37, df=8, 321, P<0.001, covariate = total number of mirids found on a plant).

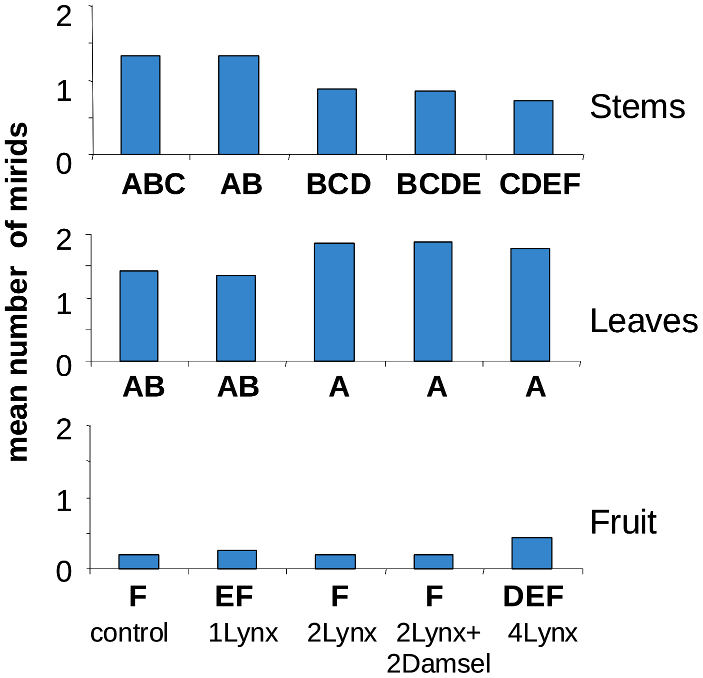

Fig. 8. The effect of predator density on the number of mirids standing on stems, leaves, or fruit (with total mirids on the plant as a covariate, l.s.d.=0.45). With higher predator pressure there were more mirids standing on leaves.

The behaviour of the predators also varied significantly between treatments (Fig. 10). Damsel bugs were more likely to be found on leaves (74% of sightings) than stems or fruit (26%; stems and fruit were lumped together because of sparse data on fruit). Lynx spiders at high densities (4 lynx spiders/plant) were more likely to be found on stems (57%) while at densities of 2 lynx spiders/ plant they were more likely to be found on leaves (69%) (ANOVA: df =4, 173, treatment x substrate: F= 5.12, P<0.001). When hunting, lynx spiders often adopted a predatory stance (Fig. 6), 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, please go to: http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/SuckingPestPublications/Mirid_Predation_by_Lynx_Spiders.aspx

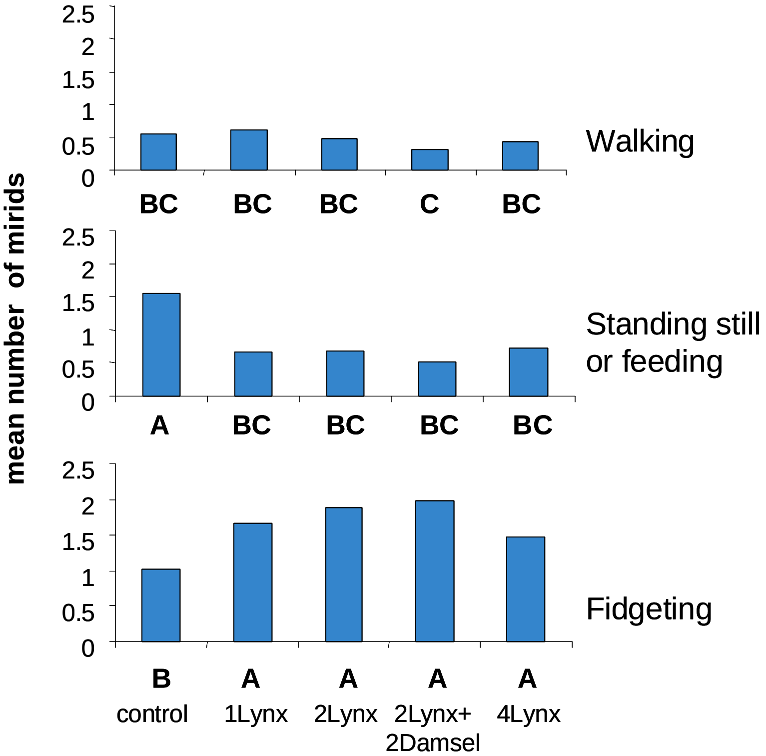

Fig. 9. The effect of predator density on the number of mirids walking, standing still or feeding, or fidgeting (with total mirids on the plant as a covariate, l.s.d.=0.48). In the presence of any predator mirids were less likely to stand still or feed.

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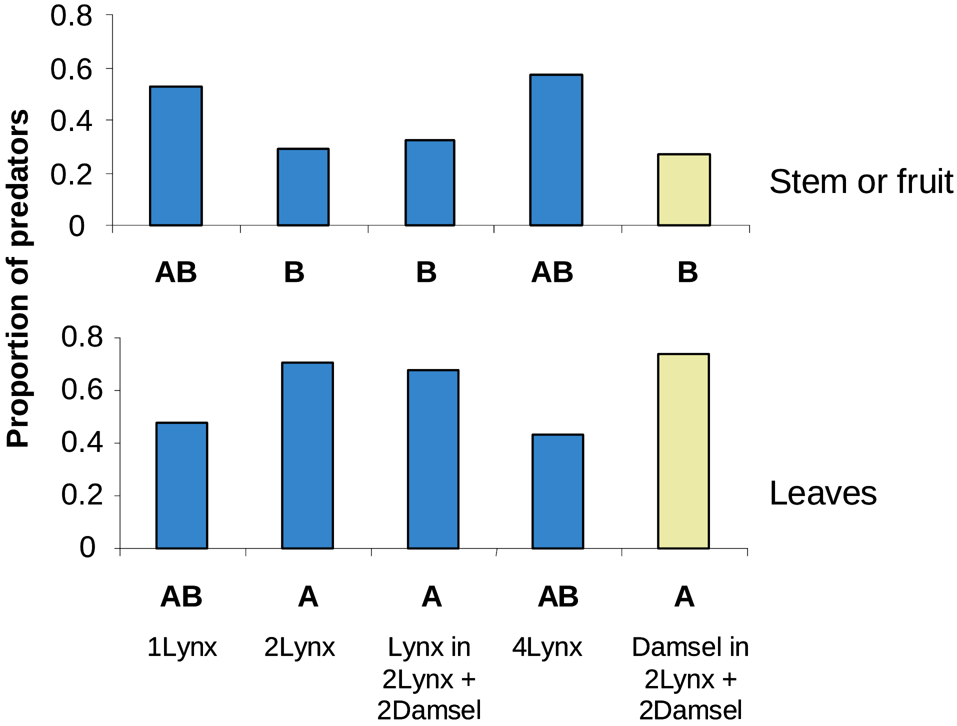

Fig. 10. The effect of predator density on the proportion of predators standing on stem and fruit, or leaves. Damsel bugs were more likely to stand on the leaves (LSD(1%)=0.33).

More damsel bugs were found at the top of the plant (Fig. 11), while lynx spiders were significantly more likely to be at the top of the plant in the presence of damsel bugs than in any other treatment (ANOVA (square root of proportion found): df = 8, 310; treatment x location: F=6.7, P<0.001). 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 these spiders varied in their preference for the top of the plant with respect to the different treatments. We found that they did respond differently to the treatments, following the same pattern as the test groups with more individuals moving to the top of the plant in the presence of damsel bugs (REML, W=36.9, df=3, P<0.001; average proportion of 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).

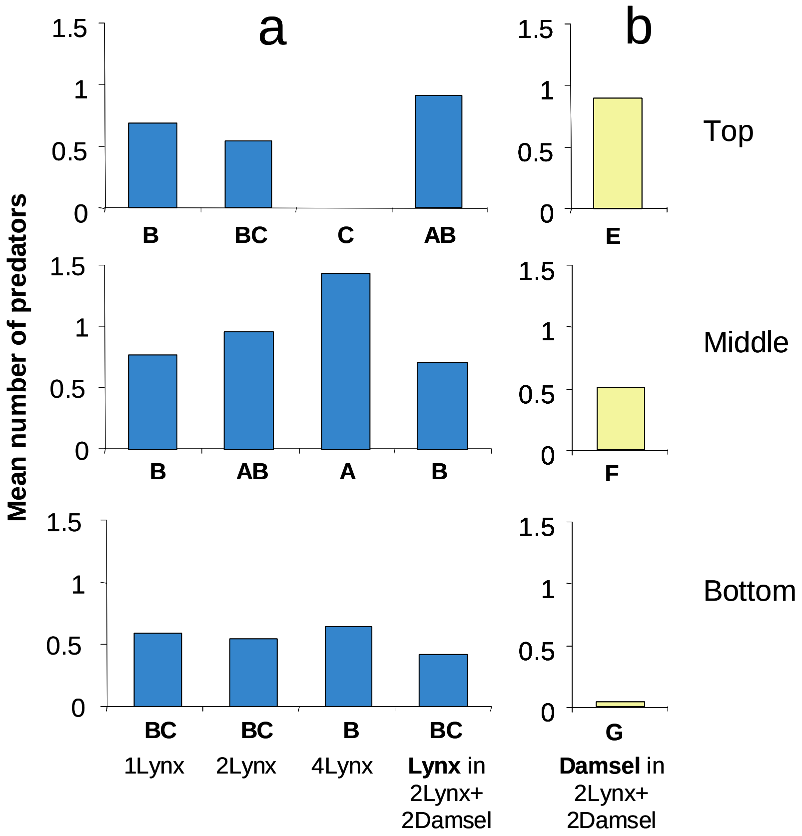

Fig. 11. The effect of predator density on the percentage of predators at the top, middle or bottom of the plant (analysis conducted on the square root transformation of proportions, .l.s.d (1%) =0.16; the same letter below the histogram indicates responses not significantly different from each other in the analysis of proportions). Two Lynx spiders were more likely to be at the top to the plant in the presence of damsel bugs than either on their own, or with two other lynx spiders (4Lynx treatment).

Discussion

1. Time budgets

In the time budget experiment, it was found that mirids were more active during the night rather than during the day and at dawn. Mirids were found to be increasingly active during the night with the most activity at dawn. For IPM purposes, the nocturnal activity of mirids may affect the success of using beneficial spiders and insects to control their numbers. Nocturnal predators may have a higher chance of both catching mirids and disrupting their feeding behaviour, which damages the cotton.

2. The effect of chemical cues on mirid behaviour.

In the nocturnal experiment, mirids did not respond to chemical cues. However, in the diurnal experiment, which also contained a cotton square, mirids in Lynx spider treatments were significantly less likely to stand still, and more likely to walk than those in Control treatments, whereas there was no significant difference between mirid behaviour in Damsel bug and Control treatments. Thus mirids were responding to the presence of predators without encountering them, but this change was only significant in the Lynx treatment. 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 had less damage with the same number of mirids. The same reduction in “fidgeting” in the presence of predators was also seen. In the field it may also lead to mirids leaving an area with a high number of predators, especially lynx spiders, thus reducing mirid pressure. This needs to be tested.

3. Behavioural effects of lynx spiders and damsel bugs on mirids.

We found that mirids did alter their behaviour in the presence of lynx spiders and damsel bugs. First, the number of mirids at the top of the plant increased with predator density. However, low densities of lynx spiders saw an increase in the number of mirids in the middle of the plant, which only dropped significantly at high lynx spider densities. If anything, this should have increased foraging pressure on bolls (which are more common in the middle and bottom section of the plant) in Mirid+Lynx cages in comparison to the All cages.

Second, mirids were less likely to stand on the stems as predator density increased. As this is a popular location for lynx spiders to ambush prey, this could be anti-predatory behaviour by the mirids against the lynx spiders.

Third, mirids were also more likely to “fidget” in predator treatments (although this dropped back a bit with high lynx densities), while the presence of any predators reduced the number of mirids standing still or feeding. Thus all predator densities had a behaviourally mediated effect suppressing mirid foraging behaviour.

Predators also altered their behaviour in the different treatments. Damsel bugs were significantly more common in the top of the plant than elsewhere on the plant. Lynx spiders were significantly more common in the middle of the plant, except when in the presence of damsel bugs. The two lynx spiders in the 2 Lynx + 2 Damsel bug treatment were more common in the top of the plant than in the lower density treatment of 2 lynx spiders only, or the higher density treatment of 4 lynx spiders. So predator density was not causing this result. The movement of lynx spiders to the top of the plant could leave the fruit lower down more exposed to mirid attack.

Conclusions

These experiments have revealed much about the behaviour of mirids and their responses to two of their predators, Lynx spiders and damsel bugs.

Mirids were found to be more active during the night, with the highest activity level at dawn. This potentially means that mirids are likely to be more protected from sprays applied during the day, however, this may change depending on the number of predators in the cotton. It was found that mirids do alter their behaviour in the presence of lynx spiders and damsel bugs. High predator density on the plant resulted in mirids moving to the top of the plant.

Previous experiments conducted during the daytime indicated that mirids responded to chemical cues, with the chemical cues of Lynx spider treatments resulting in the mirids walking more and less likely to stand still. Mirids were found to respond to the presence of Lynx spiders, but not to the presence of damsel bugs or the control treatment. However, in the nocturnal experiment conducted here, mirids do not respond to chemical cues. This is important considering that the time budget experiment demonstrated that mirids are more active during the night, indicating that there may be other mechanisms by which mirids detect their predators, and that their responsiveness to chemical cues may be influenced by their diurnal rhythms.

These results have implications for IPM in cotton with a view to improving Integrated Pest Management Programmes. The results show that mirids do alter their behaviour in the presence of predators. That is, lynx spiders can suppress mirid feeding simply by being present on the plant. By encouraging these beneficial animals in the cotton crop, and by avoiding chemicals which will reduce the numbers of the beneficials, damage by mirids can be suppressed, reducing pressure on the need to spray to control mirid damage.

Acknowledgements

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