CSIRO Plant Industry
Program T ' Cotton management and improvement'

FINAL REPORT

Project title: **Supporting IPM for future cotton systems**

Project code: **1.01.07 (CSP165C)**

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A final report prepared for the Cotton Catchment Communities CRC and the Cotton Research and Development Corporation

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FINAL REPORT 2008

Part 1 - Summary Details

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Project Title: Supporting IPM for future cotton systems

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Part 4 – Final Report Executive Summary

Supporting IPM for future cotton systems

This project has addressed issues that have emerged with the widespread adoption of Bollgard II cotton, and the resulting reduction in insecticide use. Specific objectives and outcomes are described below.

1) *The fit of new insecticides into integrated pest management.* Selection of insecticides can have a big influence on both control of the target pest as well as on beneficials, and the risk of secondary pest outbreaks. We found that low rates of fipronil provided strong efficacy against mirids, with or without salt, and were significantly more selective that the full rates of fipronil against beneficials, though still with a risk of flaring mites. Low rates of indoxacarb alone provided poor efficacy against mirids but the addition of salt or canopy oil boosted this to efficacy equivalent of the full rate with low risk to beneficials or risk of flaring mites. Altacor (rynaxypyr) a new insecticide for *Helicoverpa* control was highly efficacious and selective against many beneficials indicating a good IPM fit. These results have been made available to industry via the Cotton Pest Management Guide, to assist pest manager in spray choices, and to industry to help in registration, thereby ensuring availability of new insecticides or uses of insecticides to industry.

2) *Defining the pest status of emerging pests*

   a) Management of thrips on seedling cotton. Tobacco thrips, *Thrips tabaci*, is still the dominant species. It was controlled moderately well at some sites but poorly at others, which could indicate resistance. At some locations the western flower thrips, *Frankliniella occidentalis*, is also abundant and poorly controlled by available options indicating insecticide resistance. Control of thrips is problematic because damage is often cosmetic, plants will recover without loss, and because thrips are also predators of spider mites. Nevertheless in cooler regions, where control is justified, management of WFT may be difficult. Monitoring of thrips population composition early in the season and determination of resistance profiles for both WFT and *T. tabaci* has been initiated in conjunction with Dr Grant Herron (NSWDPI) and CSD.

   b) Late season pest damage from thrips and jassids. These pests often build to levels causing significant damage to leaves on maturing cotton. We found that late season damage to leaves is only likely to reduce yield in crops with high yield potential and if the damage is very severe and prolonged before cut-out. High yielding crops are likely more affected but even they show strong compensation at yield levels up to 14 b/ha.

   c) The effect of mirid sprays on secondary pests. We found that controlling mirids with the most popular insecticide (fipronil) increases the risk of causing mite outbreaks, which would then require additional control. Our results also suggest that in some situations Bollgard II crops are more at risk – this deserves further investigation. Nevertheless, the results highlight the need to have good mite sampling protocols in place in Bollgard II crops especially if OP's, SP's or fipronil are used to control mirids.

3) *Develop a new aphid sampling strategy and thresholds.* These were developed and extended to industry. They will provide a more rational basis for deciding when the occurrence of this pest justifies control and when beneficials are providing adequate control. This information has been linked with new information on the aphid borne disease cotton bunchy top, to provide pest managers with a holistic approach to managing both the pest and the disease.

4) *Sporadic pests.* Information and publication to help manage the pale cotton stainer was completed and will help industry to manage this pest in the future. In particular thresholds for lint damage are now available. These indicate that for stained locks the threshold is >50% of bolls with all locks damage, and for tight-locked bolls it's > 20% of bolls with all locks damaged. We also studied the feeding behaviour and have a better understanding of the damage symptoms.

This project has provided new information to help pest managers to make better decisions about management of emerging pests. Outcomes have been largely delivered to industry through a range of presentations, published resources and the WWW. Benefits to the industry are more rational decisions on the need to control pests, and awareness of risks for different control options. This will contribute to reduced pesticide use with flow-on economic, social and environmental benefits.

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Background

This project built on the outcomes of a series of projects dating back to the late 1980's, most recently CSP147C 'Incorporating aphids, insecticides and early season plant compensation into IPM'. The emphasis throughout these projects was on supporting the development of IPM systems and their practical application by providing knowledge across three main themes (i) the selectiveness of insecticides and their risk of causing pest resurgence, which helps determine their fit in IPM systems, (ii) understanding the capacity of cotton to tolerate damage and hence not require spraying, and (iii) understanding the effect of pests on the yield and fibre quality of cotton, so that accurate pest thresholds and sampling strategies can be developed. This project extended the knowledge gained in these past projects by considering;

a) The economic significance of emerging pests. As the proportion of the cotton industry growing Bollgard II varieties has increased there has been be a corresponding decrease in insecticide use. This helped the survival of beneficial populations, which contributed to the control of pests, but, also allowed some pests such as jassids, thrips (late season) and aphids to establish and increase to levels that may be economically damaging. This situation was further complicated by the invasion of cotton regions by western flower thrips, often in very high numbers late in the season. This species is a mite predator but also causes significant leaf damage.

Little was known about the economic significance of mid season jassid damage or late season thrips damage. Application of insecticides to control these pests may be not be economical and result in reduction in beneficial numbers, in turn leading to secondary pest outbreaks. However, in high yield crops these insecticides may be economically justified but their use needs to be balanced against other risks. Due to the difficulty of obtaining reliable infestations of jassids or thrips late season, we completed experiments simulating a worst case scenario to test if there is any chance that these pests could affect yield. These experiments involved removing leaves from different layers of the plant, simulating total loss of photosynthetic capacity due to extreme jassid numbers. In this case cutting off the top 25cm of the terminal or top 6 main stem leaves only to simulate late season thrips infestation caused extreme damage to the plant terminal. In both cases yield losses occurred, indicating that jassids and thrips could be economic pests under some circumstances.

Recent data collected by Steve Yeates (CSIRO PI) indicates that about 60 % of the crop yield is produced after the last leaf has developed – hence in high yielding crops the upper leaves may be more important in contributing to yield than previously thought. This project researched thresholds for jassids and late season thrips using both actual and simulated pest damage with the ultimate aim of developing thresholds based on pest abundance or damage symptoms. Additionally, during seasons with more winter rainfall and high proportions of cotton planted to BGII a range of other pests have emerged. For instance, the green stink bug, *Plautia affinis*, has shown up as a potential problem in the Gwydir Valley and in 2007-08 the pale cotton stainer (*Dysdercus sidae*) was a widespread problem. As the opportunity permitted we did preliminary investigation of these emerging pests to provide industry with information to help make better decisions about control.

b) The non-target effects of new insecticides and new low beneficial impact options and risk for resurgence of secondary pests. In the early 1990's research in predecessors of this project showed clearly the effect of early spraying with broad-spectrum insecticides on beneficial populations and the increased risk of outbreaks of spider mites or aphids. The research developed into a regular series of experiments to evaluate the efficacy, non-target effects and

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pest resurgence risk for all current and new insecticides. The outcomes have been used, along with results from other researchers, to develop look up charts showing the effects of each insecticide on non-target species, their pest resurgence risk and their impact on bees (see p. 23 Cotton Pest Management Guide 2004-05). This research was complimented by studies in other projects, such as that of Viliami Heimoana (NSW DPI – DAN160C and new submission) which has ceased. In this project we have added additional value to the experiments by artificially infesting experiments with mites and aphids to provide clearer data on the resurgence risk for these pests.

We also screened new insecticides or miticides and evaluated some of the other softer options such as the petroleum spray oils and salt plus insecticide mixtures for efficacy, non-target impacts and secondary pest resurgence. This was done with input from Moazzem Khan (QDPI&F – mirid control), and added the extra feature of secondary pest resurgence. The data has been used to provide growers and consultants with up-to-date information on the IPM fit of new chemistry, insecticides plus adjuvants and new uses of old chemistry.

c) Factors influencing the abundance of aphids. During a prior project we developed a good understanding of the effect of aphids on cotton yields, and showed that early infestations were capable of causing significant losses if not controlled (30 – 50%). However three areas where there were gaps were (i) the effect of HPR on aphids (ii) the value of beneficials at limiting increase of incipient aphid populations and (iii) the effect of crop nutrition on aphid abundance. Though we intended to investigate these issues, problems prevented completion of this work (discussed below).

This project supported the salary of Simone Heimoana (Senior Technical Officer) and a portion of the salary of Deon Cameron (Technical Assistant), Ammie Kidd (Technical Assistant) and Dee Hamilton (Technical Assistant). It also provided a vehicle and operating costs to complete the research.

Objectives

- Determine the non-target effects of new insecticides and new low beneficial impact options and the risk for resurgence of secondary pests

- Define the economic significance of emerging pests such as jassids and late season thrips.

- Understand the effect of variety, crop agronomy and predation on development of aphid populations.

NB. In the first year of this project we realised that experiments manipulating aphid densities were going to be very difficult. This was because the widespread adoption of Bollgard II cotton led to a dramatic reduction in insecticide use. This allowed beneficial populations to survive and build, making initiating and sustaining aphid outbreaks very difficult. We contacted Dr Ian Taylor (CRDC) and agreed to scale down objective three, and focus more effort in the area of emerging pests in Bollgard II. Hence a new objective could be:

- To define the effect of application of insecticides to emerging pests on the management of secondary pests in Bollgard II dominated cotton systems.

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Methods and Results

1. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

a. Determine the non-target effects of new insecticides and new low beneficial impact options and the risk for resurgence of secondary pest
This research used a protocol developed in 1993-94 and used consistently ever since. Large replicated experiments were done in each year of the project. In each experiment seven new insecticides or miticides were evaluated for their efficacy, non-target effects and risk of causing resurgence of secondary pests (mites or aphids). Over the duration of this project we have maintained ongoing communication with the range of Agrichemical companies to keep track of developments. Due to the small cotton insecticide market, the number of new products being considered for registration in cotton has declined dramatically, and many of the products we evaluated in a previous project (CSP147C) have not made it to market. Nevertheless, our research is valuable in helping products through registration and providing industry with independent information so we have to take the risk that some products may not be registered. As a result of these discussions we have evaluated two new compounds;

(i) Altacor (Rynaxypyr – Dupont), a new Lepidotpera targeting insecticide. Our research indicated that this product provides very good efficacy against *Helicoverpa* spp, and is relatively selective against many beneficials. Our report to Dupont has been used in their registration submission and in formulating some of the wording on the label (a copy of the reports can be provided to CRDC / Cotton CRC if requested)
(ii) Movento (BYI 08330 240 SC - Bayer) an experimental product targeting sucking pests. This product has not yet been progressed to market.

Over the past 5 years there has been strong interest by industry in options to control sucking pests - especially mirids - that are less expensive, efficacious and more selective. An approach that has been used is to reduce rates and add salt (NaCl) or Canopy oil to help maintain efficacy. The reduced rate is cheaper and theoretically more selective against beneficials. Work by Moazzem Khan (QDPI&F) in cotton and by Hugh Brier (QDPI&F) in pulses and grain legumes suggests that these row rate plus additive compilations also provide better efficacy than the low rates alone. We wanted to include these options in the 'Impact of insecticides and miticides on predators in cotton' table in the Cotton Pest Management Guide, so we evaluated them as well in our standard format. The options considered so far were selected based on common use in the case of fipronil (Regent) or potential to be highly selective in the case of indoxacarb (Steward), and included all of the combinations required:

(i) Low rate Regent (fipronil @ 8 g ai/ha) alone
(ii) Low rate Regent with salt (@ 10g/l)
(iii) Full rate Regent (fipronil @ 25 g ai/ha)
(iv) Low rate Steward (indoxacarb @ 60 g ai/ha)
(v) Low rate Steward + salt
(vi) Low rate Steward + canopy at 2% v/v
(vii) Full rate Steward (indoxacarb @120 g ai/ha)
(viii) Salt alone
(ix) Canopy alone

These combinations were evaluated for two years. We found that the low rates of

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fipronil provided strong efficacy against mirids, with or without salt, and were significantly more selective that the full rates of fipronil against beneficials. With indoxacarb we found that the low rate alone provided poor efficacy against mirids. However, the addition of salt or canopy oil boosted this to efficacy equivalent of the full rate. A particular outcome is that low rates of indoxacarb plus salt or canopy oil provide good control of mirids with low risk to beneficials or risk of flaring mites. In contrast, low rates of fipronil, either alone or with salt provide good mirid control and are more selective than full rates, but still carry a high risk of flaring mites. The results of these experiments have been reported in detail to Nufarm (fipronil), Dupont (indoxacarb) and to Caltex (Canopy Oil). The report has been used by Dupont in a submission for registration of the lower rates of fipronil plus salt against mirids (copies of the reports can be provided to CRDC / Cotton CRC if requested).

The information summarising effects of the new registered compounds (e.g. Altacor) and the lower rates of fipronil or indoxacarb have been incorporated into the 'Impact of insecticides and miticides on predators in cotton' table and provided to Tracey Farrell (NSWDPI) for inclusion in the Cotton Pest Management Guide (Table 1). This ensured that this reference document was up-to-date each year for the cotton industry. Evaluation of dimethoate at low rates and two new biopesticides is underway, but as only one year of data is available results are not reported yet.

b.   Define the economic significance of emerging pests such as jassids and late season thrips.

Thrips early season.
In the early 2000's the western flower thrips (WFT), *Frankliniella occidentalis*, was recorded from cotton at St George, and subsequently other cotton regions. An initial evaluation of resistance in a single strain of WFT from cotton confirmed that it was resistant to a range of control options. Since then we have regularly assisted CSD with counting and identification of thrips in their early season at-planting insecticide and seed treatment evaluations. During the course of this project we have identified the adult thrips species from some of the treatments to better interpret the results, especially where we found high survival of thrips on treatments that we expected to control them effectively. The experiments essentially compare a range of seed treatments and at-planting insecticides, including imidacloprid (Gaucho, Genero), Imidacloprid plus ? (Amparo), thiodicarb plus fipronil (Semevin Super), thiamethoxam (Cruiser) aldicarb (Temik), carbosulfan (Marshall). Here we report on a selected subset of treatments for 2006/07 and 2007/08.

Results are shown in Tables 2 and 3. Summarising these results; at most sites *Thrips tabaci* is the most common thrips species on seedling cotton. WFT is the next most abundant, followed by *F. schultzei*, but both are usually less than about 10% of the population. Across all sites, none of the treatments have provided strong control of either thrips adults or larvae, the best result being Greenbah 07-08 (Table 3), where about 90% control was achieved on one date with phorate (Thimet) and imidacloprid. Poor control of adults is to be expected as there is continual 're-invasion' of treated plots which can mask efficacy. However, the poor control of larvae is surprising and control is certainly poorer than in the past, especially with aldicarb (Temik) e.g. see (Sadras and Wilson 1998) where highly significant control in the range 50 – 95% was often achieved.

The identification of adult thrips suggests that while *T. tabaci* is suppressed by the

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treatments, at least in some experiments, control of WFT by either aldicarb or imidacloprid is generally poor e.g. ACRI ,Wee Waa 06-07, CSD Moree 06-07 (all in Table 2), Kummerow 07-08, Narromine 07-08 (Table 3). Looking at the larval populations, where adult control was poor, larval control was also generally poor e.g. Wee Waa 06-07 (Table 2) where on some dates larval numbers where higher in insecticide treated plots than in the control. At Kummerow 07-08 (Table 3), larval control was very poor, and larvae were as, or more, abundant in aldicarb or imidacloprid treated plots – probably reflecting the higher proportion of WFT at this site, and poor control of the larvae of this species due to resistance. At Narromine 07-08, WFT constituted about 40% of the adult population, and control of larval thrips, though statistically significant was fairly poor.

The results suggest that (i) at the sites surveyed none of the treatments worked as well as would be expected (ii) *T. tabaci* predominates early season, though WFT can be locally abundant (iii) control of WFT adults and larvae appears to be poor – but this is confounded by the poor performance of the insecticides at almost all sites. Nevertheless, if WFT were to become prevalent early in the season more frequently, the data from Kummerow 07-08 and Narromine 07-08 suggest that this species would be difficult to control with existing seed treatment or at-planting options. There are effective options such as spinosad (Tracer) but this would need to be managed carefully to avoid selecting for resistance. We are following this research up in 2008-09, and we have arranged for samples of thrips from the Darling Downs (Kummerow) to be collected by John Marshall and sent to Dr Grant Herron (NSW DPI) to establish their resistance profile. The cause of poor efficacy is puzzling – it may be related to growing conditions, but the possibility that *T. tabaci* also has some level of resistance would be easy to test and we have discussed this need with Dr Herron.

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Table 1. Effect of insecticides and miticides on beneficials and pest resurgence in cotton, based on data derived from this project and from collaborators (Robert Mensah - NSW DPI*, Moazzem Khan – QDPI&F, Martin Dillon – formerly CSIRO Entomology, Mark Wade – formerly QDPI&F and UQld, Brad Scholz – QDPI&F, Dave Murray – QDPI&F*, Viliami Heimoana – NSW DPI and Richard Lloyd – QDPI&F*, Jonathan Holloway – Formerly NSW DPI). This version will be published in the Cotton Pest Management Guide 2008/09.* Also Cotton Catchment Communities CRC. New additions indicated with **bold** lines.

Insecticides (in increasing rank order of impact on beneficials)	Rate (g ai/ha)	Helicoverpa	Mites	Mirids	Aphids	Thrips	Persistence[8]	Overall Ranking[10]	Total[1]	Red&Blue beetle	Minute 2-spt lady beetle	Other lady beetles	Total[2]	Damsel bugs	Big-eyed Bugs	Other Predatory bugs	Apple Dimpling	Lacewing adults	Spiders	Total (wasps)	Trichogramma	Ants	Thrips	Mite (resurgence)	Aphid (resurgence)	Helicoverpa (resurgence)	Toxicity to bees[14]
Bt[11]		✓					very short	very low	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL				VL
NP Virus		✓					very short	very low	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL	VL				VL
Pirimicarb	250				✓		short	very low	VL	VL	VL	VL	L	L	M	VL	VL	VL	VL	VL	M	VL	L				VL
PSO (Canopy)[16]	2%	✓			✓		short	very low	VL	L	L	L	VL	VL	VL	VL	VL	VL	L	VL	VL	H	VL				VL
Methoxyfenozide	400	✓					medium-long	very low	L	VL	VL	L	L	L	L	VL	VL	VL	VL	VL	VL	VL	VL				VL
Etoxazole	38.5		✓				short	low	VL	VL	-	L	VL	VL	VL	VL	VL	VL	M	L	VL	VL	L				VL
Indoxacarb (low)	80			✓			medium	low	L	L	H	M	VL	L	-	L	H	M	VL	VL	VL	H	VL		+ve		-
Indoxacarb (low+salt)	80			✓			medium	low	L	L	H	M	VL	L	-	L	H	M	VL	L	VL	H	VL				-
Indoxacarb (low+Canopy)	80			✓			medium	low	L	L	H	M	VL	L	-	L	H	M	VL	L	VL	H	VL		+ve		-
Rynaxypyr®	52.5	✓					long	low	L	M	L	L	VL	VL	VL	-	VL	VH	VL	L	L	L	VL		+ve		-
Dicofol[3]	960		✓				long	low	L	-	-	-	L	-	-	-	L	-	-	-	-	-	VL				VL
Amorphous silica[17]	2500	✓					short	low	L	L	-	M	M	-	VL	-	-	L	L	L	-	M	VL				-
Spinosad	96	✓					medium	low	VL	M	L	VL	M	L	H	VL	L	VL	VL	M	H	H	H	+ve			H[15]
Diafenthiuron	350		✓		✓		medium	low	M	H	VL	M	L	M	VL	L	H	VL	L	L	VL	H	L			+ve	M
Pymetrozine	150				✓		short	low	M	M	M	M	L	M	M	VL	H	M	L	L	L	M	VL				VL
Fipronil (v. low)	8			✓			medium	low	L	L	L	VL	L	M	-	L	M	L	M	L	-	VH	L	+ve			VH
Fipronil (v. low + salt)	8			✓			medium	low	L	L	L	VL	L	-	-	L	M	L	M	-	-	VH	L	+ve	+ve	+ve	VH
Indoxacarb[13]	127.5	✓		✓			medium	low	H[14]	L	VH	VH	L	M	L	L	VH	M	VL	L	VL	VH	VL		+ve		H[15]
Abamectin	5.4	✓[6]	✓				medium	moderate	L	M	H	VL	M	L	M	M	H	VL	M	M	M	H	M				H
Emamectin	8.4	✓					medium	moderate	L	VL	M	M	H	H	H	H	H	L	M	M	M	VL	M				H
Endosulfan (low)	367.5	✓		✓	✓	✓	medium	moderate	M	VL	VH	M	M	M	M	L	H	L	M	VL	-	VH	H				M[15]
Propargite	1500		✓				medium	moderate	M	H	H	M	M	VL	VL	VL	L	VL	M	M	H	H	M		+ve	+ve	L
Acetamiprid	22.5				✓		medium	moderate	M	M	M	H	M	M	M	M	VH	L	VL	L	H	VH	VH				M[15]
Amitraz	400	✓	✓[9]		✓[9]		medium	moderate	H	M	VH	H	L	-	-	-	-	VL	M	L	L	H	M				L
Fipronil (low)	12.5			✓		✓	medium	moderate	L	L	H	L	L	H	L	L	VH	L	M	M	M	VH	VH	+ve	+ve	+ve	VH
Chlorfenapyr (low)	200	✓	✓				medium	moderate	M	L	VH	VL	M	VL	H	H	VH	L	L	M	VH	H	M				H
Thiamethoxam	100				✓		medium	moderate	H	H	H	H	M	M	M	M	H	M	VL	M	H	VH	H	+ve		+ve	H
Endosulfan (high)	735	✓		✓	✓	✓	medium	moderate	M	VL	M	M	M	M	M	M	H	L	M	L	VH	VH	H				M[15]
Fipronil (high)	25			✓		✓	medium	moderate	L	VL	H	L	M	H	H	L	VH	L	M	M	VH	VH	VH	+ve		+ve	VH
Imidacloprid	49			✓	✓		medium	moderate	H	L	VH	H	H	M	M	M	VH	L	L	L	M	H	H	+ve		+ve	M
Methomyl	169	✓					very short	high	H	L	VH	VH	M	L	VH	L	VH	M	M	M	H	H	H	+ve			H[15]
Thiodicarb	750	✓					long	high	VH	M	VH	VH	M	M	L	M	VH	VL	M	M	M	M	H	+ve	+ve		M[15]
Chlorfenapyr (high)	400	✓	✓				medium	high	H	M	VH	L	H	H	H	H	VH	L	M	M	VH	VH	M		+ve		H
OP's[5]		✓	✓	✓	✓	✓	short-medium	high	H	M	H	H	H	M	H	H	VH	L	M	H	H	VH	H	+ve			H
Carbaryl[3]		✓					short	high	H	-	-	-	H	-	-	-	-	-	-	-	-	-	H	-		-	H
Pyrethroids[4]		✓	✓[7]		✓[7]		long	very high	VH	-	-	-	VH	-	-	-	VL	VH	VH	VH	VH	VH	VH	+ve	+ve	+ve	H

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1. Total predatory beetles – ladybeetles, red and blue beetles, other predatory beetles
2. Total predatory bugs – big-eyed bugs, minute pirate bugs, brown smudge bugs, glossy shield bug, predatory shield bug, damsel bug, assassin bug, apple dimpling bug
3. Information; Citrus pests and their natural enemies, edited by Dan Smith; University of California Statewide IPM project, Cotton, Selectivity and persistence of key cotton insecticides and miticides.
4. Pyrethroids; alpha-cypermethrin, cypermethrin,beta-cyfluthrin, cyfluthrin, bifenthrin, fenvalerate, esfenvalerate, deltamethrin, lambda-cyhalothrin,
5. Organophosphates; dimethoate, omethoate, monocrotophos, profenofos, chlorpyrifos, chlorpyrifos-methyl, azinophos ethyl, methidathion, parathion-methyl, thiometon
6. *Helicoverpa punctigera* only.
7. Bifenthrin is registered for mite control; alpha-cypermethrin, beta-cyfluthrin, bifenthrin, deltamethrin and lambda-cyhalothrin are registered for control of mirids
8. Persistence of pest control; short, less than 3 days; medium, 3-7 days, long, greater than 10 days.
9. Suppression of mites and aphids only.
10. Impact rating (% reduction in beneficials following application, based on scores for the major beneficial groups); VL (very low), less than 10%; L (low), 10-20%; M (moderate), 20-40%; H (high), 40-60%; VH (very high), > 60%. A '-' indicates no data available for specific local species.
11. *Bacillus thuringiensis*
12. Pest resurgence is +ve if repeated applications of a particular product are likely to increase the risk of pest outbreaks or resurgence. Similarly sequential applications of products with a high pest resurgence rating will increase the risk of outbreaks or resurgence of the particular pest species.
13. Very high impact on minute two-spotted ladybeetle and other ladybeetles for wet spray, moderate impact for dried spray.
14. Data Source: British Crop Protection Council. 2003. The Pesticide Manual: A World Compendium (Thirteenth Edition),. Where LD50 data is not available impacts are based on comments and descriptions. Where LD50 data is available impacts are based on the following scale: very low = LD50 (48h) > 100 ug/bee, low = LD50 (48h) < 100 ug/bee, moderate = LD50 (48h) < 10 ug/bee, high = LD50 (48h) < 1 ug/bee, very high = LD50 (48h) < 0.1 ug/bee. Refer to the Protecting Bees section in this booklet.
15. Wet residue of these products is toxic to bees, however, applying the products in the early evening when bees are not foraging will allow spray to dry, reducing risk to bees the following day.
16. May reduce survival of ladybeetle larvae – rating of moderate for this group.
17. May be detrimental to eggs and early stages of many insects, generally low toxicity to adults and later stages.

DISCLAIMER Information provided is based on the current best information available from research data. Users of these products should check the label for further details of rate, pest spectrum, safe handling and application. Further information on the products can be obtained from the manufacturer.

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Table 2. Efficacy of at-planting insecticide treatments against thrips, CSD and CSIRO Experiments, 2006/07. * = significantly different from control at p < 0.05.

| Site | Date | Adults/plant | | | | | | Larvae/plant | | |
| | | *T. tabaci* | | | *F.cccidentalis* | | | | | |
		Control	Temik	Gaucho	Control	Temik	Gaucho	Control	Temik	Gaucho
ACRI 06/07	1/11/2006	11.0	6.8*	-	1.0	3.3*	-	31.2	5.4*	-
	22/11/2006	1.4	1.6	-	0.1	0.2	-	8.5	4.1	-
	14/12/2006	2.5	2.9	-	0.8	1.0	-	22.9	25.5	-
	P value	0.007			P value	0.039		P value	0.001	
	LSD	2.1			LSD	1.28		LSD	7.1	
Greenbah 06/07	1/11/2006	6.2	6.8	7.8	0.5	0.3	0.6	11.3	5.1	12.1
	8/11/2006	5.4	3.4	7.6	0.6	0.2	0.0	12.6	4.1*	7.6
	15/11/2006	3.8	4.5	3.5	0.3	0.3	0.4	9.7	7.8	12.8
	22/11/2006	1.2	1.8	1.6	0.2	0.2	0.1	28.5	18.7*	38.4*
	29/11/2006	2.3	1.8	2.1	0.0	0.2	0.3	3.5	5.1	4.0
	6/12/2006	2.5	3.1	4.1	0.0	0.0	0.1	4.4	2.9	6.8
	14/12/2006	2.2	3.3	1.9	0.3	0.0	0.9	15.7	14.0	18.0
	20/12/2006	2.6	2.1	3.0	0.2	0.2	0.4	14.5	17.0	19.5
	P value	0.3			P value	0.3		P value	0.028	
	LSD	ns			LSD	ns		LSD	6.9	
WeeWaa 06/07	13/10/2006	3.06	1.58	0.52	0.34	0.45	0.74	0.27	0.30	0.13
	20/10/2006	6.77	5.00	6.70	0.00	0.00	0.00	3.27	2.33	2.53
	27/10/2006	5.27	5.49	4.80	0.17	0.24	0.00	7.17	4.80	10.40*
	6/11/2006	4.53	3.32	4.96	0.00	0.05	0.24	27.97	7.90*	14.90*
	P value	0.4			P value	0.6		P value	0.0001	
	LSD	ns			LSD	ns		LSD	3.9	
CSD Moree	3/10/2006	1.5	1.1	1.1	0.5	0.3	0.2	8.0	2.7	1.8
	19/10/2006	15.6	17.6	15.2	0.6	0.6	0.2	19.1	14.9	6.9
	1/11/2006	1.9	3.1	2.8	0.3	0.3	0.2	18.6	15.0	11.8
	P value	0.9			P value	0.8		P value	0.8	
	LSD	ns			LSD	ns		LSD	ns	

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Table 3. Efficacy of at planting insecticide treatments against thrips, CSD and CSIRO Experiments, 2007/08. * = significantly different from control at p < 0.05.

| | | Adults/plant | | | | | | | | | Larvae/plant | | |
| | | *T. tabaci* | | | *F. occidentalis* | | | *F. schultzei* | | | | | |
Site	Date	Control	Temik	Gaucho	Control	Temik	Gaucho	Control	Temik	Gaucho	Control	Temik	Gaucho
Kummerow 07/08	15/11/2007	0.7	0.3	1.0	3.2	3.9	3.5	-	-	-	9.1	15.6	6.9
	22/11/2007	4.5	2.1	3.4	0.5	1.3	1.7	-	-	-	8.5	8.2	17.3
	P value	0.6			P value	09					P value	0.03	
	LSD	ns			LSD	ns					LSD	8.9	
			Thimet			Thimet			Thimet			Thimet	
Greenbah 07/08	23/10/2007	1.0	0.7	0.7	0.0	0.0	0.0	0.3	0.1	0.1	0.0	0.0	0.0
	30/10/2007	4.3	2.1	2.4	0.1	0.1	0.2	0.7	0.4	0.2	12.4	1.3*	0.8*
	6/11/2007	1.2	1.4	1.7	0.0	0.0	0.1	0.1	0.5	0.2	9.2	3.0*	1.9*
	P value	0.3			P value	0.7		P value	0.3		P value	0.001	
	LSD	ns			LSD	ns		LSD	ns		LSD	2.6	
Boolooroo 07/08	30/10/2007	1.0	1.3	1.0	0.1	0.0	0.0	0.0	0.2*	0.1*	0.8	0.6	0.1
	8/11/2007	0.1	0.3	0.3	0.1	0.0	0.0	0.0	0.0	0.0	1.2	1.0	0.3
	14/11/2007	0.2	0.2	0.7	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.7	1.3
	23/11/2007	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
	P value	0.8			P value	0.4		P value	0.031		P value	0.8	
	LSD	ns			LSD	ns		LSD	0.08		LSD	6.9	
ACRI 0708	6/11/2007	1.9	0.2		2.1	2.4		0.4	0.2		5.2	4.3	
	13/11/2007	1.3	0.9		0.8	1.2		0.1	0.2		3.7	4.6	
	21/11/2007	5.7	5.2		1.4	1.5		0.1	0.8		1.5	1.7	
	P value	0.17			P value	0.9		P value	0.017		P value	0.34	
	LSD	ns			LSD	ns		LSD	0.44		LSD	3.9	
Narromine 0708	11/11/2007	9.0	10.8		6.2	7.7		6.0	7.6		14.0	4.9*	
	18/11/2007	5.6	7.3		2.4	0.0		3.3	2.3		2.8	1.9	
	27/11/2007	2.0	0.3		0.3	0.7		0.0	0.0		1.2	0.5	
	P value	0.6			P value	0.6		P value	0.3		P value	0.008	
	LSD	ns			LSD	ns		LSD	0.44		LSD	3.7	

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Late season damage due to thrips.

Since the advent of Bollgard II we have received frequent calls from consultants about late season populations of thrips on cotton. In general these late season populations, which are mostly WFT or *F. schultzei*, are not regarded as a problem and they actually assist in reducing the risk of mite outbreaks as both adults and larval thrips consume mite eggs. However, damage can sometimes be severe, especially to the upper leaves, which receive the most sunlight and which are most photosynthetically active (see Figure 1a-c). Some consultants (e.g. Mike Stone, Moree) have also expressed concern that high numbers of thrips larvae in flowers may cause flower abortion.

Figure 1. Leaves showing damage from late season thrips populations (a) from Mal Pritchard at Hillston (b) from Geoff Rudd at Dalby (c) from Togo, Wee Waa. In all cases these were upper leaves and the crop manager had expressed concern about the level of damage.

We have investigated this in several ways. Firstly we completed a small field experiment as part of a previous project that showed that loss of upper leaves late in the season could affect yield. Secondly, we established that thrips damage does reduce photosynthesis and could therefore potentially affect yield. Thirdly we set up experiments where portions of leaf area were removed to simulate severe damage to leaves and finally we developed a spray, based on spray-rig applied glacial acetic acid, which causes burns on the leaf that simulate the effects of thrips feeding. The latter two approaches both simulate a loss of photosynthetic activity which could then affect yield. These experiments are reported on below:

Effect of leaf removal on yield

Experiments were conducted over three years in collaboration with Stephen Yeates who provided valuable discussions on the treatments to be imposed. Our aim was to test how much leaf area needed to be removed in order for yield to be affected. We hypothesised that crops with high yield potential would be more likely to be at risk from yield loss due to late damage. This strategy was taken because experiments to manipulate thrips densities in field crops failed as thrips populations were variable at the particular sites chosen.

In the first year, 2005-06 we did two experiments focussing on damage after cut-out. In the first experiment we removed the leaves from the top 6 mainstem nodes at cutout, cutout plus 10 days, plus 20 days, plus 30 days and plus 40 days and had an undamaged control (6 treatments x 5 replicates, RBD). In the second experiment, damage treatments were superimposed on an experiment where flower retention had been manually manipulated to generate higher (85%) and lower (74%) retention levels. The results of Experiment 1 (Table 4) showed that none of the damage treatments affected maturity or yield, and those of Experiment 2 showed no

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significant effect of damage on yield regardless of retention level (though there was a trend for plots receiving damage at 40 days after cut-out to have higher yield).

Table 4. Effect of removal of all leaves from the top 6 nodes of cotton plants at cut-out and dates after that, ACRI, 2005/06.

| Treatment | Experiment 1 | | Experiment 2 | |
| | | | Yield (b/ha) | |
	Yield (b/ha)	Maturity (DAS)	High retention	Low retention
Control	10.9	162.5	9.716	10.112
Cutout	10.4	158.1	9.939	10.058
Cutout + 10d	10.3	157.7	9.952	9.604
Cutout + 20d	10.4	161.6	9.712	9.832
Cutout + 30d	10.3	161.9	-	-
Cutout + 40d	11.2	161.7	10.682	10.696
P value	0.68	1.12	P retention	0.76
LSD (0.05)	ns	ns	P time	0.03
			(LSD 0.05)	0.64
			P R x T	0.82

Based on the results of the earlier experiments we realised that some more extreme damage treatments would be required to determine the point at which leaf damage translated into yield loss – important in defining a threshold. We decided to implement two damage levels (i) removal of all leaves from the top 6 nodes or (ii) removal of all leaves from the top 9 nodes (Figure 2). In both cases this included leaves on the mainstem and fruiting branches.
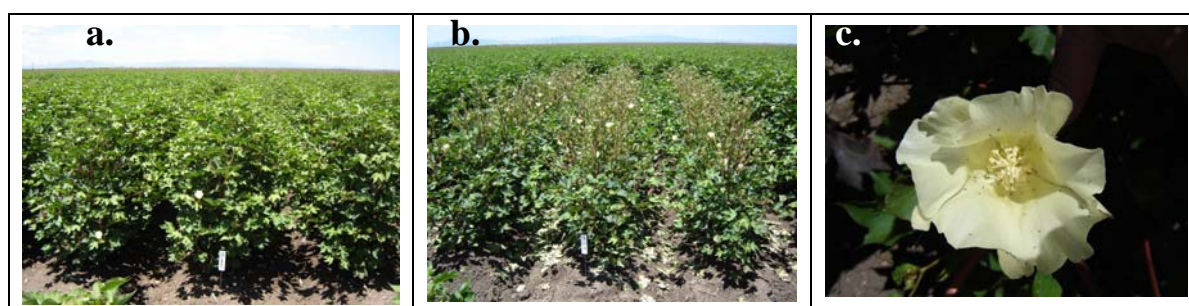
Figure 2. Plots from damage experiments (a) undamaged (b) leaves removed from top 9 nodes (c) thrips in flower, Field A3, ACRI, 2007-08.

We also decided to refocus the timing of damage to include some earlier treatments with the first damage at peak flowering, the second three weeks later (end of flowering), the third at cut-out and the fourth at cut-out plus two weeks. This experiment was completed in 2006-07 and 2007-08 using a randomised block design

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with four replications, see Figure 3. The two experiments were analysed in a single ANOVA with year, node and time as terms. As there was only a single control treatment this was replicated in the analysis to provide a control for each node level, allowing for a balanced design.

33 Flower +3wks 9	**34** During Flowering 6	**35** Cutout 6	**36** Control
29 During Flowering 9	**30** Flower +3wks 6	**31** Cutout +2 6	**32** Flower +3wks 9
25 Cutout +2 9	**26** Control	**27** Flower +3wks 9	**28** Cutout 9
21 During Flowering 6	**22** Cutout 6	**23** Cutout 9	**24** Cutout +2 9
17 Cutout 9	**18** During Flowering 9	**19** Flower +3wks 6	**20** During Flowering 6
13 Cutout+2 6	**14** Cutout+2 9	**15** Control	**16** Cutout+2 6
9 Cutout 6	**10** Cutout 9	**11** Cutout+2 9	**12** During Flowering 9
5 Control	**6** Flower+3wks 9	**7** During Flowering 6	**8** Flower +3wks 6
1 Flower +3wks 6	**2** Cutout +2 6	**3** DuringFlowering 9	**4** Cutout 6

6 m long

(8 rows wide, central 3 damaged)

Figure 3. Layout of simulated thrips damage experiments in 2006-07 and 2007-08.

The results are shown in Table 4, but because the analysis is complex the results are reported more fully below. ANOVA showed significant effects of year as 06-07 had a lower yield than 07-08 (2006-07, 9.0 bales/ha; 2007-08;12.5 bales/ha, p = 0.016). Node (p < 0.001) and time of damage (p < 0.001) also had significant effects, but also strong contributions to interactions. Year by node was just significant, indicating a marginally different response between years (p = 0.05). The difference in yield between damage to six or nine nodes was bigger in 07-08, due largely to the difference in yield levels between years. The effect of time of damage varied between years (p = 0.002), with the earlier damage reducing yield more significantly as expected, but the effect being more significant in 07-08 when yields were higher (Figure 4). The interaction of time of damage by nodes damaged was also significant (p < 0.001). As expected, removal of the leaves from the top 6 nodes affected yield less than removal of leaves from the top 9 nodes and later damage did not significantly affect yield (Figure 5). Maturity was not affected by any of the treatments and did not differ between years (Table 4).

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Overall the results indicate that cotton yield is sensitive to damage to the upper leaves, though it requires quite high damage. It is interesting that at the times when thrips are most likely to be in high abundance on cotton (end of flowering, cut-out, cutout plus 2 weeks) the higher yielding crops appear more sensitive, and damage quite late, can be important. These experiments allow us to focus in future on later damage timings and partial leaf removal which will more accurately simulate real damage.

As an additional component in this experiment we also included some extra treatments in spare rows of some plots. These treatments included flower removal, which was in response to suggestions that high densities of thrips may cause flower abortion. There is evidence for this overseas so we asked the question "Assuming thrips do cause flower abortion what would be the consequences for yield?". This was applied at the second damage timing (end of flowering) where 3 days worth of flowers were removed from both controls, and the 6 and 9 node leaf removal treatments. We removed tomorrow's flowers (candle wick stage square), today's flowers, and yesterday's flowers at the same time as the leaves were removed and again 3 days later, simulating complete flower abortion for a week.

The results (Table 5) showed that removal of flowers for 1 week did not affect yield ($p = 0.12$), but node of damage did affect yield (control, 9.71; 6 nodes, 9.22; 9 nodes, 7.57 b/ha). The interaction was not significant. This suggests that crops were able to compensate for flower loss, whether they had leaf damage or not. Crop maturity was not affected by flower removal, or the interaction between flower removal and node, but node alone was significant with the lower yielding node 6 and node 9 damage treatments maturing significantly earlier (control, 160.68; 6 nodes, 158.44; 9 nodes, 152.70 days after sowing to 60% bolls opened) (Table 5). Some of this effect could be due to the more open canopy which would allow more light and heat into the crop.
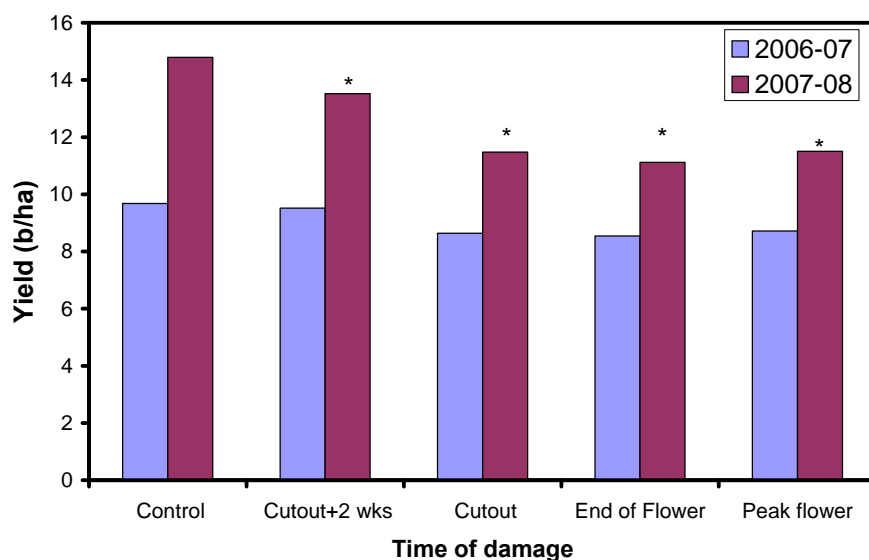
Figure 4. Earlier imposition of damage reduces yield more. This effect was stronger in 2007-08 when yield was higher. Asterisks indicate treatments significantly different from the control for each year separately.
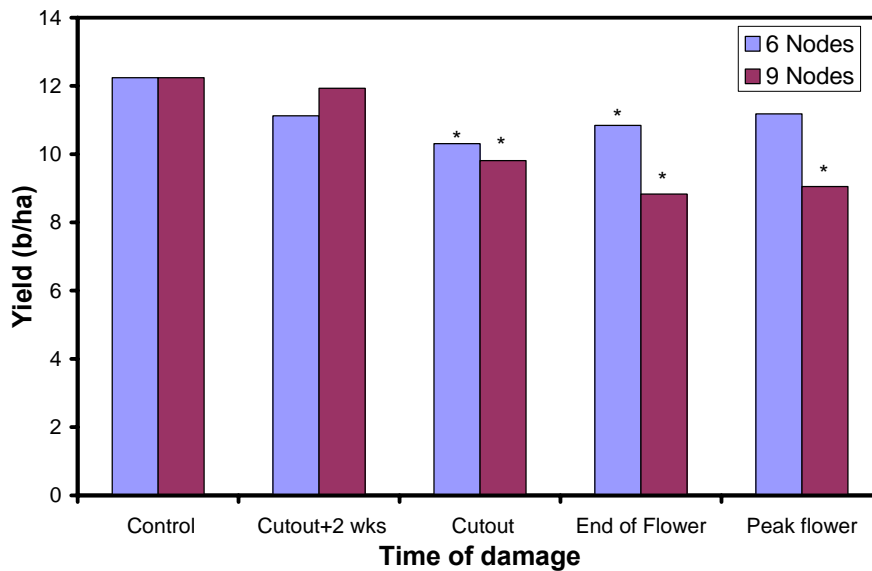
Figure 5. Earlier imposition of damages reduces yield more when imposed for 9 nodes than 6 nodes. Asterisks indicate treatments significantly different from the control for each year separately.

Table 4. Effect of removal of all leaves from the top 6 or top 9 nodes of cotton plants at a range of growth stages, ACRI, 2006-07 and 2007-08.

Treatment		Yield (b/ha)		Maturity DAS	
Timing	Nodes removed	2006-07	2007-08	2006-07	2007-08
Control (undamaged)	0	9.7	14.8	158.3	159.8
Peak Flower	6	9.9	12.5	157.9	155.4
Peak Flower	9	7.6	10.5	157.2	162.9
End flowering	6	9.1	12.6	158.2	157.5
End flowering	9	8.0	9.6	151.7	153.3
Cutout	6	8.8	11.8	157.5	162.6
Cutout	9	8.5	11.1	160.5	159.6
Cutout plus 2 weeks	6	8.6	13.7	159.4	162.3
Cutout plus 2 weeks	9	10.5	13.4	155.4	158.4

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Table 5. Effect of flower removal near the end of the flowering period on yield of cotton with or without leaves removed from the top 6 or 9 nodes ACRI, 2007/08

Nodes damaged	Flowers removed					
	Yes	No	Yes	No	Yes	No
	Mean number of flowers removed/m		Yield b/ha		Maturity (days after sowing to 60% bolls open	
0	16.2	0	10.8	8.6	161.6	159.8
6	18.0	0	9.7	8.8	159.4	157.5
9	18.1	0	7.4	7.7	152.1	153.2

Effect of 'burn spray' on yield

This experiment, the first in a series, was also designed to mimic the effect of late thrips damage (i.e. loss of photosynthetic activity of leaves), given that we were unable to do this with actual thrips populations. The aim was to overcome a deficiency of the leaf removal experiments – when leaves are removed completely more light is allowed into the lower canopy. Real thrips damage does result in smaller and less functional leaves, so there would be less shading of lower leaves, but still more shading than complete leaf removal. We used a spray consisting of diluted glacial acetic acid (10%) and Canopy crop oil (10%), plus xantham gum (0.25%) as a sticker to cause damaged areas on leaves by creating burns. This reduced leaf area would have reduced photosynthetic activity, similar to thrips damage, but without the leaves falling off completely, In a preliminary experiment this approach was quite effective. (Figure 6)

Figure 6. Effect of 'burn spray' on cotton leaves, Field A3, ACRI, 2007-08.

The experiment was set up to mimic possible field damage, with sprays being applied on three dates: (2 weeks before cut-out, at cutout and 2 weeks after cutout. We used a progression where on the first date we had the combination of sprays x dates (see Table 6). We established that the burned areas have significantly reduced photosynthetic capacity as do areas of leaf damaged by thrips – hence this technique is a reasonable approximation of pest damage (Figure 7).

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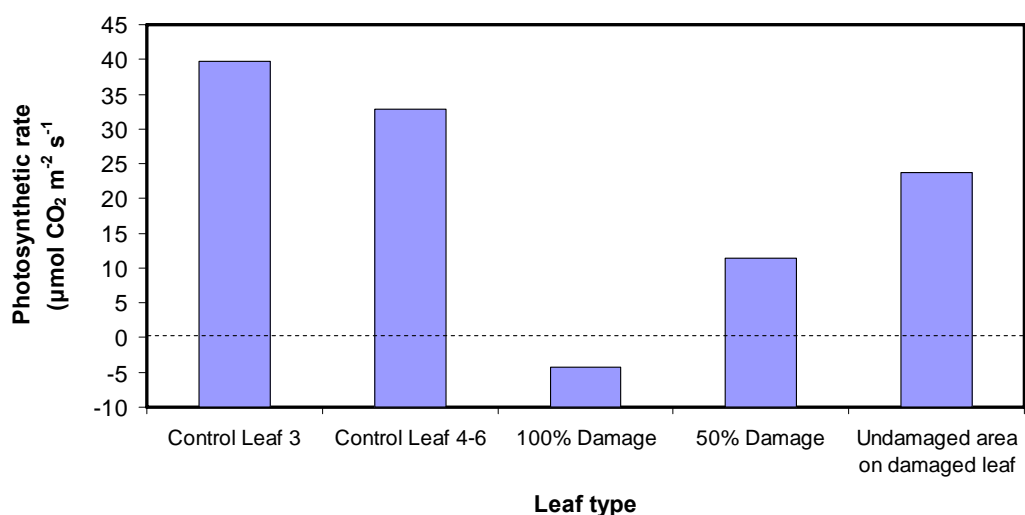
Figure 7. Effect of leaf burn damage on photosynthesis. Control leaves have no damage.

The sprays were applied with a spray rig using 5 flat fan nozzles per row. We quantified carefully the amount of leaf area damaged for each different treatment and also assessed if the damage caused differences in light penetration (data not shown). We also developed a system using a digital imaging program to select the different coloured damaged areas and automatically estimate the leaf area and damaged area for leaves sampled from each plot – this allowed us to calibrate our damage scores in the field to an actual leaf area damaged (data not shown).

The results were similar to the leaf removal experiment and show that earlier damage causes a greater reduction in yield (Table 2), especially with high yield (e.g. controls had 12.9 b/ha). Interestingly even the late damage with a single spray caused a reduction in yield – similar to the leaf removal experiment. A further experiment with a reduced spray rate or coverage would be valuable in helping to define the interaction between damage severity and timing.

Table 6. Effect of single and repeated 'leaf burn' sprays at different stages of growth on yield (b/ha) of cotton, ACRI, 2007/08. Asterisk indicates treatment significantly different to Control (ANOVA, LSD 0.05).

	Sprays applied			
Date of first spray	0	1	1 + 2	1 + 2 + 3
Control	12.9			
2 weeks before cutout		10.1*	9.2*	9.3*
Cutout			12.0	12.0
2 weeks after cutout				12.3

Late Season Jassid Damage
These experiments were designed to help understand the outcomes of damage from mid to late season jassids. These pests typically begin in the lower canopy, progress through the mid canopy and, when severe, cause damage to upper leaves. We have previously shown that severe jassid damage (80%) of upper leaf area damaged, can reduce photosynthesis of upper leaves by about 20%. However, the same damage on mid canopy leaves produced a much smaller decline of about 3%, mainly because photosynthesis in the shaded leaves is lower to start with. We tried to manipulate jassid population in the field on several occasions but were unable to generate sufficient differences in jassid density. However, when later sampling field for aphids

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and mites for resistance testing at a range of locations throughout the industry we frequently encountered fields with pronounced jassid damage especially along the edges.

As a backup method to obtain some understanding we carried out two experiments where we removed all of the leaves from the lower 1/3 of the crop, the middle, the top, and combinations of these strata e.g. bottom+middle, bottom+top, middle+top, and bottom+middle+top (see Figure 8 and 9). This essential asks the question 'if jassid damage was so severe that a particular strata was not contributing to photosynthesis what would be the effect on yield?'. The damage was implemented in early January in the first experiment in 2005-06, and in early January and early February (separate treatments) in 2006-07. We collected the leaves we removed from each stratum and measured the total leaf area removed for each treatment, and also harvested the bolls separately from each stratum so we could understand how loss of leaf area affected the bolls in each stratum, single or in combination (data not shown here). In 2006-07 we used longer plots of 6m which were machine harvested to provide a more accurate estimate of damage effects.

	D1 BT	D1 BMT	D1 MT	D1 Bottom
	D2 Top	Control	D2 Bottom	D2 BM
	D1 MT	D2 Middle	D2 BT	D1 BT
	D2 BMT	D1 BM	D1 Middle	Control
	D1 Bottom	D2 BM	D1 BMT	D2
	D2 MT	D1 BT	D2 BMT	D 2 Top
	D2 Middle	D2 Bottom	D1 Bottom	D1 BM
	D1 Top	D1 Middle	Control	D2 BMT
	D2 BM	D2 BMT	D2 MT	D1 Top
	D2 BT	D1 MT	D2 Middle	D2 BT
	D1 BMT	D1 Top	D2 Top	D1 MT
	Control	D2 MT	D1 BM	D1 Middle
	D1 BM	D2 BT	D2 BM	D2 Bottom
6m	D2 Bottom	D1 Bottom	D1 BT	D2 MT
long	D1 Middle	D 2 Top	D1 Top	D1 BMT
	Rep 1 (8 rows wide, central 3 damaged)	Rep 2	Rep 3	Rep 4

Figure 8. Layout of simulated jassid damage experiments in 2006-07 and 2007-08.

Figure 9. Examples of treatments in jassid damage simulation experiments (a) control (b) removal of leaves from bottom, middle and top strata (c) removal of leaves from bottom and top strata, leaving the middle – removed leaves can be seen in the furrow. A 1m section of removed leaves was retained to estimate the leaf area removed. Block 18, ACRI, 2006-07.

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Analysis of the total yield for each treatment (Figures 10 and 11) showed that the experiments gave very similar results. Loss of leaf area from the bottom has almost no effect on yield. Loss from the top or middle reduced yield. Additional loss of bottom leaves to loss of middle or top leaves maked little difference. This suggests jassid damage to lower leaves is unlikely to reduce yield no matter how severe. However, damage to middle or top leaves, singly or in combination is more significant. However, these results must be tempered by the fact that jassid damage doesn't reduce photosynthesis very much, even when severe. Earlier research by Lei, Reddall and Wilson found that leaves in the upper canopy, with 80% of the upper leaf surface area damaged had photosynthesis reduced by only 20%. This appeared to be because the jassids only damage part of the way through the leaf, leaving the lower half of the leaf still functional. In contrast leaves in the lower canopy with similar damage levels had photosynthesis reduced by about 5%, because the rates were low already. So, damage would need to be very severe and prolonged to reduce yield, even at high yields (as obtained here).
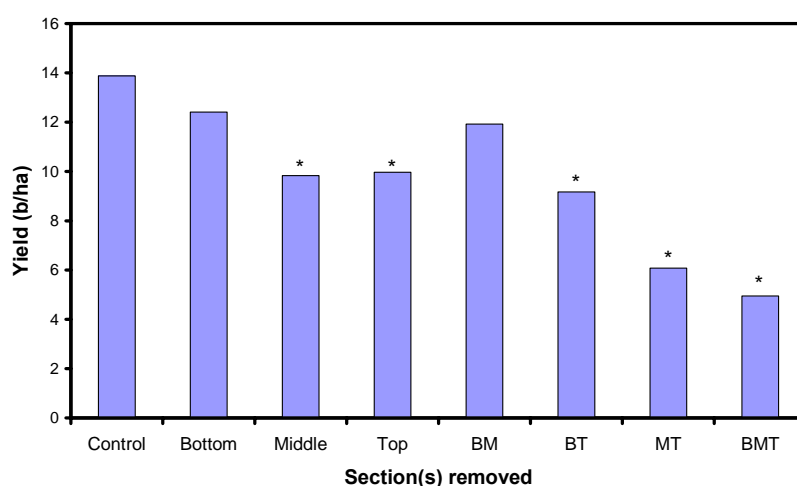
Figure 10. Effect of removing all of the leaves from a section of the crop canopy on yield, ACRI, 2005-06. Damage imposed on 4 January, early flowering. Asterisk indicates significantly different from control (ANOVA, LSD 0.05).
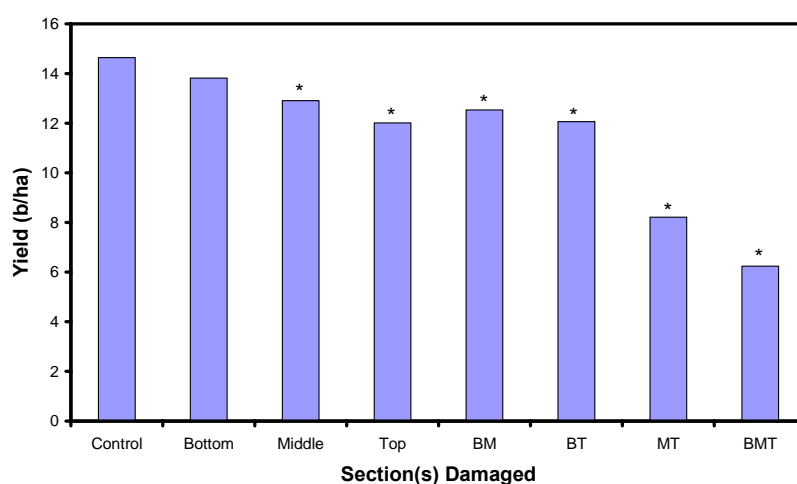
Figure 11. Effect of removing all of the leaves from a section of the crop canopy on yield., ACRI, 2006-07. There were 2 damage times (8 January early flowering, and 7 February late flowering) but the effect of damage timing was not significant (p > 0.05) so the combined values are shown. Asterisk indicates significantly different from control (ANOVA, LSD 0.05).

Real jassid damage

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We had difficulty locating suitable sites for experiments with jassids in advance of a problem – usually we heard about sites after damage had already occurred. When we decided on a predetermined site the jassid population invariably failed to develop to the extent we wanted. Nevertheless, we persevered and complete an experiment in 2006-07. In this experiment, Dr Mary Whitehouse indicated that she had seen areas with jassid damage in her cotton, and was prepared to allocate us a section. We set the experiment up on the 18th January, with four treatments: no control, control now, control 3 weeks later and control 5 weeks later. Diafenthiuron (Pegasus) was used to control jassids. This design was chosen to allow progressive accumulation of damage. We sampled immediately before the first spray event, then each week thereafter. From each plot we scored the damage on the upper and lower surfaces of leaves at node 3, 6, and 10 on 10 plants per plot (see Figure 12 and Table 7), and also sampled the jassids in each plot with a sweep net (12 sweeps across the centre two rows). At the end of the season we machine harvested the central two rows of each plot (each plot was 10m long and 8 rows wide).

Figure 12. Left, the section of field including the jassid damage experiment and right, the damage scoring system used.

Table 7. Damage score used to rank jassid damage to leaves.

Percentage Damage	Score
0 to 10	1
11 to 20	2
21 to 40	3
41 to 60	4
61 to 80	5
81 to 100	6

The jassid damage built slowly, and then declined. Analysis showed a significant overall difference in scores but only between the Control 1 (1.4) and the rest ($\approx$ 1.72)($p = 0.004$), and an overall increase then decline in scores from 0.7 to 2.2 to 1.7 ($p < 0.001$) (Figure 13). The interaction between treatment and date was not significant. The sweep netting indicated differences between treatments with a peak of $\approx$ 80 jassids per 12 sweeps in the Control 1 treatment and up to $\approx$ 140 in the no control treatment ($p = 0.001$)(Figure 14). Analysis of yields (machine harvested) showed no effect of jassids on yield, suggesting that at these low levels of damage (average high score about 2.2 about 20 % of leaf surface damaged (see Figure 12) there was no
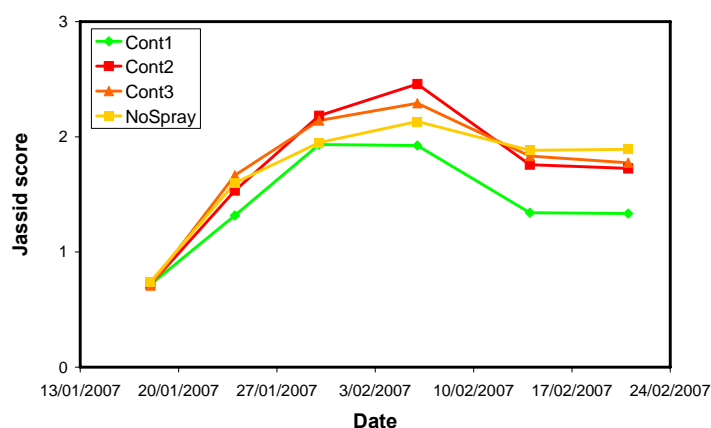
effect on yield.

Figure 13. Build up of jassid damage in plots, River Block 5, ACRI, 2006-07.
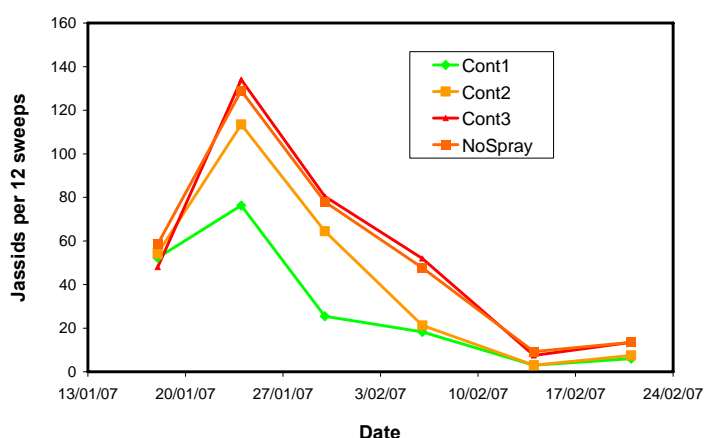

Figure 14. Build up of jassid damage in plots, River Block 5, ACRI, 2006-07.
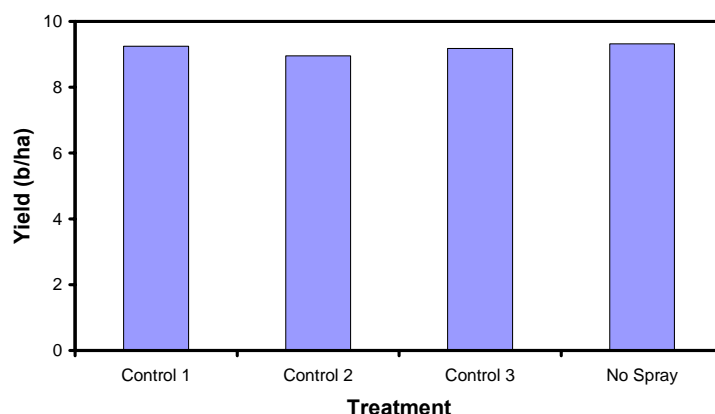

Figure 15. Effect of jassid damage on yield, River Block 5, ACRI, 2006-07.

b. Mirid management

Mirids have emerged as the major pest in Bollgard II cotton, which now accounts for about 80+ % of the planted area. Crops are regularly sprayed 1 or more times to control mirids, with an average of 2-3 but as many as 5 applications in a season. Fipronil (Regent) and dimethoate (Rogor) are the most commonly used insecticides, often at lower rates with the addition of salt or Canopy oil. We know from the work of our earlier projects that the use of these insecticides can potentially lead to increases in abundance of secondary pests, especially mites, but also whitefly and OP resistant

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cotton aphids in the case of dimethoate.

Consultants often note that they spray their Bollgard II crops first for mites. We have put this down to the fact that Bollgard II crops are sprayed with products that 'flare' mites, whereas conventional crops are sprayed with endosulfan, abamectin and emamectin especially early season which would tend to suppress mites. However, in the glasshouse and in other research with CSIRO funded Summer Scholarship students (Lauren Cave, 2005-06; Petra Norman, 2006-07) we have seen indications that mite populations may develop faster in Bollgard II cotton, though we are uncertain as to the mechanism – it does not appear to be related to differences in food quality of Bollgard or conventional cotton.

To help answer this question we designed two field experiments, in collaboration with Dr Mary Whitehouse and Dr Sharon Downes (CSIRO Entomology). Dr Whitehouse undertook detailed beatsheet sampling of the beneficial complex (data not reported here) and Dr Downes carried out detailed sampling of *Helicoverpa* abundance (data not reported here). In 2007-08 the plots were also used by PhD student Lu Baoqian as part of his research into thresholds for *Helicoverpa* spp. in Bollgard II cotton.

The experiments were laid out as a randomised block designs with two treatments specified as crop type and insecticide. Crop type was either Sicot 80RF or Sicot 80BRF while insecticide was either untreated or sprayed with fipronil (@ 25 g ai/ha) in 2006-07)(see Figure 16) and in 2007-08 was untreated or sprayed with either fipronil or thiodicarb (Larvin @ g ai/ha). We mass reared two-spotted spider mites in a glasshouse on cotton seedlings and used these to infest all plots with a moderate density of mites (2-3 seedlings /m). We sampled immediately before the first spray, then at least once between sprays. Sprays were applied 3 times with an interval of about 7-10 days depending on conditions (irrigation, rain, wind).

13 Conventional	14 SPRAY BG II	15 SPRAY Conventional	16 BG II
9 SPRAY BG II	10 Conventional	11 SPRAY BG II	12 Conventional
5 BG II	6 SPRAY Conventional	7 Conventional	8 SPRAY BG II
1 SPRAY Conventional	2 BG II	3 BG II	4 SPRAY Conventional
12 rows wide and 20 m long	12 rows	12 rows	12 rows

Figure 16. Layout of Mirid management x Mite experiments, ACRI, 2005-06.

The results for the 2006-07 experiment are quite surprising as in the sprayed plots the build up of mites was significantly faster in the Bollgard II plots than in the conventional plots. In 2007-08 there were also strong effects due to spraying, with plots treated with thiodicarb or fipronil having significantly more mites; however, there were no variety effects or variety by treatment interactions (Figure 17).

Across all of our experiments we have sometimes found more mites in insecticide treated Bollgard II plots than in conventional plots (2/5 experiments), but this has not

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been consistent. We are uncertain as to the cause of this inconsistency, but it does suggest that factors other than just insecticide application are, at least in some situations, also important. However, the results do highlight the tremendous biological control of mites – despite us infesting all plots with mites the populations were naturally controlled in the unsprayed plots. In contrast, if cotton was sprayed regularly with fipronil to control mirids it is likely that there will be mite outbreaks. Many consultants are already anticipating this problem and adding abamectin, which is very effective and inexpensive, to pre-emptively control mites. Such applications, however, do increase the risk of selection for resistance in mites and an approach that did not increase the risk of mite outbreaks in the first place would be better.
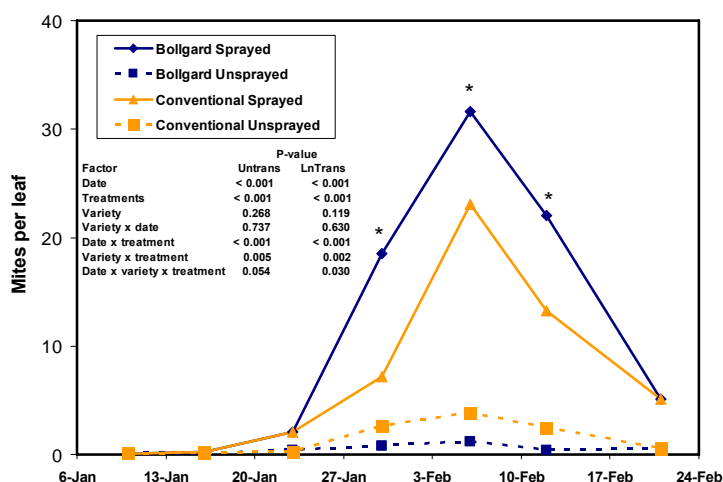
Figure 17. Build up of spider mites in plots of conventional or Bollgard II cotton either unsprayed or sprayed with fipronil 3 times at about 7-10 d intervals, Block 18, ACRI, 2006-07. Asterisks indicate treatments significantly different according to the untransformed analysis for the 3 way interaction, e.g. sprayed Bollgard II and conventional differ on 3 dates, unsprayed treatments don't.
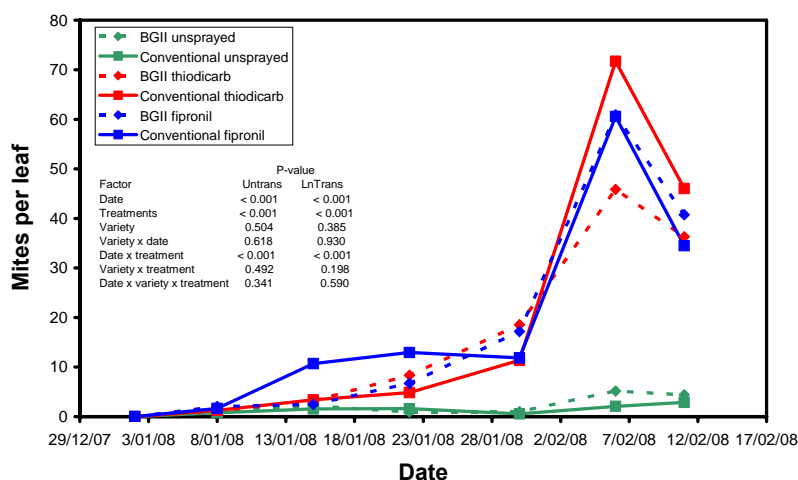
Figure 18. Build up of spider mites in plots of conventional or Bollgard II cotton either unsprayed or sprayed with fipronil or thiodicarb 3 times at about 7-10 d intervals, Field 1, ACRI, 2007-08.

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c. Pale cotton stainer (*Dysdercus sidae*)

    In 2007-08 the pale cotton stainer (PCS) emerged as a significant pest, for the first time in the modern cotton industry in Eastern Australia. This problem was detected through consultants requesting information from Tracey Farrell (NSW DPI) or Lewis Wilson early in January. Inspection of fields revealed significant densities of PCS in some fields (e.g. up to 6-7/m) and significant damage to some bolls – as indicated by distortion and staining of early open bolls.

    We did a quick search of the available literature – especially 'Insect Pests of Cotton' by Tunstall and Matthews, and realised that this pest could be quite damaging – causing significant damage to lint. Particularly significant is that this pest feeds on both developing and mature seeds and will attach to bolls too mature for other sucking pests (e.g. mirids), and will also attack open bolls – hence the period of potential damage is wide. To help industry deal with this problem we assembled some information on the life cycle of the pest, control options, sampling issues, thresholds (based on data by Dr. Moazzem Khan), thresholds for lint damage to cause discounts (see below). This was compiled into a 'Pest Profile' on this pest and was sent widely throughout the industry.

    The thresholds for lint damage were derived by collecting damaged and undamaged bolls. Separating the damaged bolls into stained, tight-locked or undamaged locks. We ginned these locks in a micro gin and took the samples to Australian Classing Services. The classing produced clear evidence of potential for discounts from stainers (Table 8). Allowing for the ratio of damaged to undamaged lock we developed two tables that indicate the proportion of bolls that need to be damaged to have potential for an overall discount.

    Following this research we wanted to investigate the symptoms of the damage caused by this pest as this would help consultants to detect it early in the field. We found we could distinguish between male and female PCS by the presence of bands on the abdomen. We caged adult stainers on relatively mature bolls in the field (bolls > 20 days old) which would not be attractive to other sucking pests.

    We found that the symptoms of damage are difficult to see – the PCS pierces the boll wall with a very fine stylet that leaves only a very tiny puncture mark on the outside of the boll – almost impossible to see even with a hand lens. On the inner boll wall the damage is evident as small black marks, easily missed. Shortly after feeding the lint appears undamaged, but if the seed are dissected out and cut open there is clear evidence of damage by female stainers but not by males. This information has been extended to industry via the CCA AGM and the CRC Science Forum in 2008. It makes managing stainers more difficult because damage is difficult to detect without opening bolls and inspecting the internal walls very carefully as well as cutting seeds open to check for damage. We intend to follow this up in 2008-09 by caging different ages of bolls undamaged by other sucking pests with known numbers of stainers and developing a better understanding of the damage symptoms and rate.

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Figure 19. Clockwise from top right, adult pale cotton stainer, undamaged boll (left) and damaged boll (right) showing staining, nymphal stage of PCS, damaged boll being fed on by a mating pair of PCS.

Table 8. Effect of PCS on grade of lint.

Boll type	Lock type	Colour	Spotting		Discounts
Damaged	Stained	3	2		22 = $15
	Tight	4	4		32 = $48
	Undamaged	2	1		33 = $78
Undamaged	Undamaged	1	1		43 = $120
					44 = >$120

Table 9. Lint grade as affected by proportion of bolls and locks per boll by pale cotton stainer damage.

Staining

Proportion of bolls affected	Damaged locks per boll			
	1	2	3	4
0.00	11	11	11	11
0.10	11	11	11	11
0.20	11	11	11	11
0.30	11	11	11	21
0.40	11	11	21	21
0.50	11	21	21	22
0.60	11	21	21	22
0.70	11	21	21	22
0.80	11	21	21	32
0.90	11	21	21	32
1.00	21	21	22	32

Tight locked (damage + disease)

Proportion of bolls affected	Damaged locks per boll			
	1	2	3	4
0.00	11	11	11	11
0.10	11	11	11	11
0.20	11	11	11	22
0.30	11	11	22	22
0.40	11	11	22	22
0.50	11	22	22	33
0.60	11	22	22	33
0.70	21	22	22	33
0.80	21	22	33	33
0.90	21	22	33	44
1.00	21	32	33	44

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Figure 20. A. male (left) and female (right) adult pale cotton stainers (*Dysdercus sidae*) B. adults stainer feeding, showing fine stylet C. tiny puncture mark D. tiny black marks on inside boll wall caused by feeding of males and females E. seeds fed on by females showing brown damaged areas F. seeds from bolls fed on by males show no sign of damage.

d. Aphids and yield

As part of the previous project (CSP147C) the effect of aphids on yield of cotton was further quantified in an experiment in 2005-06. However, due to the difficulty in generating outbreaks of aphids reliably in small plots this work was curtailed. Effort was then put into developing a relationship between aphids and yield loss from previous years' data that could be used to assist consultants and growers in the field. Data from the 2005-06 season was used to validate the equation, then included in the data set and used to improve the equation slightly.

We developed a new sampling technique as aphids are challenging to sample; they are patchy in distribution, small and too numerous to count quickly. The current recommendation for aphid sampling uses a presence/absence sampling system. We found that this technique provides a poor estimation of the actual aphid population in

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fields. To overcome this problem we developed a simple scoring system which involves scoring the density of aphids on the 3rd or 4th main-stem node below the terminal as;

0 = no aphids
1 = 1-10 aphids
2 = 11-20 aphids
3 = 21-50 aphids
4 = 51-100 aphids
5 = >100 aphids

An illustration showing these aphid densities is given in Figure 1. After counting aphids the first few times we quickly became confident at estimating abundance. About 20 leaves are required for accurate estimates of aphid density within a region of a field. In most fields at least 4 samples should be taken.
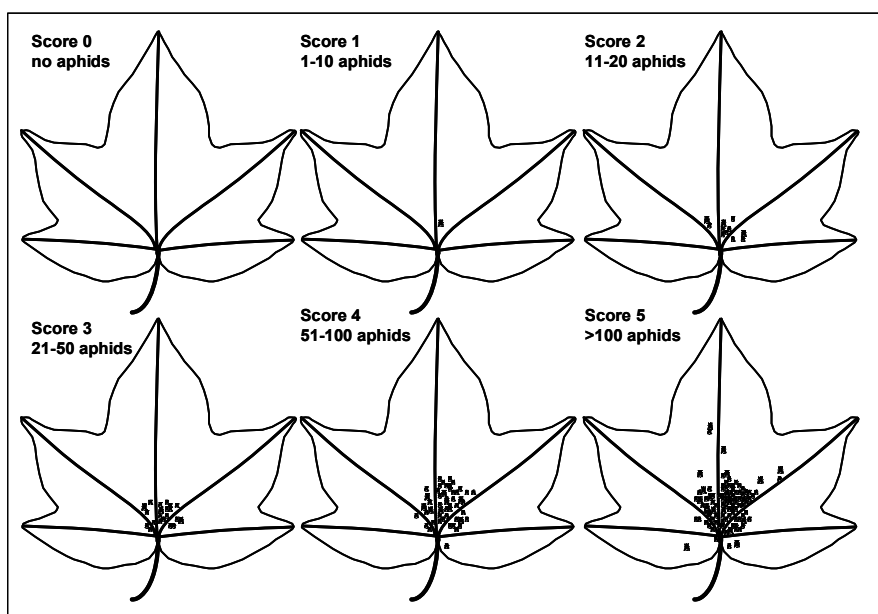
Figure 1. Representation of the aphid scoring system

Using score data from four years of experiments we calculated the average aphid score (AAS) for each sample date (total score / number of leaves sampled). To account for the build up of aphids over time we used the following formula to calculate the sample aphid score (SAS);

SAS = (Previous score x days since last score) + ((current score – previous score) x days since last score/2)

We accumulated this score across dates to give a 'cumulative season aphid score' (CSAS). A statistical equation was developed that predicted the % yield loss from the CSAS and the time remaining from the date the aphid population was first found until 60% bolls open.

Yield loss = $100 - ((-0.0369 \times CSAS \times (TRem/SL) + 10.11))^2$

Where Trem – is the time remaining in the season from the current sample date to 60% of boll open in days and SL – is the season length in days from planting to defoliation. This relationship predicted yield loss with a high degree of accuracy, accounting for approximately 85% of the variability.

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We developed simple guidelines, with help from Tracey Farrell, Simone Heimoana and Tanya Smith to use the sampling strategy and threshold equation to help improve aphid management:

(i) Fields should be sampled in several locations as aphids tend to be patchy. At each location collect 20 leaves from the 3 or 4th mainstem node below the terminal, taking one leaf per plant. Score each leaf using Figure 2 as a guide. The same leaves could also be used to score for mites and whitefly. When counting, do not include the pale brown bloated aphids as these are parasitized.

(ii) Sum the scores and divide by the number of leaves sampled to calculate the average aphid score (AAS). A hand tally counter helps with tallying the score (enter 'hand tally counter' into Google, check 'Australian sites'). Use Table 10 to convert the AAS into a SAS. For the first assessment of the season assume the 'score last check' was '0'. Find the value in the table where 'this check' and 'last check' intersect. Multiply this value by the number of days that have lapsed between checks. If this is the first assessment of the season, this value is the first CSAS. The CSAS is a cumulative score, so as the season progresses add the checks value to the previous value to give the updated CSAS.

(iii) Table 11 shows the potential yield loss from aphid populations beginning at different stages of the season for a range of CSAS. Use the date that you first found aphids, expressed as days from 60% of bolls open, as your 'Time remaining'. For that 'Time remaining' look down until you reach the line that approximates your current CSAS – this is the yield loss that the aphid population has caused (a worked example is given in Table 3). The value of the crop and the cost of control should be used to determine how much yield loss can be tolerated before intervention is required. Not controlling non-economic aphid populations saves money and also allows beneficials the chance to build and control aphids and other pests. Crop sensitivity to yield loss from aphids declines as the crop gets older, e.g. a higher CSAS is required before yield is affected. If aphids are controlled, either by a spray applied for aphids or against another pest, or if there are two aphid checks in a row with no aphids found, reset the CSAS to zero. Begin accumulating again when sampling recommences and you first get a non-zero aphid score, using this date as the new 'Time remaining'. This information has been captured in the Cotton Pest Management Guide, in an article in the Australian Cottongrower and in a webtool under development 'The Aphid Yield-Loss Estimator' (with Loretta Clancy).

Table 10. Look-up chart to help convert the average aphid score (AAS) to a sample aphid score (SAS). Look up the appropriate value for the current and previous score. Multiply this number by the number of days between the checks to give the SAS (e.g. a score of 1 last week and a score of 2 this week would be 1.5 x 7 days = 10.5).

Average aphid score *last* check	Average aphid score *this* check								
	0.0	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0
0	0.0	0.3	0.5	0.8	1.0	1.3	1.5	2.0	2.5
0.5	0.3	0.5	0.8	1.0	1.3	1.5	1.8	2.3	2.8
1.0	0.5	0.8	1.0	1.3	1.5	1.8	2.0	2.5	3.0
1.5	0.8	1.0	1.3	1.5	1.8	2.0	2.3	2.8	3.3
2.0	1.0	1.3	1.5	1.8	2.0	2.3	2.5	3.0	3.5
2.5	1.3	1.5	1.8	2.0	2.3	2.5	2.8	3.3	3.8
3.0	1.5	1.8	2.0	2.3	2.5	2.8	3.0	3.5	4.0
4.0	2.0	2.3	2.5	2.8	3.0	3.3	3.5	4.0	4.5
5.0	2.5	2.8	3.0	3.3	3.5	3.8	4.0	4.5	5.0

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Table 12. Predicted % yield loss based on time remaining in the season from the time aphids were first found in regular checks and the cumulative seasonal aphid score. This table is for a central region with a season length of 165 days from sowing to 60% of bolls open. The decision to control should take into account potential yield loss as well as control costs, impact on beneficials and selection for resistance. In the table a yield loss threshold of ≥4% is used so aphids would be controlled once the red zone is reached. * If aphids are controlled, either by a spray applied for aphids or against another pest, or if there are two aphid checks in a row with no aphids found, reset the CSAS to zero. Begin accumulating again when sampling recommences and you first get a non-zero aphid score, using this date as the new 'Time remaining'.

Cumulative Season Aphid Score*	Time Remaining (days) (Time from when aphids were first recorded until 60% of bolls open)*									
	100	90	80	70	60	50	40	30	20	10
0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
10	2.3	1.8	1.4	0.9	0.5	0	0	0	0	0
15	4.5	3.8	3.1	2.5	1.8	1.2	0.5	0	0	0
20	6.6	5.8	4.9	4.0	3.1	2.3	1.4	0.5	0	0
25	8.8	7.7	6.6	5.5	4.5	3.4	2.3	1.2	0	0
30	10.9	9.6	8.4	7.1	5.8	4.5	3.1	1.8	0.5	0
35	13.0	11.5	10.1	8.6	7.1	5.5	4.0	2.5	0.9	0
40	15.1	13.4	11.7	10.1	8.4	6.6	4.9	3.1	1.4	0
50	19.1	17.1	15.1	13.0	10.9	8.8	6.6	4.5	2.3	0
60	23.1	20.7	18.3	15.9	13.4	10.9	8.4	5.8	3.1	0.5
80	30.8	27.8	24.7	21.5	18.3	15.1	11.7	8.4	4.9	1.4
100	38.0	34.4	30.8	27.0	23.1	19.1	15.1	10.9	6.6	2.3
120	44.8	40.8	36.6	32.2	27.8	23.1	18.3	13.4	8.4	3.1

Outcomes

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

a. The effect of insecticides on target and non-target species has been quantified and made available to industry.

b. The effect of seed treatments on thrips and the abundance of western flower thrips has been quantified.

c. We have made progress in understanding the effect of late season thrips or jassid populations on yield of cotton.

d. The studies regarding the effect of mirid control options on mites illustrate the risk of

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flaring mite populations very clearly.

e. Information and publication to help manage the pale cotton stainer was completed.

f. A new aphid sampling strategy and thresholds were developed and extended to industry.

3. Please describe any:-
    a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

    (i) Development of look-up tables for establishing if aphids will affect yield loss and a new webtool.

    (ii) Development of look up charts to understand effects of insecticides on beneficials.

    b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

    (i) Development of relationship between aphid population dynamics and yield loss is a first for cotton, and is significant because it takes into consideration the time of the crop cycle at which the aphids occur. This problem has challenged pest managers for many years.

    (ii) Development of the 'burn spray' is a novel solution to a problem – it may have other applications, such as in knock down of regrowth and in other studies considering the effect of leaf damage on yield or fibre quality.

    (iii) The application of digital imaging to estimate leaf area and area damaged is a first for Australian cotton. This was done using off-the–shelf software by Simone Heimoana and Dr. Xavier Sirault (CSIRO Plant Industry, Canberra),

    c) required changes to the Intellectual Property register.
       Nil

Conclusion

4. 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?

    1. Selection of insecticides can have a big influence on both the control of the target pest as well as beneficials, and the risk of secondary pest outbreaks. We have provided tools to help with the decision making process, helping pest mangers in understanding the fit of each insecticide in IPM, and the likely consequences for beneficials and flaring of secondary pests. Better selection of the available control options will be the outcome. A particular outcome is that low rates of indoxacarb plus salt or canopy oil provide good control of mirids with low risk to beneficials or risk of flaring mites. In contrast, low rates of fipronil, either alone or with salt provide good mirid control, are more selective than full rates, but still carry a high risk of flaring mites.

    2. Poor control of thrips early in the season may be affected by the presence of WFT. This has some important implications, especially poor performance of some control options, and the likely failure of the available 'at-planting' options (seed treatments) to provide adequate control of western flower thrips. One outcome of this research will be

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the ongoing monitoring of thrips population composition early season and determination of resistance profiles.

3. Late season pests that damage leaves are only likely to reduce yield in crops with high yield potential and if the damage is very severe. Our research shows that late season damage can reduce yield if it is extreme. High yielding crops are likely more affected but even they show strong compensation at the yield levels studied (up to 14 b/ha).

4. Management of mirids can affect the risk of mite outbreaks. However, they also suggest that in some situations Bollgard II crops are more at risk – this deserves further investigation. Never-the-less the results highlight the need to have good mite sampling protocols in place in Bollgard II crops especially if OP's, SP's or fipronil are used to control mirids.

5. The provision of existing and new research data on the pale cotton stainer will help industry to manage this pest in the future. In particular thresholds for lint damage are now available and we have a better understanding of the damage symptoms and outcomes.

6. The provision of a new aphid sampling strategy and threshold will provide a more rational basis for deciding when occurrence of this pest justifies control and when beneficials are providing adequate control. This information has been linked with new information on the aphid borne disease cotton bunchy top, to provide pest managers with a holistic approach to managing both the pest and the disease.

Extension Opportunities
5. Detail a plan for the activities or other steps that may be taken:

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

Extension of key component of this project has been ongoing via active communication with individuals in the Cotton CRC Extension Network, the CCA, TIMS and CSD. Information on non-target effects of insecticides was used to update the 'Effect of insecticides and miticides on beneficials' table published annually in the Cotton Pest Management Guide. Further, reports provided to agrichemical companies have been used in registration packages – helping the industry to access these new products. We have developed new thresholds and sampling strategies for aphids and these are being extended to industry via a new webtool, the Cotton Pest Management Guide, updated aphid reviews and the Cotton Grower magazine. Information on pale cotton stainer was developed into a review and distributed widely within the industry.

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

We will continue to make use of the wide range of approaches we are currently using. Constant turn-over of extension staff is a major problem as good working relationships just get developed then the process starts again.

(c) for future research.

Goals for future research have been expanded on in a new Cotton CRC project, funded by CRDC, 'IPM in Bollgard II: coping with changes in pests and climate', which has the goals:

1. Research the seasonal abundance and ecology of GVB and WFT to better understand

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and improve management.

2. Investigate the efficacy and IPM fit of biopesticides (with Dr Mensah, NSWDPI), new chemistries, reduced rates with adjuvants and novel technologies.

3. Develop understanding of the effect of emergent pests, such as late season jassids, thrips and pale cotton stainers on cotton productivity using techniques developed in CRC89 and develop guidelines for industry.

4. Finalise field experimentation with CBT spread in cotton and explore the transmissibility of some of the apparent 'diseases' that are sporadically found in cotton.

The project will also ensure the cotton industry maintains core skills with aphid identification and cotton bunchy top epidemiology (Ms Tanya Smith) and in identification of thrips, plant responses to pest damage and manipulation of mite populations (Ms Simone Heimoana). This project will also allow ongoing collaboration with Dr Grant Herron and Dr Martin McLoon (NSW DPI) and Dr Flavie Vanlerberge-Massutti and Jerome Carletto (CIRAD France) to use microsatellite markers to characterise the aphid clones in cotton and link with resistance.

8. 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)

A selection of the publications below is included with this report.

Journal Papers
- Whitehouse, M.E.A., **Wilson, L.J.** and Fitt, G.P. (2005) A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. Environmental Entomology 35: 1224-1241
- Whitehouse, M.E.A., **Wilson, L.J**. and Constable, G.A. (2007) Target and non-target effects on the invertebrate community of VIP cotton, a new insecticidal transgenic. Aust. J. Agric. Res. 58: 273-285.
- Ali, A., Reddall, A., Roberts, J., **Wilson L. J**. and M. A. Rezaian (2007) Cytopathology, mode of aphid transmission and search for the causal agent of cotton bunchy top disease. Journal of Phytopathology 155, 220–227.
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B. Have you developed any online resources and what is the website address?

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Cotton pest management guide (http://www.dpi.nsw.gov.au/agriculture/field/field-crops/fibres/cotton/cotton-pest-management-guide)

Mirid ecology in Australian cotton (http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/Sucking_Pest_Publications.aspx)

Mirid management in Australian cotton (http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/Sucking_Pest_Publications.aspx)

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Integrated pest management guidelines for cotton production systems in Australia
(http://www.cotton.crc.org.au/Assets/PDFFiles/IPMGL05/IPMGLFor.pdf)

Pests Profiled: Pale Cotton Stainer
(http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/Sucking_Pest_Publications.aspx)

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Photosynthetic Response of Cotton to Spider Mite Damage: Interaction with Light and Compensatory Mechanisms

A. A. Reddall, L. J. Wilson,★ P. C. Gregg, and V. O. Sadras

ABSTRACT

We investigated the photosynthetic responses of cotton (*Gossypium hirsutum* L.) leaves to two-spotted spider mite (*Tetranychus urticae* K.) damage. Light-response curves of mite-infested (+M) and uninfested (– M) leaves diverged as mite populations increased. At 17 adult female mites per leaf, photosynthetic rate of +M leaves at photosynthetic photon flux density of about 1600 μmol m^{-2} s^{-1} was halved from 31 μmol CO_2 m^{-2} s^{-1} in –M to 16 μmol CO_2 m^{-2} s^{-1} in +M but there was no effect on either respiration or apparent maximum quantum yield. This has important implications when comparing the response to mites of individual leaves versus canopies. In the field (i) photosynthesis declined with crop age, but the rate of decline was faster in mite-infested leaves, and (ii) mite damage progressed downward in the canopy and from basal to distal leaf positions. We found no evidence of within-leaf (i.e., basal vs. distal section) or within-plant (top vs. mid or bottom leaf) increases in photosynthesis in compensation for mite damage, except for a minor enhancement of photosynthesis in bottom leaves of mite-infested crops due to greater light penetration in canopies severely defoliated by mite damage.

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The two-spotted spider mite (*Tetranychus urticae* K.) is an important secondary pest of cotton in Australia, with potential to cause severe reductions in lint and oil yield and in lint quality (Sadras and Wilson, 1996; Wilson, 1993; Wilson et al., 1991). Mites are known to reduce photosynthetic rate in a number of plant species including almonds [*Prunus dulcis* (Mill.) D.A. Webb.], oranges [*Citrus sinensis* (L.) Osbeck], tomato (*Solanum lycopersicum* L.), and cotton (*Gossypium hirsutum* L.) (Bondada et al., 1995; Hare et al., 1989; Royalty and Perring, 1989; Youngman and Barnes, 1986). In cotton, photosynthetic responses to mites have been researched from cytological (Bondada et al., 1995) to whole–canopy levels (Sadras and Wilson, 1997a). Most studies of mite effects on leaf-level photosynthesis, however, measured light-saturated photosynthetic rate. Understanding the effect of mites in partially shaded leaves is important because: (i) most leaves in the crop canopy are exposed to below–saturation light intensity, and (ii) in typical irrigated cotton crops in Australia, mites initially concentrate in nodes 3 to 5 below the main plant apex, but as populations

build they move down to nodes 5–15 below the terminal (Wilson and Morton, 1993).

Equation [1] relates net photosynthetic rate (P_{net}) and light incident at the leaf surface (Q):

$$P_{net} = R + P_{max} \star [1-exp(-b \star Q)] \qquad [1]$$

where R, the gas exchange rate at $Q = 0$, is taken as a measure of dark respiration, b is a fitted parameter, related to curvature, which multiplied by P_{max} gives an estimate of the apparent maximum quantum yield, and P_{max} is the light-saturated photosynthetic rate (Constable and Rawson, 1980; Connor et al., 1993; Peek et al., 2002). Elucidating the mechanisms of mite effects on leaf photosynthesis in terms of the parameters of the light-response curve is essential to link leaf and canopy photosynthetic responses. Reduction in P_{max} would be more important in young, well-lit leaves at the top of the canopy, whereas reduced apparent maximum quantum yield would be more important in leaves which are under a prevalent nonsaturating regime. Putative increase of respiration in mite-damaged leaves is particularly important for the low-lit "overdraft" layer of the canopy, where leaves are predominantly below their compensation point (Thomas and Sadras, 2001).

There are also knowledge gaps in relation to compensatory photosynthesis in mite-infested cotton. The relationship between radiation-use efficiency and intensity of mite infestation found by Sadras and Wilson (1997a) suggested some degree of compensation for mite damage. Nowak and Caldwell (1984) indicate that within-plant compensation occurs in herbivore-infested plants when the photosynthetic rate of undamaged leaves or undamaged portions of damaged leaves increases in relation to foliage of the same age on uninfested plants. These compensatory responses to herbivory may be mediated by one or more of the following processes: (i) changes in assimilate transport or use that overcome inhibition caused by accumulation of assimilate in leaves, (ii) reduction in leaf area which may improve water availability to undamaged leaves, (iii) increased chlorophyll content of remaining or new leaves, (iv) increased cytokinin level that could enhance CO_2 fixation and delay senescence of undamaged leaves (Trumble et al., 1993). There is also evidence for other mechanisms of increased crop photosynthesis in response to herbivory that involve changes in canopy structure and light distribution (Sadras, 1996; Holman and Oosterhuis, 1999). In comparison to undamaged controls, Sadras (1996) found a 20 to 27% increase in radiation-use efficiency of well-fertilized, low density cotton crops where reproductive structures were manually removed to simulate damage by *Helicoverpa* spp. More recently, Holman and Oosterhuis (1999) confirmed this response in a study where increased photosynthesis was measured in mid canopy leaves (node 8 below terminal) or whole canopies in response to loss of flower buds, which triggered a changed canopy structure

allowing a better light distribution. Some studies have shown no evidence of compensatory photosynthesis; for instance, Lei and Wilson (2004) found that the photosynthetic rate of cotton seedlings damaged by thrips was no different from undamaged plants and therefore could not explain the compensatory growth that occurred in damaged plants.

However, spider mites are mesophyll feeders, and their mode of action is fundamentally different from that of the herbivores investigated in the previous studies, which were predominantly defoliators or cause shedding of reproductive structures. Reddall et al. (2004) found no evidence of increased photosynthetic rate in undamaged portions of mite-infested leaves in the upper canopy, but the possibility of compensation in lower leaves was not reported. Spider mite damage can result in reduced leaf size, internode length, and plant height, and if damage is severe, loss of upper leaves (Reddall, 2000). This could alter the canopy structure, changing the light environment, and allowing more light to mid and lower canopy leaves, resulting in elevated photosynthetic rate of those leaves compared with similar aged leaves on undamaged plants.

The aims of this study were to (i) quantify the effect of mites on the parameters of the light-response curve of photosynthesis (Exp. 1), (ii) characterize the spatial and temporal patterns of mite effects on photosynthesis, and (iii) seek evidence for compensatory photosynthesis by comparison of rates in basal (preferred by mites) and distal leaf sections, and in leaves at two to three positions in the canopy (Exp. 2 and 3). This information will be valuable in understanding how mite damage at the leaf level translates to effects at the canopy level, especially taking into account the possibility of compensatory photosynthesis which has received little attention in cotton–arthropod studies. In future, this information will also assist efforts to model the effects of mites on cotton growth, yield, and fiber quality via effects on radiation use efficiency (Sadras and Wilson, 1997b), which may be valuable in pest management decisions for mite control.

MATERIALS AND METHODS

All experiments were done at the Australian Cotton Research Institute, Narrabri (30°S, 150°E), NSW, Australia.

Experiment 1: Light-Response Curves of Mite-Damaged Leaves

Cotton cv. Nucotn 37 was grown in 10-L pots filled with top-soil from a cracking gray clay Vertisol. Three seeds were sown per pot, on 7 Dec. 1998, and were later thinned to one seedling per pot. Forty pots were arranged in the open in 10 rows spaced 0.4 m apart from the pot perimeters, with 0.6 m between each pot within each row, yielding a density of 5 plants m⁻². Plants were well fertilized and fully watered with an automated dripper system.

The pots were assigned randomly to one of two treatments: control, no mites (−M) or plants artificially infested with mites

(+M). The +M treatment was established 70 d after sowing (DAS), when four adult female mites were placed on the first fully expanded mainstem leaf from the top of the plant (L1 leaf). This leaf position, usually the fourth node from the top, is most likely to contain the highest mite density in naturally infested cotton (Wilson and Morton, 1993). A fine brush was used to transfer each mite individually from mite–infested cotton seed-lings, reared in a glasshouse, to the plants. The mite population of each plant was assessed weekly by counting the number of adult female mites on the L1 leaves. Adult female mites provide a good surrogate for the total mite population (Carey, 1983). Full details and justification of mite sampling procedures are given in Wilson and Morton (1993). Acaricide (Agrimec [18 g L^{-1} abamectin, Syngenta Crop Protection Australia, Sydney, Australia] applied at 0.09 g ai abamectin L^{-1}) was sprayed when necessary to control mites in the −M plants.

Photosynthesis was measured at 65, 81, and 94 DAS, on clear, sunny days between 1030 and 1530 h. Four plants were randomly selected from each treatment and gas exchange and photosynthetic photon flux density (PPFD) measurements were taken from the basal section of an L1 leaf from each plant using the Li-Cor, LI-6400 (Lincoln, NE) portable photosynthesis system with a clear leaf chamber covering an area of 6 cm^2 which was clamped onto the leaf, as described by Reddall et al. (2004). Photosynthetic rate was measured in leaves exposed to full sunlight (PPFD > 1600 µmol m^{-2} s^{-1}) and in leaves shaded with a combination of black tulle, black shade cloth, and heavy duty black plastic reducing average PPFD to 1307, 950, 675, 379, 21, and 0 µmol m^{-2} s^{-1}. Shading material was mounted on 50 by 50 cm steel frames placed over the target leaf. To allow stomata to adjust to the shaded conditions each incremental level of shade was maintained for 20 min before the measure-ments were made, beginning with full sunlight and working progressively to the lowest PPFD (Petersen et al., 1991).

Experiments 2 and 3: Temporal and Spatial Patterns of Photosynthesis and Compensation

The effect of mite damage on the photosynthetic rate of leaves of differing age and position in the canopy profile was inves-tigated in field crops over two seasons (Season 1, 1996–1997; Season 2, 1997–1998). The experiments have been described by Reddall et al.(2004). Briefly, Nucotn 37 cotton crops were grown during two seasons under high input crop agronomy. There were two mite treatments, −M, control (no mites), and +M, crops artificially infested with mites, in a randomized block design with four replications (eight plots). The cotton was sown on beds 1 m apart at about 12 plants m^2 and each plot was eight rows by 18 m (Season 1) and eight rows by 15 m (Sea-son 2). Details of mite infestation methods and management are in Wilson (1993). Briefly, mites were mass reared on cotton seedlings in a glasshouse then transferred to the +M plots at a density of about five infested seedlings per meter to initiate a field infestation. The exact number of mites transferred per plant or square meter was not assessed; this is unnecessary as the number of mites established per leaf was recorded during each experiment (see below). As the mites can be transferred from plot to plot on clothing and by air currents there was always the

risk of contamination of −M plots with mites, and a selective acaricide (Agrimec applied at 5.4 g ai abamectin ha^{-1}) was used to control these unwanted infestations as needed.

Measurements of mite abundance, photosynthetic rate, PPFD, and relative chlorophyll content were made on a weekly basis. In Season 1, we measured these parameters on leaves in three positions: L1, defined as before; L2, at the 14th node from the base of the plant, and L3 at the 10th node from the base. Positions L1 and L2 were sampled in the second season. Data for L1 leaves were partially discussed in Reddall (2000). This provided a leaf near the top of the plant, which is the preferred feeding position of mites (Wilson and Morton, 1993), and also in the mid (L2) and lower (L3) canopy where mites are less abundant and where some within–plant compensation for mite damage to upper leaves (e.g., L1) could occur. The position of the L1 leaf measured remained constant relative to the plant ter-minal but was at a progressively higher node position in relation to the cotyledons as the season progressed. Because the leaf that occupied this position changed from week to week the fate of particular leaves over time was not studied. Instead, a new plant was tagged in each plot each week (i.e., mite numbers, relative chlorophyll content, and gas exchange were measured on three leaves [L1– L3] in Season 1, or two leaves [L1 and L2] in Season 2 on each of four plants per plot, the following week similar measurements were made on four new plants in that plot and so on). On each measurement date the number of adult female mites was counted on the underside of each leaf measured.

Measurements of gas exchange and relative chlorophyll content were taken from the basal (near the junction with the petiole) and distal leaf portions (near the leaf edge farthest from the petiole junction) of each leaf as described in Reddall et al. (2004) to assess within–leaf compensation. At each position gas exchange variables were measured with the LI-6400 portable photosynthesis system with a clear leaf chamber covering an area of 6 cm^2. Measurements were taken within the period of 3 h either side of solar noon using ambient light when the PPFD reaching the adaxial leaf surface of the L1 leaves was greater than 1600 µmol m^{-2} s^{-1}. Chamber conditions used ambient CO_2, the chamber temperature was set to 2°C below ambient to allow for slight heating when the chamber is clamped on the leaf (usually between 25 and 35°C) and relative humidity was controlled to between 60 and 70% using air flow rate and moisture scrubbers. While measurements were being taken, the L1 leaves were held perpendicular to the sun, while L2 and L3 leaves were measured in their natural position at the ambient light level, which was often considerably less than that of the L1 leaves. This was done to estimate the effects of mites on leaves in the light environment in which they were functioning, which meant that L2 and L3 leaves would often be functioning at a lower level than L1 leaves due to less light. This approach also avoided long acclimation times (>30 min) required if the L2 or L3 leaves had been exposed to higher light levels to estimate their maximum photosynthetic capacity. Photosynthetic rates were measured approximately 2 min after the chamber was placed over the leaf to allow stabi-lization of readings. PPFD was measured at the same time as photosynthesis using the sensor on the LI-6400. For both the gas exchange parameters and PPFD, each measurement was the average of five consecutive readings, taken sequentially at 2-s intervals. Leaf relative chlorophyll content was measured using a

SPAD 501 chlorophyll meter (Minolta, Osaka, Japan), which has been tested in a number of plant species, including cotton (Wood et al., 1992).

Relative chlorophyll content was measured in basal and distal portions (average of five separate measurements) of the same mite-infested and control leaves used for photosynthesis measurements. Data was analyzed and presented in SPAD units.

Statistical Analyses

Genstat 8 (Payne et al., 2005) was used for all statistical analysis except the fitting of the light curves for Experiment 1. Effect of mite treatments (+M vs. −M) on all response variables was assessed with analysis of variance. Nonlinear regression in S-PLUS (Insightful Corporation, 2001) was used to fit and compare nonlinear curves (Eq. [1]) for data from Experiment 1.

RESULTS
Light-Response Curves of Mite-Damaged Leaves

Once established, mite colonies grew exponentially to an average of 17 adult female mites per leaf at L1 whereas acaricide treatment controlled mites effectively in controls (Fig. 1). The photosynthetic response to PPFD was not significantly different between +M and −M treatments at 65 DAS, before mite addition, and at 85 DAS when mite populations in infested plants averaged four adult female mites per leaf (Fig. 2). At 94 DAS, when mites in +M leaves averaged 17 adult female mites per leaf, the light curves of the +M and −M plants diverged substantially. This was tested by fitting a general nonlinear model of Eq. [1] to all data (+M and −M), then adding a separate term for each treatment for each parameter (P_{max}, R, and b) and testing if this significantly improved the fit of the model. The analysis indicated that the fitted parameters P_{max} and b were significantly different between the +M and −M treatments ($P < 0.001$ in both cases) while R was not

(Fig. 2). The apparent maximum quantum yield (P_{max} b) was the same for both treatments (0.04 mol CO_2 mol^{-1}). At 94 DAS the photosynthetic rate of +M leaves was significantly less than that of −M leaves, for a given PPFD level. Estimated P_{max} in +M treatments was 50% lower than in −M treatments.

Temporal and Spatial Patterns of Photosynthesis and Compensation

Acaricide treatments were effective in controlling mites in the −M treatment (data not shown). Once established, mite colonies tended to grow exponentially in +M crops (Fig. 3). Start of rapid colony growth and peak numbers both were delayed in the bottom of the canopy compared with the top (e.g., compare L2–L3 to L1).

Figures 4 and 5 show the dynamics of leaf photosynthesis accounting for variation in leaf position in the canopy, and for basal and distal leaf sections. Photosynthesis declined with crop age in all leaves. The rate of decline, however, was faster in mite-infested leaves, particularly in Season 1. Mite effects were first detected in apical leaves, and progressed downward in the canopy. Within leaves, photosynthetic reduction attributable to mites was detected first, and was more pronounced, in basal leaf positions, where mites prefer to establish and feed. Late in Season 1, the photosynthetic rate of leaves at the bottom of the canopy (L3) was significantly greater in +M crops than in controls. This was associated with defoliation of the upper section of the canopy (Reddall, 2000) and consequent increase in PPFD incident at the bottom of mite-damaged crops (Fig. 6). Mites reduced the relative chlorophyll content of cotton leaves in both seasons but only in the basal positions where colonies were established (Fig. 7, 8). Late in Season 2, bottom leaves of mite-infested crops had greater relative chlorophyll content than leaves in control crops.

DISCUSSION

A Mitscherlich-type model provided physiologically adequate descriptions of the response of net photosynthesis to irradiance, and captured important changes in leaf performance in response to mites. All three parameters of the curve obtained from our pot-grown plants agreed closely with those reported for irrigated cotton crops (Milroy and Bange, 2003). The main effect of mites was to reduce light-saturated photosynthesis, with no effects on either respiration or apparent maximum quantum yield. The lack of mite effects on leaf photosynthesis at low light intensity, i.e., PPFD below 400 µmol m^{-2} s^{-1}, may be related to their feeding habit, where damage is initially caused on the abaxial leaf surface, within the spongy mesophyll layer (Welter, 1989). Evans et al. (1993) has shown that there is a gradient in light absorption, and hence photosynthetic capacity,
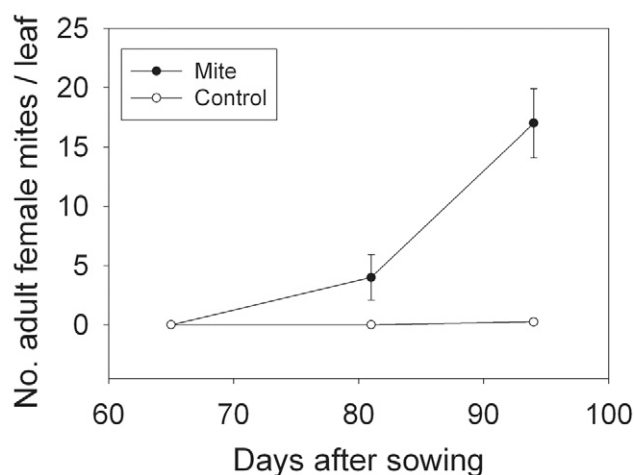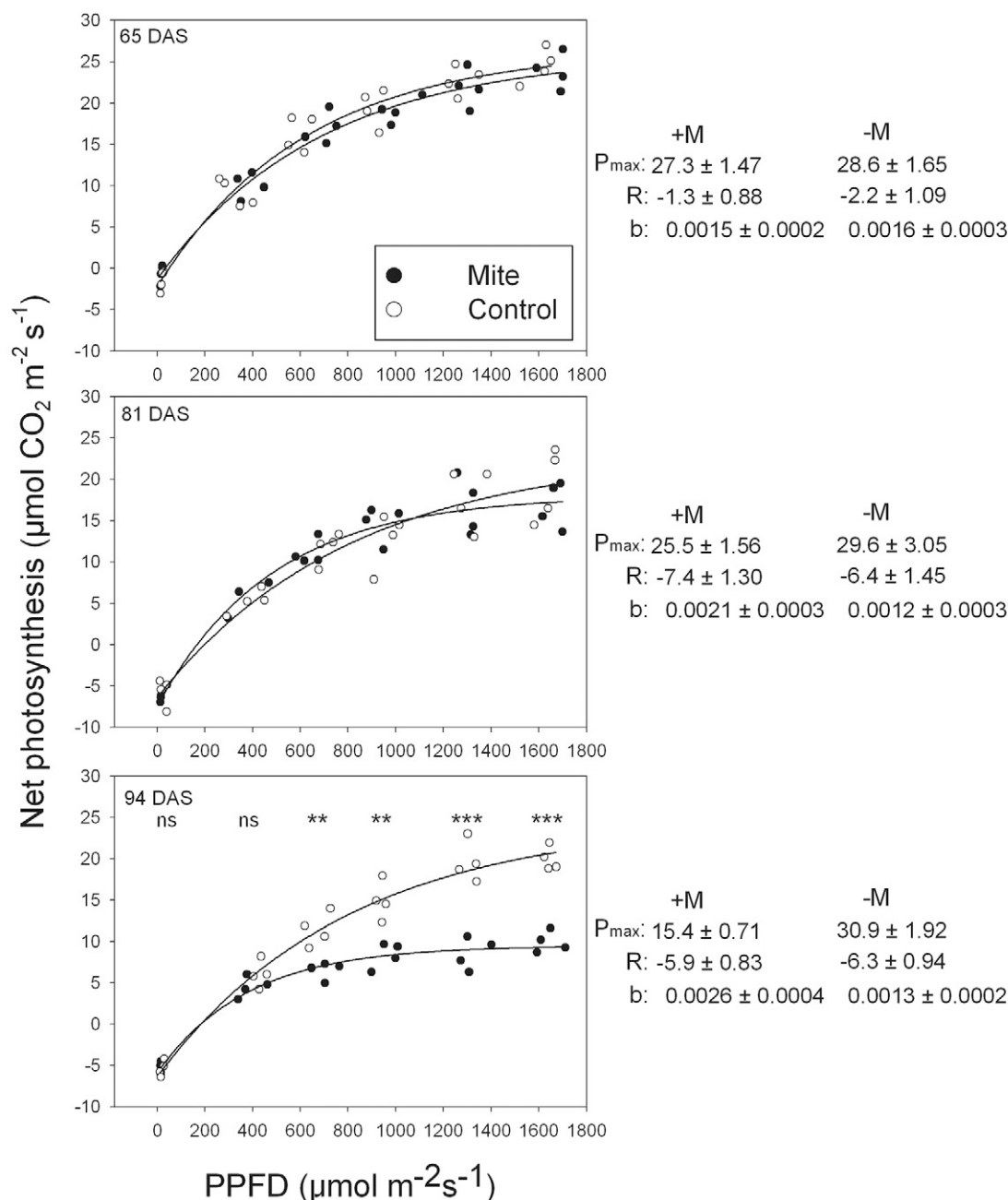

Figure 1. Number of adult female *T. urticae* per leaf for L1 leaves in mite-infested and control plants grown in pots in the field. Error bars 2 SEM, and are not shown when smaller than the symbol.

Figure 2. Relationship between net photosynthesis and photosynthetic photon flux density (PPFD) for mite-infested (+M) and control (−M) leaves of cotton plants grown in pots in the field. Effect of mite treatment for each PPFD level is ns = not significant; ** $P < 0.01$; *** $P < 0.001$. Boxes show parameters (mean ± SE) of the light-response curve (Eq. [1]), i.e., light-saturated photosynthesis (P_{max}, μmol CO_2 m^{-2} s^{-1}), respiration (R, μmol CO_2 m^{-2} s^{-1}), and a fitted parameter of curvature [b, (μmol m^{-2} s^{-1}) $^{-1}$]. DAS, days after sowing.

through *Eucalyptus* leaves which affects the shape of the light-response curves. It is possible that at low light levels, most light is being absorbed by the undamaged adaxial layers (epidermis and palisade cells) of mite-damaged leaves, hence there is little or no effect on photosynthesis compared with undamaged leaves. As light levels increase and there is greater penetration of light through the leaf, the effect of mite damage to spongy mesophyll means these cells, in damaged areas, are less active than similar cells on undamaged leaves.

The lack of photosynthetic response to mites under low light may have implications for partially shaded leaves in the canopy profile, and is important for scaling-up growth calculations from leaf to canopy. The level of light at which mite damage results in a reduction in photosynthesis, however, may be variable as light-response curves may differ at different canopy positions (Campbell et al., 1992), with lower/older and very young cotton leaves higher in the canopy reaching light saturation at lower irradiance than fully expanded leaves in the upper mid canopy (Constable and Rawson, 1980).

As both the leaf and mite populations were included in the LiCor 6400 chamber in all experiments we calculated the likely CO_2 contribution of the mites to see if it could significantly affect photosynthesis calculations.
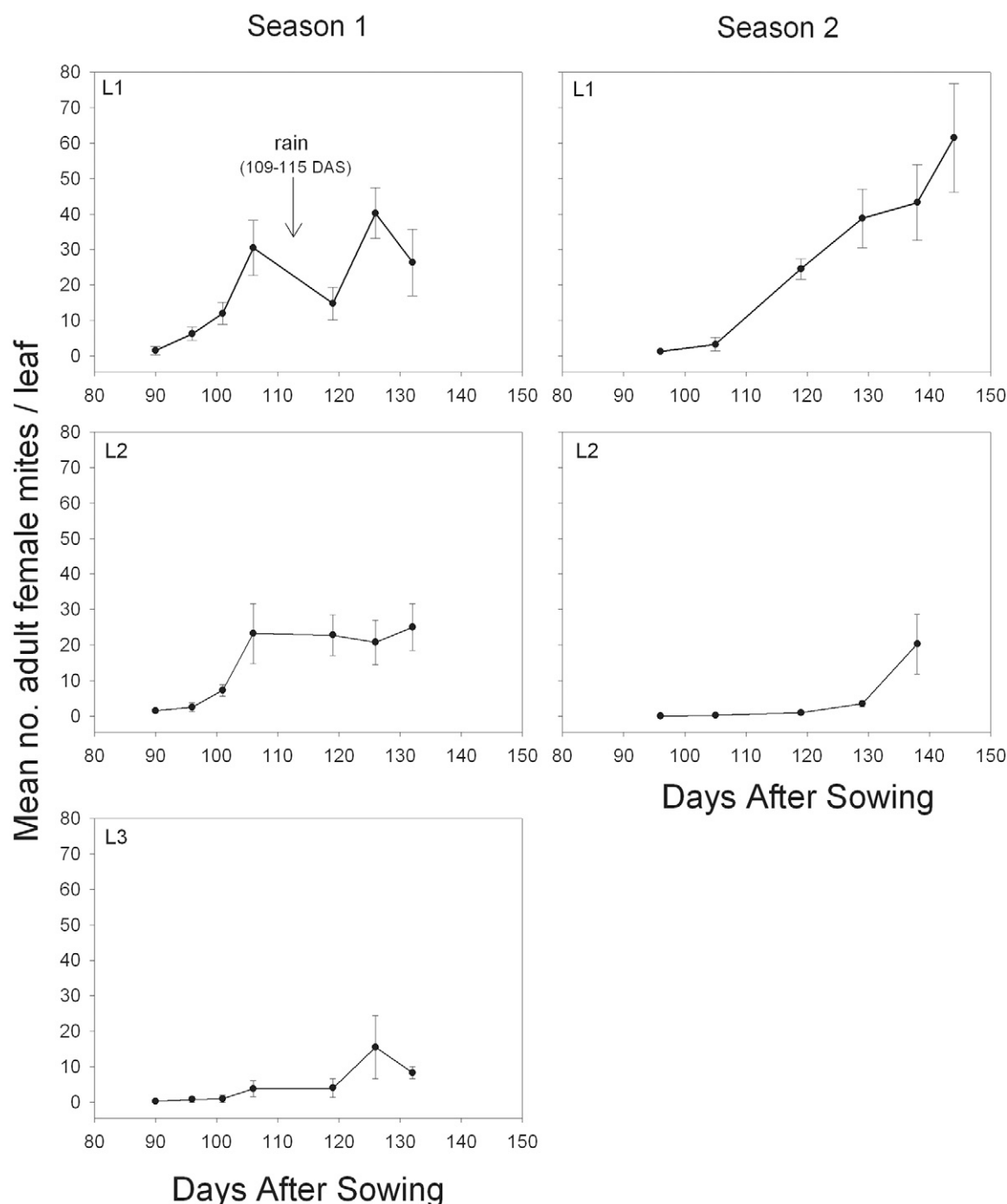
Figure 3. Number of adult female *T. urticae* in top (L1), medium (L2), and low (L3) canopy positions of mite-infested crops during two seasons. Error bars are 2 SEM and are not shown when smaller than the symbol. DAS, days after sowing.

Estimates of the respiration rate (nL O_2 μg live weight^{-1} h^{-1}) of mite adults, nymphs, and eggs of *T. cinnabarinus* Boisduval, which is closely related to *T. urticae*, were obtained from Thurling (1980). These were converted to micromoles of CO_2 per microgram live weight per hour, then to micromoles of CO_2 per mite stage per hour. The peak density of adult female mites at 94 DAS was about 17 mites leaf^{-1}. Using the stable age distribution found by Carey (1983) we calculated the number of mite nymphs and eggs expected for a population of

17 female mites (males are not abundant), then multiplied this up to a square meter assuming an average fully expanded cotton leaf has an area of about 70 cm^2 (Wilson, 1994). This provided an estimate of micromoles of CO_2 for each mite stage per square meter per hour, which was then divided by 3600 to provide CO_2 production in micromoles of CO_2 for each mite stage per square meter per second. This was totaled across mite stages to yield a value of 0.0026 μmol CO_2 m^{-2} s^{-1} which is about 1/10,000th of the CO_2 (31 μmol CO_2 m^{-2} s^{-1}) exchanged by control leaves at 94 DAS (e.g.,
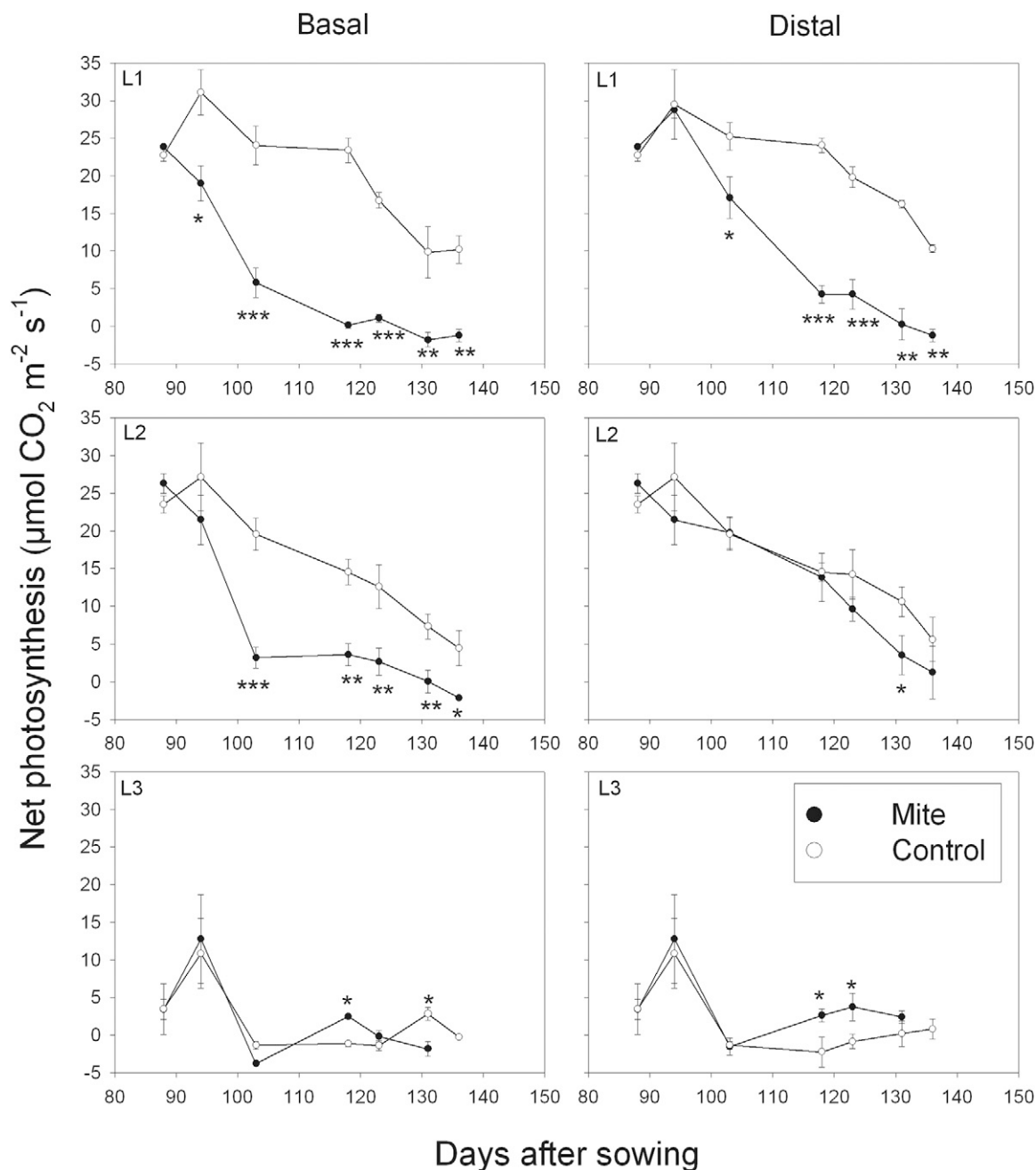
Figure 4. Net photosynthetic rate of leaves in top (L1), medium (L2), and low (L3) canopy positions in Season 1. Measurements were taken in basal and distal leaf sections. Error bars are 2 SEM and asterisks indicate mite effects: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

0.0026/31), so the contribution of mite respiration to the results was trivial, at a density of 17 adult mites leaf^{-1} in the light response experiment, or even at the peak of 60 adult females leaf^{-1} at L1 in Season 2.

Wilson (1993) and Wilson and Morton (1993) characterized mite distribution in canopy profiles and individual leaves through the crop cycle for typical irrigated cotton crops in Australia. At the canopy level, colonies are usually established around the fourth node from the top (L1 in this paper), and move downward later in the season. At the level of individual leaves, colonies are first established in basal leaf sections, where they are relatively protected under the thicker bound-

ary layer associated with thicker leaf veins. The spatial and temporal dynamics of leaf photosynthesis in our field experiments (Fig. 4 and 5) reflected the combined effect of crop ontogeny and mite dynamics. Photosynthesis declined with crop age, but the rate of decline was generally faster in mite-infested leaves, particularly the L1 leaves in the upper canopy. The "wave" of mite damage, as reflected in reduced photosynthesis and relative chlorophyll content, progressed downward in the canopy and from basal to distal leaf positions. Though mites reduced photosynthesis in lower leaves (L1 and L2) the effect of lower light intensity, due to shading was probably the most limiting factor, especially for L3
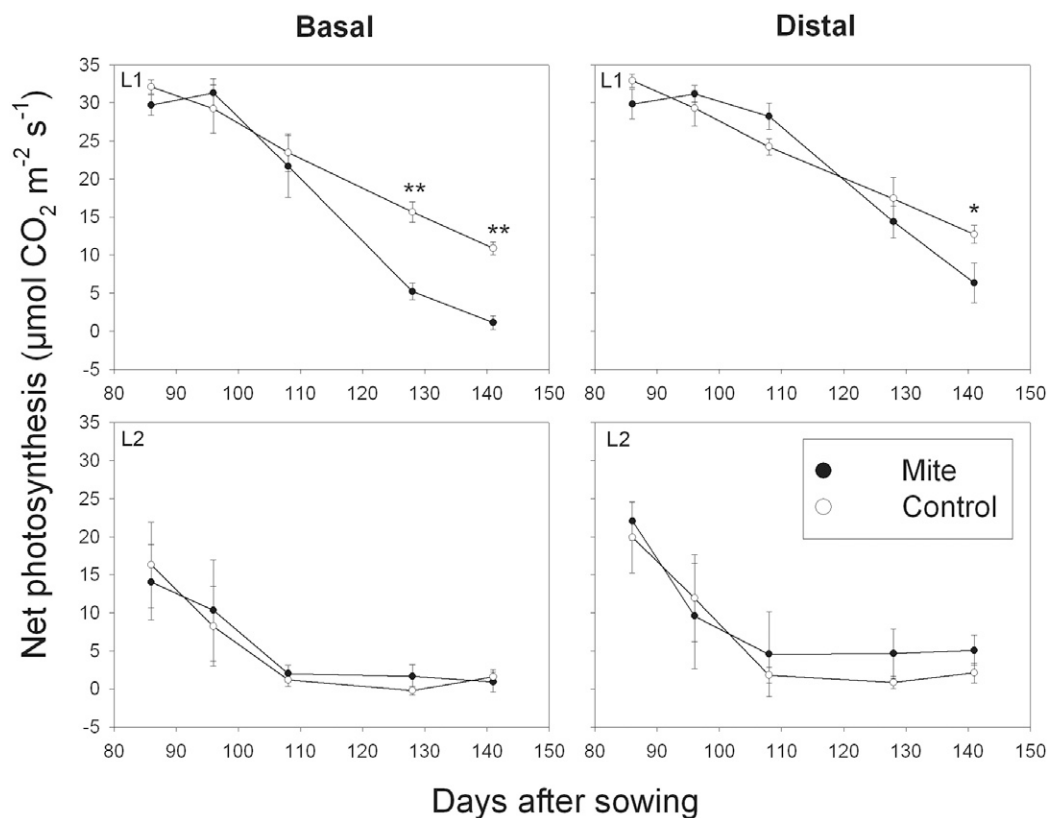
Figure 5. Net photosynthetic rate of leaves in top (L1), and medium (L2) canopy positions in Season 2. Measurements were taken in basal and distal leaf sections. Error bars are 2 SEM and asterisks indicate mite effects: *$P < 0.05$; **$P < 0.01$.
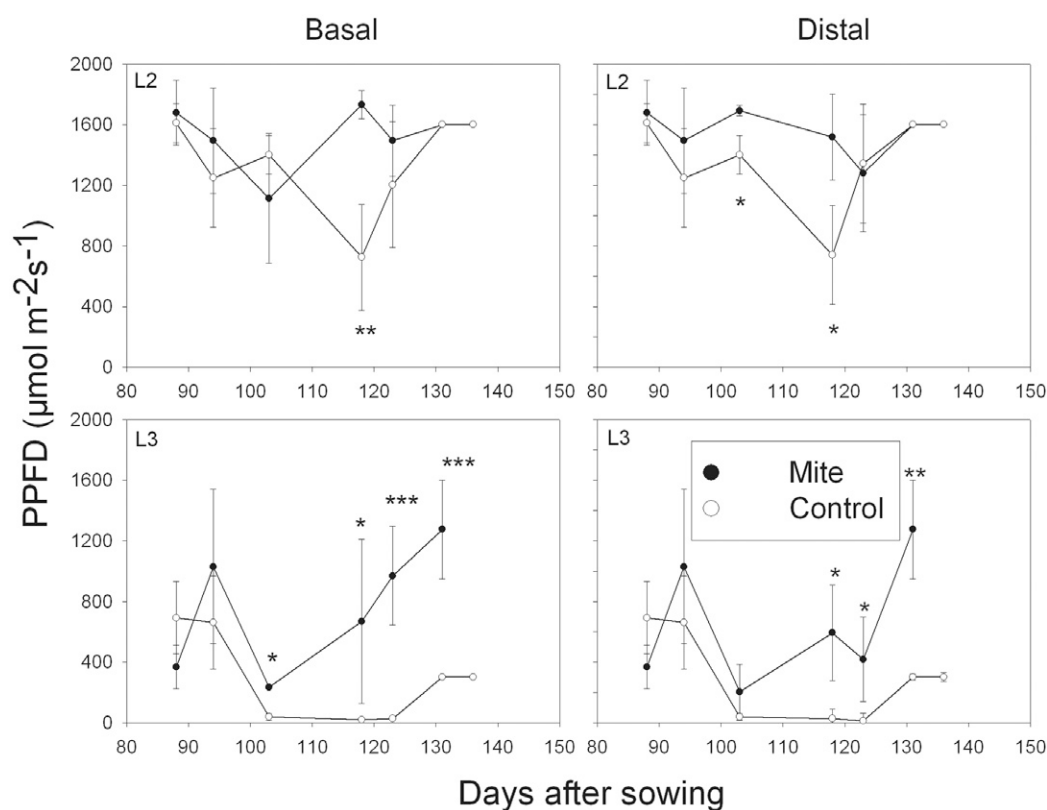
Figure 6. Dynamics of photosynthetic photon flux density (PPFD) incident at the leaf surface for leaves in mid (L2) and bottom (L3) canopy positions in mite-infested (+M) and uninfested controls (–M) in Season 1. Measurements were taken in basal and distal leaf sections. Error bars 2 SEM, and asterisks indicate significance on mite effects: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
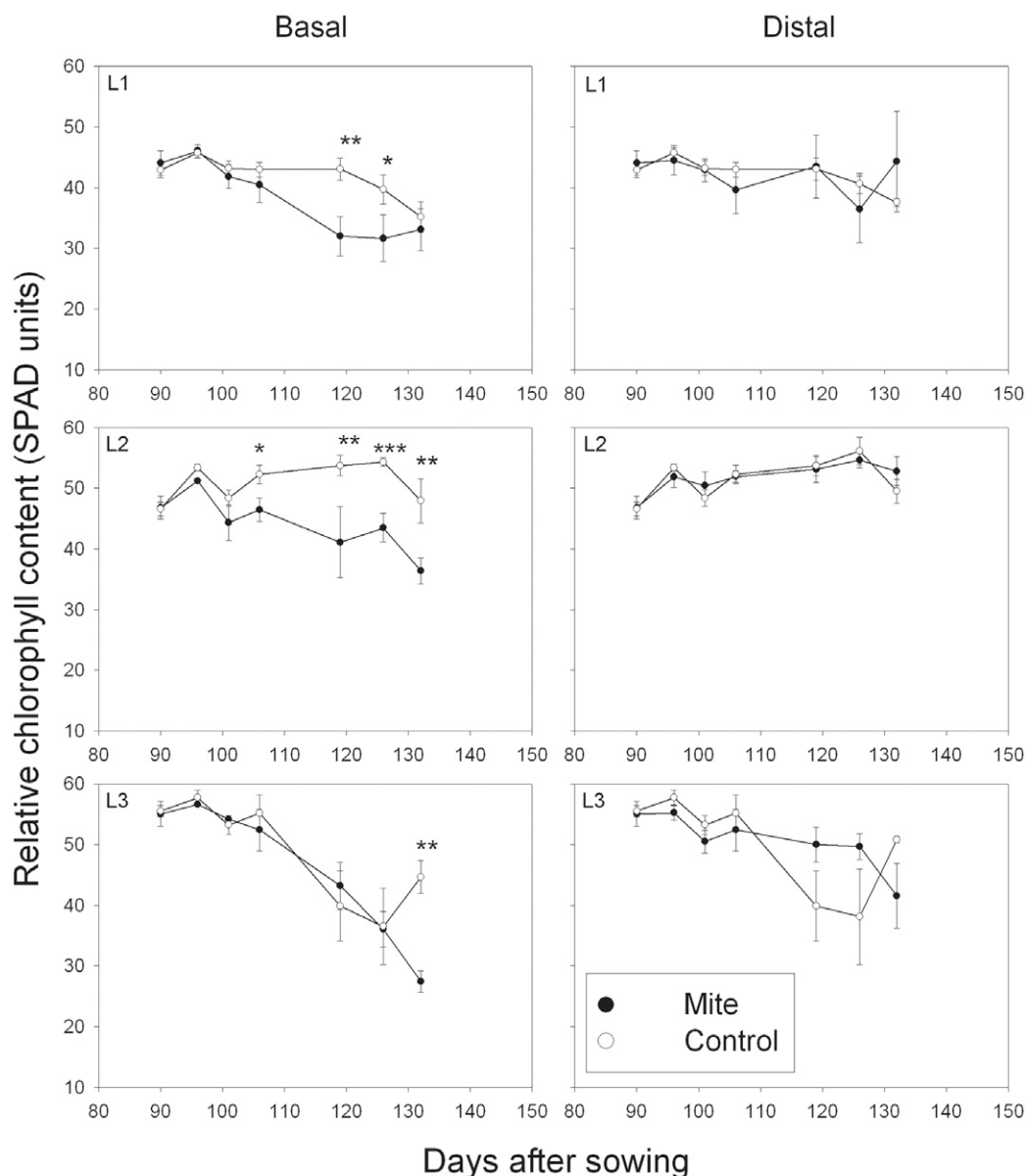
Figure 7. Relative chlorophyll content of leaves in top (L1), medium (L2), and low (L3) canopy positions of mite-infested (+M) and control (−M) crops in Season 1. Measurements were taken in basal and distal leaf sections. Error bars are 2 SEM and asterisks indicate mite effects: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. See "Methods" for an explanation of SPAD units.

leaves. Temporal and spatial patterns in photosynthesis thus closely reflected the temporal and spatial distribution of mites in both the canopy profile and within leaf sections and also leaf position, with L2 and L3 leaves having lower photosynthesis than L1 leaves due to less light.

The response of canopy radiation use efficiency (i.e., biomass per unit intercepted radiation) to mite infestation reported by Sadras and Wilson (1997a) had two phases. Little or no response for infestations below about 20 adult females per leaf, measured at L1, followed by a sharp reduction in canopy radiation use efficiency for more intense infestations. Here we measured photosynthetic rate in basal and distal leaf sections, and in leaves from upper (L1), middle (L2), and lower (L3) sections of the profile. These direct measurements revealed no compensatory photosynthesis in the early stages of infestation.

Distal leaf sections had similar relative chlorophyll concentration and similar photosynthetic rates in +M and −M plants. For most of the growing season, the photosynthetic rate of undamaged leaves in damaged plants (leaves L2 and L3) was similar to that in their uninfested counterparts. These results therefore indicate that the stability of crop photosynthesis reported by Sadras and Wilson (1997a) for the early stages of mite infestation is not due to within-plant or within-leaf increases in photosynthesis in undamaged areas. Instead it is likely that at low levels of mite damage a relatively small proportion of the crop leaves are affected—hence reduction in photosynthesis, though significant on the leaves affected, is not significant when viewed at the crop level. As mite populations increase they spread not only to lower main-stem leaves but also to secondary leaves on both vegetative and fruiting branches
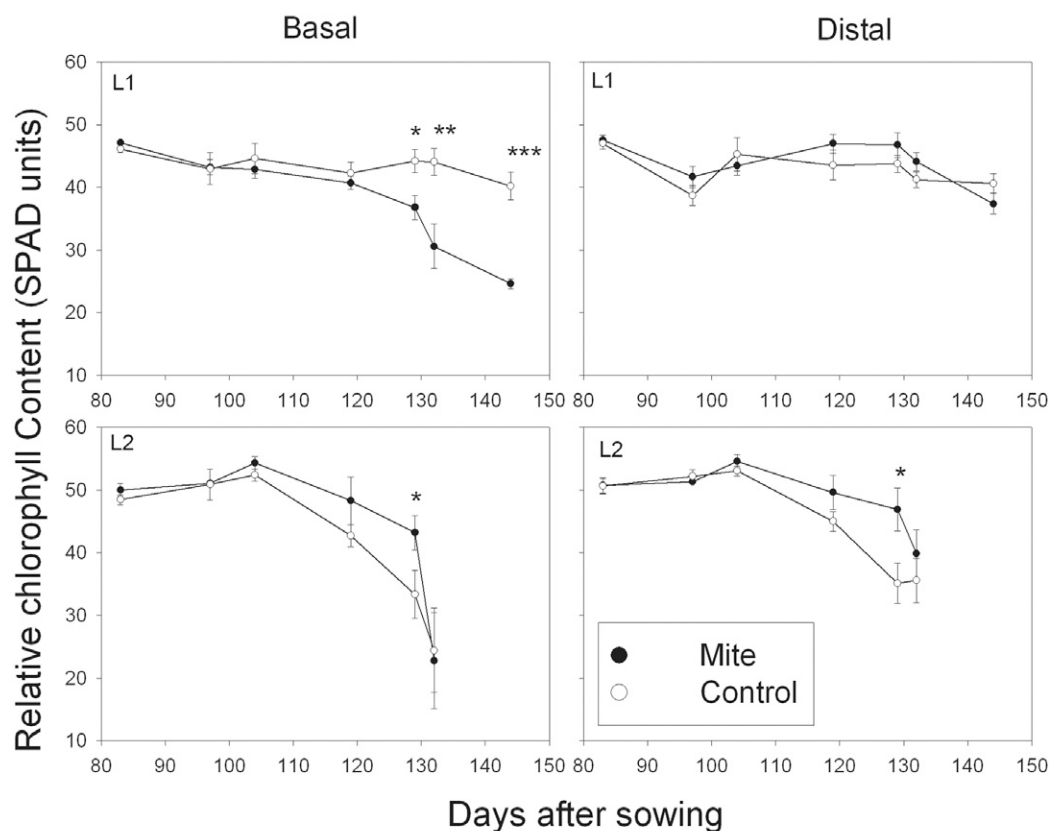
Figure 8. Relative chlorophyll content of leaves in top (L1), medium (L2), and low (L3) canopy positions of mite-infested (+M) and control (−M) crops in Season 2. Measurements were taken in basal and distal leaf sections. Error bars are 2 SEM and asterisks indicate mite effects: *P < 0.05, **P < 0.01. See "Methods" for an explanation of SPAD units.

(Carey, 1982). Hence, it is likely that an increasing mite population leads to damage on an increasing proportion of leaves, and to a larger effect on crop photosynthesis.

Compensatory photosynthesis was found at the crop level in advanced stages of mite infestation, when upper portions of the crop (e.g., L1 and young and fully expanded leaves in this zone of the canopy) were severely damaged, causing rapid leaf senescence. This allowed for greater PPFD incidence in and regreening of bottom leaves, which accounted for their increased photosynthetic rate. Seventy-five percent of the variation in the rate of photosynthesis of bottom leaves in +M and −M plants was explained by variation in PPFD (data not shown). The contribution of this compensatory mechanism to whole-plant C economy is likely to be negligible, as the photosynthetic rate of leaves at the bottom of the canopy was very small. The regreening at the bottom of the open canopy following severe mite infestation, however, may be important in other systems, such as perennials that could therefore initiate active regrowth.

Acknowledgments

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A Comparison of Arthropod Communities in Transgenic *Bt* and Conventional Cotton in Australia

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ABSTRACT   Transgenic *Bacillus thuringiensis* (*Bt*) cotton has had a major impact on the Australian cotton industry by largely controlling lepidopteran pests. However, it also may have other impacts on the invertebrate community that need to be identified. We compared the canopy invertebrate community in sprayed conventional, unsprayed conventional, and unsprayed *Bt* cotton over three seasons using suction sampling methods. We found that the diversity or species richness of the beneficial communities was reduced in the sprayed crops at two sites. Although spraying had the strongest effect on the community, there was a slight difference between the total community in unsprayed conventional and *Bt* crops, with crop type accounting for 4.5% of the variance between these communities. Out of over 100 species groups examined, the most consistent differences between unsprayed *Bt* and conventional communities were higher numbers of *Helicoverpa* in conventional crops (as would be expected) and slightly higher numbers of Chloropidae and Drosopillidae (Diptera), damsel bugs (Hemiptera, Nabidae), and jassids (Hemiptera, Cicadellidae) in conventional crops. With the advent of Bollgard II and the possibility that 80% of the cotton crop in Australia could be transgenic, the effects of these small differences in the transgenic and conventional communities should be monitored over the long-term to assess if any modifications to cotton management practices need to be made.

KEY WORDS   *Bacillus thuringiensis*, *Helicoverpa*, Cry1Ac, beneficals, biodiversity

LEPIDOPTERAN SPECIES, particularly *Heliothis* and *Helicoverpa* species, are key pests of cotton worldwide, capable of dramatically reducing cotton yield through damage to flower buds (squares) or maturing fruit (bolls) (Luttrell et al. 1994). Control of these pests has often relied on the use of broad-spectrum insecticides, which disrupt beneficial populations, often leading to pest resurgence and outbreaks of secondary pests, as well as risks of off-farm movement of pesticides and environmental contamination. Therefore, these pests have been a major challenge to the development of integrated pest management (IPM) systems in cotton.

IPM practitioners have long sought alternatives that are efficacious against lepidopteran pests, selective against beneficials and with low mammalian toxicity or environmental risk. One such option has been the bacterium *Bacillus thuringiensis* variety *kurstaki* Berliner (*Bt*), which has been cultured commercially and formulated as a biopesticide spray against *Heliothis*/*Helicoverpa* for over 30 yr (Van Rie 2000). The sprayed formulations contain a number of Cry toxins as well as the infective spore. Unfortunately, although the spray is selective against most beneficial groups and has low mammalian toxicity and environmental risk, its efficacy is variable and generally poor compared with conventional insecticides, and this has limited its use and value for IPM in cotton.

One *Bt* protein, the Cry IAc δ endotoxin, also has been available commercially in genetically modified cotton (*Bt* cotton) since 1996 (Perlak et al. 2001). Here we use *Bt* cotton to designate plants expressing only the Cry1Ac protein (known as Ingard in Australia and Bollgard® elsewhere in the world). In contrast to *Bt* sprays, *Bt* cottons have provided much more consistent control of *Heliothis*/*Helicoverpa* spp. and have had a major impact on cotton production wherever they have been commercially adopted by significantly reducing pesticide inputs (Benedict and Altman 2001, Fitt and Wilson 2000, Perlak et al. 2001, Qaim 2003). As a result, *Bt* cotton has provided a valuable tool for developing IPM strategies in cotton (Wilson et al. 1998, Fitt and Wilson 2000, Wu 2001).

The sprayable form of *Bt* differs from the transgenic form. The *Bt* spray contains Cry proteins, present in a nonactivated form that must be activated in the insect's gut to be toxic, whereas *Bt* cotton has a truncated form of one insecticidal protein (Cry IAc) that does not require additional activation (Van Rie 2000). *Bt*

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sprays are UV susceptible, breaking down quickly, and because coverage is variable, the toxic components in the spray are not delivered to the target in a consistent dose. In contrast, in *Bt* cotton, the *Bt* is present throughout the growing season, although parts of the plant express different amounts of *Bt*, and the overall concentration of *Bt* declines as the season progresses (Fitt et al. 1994, 1998). Because of these differences, it is possible that the Cry1Ac protein produced in the plant interacts differently with the arthropod community than do *Bt* sprays.

Bt cotton will alter the arthropod community directly by reducing the abundance of *Helicoverpa* spp. (Hoffmann et al. 1992, Jenkins 1994) and some other lepidopteran species (Wilson et al. 1992, Flint et al. 1995). *Bt* cotton may also have indirect, although expected, effects on the abundance of predators and parasitoids that specialize on larvae of *Helicoverpa* spp. or other lepidopteran species controlled by Cry1Ac (Luttrell et al. 1994, Fitt and Wilson 2000). Whether such indirect effects extend to other, nontarget organisms is less clear and was the main rationale for this work. Hilbeck reported a tritrophic effect in laboratory studies of *Bt* corn, where lacewings [*Chrysoperla carnea* (Stephens)] that were fed on the caterpillar *Spodoptera littoralis* (Boisduval) that had fed on Cry1Ab had reduced survival compared with controls *Bt* corn containing the toxin Cry1Ab (Hilbeck et al. 1998, 1999). Lacewings were not affected when they fed on mites that had fed on *Bt* corn even though the mites contained more *Bt* toxin than the larvae, suggesting that there was an interaction between the toxin and the caterpillar (Dutton et al. 2002, 2003) that reduced the suitability of this already low-quality prey. Romeis et al. (2004) showed no direct effect of *Bt* protein on lacewings and showed clearly that the effects reported by Hillbeck were caused by reduced prey quality. Furthermore, because lacewings prefer aphids (which retain little or no *Bt* in their bodies) rather than caterpillars as prey (Meier and Hilbeck 2001), it is unclear whether the tritrophic effect observed in the laboratory would translate into lower numbers of this predator in *Bt* maize fields or in *Bt* cotton fields.

With the introduction of Bollgard II® cotton, which has two *Bt* genes (Cry1Ac and Cry2Ab), the majority (≈70% in 2004) of the cotton crop in Australia is now transgenic (A. Hurst, personal communication). Under these conditions, even a small difference in the invertebrate community could have a compounding effect. For instance, subtle effects on a component of the beneficial fauna, not easily detected in small plot research, may become more significant in the survival of a particular organism as the scale of *Bt* cotton production increases. In addition, changes in abundances of other animals that are neither pests nor beneficials could influence pest and beneficial abundances through the food web. Thus, it is important to look for changes in the whole community

as well as changes in the beneficials. If there is a change in species composition in cotton, this could influence how cotton is managed using an IPM approach.

The aim of this study was to establish if the insect community in transgenic *Bt* cotton differs from that in unsprayed conventional cotton and, further, to compare these with the community found in the conventionally sprayed cotton system. Preliminary analysis of these data (Fitt and Wilson 2002) reported little numerical difference in the abundance of key beneficial and pest groups between unsprayed *Bt* cotton (Ingard; Cry 1Ac), stacked *Bt* cotton (Cry1Ac + Cry2Aa; a forerunner of later Cry1Ac/Cry2Ab combinations now commercialized as Bollgard II®), or conventional cotton. Sprayed cotton, in contrast, had significantly reduced beneficial populations. In this analysis, the aim was to more thoroughly explore the data set using ordination techniques to examine "whole of community" patterns and to ask the following: (1) are there functional groups within invertebrate families that are more affiliated with *Bt* or conventional cotton; (2) are there specific species more affiliated with *Bt* or conventional cotton; and (3) if there is no significant change in individual species, does the overall community structure of *Bt* and conventional cotton differ?

Materials and Methods

Study Sites and Agronomic Management. Experiments were carried out on three commercial cotton farms: Doreen (30°00′, 149°17′) in the Namoi Valley, Auscott Ewenmar "Ewenmar" (31°42′, 147°56′) in the Macquarie Valley, and Auscott Narrabri "Auscott" (30°12′, 149°33′) in the Namoi Valley (see Table 1 for details). Fields were selected because they were relatively isolated from other sprayed cotton, therefore reducing the chance of insecticide drift across the unsprayed areas. All the experiments involved fertilized, irrigated cotton grown on beds 1 m apart with agronomic practices that followed commercial "best practice."

Experiments were conducted over three seasons (1995/96, 1997/98, 1998/99). Doreen and Ewenmar were sampled in 1995/96, Doreen in 1997/98, and Auscott in 1998/99 (Table 1).

At each site there were three or four treatments: unsprayed conventional cotton, unsprayed *Bt* cotton (Ingard, Cry1Ac only), unsprayed stacked *Bt* cotton (Cry1Ac + Cry2Aa), and sprayed conventional cotton (Fig. 1). In the 1995/96 and 1997/98 seasons, the unsprayed conventional, unsprayed *Bt*, and unsprayed *Bt* stacked plots (included in 1997/98) were replicated twice, whereas during the 1998/99 season, all three unsprayed treatments were replicated three times. The sprayed conventional cotton treatment was not replicated in any year, although there was replication of the sampling effort. Replication of the insecticide sprayed treatment among the unsprayed treatments would have greatly increased the risk of disruption of the unsprayed treat-

Table 1. Cotton varieties and constructs used at the different sites

Season	Farm	Plot size (ha)	Planting date	Treatment	Cotton variety	*Bt* gene	*Bt* construct
1995/96	Doreen	5.17 and 4.8	12th Oct	Unsprayed *Bt*	DPL5415	Cry1Ac	MON531
		1.0 and 1.2	12th Oct	Unsprayed Conventional	DPL5415	—	—
		40.0	12th Oct	Sprayed Conventional	DPL5415	—	—
1995/96	Ewenmar	5.4 and 5.3	27th Oct	Unsprayed *Bt*	Sicala V2i	Cry1Ac	MON757
		2.4 and 1.6	27th Oct	Unsprayed Conventional	Sicala V2	—	—
		80.0	6th Oct	Sprayed Conventional	Sicala V2	—	—
1997/98	Doreen	1.9	15th Oct	Unsprayed *Bt*	Siokra V15i	Cry1Ac	MON757
		1.9	15th Oct	Unsprayed *Bt* stacked	Siokra V15 Stacked	Cry1Ac Cry2Aa	MON757 MON1849
		1.9	15th Oct	Unsprayed Conventional	Siokra V15	—	—
		4.56	15th Oct	Sprayed Conventional	Siokra V15	—	—
1998/99	Auscott	1.2	30th Sept	Unsprayed *Bt*	Siokra V15i	Cry1Ac	MON757
		1.2	30th Sept	Unsprayed *Bt* stacked	Siokra V15 Stacked	Cry1Ac Cry2Aa	MON757 MON1849
		1.2	30th Sept	Unsprayed Conventional	Siokra V15	—	—
		3.2	10th Oct	Sprayed Conventional	Siokra V15	—	—

ments with insecticide drift. This risk is exacerbated late in the season when the frequency of irrigation and the dense crop canopy preclude the use of a ground sprayer and necessitates aerial application of insecticides.

Pests in the sprayed portion of the field were managed by a professional cotton consultant who checked the field every 3–4 d and advised the grower when the crop needed spraying and the most appropriate insecticide to apply. In the 1995/96 season, the unsprayed plots were sprayed with a selective aphicide, Pirimicarb (Pirimor at 500 g/ha) to control aphids (*Aphis gossypii* Glover) that would have caused economic damage to the cotton line through honey dew contamination if left unchecked. These applications occurred on 22 February at Doreen (before the fourth to last sample) and 13 February at Ewenmar (before the fourth to last sample).

Sampling. To assess insect abundance in the crop canopy, we used a suction sampler (mini Blower Vac, Homelite B180v; Ryobi Technologies, Milperra, Australia). Samples were taken weekly (1995/96) or fortnightly (1997/98, 1998/99) from the central rows of the replicated plots. In the sprayed plots, samples were taken from two (1995/96, 1997/98) or three sites (1998/99) within the sprayed plot. Sampling began at seedling emergence and continued until ≈20% of the bolls had opened. At each replicated plot or sampling site we took five (1995/96, 1997/98) or three (1998/99) replicate suction samples, each of 10 m along a row.

To sample the cotton using the suction sampler, a single pass was made over the cotton while it was young, but for larger plants, the suction sampler was swept back and forth three times from the bottom of the plants to the top in a zigzag pattern. This was done to ensure that all strata of the plant were sampled. Collected samples were taken back to the laboratory where they were killed and counted under a dissecting microscope.

Taxonomy. The 1995/96 samples were identified at least to family and often to species for most insects and to order for most other invertebrates. Identifications were conducted using a reference collection of cotton insects at ACRI, Narrabri, or were sent for identification to the Australian National Insect Collection, Commonwealth Scientific and Industrial Research Organization Entomology, Canberra (where voucher specimens are located). Because of the large number of "unknown" insect species and the chance that many of these could have been the same species, we analyzed these samples at the level of family for most insects and order for most other invertebrates (Table 2). For the 1997/98 and 1998/99 samples, we applied a standard suction sampling classification scheme used in Australia where only key pests, predators, and parasites were identified to species level, sometimes even to developmental stage, and other insect species were identified only to order (Room and Wardhaugh 1977, Pyke and Brown 1996, Deutscher et al. 2005; Table 2). The animals were classified as predators, pests, or others.

Statistical Analysis. Because diversity indices differ in their strengths and weaknesses, it is unwise to rely on one index (Tothmeresz 1995). Consequently, we
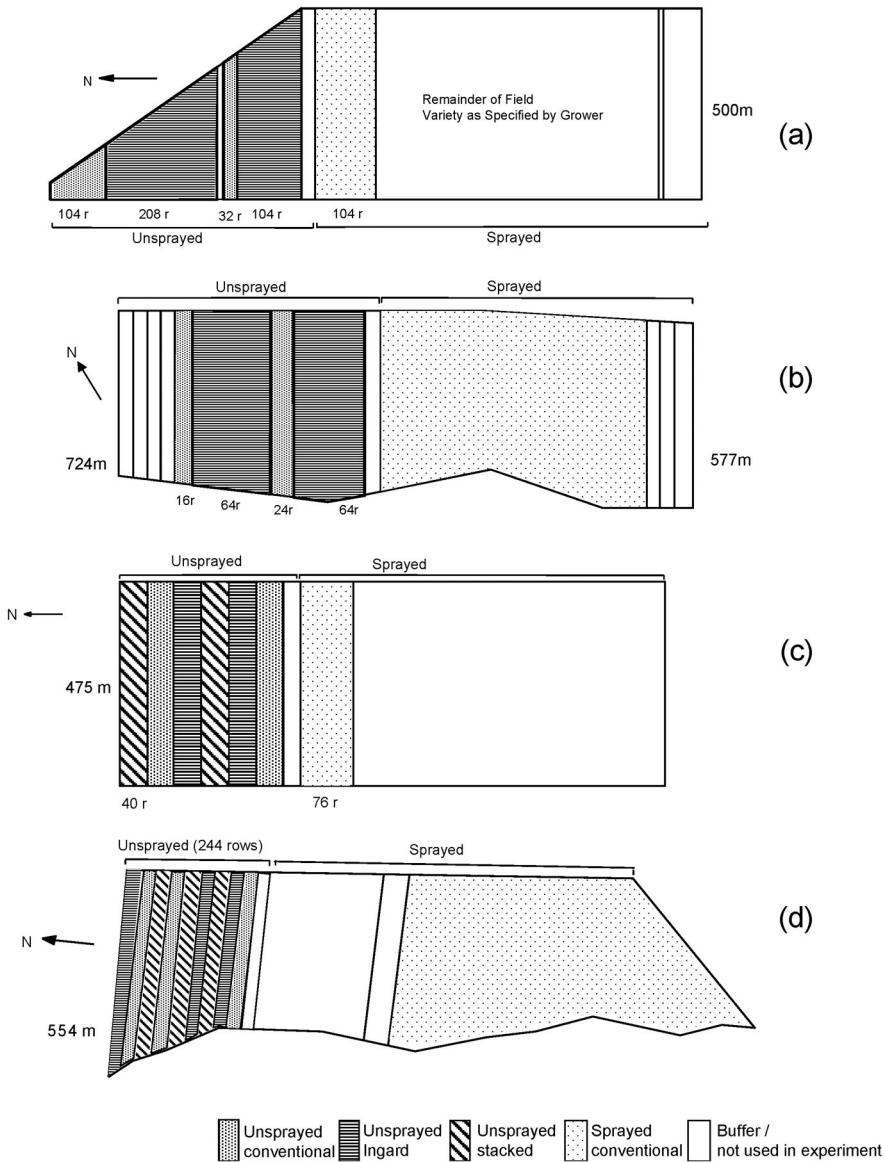
Fig. 1. Layout of the fields used in this analysis. The shaded sections are those used in the experiments. (a) Doreen, 1995/96 season. (b) Ewenmar, 1995/96 season. (c) Doreen, 1997/98 season. (d) Auscott, 1998/99 season.

used the Simpson index (SI; Simpson 1949) and Shannon Weaver index (SW; Shannon and Weaver 1949), both of which are members of Renyi's diversity index family, to measure diversity, and rarefaction curves (Sanders 1968) to measure species richness of beneficial populations. Diversity indices of the beneficial communities in each plot for each date were compared for each site using repeated-measure analyses of variance (ANOVAs), calculated using the program GENSTAT (Payne 2000). When a significant difference was detected, we compared indices using the LSD. To calculate the rarefaction curves, we used

the program developed by S. M. Holland, which is available at www.uga.edu/≈strata/software/AnRare Readme.html. The Simpson index was corrected for sample-size bias $\{SI = \Sigma[(n^2 - n)/(N^2 - N)]\}$ and modified ($-lnSI$) following Rosenzweig (1995) so that the units increase with an increase in diversity. The Simpson index is more sensitive to dominant species, whereas the Shannon Weaver index ($H = -\Sigma p_i ln p_i$) is more sensitive to rare species.

The data were examined using principle response curve (PRC) analysis, which is a multivariate method for the analysis of repeated measures and is designed

Table 2. All the animal groups identified in the surveys, including their common name (when present) and the number of individuals found at each site

Role	Order	Family	Genus	Common name	Other characteristics	Ewenmar 1995/96 Totals	Doreen 1995/96 Totals	Doreen 1997/98 Totals	Auscott 1998/99 Totals
P	Acarina	Tetranychidae		Mites	Adult	1	3,964	39	2,247
P	Acarina	Tetranychidae		Mites	Juvenile			17	671
P	Acarina			Brown mites					7
B	Araneae	Clubionidae		Sac spider		180			
B	Araneae	Oxyopidae		Lynx spider		167			
B	Araneae	Salticidae		Jumping spider		63			
B	Araneae	Theridiidae		Tangle-web spider		45			
B	Araneae	Thomisidae		Crab spider		21		16	209
B	Araneae	"Unknown"		Spiders		1,701	1,317	1,251	1,911
P	Blattodea			Cockroaches			3		
O	Coleoptera	Anthicidae				271	93	15	
B	Coleoptera	Cantharidae				1			
B	Coleoptera	Carabidae		Ground beetles/tiger beetles		19	13		
P	Coleoptera	Cerambycidae		Long horn beetles		9			
P	Coleoptera	Chrysomelidae		Flea Beetle		226	1,265	85	244
B	Coleoptera	Coccinellidae	"Unknown"	Lady beetles		283	307	99	170
B	Coleoptera	Coccinellidae	Diomus noescens (Blackburn)	"Minute two-spotted ladybird"				88	86
P	Coleoptera	Curculionidae		Weevils		38	70		34
P	Coleoptera	Elateridae		Wire worms			5		
O	Coleoptera	Languriidae				4			
O	Coleoptera	Lathridiidae				598	217		
B	Coleoptera	Melyridae	Dicranolaius bellulus (Guérin-Méneville)			692	123	110	73
O	Coleoptera	Mycetophagidae		Hairy fungus beetles		13	20		
O	Coleoptera	Nitidulidae	Carpophilus	Dried fruit beetles		191	159		3
O	Coleoptera	Phalacridae				23	264		
O	Coleoptera	Staphylinidae		Rove beetles		6	4		
P	Coleoptera	Tenebrionidae		Flour beetles		1	2		146
B	Coleoptera	"Unknown"			Predatory	2	2	256	581
P	Coleoptera	"Unknown"			Pests			25	19
B	Coleoptera	"Unknown"							
B	Dermaptera			Earwigs					
B	Dermaptera	Labiduridae		Earwigs		1	3	1	
P	Diptera	Agromyzidae			Leaf-mining flies				
O	Diptera	Anthomyiidae					3		
O	Diptera	Cecidomyiidae				20	4		
O	Diptera	Ceratopogonidae				4	23		
O	Diptera	Chironomidae				102	794		
O	Diptera	Chloropidae				1,862	11,196		
P	Diptera	Dolichopodidae				10,802	2		
O	Diptera	Drosophilidae		Fruit flies		5	188		
O	Diptera	Empididae				460	10		
O	Diptera	Ephydridae				10	21		
O	Diptera	Fergusoninidae				29	1		
O	Diptera	Heleomyzidae				5	21		
O	Diptera	Lauxaneidae					30		
O	Diptera	Muscidae		House flies		18	35		
O	Diptera	Phoridae				11	20		

	Order	Family	Species	Common name				
O	Diptera	Pipunculidae			3			
O	Diptera	Platystomatidae			31	1		
O	Diptera	Psychodidae			3	8		
O	Diptera	Pyrgotidae			2	3		
O	Diptera	Scatopsidae						
O	Diptera	Sciaridae			150	478		
B	Diptera	Syrphidae		Hoverfly	3	3	4	2
O	Diptera	Tephritidae			1	5		
O	Diptera	Teratomysidae						
O	Diptera	Tipulidae			1	2		
B	Diptera	Tachinidae		Parasitoid flies	453	65	1,476	4
O	Hemiptera	Aleyrodidae	"Unknown"	Whitefly	14	18		2,781
O	Hemiptera	Alydidae				1		2,759
B	Hemiptera	Anthocoridae	*Orius* spp	Pirate Bug			15	53
P	Hemiptera	Aphididae		Aphids	25,171	72,404	9,128	158,044
P	Hemiptera	Cicadellidae		Jassids — Adults	9,450	9,252	3,827	8,826
P	Hemiptera	Cicadellidae		Jassids — Juveniles			6,601	18,008
P	Hemiptera	Scutelleridae	*Tectocoris*		19	9		1
O	Hemiptera	Pyrrhocoridae	*Dysdercus sidae* Montrouzier	"Cotton stainer"	4			
O	Hemiptera	Lygaeidae	"Unknown"	"Unknown"				
B	Hemiptera	Lygaeidae	*Geocoris lubra* Kirkaldy	"Bigeyed bug"	351	257	124	17
P	Hemiptera	Lygaeidae	*Nysius vinitor* Bergroth				39	361
P	Hemiptera	Lygaeidae	*Oxycarenus luctuosus* (Montrouzier)				24	23
P	Hemiptera	Miridae	"Unknown"		887	675	131	
B	Hemiptera	Miridae	*Campylomma liebknechti* (Girault)	Adult			131	
B	Hemiptera	Miridae	*Campylomma liebknechti* (Girault)	Juvenile			8	
P	Hemiptera	Miridae	*Creontiades dilutus* (Stål)	Adults			9	
P	Hemiptera	Miridae	*Creontiades dilutus* (Stål)	Juvenile			4	
B	Hemiptera	Miridae	*Deraeocoris signatus* (Distant)	Brokenback bug			22	34
B	Hemiptera	Miridae	*Taylorilygus pallidulus* (Blanchard)					
B	Hemiptera	Nabidae	*Nabis kinbergii* Reuter	"Damsel bug"	181	90	9	349
P	Hemiptera	Pentatomidae	*Nezara viridula* (Linnaeus)	Southern green stink bug — Adults	21	6		6
P	Hemiptera	Pentatomidae	*Nezara viridula* (Linnaeus)	Juvenile				88
P	Hemiptera	Pentatomidae	*Nezara viridula* (Linnaeus)		2	2		
B	Hemiptera	Pentatomidae	*Cermatulus nasalis* (Westwood)				4	15
B	Hemiptera	Pentatomidae	*Oechalia schellengergii* (Guérin-Méneville)					
O	Hemiptera		"Unknown"	Predatory	29		53	6
B	Hemiptera		"Unknown"					
P	Hemiptera		"Unknown"	Pests			2	15
O	Hymenoptera	Agaonidae			3	3		
O	Hymenoptera	Aphelinidae				6		
B	Hymenoptera	Apidae		Bees	15	1		
O	Hymenoptera	Bethylidae			10	13		
B	Hymenoptera	Braconidae		Parasitoid wasps	350	93		
B	Hymenoptera	Ceraphronidae			4	2		
B	Hymenoptera	Chalcididae		Parasitiod wasps	16	5		
O	Hymenoptera	Cynipidae	*Eucoilidea*		7			
O	Hymenoptera	Diapriidae		Aphid hyperparasite	28	40		
O	Hymenoptera	Elasmidae			1	2		

Table 2. Continued.

Role	Order	Family	Genus	Common name	Other characteristics	Ewenmar 1995/96 Totals	Doreen 1995/96 Totals	Doreen 1997/98 Totals	Auscott 1998/99 Totals
O	Hymenoptera	Eucoilidae					8		
O	Hymenoptera	Encyrtidae				102	56		
O	Hymenoptera	Eulophidae	"Unknown"			346	360		
O	Hymenoptera	Eulophidae	*Hemiptarsenus*		Hyperparasitoid of Leafmining fly	7	3		
O	Hymenoptera	Eupelmidae				7	6		
O	Hymenoptera	Figitidae				4			
B	Hymenoptera	Formicidae	"Unknown"	Ants		62	469	342	13
B	Hymenoptera	Formicidae	*Iridomyrmex*						84
B	Hymenoptera	Formicidae	*Paratrechina*						1
B	Hymenoptera	Formicidae	*Rhytidoponera*						2
B	Hymenoptera	Halictidae		Bees		11	1		
B	Hymenoptera	Ichneumonidae	"Unknown"		Parasitoid wasps	9	4		
B	Hymenoptera	Ichneumonidae	*Lissopimpla excelsa* (Costa)	Orchard dupe	Parasitoid wasps	1			
B	Hymenoptera	Mymaridae			Egg parasitoids	349	120		
O	Hymenoptera	Mymarommatidae					1		
O	Hymenoptera	Perilampidae				2			
B	Hymenoptera	Platygastridae	"Unknown"		Egg parasitoids	21	26		
B	Hymenoptera	Platygastridae	*Telenomus*		Egg parasitoids				22
O	Hymenoptera	Pteromalidae				132	84		
O	Hymenoptera	Scelionidae				186	83		
O	Hymenoptera	Torymidae				22	2		
B	Hymenoptera	Trichogrammatidae	"Unknown"		Egg parasitoids	198	150	4	1,448
O	Hymenoptera		"Unknown"	Wasps				350	3,196
P	Isopoda			Slater		43		2	370
P	Lepidoptera	Noctuidae	*Helicoverpa*		Larvae	741		135	
P	Lepidoptera	Noctuidae	*Helicoverpa*		Eggs			48	
P	Lepidoptera	Tortricidae	*Crocidosema plebejana* Zeller			82			24
P	Lepidoptera		"Unknown"		Moth		407	7	41
P	Lepidoptera		"Unknown"						12
B	Neuroptera		"Unknown"	Lacewings	Juveniles		27	19	104
B	Neuroptera		"Unknown"	Lacewings	Eggs				10
B	Neuroptera	Chrysopidae	*Mallada*	"Green lacewing"	Adult	18		12	167
B	Neuroptera	Hemerobiidae	*Micromus tasmaniae* Walker	"Brown lacewing"	Adult	40			70
O	Odonata			Dragon fly		11	4		
P	Orthoptera	Acrididae		Locust		12		2	
P	Orthoptera		"Unknown"	Grasshoppers			33		14
P	Thysanoptera	Thripidae		Thrips		1,066	1,371		
P	Thysanoptera	Thripidae		Thrips	Adults			4,267	4,219
P	Thysanoptera	Thripidae		Thrips	Juveniles			7,350	1,303
		TOTALS				58,556	106,838	36,020	212,622

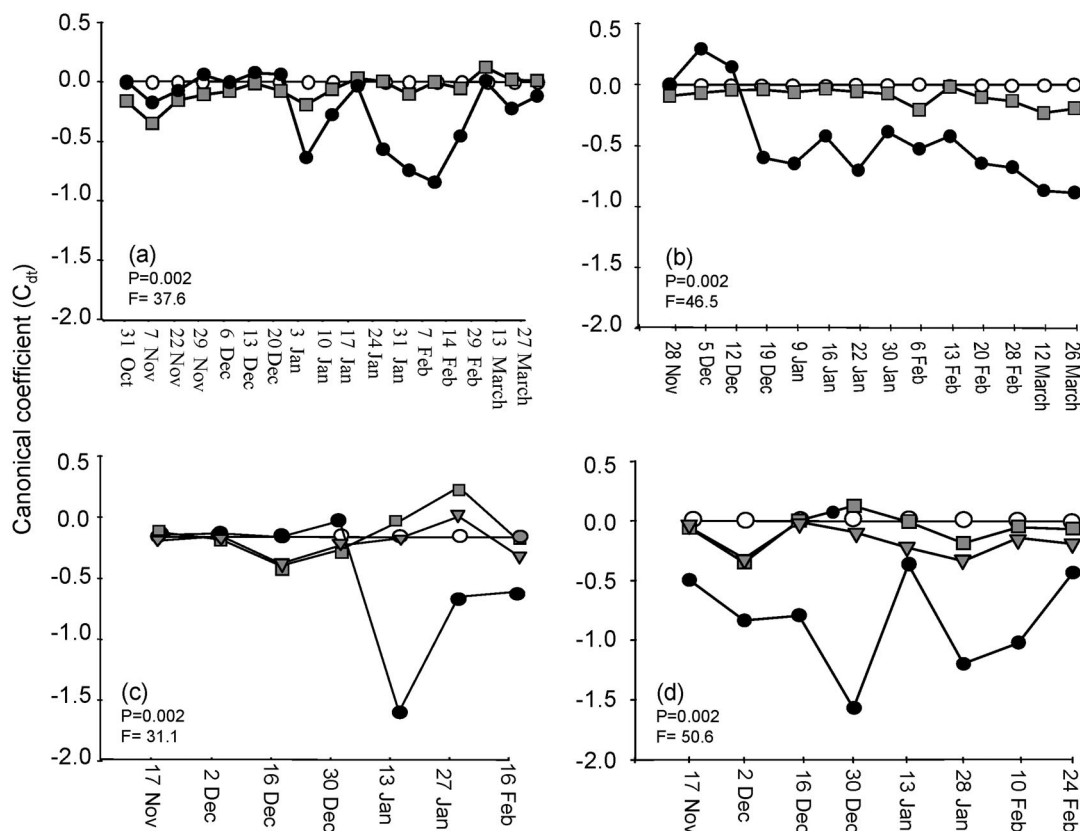P, pest; B, beneficial; O, other (i.e., not a pest or beneficial).

Fig. 2. PRCs of arthropod communities found in sprayed conventional, unsprayed conventional, and unsprayed *Bt*. At all sites, there was a significant difference between the communities (Monte Carlo simulation, 499 permutations). (a) Doreen, 1995/96 season: 80 taxa, 510 samples. (b) Ewenmar, 1995/96 season: 88 taxa, 420 samples. (c) Doreen, 1997/98 season: 43 taxa, 280 samples. (d) Auscott, 1998/99 season: 55 taxa, 288 samples. White circles, conventional unsprayed (control); black circles, conventional sprayed; gray squares, Ingard *Bt* cotton; gray triangles, stacked *Bt* cotton.

to test and display treatment effects that change across time. Treatment "curves" are presented relative to a standard, in this case unsprayed conventional cotton. The PRC is based on a partial redundancy analysis (the y-axis in a PRC is the first ordination axis "axis 1" of a redundancy analysis [RDA], whereas the x-axis is time) and was generated using the program CANOCO (ter Braak and Smilauer 2002). To test that the PRC explains significant treatment variance, we conducted permutation tests using the Monte Carlo method (available within the program CANOCO) on the first canonical axis of the RDA. To ensure all samples taken at each plot "traveled" together during each permutation, we did random permutations of the whole plots only.

Species groups with species weights that contributed to the overall community response (from PRC) were further analyzed by comparing their distribution on *Bt* (Cry1Ac only) and conventional cotton throughout the season at all four sites. Because of sparse data, insect counts from all samples per crop per date were combined ($n = 10$ samples for sites Doreen

95/96, Ewenmar 95/96, and Doreen 97/98 and $n = 9$ samples for site Auscott 98/99). To ensure the data from Auscott were comparable with the other sites, it was multiplied by $10/9_{ths}$. To meet the assumptions of normally distributed residuals, the counts of insect numbers were log-transformed. Despite combining the sample counts, there were still a number of zero counts, so one was added to the counts before transformation. Plots of the log-transformed insect counts versus time of sampling showed that no particular function could be adequately fitted to all the data. Therefore, the logged insect counts over time were modeled using smoothing splines (Verbyla et al. 1999), which uses the data to determine the shape of the response. This was done within a linear mixed model using ASREML (Gilmour et al. 2000). Each spline curve consists of a linear component (slope and intercept terms) and a nonlinear component (spline term). The fixed terms in the model are crop and day and their interaction. If the crop term is significant, the spline curves for each crop type have differing intercepts. Because so many species were tested, signifi-
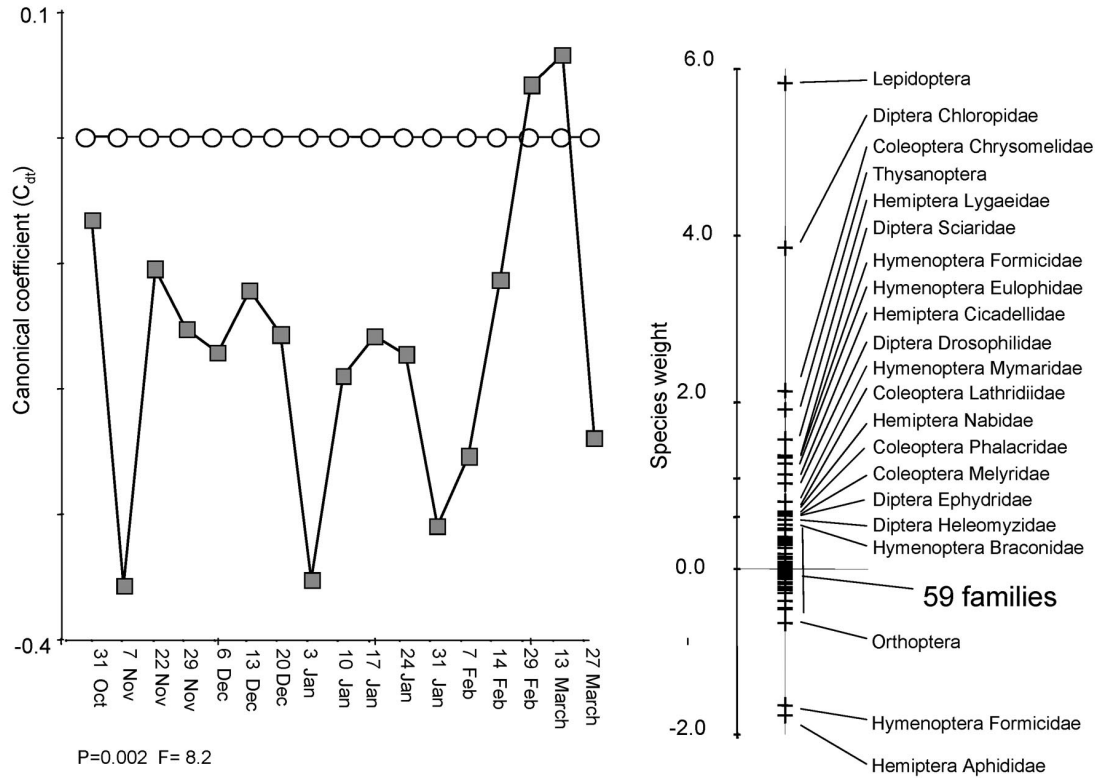
Fig. 3. PRCs and species weights of the unsprayed conventional and *Bt* cotton at site Doreen 1995/96. Taxa with species weights between −0.5 and 0.5 are not listed because these have little influence on the curves. The symbols are the same as those used in Fig. 2.
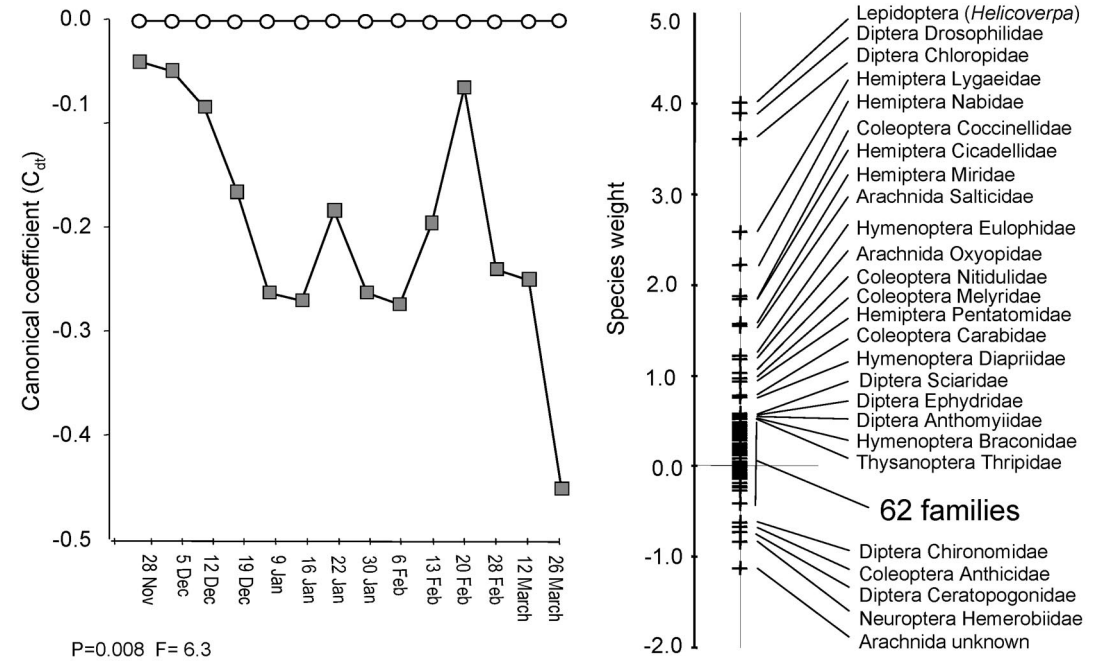

Fig. 4. PRCs of the unsprayed conventional and *Bt* cotton at site Ewenmar 1995/96. The symbols are the same as those used in Fig. 2.
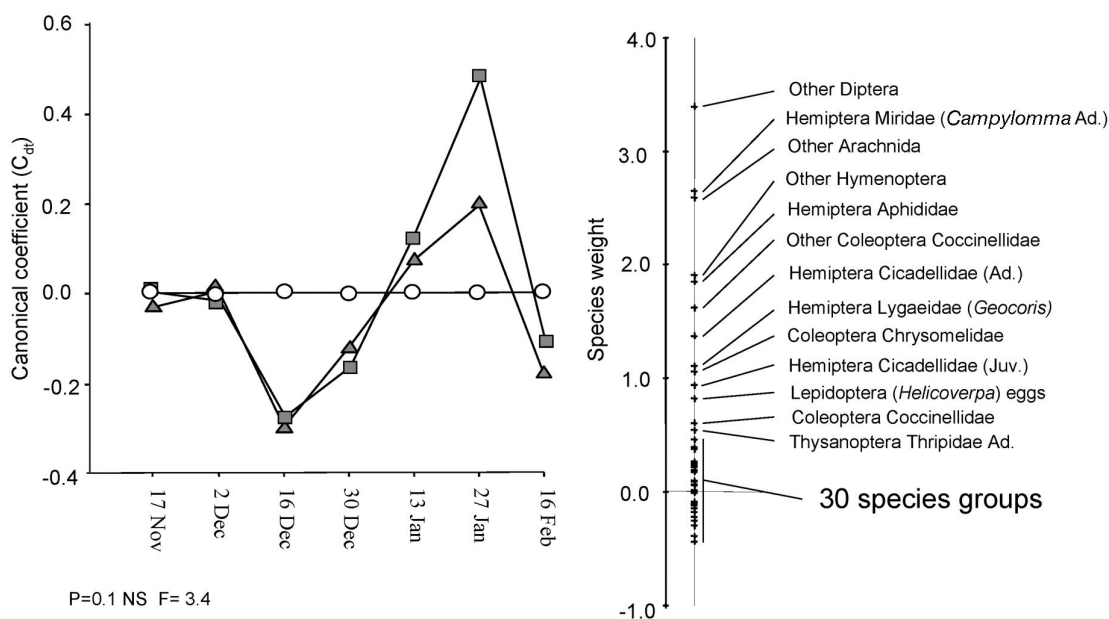
Fig. 5. PRCs of the unsprayed conventional and *Bt* cotton at site Doreen 1997/98. The symbols are the same as those used in Fig. 2.

cance for species data were accepted at 0.01, with 0.05–0.01 indicating a strong trend.

When comparing communities at the four sites, we were effectively asking the same question four times, thus possibly increasing the chance of committing a type 1 error. To correct for this, we used a modified Bonferroni procedure where *P* values were sorted from the highest to the lowest and compared with the corresponding adjusted α value (α, α/2, α/3, etc.; Haccou and Meelis 1992). If any *P* value was less than its adjusted α value, the null hypothesis was rejected.

Results

Community Differences in *Bt* and Conventional Cotton. PRCs (Fig. 2) indicated that at all four sites there was a significant difference between the communities of different crop types ($P = 0.002$; Fig. 2), with the community in the sprayed conventional cotton showing the most divergence. Crop type accounted for 8.9, 16.3, 9.5, and 16.6% of the variance for Doreen 1995/96, Ewenmar 1995/96, Doreen 1997/98, and Auscott 1998/99, respectively, of which 40.7, 44.4. 54.2 and 49.6%, respectively, of this variance was captured by axis 1. A large proportion of the variance in the communities was explained by changes during the season, because sampling dates accounted for 52.1, 33.7, 53, and 50.1% of the variance in the communities for Doreen 1995/96, Ewenmar 1995/96, Doreen 1997/98, and Auscott 1998/99, respectively.

To test if there was any influence of the Cry proteins on the communities, the sprayed treatment was removed from the analysis. This revealed a significant difference between the communities of unsprayed *Bt* and conventional treatments in three of the four data sets (Figs. 3–6; Table 3). The three communities with significant differences all had *P* values smaller than their adjusted α values (Table 3). This indicates that unsprayed *Bt* communities are significantly different from unsprayed conventional communities. In the communities with a significant effect of crop type, sampling dates explained 43–60% of the variance in the communities, whereas crop type accounted for 4.3–5.5% of the variance.

Species weights >0.5 (Figs. 3–6) are most likely to follow the abundance changes shown in the PRCs, whereas those less than -0.5 show a trend in the opposite direction (values between -0.5 and 0.5 do not contribute strongly to the community response; Van den Brink and Ter Braak 1999). Of the species groups that contributed to the changes depicted in the PRCs, *Helicoverpa* and Lepidoptera had high species weights, as expected (Figs. 3, 4, and 6). In addition the fly family Chloropidae also had high species weights (Figs. 4 and 5). As the *Bt* curves in the PRCs were negative and most of the species with high species weights were positive, these species were less abundant in unsprayed *Bt* crops compared with unsprayed conventional crops (Figs. 3–6).

Are Any Species Groups More Affiliated with *Bt* or Conventional Cotton? We tested eight general groups to see if there were consistent differences between the number of individuals on Ingard® and conventional cotton over the four sites by fitting a model of their distribution using smoothing splines. The *F* statistic and denominator degrees of freedom for the crop term (numerator df is 1) are shown in Table 4, together with the retransformed value of the spline curves for both crop types at the time midway through the experiment. Of these, we identified five general groups
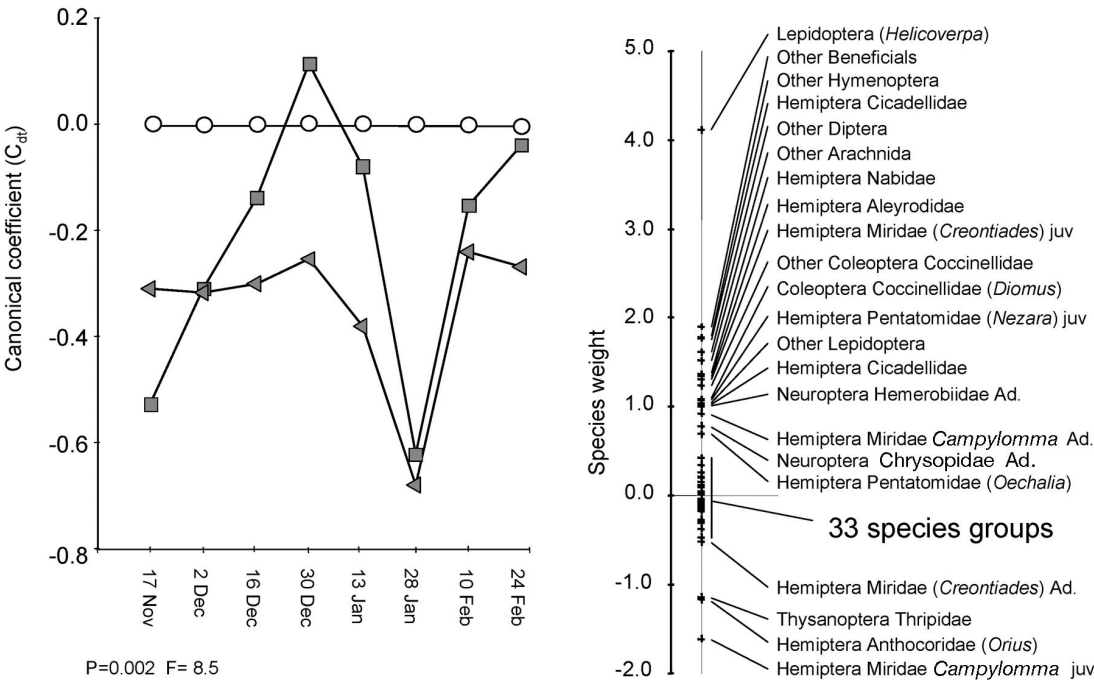
Fig. 6. PRCs of the unsprayed conventional and *Bt* cotton at site Auscott 1998/99. The symbols are the same as those used in Fig. 2.

in which $P < 0.01$, indicating that crop type significantly improved the fit of the model (Table 4; Fig. 7). For spiders and pest Hemiptera in particular, the SE of the predicted values for unsprayed conventional and *Bt* strongly overlapped. This indicates that, although there were only slight differences in the abundances of these animals in *Bt* and conventional cotton, the differences were consistent over time and between sites. For Lepidoptera and Beneficial and other Hemiptera, the SEs were more distinctive (Fig. 7).

Table 3. Characteristics of the PRC of unsprayed communities

Site	Doreen 1997/98	Ewenmar 1995/96	Doreen 1995/96	Auscott 1998/99
F value	3.4	6.3	8.2	8.5
P value (calculated using monte carlo simulation, 499 permutations)	0.1	0.008	0.002	0.002
α (calculated using improved Bonferroni procedure)	0.05	0.025	0.0167	0.0125
Variance explained by crop type	3.7%	5.2%	4.3%	5.5%
Proportion of this variance captured by axis 1	20.5%	26.7%	27.9%	30.9%
Variance explained by sampling date	56.6%	43%	55.9%	59.9%

There were 39 species groups (identified in Figs. 3–6) with high species weights. Of these, seven were discarded from further analysis because of sparse data. We modeled the distribution of individuals in Ingard® and conventional cotton over time for the remaining 32 taxa using smoothing splines. The *F* statistic and denominator degrees of freedom for the crop term (numerator df is 1) are shown in Table 5, together with the retransformed value of the spline curves for both crop types at the time midway through the experiment. Of these, we identified five taxa (two Diptera: Chloropidae and Drosopillidae; two Hemiptera: Cicadellidae and Nabidae; and one Lepidoptera: *Helicoverpa*) in which crop type improved the fit of the model (Table 5; Fig. 8). Again there was substantial overlap of the SEs of the predicted values for unsprayed conventional and *Bt* for most taxa, indicating a slight but consistent difference in the abundances of the taxa in the two crop types. *Helicoverpa* showed the least amount of overlap of the SEs.

Abundance, Diversity, and Species Richness of Beneficial Arthropods. Diversity indices of the beneficial communities (species identified in Table 2) were not affected by crop type at Doreen 1995/96 (SI: $F = 0.61$, $P = 0.55$, df = 2,27; SW: $F = 1.35$, $P = 0.27$, df = 2,30; Fig. 9a). At Doreen 1997/98, the SW index was also unaffected ($F = 0.65$, $P = 0.59$, df = 3,19), although the SI indicated that sprayed cotton was significantly more diverse than either Ingard® or stacked (SI: $F = 3.55$, $P = 0.035$, df = 3,18; LSD = 0.2165; Fig. 9c). The SI was

Table 4.   Results of a spline analysis examining the effect of "crop type" (Ingard or conventional cotton) on general groups

Role	General groups	Conventional	Ingard®	df	F	P
P	Lepidoptera	7.7	3.7	42	19.5	<0.001[b]
P	Pest Hemiptera	465.0	374.6	38	10.9	0.002[b]
P	Pest Coleoptera	3.5	2.8	40	2.67	0.11
B	Hymenoptera	22.0	21.7	30	0.03	0.874
B	Spiders	31.3	24.5	44	11.5	0.001[b]
B/O	Beneficial and other Coleoptera	21.8	17.6	21	5.79	0.025[a]
B/O	Beneficial and other Hemiptera	6.8	4.0	39	25.4	<0.001[b]
O	Diptera	112.6	62.1	39	44.9	<0.001[b]

The *F* and *P* values indicate whether adding "crop type" significantly improved the model. The conventional and Ingard values are the predicted number of individuals in 10 samples in the middle of the season.

[a] *P* value between 0.05 and 0.01 (indicates a trend).

[b] *P* value <0.01 (indicates a significant difference).

P, pest; B, beneficial; O, other.

unaffected by crop type for both Ewenmar 1995/96 (SI: $F = 3.21$, $P = 0.057$, df = 2,25) and Auscott 1998/99 (SI: $F = 2.15$, $P = 0.107$, df = 3,45), although in both cases there was a significant difference in the SW index (Ewenmar 1995/96: $F = 22.86$, $P < 0.001$, df = 2,26, LSD = 0.1166; Auscott 1998/99: $F = 21.15$, $P < 0.001$, df = 3,47, LSD = 0.1562), with diversity in sprayed cotton either significantly lower than the other crops (Auscott; Fig. 9d) or significantly lower than Ingard®, which was significantly lower than unsprayed conventional (Ewenmar; Fig. 9b). There was no consistent pattern in the rarefaction curves at the four sites.

Discussion

To date, most field studies have indicated little or no change in the beneficial community on *Bt* crops in comparison to conventional crops (Sims 1995, Orr and Landis 1997). We also found little difference in the diversity or species richness of beneficial arthropods in the unsprayed *Bt* and conventional crop types. We did find that the beneficial community in sprayed crops was significantly less diverse than that in unsprayed crops at two sites according to the SW index. This pattern, however, was not supported by the SI, which, at one site, indicated that the sprayed crop was more diverse than the *Bt* crops. Because the SW index is more sensitive to rare species, the differences in the effect of crop type on the indices suggest that spraying had a stronger affect on rarer species.

Although most differences in the communities were attributable to the effect of spraying, we did identify slight differences in the invertebrate communities found in unsprayed conventional compared with unsprayed *Bt* cotton or stacked Ingard®. These differences accounted for ≈4.5% of the variability between unsprayed conventional and unsprayed transgenic cotton.

Some difference between the invertebrate communities found in unsprayed conventional and *Bt* cotton is to be expected, given that the abundance of many lepidopteran larvae has been greatly reduced in the *Bt* cotton community. Indeed, the species weights of

Lepidoptera and *Helicoverpa* had the strongest influence on the PRCs. The potential for lower numbers of parasitoids or predators, which specialize on larvae of *Helicoverpa* spp. or other lepidopterans, could also contribute to the difference. The drop in larval density may account for the slight drop in spider numbers in *Bt* crops (Table 4; Fig. 7). Some workers report no effect of *Bt* crops on either the numbers of lepidopteran parasitoids present (Johnson et al. 1997, Wu and Guo 2003) or their activity (Johnson and Gould 1992, Orr and Landis 1997), whereas others report lower numbers of lepidopteran parasitoids in *Bt* crops (Pilcher et al. 2005). Overall, we found no consistent differences between the number of egg and larval parasitoids of Lepidoptera throughout the season, although the Eulophidae (Hymenoptera) showed a trend to be lower in *Bt* cotton (Table 5).

We found slightly lower numbers of Hemiptera in Ingard® and the stacked *Bt* cotton in comparison with unsprayed conventional cotton. Hemiptera includes damsel bugs (Nabidae, *Nabis kinbergii*) and jassids (Cicadellidae), both of which were in lower numbers in *Bt* cotton and may have influenced results (Fig. 8). Although there are reports of no change in damsel bug numbers in some *Bt* crops such as corn (Wold et al. 2001), our findings are in agreement with Naranjo (2005), who reported a reduction in the number of damsel bugs in a 5-yr study in *Bt* cotton, as did Daly and Buntin (2005) in their multi-year study on *Bt* corn. Observations in commercially grown *Bt* cotton crops in Australia have also shown lower numbers of damsel bugs compared with conventional crops (M. Dillon, unpublished data). Why there should be lower numbers of damsel bugs is unclear. Laboratory experiments have found no effect on the development, fecundity, or survival of damsel bugs when fed on Lepidoptera prey, *Spodoptera exigua* (Ponsard et al. 2003) that had been fed on *Bt* or conventional cotton. Damsel bugs are generalist predators (Snyder and Ives 2003) that may attack Lepidoptera larvae and eggs (Ehler 2004), but are also predators of aphids (Hesler et al. 2000, Elliott et al. 2002, Östman and Ives 2003)
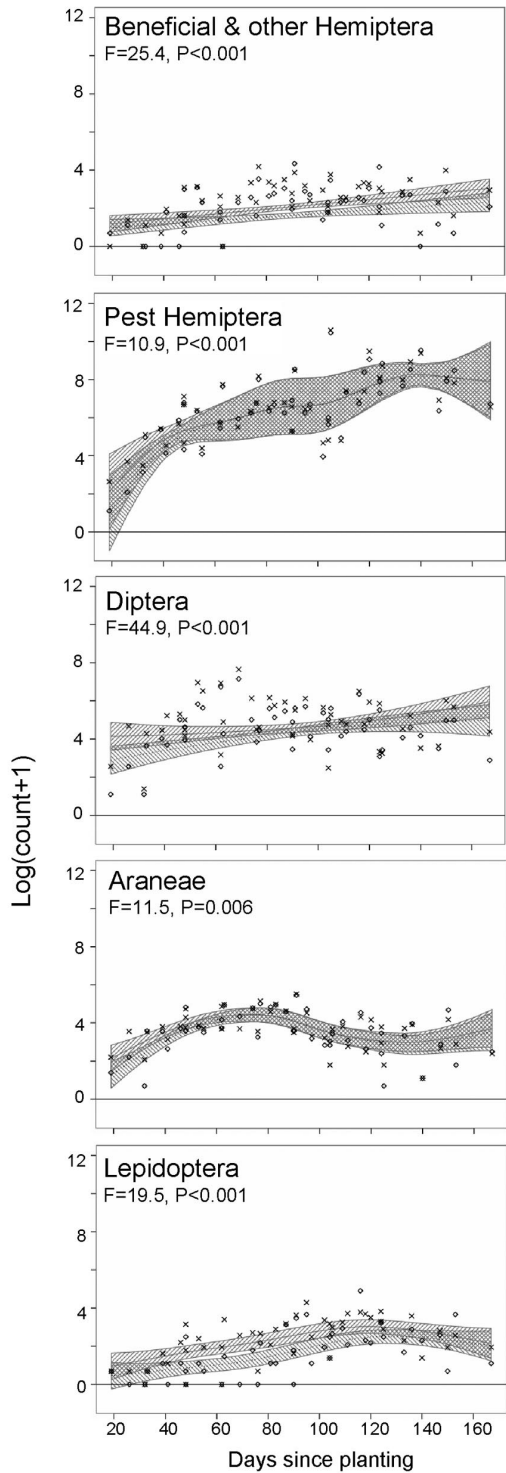
Fig. 7. Graphs of general groups at all sites modeled using smoothing splines. The plots show predicted values for both crop types versus time, with the shaded area within 1 SE of the predicted value. Forward diagonal shading, conventional; backward diagonal shading, *Bt*. Crosses are conventional data points, and circles are *Bt* data points.

Fig. 8. Graphs of selected taxa counts at all sites modeled using smoothing splines. The plots show predicted values for both crop types versus time, with the shaded area within 1 SE of the predicted value. Forward diagonal shading, conventional; backward diagonal shading, *Bt*. Crosses are conventional data points, and circles are *Bt* data points.
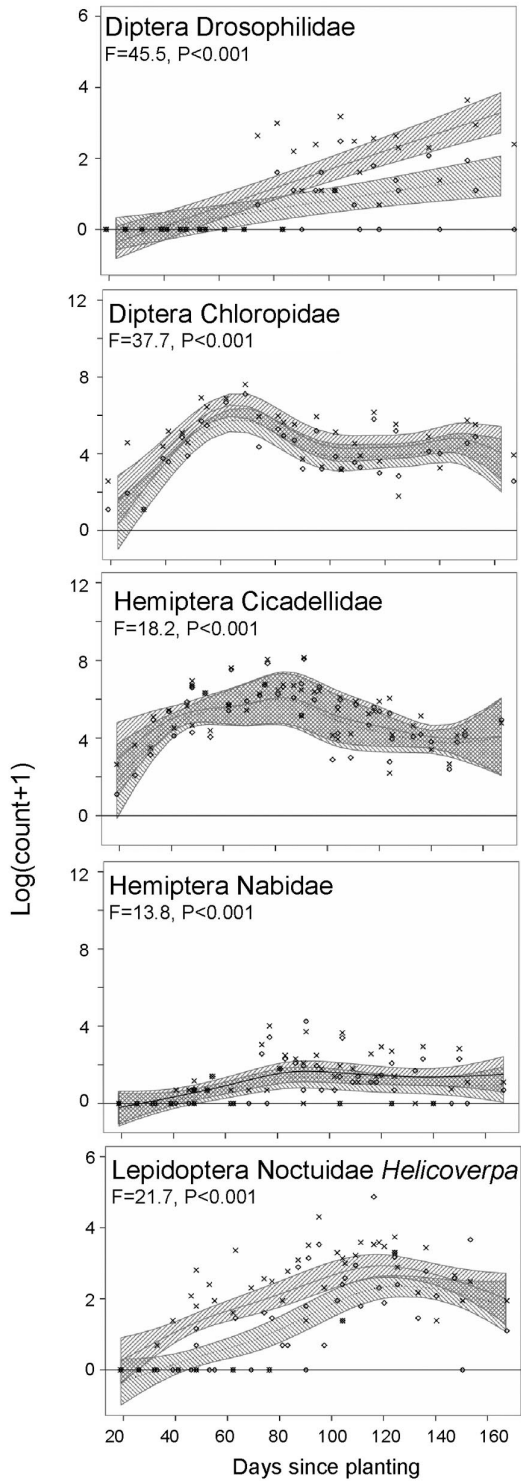
Table 5.   Results of a spline analysis examining the effect of "crop type" (Ingard or conventional cotton) on species groups identified from the PRC

Role	Site 1	2	3	4	Species groups	Conventional	Ingard®	df	F	P
B		+			Arachnida Oxyopidae	4.8	2.7	12	3.5	0.087
B		+			Arachnida Salticidae	1.9	0.7	12.5	5.7	0.035[a]
O		−			Coleoptera Anthicidae	1.2	1.2	37.3	0	0.974
P	+		+		Coleoptera Chrysomelidae	3.5	2.8	40.3	2.7	0.107
B				+	Coeloptera Coccinellidae (*Diomus*)	1.3	1.0	59.3	2	0.167
B		+	+	+	Coleoptera Coccinellidae (others)	1.3	1.0	60.7	2	0.164
O	+				Coleoptera Lathridiidae	5.0	4.4	27.4	1	0.324
B	+	+			Coleoptera Melyridae	2.5	2.1	36.1	1.5	0.233
O		+			Coleoptera Nitidulidae	1.0	0.9	53.7	0.1	0.768
O	+				Coleoptera Phalacridae	0.7	0.6	18.4	1	0.34
O		−			Diptera Ceratopogonidae	0.3	0.4	29	0.4	0.534
O		−			Diptera Chironomidae	8.2	9.3	28.6	1.9	0.177
O	+	+			Diptera Chloropidae	89.3	41.8	29	38	<0.001[b]
P	+	+			Diptera Drosophilidae	2.9	0.9	29	46	<0.001[b]
O	+	+			Diptera Sciaridae	2.6	1.8	29.1	2.5	0.126
P			+		Hemiptera Aleyrodidae	1.0	0.8	37.5	2.1	0.156
P	−				Hemiptera Aphididae	25.6	29.7	34.2	1.8	0.185
P	+	+	+	+	Hemiptera Cicadellidae	109.4	79.8	40.2	18	<0.001[b]
P/B	+	+	+		Hemiptera Lygaeidae	3.7	2.3	38	5.8	0.021[a]
B		+			Hemiptera Lygaeidae (*Geocoris*)	1.0	0.5	46.3	4.3	0.045[a]
P		+	−		Hemiptera Miridae (*Campylomma*)	8.3	7.1	46.2	2.2	0.142
P		+		+	Hemiptera Miridae (*Creontiades*)	3.0	2.7	11.2	0.7	0.41
B	+	+		+	Hemiptera Nabidae	1.8	1.1	43.9	14	<0.001[b]
P/B		+		+	Hemiptera Pentatomidae	0.7	0.4	26.1	3	0.097
B	+	+			Hymenoptera Braconidae	2.4	2.1	27.8	0.8	0.385
O	+	+			Hymenoptera Eulophidae	5.1	3.8	29.2	4.7	0.038[a]
B	−				Hymenoptera Formicidae	2.2	2.9	47.4	2.2	0.144
B	+				Hymenoptera Mymaridae	2.3	1.8	26.8	1.8	0.186
P	+	+		+	Lepidoptera Noctuidae (*Helicoverpa*)	5.6	2.3	44.1	22	<0.001[b]
B				+	Neuroptera Chrysopidae	0.4	0.5	19	1.1	0.317
B		−		+	Neuroptera Hemerobiidae	0.6	0.4	28.8	0.2	0.689
P	+	+		−	Thysanoptera Thripidae	24.9	26.7	43.1	0.5	0.485

A plus indicates sites where the species had positive species weights; a minus indicates sites where the species had negative species weights. The F and P values indicate whether adding "crop type" significantly improved the model. The conventional and Ingard values are the predicted number of individuals in 10 samples in the middle of the season.

[a] P value between 0.05 and 0.01 indicates a trend.

[b] P value <0.01 indicates a significant difference.

Site 1, Doreen 1995/96; 2, Ewenmar 1995/96; 3, Doreen 1997/98; 4, Auscott 1998/99; P, pest; B, beneficial; O, other.

and spider mites (Wilson et al. 1998). It may be that damsel bugs are more dependant on lepidopteran larvae than currently realized, which could partially explain their reduced abundance.

Jassid densities were slightly but significantly lower in *Bt* compared with conventional cotton. Because jassids are sometimes considered a pest (Deutscher et al. 2005), this could be a bonus for the grower. Nevertheless, the slight differences in jassid numbers between conventional and *Bt* cotton would be masked in commercially grown cotton by any differences in spray regimen between the two crop types.

We also found that the number of Chloropidae and Drosophillidae (Diptera) was lower in *Bt* cotton compared with conventional cotton. Why this occurred is unclear. Purified insecticidal proteins known to be effective against some Diptera include Cry4Aa1, Cry4Ba1, Cry10Aa1, and Cry11Aa1 (Benedict and Altman 2001). Cry2Aa1 (old name, CryIIA; Benedict and Altman 2001) is effective against Lepidoptera and some Diptera, including the mosquito *Anopheles quadrimaculatus* Say, and to a lesser extent, the mosquito *Culex pipiens* L., but it has no effect on other Diptera, including *Musca* and *Drosophila* (Sims 1997). CryBI is effective against the lepidopterans *Heliothis virescens* (Fabricius) and *Lymantria dispar* L., and to a lesser extent the mosquito *Aedes aegypti* L. (Donovan et al. 1988). The *Bt* cotton used in this study expresses Cry1Ac, which is specific to Lepidoptera (Sims 1995, Peferoen 1997). Even the two-gene cotton, Bollgard II, which has been recently licensed for general release in Australia, expresses Cry1Ac and Cry2Ab, both of which are specific to Lepidoptera. Thus, it is unlikely that the *Bt* gene in cotton had a direct effect on Drosophillidae or Chloropidae.

The role of Chloropidae in cotton is also unclear. The larvae of this family are reported to feed on a range biota, including bacteria, vegetative matter (both living and rotting), the eggs of other insects and spiders, beneath the skins of living frogs, and as parasites of Hymenoptera (Spencer 1986). Because Chloropidae do not seem to be pests or beneficials in cotton, its role from an IPM perspective is probably limited to providing an alternative source of food for some predators.
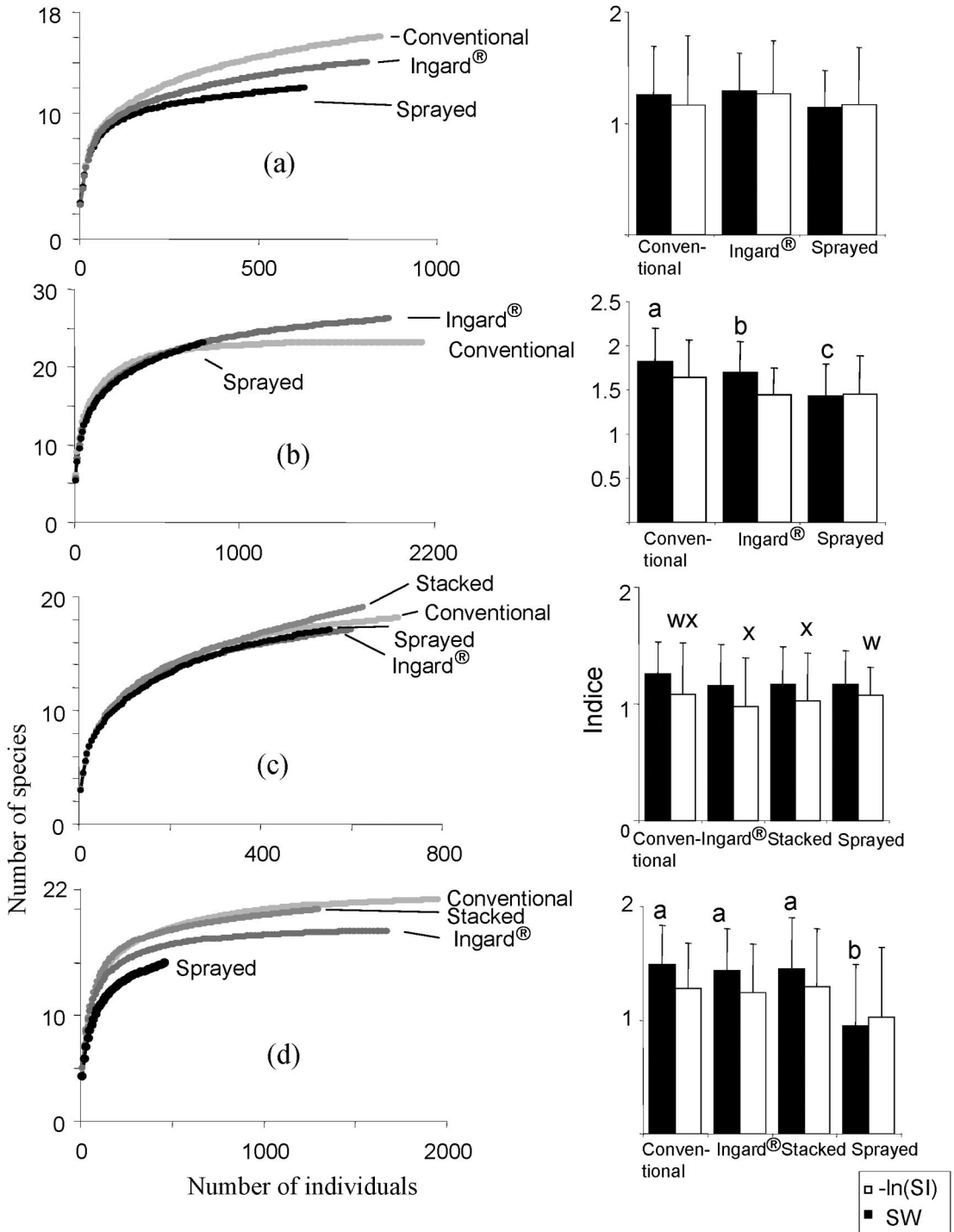
Fig. 9. Rarefaction curves and diversity indices of the beneficials found at the four sites. Rarefaction curves were calculated in increments of five specimens for sites (a) and (c), and increments of 10 specimens for sites (b) and (d). SDs are shown for the diversity indices. There was no difference in the indices calculated for sites (a) and (c). At sites (b) and (d), different letters above the histograms indicate statistical differences among the indices (a–c, SW; w,x, SI; as calculated using the LSD). (a) Doreen 1995/96. (b) Ewenmar 1995/96. (c) Doreen 1997/98. (d) Auscott 1998/99.

We found no difference in the number of green lacewings in *Bt* crops over the course of a season. Other studies that have focused specifically on lacewings have also found no effect (Orr and Landis 1997, Pilcher et al. 1997). Lepidopteran prey that have fed on *Bt* appear to be a poor-quality food source for lacewings, probably because of a change in the amino acid composition of the lepidopteran's hemolymph (Dutton et al. 2003). Lacewings readily consume other prey such as mites, which have been shown to accumulate higher levels of *Bt* toxin than lepidopteran larvae, without harm (Dutton et al. 2002, 2003).

The greatest influences on invertebrate communities in cotton are insecticide sprays, and the advent of *Bt* cotton has fostered a large drop in insecticide applications, with a 56% reduction in pesticide applications for *Helicoverpa* (Fitt 2004) and a 50% reduction in active ingredient overall. Nevertheless, when managing *Bt* cotton, it is important to understand how the dynamics of pest and beneficial species may be affected so that management practices can be adjusted if necessary. Our results indicated only a subtle shift in the arthropod community between *Bt* and conventional cotton, some of which was probably driven by the reduction in *Helicoverpa* and other lepidopterans. Our analyses did not indicate significant and consistent intrinsic effects of *Bt* cotton on key species that would warrant a different pest management approach.

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Target and non-target effects on the invertebrate community of Vip cotton, a new insecticidal transgenic

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Abstract. A new transgenic cotton producing the Vegetative Insecticidal Protein (Vip) is being developed to control Lepidopteran pests, especially *Helicoverpa* larvae. Before its introduction its efficacy against *Helicoverpa* larvae under field conditions needs to be confirmed, and any non-target effects it may have on the arthropod community need to be identified. We conducted field trials to compare the arthropod community in unsprayed conventional (Sicala 40) and Vip (Coker 312 Vip3A, event 102) cotton using visual searches, beatsheets, and suction samplers at 2 sites in Australia. At both sites, Vip controlled *Helicoverpa* larvae leading to much higher boll counts. There were no major differences in either species richness or diversity of the beneficial and non-target communities between Vip and conventional cotton, although cotton cultivar accounted for 2–7% of the variance in arthropod communities. There was no detrimental effect of Vip cotton on egg parasitoids. The number of predatory beetles and the pest mirid *Creotiades dilutus* (Stål) was higher in the Vip, although the increase in mirids was probably the result of more food (bolls) in the Vip crop. In a small plot experiment, we found higher numbers of whitefly in Vip, but this may be driven by differences in leaf hair between the cotton cultivars. Vip cotton appeared to have little effect on the arthropod community other than on *Helicoverpa*. As such it has the potential to be a useful tool in the management of *Helicoverpa* and may relieve resistance pressure on existing Bt cultivars (transgenic cotton containing genes for insecticidal Cry proteins), thereby increasing the durability of both technologies.

Additional keywords: GMO, IPM, beneficials, pests, Australia, *Trichogramma*.

Introduction

Lepidopteran species, particularly those belonging to the genera *Heliothis* and *Helicoverpa*, are key pests of cotton worldwide (Luttrell *et al*. 1994). Integrated pest management (IPM) practitioners have long sought alternatives to insecticide sprays to control these pests. Ideally, such controls should be efficacious against *Helicoverpa*, not harm beneficials, and have low mammalian toxicity and environmental risk. One such option has been the Cry1Ac δ-endotoxin produced by the bacterium *Bacillus thuringiensis* var. *kurstaki* Berliner, which has been available commercially in genetically modified cotton (Bt cotton) since 1996 (Perlak *et al*. 2001).

Bt cotton has had a major effect on cotton production wherever it has been commercially adopted by significantly reducing pesticide inputs (Fitt and Wilson 2000; Benedict and Altman 2001; Perlak *et al*. 2001; Qaim 2003). For example, Bt cotton crops in Australia received 56% fewer pesticide applications for *Helicoverpa* (Fitt 2004) that translated into a 50% reduction in active ingredient overall compared with conventionally sprayed cotton. In addition, several studies indicate that Bt cotton has minimal effect on the beneficial insect community in cotton worldwide (Sims 1995; Men *et al*. 2003; Kannan *et al*. 2004; Sisterson *et al*. 2004; Tabashnik 2004; Naranjo 2005; Whitehouse *et al*. 2005). The adoption of Bt cotton by Australian cotton growers has occurred rapidly and,

with the introduction of Bollgard II® cotton, which expresses 2 Bt genes (Cry1Ac and Cry2Ab) in 2003, the majority (over 70% in 2004; Whitehouse *et al*. 2005) of the cotton crop in Australia was transgenic Bt cotton.

High adoption rates of Bt cotton places enormous selection pressure on *Helicoverpa* spp. in Australia, increasing the risk that these pests will develop resistance to the proteins. In America the frequency of individuals carrying a resistance gene to Cry1Ac in untreated populations of *Helicoverpa zea* (Boddie) and *Heliothis virescens* (Fabricius) is ∼1 or 2 in 1000 (Gould *et al*. 1997; Tabashnik *et al*. 1997). In Australia, a resistance gene to Cry1Ac has been confirmed in a natural population of *Helicoverpa armigera* (Hübner) in Australia (R. Akhurst, pers. comm.), although the actual frequency is unknown (Akhurst *et al*. 2001). Alleles that confer resistance to Cry2Ab appear to be more common in *H. armigera* (1 in 132 alleles in initial studies; Mahon *et al*. 2004) but as at Feb. 2007, the frequency is 7 in 1684 (a Bayesian Frequency of 0.0012) alleles, with a 95% credibility interval of 0.0005–0.0021 (Downes *et al*. 2007). In addition, there have been reports of dual resistance developing in laboratory-reared *H. virescens* to both Cry1Ac and Cry2Aa (Jurat-Fuentes *et al*. 2003) although the mechanisms are quite distinct. Although the Australian cotton industry has adopted a comprehensive and pro-active resistance management strategy for Bt cotton

(Fitt 2004), the strategy would be greatly enhanced if another toxin were available that was effective against *Helicoverpa* but which did not share the same mode of action as the Bt proteins currently used.

Vegetative Insecticidal Protein (Vip) is a new class of insecticidal protein produced during the vegetative phase of the bacterium *B. thuringiensis*. Vip3A is effective against a range of Lepidopteran pests (Estruch *et al.* 1996; Donovan *et al.* 2001) where it attacks midgut receptors in target species (Lee *et al.* 2003), but the protein bears no similarity to the δ-endotoxins (Yu *et al.* 1997), indicating that Vip3A ion channels cannot be equated with those of the Cry proteins (Lee *et al.* 2003, 2006). Consequently, its use in cotton cropping systems could help reduce resistance pressure on the Bt proteins currently deployed and increase the potential life span of both technologies.

Although Vip cotton has strong efficacy against target Lepidopteran pests, such as *Helicoverpa* spp., its effect on other members of the invertebrate community is not known. The only study to date looked at the effect of Vip3A stacked with the Bt protein Cry1Ab in corn (Dively 2005). The few differences found in this study were density related in response to either a drop in prey availability or the absence of insect damage on the plants. No studies have reported on the effect of Vip in isolation on cotton invertebrate communities. Any such effect needs to be identified so that changes can be incorporated into management practices that support IPM systems for transgenic cotton varieties that express the Vip gene.

We conducted experiments at 2 sites: Narrabri, New South Wales; and Kununurra, Western Australia. Narrabri is in the Namoi Valley, which is one of the main cotton-growing regions of Australia, and Kununurra is a potential tropical production area for winter cotton production. The aim was to compare unsprayed Vip3A cotton with unsprayed conventional cotton to determine if there were differences in: (*1*) efficacy against *Helicoverpa*, (*2*) parasitism rates of *Helicoverpa* eggs and larvae, (*3*) the invertebrate communities of the crop, and (*4*) plant growth or morphology that could contribute to differences in the invertebrate community.

Materials and methods

The 2 Narrabri experiments were conducted in different fields at the Australian Cotton Research Institute (30°11′S, 149°33′E; planted 3 November 2003). In Expt 1, 4 large unsprayed plots (∼0.5 ha each) were arranged in a 2 by 2 matrix (row 1: Vip, conventional; row 2: conventional, Vip). The 2 Vip plots were Coker 312 Vip (a normal leaf variety expressing the Vip3A event 102; referred to hereafter as Vip). The 2 conventional plots were Sicala 40 (a conventional normal leaf variety). There was a buffer of 20 m of Sicala 40 all around the plots and between the plots.

The Vip gene was embedded in Coker cotton, which is a useful cultivar for establishing new lines of transgenic cotton, but it is an old cultivar not commonly used in commercial cotton production. Obtaining large quantities of conventional Coker seed to compare with Vip was not possible. Instead we used Sicala 40 as the conventional cotton, which is similar to Coker in height and maturity. Within the conventional buffer around the outside of the plots we included a small plot (4 rows by 20 m) of Coker 312 conventional cotton, which was used to compare cotton growth characteristics with the Vip cotton. The plot was

small because there was very little planting seed available of this old cultivar.

Experiment 1 was used to compare plant growth, efficacy against *Helicoverpa*, parasitism rates, and invertebrate communities in Vip and conventional cotton.

Experiment 2 at Narrabri focussed on the ability of Vip cotton and conventional cotton to support spider mite pests (*Tetranychus urticae*, Koch). The experiment used a randomised design with 3 replicates. Plots were each 6 rows wide by 20 m long, arranged in an area 18 rows by 54 m. Each plot was inoculated with mites and sampled throughout the season (from 16 December 2003 to 27 February 2004).

The Kununurra component was located at the Frank Wise Institute (FWI) (15°37′S, 128°42′E; planted 13 May 2003). The experimental field consisted of 5 plots (each 15 rows, 49 m long). All plots were in a line and included within a wider strip of conventional (Sicala 40) cotton (146 rows by 89 m). The first 4 plots (Vip – conventional – Vip – conventional) were unsprayed. The final plot of 26 rows was conventional cotton, which was sprayed twice, once on 7 July with deltamethrin (16.5 g a.i./ha) for mirid control and again on 28 July with deltamethrin (16.5 g a.i./ha) plus pirimicarb (500 g a.i./ha) to control mirids and aphids. Only the central 7 rows and 29 m of each plot (henceforth the 'sampling area') were sampled to reduce edge effects (i.e. 4 rows on each side and 10 m at each end of each plot were not sampled). Sampling was conducted over a period of 2 weeks from 30 July 2003 until 12 August 2003, when the cotton was flowering and beginning to form bolls.

At both locations the cotton was planted in rows 1-m apart and fertiliser, irrigation, and weed management implemented according to local best practice.

Plant growth

As differences in the invertebrate community may be linked to differences in plant morphology between cultivars, we assessed plant height, fruit production, and fruit distribution, as these features are important in determining the potential attractiveness to pests.

At Narrabri in Expt 1, the average height and number of nodes were recorded for 3 of the plants from each area sampled during each survey in order to assess the plant growth of each cultivar (120 plants per survey). At the end of the season we recorded the proportion of open bolls retained on 10 plants, selected at random, in each of the 4 large plots, and on 20 plants from the small Coker 312 conventional plot. We also scored whether fruit was retained at position 1 (first boll from the stem along the branch) or position 2 (second boll from the stem along the branch) of each fruiting branch, and the total proportion of bolls retained.

At Kununurra we measured plant height and boll and square production in unsprayed Vip, unsprayed conventional, and sprayed conventional cotton in between surveys. Plant height was measured for 2 randomly selected groups of 10 plants in each plot. The number of bolls and squares was counted in two 1-m samples in each plot.

Efficacy of Vip against Helicoverpa *spp.*

At Narrabri we conducted 11 visual surveys of cotton during the season, at approximately weekly intervals. Each survey consisted

of 5 visual searches in each of the 4 large plots described above, totalling 20 visual searches per survey. Each visual search consisted of a thorough search for Lepidopteran eggs and larvae in a 1-m section of cotton row. The colour of the egg (reflecting developmental stage) and the size and species of larvae (where identification was possible) were recorded.

At Kununurra we conducted 5 visual searches in each plot, as described above, and completed 2 visual surveys (20 visual searches per survey), 1 per week, during the 2 week sampling period.

Parasitism rates of Helicoverpa *spp. eggs and larvae*

At Narrabri, during the 11 visual surveys described above, *Helicoverpa* spp. eggs and larvae were collected and taken to the laboratory. To determine parasitism rates, collected eggs were housed singly in plastic cells and left to hatch. Collected larvae were placed into individual cells on artificial diet and reared through to pupation or until parasitoids emerged.

At Kununurra, *Helicoverpa* spp. eggs and Lepidopteran larvae that were found during the 2 visual surveys were collected and reared in the laboratory to determine parasitism rates using the same methods described for Narrabri.

Invertebrate communities of Vip and conventional cotton

Narrabri

Experiment 1 plots were sampled 10 times at approximately weekly intervals during the season (from 16 December 2003 to 27 Feburary 2004) using 'beatsheets' (see below), and 3 times during the season using suction samplers (mini 'Blower Vac' suction samplers, Homelite B180v). Ten beatsheets were used in each plot during each beatsheet survey (40 beatsheets per survey).

Kununurra

Beatsheets (see below) were used to sample the invertebrate communities on unsprayed Vip and conventional cotton and on sprayed conventional cotton. Ten beatsheets (randomly located) were used per plot during the 2 beatsheet surveys (50 beatsheets in total per survey).

Beatsheets sample a 1-m section of a cotton row for both pests and beneficials. For many species beatsheets give a more reliable result than visual or suction sampling (Deutscher *et al.* 2003). Sampling involved placing the edge of a yellow plastic sheet (1.5 by 2 m) below the sample row and then stretching the rest of the sheet along the ground and up over the adjacent row. Plants in the sample row were shaken vigorously 10 times using a 1-m stick, working from the base to the top of the crop canopy. The shaking caused insects and spiders to fall from the plants onto the sheet. Arthropods were counted according to a standard classification scheme used in Australia where key pests, predators, and parasites were identified to species level, sometimes even to developmental stage, and other insect species were identified to order (Room and Wardhaugh 1977; Pyke and Brown 1996; Deutscher *et al.* 2005; Table 1).

The suction sampler, which was only used at Narrabri, was included because it captured some of the smaller flying insects that may have been missed using visual or beatsheet sampling. During sampling the suction sampler was swept back and forth

from the bottom of the plants to the top in a zig-zag pattern (to ensure that all strata of the plant were sampled) over a 10-m strip of cotton plants. Five suction samples were taken in each plot during each of the 3 suction surveys (20 suction samples per survey). Suction samples were killed immediately with chloroform, cleaned in the laboratory (by removing leaf and flower debris), and then stored in vials of 70% ethanol. Fauna within samples were counted under a dissecting microscope in accordance with the standard classification scheme. The scheme focussed on species groups most relevant to cotton (Table 1); therefore, many species of small flies were not differentiated.

Development of secondary pest populations on Vip

Because pest populations could be favoured by a new cultivar, the second field experiment at Narrabri tested whether mites were better able to survive on Vip cotton in comparison with conventional cotton. As described above, we used a replicated plot design with 2 treatments (Vip or conventional Sicala 40) and 3 replicates. Mites were introduced into all plots when the plants were at about the 6-node stage by placing 6 heavily mite-infested cotton seedlings in the centre of the plot. The first infestation on 19 December 2003 died out, so we re-infested the plots with 24 infested seedlings each on 27 January 2004. We took 4 samples from each plot (after 16, 24, 31 and 37 days). Each sample consisted of the leaf at the third node below the terminal on either (*a*) each of the plants in the central 1 m (Samples 1 and 2); or (*b*) for 10 plants in the centre of the plot (Samples 3 and 4). Insects (including whitefly) and mites were washed off the leaves onto Petri dishes where they were counted.

We were concerned that the difference in whitefly numbers between conventional and Vip cotton may be due to morphological differences between the cultivars, independent of the presence or absence of Vip. We compared the hairiness of Vip and conventional Sicala 40 by counting hair clumps (a bunch of 2–5 hairs all arising from the same point) in 30-mm discs cut from the centre of the leaf at position 3.

Analysis

Statistical analysis was conducted using the programs Genstat 7 (Payne 2000) for general analyses and CANOCO 4.5 (Ter Braak and Smilauer 2002) for ordination analyses. We used principal response curve (PRC) analysis to compare the invertebrate communities throughout the season. A PRC analysis looks at treatment effects relative to a standard, usually an untreated control. It is a multivariate technique derived from redundancy analysis (RDA). It highlights the proportion of variance in the invertebrate community explained by the treatment (Vip and conventional cotton) over the course of the growing season. A PRC is drawn by plotting the canonical coefficients from the RDA for the treatments over time, thus illustrating the dynamic changes in the community composition over the course of the season. The Monte-Carlo method was used to test the significance of the deviation of the PRC for Vip cotton from the control (unsprayed conventional cotton). We used canonical correspondence analysis (CCA) as a direct analysis to compare the relationship of species with different crop types.

For all species (where $n > 30$ over the season) we tested whether we counted more individuals on Vip or conventional cotton. Whenever possible, parametric techniques were used,

Table 1. Total number of individuals found during the season at Narrabri and Kununurra using different sampling methods

Number of surveys during the season is in parentheses. Gaps in the table indicate species not sampled (in some cases, species groups were slightly different between sampling techniques). E, eggs; L, larvae; A, adults; N, nymphs; J, juveniles

Common name	Life stage	Latin name	Narrabri beatsheets (10)			Narrabri devacs (3)			Kuninurra beatsheets (2)		
			Vip	Conv.	Total	Vip	Conv.	Total	Vip	Conv.	Total
		Pests									
Helicoverpa	E	Noctuidae, *Helicoverpa* spp.	0	0	0	3	0	3	0	0	0
Helicoverpa	L	Noctuidae, *Helicoverpa* spp.	2	123	125	3	29	32	0	5	5
OTHER caterpillars	L	Lepidotera	0	0	0	2	3	5	0	11	11
OTHER moths	A	Lepidotera	10	0	10	5	7	12	1	0	1
Mirid	A	Miridae, *Creontiades* spp.	94	56	150	42	29	71	31	14	45
Mirid	N	Miridae, *Creontiades* spp.	484	322	806	27	12	39	174	70	244
Apple dimpling bug	A	Miridae, *Campylomma liebknechti* (Girault)	31	6	37	17	3	20	6	0	6
Apple dimpling bug	N	Miridae, *Campylomma liebknechti* (Girault)	10	2	12	6	3	9			
Green vegetable bug	N&A	Pentatomidae, *Nezara viridula* (L.)	4	19	23	1	0	1	0	0	0
Red banded shield bug	N&A	Pentatomidae, *Piezodorus hybneri* (Gmelin)	0	6	6	0	0	0	1	2	3
Rutherglen bug	N&A	Lygaeidae, *Nysius vinitor* Bergroth	84	40	124	5	11	16	2	1	3
Cotton seed bug	N&A	Lygaeidae, *Oxycarenus luctuosus* (Montrouzier)	41	107	148	15	10	25	418	318	736
Large orange bug	A	Lygaeidae, *Melanerythrus*	0	0	0	0	0	0	4	1	5
Stilt bug	A	Berxtidae	0	0	0	0	0	0	2	1	3
Flea beetle	A	Chrysomelidae, Halticinae (sub fam.)	0	0	0	0	0	0	1	1	2
Red shouldered leaf beetle	N&A	Chrysomelidae, *Monolepta australis* (Jacoby)	0	0	0	0	0	0	0	1	1
Thrips	L&A	Thripidae	177[A]	119[A]	296[A]	696	539	1235	26	6	32
Green jassids	N&A	Cicadellidae	1136	1356	2492	922	1263	2185	456	302	758
Other jassids	N&A	Cicadellidae							43	42	85
Aphids	N&A	Aphidae				25	116	141	0	2	2
Whiteflies	N&A	Aleyrodidae	0	0	0	848	639	1487	0	0	0
Weevils	A	Curculionidae	11	2	13	0	0	0	44	24	68
Crickets	N&A	Gryllidae, *Teleogryllus* spp.	4	0	4	0	1	1	0	3	3
		Natural enemies									
3-banded ladybird	A	Coccinellidae, *Harmonia octomaculata* (F.)	0	1	1	0	0	0	2	0	2
Common spotted ladybird	A	Coccinellidae, *Harmonia conformis* (Bosiduval)	2	2	4	0	0	0	0	0	0
Variable ladybird	A	Coccinellidae, *Coelophora inaequalis* (F.)	0	1	1	0	0	0	0	1	1
Spotted amber ladybird	A	Coccinellidae, *Hippodamia variegata* (Goeze)	69	29	98	4	7	11	0	0	0
Transverse ladybird	A	Coccinellidae, *Coccinella transversalis* F.	30	24	54	5	2	7	11	10	21
Striped ladybird	A	Coccinellidae, *Micraspis frenata* (Erichson)	0	0	0	2	0	2	0	0	0
Ladybird larvae	L	Coccinellidae	0	1	1	0	0	0	0	0	0
Minute two spotted ladybird	A	Coccinellidae, *Diomus notescens* (Blackburn)	5	3	8	1	0	1	0	0	0
Mite eating ladybird	A	Coccinellidae, *Stethorus* spp.	0	0	0	0	0	0	1	0	1
Red and blue beetle	A	Melyridae, *Dicranolaius bellulus* (Guérin-Méneville)	547	521	1068	33	66	99	1	1	2
Glossy shield bug	N&A	Pentatomidae, *Cermatulus nasalis* (Westwood)	0	1	1	0	0	0	0	0	0
Predatory shield bug	N&A	Pentatomidae, *Oechalia schellenbergii* (Guérin-Méneville)	0	5	5	0	0	0	1	4	5
Big eyed bug	N&A	Lygaeidae, *Geocoris lubra* Kirkaldy	116	76	192	37	28	65	17	13	30
Damsel bug	N&A	Nabidae, *Nabis kinbergii* Reuter	275	281	556	45	54	99	2	5	7
Pirate bug	N&A	Anthocoridae, *Orius* spp.	8	3	11	14	0	14	0	0	0
Brown smudge bug	N&A	Miridae, *Deraeocoris signatus* (Distant)	108	129	237	37	50	87	1	0	1
Broken backed bug	N&A	Miridae, *Taylorilygus pallidulus* (Blanchard)	0	2	2	0	1	1	0	0	0

(*Continued next page*)

Table 1. (*continued*)

Common name	Life stage	Latin name	Narrabri beatsheets (10)			Narrabri devacs (3)			Kuninurra beatsheets (2)		
			Vip	Conv.	Total	Vip	Conv.	Total	Vip	Conv.	Total
Brown lacewing	A	Hemerobiidae, *Micromus tasmaniae* Walker	2	3	5	0	0	0	9	4	13
Brown lacewing	L	Hemerobiidae, *Micromus tasmaniae* Walker	1	4	5	0	2	2	3	1	4
Green lacewing	A	Chrysopidae, *Mallada* spp.	5	1	6	3	4	7	1	0	1
Green lacewing	L	Chrysopidae, *Mallada* spp.	9	5	14	1	3	4	3	2	5
Ants <2mm small	A	Formicidae	54	43	97	0	2	2	6	5	11
Ants 3–4mm large	A	Formicidae	143	98	241	112	111	223	4	9	13
OTHER ants	A	Formicidae	5	0	5	2	0	2	0	0	0
Orb weaving spider	J&A	Araneidae/Tetragnathidae	11	17	28	3	6	9	9	20	29
Yellow night stalker Spider	J&A	Clubionidae, *Cheiracanthium*	26	26	52	3	0	3	13	9	22
Wolf spider	J&A	Lycosidae	20	45	65	1	0	1	0	3	3
Lynx spider	J&A	Oxyopidae	422	392	814	108	112	220	8	9	17
Jumping spider	J&A	Salticidae	61	63	124	7	11	18	13	5	18
Tangle web spider	J&A	Theridiidae/Linyphiidae	65	125	190	13	11	24	13	14	27
Flower/crab spider	J&A	Thomisidae	15	8	23	8	1	9	0	1	1
Cycloctenidae spider	J&A	Cycloctenidae	0	0	0	0	0	0	8	6	14
OTHER spiders	J&A		146	139	285	36	32	68	2	4	6
Trichogramatids	A	Trichogrammatidae				3	5	8			
Microplitis	A	Braconidae, *Microplitis*				0	1	1			
Parasitoid wasp	A		0	2	2				2	0	2
Others											
Pollen beetle	A	Nitidulidae, *Carpophilus* spp.	314	170	484	43	43	86	1	0	1
OTHER wasps	A		37	71	108	28	45	73			
Flies	A		851	836	1687	3200	4639	7839			
OTHER wasps/flies	A								5	1	6
OTHER bugs	N&A		106	129	235	24	22	46	1	0	1
OTHER beetles	L&A		639	417	1056	132	159	291	6	5	11
			6003	5712	11715	6522	8082	14604	1352	936	2288

[A]Only presence or absence noted in individual beatsheet samples; 200 samples in total for Vip and for conventional.

especially ANOVA, but where the data could not be normalised through transformation we used non-parametric techniques such as the Mann–Whitney U test or Friedman ANOVA. For statistical analysis of *Helicoverpa*, aphid, and jassid data, the ANOVA was a split plot with sample date the main plot, Vip/Conventional the subplot, and 10 beatsheet samples per plot pooled to give 1,360 d.f. For other species where numbers were low, the 10 beatsheets were grouped to give 1,9 d.f. Because of the large number of species tested, we were concerned about committing a type I error. To correct for this our α value was modified to 0.01, and $0.05 > P > 0.01$ was used to indicate a strong trend.

As diversity indices differ in their strengths and weaknesses, it is unwise to rely on one index (Tothmeresz 1995). We compared the species diversity of beneficial and non-target arthropods in the beatsheet communities using 2 diversity indices, the Simpson index (SI; Simpson 1949) and the Shannon-Weaver index (SW; Shannon and Weaver 1949), which are part of Renyi's diversity index family (Tothmeresz 1995) and give a measurement of evenness (a measure of the distribution of relative abundances of different taxa in a sample) and richness (the number of species in a sample). The Simpson index was corrected for sample-size bias $(SI = \Sigma[(n^2 - n)/(N^2 - N)])$ and modified $(-\ln SI)$ following Rosenzweig (1995) so that the units increase with an increase in diversity. The Simpson index is more sensitive to dominant species, whereas the Shannon-Weaver index $(H = -\Sigma p_i \ln p_i)$ is more sensitive to rare species.

Results

Plant growth

At Narrabri there was no significant difference between Vip or conventional cotton in height for any of the sample dates (ANOVA; $F = 0.38$; d.f. $= 1,8(1)$; $P = 0.555$, mean: Vip $= 0.67$ m, Sicala $40 = 0.66$ m) nor in node number (ANOVA; $F = 1.26$; d.f. $= 1,9$; $P = 0.29$; mean: Vip $= 16.1$, conventional $= 15.8$). Plants in Vip plots retained more bolls (44%) than either those in Sicala 40 conventional (15%) plots or those in the small Coker conventional (24%) plots (ANOVA; s.e.d. $= 5\%$; $P < 0.001$). Vip retained more bolls at position 1 on fruiting branches (58%) than either Coker conventional (22%) or Sicala 40 conventional (15%) (ANOVA; s.e.d. $= 7\%$; $F = 5.01$; $P = 0.018$).

At Kununurra, unsprayed Vip plants were taller than those in the unsprayed conventional plots (ANOVA; $F = 307$; d.f. $= 1,39$; $P < 0.001$; Vip mean height $= 0.90$ m, conventional mean height $= 0.63$ m). The sprayed conventional cotton had a mean height of 0.69 m but was not included in ANOVA because there were fewer replicates. Plants in the unsprayed Vip plots also had more bolls and squares than those in the unsprayed conventional plots (Mann–Whitney U test; $U = 0.0$; $P = 0.029$; $n = 4,4$; mean number of bolls: Vip $= 21/m^2$, conventional $= 1/m^2$; mean number of squares: Vip $= 41/m^2$, conventional $= 8/m^2$). The average number of bolls and squares in the 2 sprayed samples was 1 boll/m^2 and 41 squares/m^2.

Efficacy against Helicoverpa *spp.*

At Narrabri, 401 *Helicoverpa* spp. eggs were recorded during visual surveys, of which 164 eggs were found on Vip plots. There was no difference in the number of eggs found on unsprayed conventional or Vip plots (Mann-Whitney U test; $n = 44$; $U = 179.5$; $P = 0.14$; mean number of eggs (per sample date): conventional $= 10.77$, Vip $= 7.46$).

In total 156 *Helicoverpa* spp. larvae were found during visual surveys. Of these, only 2 were found in Vip plots (Fig. 1).

At Kununurra in 2 visual surveys, 163 *Helicoverpa* eggs were found, a higher proportion of which was found in the unsprayed Vip plots compared with unsprayed conventional plots (Mann–Whitney U test; $U = 121$; $n = 40$; $P = 0.033$; mean number of eggs per m in Vip: 4.3, unsprayed conventional: 2.1, sprayed conventional not part of analysis: 3.6).

Of the 13 larvae collected at Kununurra, 5 were *Helicoverpa* larvae, 4 were rough bollworm (*Earias huegeliana* Gaede), and 4 were loopers [*Anomis flava* (Fabricius)]. Of these larvae only one, a rough bollworm, was found in a Vip plot.

Parasitism rates of Helicoverpa *spp. eggs and larvae*

Narrabri

Of the 360 eggs collected from the field for rearing, 204 yielded caterpillars and 11 yielded parasitoid wasps. The remainder failed to hatch. Eight of the 11 eggs that yielded parasitoids were collected from conventional plots and of these, one yielded a *Telenomus* sp. and 7 yielded a total of 19 *Trichogramma* sp. Of the 3 eggs collected from the Vip plots that yielded parasitoids, one yielded a *Telenomus*, and 2 yielded a total of two *Trichogramma*. There was no significant difference between Vip and conventional plots in the proportion of eggs that produced parasitoids (6% of the hatched eggs collected from conventional plots, 3% of the hatched eggs collected from Vip produced parasitoids; Fisher's exact test: $P = 0.4$).

Of the 207 larvae (from both beatsheet and visual surveys) collected for rearing, 112 yielded moths and 20 yielded parasitoid wasps (8 *Microplitis demolitor* Wilkinson, 7 *Heteropelma scaposum* (Morley) and 5 tachinid flies). The rest either died as larvae or pupae. Of the larvae collected,
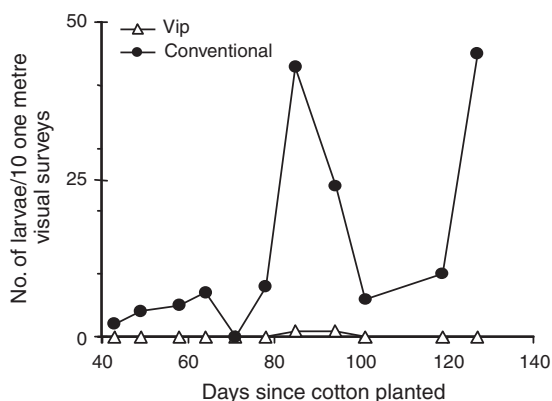

Fig. 1. The mean number of *Helicoverpa* larvae found per 10 one metre visual samples in Vip and conventional plots throughout the season at Narrabri.

only 2 were collected from Vip plots, and both of these yielded *H. armigera* moths. Of the 130 emergences (moth or parasitoid) from larvae collected from conventional plots, 15.4% yielded parasitoids. *H. punctigera* (47.7%) was more common than *H. armigera* (36.9%).

Kununurra

A higher proportion of the eggs found in Vip plots were attacked by *Trichogramma* egg parasitoids in the first survey than those in the unsprayed conventional plot (Fishers exact test: 2-tailed significance level, $P = 0.005$). In the Vip plots, 38 (95%) of the 40 eggs that hatched yielded 104 *Trichogramma*, whereas 11 (61%) of the 18 eggs from conventional plots that hatched yielded 28 *Trichogramma*. Three of the 5 eggs collected from the sprayed conventional plot yielded 4 *Trichogramma*. There was no difference in the parasitism rate of eggs in Vip and conventional crops in the second survey as in both crops all eggs were parasitised by *Trichogramma* (Vip, 14 eggs; unsprayed conventional, 9 eggs). The Vip eggs yielded 38 *Trichogramma*, and the conventional eggs yielded 31 *Trichogramma*. Of the 5 eggs collected from sprayed conventional cotton, 3 were parasitised, producing a total of 4 *Trichogramma*. It was common for over 3 *Trichogramma* to emerge from the same egg. The most to emerge from one egg was 8.

Invertebrate communities of Vip and conventional cotton

Narrabri beatsheets

Over 12 000 invertebrates were sampled throughout the season using the beatsheet sampling technique (Table 1). Of these, 125 were *Helicoverpa* larvae, which were significantly less abundant in the Vip plots (2 larvae) than in the conventional plots (123 larvae) (ANOVA split plot design; $F = 1473$; d.f. $= 1,1$; $P = 0.017$).

There was no difference between Vip or conventional cotton in arthropod abundance per beatsheet on any of the sample dates (ANOVA; $F = 0.25$; d.f. $= 1,189$; $P = 0.621$; mean number per Vip beatsheet $= 30.6$, mean number per conventional beatsheet $= 31.0$).

There was no difference in the diversity of beneficials and non-target organisms as measured by beatsheets between crops using the –ln Simpson index (ANOVA; $F = 0.66$; d.f. $= 1,159$; $P = 0.418$; mean: Vip $= 1.64$, conventional $= 1.61$) but there was a difference in diversity between crops as measured by the Shannon-Weaver index (ANOVA; $F = 8.57$; d.f. $= 1,178$; $P = 0.004$; mean: Vip $= 1.58$, conventional $= 1.49$; Fig. 2), although this difference explained only 0.72% of the variability in diversity.

The most prominent group of invertebrates collected was jassids (Cicadellidae), followed by flies and the red and blue beetle *Dicranolaius bellulus* (Guérin-Méneville) (Table 1). Other beetles (a category for those not identified to species), mirids (*Creontiades* spp.), lynx spiders (Oxyopidae), and damsel bugs (*Nabis kinbergii* Reuter) were also very common.

We compared the overall arthropod community in Vip and conventional cotton across the season using a principal response curve (PRC). Seasonal differences accounted for 34.3% of the total variance. Although there was a significant difference between the communities within the
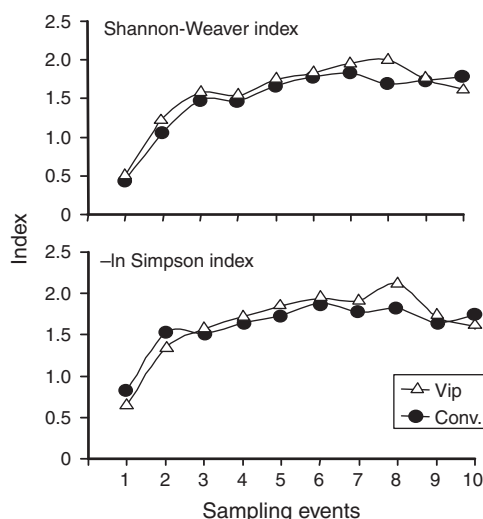
Fig. 2. Mean –ln Simpson index and Shannon-Weaver diversity indices of beneficials and non-target organisms in beatsheet samples throughout the season. Some beatsheet samples could not be included in the analysis because of sparse data.

2 crops (Monte Carlo simulation, F-ratio $= 11.9$; $P = 0.005$; 199 permutations, Fig. 3), crop type accounted for only 5.2% of the variance.

To identify which species or groups (apart from *Helicoverpa* spp. and other Lepidoptera) may be influencing the pattern, we compared general groups of species found in the Vip and conventional cotton (see rows labelled G in Table 2). Of these, only beetles (predators and others) showed a strong trend to be more common in the Vip cotton.
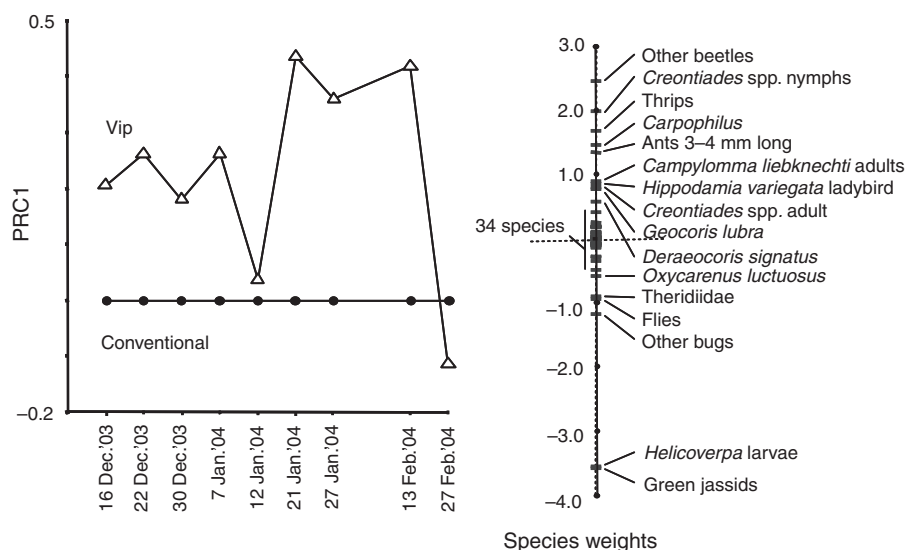
Species 'weights' were generated as part of the PRC analysis. Weights greater than 0.5 (Fig. 3) are most likely to follow the abundance changes shown in the PRC, whereas those less than -0.5 show a trend in the opposite direction (values between -0.5 and 0.5 do not contribute strongly to the community response; Van den Brink and Ter Braak 1999). Of the taxa with significant species weights, *Helicoverpa* larvae (Table 2), jassids (Fig. 4), and tangle web spiders (Theridiidae; Table 2, Fig. 4) were significantly more common in conventional plots, whereas the mirids (*Creontiades* spp.; Table 2, Fig. 4), the spotted amber ladybird *Hippodamia variegata* (Goeze) (Table 2, Fig. 4), and the pollen beetle (*Carpophilus* sp.; Fig. 4) were significantly more common in Vip plots. There was a strong trend for the apple dimpling bug [*Campylomma liebknechti* (Girault)] and the rutherglen bug (*Nysius vinitor* Bergroth) to be more common in Vip cotton (Table 2), whereas wolf spiders (Lycosidae) showed a tendency to be associated with conventional cotton (Table 2), although neither wolf spiders nor *N. vinitor* contributed strongly to the PRC.

Narrabri suction samples

There was no difference between Vip or conventional cotton in arthropod abundance per devac. on any of the survey dates (ANOVA; $F = 1.17$; d.f. $= 1,29$; $P = 0.289$; mean number per Vip suction sample $= 217$, mean number per conventional suction sample $= 269$).

The most prominent groups of invertebrates collected were flies, jassids, whiteflies (Aleyrodidae), and thrips (Table 1), although ants, lynx spiders, and other beetles were also very common.

The overall arthropod communities in Vip and conventional cotton were compared across the season using a PRC (Fig. 5). We found that the structure of arthropod communities sampled

Fig. 3. PRC of the conventional and Vip plots as sampled by beatsheets throughout the season (the conventional is the control) and the species weights that most contributed to this pattern (species weights with positive values followed the Vip PRC, whereas negative values showed the opposite trend). There was a significant difference in the conventional and Vip communities (Monte Carlo test: F-ratio $= 11.9$; $P = 0.005$; 199 permutations), although only 5.2% of the variance in the data is explained by crop type.

Table 2. Arthropod groups tested, where $n > 30$, to see if they were more common in Vip or conventional cotton

F-statistic for ANOVA; *U*-statistic for Mann–Whitney test. *P* values in bold indicate samples that the ordination diagrams indicated were more associated with either Vip or conventional crops (see text). An empty space beside an animal group means that $n < 30$ or the group was not sampled in that survey (e.g. web spiders instead of tangle web spiders). Vip, Mean/average rank in Vip per beatsheet/devac; conv, Mean/average rank in conventional; E, eggs; L, larvae; A, adults; N, nymphs; J, juveniles

Common name		Life stage	Latin name		Narrabri beatsheets d.f.	Test statistics	Vip	Conv.	P-value	Narrabri devacs d.f.	Test statistics	Vip	Conv.	P-value	Kununurra d.f.	Test statistics	Vip	Conv.	P-value
Helicoverpa		L	*Helicoverpa* spp.	P	1,360	F=121	0.01	0.7	**<0.001**	1,51	18.2	0.1	1	**<0.001**					
Cotton seed bug		N&A	Lygaeidae, *Oxycarenus luctuosus*	P	n=36	U=104	2.0	5.3	**0.068**						1,38	F=4.18^A	10.5	8	0.048*
Apple dimpling bug		N&A	Miridae, *Campylomma liebknechti*	P	1,9	F=7.03	3	2	0.026*										
Whiteflies		N&A	Aleyrodidae	P						1,27	F=1.71^A	2.8	2.1	**n.s.**					
Aphids		N&A	Aphidae	P						n=20	U=17.5^E	2.5	11.1	0.011*					
Green Jassids		N&A	Cicadellidae	P	1,360	F=19.1	5.7	7.0	**<0.001**	1,2	F=7.9	31	42	n.s.	1,38	F=15.2	12.5	8.6	**<0.001**
Mirids		N&A	Miridae, *Creontiades* spp.	P	1,360	F=18.4	2.9	2.1	**<0.001**	1,51	F=7.3	2.3	1.4	**0.009**	1,38	F=52.3^C	5.1	2.1	**<0.001**
Weevils		L&A	Curculionidae	P											n=80	U=648	1.1	0.6	**n.s.**
Thrips		L&A	Thripidae	P	1,9	F=8.26^A	0.9	0.6	**n.s.**	1,27	F=2.66	23	18	n.s.	n=80	U=582	0.7	0.2	**0.036***
all Shield bugs		N&A	Hemiptera, Pentatomidae	P	1,5	F=4.29^B	0.3	3.1	0.051										
Rutherglen		N&A	Lygaeidae, *Nysius vinitor*	P	1,9	F=4.4	0.4	0.2	0.036*										
Pest bugs		N&A	Hemiptera	P	1,9	F=0.00^A	3.7	3.1	n.s.										
G Beneficial bugs		N&A	Hemiptera	B	1,9	F=0.32^A	2.5	2.7	n.s.										
G Big-eyed bug		N&A	Lygaeidae, *Geocoris lubra*	B	1,9	F=64.4	0.58	0.4	0.079	1,51	F=0.85^C	1.2	0.9	**n.s.**	1,1	F=205^C	0.60	0.52	0.044*
Damsel bugs		N&A	Nabidae, *Nabis kinbergii*	B	1,9	F=13.5	1.4	1.5	n.s.	1,2	F=0.17	1.5	1.8	n.s.					
Brown smudge bug		N&A	Miridae, *Deraeocoris signatus*	B	1,9	F=1.0^A	0.54	0.65	**n.s.**	1,2	F=0.45	1.2	1.7	n.s.					
G Beetles		L&A	Coleoptera	B	1,9	F=6.27	0.8	0.6	0.034*										
Red & blue beetles		A	Melyridae, *Dicranolaius bellulus*	B	1,9	F=0.67^C	2.7	2.7	n.s.	1,51	F=6.8	1.1	2.2	0.012*					
Spotted amber ladybird		A	Coccinellidae, *Hippodamia variegata*	B	1,9	F=10.73	0.4	0.1	0.01**	1,2	F=0.37	3.6	3.7	n.s.					
Transverse ladybird		A	Coccinellidae, *Coccinella transversalis*	B	1,9	F=0.84	0.2	0.1	n.s.										
G Ants		A	Formicidae	B	n=36	U=127	2.8	2.3	**n.s.**	1,2	F=0.37	3.6	3.7	n.s.					
Ants 3–4 mm large		A	Formicidae	B															
G Spiders		J&A	Araneidae	B	1,9	F=4.42	3.8	4.5	0.065	1,2	F=0.37	3.6	3.7	n.s.	n=80	U=679	0.55	0.85	**n.s.**
web spiders		J&A	Araneidae	B															
Tangle web spider		J&A	Theridiidae/Linyphiidae	B	1,9	F=44.1	0.3	0.8	**<0.001**										
Wolf spider		J&A	Lycosidae	B	1,9	F=6.24^C	0.1	0.2	0.034*										
Lynx spider		J&A	Oxyopidae	B	1,9	F=0.01	2.1	2.1	n.s.	1,2	F=0.37	3.6	3.7	n.s.					
Jumping spider		J&A	Salticidae	B	1,9	F=1.4^A	0.3	0.3	n.s.										
Yellow night stalker		J&A	Clubionidae, *Cheiracanthium*	B	1,9	F=1.6	0.1	0.1	n.s.										
G Flies		A	Diptera	B	1,9	F=0.00	4.3	4.3	**n.s.**	1,2	F=2.8	107	155	n.s.					
G Wasps		A	Hymenoptera	B	3	F=1.90^D	2.7	2.2	n.s.										
Pollen beetle		A	Nitidulidae, *Carpophilus* spp.	O	1,5	F=32.36^B,C	3	1	**0.002**	1,2	F=0	1.4	1.4	n.s.					

**P < 0.01; *: a trend (0.05 > P > 0.01), G, general groups; B, beneficial; P, pest; O, other.

^A Ln transformed. ^B First 4 dates not analysed because no individuals were present. ^C Square root transformed. ^D Friedman one-way ANOVA between blocks. ^E Survey 3 only.
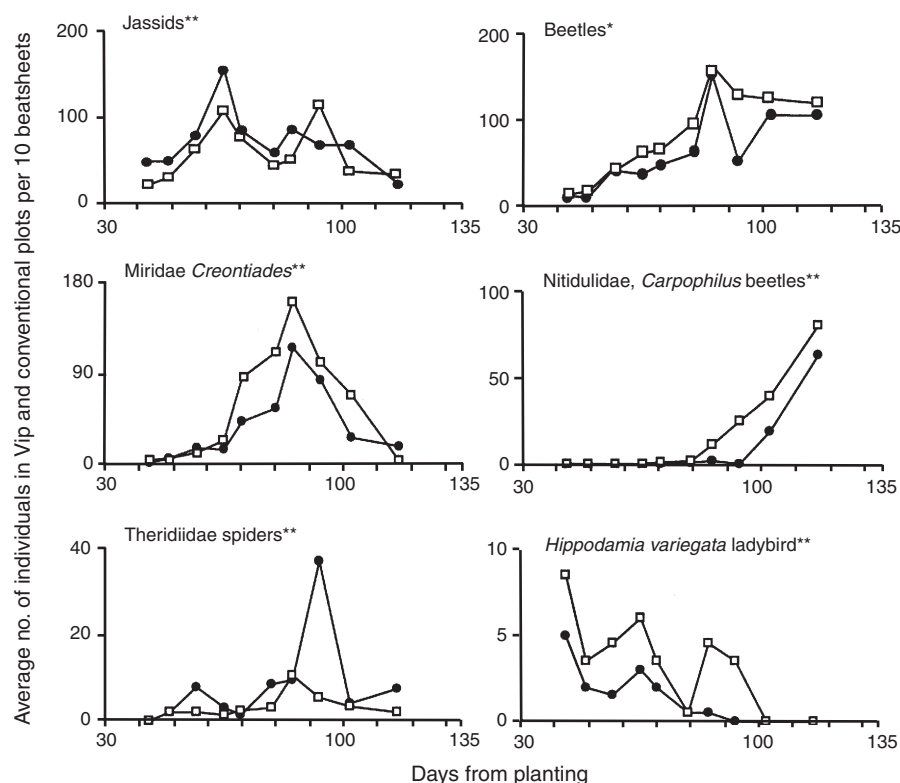
Fig. 4. A comparison between the number of individuals found in beatsheets on conventional (closed circles) and Vip (open squares) plots in invertebrate groups which most contributed to the PRC in Fig. 4. All species groups showed a significant difference (**, where $P < 0.01$) or a trend (*, where $0.05 > P > 0.01$) between the number of individuals on the 2 crop types (Table 2), *Creontiades* includes both adults and nymphs.

by suction samplers, like that sampled by beatsheets, was strongly influenced by seasonal variance, which accounted for 30.8% of the total variance. Although there was a significant difference between the Vip and conventional cotton communities (Monte Carlo simulation; F-ratio $= 2.5$; $P = 0.025$; 199 permutations), this accounted for only 6.8% of the variance.

Twenty three species groups had species weights that strongly contributed to the Vip PRC (Fig. 5). Of these, 7 had $n > 30$ (mirids, *Helicoverpa* larvae, aphids, the red and blue beetle (*D. bellulus*), big-eyed bugs (*Geocoris lubra* Kirkaldy), thrips, and whiteflies). Mirids were significantly more common in Vip plots and *Helicoverpa* larvae were significantly more common in conventional plots, while aphids and *D. bellulus* showed a trend to be more common in conventional plots (Table 2). Of the 32 *Helicoverpa* larvae sampled, only three were found on Vip cotton.

Kununurra beatsheets

Due to uneven replication the counts were analysed using a one-way ANOVA to compare the means for each of the 5 plots. There was a significant difference between the number of individuals in beatsheets taken from the 5 plots (ANOVA; $F = 48.39$; d.f. $= 4, 76$; $P < 0.001$). Extremely low numbers of

arthropods were found in the sprayed plot (mean $= 2.9/m^2$) and significantly lower numbers were found in the conventional plots (means $= 23.6/m^2$ and $23/m^2$) compared to the Vip plots (means $= 34.3/m^2$ and $33.4/m^2$, l.s.d. for comparison between all plots $= 5.12$).

The Kununurra beatsheet samples differed from those in Narrabri in that cotton seed bugs were particularly common (over 700 were collected, Table 1). A CCA (with crop type as the environmental variable) indicated that crop type explained 2.1% of the total variation in the unsprayed communities with the 2 surveys combined (Monte Carlo simulation; F-ratio $= 1.7$; $P = 0.006$; 499 permutations; eigenvalue $= 0.047$; Fig. 6). The CCA indicated that 3 taxa with more than 30 individuals (the mirids *Creontiades* spp., thrips, and weevils) appeared to be more strongly associated with Vip than with conventional cotton. Of these, the mirids were significantly more common in Vip crops (Table 2) and thrips showed a strong trend to be more common in Vip.

Although the CCA diagram indicated that green jassids and the bug *Oxycarenus luctuosus* (Montrouzier) were not more associated with Vip cotton with respect to the other invertebrates, we found that in absolute numbers, green jassids were significantly more common in Vip, while *O. luctuosus* showed a strong trend to be more common in Vip (Table 2).
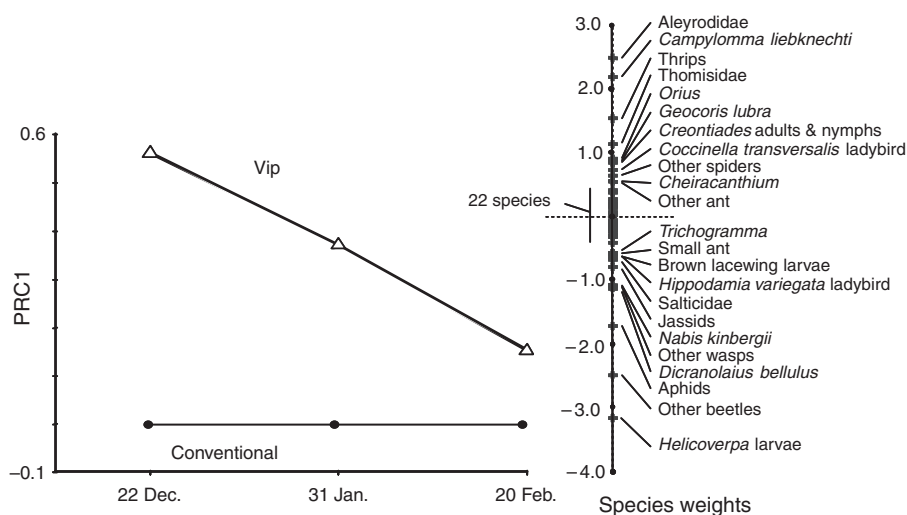
Fig. 5. PRC of the conventional and Vip plots as sampled by suction samples throughout the season (the conventional is the control) and the species scores that most contributed to this pattern. There was a significant difference in the conventional and Vip communities (Monte Carlo test; F-ratio = 2.5; $P = 0.025$; 199 permutations), although only 6.8% of the variance in the data is explained by crop type.
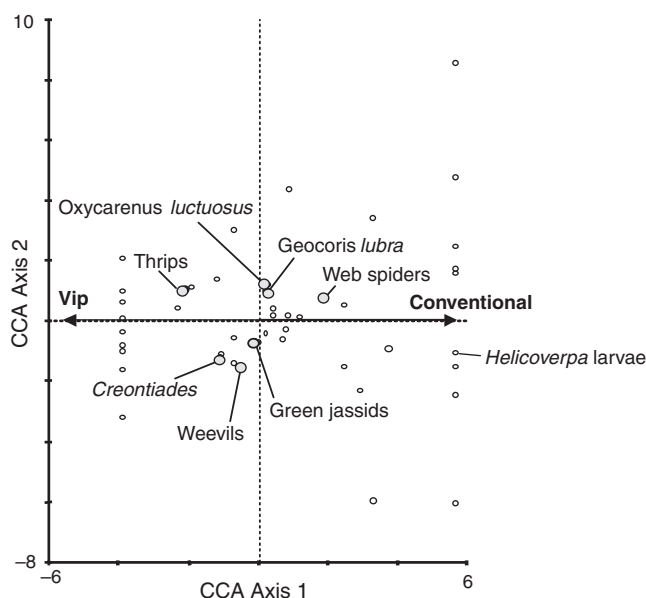

Fig. 6. CCA of species (excluding samples from sprayed plot) in both Kununurra surveys with respect to their association with unsprayed Vip or conventional cotton (axis 1). Those species towards the left are more strongly associated with the Vip crop, while species to the right are strongly associated with the conventional crop. The 6 most common species are highlighted. *Creontiades* includes both adults and nymphs.

Development of secondary pest populations on Vip

In the second field at Narrabri inoculated with mites, we sampled 4 times during the season and found no significant difference between the number of mites on Vip and on conventional cotton (ANOVA with square-root transformation; $F = 0.01$; d.f. = 1,19; $P = 0.9$; mean number of mites per sample: Vip = 16.7, conventional = 16.1). There were, however, significantly more juvenile whitefly (*Bemisia tabaci* (Gennadius) B-biotype) on

the Vip cotton (ANOVA with square-root transformation; $F = 21.41$; d.f. = 1,19; $P < 0.001$; mean number of juvenile whitefly per sample: Vip = 64.2, conventional = 16.5).

Hairiness

No difference was found in the number of hairs on leaves at position 3 on Vip compared with conventional Sicala 40 (Mann-Whitney U test; U = 9; $P = 0.18$; $n = 24$; mean number of hairs: Vip = 44 hairs/disc, conventional = 35 hairs/disc).

Discussion

Plant growth

Although most parameters used to compare the growth of Vip and conventional cotton in Narrabri showed no differences by the end of the season, Vip plants retained more bolls. This may explain the higher number of mirids in Vip cotton because young squares (flower buds) and bolls are a food source for mirids. As our data suggest that Vip is highly efficacious against *Helicoverpa* spp. and other Lepidotera, these pests would cause less pest-induced shedding of young squares and bolls in the Vip cotton. Hence, there is likely to have been more squares, flowers, and bolls most of the time in the Vip cotton, possibly also explaining why other members of the invertebrate community that use the fruiting structures, such as the pollen beetles, were also more common in Vip cotton.

In Kununurra, Vip plants were taller and set more bolls during the survey period than either the unsprayed or sprayed conventional plots (the lower height and fewer bolls of the sprayed conventional cotton may be because it was not sprayed often enough to effectively prevent all damage). As Vip plants were bigger and retained more fruit, they may have been more attractive and apparent to insects than conventional plants. This may explain the larger number of arthropods and the heavier *Helicoverpa* egg-lays in the Vip plots at Kununurra.

It may also explain why eggs in the Vip cotton suffered higher parasitism rates.

Efficacy

Beatsheets, visual surveys, and suction samples all showed that *Helicoverpa* spp. had an extremely low survival rate on Vip cotton. This is despite comparable egg-lays between Vip and conventional cotton in Narrabri, and higher egg-lays in Vip crops in Kununurra. These results show that Vip cotton was very effective at controlling *Helicoverpa* spp. larvae under field conditions at both Narrabri and Kununurra.

Parasitism rates

Egg parasitism rates were low at Narrabri. Only 3–6% of the eggs collected yielded parasitoids, and of these there was no significant difference in the parasitism rate of Vip or conventional cotton. At Kununurra, the parasitism rate was much higher. More eggs were laid on Vip plants, but a higher proportion of these eggs was attacked by parasitoids compared with those in conventional plots. These results show that there is no detrimental effect of Vip cotton on egg parasitoids. Larval numbers were extremely low on the Vip cotton so it was not possible to compare larval parasitism rates. However, larval parasitism rates in the unsprayed conventional cotton at Narrabri averaged at ∼15%.

Arthropod community

Sampling with both beatsheets and suction samples revealed a small but consistent difference between communities on unsprayed Vip and conventional crops of 2–7%. Some differences between the communities are to be expected as reducing *Helicoverpa* spp. and other Lepidoptera in the Vip cotton would alter the community directly (fewer Lepidopterans) and indirectly (fewer Lepidopteran-specific predators and parasites).

Nevertheless, we found several patterns in the beneficial community that cannot easily be explained and may be worthy of further investigation. First, it is unclear why tangle web spiders, a generalist predator, were less abundant in Vip compared with conventional cotton in Narrabri beatsheet samples. In addition, there was a strong trend for some Coleopteran species to be more common in the Vip beatsheet samples from Narrabri, in particular pollen beetles and spotted amber ladybirds. The beetle *Carpophilus* spp. feed in flowers, so greater numbers of these animals could be due to increased flowering rates in Vip cotton, due to less loss of squares following damage by *Helicoverpa* spp. larvae. However, it is unclear why the coccinelid *H. variegata* should be more common in Vip cotton. They feed on aphids whose numbers were lower in Vip cotton, but they also feed on whitefly (Gerling *et al*. 2001) whose numbers were not reduced in Vip, and were even more common in the Vip plots in Expt 2 at Narrabri.

Damsel bugs (*Nabis kinbergii* Reuter) are one of the few generalist predators whose numbers are often lower in Bt cotton and Bt corn crops (Daly and Buntin 2005; Naranjo 2005; Whitehouse *et al*. 2005). Why damsel bug numbers are often lower in Bt crops is unclear. Damsel bugs prey on a range of small insects including aphids, mites, whitefly, and *Helicoverpa* spp., leading Whitehouse *et al*. (2005) to suggest that their lower

numbers in Bt cotton may be due in part to lower numbers of Lepidopteran larvae. This argument was not supported by the current study, which showed no significant difference in the number of damsel bugs in Vip and conventional cotton, despite a big difference in the number of Lepidopteran larvae.

Among pest species, we found a strong trend for fewer jassids in the Vip crop in the Narrabri beatsheet data, but higher numbers in the Vip crop at Kununurra. The Vip plots were much more attractive than the conventional plots at Kununurra (they were taller, more lush, and had more squares), possibly explaining the higher numbers of jassids in the Vip at this site. The Kununurra CCA diagram supports this argument; all arthropods were more abundant in the Vip crop, and jassids were no more associated with Vip plots than any other arthropod.

Aphids in Narrabri devac. data samples were less common in Vip crops, possibly reflecting the higher numbers of spotted amber ladybirds in the Vip crop. There was no effect of Vip on mites (small plot, Narrabri) or thrips (small plot data and devac. data, Narrabri). The higher number of mirids in the Vip plots is probably due to the higher number of bolls and flowers in the Vip crop at both sites.

We found a large increase in the number of whitefly on Vip plants in Expt 2 at Narrabri, although this finding was not supported by the devac. samples from the larger plots in Expt 1. Coker 312 is generally hairier than the local delta-smooth varieties such as Sicala 40, and whitefly are known to be more numerous on more hairy cotton leaves (Fitt 1996; Chu *et al*. 1999, 2003). Although we did not find any difference in the hairiness of the leaves of Vip and conventional Sicala 40, these counts were done late in the season where differences may be less pronounced. Whitefly is an important secondary pest in the northern cotton-growing regions of Australia, which can be difficult to manage. If this difference occurred consistently in Vip crops then a change in crop management could be required.

There was little difference in species diversity or species richness of beneficial and non-target arthropods between Vip and conventional cotton. The Shannon-Weaver index, which is sensitive to rare species, indicated that Vip cotton was more diverse, but the percentage of variation explained was very small (0.7%). Overall it appears that the structure of the beneficial community was little affected by Vip cotton.

In summary, there was only a slight difference between the arthropod communities in Vip and conventional cotton, most of which could be explained by the lack of Lepidopterans, and the resulting healthier Vip plants. The need to use 2 cultivars (Coker and Sicala 40) may also have contributed to the slight difference. It appears that this technology would be a good complement to the existing Bt-cotton technology, especially if implemented in such a way as to reduce the risk of resistance developing to either technology.

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Pest Profile

Pale cotton stainers, *Dysdercus sidae*

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Cotton stainers are recognised as occassional pests of cotton in Australia. Economic damage is unusual because of their;

- **incidental control when using broad spectrum insecticides for other pests;**
- **inability to survive; temperatures above 40°C.**
- **need for free water to be present.**

However in mild seasons Bollgard II® crops maybe a favourable environment for cotton stainers and they may need to be managed.

Figure 1 (above); D. sidae first instar.

Figure 2 (above); D. sidae third instar.

Figure 3 (below); D. sidae fourth instar

Figure 5 (below); D. sidae adult.

Cotton stainer bugs belong to the Pyrrhocorid group of bugs and are pests of cotton around the world. A range of species are involved, each slightly different in appearance and biology. In Australia two species have been recorded from cotton, the cotton stainer (*Dysdercus cingulatus*) and the pale cotton stainer (*Dysdercus sidae*), which is generally the most common. These species look very similar but the pale cotton stainer is generally a duller brown (see Figure 5, next page).

Lifecycle

Females lay batches of ~100 creamy white eggs in shallow depressions in the soil (Figure 6),

under debris or occasionally on the undersides of cotton leaves low in the canopy. Eggs change to orange as they near hatching. The time taken can vary from 5 days at 30°C to 13 days at much cooler or warmer temperatures.

Young cotton stainers moult

Figure 6; D. sidae eggs (1 mm diameter) laid on the soil surface.

through five nymphal stages before reaching adulthood. The indicative size of a cotton stainer at each stage of development is;

First Instar   2–3 mm   (Figure 1)
Second Instar   4–5 mm
Third Instar   6–8 mm   (Figure 2)
Note the small wing buds.
Fourth Instar   9–11 mm   (Figure 3)
Note the larger wing buds.
Fifth Instar   12–14 mm
Adult   15 mm   (Figure 4)

Cotton Catchment Communities CRC

First instars remain together and can be observed on the ground near to where they have hatched.

Second instars may disperse in search of food. Their stylets are not yet long enough to penetrate unopen bolls and reach the seed within. Instead they look for ripe, exposed seed or decaying seed. When free water is present they are able to penetrate quite hard, dry seeds. They may be seen to congregate around suitable sourses of food.

Once the third instar is reached, they are able to commence feeding on developing seed within the bolls. From this stage onward the nymphs will disperse to feed and congregate to moult.

Two days after the final moult, adults begin to mate. Fertility will be highest when the temperature is 30°C and low at temperature extremes.

The availability of water and nectar is important for feeding and development during all growth stages. Adults can survive on water alone for several weeks when food is scarce.

There is no resting stage in winter. Survival is dependent on them finding food, water and shelter from frost. Of the cotton stainers,
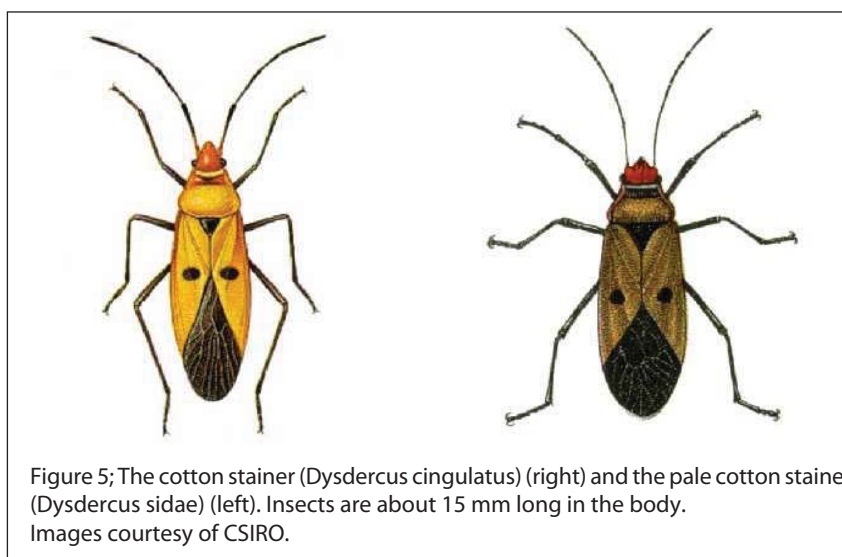
Figure 5; The cotton stainer (Dysdercus cingulatus) (right) and the pale cotton stainer (Dysdercus sidae) (left). Insects are about 15 mm long in the body. Images courtesy of CSIRO.

D. sidae is one of the most frost tolerant species, being able to survive subzero conditions for up to 7 days.

Arrival in Cotton

Usually cotton becomes infested by adults that fly into fields around the time of first open boll, though sometimes, perhaps due to seasonal conditions populations can be found early, during boll maturation. Flights of up to 15 km have been recorded. Adults will mate soon after arrival. The expanding population of developing nymphs will be the cause of economic damage.

Damage

Pale cotton stainers use their strong piercing/sucking mouthparts, shown in Figure 7, to feed on developing and mature cotton seed. Seed weight, oil content and seed viability all decline as a result of cotton stainer feeding. Loss of seed viability can be substantial so should be a careful consideration in pure seed crops.

In bolls up to two weeks old severe attacks can kill developing seeds leading to boll shedding. Where feeding is less severe damaged bolls are retained and lint yield may also be reduced as a secondary effect of feeding. Tightlock, shown in Figure 8, can result around damaged seeds, preventing the lint

Other species that may be confused with cotton stainers are Graptostethus servus, seed eating bug (left) and Leptocoris mitellata, the red eyes bug (right). Note that their wings are generally dark, rather than being orange with a distinct dark spot. G. servus is a pest of sorghum, soybeans, mungbeans and cowpes. L. mitellata has a wide host range including fruit trees and many garden plants. They are attracted to light and will often shelter in large numbers around houses.

Little is known of the behaviour of these species in cotton. If they are present, ensure bolls are monitored for signs of plant bug damage.

Photo; Jo Wessels.

Photo; Jo Wessels.

from fluffing out as the boll opens.

Unlike mirids and other plant bugs, pale cotton stainers are able to continue feeding on bolls during their later stages of development.

As bolls open stainers will feed on the mature seeds. 'Bald patches' where there is less lint on the seed may become evident as the lint fluffs out. Shown in Figure 9, several seeds within each lock may be affected or only one or two seeds in the boll. At present it is unclear whether this damage is a result of feeding after boll opeing or from earlier feeding during seed development.

Yellow staining of the lint has also been observed. Shown in Figure 10, it is thought to occur as a consequence of watery faeces being deposited on the lint while bugs are feeding in the open bolls.

Overseas, staining of cotton lint has occurred as a result of feeding in young bolls. The bugs transmit a fungal pathogen during feeding causing a reddening of the lint. This has not been documented to occur in Australia.

Staining can also occur as a result of bugs being squashed during picking.

Monitoring for damage

Bolls of varying ages should be cut open to confirm and monitor for signs of damage. Study done by QDPI&F, Kingaroy entomologist Moazzem Khan showed pale cotton stainer bug caused damage to developing bolls that was similar to that of green vegetable bug (GVB). This includes a black spot on the outside of the boll, warty growths inside boll wall and brown coloured lint.

The mild, wet conditions that favour the survival of pale cotton stainers in cotton will also favour the occurrence of secondary infections by yeasts, *Alternaria* and bacteria in cracked bolls.

www.cottoncrc.org.au

These infections can cause tightlock and lint staining. The presence of pale cotton stainers when such damage occurs may be coincidental.

Monitoring for the presence of bugs

Distribution through the field and through the canopy can be quite patchy. To avoid under/over estimating abundance ensure sampling occurs at multiple sites spread throughout the field.

The beat sheet is a suitable sampling method to monitor the bugs, but as some growth stages favour the lower canopy, visual searching is also a good complementary technique.

First instar nymphs tend to be found on the soil or very low in the canopy. Young nymphs are gregarious and will tend to stay in clusters until about the third instar, making their distribution quite patchy. At the open boll stage nymphs are more visible than in earlier stages of the crop. However they may also hide in the fluffy lint.

Older nymphs tend to be found in the lower to mid canopy and as open bolls appear they will often been seen in the bolls feed on the exposed seeds.

Adults can be distributed through the crop at low densities, often in mating pairs, but sometimes they can also be found in quite dense

clusters of mating pairs. Cotton stainers tend to hide during the heat of the day, sometimes in partially opened bolls. They are not easily observed at this time.

Once pale cotton stainers are observed, monitor developing and mature bolls for signs of damage.

Alternate hosts

Pale cotton stainers are considered to be Malvaceae feeders for development. In Australia they have been observed in Malvaceae such as

Figure 7; Pale cotton stainers use their strong probiscus to feed on seed in large bolls and in open cotton.

Figure 9; 'Bald patches' on mature cotton seeds due to pale cotton stainer feeding.

Figure 8; Tightlock associated with pale cotton stainer feeding during boll development.

Figure 10; Lint staining caused by pale cotton stainers feeding on mature seeds after boll opening.

Gossypium spp., *Malvastrum* spp., *Malva* spp., *Hibiscus* spp. (e.g. Norfolk Island hibiscus), *Sida* spp. and *Abutilon* spp. Adults may be found on a range of species, including *Brachychiton* spp. (eg Illawarra flame tree), *Ceiba pentandra* (Kapok), *Pennisetum* spp. (native grasses), *Geijera parviflora* (Wilga) and in *Sorghum* spp.

Natural Enemies

A range of natural enemies such as Tachinids (parasitic flies) and predatory reduvid bugs (e.g. assassin bugs) have been recorded in Africa. However, they have mainly exerted pressure when cotton stainers have been feeding on native hosts rather than in cropping situations. The role of natural enemies in the control of developing populations of pale cotton stainers in Australia has not been studied.

Action Threshold during Boll Development

When adults and nymphs are observed in the crop and damage to developing bolls is detected, an action threshold of 3 pale cotton stainers/m is recommended. This threshold is based on the relationship between cotton stainer damage and the damage caused by other plant bugs (see Figure 11). The figure shows that pale cotton stainer bugs caused only one third as much boll damage as green vegetable bugs. Since the action threshold for green vegetable bug is 1/m, the action threshold for pale cotton stainer bug should be 3/m.

Both nymphs (usually 3rd to 5th stage nymphs) and adults cause similar amounts of damage.

Action Threshold after First Open Boll

When adults and nymphs are observed feeding in open bolls, the threshold must consider the potential for quality downgrades of the lint as well as the loss of

Figure 11; Comparative damage to 10 day old cotton bolls by different plant bugs.

GVB – green vegetable bug
GSB – green stink bug
BSB – brown stink bug
HRLQB – harlequin bug
RBSB – red banded shield bug
CSB – pale cotton stainer bug

Source: Moazzem Khan, QDPI&F, 2004 unpublished data.
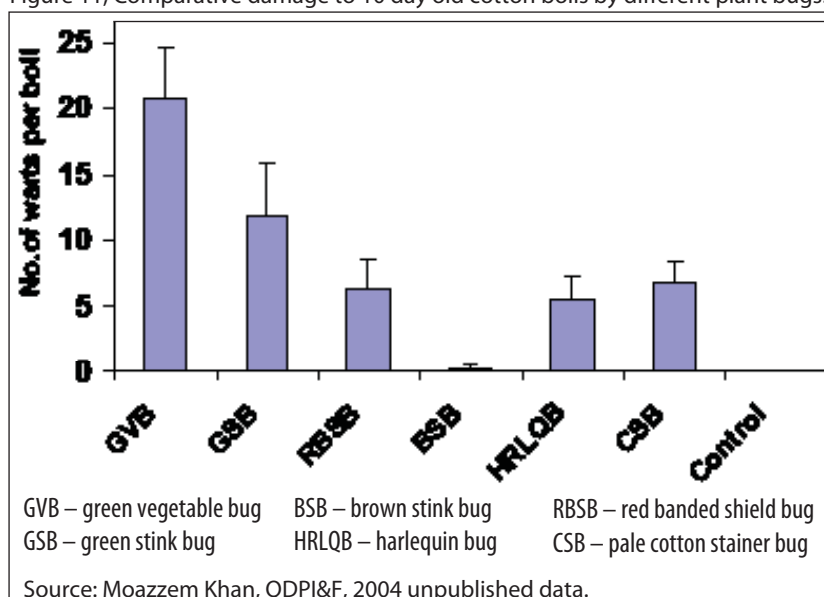
seed weight and seed viability.

Where staining is observed as in Figure 9, a threshold of 30% of bolls affected should be used to prevent a colour downgrade.

Reaction to Insecticides

As an occasional pest, there are few products registered for their control. The synthetic pyrethroids lambdacyhalothrin (Karate Zeon ®, Matador ®and gamma-cyhalothrin (Trojan ®) are registered; check the labels of these products for more information. However their status as an occasional pest is influenced by their susceptibility to insecticides used for the control of *Helicoverpa* and other pests. Cotton stainers will be incidentally controlled when synthetic pyrethroids, carbamates such as carbaryl or organophosphates such as dimethoate are used.

Worldwide there are few records of resistance to insecticides developing in the field, however cotton stainers will react to selection pressure under laboratory conditions. Any decision to use broad spectrum insecticides such as SPs should take into account their impact on beneficial insects. Particularly in the Darling Downs and St George the risk of flaring whitefly and

other secondary pests should be considered.

Controlling Cotton Stainers

As there is no resting stage in the cotton stainer's lifecycle, cultural controls between cotton seasons assist greatly in limiting population development.

Fuzzy cotton seed used for stockfeed is an important alternate source of food for cotton stainers. Avoid storing fuzzy seed in exposed places where cotton stainers can access this food source over long periods.

Controlling ratoon cotton and cotton volunteers is important for limiting cotton stainer's access to alternate food source.

Further Information

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Cotton insects

Aphid ecology in cotton

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Aphids, once only a secondary pest in cotton are now a major problem for growers in some regions. Problems associated with aphid in Australian cotton include vectoring of cotton bunchy top disease, yield reductions from large early season infestations and late season effects of honeydew on fibre quality. Aphids ability to reproduce asexually has an impact on resistance management as does their ability to overwinter on non cotton hosts.

Picture1: Leaf affected by Aphid honeydew (left)

Introduction

This is a companion document to 'Strategies to manage aphids in cotton' available from the Cotton CRC web site.

Aphids, once considered only a secondary pest in cotton are now a major problem for growers in some regions. Three species are commonly found on cotton, the cotton aphid (*Aphis gossypii*), green peach aphid (*Myzus persicae*) and cowpea aphid (*Aphis craccivora*). The cotton aphid is the most common. Occasionally there are early or late season infestations of green peach aphid, which die off during hot periods. Cowpea aphid is sometimes found on seedling cotton in late spring after legume crops die off, but rarely establishes effectively in cotton plants.

Damage

Aphids insert their stylets into leaf or terminal tissues of plants and probe until they contact a phloem vessel. The phloem is the tissue that distributes the products of photosynthesis (assimilates) required for plant growth throughout the plant. The sap in the phloem is under pressure and is basically 'forced' into the aphids, which regulate the flow. Phloem sap is rich in sugars, but poor in amino acids which aphids need for growth. To accumulate enough amino acids for growth the aphids 'pass' a lot of excess sugar, which is excreted onto plants as a shiny sugar-rich deposit known as 'honeydew'. Honeydew encourages the growth of sooty moulds on leaf surfaces. Aphid feeding causes economic damage to cotton in four ways;

1. Competition with young growth and developing fruit (squares and bolls) for assimilate. If this is beyond the capacity of the plant to compensate, reduction in growth is likely.

2. Reduced photosynthesis due to the presence of aphids on leaves. The cause of

this effect is not well understood but could be due to a number of factors including; the damage caused by insertion of stylets, (especially when there are many aphids), the effects of assimilate depletion or the effects of saliva secreted in to the plants by the aphids.

3. Secretion of honeydew (Picture:1) onto leaves also reduces photosynthesis.

4. Late season aphid infestations result in honeydew contaminating lint, making it sticky and discoloured. Severe downgrading of sticky lint may result because of the difficulties of processing it in high speed spinning machinery.

Cotton aphids generally prefer to feed on the terminals, young leaves and fruit, sites where the supply of assimilate is high. Damage symptoms from cotton aphids initially appear as crinkled and curled leaves, with the margins of the leaves curling downwards (Picture 2).

Prolonged high populations of cotton aphid will lead to a dramatic shortening of internodes, severely reduced leaf size, leaf / fruit loss and

Picture 2. Severe aphid damage results in wrinkling, stunting and cupping of leaves. Younger leaves may show a yellow margin and reddened patches may appear on leaves. Photo: L. Wilson

obvious yellowing or mottling of young leaves. This yellowing or mottling often occurs on areas of leaves heavily damaged by aphids or can occur evenly around the margins of leaves and should not be confused with the angular mottling found with Cotton Bunchy Top (see Picture 4).

Pre-squaring cotton appears to be able to fully compensate for aphid damage as long as the aphid feeding ceases. However, prolonged high population levels up to cut-out (when fruit production slows or stops) can cause substantial damage and reductions in yield. Populations in excess of 90% of plants infested with aphids for 3 or more weeks are likely to result in economic loss. (see companion article ' Strategies to manage aphids in cotton' for details).

Biology Ecology and Insecticide Resistance

In Australia, the life cycle of aphids is quite different to that of other cotton insects. Cotton aphids (*Aphis gossypii*) are all females, there are no males and therefore no sexual reproduction. Females give birth to live female young which are clones of themselves, inheriting all of their

Suitable host plant (e.g. Cotton) - Population build-up | **Migration to new host plants**

Cycle continues, adult to adult in about 5-7 days in summer. Adults birth 4-6 young per day. Short range dispersal by walking (i.e. leaf-leaf, plant to plant).

Non – winged Adults (Apterae)

Changing Conditions
- crowding
- poor food quality
- short day-length

Adult produces young which which develop on the host into winged adults

1st instar
2nd instar
3rd instar (wing buds visible)
4th instar (wing buds visible)

Winged adult (Alate)

Good Conditions
- quality feed
- low population density

1st instar (Live young)

If suitable host then winged adult begins producing young which grow into non-winged adults

2nd instar

3rd instar

Winged aphids leave host plant and migrate to new hosts. Settles and test feeds on potential hosts. Migration can be short-range (i.e. between plants within a field) or longer-range (i.e. between fields/farms/regions).

If host is unsuitable the aphid continues migration.

KEY POINTS
- All females
- All clones (range of clones in a region)
- No sexual reproduction
- Live young (no eggs)
- No diapause – in winter reproduce very slowly on available hosts

Summer - many generations
Winter   - fewer generations

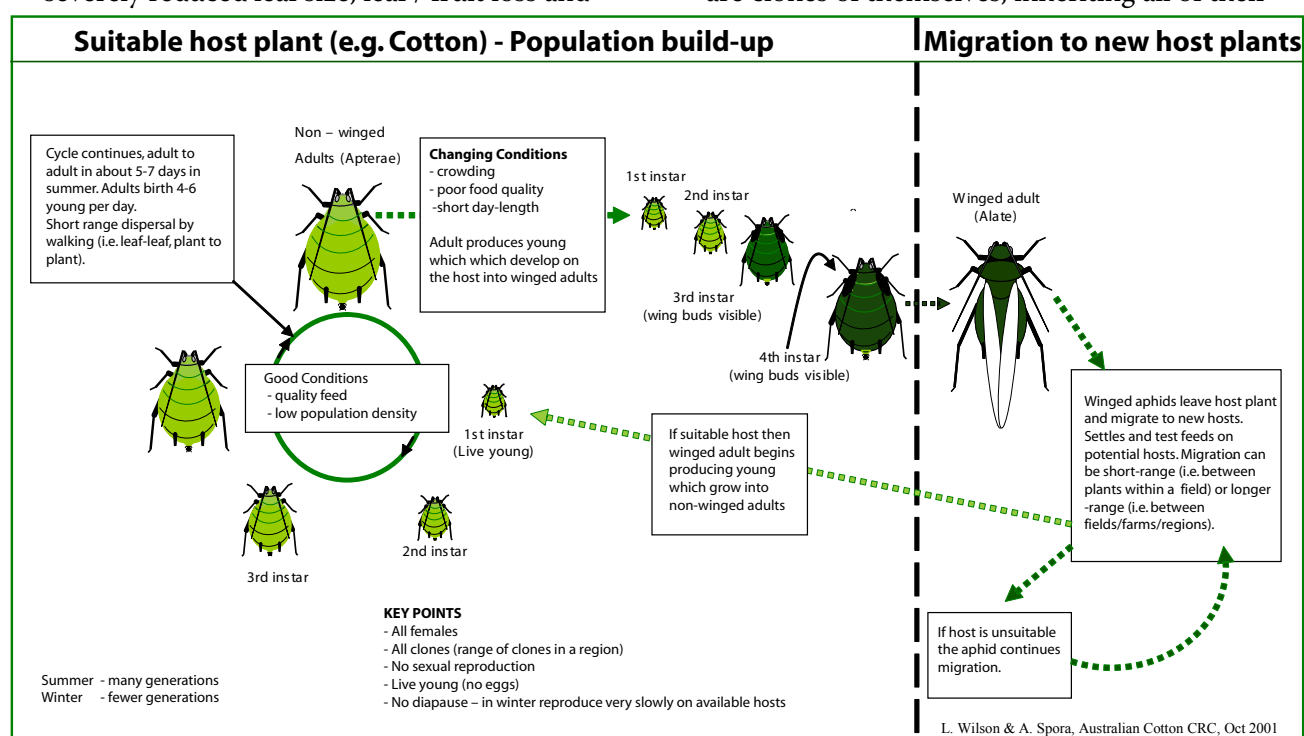L. Wilson & A. Spora, Australian Cotton CRC, Oct 2001

Figure 1: Life cycle if cotton aphid

characteristics including insecticide resistance. A female aphid can produce live young at the rate of 4 - 6 per day, which in summer can mature through four nymphal stages into adults in 4 - 7 days. They can immediately begin producing live young. Female aphids have within them many live young at various stages of development. These cloned offspring already have clones developing within them before they are born. This is how so many generations can be produced in such short periods. Populations can explode if conditions such as food quality and climate are favourable.

Biology, Ecology and Colonisation of Cotton

Colonisation of a new host plant usually occurs by the winged (alate) adult. Aphids will settle on the plant and test feed. If the plant is unsuitable the winged adult will resume the "flight, settle and test feed' pattern, until it finds suitable food or dies. If the plant is suitable, production of live young commences quickly. These mature through four nymphal stages into wingless adults (apterae). The wingless cycle will continue until some aspect of their environment triggers the switch to the production of dispersing (winged) forms. This switch can be caused by declining food quality, for instance if the host plant is senescing, by overcrowding or by reduced day-length.

Picture 3. Cotton aphids on a leaf. Note wide variation in colour and globula shape. Centre left is a predatory larvae of a hoverfly.
Photo: C. Mares

Once triggered to disperse, the adult aphid produces live young which develop wing-buds. These nymphs mature into winged adults, which then fly off to find a new host plant. This could entail only a short flight, to another uncolonised plant in the same cotton field, or depending on wind currents, a longer flight to a new crop or weed host further away.

Resistance in aphid populations

Aphid populations in a region will consist

of a number of different clones. These clones will appear identical but as there is no sexual reproduction the clones are essentially separate sub-populations. Different clones may display differences in biological features, for instance a degree of specialisation toward a particular type of host plant. They can also vary in their resistance to insecticides.

It is likely that there is a range of resistant and susceptible clones in a region. Normally when resistance develops in an insect population there is some penalty in growth or reproduction in that population. In the absence of insecticide selection the resistant individuals are not favoured, though this is not always the case, and some resistant aphid clones appear as well adapted as non-resistant clones. Table 1 summarises current resistance in aphids found in Australian cotton.

When aphids are treated with an insecticide (aphicide) the aphids of resistant clones survive and those of susceptible clones die. This leaves a population that is highly resistant which can continue to develop rapidly in the field. Due to the clonal nature of aphid populations one field can have resistant aphids while the adjacent field can have susceptible aphids.

Table 1 Resistance notes for major aphid species found in cotton

Aphid species	Resistance
Cotton Aphid	Patchy resistance and cross resistance to a broad range of aphicides. In particlular resistance to dimethoate or omethoate and cross resistance to pirimicarb is common. Resistance to profenofos, chlorpyrifos ethyl and methyl, pyrethroids and endosulfan is also found in some populations. Patchiness of resistance indicates that basic measures like farm hygeine can still limit resistance spread. See Infomation Sheet: Strategies to manage aphids in cotton for latest details.
Green peach aphid	Resistance to OP's and Pirimicarb. Resistance levels are lower compared to cotton aphid See Infomation Sheet: Strategies to manage aphids in cotton for latest details.
Cowpea Aphid	No known resistance.

Overwintering

In winter, cooler temperatures slow the growth rate of aphids dramatically. In Australian cotton regions, neither cotton aphids, nor green peach aphids nor cowpea aphids have an overwintering form. Instead they persist through winter in small scattered populations on whatever suitable host plants are available. In spring as temperatures increase aphid populations begin to build rapidly again.

Predators parasites, parasitoids and pathogens

Beneficial insects play an important role in aphid control at the stage before aphid numbers begin to increases exponentially. Disruption of beneficial populations by some insecticides can lead to earlier, more severe aphid outbreaks.

Aphids are bread and butter for many predators in cotton. Major predators of aphids include the larvae of the hoverfly (Syrphid) and lacewings , and nymphal and adult stages of ladybirds (white collared , transverse, variable), red and blue beetle and the brown smudge bug.

Parasites of aphids include small wasps (*Aphidius colemani* and *Lysiphlebus testaceipes*) that sting developing aphids, inserting an egg which hatches into a larvae that grows and matures in the aphid, resulting in the pale bloated aphid mummies often seen on cotton leaves.

Natural variability in predator and parasite abundance means that some will be more important in different seasons.

During periods of heavy rainfall fungal pathogens can also take a toll on infestations

Cotton aphid

The cotton aphid varies widely in colour. The winged adults are typically black, but the wingless stages can vary from pale yellow through to dark green, brown and dull black (Picture 3). The wingless forms have a typically bulbous round shape.

The development of cotton aphid is favoured by warm temperatures and this species does well on cotton through the peak growing period.

Cotton aphid has a broad host range and has been recorded on members of the following families; Fabaceae (legumes, lucerne, medic), Solanaceous weeds (datura, ground cherry, nightshades), Cucurbitaceae (paddymelon), Malvaceae (bladder ketmia, marshmallow) and Asteracae (sunflower, capeweed, daisies, thistles, bathurst burr). A more complete host range can be found in the IPM Guidelines, or Cotton CRC website (see Useful Documents and Links at the end of this document).

Cotton aphid and Cotton BunchyTop (CBT)

The cotton aphid is the only known vector of cotton bunchy top disease.

Symptoms of CBT include reduced plant height, leaf surface area, petiole length and internode

Figure 4: Leaf symptoms of Cotton Bunchytop in the field

length. Pale, angular patterns on the leaf margins are often observed with the remainder of the leaf blade usually dark green in colour. These darker leaves have a leathery and sometimes glossy texture when compared to those on healthy plants. Typically, the pale angular patches in field-grown cotton turn red as leaves age. Boll development is also affected, with bolls often less than half the size of healthy bolls.

Host plants for CBT include volunteer cotton and marshmallow and potentially other malvaceous weeds.

Green peach aphid

Picture 5: Green peach aphid, note pale green/yellow colour and tubercules with indented 'U' shape between them (see Figure 1). Photo: L. Wilson

The green peach aphid is a pale yellow-green and is more tear-drop shaped than the cotton aphid (Picture 4). Colonies tend to be uniform in colour compared with cotton aphid. Seen with a hand lens or microscope, green peach aphid has a small tubercle at the junction of the antenna and head, which is absent in cotton aphid and cowpea aphid. The area between these tubercles is 'U' shaped in green peach aphid whereas it is flat in cotton aphid. Also, the green peach aphid has a pair of long, pale, tube-like siphunculi at the tip of its abdomen, whereas those of cotton aphid are quite short and usually dark (see Figure 2).

The green peach aphid causes far more severe effects on plant growth at much lower densities than coton aphid. Symptoms include yellowing of young leaves and the terminal and severe reductions in internode length and leaf / fruit size. Plants generally recover quickly if the green peach aphid numbers decline due to hot weather, beneficial insect activity or insecticides.
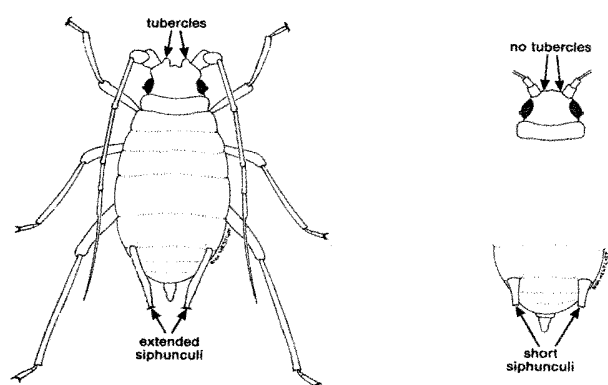
Figure 2. Green peach aphid (left) and cotton aphid (right). - extracted from Forrester and Wilson 1988.

Fortunately this pest rarely establishes well on cotton.

The green peach aphid prefers cooler conditions. It is sometimes found on cotton early in the season but populations do not usually persist once hot conditions commence. Green peach aphid also has a wide host range and is often found on members of the following families. Fabaceae (legumes, lucerne and lupins), Asteraceae or all Brassica sp. They are also often found on peach and plum trees.

Cowpea aphid

The cowpea aphid is very similar to the cotton aphid in appearance. However, the wingless adults of this species are a shiny black, in contrast to cotton aphid, which is always a dull colour.

Picture 6: Cowpea aphid showing shiny black wingless adults Photo J. Wessels

Cowpea aphid will colonise a range of hosts but prefers legumes and is often found on medics. This species is often found on cotton early in the season but seldom establishes, though it may sometimes produce a small number of offspring. More common hosts include the Cucurbitaceae, Asteraceae, and Fabaceae families.

Other species of aphids on cotton

A range of other aphid species are occasionally found on young cotton. These are mostly the winged forms of species that have migrated from other hosts, especially leguminous weeds. These include pea aphid (*Acyrtosiphon pisum*), blue green aphid (*Acyrtosiphon kondoi*) (Picture 7) and the spotted alfalfa aphid (*Therioapihs trifolii*).

Picture 7: Winged adult of blue green aphid, (Photo NSW Agri-culture)

These species can settle on cotton to test feed but will not normally establish. Populations of winged adults on seedling cotton may initially be high but will usually decline quickly over two or three weeks. A wide range of beneficial insects also enters cotton crops at this time.

Winged forms of the corn aphid (*Rhopalosiphum maidis*) and the oat or wheat aphid (*Rhopalosiphum padi*) may also migrate from grasses, cereals or sorghum into cotton but do not establish. *Aphis spiraecola* (apple aphid) is also sometimes found on cotton , and probably originates from certain Asteraceae such as *Conyza* spp. (fleabane) or Chrysanthenum.

Soil aphids

Bean root aphid (*Smynthurodes betae*) is a rare aphid pest that feeds on the roots of cotton seedlings (Picture 8).

Death of seedlings can occur and bean root aphid damage may be mistaken for seedling disease. The aphids are small, pale, globular and wingless.

The presence of aphids can be detected by carefully separating the soil away from the roots of seedlings. Aphids can be found on the roots at a depth of about 10 cm and they are tended

by ants which construct small chambers to allow movement of aphids around the roots. The chambers are covered in a white, waxy dust from the aphids. Infestations so far have tended to occur in fields previously heavily infested with burr medic.

If infestations are discovered after seedling emergence there is no effective chemical control for the aphids. If planting cotton into seedbeds which have been infested with burr medic then granular insecticides applied to control other pests may coincidentally control bean root aphid.

Acknowledgments

Useful documents and links

Strategies to manage aphids in cotton: Companion information sheet

"Integrated Pest management Guidelines for Cotton Production systems in Australia" (IPM Guidelines) Hardcopy or COTTONpaks CD versions available from The Cotton TRC or from the Cotton CRC website:
   http://www.cottoncrc.org.au.

Cotton aphid hosts in Australia Web link:
   http://www.cottoncrc.org.au/content/Industry/ Publications/Pests_and_Beneficials/Aphids__ Bunchytop/Overwinter_host_plants_of_Cotton_ Aphid.aspx

Cotton Pest Management Guide: Published yearly by NSW DPI also web:
http://www.dpi.nsw.gov.au/agriculture/field/field-crops/fibres/cotton/cotton-pest-management-guide

Cotton insects

Strategies to manage aphids in cotton

Research: Lewis Wilson[1], Grant Herron[2], Tanya Smith[1] and Simone Heimona[1] Review Input: Rod Gordon[4], Tracey, Farrell[2], James Hill[2], David Larsen[1]
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Aphids, once a secondary pest in cotton are now a major problem for growers in some regions. Problems associated with aphid in Australian cotton include vectoring of cotton bunchy top disease if present in the crop, yield reductions from uncontrolled early season infestations and late season effects of honeydew on fibre quality .

Principles

1 Overwintering hosts

2. Varietal selection

3. Sowing

4. Sampling and thresholds

5. Predators

6. Rotation

Aphid Management
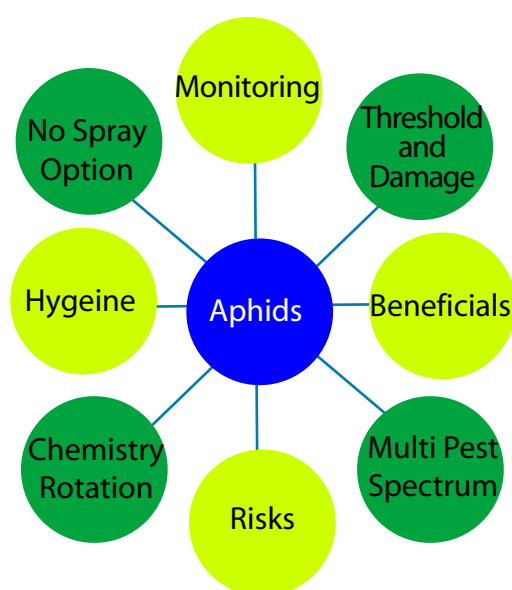
Introduction

This is a companion document to "Aphid Ecology in Cotton" available from the Cotton CRC web site.

Aphid life cycle and resistance

Resistance management for aphids is different to that for other pests that reproduce sexually. There is no mating of susceptible aphids with resistant aphids to dilute the resistance in a population as is the case with Helicoverpa.

Aphid populations in a region will consist of a number of different clones. These clones will appear identical but as there is no sexual reproduction the clones are essentially separate sub-populations. Different clones may display differences in biological features, for instance a degree of specialisation toward a particular type of host plant. They can also vary in their resistance to insecticides.

When aphids are treated with an insecticide (aphicide) the aphids of resistant clones survive and those of susceptible clones die. This leaves a population that is highly resistant which can continue to develop rapidly in the field. Due to the clonal nature of aphid populations, one field can have resistant aphids while the adjacent field can have susceptible aphids.

It is likely that there are a range of resistant and susceptible clones in a region. Normally when resistance develops in an insect population there is some penalty in growth or reproduction in that population. In the absence of insecticide selection the resistant individuals are not favoured, though this is not always the case. Recent studies with resistance strains of cotton aphid have found no evidence of a fitness penalty.

Cotton Catchment Communities CRC

Insecticide resistance in aphids in cotton

Cotton aphid

Cotton aphids are collected from cotton regions and tested for resistance to insecticides each year. Collections have found aphids resistant to organophosphates, some carbamates, endosulfan and pyrethroids. The most common resistance is to the carbamate pirimicarb, and this also confers cross resistance to dimethoate and omethoate, both older organophosphates (OPs).

This means that if a dimethoate (OP) spray fails then a pirimicarb spray will also fail. This resistance is patchy within regions and inconsistent between years. In fact resistance can vary between fields within a farm and within a single field through the season. Aphid clones resistant to pirimicarb are not necessarily resistant to all other carbamates, and they are not resistant to aldicarb, which is sometimes used at planting. There is also moderate resistance in some populations to other OPs such as profenofos and chlorpyrifos methyl, though this is a different mechanism to the resistance to dimethoate/omethoate. Aphid strains have also been collected that are resistant to pyrethroids and endosulfan, but fortunately these are rare.

Green peach aphid

This species is widely resistant to dimethoate / omethoate, profenofos and pirimicarb. However, resistance levels are generally fairly low in comparison to resistant cotton aphid clones. These insecticides may provide adequate control of green peach aphids where they will not control resistant cotton aphid. It is therefore important to distinguish between cotton aphid and green peach aphid in the field (See Aphid Ecology in Cotton information sheet for identification diagram).

Strategies to manage aphids

Aphids, cotton aphid in particular, are emerging as a difficult pest to manage due to resistance and their potential as a vector of Cotton Bunchy Top disease (CBT). In the light of these issues, aphid management needs to be reviewed to ensure that sensible thresholds are used and that the efficacy of aphicides is maintained.

There are no silver bullets for managing aphids, instead a number of integrated control tactics are recommended to help reduce aphid numbers and manage resistance.

Key Considerations

Aphid Management
- Resistance
  - OP Resistance Chemistry Rotation
- Flaring Secondary Pests
  - Whitefly Mites
- Hygiene
  - Control of Alternative Hosts

It is important to manage aphids within an integrated pest management (IPM) system that takes into account the whole year (see IPM Guidelines & Useful documents and links - at the end of this document).

Decision making in aphid management is critical to maintaining that predator/prey balance in the field. More often than not it may prove more cost effective to take the NO spray approach or use a selective insecticide. This strategy will help to maintain beneficial populations that will help to control other pests.

Key considerations in aphid management

To reduce the use of expensive chemistry and to prevent flaring of secondary pests, management will need to encompass an awareness of resistant populations, chemistry choice and rotation, crop hygiene and controlling overwintering hosts.

Control of winter aphid hosts

Aphids are able to overwinter on a number of other host crops and weeds. There is a risk that resistant clones could use these hosts to persist on farm through winter. Farm gardens can also provide habitat and host plants when conditions are dry. Growers and consultants should aim to reduce the availability of on-farm hosts over winter. Cotton regrowth and volunteer cotton, in particular, pose a high risk as they are both an overwinter aphid host and a reservoir for CBT.

This may be a particular issue if there is germination of cotton seed from fallen seed-cotton or regrowth on cotton stubs.

Winter growing crops of woolly pod vetch, canola, and lucerne will not support cotton aphid and lupins and faba beans usually support cotton aphid poorly. Be wary if these crops are infested with cotton aphid hosts such as weeds or volunteer cotton.

If farm hygiene is adequate through winter it is possible that localised aphid survival will be low and that immigrant colonising aphid clones will be susceptible in the following year. Where resistance problems have occurred in the previous season consider planting a 'non-host' rotation crop, such as a winter cereal. Control of weeds, cotton stubble and cotton volunteers between seasons is also critical.

Seasonal conditions during winter will also also effect host numbers. Higher aphid pressures early in the season could be expected following a moist warm winter that allows extensive growth of alternative hosts.

Alternative Hosts

Cotton aphid has a broad host range and has been recorded on members of the following families .

Fabaceae (legumes, lucerne[+,] medic),

Solanaceous weeds (datura, ground cherry, nightshades),

Cucurbitaceae (paddymelon),

Malvaceae (bladder ketmia, marshmallow - an alternative host for the Cotton Bunchy Top Disease),

Asteracae (sunflower, capeweed, daisies, thistles, bathurst burr).

([+]survive not thrive)

Varietal resistance

None of the current commercial varieties show resistance to aphids.

Seed treatment or 'at planting' insecticide

This strategy may help prevent the early build up of aphid populations, therefore reducing the need for foliar aphicides which may disrupt beneficial populations. It may also reduce the risk of CBT for farmers who have concerns about this disease. However, care must be taken in selecting later insecticide sprays as some seed treatments and 'at planting' insecticides are from the same groups as foliar sprays used for aphid control and there is the potential for prolonged selection with one insecticide group. Check the insecticide label to see that the first foliar insecticide is not the same group as the at-planting insecticide or seed treatment (see Table 1).

 Insecticides used to control other pests may also select for resistance in sub-threshold aphid populations. This may then render those products ineffective for aphid control later in the season. This is a particular risk with dimethoate

Picture 1: Marshmallow, *Malva parviflora,* one of a large number of weed species on which cotton aphid can overwinter. this species is also an alternative host for Cotton Bunchy Top Disease Photo WEEDpak

/omethoate being used to control mirids.

Beneficials.

A range of parasitoids and predators will help to reduce aphid survival. Predators of aphids include; lady beetle larvae, damsel bugs, big-eyed bugs, and the larvae of green lacewings and hoverflies. Wasp parasites *Aphidius colemani* and Lysiphlebus testacipes can mummify and kill aphids (mummified aphids appear as bloated pale brown aphids which do not move).

Beneficials can be very important in keeping aphid populations under control when aphid densities are low. Disruption of these beneficials with a broad spectrum insecticide releases aphid populations from this important source of mortality and populations can increase very quickly.

Once aphid populations begin to increase rapidly it is the more specific beneficials that will eventually control them,

Picture 2: Hoverfly Larvae feeding on cotton aphid

such as ladybeetles, hoverfly larvae and the parasitoids. However, aphids may have already caused economic damage before the beneficials bring them under control, especially if the crop is sprayed with further disruptive insecticides. Those insecticides with the greatest risk are those that are more disruptive of beneficials and have poor efficacy against aphids (Table 3).

The risk of adverse effects of drift of insecticides applied to nearby cotton on beneficial populations should be taken into account.

Selection of insecticides should

Picture 3: Ladybird larvae- effective aphid predators of aphid

consider both the target pest and the type of beneficials that are present. For example, an insecticide can have little effect on one beneficial group, such as spiders, yet be disruptive to another, such as predatory beetles (See IPM section, Table 3 of the Cotton Pest Management Guide for a complete list).

Sampling

Aphid sampling should begin from seedling emergence and be done at least weekly. Aphids generally prefer younger growth so sample the 4th or 5th mainstem leaf below the terminal. This leaf can also be used for mites and whitefly sampling. Aphids have winged and non-winged forms. Score a plant as infested only if non-winged forms are present (winged forms could be non-economic species that are just test feeding before moving on to another host). If a high proportion of plants do have winged forms

then re sample within a few days to check if they have settled and produced young. If reproducing aphids are found it is important to confirm the species (See Aphid Ecology Document), as green peach aphid causes more severe damage than cotton aphid and has a different resistance spectrum.

At the same time as sampling for pests, sample the crop for beneficial insects. See the IPM Guidelines booklet - Sampling beneficial insects and spiders or the Cotton CRC web site, for further details of aphid and beneficial sampling.

Thresholds.

Australian research has shown that if aphid population are allowed to develop to high levels (>90% plants infested) for an extended period (e.g. 2-3 weeks) then significant loss of yield can occur.

The sampling scoring & conversion system explained on page 5 allows an estimate to be made of yield loss caused by early infestations. This can be compared to cotton price and control option costs to determine if a spray is required.

The system requires that regular samples are taken, averaged and converted to an aphid score and recorded with the sample date.

Table 1a can be used to calculate the Seasonal Aphid Score for each individual check. This score is accumulated as the season progresses.

The seasonal aphid score is added to the accumulation of seasonal aphid scores and compared to the amount of time remaining to 60% open cotton in Table 1b to estimate the yield loss that aphids have caused.

Once cotton has open bolls, the threshold should be changed to 50% of plants infested with aphids or 10% of plants infested if honeydew is present to prevent contamination of lint.

Record keeping and calculation of these Scores can be simplified by using the Aphid Yield Loss Estimator in CottASSIST on the web. The Tool allows users to keep records for multiple crops

on multiple farms throughout the season. After initial set up, the user enters the Average Aphid Score from Step 2 and the date of each check.

The Tool then calculates the Scores and tracks the estimate of yield loss. Find CottASSIST on the 'Industry' home page on the Cotton CRC website.

Managing aphids and CBT disease

Should managers manage aphids for yield loss or disease transmission? In the past spraying aphids at very low thresholds to prevent disease transmission resulted in rapid selection of aphids for resistance to key control options. Our research shows that the rate of spread of CBT in fields is generally slow, so growers can primarily manage aphids to avoid yield loss from feeding rather than to prevent them spreading disease.

Picture 4: Aphid colony showing brown bloated parasitised mummies (Photo Peter

The risk from CBT is generally low, for three reasons;

(1) CBT is poorly transmitted. If just one CBT-infected aphid colonizes a plant, the transmission rate is about 5% (e.g 1 in 20 plants become infected). Three or more infected aphids per plant pushes this to ~40%, but higher levels require even more infected aphids.

(2) When a CBT-infected aphid feeds on a cotton plant, transmission of the disease from the aphid to the plant will happen within half an hour. A latent period then passes while the number of disease particles in the plant gradually builds. During this

time (at least 10–14 days) young aphids produced by the infected female can feed on the newly infected plant but not pick up the disease. If they move to nearby plants and start new colonies they do not carry the disease. Eventually the number of disease particles is high enough and young aphids may pick up CBT from the original plant, but if they move to, feed on and infect a nearby plant there will again be a latent period in that plant.

(3) After a female aphid settles on a plant she will produce wingless aphids. Hence movement between plants is only by crawling and fairly slow. This only changes at high aphid densities when crowding stimulates production of winged forms which can disperse further.

The combination of low transmission rates, especially when only 1 aphid colonizes a plant, the latent period and the fact that at low to moderate population densities aphids spread from plant to plant by walking means that the rate of infection and spread of CBT across fields is very slow. Furthermore, if beneficial populations are conserved they will tend to find and control aphid hotspots, so even if some plants are infected the spread is halted. If winters are fairly dry there are few hosts for aphids and populations will be small, as has occurred in recent years. In such situations the main hosts for CBT and aphids are on-farm and management of weeds and cotton ratoons, which are reservoirs for CBT, will have a big impact.

Higher risk situations are when there are many sources of the disease and aphids nearby or within the field (especially ratoon cotton), following wetter winters. Poor management of aphids after they have entered the crop can encourage a rapid increase in populations and development of winged forms which disperse across the field and spread the disease. For instance, attempting to control aphids with an insecticide to which they are resistant and

STEP 1. COLLECT LEAVES.

Fields should be sampled in several locations as aphids tend to be patchy in distribution. At each location collect 20 leaves, taking only one leaf per plant. Choose main stem leaves from 3–4 nodes below the terminal. The same leaves can be used for mite scoring.

STEP 2. SCORE LEAVES.

Score each leaf as either 0, 1, 2, 3, 4 or 5 based on the number of non-winged aphids on the leaf. As a guide, the diagrams below represent the population range for each score (After counting aphids a few times, you will quickly gain confidence in estimating abundance). Discount pale brown bloated aphids as these are parasitised. Sum the scores and divide by the number of leaves to **calculate the average aphid score**.

| Score = **0** | Score =**1** | Score = **2** | Score = **3** | Score = **4** | Score = **5** |
| No aphids | 1–10 aphids | 11–20 aphids | 21–50 aphids | 51–100 aphids | > 100 aphids |
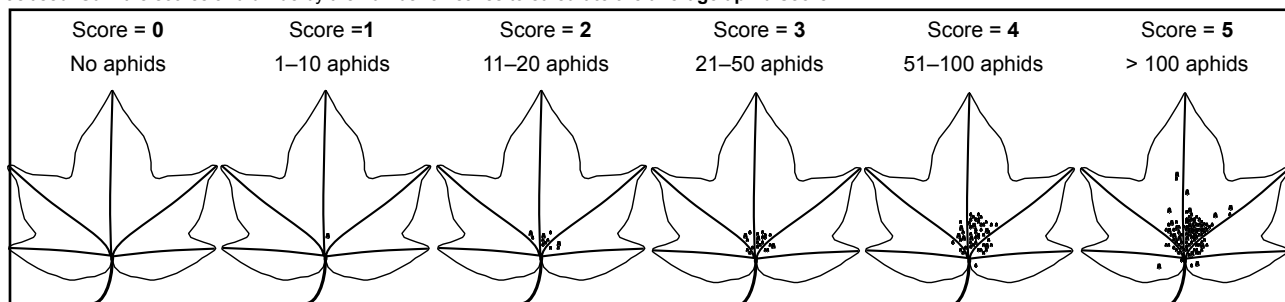
STEP 3. CONVERT TO A SEASON APHID SCORE. Table 1a

In order to estimate yield loss, the Average Aphid Score must firstly be transformed into a Sample Aphid Score and then into a Cumulative Season Aphid Score. Use the Look Up Table below to firstly convert the Average Aphid Score calculated in Step 2 to a Sample Aphid Score. This step accounts for the length of time the observed aphids have been present in the crop. If aphids are found in the first assessment of the season, assume the 'Score last check' was '0' and that it occurred 5 days ago. Find the value in the table where 'this check' and the 'last check' intersect. **Multiply this value by the number of days that have lapsed between checks.** This value is the Sample Aphid Score. As the season progresses, add this check's Sample Aphid Score to the previous value to give the Cumulative Season Aphid Score. When aphids are sprayed, or, if during the season the Average Aphid Scores return to '0' in 2 consecutive checks, reset the Cumulative Season Aphid Score to '0'. Disappearance of aphids can occur for reasons such as predation by beneficials, changes in the weather and insecticide application.

Average score *last* check	Average score *this* check										
	0	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**
0	0.0	0.3	0.5	0.8	1.0	1.3	1.5	1.8	2.0	2.3	2.5
0.5	0.3	0.5	0.8	1.0	1.3	1.5	1.8	2.0	2.3	2.5	2.8
1.0	0.5	0.8	1.0	1.3	1.5	1.8	2.0	2.3	2.5	2.8	3.0
1.5	0.8	1.0	1.3	1.5	1.8	2.0	2.3	2.5	2.8	3.0	3.3
2.0	1.0	1.3	1.5	1.8	2.0	2.3	2.5	2.8	3.0	3.3	3.5
2.5	1.3	1.5	1.8	2.0	2.3	2.5	2.8	3.0	3.3	3.5	3.8
3.0	1.5	1.8	2.0	2.3	2.5	2.8	3.0	3.3	3.5	3.8	4.0
3.5	1.8	2.0	2.3	2.5	2.8	3.0	3.3	3.5	3.8	4.0	4.3
4.0	2.0	2.3	2.5	2.8	3.0	3.3	3.5	3.8	4.0	4.3	4.5
4.5	2.3	2.5	2.8	3.0	3.3	3.5	3.8	4.0	4.3	4.5	4.8
5.0	2.5	2.8	3.0	3.3	3.5	3.8	4.0	4.3	4.5	4.8	5.0

STEP 4. ESTIMATE POTENTIAL YIELD REDUCTION. Table 1b

Use the table to estimate the yield loss that aphids have already caused. The 'Time Remaining' in the season needs to be determined the first time aphids are found in the crop. The data set is based on 165 days from planting to 60% open bolls. If for example aphids are first found 9 weeks after planting, the Time remaining would be ~100 days. As the Season Aphid Score accumulates with each consecutive check, continue to read down the '100' days remaining column to estimate yield loss. When aphids are sprayed, or, if aphids disappear from the crop then reappear at a later time, reassess the time remaining based on the number of days left in the season at the time of their reappearance. Crop sensitivity to yield loss declines as the crop gets older. The estimate takes into account factors that affect the rate of aphid population development, such as beneficials, weather and variety. Yield reductions >4% are highlighted, however the value of the crop and cost of control should be used to determine how much yield loss can be tolerated before intervention is required.

Cumulative Aphid Score	Days until 60% open									
	100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**
0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
10	2	2	1	1	1	0	0	0	0	0
15	5	4	3	3	2	1	1	0	0	0
20	7	6	5	4	3	2	1	1	0	0
25	9	8	7	6	5	3	2	1	0	0
30	11	10	8	7	6	5	3	2	1	0
40	15	13	12	10	8	7	5	3	1	0
50	19	17	15	13	11	9	7	5	2	0
60	23	21	18	16	13	11	8	6	3	1
80	31	28	25	22	18	15	12	8	5	1
100	38	34	31	27	23	19	15	11	7	2
120	45	41	37	32	28	23	18	13	9	3

Table 2: Aphid control chemistry

Table 1 Impact of Aphicides on predators in cotton and registrations on other pests

Adapted from "Impact of insecticides and miticides on predators in cotton" Cotton Pest Management Guide

Insecticides (in increasing rank order of impact on beneficials when used as a foliar application)	Rate (g ai/ha)	Aphids S=Seed, soil F=foliar, P=At Plant	Helicoverpa	Mites	Mirids	Thrips (target)	Persistence	Total Predatory Beetles	Red and blue beetle	Minute 2-spotted lady beetle	Other lady beetles	Total Predatory bugs	Damsel bugs	Big-eyed bugs	Other predatory bugs	Apple dimpling bug	Lacewing adults	Spiders	Total (wasps)	Trichogramma	Ants	Thrips	Mite (resurgence)	Helicoverpa (resurgence)	Toxicity to bees	Insecticide mode of action group	Withholding Period - Days	Notes
Aldicarb		P		✓	✓	✓	medium–long	VL				VL						VL	VL		VL	VH				1A		At planting only
Imidadoprid *see below*		S			✓	✓	medium	VL				VL						VL	VL		VL	VH			M	4A		When applied as seed dressing only - see foliar below
Phorate		P		✓	✓	✓	medium–long															VH				1B		At planting only
Thiamethoxam *see below*		S			✓	✓	medium															VH			H	4A	0	When applied as seed dressing only - see foliar below
Pirimicarb	250	F					short	VL	L	VL	VL	L	L	M	VL	VL	VL	VL	VL	M	VL	L			VL	1A	21	Thorough spray coverage essential- Cross resistance 1B
PSO (Canopy) [16]	2 %	F	✓	✓			short	VL	L	L	VL	L	VL	VL	VL	VL	VL	VL	VL	VL	H	VL			VL		1	Supression only
Diafethiuron	350	F		✓			medium	M	H	L	M	L	M	L	L	H	M	M	L	L	H	L	+ve		VL	12A	35	Apply before damage occurs. Flares whitefly
Pymetrozine	150	F					short	M	M	M	M	M	M	L	VL	H	M	M	L	I	M	VL			VL	9B	28	Apply to an actively growing crop
Endosulfan (low)	367.5	F	✓		✓	✓	medium	M	VL	VH	H	M	M	M	L	H	L	M	VL	I	VH	H			M[15]	2A	35	Apply at first sign of infestation
Acetamiprid	22.5	F	✓		✓		medium	M	M	VH	H	M	M	H	M	VH	L	VL	L	H	VH	VH			M[15]	4A	10	Ensure good coverage
Amitraz (supression only)	400	F		✓		✓	medium	H	M	VH	M	L	I	I	I	H	VL	M	M	L	H	M	+ve		L	19a	21	Suppression only
Thiamethoxam	100	F	✓		✓		medium	H	H	VH	H	M	M	M	H	H	M	M	M	H	VH	H	+ve	+ve	H	4A	0	
Endosulfan (high)	735	F	✓		✓	✓	medium	M	VL	VH	M	M	M	M	M	H	VL	M	L	VH	VH	H	+ve		M[15]	2A	35	Suppression only
Imidadoprid	49	F			✓	✓	medium	H	L	VH	H	M	M	M	H	VH	L	M	L	M	VH	H	+ve	+ve	M	4A	91	Add pulse penetrant at 0.2% NB Long witholding
OP's: Chlorpyrifos, Chlorpyrifos-methyl, Disulfoton, Dimethoate, Omethoate, Parathion Methyl, Profenofos	S F / F / S F / F / F / F / F		✓	✓	✓	✓	short–medium	H	M	VH	H	H	M	H	H	VH	L	M	H	M	VH	H	+ve		H	1B	14-28	OP resistance Cross resistance 1A

DISCLAIMER. Information provided is based on the current best information available from research data. Users of these products should check the label for further details of rate, pest spectrum, safe handling and application. Further information on the products can be obtained from the manufacturer.

TABLE NOTES

1. Total predatory beetles – ladybeetles, red and blue beetles, other predatory beetles.
2. Total predatory bugs – big-eyed bugs, minute priate bugs, brown smudge bugs, glossy shield bug, predatory shield bug, damsel bug, assassin bug, apple dimpling bug.
4. Pyrethroids; cypermethrin, alpha-cypermethrin, betacyfluthrin, bifenthrin, fenvalerate, esfenvalerate, deltamethrin, lamda-cyhalothrin.
8. Persistence of pest control, short, <3 days; medium, 3–7 days, long, >10 days.
9. Impact rating (% reduction in beneficials following application, based on scores for the major beneficial groups);

VL	(very low), less than 10%;
L	(low), 10–20%;
M	(moderate), 20–40%;
H	(high), 40–60%;
VH	(very high), > 60%. A '–' indicates no data available for specific local species.

12. Pest resurgence is +ve if repeated applications of a particular product are likely to increase the risk of pest outbreaks or resurgence. Similarly sequential applications of products with a high pest resurgence rating will increase the risk of outbreaks or resurgence of the particular pest spp..
13. Very high impact on minute two-spotted ladybeetle and other ladybeetles for wet spray, moderate impact for dried spray.
14. Data Source: British Crop Protection Council. 2003. The Pesticide Manual: A World Compendium (Thirteenth Edition). Where LD50 data is not available impacts are based on comments and descriptions. Where LD50 data is available impacts are based on the following scale: very low = LD50 (48h) > 100 ug/bee, low = LD50 (48h) < 100 ug/bee, moderate = LD50 (48h) < 10 ug/bee, high = LD50 (48h) < 1 ug/bee, very high = LD50 (48h) < 0.1 ug/bee. Refer to the Protecting Bees section in this booklet.
15. Wet residue of these products is toxic to bees. Applying the products in the early evening when bees are not foraging will allow spray to dry, reducing risk to bees the following day.
16. May reduce survival of ladybeetle larvae – rating of **M** for this group.

CHEMISTRY NOTES

Pirimicarb : A very selective insecticide that basically only controls aphids and has little negative effect on beneficials. There is resistance to this insecticide that confers cross resistance to dimethoate and omethoate. A test kit is available to test aphids for this type of resistance and this information can be valuable when selecting control options.

PSO: Petroleum spray oil. These products require good coverage to provide control. They can be added to insecticides applied against other pests to help suppress populations of aphids and mites. They are only effective as a stand-alone option when aphid populations are relatively low.

Diafenthiuron : Provides good control of aphids, including strains resistant to pirimicarb and OPs, and mites, and suppresses silver leaf whitefly populations. Has low negative effect on beneficials. Requires a closed canopy to obtain best results from fumigant action.

Pymetrozine: Moderately selective aphicide which will control pirimicarb/OP resistant aphids. Requires actively growing cotton for best results.

Endosulfan: Moderately selective insecticide. Is unlikely to flare mites or whitefly.

Acetamiprid: Same chemical group as imidacloprid and thiamethoxam. Most selective compound of this group. Provides effective control of aphids and can be used late in the season due to short with-holding period (10d). Will control pirimicarb/OP resistant aphids.

Thiamethoxam: Same chemical group as imidacloprid and acetamiprid. Very selective as a seed treatment, moderately disruptive to beneficials as a spray. Can increase the risk of mite outbreaks as it controls thrips, which eat mite eggs as well as damage the plant, but not mites. Controls pirimicarb/OP resistant aphids. No withholding period so can be used very late.

Imidacloprid: Same chemical group as acetamiprid and thiamethoxam. Effective and soft on beneficials as a seed treatment. Can increase the risk of mite outbreaks as it controls thrips, which eat mite eggs as well as damage the plant, but not mites. Less effective, and moderately disruptive to beneficials as a foliar spray. Long withholding period

OP's: High level resistance to omethoate and dimethoate is common and confers cross resistance to pirimicarb. Moderate resistance to profenofos and chlorpyrifos occurs occasionally, resulting in poor control. OPs are disruptive of beneficial populations and will increase the risk of mite or whitefly outbreaks. Use of OPs against other pests will select for resistant aphids even if populations are below threshold.

Table 2: Seed Treatment Component Descriptions

Trade Name/s	Active - Ensure rotation with foliar sprays	
Amparo™	Imidacloprid, (neonicotinoid)	Group 4A
	Thiodicarb (carbamate)	Group 1A
Gaucho Genero™	Imidacloprid: (neonicotinoid)	Group 4A
Cruiser®	Thiamethoxam: (neonicotinoid)	Group 4A
Lorsban® 30 Flowable	Chlorpyrifos: (OP)	Group 1B

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Genero™ is a trademark of eChem Crop Protection Products

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Table 3: Pesticides registered for other pests that may encourage aphid resurgence

Insecticides (in increasing rank order of impact on beneficials)	Rate (g ai/ha)	Target pest(s) Helicoverpa	Mites	Mirids	Thrips	Pest resurgence[12] Mite	Aphid	Helicoverpa
Indoxacarb (low)	80			✓			+ve	
Indoxacarb (low + Canopy)	80			✓			+ve	
Fipronil (v. low + salt)	8			✓		+ve	+ve	+ve
Indoxacarb[13]	127.5	✓		✓			+ve	
Propargite	1500		✓				+ve	+ve
Fipronil (low)	12.5			✓	✓		+ve	+ve
Thiodicarb	750	✓					+ve	+ve
Chlorfenapyr (high)	400	✓	✓				+ve	
Pyrethroids[4]		✓	✓[?]	✓[?]		+ve	+ve	+ve

* Important Use of Pesticides
Pesticides must only be used for the purpose for which they are registered and must not be used in any other situation or in any manner contrary to the directions on the label.

Some chemical products have more than one retail name. All retail products containing the same chemical may not be registered for use on the same crops. Registration may also vary between States. Check carefully that the label on the retail product carries information on the crop to be sprayed.

This publication is only a guide to the use of pesticides. The correct choice of chemical, selection of rate, and method of application is the responsibility of the user.

Pesticides may contaminate the environment. When spraying, care must be taken to avoid spray drift on to adjoining land or waterways. Residues may accumulate in animals fed any crop product, including crop residues, which have been sprayed with pesticides. In the absence of any specified grazing withholding period(s), grazing of any treated crop is at the owner's risk.

which decimates beneficials. However, even in this situation the 'infection, colonisation, latent period' cycle will occur so prompt control of the aphids would prevent significant yield loss.

Aphids and insecticides

Insecticides that can be used for aphid control and their effect on beneficial insects can be found in Table 2.

A list of seed dressings and there active groups can be found in Table 3. It is important to ensure that alternative chemical groups are used even if they are applied in different forms.

Rotation of insecticides

As aphids have developed resistance to some insecticides it is essential that we try to preserve the efficacy of existing aphicides. This requires a combination of resistance management and improved management of aphid populations. Insecticides used to control aphids (aphicides) act on the aphids in different ways. Each insecticide belongs to a 'mode of action' group, which describes the way the insecticide acts within the insect to kill it. In general, if insects develop resistance to one insecticide from a given mode of action group they will be resistant to other insecticides in that group as well. For this reason it is important to alternate between mode of action groups rather than repeatedly using insecticides from the same group. Mode of action groups for aphicides are listed in Table 1.

Our current resistance management strategy for aphids hinges on four main points;

1. A maximum of 2 sprays of any registered aphicide mode of action group against aphids, unless the product is otherwise restricted.
2. Rotation of chemistry, that is, do not use the same chemical group consecutively.
3. The first aphicide spray should not be from the same chemical group as any seed treatment or at-planting

insecticide used that also controls aphids, see Tables 1 and 2.

4. There is cross-resistance between carbamate (group 1A) and organophosphates (group 1B) and therefore they should be considered as the same group for aphid control. However, note that aphids resistant to pirimicarb, dimethoate and omethoate are not resistant to aldicarb, so this product can be treated as if it were a different mode of action for resistance management.

It is important not to follow a failure with a given product with another product from the same group. The current aphicides registered for use in cotton and their chemical groups are listed in Table 1 and in the Cotton Pest Management Guide. If there is a spray failure the follow-up spray should use an active ingredient from a different chemical group.

New control technology

Biopesticides.
A new fungal control for aphids will be evaluated in large scale trials in the 2008-09 season.

Semiochemicals
Semiochemicals are essentially 'signalling' chemicals. They can be produced by plants or animals.

Research is being conducted into their effectiveness in aphid management in the cotton system.

Useful documents and links

Aphid Ecology in Cotton: Companion information sheet

Integrated Pest Management Guidelines for Cotton Production systems in Australia (IPM Guidelines) hardcopy or COTTON-paks CD version available from The Cotton TRC or from the CRC website: http://www.cottoncrc.org.au.

Cotton aphid hosts in Australia Web link: http://www.cottoncrc.org.au/content/Industry/Publications/Pests_and_Beneficials/Aphids__Bunchytop/Overwinter_host_plants_of_Cotton_Aphid.aspx

Cotton pest Management guide: Published yearly by NSW DPI also web: http://www.dpi.nsw.gov.au/agriculture/field/field-crops/fibres/cotton/cotton-pest-management-guide