Cotton Catchment Communities CRC

FINAL REPORT

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

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Project Title: IPM in Bollgard II: coping with changes in pests and climate

Cotton CRC Program: The Farm

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Part 4 – Final Report Executive Summary

IPM in Bollgard II: coping with changes in pests and climate

This project supports the ongoing development of IPM in cotton by targeting emerging pest issues, and inappropriate management which may threaten IPM. Key outcomes were:

1. Green vegetable bug (GVB) uses broad leaf weeds as hosts on cotton farms and in refuge areas. GVB prefer to feed and oviposit in legume crops such as mungbean, pigeon pea and soybean. Management of these weeds and crops on farms could influence risks of problems in cotton. Parasitism rates by the egg and nymphal/adult parasites are generally low.

2. Information summarising effects of the new registered compounds (e.g. Shield) and the lower rates of dimethoate has been incorporated into the 'Impact of insecticides and miticides on predators in cotton' table in the Cotton Pest Management Guide 2010-11.

3. Leaf damage resulting in reduced leaf area at or after cutout is unlikely to affect yield unless it is high – probably > 50% leaf loss in the upper canopy (top 6-9 nodes). Damage in the boll fill period before cutout may reduce yield. A tentative leaf loss threshold of 30% to 40% could be used. Results are relevant in assessing effects of leaf loss due to locusts and cluster caterpillar.

4. The efficacy and IPM fit of two fungal biopesticides BC639 and BC667 was evaluated. Both reduced abundance of aphids compared with the control by about 10-50% but the results were erratic and slow. However, the bio-pesticides are more selective than most commercial options – hence the conservation of beneficials may be greater.

5 The spread of CBT from the transplant colonies (= ratoon plants) was greater than from the inoculation colonies (= influxes from host outside the field). Transmission rate increased from < 10% with 1-2 aphids to > 50% with 5-15 aphids. If single aphids infest plants the latent period is 3 to 3.5 weeks but could be a little as 9 days with greater infestations. In the latter case, early management of aphids would be required to reduce the risk.

6. Pale cotton stainer (PCS) females are more damaging than males or mating couples. Females caused up to 50% yield loss and reduced germination success when feeding on young bolls. Feeding on older bolls did not reduce boll weight, but did affect boll opening, harvestability and germination.

7. *Bemisia tabaci* B-Biotype dominated whitefly populations during 2008-09, with virtually no *B. tabaci* Eastern Australian Natives, few greenhouse whitefly (*Trialeurodes vaporariorum*) and no *B. tabaci* Q-Biotype found. Volunteer and ratoon cotton, sowthistle, marshmallow, turnip weed, noogoora burr and paddy melon are hosts through winter.

This project provides new information to make better decisions about management of emerging pests. Many outcomes have been delivered to industry through 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 to obtain a better management balance between control and environment.

Part 3 – Final Report

Background

Since the widespread uptake of BGII, pest management in cotton has changed dramatically, creating new challenges for IPM. Green mirids are now the major pest in cotton. They are being controlled using broad-spectrum insecticides, often at numbers well below threshold. Other pests, which we know little about, are also increasingly found,. Thrips and jassids are causing late season damage, but we are unsure if this damage is economically important. Green vegetable bug (GVB) is often found and difficult to control yet we know little of its ecology. Western flower thrips (WFT) has entered cotton but we know little of its ecology or risk of resistance development.

We found that the use of broad-spectrum insecticides to control mirids dramatically increases the risk of secondary pest outbreaks, especially spider mites. This risk has been hidden by drought over recent years as low winter rainfall means fewer hosts for mite survival, leading to lower populations on cotton. Current mirid management combined with wetter winter conditions could lead to significant problems with mites and other secondary pests. In addition, there are large areas of sorghum and sometimes maize being grown. These crops are excellent hosts for mites, and cotton nearby is at high risk of influxes as these crops mature. This risk is magnified if the cotton has been sprayed for mirids and is devoid of beneficials.

To ensure that we have effective IPM for the future we need to: define the pest status of late season pests, understand the ecology of key emerging pests, develop selective controls and understand the interactions between mirid management and secondary pests. This research will allow our IPM to be prepared for current and future challenges. Our team (Wilson, Heimoana and Smith) has unique skills of value to industry, especially in pest/plant interactions (compensation, thresholds), pest ecology, epidemiology of insect borne diseases and non-target effects of insecticides.

Objectives

This project combined two projects that were in place already, ‘Emerging Pests: Developing Knowledge for GVB and Aphids’ and ‘Supporting IPM for future cotton systems’. The former was in the first year of a 3 year project, the latter was in the final year of a three year project. In response to a PRP to continue the IPM project CRDC asked that we combine these two projects for better integration. This was done and the aims below developed to build on the issues identified above.

- *Research the seasonal abundance and ecology of GVB.* This objective has been completed, though a small extra phase of sampling will carry on into 2010-11 in a new project.
- *Crop host use by GVB.* This objective was completed and the abundance of GVB has monitored over two years in a sequence of summer and winter crops.
- *Investigate the efficacy and IPM fit of biopesticides (with Dr Mensah, NSWDPI), new chemistries, reduced rates with adjuvants and novel technologies.* This objective has been completed. We investigated the efficacy and non-target effects of two new compounds from agrochemical companies, the effects of reduced rates of dimethoate with or without salt and the effects to two potential fungal bio-pesticide compounds (BC639a and BC667).

- *Efficacy of Biopesticides against aphids.* Completed. We investigated in more detail the efficacy of these bio-pesticides against aphids.
- *Effect of emergent pests on cotton productivity.* This objective has been achieved. The effects of late season damage on cotton yield was explored in field experiments over the two seasons, building on the outcomes of earlier research. In addition the effect of pale cotton stainers on boll development and yield was investigated.
- *CBT spread and transmissibility of apparent 'diseases'* will compare the spread of aphids from ratoon plans versus influxes, and to define more clearly the latent period of the disease (year 1).
- *New Emergent pest issue.* Completed. During the course of this project we had the first outbreaks of silver leaf whitefly in the central and southern cotton areas. Wilson co-ordinated a response to this problem – organising field days and collating information from experienced researchers in central Qld and CSIRO. Initial surveys of host use by SLW were begun and we provided collections to Ms Zara Hal to test for the presence of the Q-Biotype of *Bemisia tabaci*. We also continued research with pale cotton stainer to better understand its potential effect on cotton yield.

The project also ensured that the cotton industry maintained core skills with aphid identification and cotton bunchy top epidemiology (Ms Tanya Smith) and with identification of thrips and manipulation of mite populations (Ms Simone Heimoana). It also allowed ongoing collaboration with Dr Grant Herron and Dr Martin McLoon (NSW DPI) in the collection and resistance testing of spider mites and aphids from cotton regions and with the former as well as Dr Flavie Vanlerberge-Massutti[1] and Jerome Carletto (CIRAD France) in the use of microsatellite markers to characterise the aphid clones in cotton and link with resistance.

The project team for this research was:

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[1] Though not discussed further, the research with Flavie and Jerome is complete. Grant Herron and Yizhou Chen are currently putting together the analysis of the microsatellite data.

Methods and Results

1. Ecology of GVB.

We initially visited Dr Don Sands, Dr Marc Coombes, Dr Nancy Schellhorn and Dr Felix Bianchi (CSIRO CES, Indooroopilly) to discuss the aims of our research, review past research and review current research of Schellhorn and Bianchi relating to use if native vegetation remnants by pests and beneficials of cotton. We also discussed with Dr Moazzem Khan his experiences with sampling GVB in terms of host use, overwintering and establishment of a culture. These discussions were invaluable in planning our research.

Based on these discussions we set up sample sites in three main areas: Merah North, Breeza and Moree (Figure 1). Each site included winter/summer cropping, and populations of non-cultivated hosts, including native remnant areas. Amongst these sites Moree tended to be a less diverse site, with limited host species and cropping predominantly cotton/wheat in a hot region, Merah North was more diverse and with more non-cultivated hosts in a hot region and Breeza was the most diverse with a wide range crops, including soybean and maize, as well as cotton and wheat, and a diverse range of non-cultivated hosts (though only minor native vegetation areas) and tended to be slightly wetter and cooler.

Hence within each site there were several defined sampling areas. Each site was visited every three weeks, weather permitting. At each visit we sampled vegetation, estimated its % cover (including trees) and recorded numbers of GVB adults and nymphs (Figure 2), parasitism of adult GVB by the tachinid fly, *Trichopoda giacomelli*, and sampled sheltered locations for the 'bronze' overwintering forms. These forms start as green forms and gradually become darker, developing a rusty hue. They usually seek sheltered locations which are protected from the elements. They usually become active again in spring and resume their green colour and feeding. Artificial hides were developed to help track diapause and a culture established to provide sentinel egg rafts which were placed in the field to understand egg predation and parasitism. Examples of sites are shown in Figure 2.

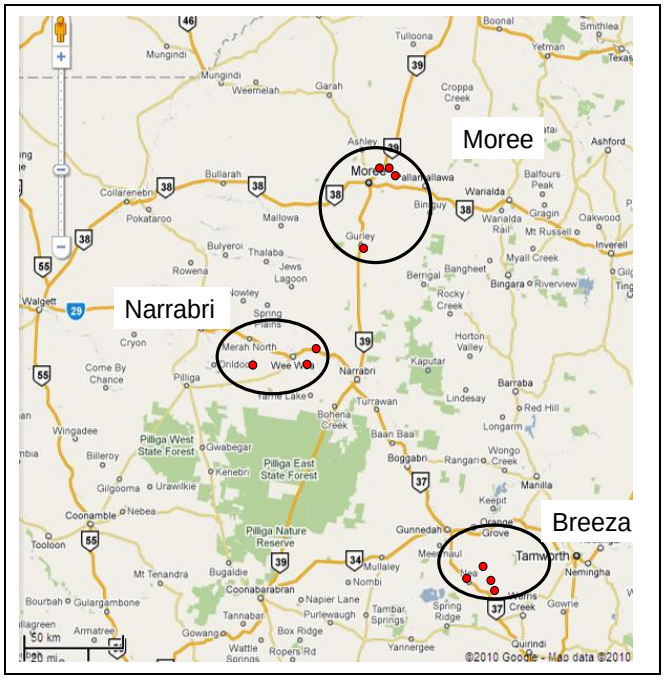

Figure 1. Location of sampling areas.

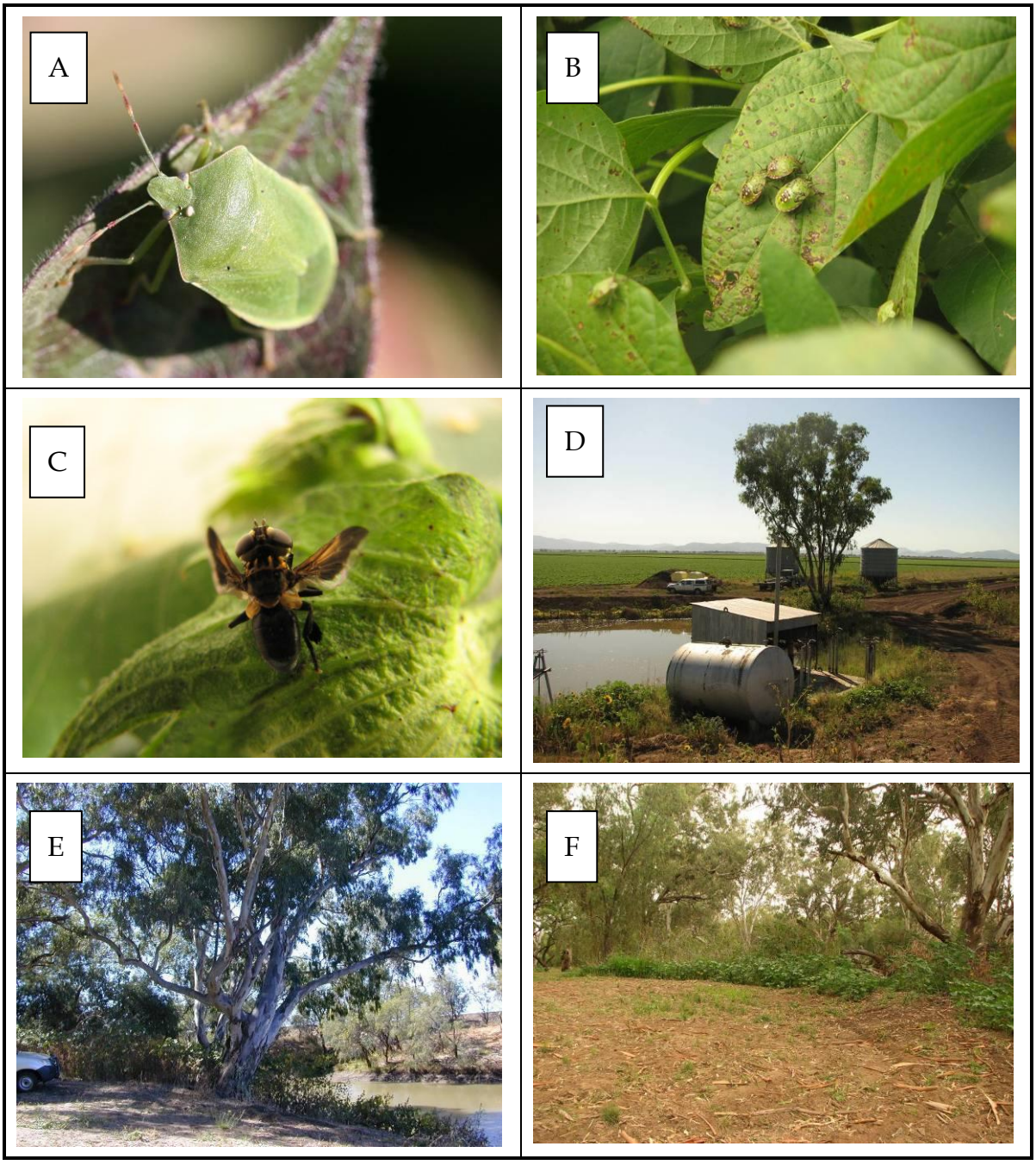

Figure 2. A. - Adult green vegetable bug with egg of the tachinid parasite *Trichopoda giacomelli*. B. - Nymphs of green vegetable bug. C. Adult of the tachinid parasite *Trichopoda giacomelli* D. – an on-farm sample site. E and F. – refuge sites adjacent to farms showing mix of native vegetation and also weeds.

Over the 3 years we found that abundance varied widely between sites and between years. The site at Breeza had the highest abundance while Moree consistently had the least (though this was a good site for presence of the smaller green stink bug, *Plautia affinis*). Active green adults and nymphs were more abundant in summer though there was high variability. Nymphs were not found for most of June-August indicating that although some of the 'green' forms of the adults were still active at this time there was little if and egg laying. Sex ratios where close to 50:50 across all sites.

The bronze overwinter forms were readily found in May – early August. These were very common under the hardened partially sloughed off bark of eucalyptus species especially River Red Gums (*Eucalyptus camaldulensis*). However, they used whatever protected areas there were available and could be found under hardened mud on tires of parked tractors, under tarpaulins and under pieces of loose wood on protected walls. In many cases other pest species were also present such as pale cotton stainer.

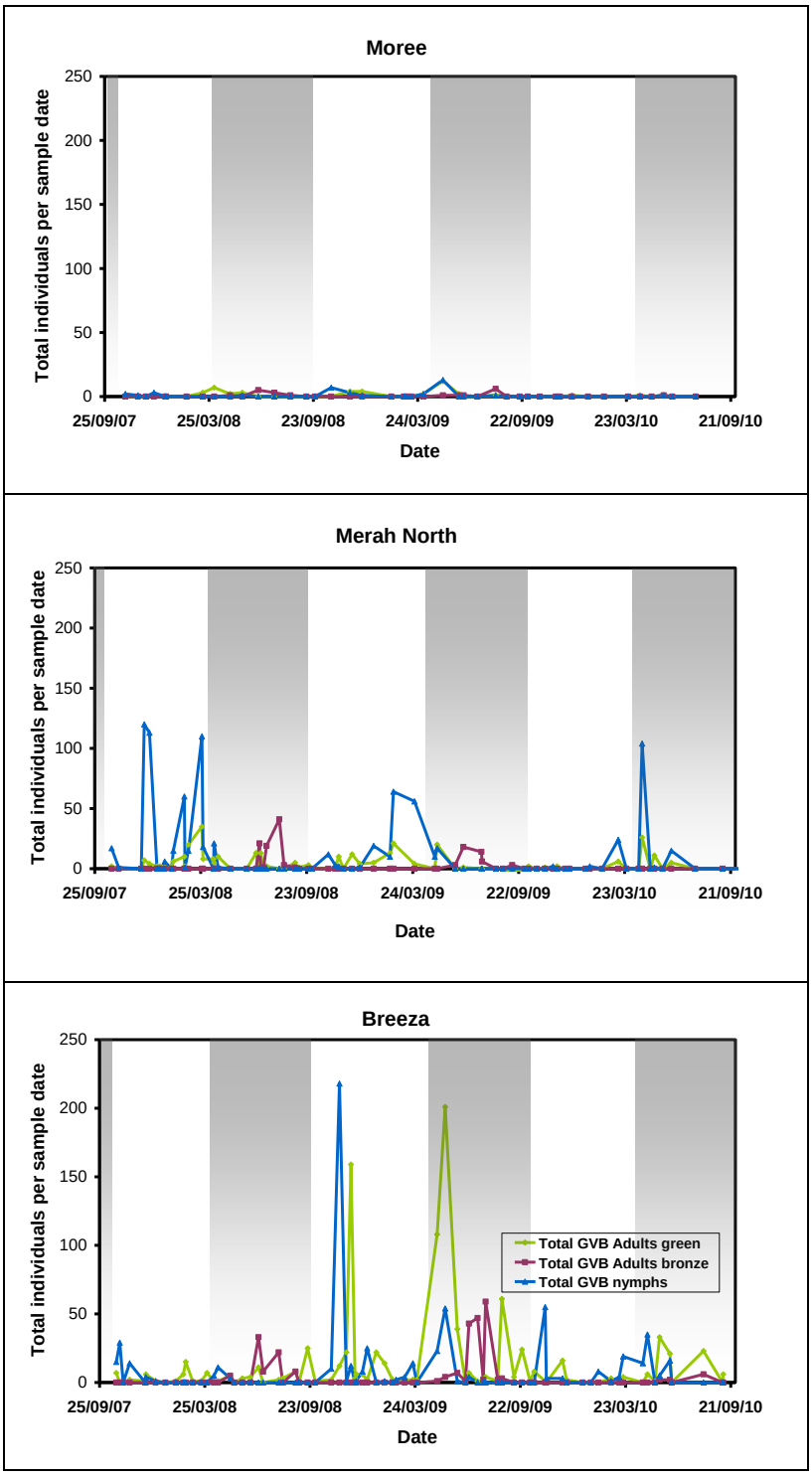

Figure 3. Seasonal abundance of adults, nymphs and diapause forms of green vegetable bug in each sampling area.

Figure 4. Bronze forms of green vegetable bug (left) can often be found under the bark of river red gums (right).

The parasite *T. giacomelli* was introduced into Australia from Argentina by CSIRO and DEEDI in 1996. This is a small brown fly, about 8mm long. It lays its egg on the outside of adult GVB (also on nymphs but less commonly). Parasitism rates of nymphs are difficult to estimate as the egg shell is shed when the nymph moults. The egg (s) hatches, in summer usually within days, and the maggot(s) enters the adult and consumes fatty tissues and reproductive structures. Feeding by the maggot(s) can cause death but often the main effect is dramatically reduced egg production.

The only way to determine if nymphs were parasitised was to collect and dissect them. However, due to often low number we decided that this could be to disruptive of the total population at ours sites. Hence we recorded the presence of the eggs or shells on adults and nymphs, though we rarely saw eggs on nymphs. We were especially interested in parasitism rates of the bronze forms as these are a significant portion of the spring populations.

Parasitism varied between sites. For the green form we found higher parasitism percentages at the Moree site (25.0%) even though the abundance of GVB was low. Parasitism rates at Merah North averaged 11.1% and at Breeza 10.5%. However, parasite activity was much more frequent at Breeza and Merah North where GVB numbers where higher and more consistent (Figure 5). For the bronze form parasitism rates were generally very low (Breeza; 1.2%, Moree; 5.0%, Merah North; 3.1%) and this is disappointing for potential biological control.

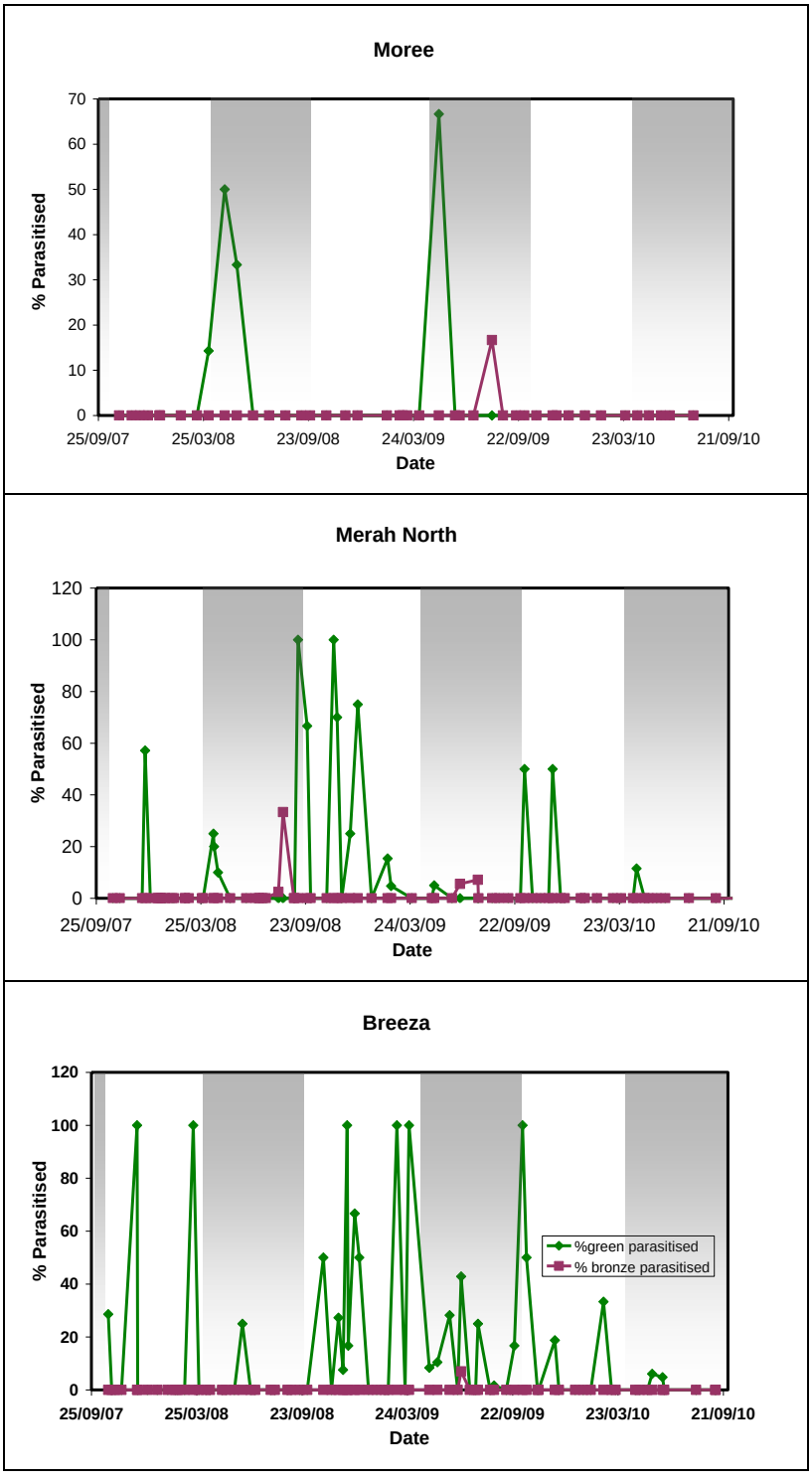

Figure 5. Parasitism rates of green vegetable bugs in the green or bronze form by the tachinid parasite.

We were interested to understand host use in these sites, especially to identify which host species were most important in maintaining GVB populations. This is not straightforward as the plants vary in size, time of availability and abundance as well as attractiveness to GVB. For instance a plant could be small, rare and highly attractive to GVB or large, common and moderately attractive – the latter is probably more significant in terms of overall pest abundance, but the former may be an important refuge host in drought conditions.

A simple approach that we developed to assess the importance of hosts was to plot the total number of GVB adults or nymphs recorded from a particular host against the number of times that GVB nymphs or adults were recorded as present on that host, out of a total of 43 sample dates over the three sites and 2.5 years. This provides a simple way to view both the abundance of the host which is reflected in the frequency with which GVB are recorded on it and its suitability as reflected by the total abundance of GVB found. This was done for the three sites combined over the three years and the data was separated into the four seasons with 43 sample dates for Summer, 35 for Autumn, 32 for Winter and 34 for Spring.

The results for adults showed that key hosts for adult GVB are *Rapistrum rugosum*, *Ricinus communis*, *Solanum nigrum*, *Lamium amplexicaule*, *Sonchus oleraceus*, *Malva parviflora*, *Ammi majus*, *Gossypim hirsutum* volunteers (some of these are shown in Figure 6, and see graphs in Figure 7). Key hosts for nymphs were *Rapistrum rugosum*, *Ricinus communis*, *Solanum nigrum*, *Urtica incisa*, *Xanthium occidentale*, *Lactuca seriola*, *Rumex crispus*, *Malva parviflora*, *Gossypim hirsutum* volunteers (Figure 8). It is worth noting that several weeds feature predominantly all of the time, both for adults and nymphs - especially *Rapistrum rugosum*, *Ricinus communis*, *Solanum nigrum* and *Malva parviflora*.

These hosts are all introduced weed species. We recorded GVB on these hosts in the native vegetation remnant areas – but recorded very few GBV on native hosts in these areas. Is this positive or negative for pest management? Weeds in native vegetation remnants may provide refuge areas for GVB and particularly for parasites that are relatively specific to these and similar bug species. However, the generally relatively low rates of parasitism suggest that this is not always happening – for reasons that we don't really understand. In these situations the weeds are really a contaminant that possibly reduces the value of the native vegetation remnants, and their removal could be beneficial.

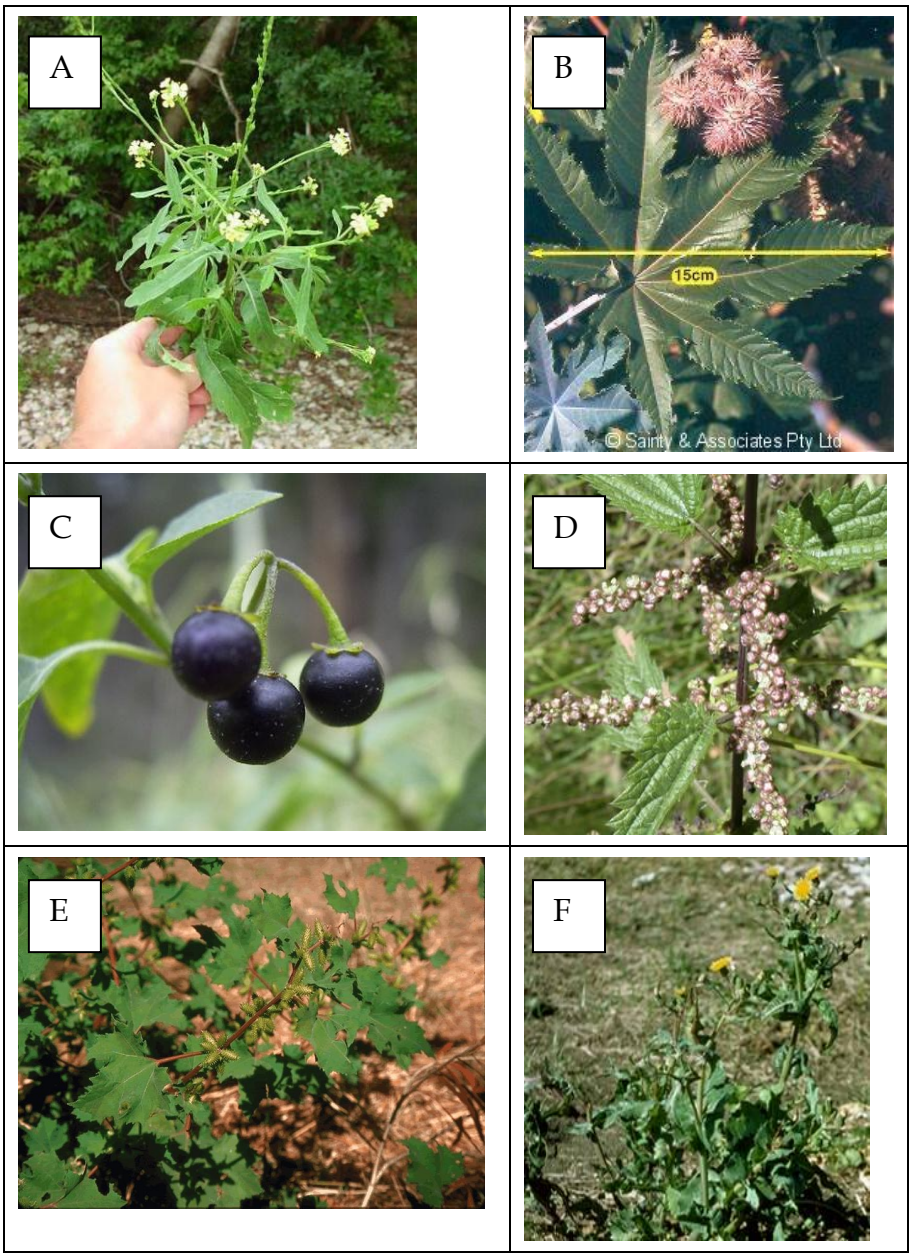

Figure 6. Some of the important hosts of green vegetable bug at the sample sites included: a) *Rapistrum rugosum* (wild turnip) b) *Ricinus communis* (castor oil) c) *Solanum nigrum* (blackberry nightshade) d) *Urtica incisa* (stinging nettle) e) *Xanthium occidentale* (noogoora burr) f) *Sonchus oleraceus* (sowthistle).

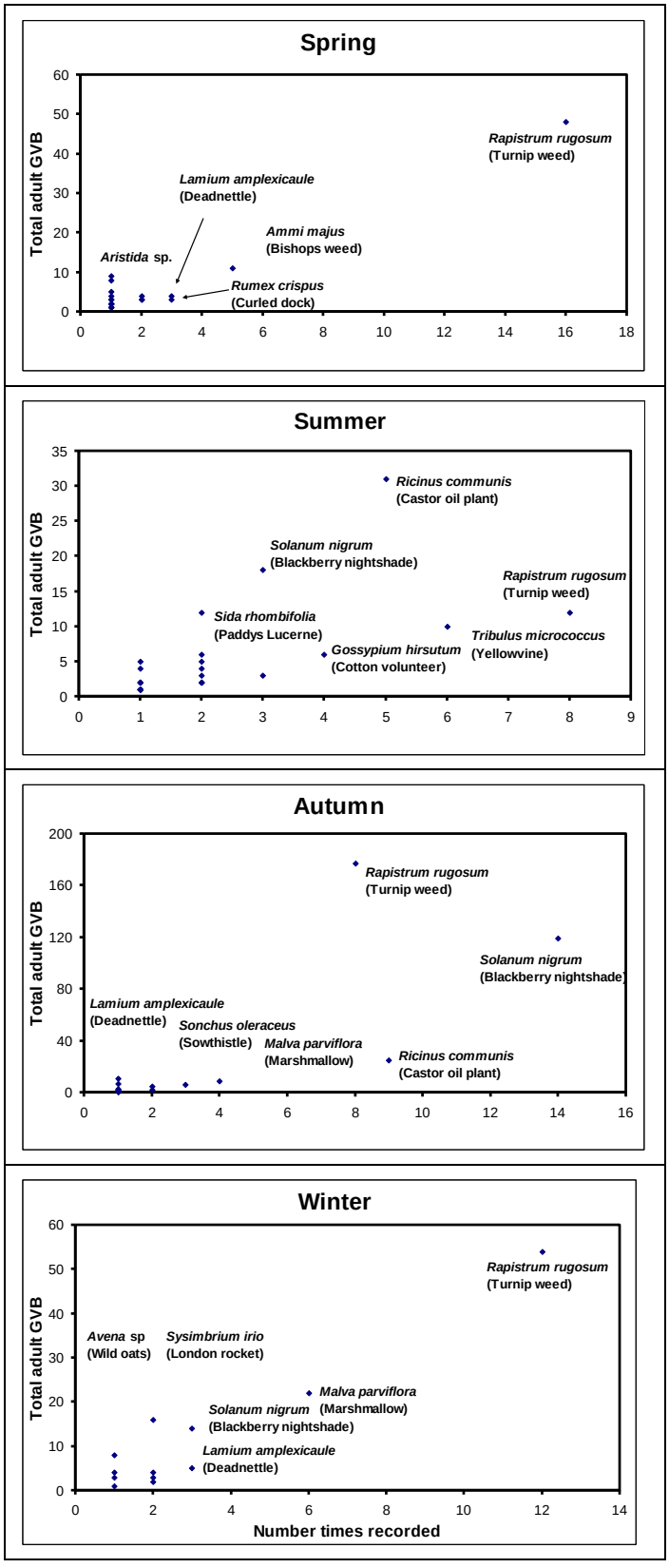

Figure 7. Preferred hosts for adult green vegetable bug based on the number of times they were recorded on that host and the total numbers recorded on that host. Hosts in the upper right are likely to be highly significant.

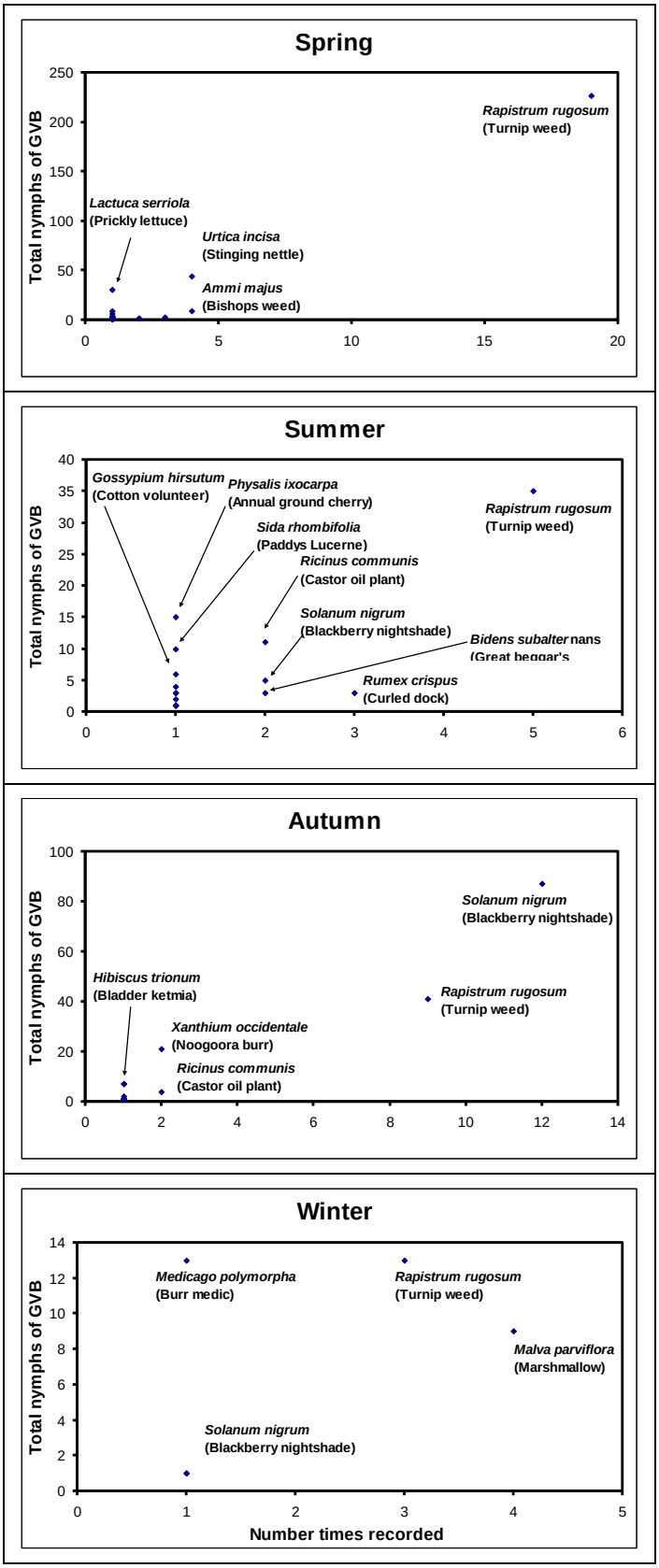

Figure 8. Preferred hosts for nymphs of green vegetable bug based on the number of times they were recorded on that host and the total numbers recorded on that host. Hosts in the upper right are likely to be highly significant.

2. Host use by GVB

We established small plots of winter and summer crops. In the first summer crops were planted in Block 18 at ACRI, each crop was a strip 4 rows by 100m in length. After that we moved to Block 17 and established small plots of winter and summer crops in a replicated design. Each plot was 8 rows by 20 m, with 3 replicates. The first summer crops were established in October 2008 and this was repeated in October 2009. Summer hosts included cotton, sunflower (*Helianthus annus*), corn (*Zea mays*), pigeon pea (*Cajanus cajan*), soybean (*Gycine max*), and mung bean (*Vigna radiata*). The first winter crops were established in June 2009 and this was repeated in June 2010. Winter crops included canola (*Brassica rapa*), field pea (*Pisum sativum*) and wheat (*Triticum aestivum*). Lucerne (*Medicago sativa*) was included as a perennial treatment. Samples were made every 3-4 weeks and included visual scouting of 1m sections in each plot and sweep net samples (20 sweeps per plot).The data show quite a strong pattern of host use and GVB abundance. The sweep net and visual samples both show that adults tended to colonise mungbean first, then use cotton or soybean and finally move onto pigeon pea (Figure 9). Small populations could be found on lucerne even through winter indicating this could be a source of continuity.

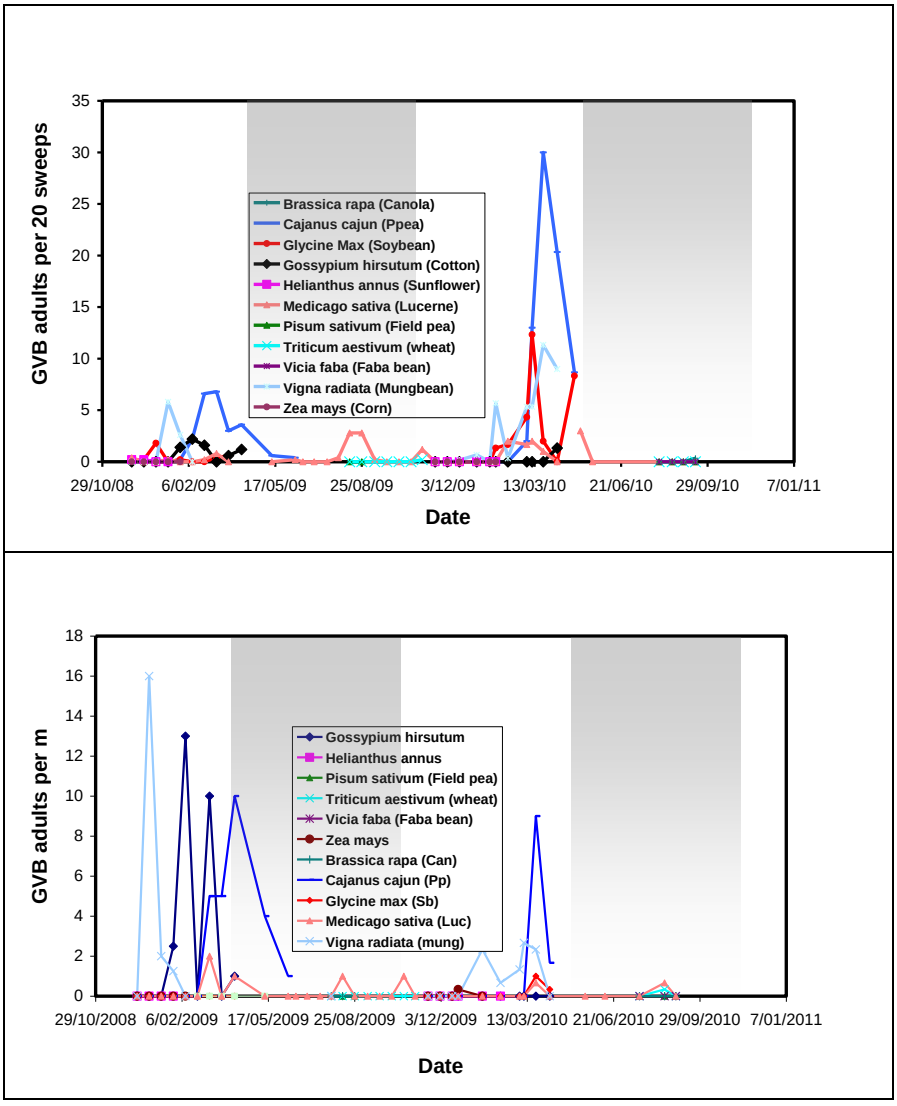

Figure 9. Crop host use by adult green vegetable bug, top is sweep samples bottom is visual samples.

Nymphs showed a slightly different pattern (Figure 10). Similar to adults, populations of nymphs began to build on mung beans, then as these finished populations increased in soybean then finally pigeon pea. Notably few nymphs were found in cotton. This suggests that compared with the legumes cotton is less preferred as an oviposition site especially early on when there are no bolls. It raises the possibility to use small areas of legumes as trap crops, which could be used to capture spring populations of GVB where they and the nymphs could be controlled and reduce risks to nearby cotton.

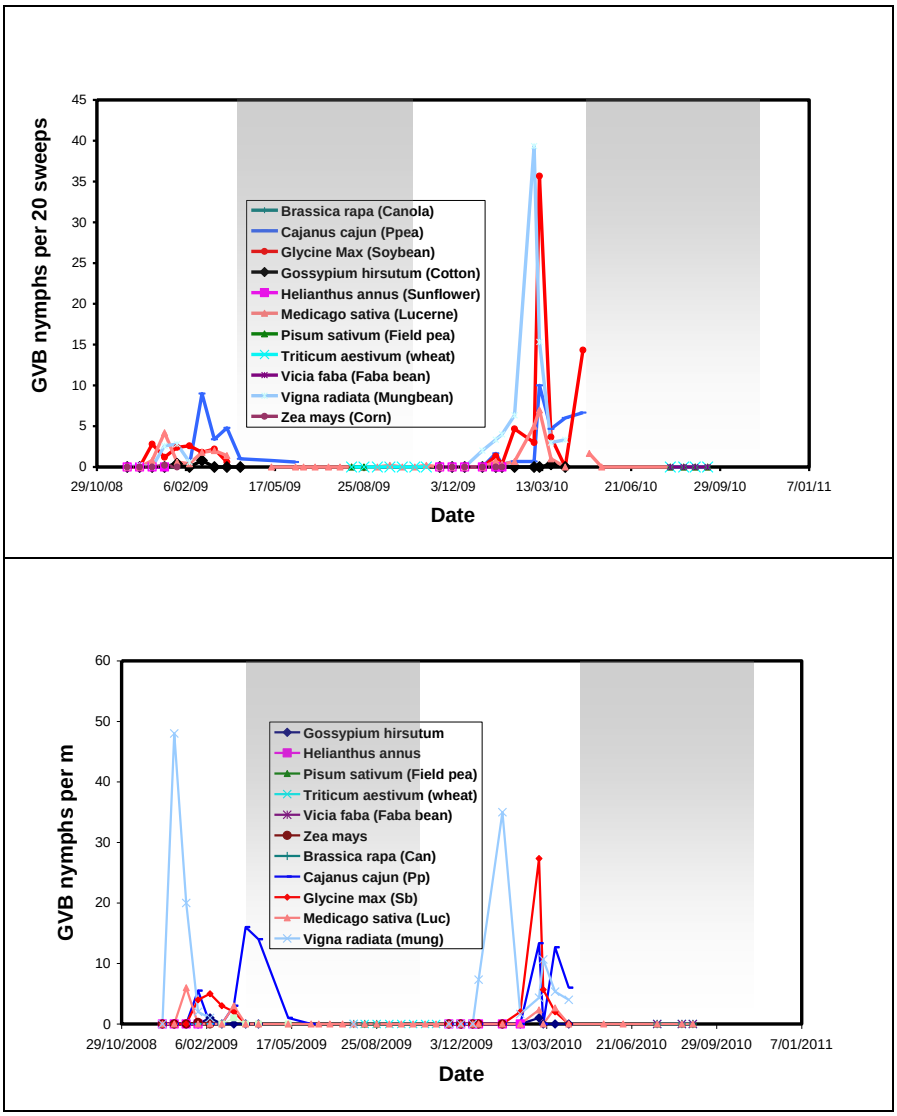

Figure 10. Crop host use by nymphal green vegetable bug, top is sweep samples bottom is visual samples.

To add further value to the research with crop hosts we established a GVB culture in the laboratory. This was relatively straightforward though time consuming to manage. We used the culture to generate egg rafts that could be placed in the field to obtain an estimate of egg parasitism rates (Figure 11). Egg rafts were collected from the culture, the eggs counted then the raft placed into the field within 24hr – the literature suggested that rafts older than this age are less acceptable to parasitoids. The eggs were left in the field for about 48 hours then collected (before they hatched) to assess for parasitism. Parasitism rates were generally

modest, with an average of <10% of eggs parasitised, and a peak of about 35 % of eggs parasitised (Figure 12).

Figure 11. Clockwise from top left, GVB culture cages, close up of culture showing corn and beans used as food, Egg raft attached to fibreglass pole within the crop, *Trisolcus basislis* (egg parasite).

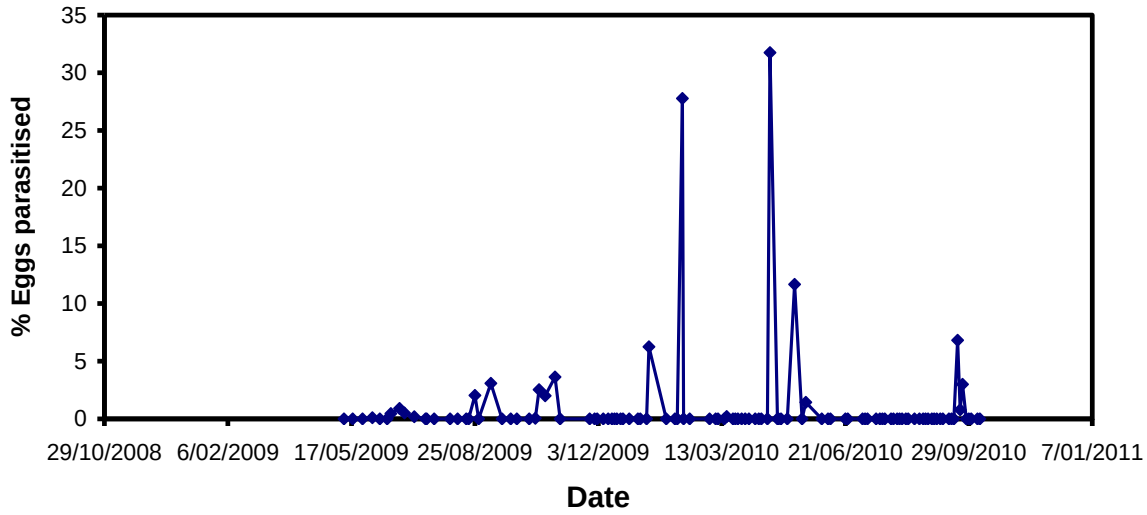

Figure 12. Parasitism of green vegetable bug eggs.

Combined, the surveys of non-cultivated and cultivated hosts show that it is the introduced weeds and crops that are the key hosts for GVB. Careful weed management on farm and in remnant vegetation areas may reduce overall population size. Regions most at risk from this pest will be those where the cropping system includes spring legumes such as mung bean or early sown soy bean, or near areas of native vegetation heavily contaminated with weeds. Since this work was complete we have had reports from consultants of populations of GVB in sorghum crops. This will be followed up though the summer of 2011 in another project. Though we have only presented data here for GVB, as this was the target of the work, we also collected similar data for other potential pests such as the green stink bug (*Plautia affinis*), red banded shield bug (*Piezodorus hybneri*) and green mirid (*Creontiadies dilutus*).

3. Efficacy and IPM fit of biopesticides, new chemistries and novel technologies.

This research uses 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. 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 the following compounds;

(i) Dimethoate at reduced rates (80 g ai/ha) with salt (10 g/l NaCl) or without salt was compared with the full rate alone (200 g ai/ha). This finished off research that was started at the end of a previous project. The need for this was that growers and consultants were looking for options to control mirids that were cheap with low negative risk to beneficials. Our research showed that reducing the rate of dimethoate did increase selectivity, compared with the full rate) and provide effective control of mirids. However, even the reduced rate of dimethoate had a substantially greater risk to beneficials than reduced rates of indoxacarb (60 g ai/ha) plus salt or fipronil (8 g ai/ha) plus salt. Low rates of dimethoate plus salt significantly increased the risk of cotton aphid outbreaks in the second year of testing.

(ii) Initial experiments with BC639a (*Aspergillus fumigatus*, note this was formerly thought to be a *Metarhizium* strain) and BC667 (*Beauvaria* spp.). In 2008-09 we included BC639 and BC667 both at 500 ml/ha. In 2009-10 the decision had been made not to progress BC667 so we dropped it and included two rates of BC639. A confidential report on the evaluations to date was prepared and provided to NSW I&I.

(iii) We completed the first year of evaluation of a new compound from Dupont which targets *Helicoverpa* spp. and silver leaf whitefly (DPX-HGW86). A confidential report on the first year of evaluation was prepared and provided to Dupont.

(iv) We completed the first year of evaluation of a new compound from Dow which targets aphids, silver leaf whitefly and mirids (GF-2032). A confidential report on the first year of evaluation was prepared and provided to Dupont.

(v) In a previous project we investigated non-target effects and efficacy of clothianidin, a compound from the neonicotinoid group. Clothianidin has since been registered and we have now completed the analysis to evaluate its IPM fit.

The information summarising effects of the new registered compounds (e.g. Shield) and the lower rates of dimethoate have been incorporated into the 'Impact of insecticides and miticides on predators in cotton' table and provided to Yvette Cunningham (Cotton CRC) and Susan Maas (DEEDI Qld) for inclusion in the Cotton Pest Management Guide 2010-11 (see Table 19in that book) and Figure 13 below. This ensured that this reference document was up-to-date each year for the cotton industry. Evaluation with the two new compounds mentioned above continues. Work with the biopesticides has stopped due to a decision not to proceed with BC639 and to return to BC667. Investigation with this biopesticide will resume in 2011-12. We have also begun evaluation of Plant X extract.

Figure 13. Non-target effects of all available insecticides and miticides, 2010-11.

Insecticides (in increasing rank order of impact on beneficials)	Rate (g ai / ha)	Target Pest(s): Helicoverpa	Mites	Mirids	Aphids	Thrips	Silverleaf whitefly	Persistence[8]	Beneficials: Overall Ranking[10]	Predatory beetles: Total[1]	Red & Blue beetle	Minute 2-spt lady beetle	Other lady beetles	Predatory bugs: Total[2]	Damsel bugs	Big-eyed Bugs	Other Predatory bugs	Apple Dimpling	Lacewing adults	Spiders	Hymenoptera: Total (wasps)	Eretmocerus[19]	Trichogramma	Ants	Thrips	Pest resurgence[12]: Mite	Aphid	Helicoverpa	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	M	VL	L				VL
PSO (Canopy)[16]	2%	✓			✓			short	very low	VL	L	L	VL	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
Pyriproxyfen	50						✓	long	very low	M	-	M	M	VL	-	-	-	-	L	VL	VL	L	VL	VL	VL				L
Etoxazole	38.5		✓					short	low	VL	VL	-	L	VL	VL	VL	VL	VL	VL	M	L	-	VL	VL	L				VL
Indoxacarb (low)	60			✓				medium	low	L	L	H	M	VL	L	-	L	H	M	VL	VL	-	VL	H	VL		+ve		-
Indoxacarb (low+salt)	60			✓				medium	low	L	L	H	M	VL	L	-	L	H	M	VL	L	-	VL	H	VL				-
Indoxacarb (low+Canopy)	60			✓				medium	low	L	L	H	M	VL	L	-	L	H	M	VL	L	-	VL	H	VL		+ve		-
Rynaxypyr®	52.5	✓						long	low	L	M	M	L	VL	VL	VL	L	VL	VH	VL	L	-	L	L	VL		+ve		-
Dicofol[3]	960		✓					long	low	L	-	-	-	L	-	-	-	L	-	L	-	M	-	-	VL				VL
Amorphous silica[17]	2500	✓						short	low	L	L	-	M	M	-	VL	-	L	L	L	L	-	-	M	VL				-
Spinosad	96	✓						medium	low	VL	M	L	VL	M	L	H	VL	L	VL	VL	M	H	H	H	H	+ve			H[15]
Diafenthiuron	350		✓		✓		✓	medium	low	M	H	VL	M	L	M	VL	L	H	VL	L	L	H	VL	H	L			+ve	M
Pymetrozine	150				✓			short	low	M	M	M	M	L	L	L	VL	H	M	L	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	M	-	L	M	L	M	L	-	-	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]
Spirotetramat	96				✓		✓	medium	moderate	M	L	H	H	VL	VL	VL	VL	M	VH	M	M	-	M	M	M				-
Abamectin	5.4	✓[6]	✓					medium	moderate	L	M	H	VL	M	L	M	M	H	VL	M	M	H	M	H	M				H
Emamectin	8.4	✓						medium	moderate	L	VL	M	VL	H	H	H	H	H	L	M	M	-	M	VL	M				H
Dimethoate (low)	80		✓[18]	✓	✓[18]	✓		short	moderate	M	L	H	H	M	L	-	H	M	M	L	M	-	M	H	M	+ve	+ve	+ve	H
Dimethoate (low + salt)	80		✓[18]	✓	✓[18]	✓		short	moderate	M	L	H	H	M	L	-	H	M	M	L	M	-	M	H	M	+ve	+ve	+ve	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	H	VL	VL	L	VL	M	M	L	H	H	M		+ve	+ve	L
Acetamiprid	22.5				✓			medium	moderate	M	M	VH	H	M	M	H	M	VH	L	VL	L	VH	H	VH	VH				M[15]
Clothianidin (low)	25			✓				medium	moderate	M	VL	-	H	L	M	VL	VL	H	H	M	M	H	M	VH	VL			+ve	-
Amitraz	400	✓	✓[9]		✓[9]			medium	moderate	H	M	VH	H	L	-	-	-	H	VL	M	M	H	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	H	H	M	VL	M	-	H	VH	H	+ve		+ve	H
Endosulfan (high)	735	✓		✓	✓	✓		medium	moderate	M	VL	VH	M	M	M	M	L	H	L	M	L	H	VH	VH	H				M[15]
Fipronil (high)	25			✓		✓		medium	moderate	L	VL	H	L	M	H	H	L	VH	L	M	M	-	M	VH	VH	+ve		+ve	VH
Imidacloprid	49			✓	✓			medium	moderate	H	L	VH	H	H	M	H	L	VH	L	L	L	VH	M	H	H	+ve		+ve	M
Clothianidin (high)	50			✓				medium	moderate	H	VL	-	VH	M	M	L	VL	H	H	M	M	VH	H	VH	VL	+ve		+ve	-
Methomyl	169	✓						very short	high	H	L	VH	VH	M	L	VH	L	VH	M	M	M	VH	H	H	H	+ve			H[15]
Thiodicarb	750	✓						long	high	VH	M	VH	VH	M	M	L	L	VH	VL	M	M	-	M	M	H	+ve	+ve		M[15]
Dimethoate (high)	200		✓[18]	✓	✓[18]	✓		short	high	M	M	H	H	M	H	-	H	H	VH	M	H	H	H	VH	M	+ve		+ve	H
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	VH	H	VH	H	+ve			H
Carbaryl[3]								short	high	H	-	-	-	H	-	-	-	-	-	-	-	-	-	-	H	-	-	-	H
Pyrethroids[4]		✓	✓[7]	✓[7]			✓[7]	long	very high	VH	-	-	-	VH	-	-	-	VH	VH	VH	VH	VH	VH	VH	VH	+ve	+ve	+ve	H

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; omethoate, monocrotophos, profenofos, chlorpyrifos, chlorpyrifos-methyl, azinophos ethyl, methidathion, parathion-methyl, thiometon
6. *Helicoverpa punctigera* only.
7. Bifenthrin is registered for mite and silverleaf whitefly 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.
18. Will not control organophosphate resistant pests (e.g. mites, some cotton aphid (*Aphis gossypii*) populations
19. Rankings for *Eretmocerus* based on data for *E. mundus* (P. De Barro, CSIRO, unpublished) and for *E. eremicus* (Koppert B.V. , The Netherlands (http://side-effects.koppert.nl/#))

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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4. Efficacy of biopesticides against cotton aphids.

A component of this project has a linkage with Dr Robert Mensah to evaluate efficacy of biopesticides against cotton aphids. The research relied on being able to reliably establish field outbreaks of cotton aphids. To do this we mass reared aphids in the glasshouse and in large field cages and transferred them to cotton plots. However, we have increasingly experienced problem with this approach due to beneficial insects rapidly locating the plots and consuming the aphids.

For this project we worked around this problem by using field cages to exclude beneficials (at least partially). The cages were each 3m x 2 rows. For these experiments we used a replicated design with one treatment per cage and 3 replications. Cages were infested with aphids, which were allowed to establish and spread for one or two weeks or in experiments 2 and 3 reinfested, then pre-treatment counts were made, sprays applied and post treatment sampling started then sprays reapplied. Sampling was done at about 3 days, and 7 days after spray application and at about 7 day intervals thereafter between spray events. Sprays were applied with a hand held sprayer.

We did three experiments, Experiment 1 in 2007-08, Experiment 2 in 2008-09 and experiment 3 in 2009-10. Data from Experiment 1 is included because this project included the first year of 'Emerging Pests: Developing Knowledge for GVB and Aphids'. The 'multiple application' approach was used because earlier research had indicated that these biopesticides only provided modest control (30-60%) compared with a commercial synthetic insecticide (usually > 95%). This is probably related to the lack of system uptake of the fungal biopesticides so they must contact the target – which is difficult with aphids as most are on the undersides of leaves. All commercial aphicides in cotton are either fumigant, trans-laminar or systemic to overcome this challenge.

In Experiment 1 we included BC639a (*Aspergillus spp*, note this was formerly thought to be a *Metarhizium* strain) and BC667 (*Beauveria* spp.). In Experiment 2 we were advised to only progress with BC639. In the Experiment 3 we attempted to evaluate if initial application when aphid population densities were lower was beneficial, e.g. by preventing population build up in the first place so high efficacy is not required. The treatments for each experiment are provided in Tables 1-3. Acetamiprid at half the registered rate was used as a standard.

Statistical analyses were run using Genstat 12 for PC. All beneficial or pest counts were transformed (ln + 1) prior to analysis to stabilise the mean/variance relationship. The pre-spray counts were analysed separately and if significant (Experiment 3) were used as a co-variate in later analysis. Analysis of variance (or co-variance for Experiment 3) was used to test for treatment differences over the duration of the experiment with terms for block, treatment, date and treatment by date interaction included. Where the interaction term was significant the interaction mean square value was used as the error term to test for main effects of treatment over and above its contribution to the interaction effect. Fisher's protected least significant difference test was used to separate means for insecticide treatments from those of the control.

Experiment 1 showed that the two lower rates of BC639 did not provide consistent suppression of aphids. The 1 litre rate did provide control on some dates and BC667 provided control on one date. Overall BC639 and BC667 tended to reduce abundance of aphids compared with the control by about 10-50% but the results were erratic, especially compared with the commercial standard.

In Experiment 2 three applications of the 0.5 litre/ha rate of BC639 provided moderate control of aphids, ranging between about 10-50%. The 1 litre/ha rate provided more consistent control of between about 15-60% peaking at 82% on one date. The commercial standard provided > 95% control.

In Experiment 3 application of BC639 to cotton with high aphid densities resulted in a similar level of control as at lower densities though it took longer for numbers to reduce to below that of the untreated. Again the biopesticide provided modest control compared with the commercial standard, but repeated applications did prevent aphid populations from developing.

Overall the results suggest that BC639 will provide suppression of aphid populations if used in a program basis – e.g. perhaps included when sprays are applied against other pests. BC639 used this way may prevent aphid populations from developing to high abundance, but is not likely to provide the fast acting strong knockdown of a commercial aphicide. However, it is important to consider the fact that earlier data shows that BC639 is far more selective than acetamiprid – hence the conservation of beneficial or 'bio-residual' of the biopesticide may be greater and further contribute to suppression of aphids and other pests.

Table 1. Experiment 1 - Aphid infestation and spray dates for all treatments, ACRI, 2007-2008

Treatment	Rate	Infestation 1	Spray 1	Spray 2
BC639	0.5 L/ha	21/01/2008	08/02/2008	22/02/2008
BC639	0.75 L/ha	21/01/2008	08/02/2008	22/02/2008
BC639	1.0 L/ha	21/01/2008	08/02/2008	22/02/2008
BC667	1.0 L/ha	21/02/2008	08/02/2008	22/02/2008
Acetamiprid	0.05 L/ha	21/02/2008	08/02/2008	22/02/2008
Control	*	21/02/2008	08/02/2008	22/02/2008

Table 2. Experiment 2 - Aphid infestation and spray dates for all treatments, ACRI, 2008-2009

Treatment	Rate	Infestation 1	Infestation 2	Spray 1	Spray 2	Spray 3
BC639	0.5 L/ha	28/01/2010	06/02/2010	10/02/2010	24/02/2010	12/03/2010
BC639	1.0 L/ha	28/01/2010	06/02/2010	10/02/2010	24/02/2010	12/03/2010
Acetamiprid	0.05 L/ha	28/01/2010	06/02/2010	10/02/2010	24/02/2010	12/03/2010
Control	*	28/01/2010	06/02/2010	10/02/2010	24/02/2010	12/03/2010

Table 3. Experiment 3 - Aphid infestation and spray dates for all treatments, ACRI, 2009-2010

Treatment	Rate	Infestation 1	Infestation 2	Spray 1	Spray 2	Spray 3
BC639 Early	1.0 L/ha	11/01/2010	19/01/2010	25/01/2010	08/02/2010	22/02/2010
BC639 Late	1.0 L/ha	21/01/2010	*	25/01/2010	08/02/2010	22/02/2010
Acetamiprid Early	0.05 L/ha	11/01/2010	19/01/2010	25/01/2010	08/02/2010	22/02/2010
Acetamiprid Late	0.05 L/ha	21/01/2010	*	25/01/2010	08/02/2010	22/02/2010
Control	*	11/01/2010	19/01/2010	25/01/2010	08/02/2010	22/02/2010

Table 4. Average aphids per leaf from counts on twenty leaves for each cage in each treatment. Difference between control and treatments, expressed as a percentage and recorded over two spray periods, Experiment 1, ACRI, 2007-2008.

Treatment	Formulation	Rate	07/02/2008 (Pre Treatment)	13/02/2008		15/02/2008		18/02/2008		22/02/2008		25/02/2008	
		L/ha	Mean[1]	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%
BC639	1x10^7 spores/mL	0.5	18.4	77.85	-5.1	122.95	38.7	117.21	-6.9	105.76	25.8*	89.15*	113.2
BC639	1x10^7 spores/mL	0.75	13.77	57.06	-30.4	52.45	-40.8	77.97	-38.1	75.54	-10.2*	94.52*	126.1
BC639	1x10^7 spores/mL	1.0	19.31	52.12*	-36.5	93.99	6.0	128.93	2.4	93.04	10.6	68.28	63.3
BC667	1x10^7 spores/mL	1.0	6.05	52.48	-36.0	71.05	-19.9	82.38	-34.6	62.62	-25.6	53.40	27.7
Acetamiprid	225 g ai/L	0.05	8.25	0.07*	-99.9	0.00*	-100.0	0.12*	-99.9	0.04*	-99.9*	1.15*	-97.3
Control			9.18	82.03	0.0	88.66	0.0	125.91	0.0	84.13	0.0	41.81	0.0
P value			ns	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
LSD			ns	0.53		0.58		0.51		0.52		0.56	

27/02/2008		29/02/2008		03/03/2008		07/03/2008		14/03/2008	
Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%
116.68*	94.6	118.65	-5.5	163.06	79.6	85.00*	-54.7	246.44*	198.0
36.93	-38.4	68.66	-45.3	69.05	-23.9	121.87	-35.0	98.44	19.0
87.06	45.2	59.76	-52.4*	71.45	-21.3	98.13	-47.7	54.42	-34.2
87.39	45.8	63.57	-49.4*	79.94	-11.9	139.33	-25.7	226.07*	173.4
0.07*	-99.9	0.20	-99.8*	0.81*	-99.1	0.34*	-99.8	0.79*	-99.1
59.96	0.0	125.56	0.0	90.78	0.0	187.53	0.0	82.70	0.0
< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
0.64		0.66		0.66		0.75		0.82	

1. Mean values are the average number of insects per leaf from twenty leaves per treatment, back transformed from ln of mean

Table 5. Average aphids per leaf from counts on thirty leaves for each cage in each treatment. Difference between control and treatments, expressed as a percentage and recorded over three spray periods, Experiment 2, ACRI, 2008-2009.

Treatment	Formulation	Rate	10/02/2009 ↓ (Pre Treatment)	13/02/2009		17/02/2009		24/02/2009 ↓		28/02/2009	
			Mean[1]	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%
BC639	1*10[7] spores/mL	0.5 L/ha	35.63	12.71	-34.7	12.52*	-47.7	41.44	-41.5	70.59*	-54.4
BC639	1*10[7] spores/mL	1.0 L/ha	39.13	15.04	-22.8	20.61	-13.9	53.04	-25.1	67.44*	-56.4
Acetamiprid	225 g ai/L	0.05 L/ha	30.23	0.05*	-99.8	0.0*	-100.0	0.00*	-100.0	0.00*	-100.0
Control			39.93	19.48	0.0	23.94	0.0	70.85	0.0	154.67	0.0
P value			ns	< 0.001		< 0.001		< 0.001		< 0.001	
LSD			ns	0.68		0.52		0.55		0.53	

03/03/2009		10/03/2009 ↓		15/03/2009		19/03/2009		26/03/2009	
Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%
75.08*	-51.5	63.36*	-57.8	56.00	-56.8	78.91	-25.7	197.83	-6.7
59.68*	-61.4	58.20*	-61.3	22.19*	-82.9	57.44*	-45.9	103.77*	-51.1
0.02*	-100.0	0.00*	-100.0	0.00*	-100.0	0.02*	-100.0	0.00*	-100.0
154.69	0.0	150.20	0.0	129.57	0.0	106.25	0.0	212.12	0.0
< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
0.52		0.50		1.05		0.69		0.52	

1. Mean values are the average number of insects per leaf from twenty leaves per treatment, back transformed from ln of mean

Table 6. Average aphids per leaf from counts on twenty leaves for each cage in each treatment. Difference between control and treatments, expressed as a percentage and recorded over three spray periods, Experiment 3, ACRI, 2009-2010.

Treatment	Formulation	Rate	25/01/2010 (Pre Treatment)	28/01/2010		01/02/2010		08/02/2010		10/02/2010		15/02/2010	
			Mean[1]	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%
BC639 Early	1*10[7] spores/mL	1.0	51.76	28.26	-28.7	27.37	-35.9	231.71*	139.7	176.19	102.5	107.50	-36.8
BC639 Late	1*10[7] spores/mL	1.0	210.12*	130.51*	189.5	87.76	105.7	103.28	6.9	90.76	4.3	17.01*	-90.0
Acetamiprid Early	225 g ai/L	0.05	26.93*	0.09*	-62.9	0.17*	-99.6	0.25*	-99.8	0.00*	-100.0	0.00*	-100.0
Acetamiprid Late	225 g ai/L	0.05	103.10	1.31*	42.0	0.17*	-99.6	0.52*	-99.5	0.07*	-99.9	0.04*	-99.9
Control			72.59	29.27	0.0	42.68	0.0	96.65	0.0	87.00	0.0	170.20	0.0
P value			0.002	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
LSD			0.97	0.99		0.86		0.83		0.83		0.49	

22/02/210		24/02/2010		02/03/2010		15/03/2010		29/03/2010	
Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%	Mean[1]	%
66.97	-25.6	34.30*	-55.1	9.37*	-70.0	1.09*	-94.9	4.84*	-92.5
7.49*	-91.7	5.57*	-92.7	2.97*	-90.5	4.37*	-79.4	7.19*	-88.9
0.00*	-100.0	0.04*	-100.0	0.00*	-100.0	0.00*	-100.0	-	-
0.00*	-100.0	0.00*	-100.0	0.00*	-100.0	0.72*	-96.6	-	-
89.99	0.0	76.32	0.0	31.26	0.0	21.23	0.0	64.93	0.0
< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
0.58		0.63		0.60		0.76		0.94	

1. Mean values are the average number of insects per leaf from twenty leaves per treatment, back transformed from ln of mean

5. Effect of emergent pests on cotton productivity.

Reduced insecticide use in Bollgard II cotton has allowed some pests, not controlled by the Bt proteins, to survive and sometimes build in abundance to potentially damaging levels late season. High populations of tomato thrips (*Frankliniella schultzei*), western flower thrips (*Frankliniella occidentalis*) and vegetable leaf hopper (*Austroasca viridigrisea*) can damage upper leaves of mature plants (Figure 1a, b). Thrips are also important predators of mite eggs (Figure 1c). The effect of this damage is not understood. Unnecessary spraying to prevent it may incur costs and lead to mite outbreaks, while lack of control could result in yield loss.

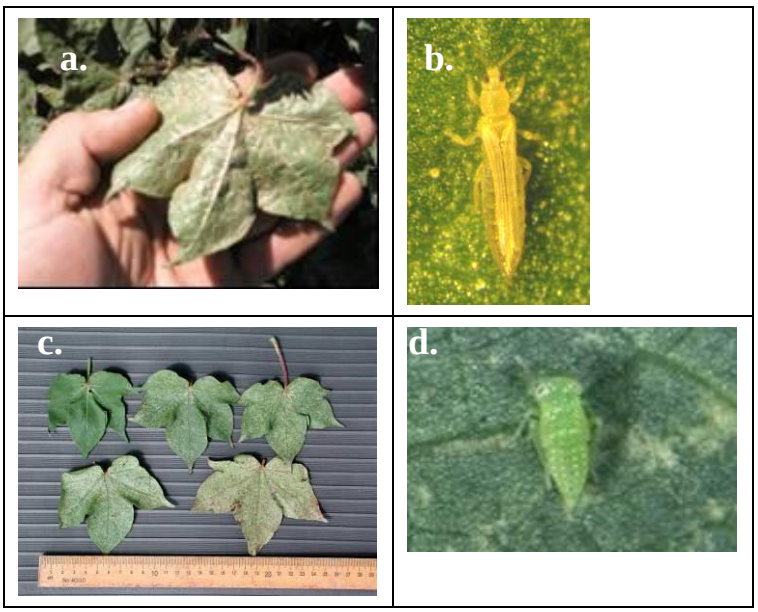

Figure 12. (a) Leaves showing damage from late season thrips populations from Mal Pritchard at Hillston (b) thrips (c) leaves damaged by jassids and (d). a jassid nymph. In (a) and (c) the crop manager had expressed concern about the level of damage.

Effect of leaf area loss

Earlier experiments considered the effect of removal of all leaves from the top 6 or 9 nodes at four times of the season: the first at early flowering, the second three weeks later (peak flowering), the third at cut-out and the fourth at cut-out plus two weeks. These experiments were completed in 2006-07 and 2007-08 and indicated that cotton yield is sensitive to damage to the upper leaves, though it requires quite high damage before yield is affected (See Final Report for CSP 165C). The results showed 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 crop (2007-08) appeared to be more sensitive, and damage quite late resulted in yield loss. These experiments suggested we should focus in future on later damage timings and partial leaf removal which will more accurately simulate real damage.

In this project we sought to further refine this understanding by including a less severe leaf damage treatment. We limited damage to the top 6 nodes only, as this is more realistic, and included high damage (total leaves removed) and moderate damage (50% of each leaf removed by cutting it off with scissors). This experiment used a replicated design with four replications and was complete in 2008/09 and repeated in 2009/10. Plots were each 8 rows by 6m, but only the central 3 rows were damaged. Hand harvests were carried out to look at effects on crop maturity and a machine harvesting of the central row aimed to obtain an accurate estimate of yield.

Figure 13. Plots from damage experiments (a) undamaged (b) 50% leaf removal (c) 100% leaf removal, Field A3, ACRI, 2008-09.

Data were analysed using Genstat 12. REML was used to allow for the unbalanced design of the experiment. There was a significant difference ($P<0.003$) between years in overall yield levels, with yield averaging 10.9 b/ha in 2008-09 and 14.4 in 2009-10. There was also a significant effect ($P<0.002$) due to the % removed, 100% removal (12.3 b/ha) generally having lower yield than the 50% removal (13.1 b/ha) or control (12.7 b/ha) which were similar. There was a significant interaction between year and the timing at which damage was inflicted ($P<0.002$) (Figure 14), however, this really just reflected the strong year effect. The interaction was due to smaller differences between years for the late damage (cutout and cutout plus 2 weeks) than the other treatments. There was also a marginally significant interaction of timing of damage and % of leaf removed ($P = 0.058$) which showed that removal of 50% of leaf area from the top 6 nodes did not reduce yield but removal of 100% did, if it was done early (Figure 15). The crop duration varied significantly between years ($P<0.001$) with crop development much quicker in 2008/09 at 155 days from sowing, compared to 189 days from sowing in 2009-10. However there were no effects of any damage treatments or interactions.

The results there suggest that cotton crops are reasonable tolerant of reduction in leaf area from the top 6 nodes especially later in the season. One of the possible mechanisms is that loss of leaf area in the top of the canopy allows more light to other leaves lower in the canopy which may then increase their contribution to plant assimilate supply than they would have otherwise. These results have been of

particular value in the 2010-11 season when assessing possible effects of leaf loss due to locusts and cluster caterpillar. The data collected over the past 4 seasons (see also data in CSP165C where removal from each third of the canopy was also completed as well a removal of leaves from the top 6 or 9 nodes) allowed confident explanation to consultants of risks from such damage.

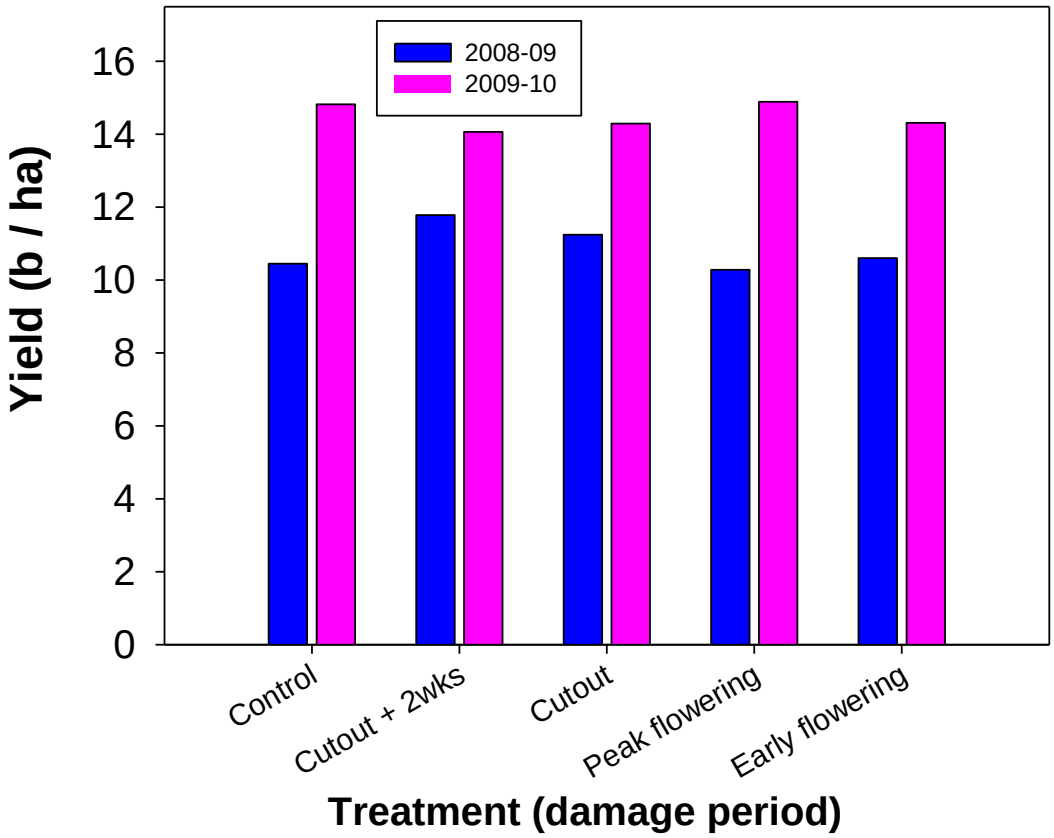

Figure 14. Difference between years in response of cotton yield to simulated late season leaf damage.

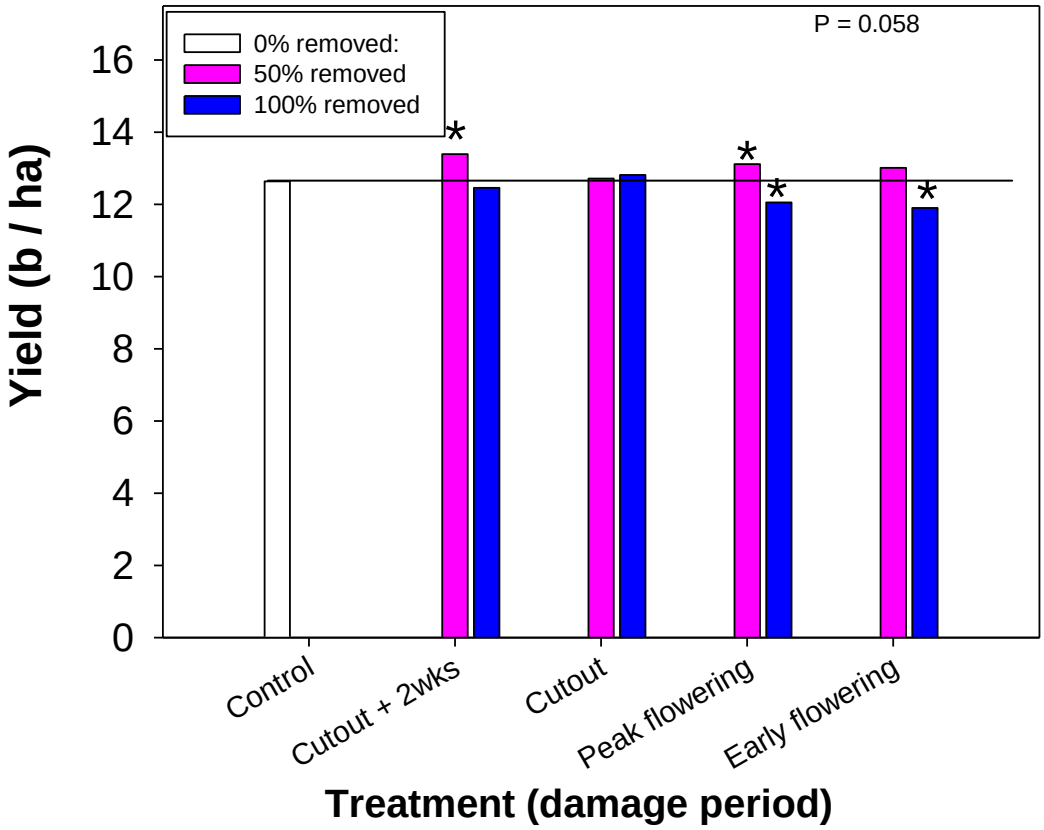

Figure 15. Earlier imposition of damages reduces yield more when0, 550 or 100% of leaf tissue was removed from the top 6 nodes. Asterisks indicate treatments significantly different from the control for each year separately – note that for 50% damage this may mean yield greater than the contro..

Effect of flower removal

As an additional component in this experiment we also included some extra treatments in spare rows of some plots in 2008-09 and 2009-10 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?.

We also included these treatments in earlier versions of these experiments (see Final Report for CSP165C). In those studies flower removal 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. Those studies showed that removal of all flowers for 1 week did not affect yield ($p = 0.12$) and crops were able to compensate for flower loss, whether they had leaf damage or not. Crop maturity was not affected by flower removal.

We also included these treatments in the experiments for this project - considering specifically the interaction between flower removal and percentage of leaf area lost. Flower removal was applied at the second (end of flowering) and third damage timings (cutout). In each case 3 days worth of flowers were removed from both controls, and the 50% and 100% leaf removal treatments on two occasions about 3

days apart. On each date 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 by having two dates we simulated complete flower abortion for a week.

The yield results for both experiments showed that removal of flowers had no effect on yield whether this occurred at peak flowering or cutout. Flower removal overall did result in slightly earlier maturity of cotton in 2009-10, but there was no interaction with time of damage or % of leaf area damaged.

Combining all of the flower removal experiments it appears that short burst of complete flower loss are unlikely to affect yield. Given that the damage inflicted was significantly higher than that likely from thrips it would seem very unlikely that thrips populations would cause sufficient damage to flowers to reduce yield.

Table7. Effect of flower removal near the end of the flowering period or at cutout on yield of cotton with or without leaves removed from the top 6 or 9 nodes ACRI, 2008/09 and 2009/10

			Flowers removed					
			Yes	No	Yes	No	Yes	No
Year	Time of damage	% leaf area removed	Mean number of flowers removed/m		Yield b/ha		Maturity (days after sowing to 60% bolls open	
2008/09	Peak Flowering	50%	15.0	0	10.1	9.7	157.0	157.1
		100%	9.0	0	7.4	9.3	151.4	150.5
	Cutout	50%	2.0	0	9.8	9.2	159.6	156.6
		100%	0.8	0	9.4	9.9	156.3	153.2
2009/10	Peak Flowering	0	2.7	0	13.2	11.6	180.8	188.0
		50%	1.5	0	13.1	11.8	181.5	192.4
		100%	1.5	0	11.7	10.7	179.6	189.3
	Cutout	0	13.8	0	13.0	11.6	179.0	188.0
		50%	14.4	0	12.3	11.7	177.1	188.6
		100%	16.2	0	10.5	10.6	176.7	183.1

Effect of 'burn spray' on yield

We developed a damage spray, based on spray-rig applied glacial acetic acid, which causes necrotic burns on the leaf that were aimed to simulate the effects of thrips feeding as a loss of photosynthetic activity of leaves. This was designed to mimic the effect of late thrips damage given that we were unable to do this with actual thrips populations. This method was developed to overcome a potential 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 16). In a previous project 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 16. 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. These dates are roughly analogous to the last three dates in the leaf removal experiments described above. We used a progression where on the first date we had the combination of sprays x dates (see Table 6).

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 damage caused by the burn sprays was assessed by ranking 4 leaves from node 1,3,6,9 and 12 below the terminal from each plot. The ranking was 0, 1 = 1-5%, 2=6-10%, 3 = 11-25%, 4 = 26 – 50%, 5 = 51 – 75%, 6 = 75 – 100%. An example of the distribution of damage over progressive spray applications is shown in Figure 17, which shows the damage in the control (no spray) and the Treatment that was sprayed on all three dates. The scores were converted back to average % damage. This shows clearly the high level of damage that resulted and also the distribution well into the canopy. The small amounts of damage on the controls were due to thrips, jassids or disease.

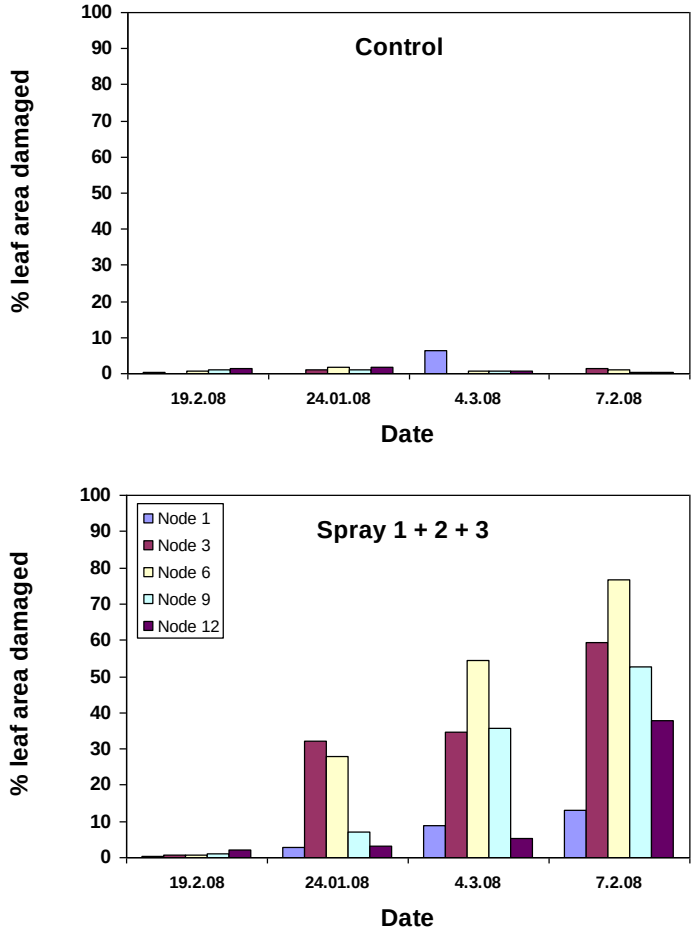

Figure 17. An example of the distribution of damage from the burn spray. Values are the % of leaf area damaged for nodes 1, 3, 6, 9 and 12, counting down from the top of the plant. The 'Control' was not sprayed, the 'Spray 1 + 2 + 3' was sprayed three times – at 2 weeks before cut-out, at cutout and at 2 weeks after cutout.

To simplify presentation we used only the scores for nodes 3, 6 and 9, which were generally fairly similar. These were averaged and converted from scores back to average % damage. The data are shown in Figure 18. Where only the first spray was applied damage increased to a peak of about 30-50%, where the first and second were applied a peak of about 60-70% and the first , second and third a peak of about 70 – 80%. However, in 2008-09 a problem with the spray rig resulted in a more concentrated solution being sprayed with a resulting very high damage very quickly in all of the first spray treatments.

Damage in treatments that received only the second spray began about 2 weeks later and peaked at only about 20-30% leaf area, those including spray two and three peaked at about 40-50% while those only receiving the third spray peaked later at about between 5-40%. However it should be noted that as this damage occurred to a large proportion of the canopy (eg see Figure 17) – even in some cases down to nodes 9 and 12 the impact on the crop was visually quite striking and this can be seen in the background leaves in Figure 16.

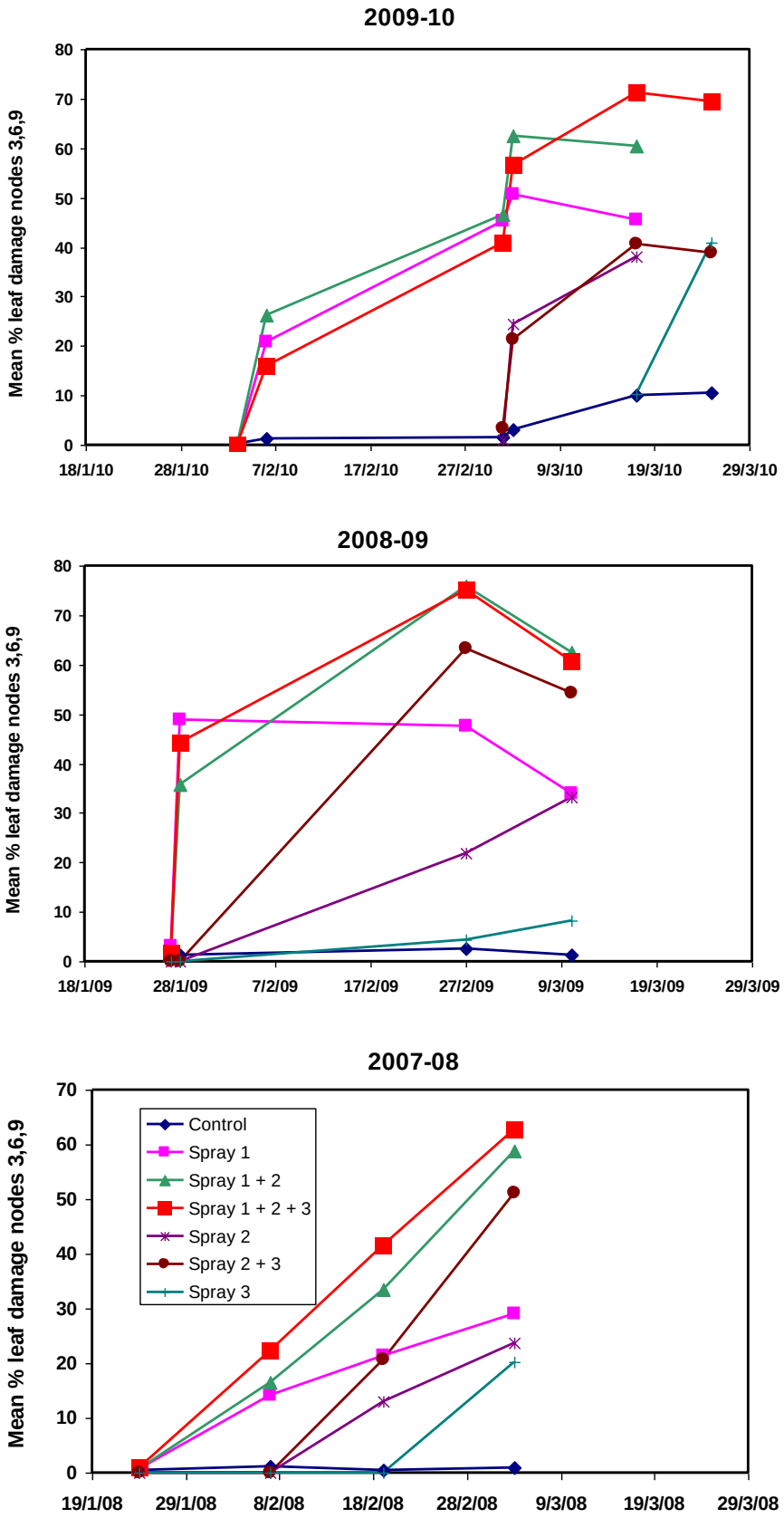

Figure 18. Proportion of leaf area damaged for the different burn spray treatments in experiments in 2007-08, 2008-09 and 2009-10. Data are the mean damage for leaves at nodes 3, 6 and 9 counting down from the terminal.

We were also interested if damage to the leaves reduced expansion and resulted in reduced leaf area, as this is what often happens with thrips damage (but less so with jassid damage). Data were only collected for the 2007-08 and 2009-10 experiments (Figure 19). The leaf area of the damaged leaves scored for damage was assessed using the Licor leaf area (planimeter) measurer. This shows that damage, especially any of the treatments that involved the first damage, which occurred at a time when there would be considerable subsequent growth of the upper leaves as well as growth of several new leaves, had a clear affect growth and expansion of leaves and reduced leaf size. The later applied treatments, when leaf expansion and production of new leaves had practically ceased had low or no effects on leaf area. This is more similar to the late development of jassid damage, which begins low in the canopy and moves upwards, hence at cut-out the reduced plant growth means the damage from the jassids catches up and the upper leaves become damaged.

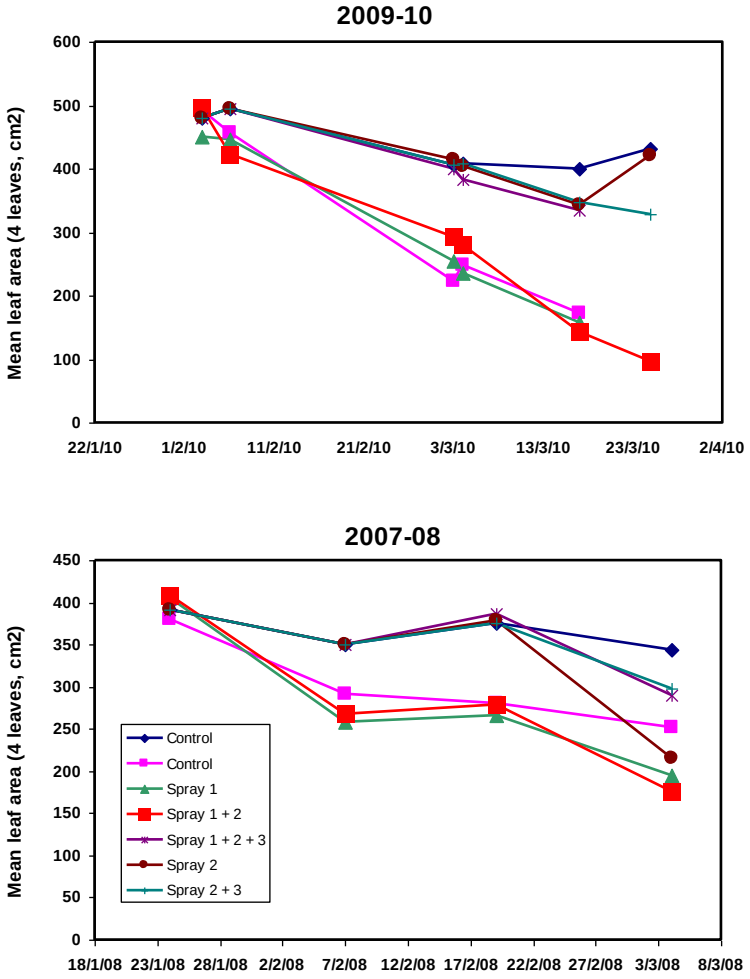

Figure 19. Measured leaf area (average of 4 leaves) for nodes 3, 6 and 9 for different burn spray treatments in experiments in 2007-08 and 2009-10.

Yield data for all three experiments are shown in Table 8. The results show that leaf damage applied two weeks before cut-out generally reduced yield – which is similar to the late flowering treatment in the leaf damage experiments. Sprays applied at cut-out reduced yield significantly only in the 2009 experiment. Sprays applied at two weeks after cut-out did not affect yield. These results confirm those of the leaf removal experiments that cotton can withstand some leaf damage from mid-late

season without loss of yields. Damage from cutout onwards and later is unlikely to affect yield unless it is high – probably > 50% leaf loss in the upper canopy (top 6-9 nodes). However, damage in the boll fill period before cutout may reduce yield. Further analysis is required to refine a threshold of damage and this is a milestone in CRC Project 1.01.30. Conservatively a leaf area loss threshold of about 30% could be used as a starting point based on the single spray 2 weeks before cutout treatment in 2007-08.

Table 8. Effect of single and repeated 'leaf burn' sprays at different stages of growth on yield (b/ha) of cotton, ACRI, 2007/08. Within each year, asterisks indicate treatment significantly different to Control (ANOVA, LSD 0.05).

		Sprays applied			
Year	Date of first spray	0	1	2	3
2008	Control	12.9			
	2 weeks before cutout		10.1*	9.2*	9.3*
	Cutout		12.0	12.0	
	2 weeks after cutout		12.3		
2009	Control	10.4			
	2 weeks before cutout		6.5*	6.1*	5.7*
	Cutout		8.9*	9.1*	
	2 weeks after cutout		10.1		
2010	Control	9.0			
	2 weeks before cutout		7.4	7.0*	7.1*
	Cutout		8.3	9.6	
	2 weeks after cutout		10.4		

6. CBT spread and latent period.

This research focuses on two primary questions (i) how does CBT spread in field and (2) how is this influenced by the intensity of colonisation (e.g. how many CBT carrying aphids settle on a plant).

Question 1. How does CBT spread in cotton fields. Previous data suggests that the spread of the disease is slower if it is introduced to the field by migrant aphids and faster if ratoon plants affected with CBT and aphids are present within the field. However, this conclusion was reached with data from two separate experiments. In these experiments either the central plant of a plot of cotton (24m x 24r) was infested with about 200 aphids that had been reared on a CBT affected cotton plant, or a potted plant with CBT symptoms that was also infested with aphids was placed into the centre of the plot. The spread of the aphids or the disease was monitored each week by scoring plants progressively out from the centre plant and assessing the furthest point that aphids or disease could be found. In the first experiment (2005-06) we infested the central plant with CBT carrying aphids, in the second year we placed an infested CBT affected plant in the centre of plots. The spread of aphids and disease was faster in the second year, implying that ratoon plants in field are a high risk, more so than aphids colonising from outside of fields. We decided to test this by comparing both types of CBT infestation in the same experiment and this was done in 2007-08, 2008-09 and 2009-10. In the 2009-10 experiment we used cages to try to reduce the effect of natural enemies on the development of aphid populations. The cages were 3m x 3 rows, so this restricted assessment of spread along the row to a maximum of 1.5m from the central plant.

The data for 2008-09 show that aphids spread away from the site of inoculation or transplant, slowly at first then quite rapidly after about 4 weeks. There was no obvious difference between the rate of spread for the transplant vs inoculation aphids. The rate of spread of CBT across rows was similar to the aphids but slightly lagged as would be expected given there is a delay between a plant being infected with CBT and it showing symptoms. The spread of CBT from the transplant colonies was greater than from the inoculation colonies. Looking at spread along rows the rate of spread was again fairly similar between inoculation and transplant colonies. The rate of spread of CBT was initially a little faster on the transplant colonies but by the end of the experiment there was no difference.

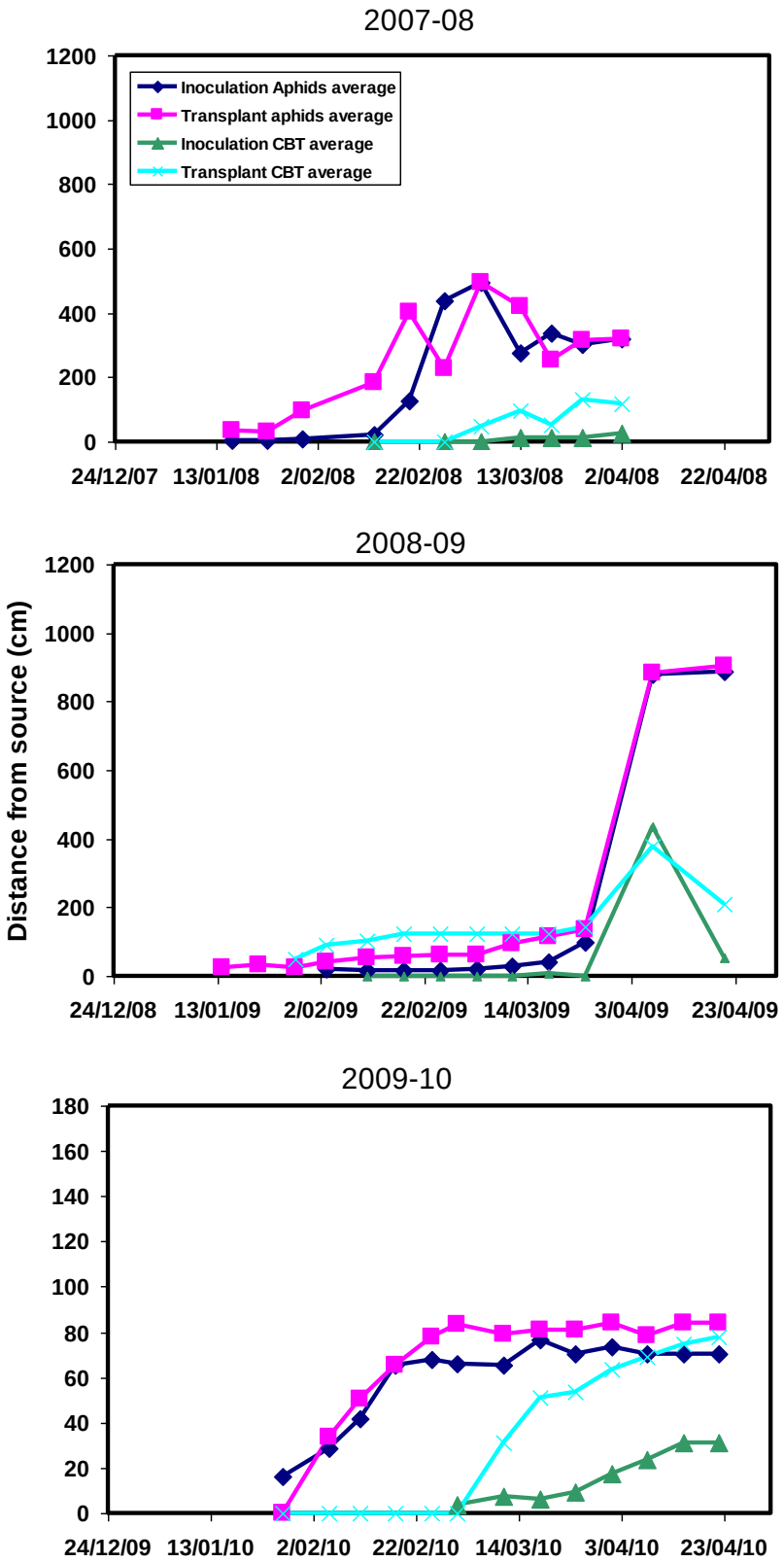

Figure 20 Average distance aphids moved away from the central release site when released by placing then +CBT aphids on the central plant (inoculation) or by transplanting a +CBT plant with aphids on it into the centre of the plot. The spread of CBT away from the central plant or transplant plant. Distance is cm along the central row of the plot.

Question 2. When an aphid carrying CBT settles and feed on a cotton plant the virus transmitted to the plant must first successfully survive the plants natural defences and secondly begin to replicate within the plant. It is these replicated virus particles that will be taken up by the offspring of the founding aphid(s) and mean that they will then be capable of transmitting disease to new plants. There is usually a lag between the infection of the plant with the disease and the time at which there are sufficient replicated virus particles and this is know as the latent period (see Figure 21). We would predict that a plant that is infested with more aphids carrying CBT will replicate virus in greater amounts faster and hence the latent period would be shorter. A shorter latent period means it is more likely that the offspring of the founders will be carrying the disease when they move to adjacent plants and this should increase the rate of spread of the disease. Hence it is important to understand.

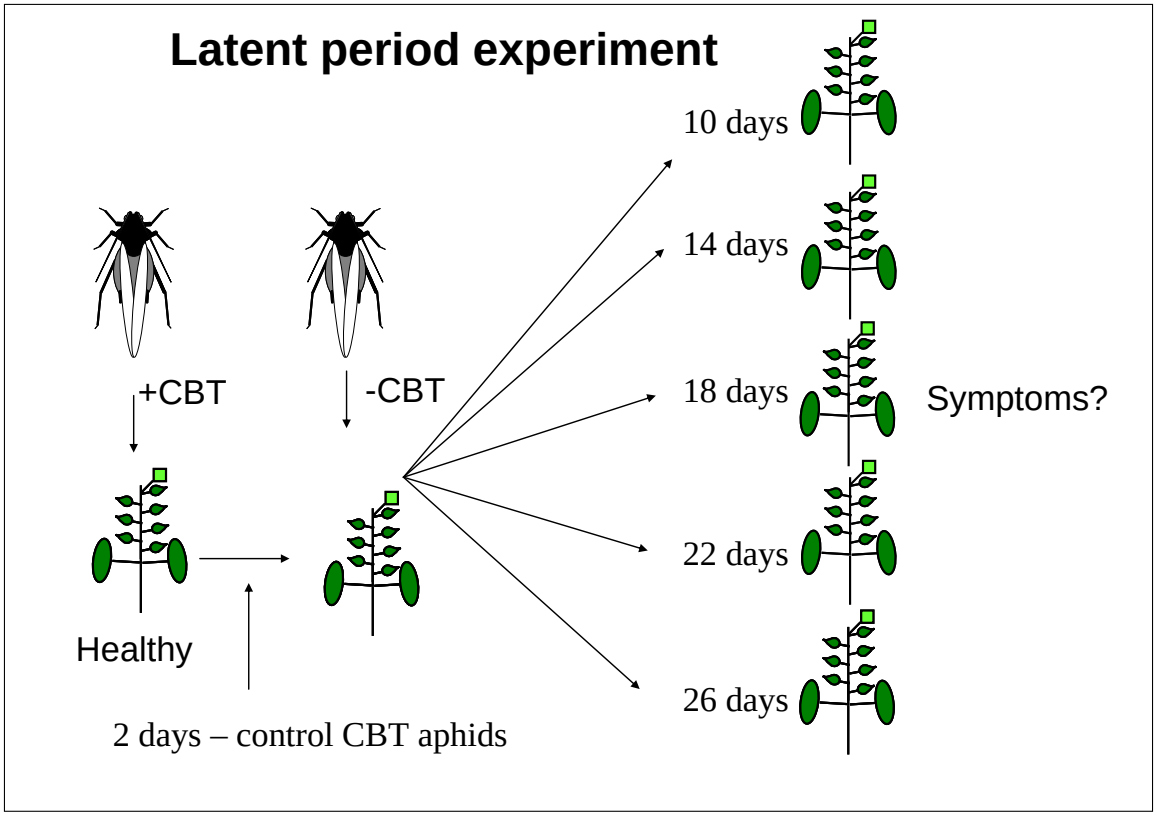

Figure 21. Conceptual design of latent period experiments. By varying the number of +CBT aphids placed on the healthy plants (source plants) we can investigate the relationship between the number of aphids colonising a plant and the duration of the latent period.

We investigated the latent period is a series of experiments – some of which were done before this project and some within the project. Essentially we maintain a culture of cotton aphid on CBT affected plants of a susceptible variety and a separate 'clean' culture on resistant plants. We sow a number of pots with a CBT susceptible cotton variety (Sicot 71BRF) these plants will become sources plants (see below). We also sow some pots with a susceptible variety - these are kept separate and are the recipient plants. The source plants are divided into treatments – which are different densities of CBT infected aphids placed on them from the colony – some treatments receive few, some more – depending on the particular experiment. These plants we call the source

plants. After two days we remove all of the aphids off the source plants and check them several times until we are sure they are completely clean. We then infest them with 'clean' aphids. At regular intervals we move a portion of these aphids off onto recipient plants – which we then monitor for the presence of CBT. The shortest interval that results in recipient plants with CBT is the 'latent period'. These are complex and long experiments to run, and occasionally will completely fail as none of the source plants will develop disease – and hence the latent period can't be defined.

The results show that in general, as the number of infesting aphids decreases the latent period increases (Table 9, Figure 22). We found a lot of variability in the experiments – sometimes only one of the 5 source plants for a given density became infested, especially at the lower aphid densities, and this confirms earlier studies that the transmission success is reduced at low aphid densities – discussed below. Nevertheless the fitted curve suggests that if single aphids infest plants the latent period is likely to be in the vicinity to 3 to 3.5 weeks (Figure 22).

Table 9. Estimated latent period and transmission success when various +CBT aphid densities were used to infect cotton plants.

Experiment	Aphid density transferred (aphids/plant)	Latent period (days)	Transmission success into source plants (%)
2006-07	150	10	-
2007-08	20	7	60
2008-09 a	20	11	80
2008-09 a	100	9	80
2008-09 b	5	25	20
2008-09 b	20	11	40
2008-09 b	100	9	100
2008-09 c	5	20	40
2008-09 c	20	9	20
2008-09 c	100	15	100

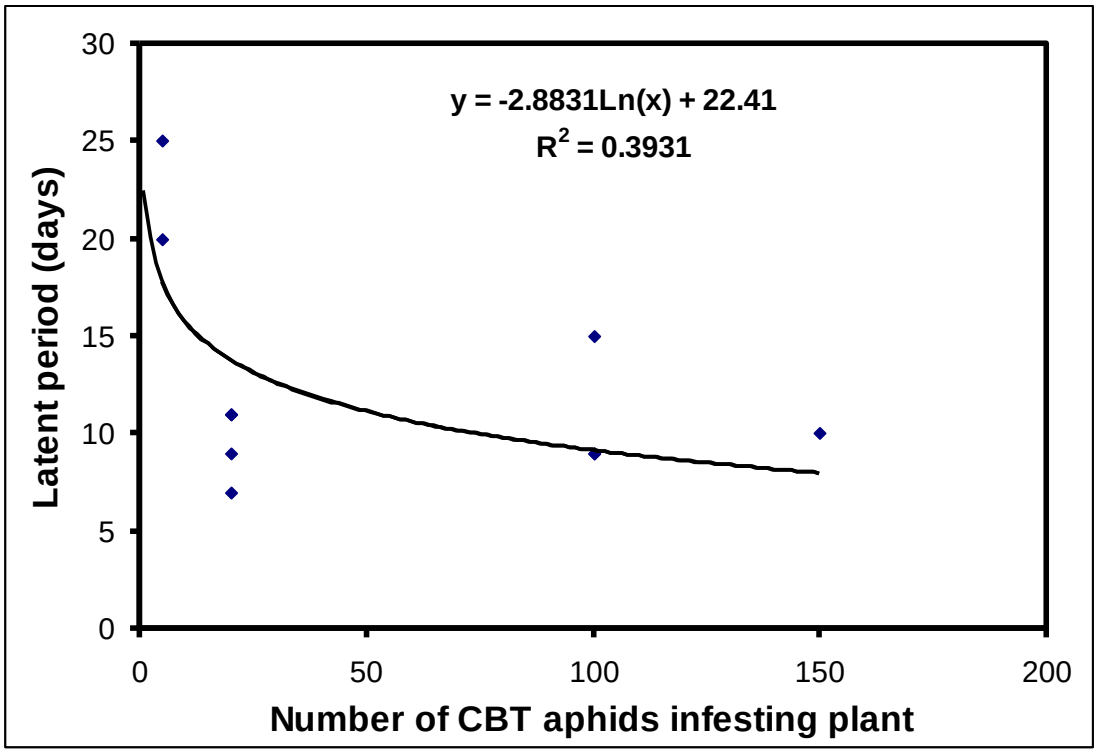

Figure 22. Relationship between infestation rate with CBT aphids and latent period.

As part of these experiments we also gained more information on the transmission rate for different densities of aphids (Table 9). This is because we infested plants with either 5, 20 or 100 aphids carrying CBT and we assessed the success rate of transmission of the disease. Combining these data with those from previous experiments shows a logarithmic response, whereby as the aphid density declines the transmission rate also declines (Figure 23).

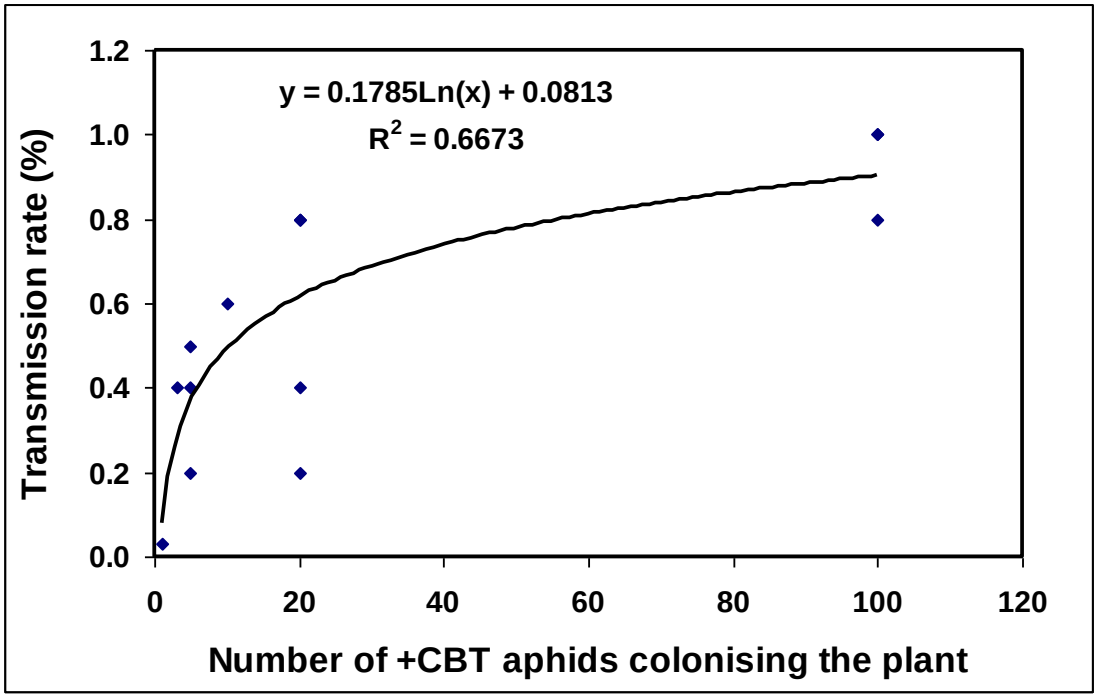

Figure 23. Relationship between number of +CBT aphids infesting a cotton plant and success of transmission of the disease.

7. Emerging pests – Pale cotton stainer and Silver leaf whitefly

a. Pale cotton stainer (*Dysdercus sidae*)(See Appendix 2)

In 2007-08 the pale cotton stainer (PCS, Figure 24) emerged as a significant pest, for the first time in the modern cotton industry in Eastern Australia. Since then it has been a sporadic pest for a small proportion of growers. Several questions that we considered included:

i. Are males, females and mated pair all equally damaging?
ii. Do PCS attack young bolls.
iii. What effect does their feeding have on boll yield, seed weight and seed viability

We collaborated with Dr Moazzem Khan, QDEEDI, in experiments to answer these questions and understand the damage caused by pale cotton stainer, *Dysdercus sidae* (PCS) to Bollgard® II cotton. We established a PCS culture and assisted Moazzem to establish one as well – this allowed us an assured supply of bugs for experiments. Three boll age groups, 5, 10/15 and 30/35 day old bolls were exposed to male and female PCS adults separately for 7 days in three experiments. In the first experiment damage symptoms were assessed destructively immediately after termination but in the other experiments damage symptoms were assessed after harvest. The damage caused by PCS to younger bolls was similar to damage caused by mirid and green vegetable bug (GVB) characterised by a black spot on the boll wall, warty growth inside the boll wall, brown coloured lint and tight lock (in severe cases). On older bolls damage symptoms were much less visible and would require bolls to be cut open and the insides walls examined for the presence of small black feeding marks (see Appendix 2). However, unlike mirid and GVB, PCS caused damage to seed which affected seed germination. For 5 and 15 day old bolls, 32 and 38 per cent of seeds failed to germinate. Younger bolls incurred significantly more damage ($P < 0.05$) than older bolls. Female PCS caused significantly more damage ($P < 0.05$) than the males. Female feeding on younger bolls contributed up to 50% yield loss. We have investigated the effects of cotton stainer on bolls of different ages, compared males, females and mated pairs and looked at outcomes for boll weights seed weight and seed viability.

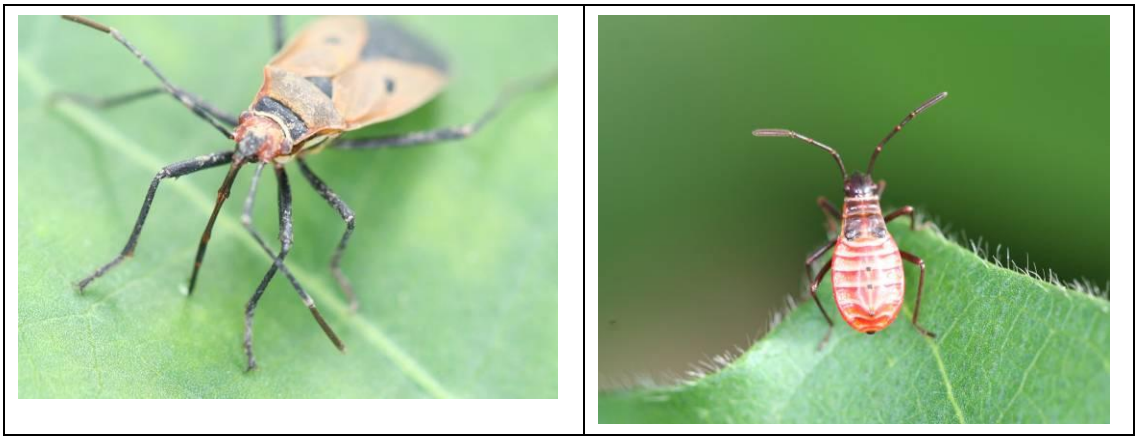

Figure 24. Adult and nymph of pale cotton stainer.

b. SLW sampling and new experiments to report

In the second year of this project Silver leaf whitefly emerged as a pest in the Namoi and Gwydir regions. It was clear that local consultants and agronomists in these areas needed support to understand whitefly biology, sampling, thresholds and management strategies. Given the lack of extension staff in these regions the project supervisor (Wilson) contacted relevant industry personnel, such as Tracey Leven at CRDC and Yvette Cunningham at ACRI and organised a Whitefly workshop at ACRI and a partner workshop at Moree (run by Tim Burley, NSW I&I). Wilson co-ordinated Dr Dave Murray and Ms Zara Ludgate to come to both workshops and provide first hand knowledge. This was well attended and helped consultants to ensure effective control and avoid problems with contaminated lint. In collaboration with Richard Sequiera, Wilson also developed some slides to provide an indication of the appropriate response for cotton with different levels of SLW and honeydew at different crop stages, as well as advice on management of cotton known to be contaminated to reduce the level of contamination. A combination of effective management and timely rainfall ensured that there were no contaminated bales reported.

In the early autumn of 2009 Robin Gunning reported the presence of the Q-Biotype of *Bemisia tabaci* in Australian cotton. Given that this Biotype is resistant to pyriproxifen, which is the cornerstone of SLW management, it could pose a significant challenge to growers and consultants and threat to Australia's reputation of clean cotton. One of these collections reported as Q-biotype was from the Namoi Valley. We sought to confirm these with additional collections which could be sent to Zara Hall at QDEEDI for confirmation by PCR. We sampled cotton and weeds from this site (Tulladunna) as well as from a range of other cotton crops and potential weed hosts throughout the lower Namoi Valley. The QDEEDI team processed these samples, 49 collections in total and found no Q-biotype. The sampling did confirm that B-Biotype totally dominated whitefly population at this stage, with virtually no *B. tabaci* Eastern Australian Native and no greenhouse whitefly (*Trialeurodes vaporariorum*). Most significantly no Q-Biotype specimens were found. Interestingly we confirmed that sowthistle, marshmallow, turnip weed, noogoora burr and paddy melon in sheltered sites were potential hosts through winter (though we would expect noogoora burr and paddy melon to die off in winter). We also found SLW readily on volunteer cotton, providing another reason for improved management of volunteer and ratoon cotton.

Table 10. Percentage of silver leaf whitefly in collections of whitefly from cotton and other hosts in the Namoi Valley, 2009. Note also that collections were tested by QDEEDI using PCR for the presence of Q-Biotype and this was not found in any samples.

Date Collected	Region	Locality	Crop	Site	SLW abundance	No. SLW (PCR)	No. Q biotype	No. Native Bemisia (PCR)	No. Adult Greenhouse (PCR)	Unknown/other	% SLW
28/01/2009	Namoi	ACRI, Narrabri	Soybeans	C1		27	0	1	1	1	90.0
9/02/2009	Namoi	Pindara East	Cotton	Near Airstrip		29	0	0	0	1	96.7
10/02/2009	Namoi	ACRI	Cotton	River 1		27	0	1	1	1	90.0
10/02/2009	Namoi	Carsons	Cotton	Field		29	0	0	0	1	96.7
10/02/2009	Namoi	Tulludunna	Cotton	Field		17	0	0	0	3	85.0
12/02/2009	Namoi	ACRI	Cotton	Simone							
28/04/2009	Namoi	ACRI	Cotton	River 1	< 3/leaf	29	0	0	0	2	93.5
28/04/2009	Namoi	Appletrees	Turnip weed	Near entrance	1-3/leaf	31	0	0	0	0	100.0
28/04/2009	Namoi	Appletrees	Turnip weed	next to cotton	< 1/leaf	32	0	0	0	0	100.0
28/04/2009	Namoi	Appletrees	Cotton	volunteer		3	0	0	0	0	100.0
28/04/2009	Namoi	Auscott Narrabri	Cotton	Field 13							
28/04/2009	Namoi	Togo	Cotton	near airstrip	5-10/leaf	32	0	0	0	0	100.0
29/04/2009	Namoi	Cumberdeen	Cotton	Volunteer	< 1/leaf	14	0	0	0	7	66.7
29/04/2009	Namoi	Glence	Sowthistle	near field	1-3/leaf	30	0	0	0	0	100.0
29/04/2009	Namoi	Glencoe	Cotton	near field		20	0	0	0	0	100.0

29/04/2009	Namoi	Glencoe	Soybeans	Near river on Wee Waa rd	3-5 /leaf	27	0	0	1	4	84.4
29/04/2009	Namoi	The Bray	Cotton	Field	< 1/leaf	23	0	0	0	6	79.3
29/04/2009	Namoi	Willawah	Cotton	Field	< 1/leaf	30	0	0	0	2	93.8
29/04/2009	Namoi	Willawah	Noogoora Burr	Edge of field	< 1/leaf	19	0	0	8	5	59.4
5/05/2009	Namoi	Belah	Sowthistle	channel	5-10/leaf	27	0	0	1	4	84.4
5/05/2009	Namoi	Le Grand	Cotton	skip row cotton	< 1/leaf	30	0	0	0	2	93.8
5/05/2009	Namoi	Le Grand	Turnip weed	channel	3-5/leaf	30	0	0	0	2	93.8
5/05/2009	Namoi	Le Grand	Marshmallow weed	channel	< 1/leaf	24	0	0	0	7	77.4
5/05/2009	Namoi	Le Grand	Sowthistle	channel	<1/leaf	28	0	0	0	3	90.3
5/05/2009	Namoi	Le Grand	C. pumilio, small crumweed	channel		23	0	0	0	7	76.7
5/05/2009	Namoi	Tulludunna	Cotton	voluntee r	< 1/leaf	21	0	0	1	5	77.8
17/06/2009	Namoi	Belah	Sonchus oleraceus & Malva parviflora	Tankstand	< 1/leaf	10	0	0	3	0	76.9
21/07/2009	Namoi	ACRI	Sowthistle	Bus Park	< 1/leaf	0	0	0	31	0	0.0
4/08/2009	Namoi	Belah	Sowthistle	C1		29	0	0	1	2	90.6
4/08/2009	Namoi	Le Grand	Sowthistle	Edge of field		1	0	0	0	0	100.0
4/08/2009	Namoi	Le Grand	Marshmallow weed	Edge of field		2	0	0	1	0	66.7
23/11/2009	Namoi	Belah	Paddy melon	Edge of field		3	0	0	0	0	100.0

17/12/2009	Namoi	ACRI	Cotton	glassho use		32	0	0	0	0	100.0
14/01/2010	Namoi	ACRI	Rock melon	Block 17		32	0	0	0	0	100.0
14/01/2010	Namoi	Narrabri	Apple	town		19	0	0	0	0	100.0
14/01/2010	Namoi	Togo	Cotton	AK, 0814		32	0	0	0	0	100.0
3/02/2010	Gwydir	Caithness	Cotton	crop		32	0	0	0	0	100.0
26/02/2010	Namoi	ACRI	Cotton	Crop		32	0	0	0	0	100.0
26/02/2010	Namoi	ACRI	Cotton	Crop		32	0	0	0	0	100.0
26/02/2010	Namoi	Belah	Sowthistle	Edge of field		32	0	0	0	0	100.0
26/02/2010	Namoi	Belah	Cotton	Crpop		32	0	0	0	0	100.0
26/02/2010	Namoi	Bronon 1	Apple	town		32	0	0	0	0	100.0
26/02/2010	Namoi	Narrabri	Apple	town		32	0	0	0	0	100.0
26/02/2010	Namoi	Narrabri	Apple	town		32	0	0	0	0	100.0
26/02/2010	Namoi	Narrabri	Apple	town		32	0	0	0	0	100.0
26/02/2010	Namoi	Togo	Cotton	Field 74		32	0	0	0	0	100.0
26/02/2010	Namoi	Togo	Cotton	crop		32	0	0	0	0	100.0
29/03/2010	Namoi	ACRI	Cotton	Crop		32	0	0	0	0	100.0
29/03/2010	Namoi	Togo	Cotton	F53-2		32	0	0	0	0	100.0

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. Non-cultivated hosts of GVB adults and nymphs were identified.
    b. Parasitism rates of GVB both in cultivated and non-cultivated hosts identified
    c. Crop host sequence showed that GVB adults and nymphs preferentially colonise legumes compared with cotton
    d. Overwinter habitats of diapausing GVB identified and parasitism rates of these bugs quantified.
    e. Efficacy and IPM fit of biopesticides, dimethoate at reduced rates with or without salt and two new insecticides was evaluated. Outcomes reported to agrichemical companies and I&I NSW.
    f. 'Impact of insecticides and miticides on beneficials' table in the Cotton Pest Management Guide was updated to include clothianidin (Shield), Spirotetramat (Movento), and low rate dimethoate.
    g. Efficacy of biopesticides BC 667 and BC669 against aphids investigated and showed moderate efficacy.
    h. The effect of late season damage on cotton productivity was investigated in leaf removal and 'burn spray' experiments allowing tentative guidelines for damage thresholds to be suggested.
    i. The effect of flower loss in addition to leaf damage was investigated and showed no additive effect
    j. The spread of aphids and CBT disease from infestations originating from influxes or from ratoon plants was compared and showed a higher risk from ratoons (transplants).
    k. The relationship between latent period and transmission efficiency and the density of colonising +CBT aphids was quantified.
    l. The damage potential of pale cotton stainer was further quantified.
    m. Information of management of sliver leaf whitefly was rapidly disseminated to industry during the first wide scale outbreaks in 2008-09.
    n. We confirmed that Q-Biotype *Bemisia tabaci* was not present in NSW cotton regions.
    o. We showed that silver leaf whitefly uses a range of non-cultivated hosts through winter, including cotton and common broadleaf weeds.
    p. The project leader, Lewis Wilson, also contributed as a committee member to the TIMS Committee, the TIMS Insecticide and TIMS BT-Cotton Technical Panels, and the Australian Cotton Industry Bio-security Committee.

3. Please describe any other information developed from research (eg discoveries in methodology, equipment design, etc):

    1. We successfully modified a rearing technique for pale cotton stainer that is suitable for production of large numbers for experiments
    2. We successfully developed a culture system for green vegetable bug that allowed us to obtain egg rafts to investigate egg parasitism rates.
    3. Mite Yield Loss Tool http://cottassist.cottoncrc.org.au/Mites/Default.aspx

No changes required to the Intellectual Property register.

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. Green vegetable bug uses a range of broad leaf weeds as key hosts on cotton farms and in refuge areas. Management of these weeds will help reduce incidence of this pest.

    2. Crop sequence experiments show that green vegetable bug prefer to feed and oviposit in legume crops such as mungbean, pigeon pea and soybean, Management of these crops on farms, especially unsprayed pigeon pea refuges, could also influence risks of problems with this pest in cotton. There is the opportunity to evaluate use of one or more of these legumes as a trap crop.

    3. Parasitism rates by the egg and nymphal/adult parasites is generally low. This would be a worthwhile area for further study, such as a PhD project to evaluate if parasitism rates could be improved to the extent that the pest does not reach damaging levels – perhaps by provision of nectar sources for the parasites

    4. Information summarising effects of the new registered compounds (e.g. Shield) and the lower rates of dimethoate has been incorporated into the 'Impact of insecticides and miticides on predators in cotton' table was provided to Yvette Cunningham (Cotton CRC) and Susan Maas (DEEDI Qld) for inclusion in the Cotton Pest Management Guide 2010-11 (see Table 19 in that book)

    5. Cotton crops are reasonable tolerant of reduction in leaf area from the top 6 nodes especially later in the season. Damage from cutout and later is unlikely to affect yield unless it is high – probably > 50% leaf loss in the upper canopy (top 6-9 nodes). However, damage in the boll fill period before cutout may reduce yield. These results have been of particular relevance in 2010-11 when assessing possible effects of leaf loss due to locusts and cluster caterpillar. Further analysis is required to refine a threshold of damage and this is a milestone in CRC Project 1.01.30. Conservatively a leaf area loss threshold of about 30% could be used as a starting point based on the single spray 2 weeks before cut0out treatment in 2007-08.

    6. Overall the fungal biopesticides BC639 and BC667 tended to reduce abundance of aphids compared with the control by about 10-50% but the results were erratic

compared with the commercial standard and did not provide the fast acting strong knockdown of a commercial aphicide. However, our data also show BC639 is far more selective than acetamiprid – hence the conservation of beneficial or 'bio-residual' of the biopesticide may be greater and further contribute to longer term suppression of aphids and other pests.

7 Combining all of the flower removal experiments showed that a short burst of complete flower loss is unlikely to affect yield. Given that the damage inflicted was significantly higher than that likely from thrips it would seem very unlikely that thrips populations would cause sufficient damage to flowers to reduce yield.

8. The spread of CBT from the transplant colonies (= ratoons plants) was greater than from the inoculation colonies (= influxes from host outside the field). The rate of spread of aphids was fairly similar between inoculation and transplant colonies. The rate of spread of CBT was initially a little faster on the transplant colonies but by the end of the experiment there was no difference. This highlights a potential risk with having sources of CBT – especially volunteer or ratoon plants on farms.

9.As the number of infesting aphids decreases the latent period increases If single aphids infest plants the latent period is likely to be in the vicinity to 3 to 3.5 weeks but if heavy infestations occur could be a little as 9 days. In the latter case early management of aphids would be require to reduce the risk of secondary spread of disease.

10. The relationship between the density of +CBT aphids infesting a plant and transmission success showed a logarithmic curve, whereby as the aphid density declines the transmission rate also declines. Aphid densities of greater than 10-15 aphids per plant will result in transmission rates > 50%.

11. Pale cotton stainer females are more damaging than males or mating couples. Younger bolls incurred significantly more damage ($P < 0.05$) than older bolls. For 5 and 15 day old bolls, 32 and 38 per cent of seeds failed to germinate. Yield loss was significant, up to 50% loss by female feeding on younger bolls. Extrapolating this result to the field requires care as growth and weather conditions are major influences on yield in the field. Further, PCS tend to be more abundant later in the season, when there are few young bolls. In this situation we found that feeding on older bolls did not reduce boll weight, but did affect boll opening and harvestability, as well as reduce germination success.

12. We confirmed that *Bemisia tabaci* B-Biotype totally dominated whitefly populations during 2008-09, with virtually no *B. tabaci* Eastern Australian Natives and few greenhouse whitefly. Most significantly no *Bemisia tabaci* Q-Biotype were found. Sowthistle, marshmallow, turnip weed, noogoora burr and paddy melon in sheltered sites were potential hosts through winter (though we would expect noogoora burr and paddy melon to die off in winter). We also found SLW readily on volunteer cotton, providing another reason for improved management of volunteer and ratoon cotton.

Extension Opportunities

5. Detail a plan for the activities or other steps that may be taken:
   (a) Steps taken:
   (i) The efficacy and IPM fit of new chemistry has been published for industry.
   (ii) Whitefly meetings were organised in summer 2009 in response to the first reports of significant SLW populations in the Namoi and Gwydir regions. Extension publications and CottonTales contributed to at the time.
   (iii) Information on managing Silverleaf Whitefly has been published.
   (iv) Wilson has spoken at a range of CRDC/CRC extension meetings including 2 (Narrabri, Gwydir) for Silverleaf Whitefly in December 2009, and 4 (Namoi, Gwydir, McIntyre and Gunnedah) in November 2010.
   (v) Wilson spoke at the CCA AGM in 2008, 2009, 2010 on a range of IPM and pest issues.
   (vi) A number of issues have been extended via the CottonTales

   (b) Steps that need to be taken:
   (i) Information of green vegetable bug parasitism rates, crop preferences, host use and overwintering habits needs to be published for industry. The concept of using a legume trap crop is probably worth exploring, though even if effective unlikely to gain traction.
   (ii) Information on the effect of late season damage, though originally targeting jassids and thrips, also has relevance to other pests and need to be published for industry. A limited amount of extension has been done via presentations to CCA.
   (iii) Information on CBT has been presented to industry but during years of low incidence received little attention. There is a substantial information base now that should be published for industry and could fairly readily be developed into a strategy to reduce the risk from this disease.
   (iv) Information from this project and previous projects on the interaction between spray choices and the risk of secondary pests needs to be revisited. Though such information was first widely publicised almost 20 years ago the expansion of cotton production means many new consultants do not really understand these concepts well.

   (c) For future research.
   This is detailed in a new submission that has been funded by CRDC via the Cotton CRC. Aims for that project are:

   1. Researching SLW ecology (hosts, parasites, survival) in central regions
   2. Researching IPM compatible management strategies for SLW and mirids
   3. Investigating breakdown of honeydew on lint and effect on fibre quality.
   4. Finalising surveys of crop and non-crop hosts for GVB
   5. Finalising thresholds for late thrips and jassids from existing data
   6. Use new techniques to evaluate the presence of cotton bunchy top disease in alternative hosts – to understand its ecology and risks.
   7. Continue to investigate the efficacy and IPM fit of biopesticides (with Dr Mensah, I&I NSW), new chemistries, reduced rates with adjuvants and novel technologies.

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

Journal Papers

- Constable GA, Llewellyn D, **Wilson L** and W. Stiller (2011) An industry transformed: the impact of GM technology on Australian cotton production. Farm Policy Journal 8: 23-41.
- Herron, G. A. and **Wilson, L. J**. (2011) Neonicotinoid resistance in *Aphis gossypii* Glover (Aphididae: Hemiptera) from Australian cotton. Australian Journal of Entomology 50: 93-98.
- Reddall AA, Sadras VO, **Wilson LJ** and Gregg PC (2010) Contradictions in host plant resistance to pests: Spider mite (*Tetranychus urticae* Koch) behaviour undermines the potential resistance of smooth-leaved cotton (*Gossypium hirsutum* L.). Pest Management Science 2011: in press
- **Wilson LJ**, Lei TT, Sadras VO, Wilson LT and Heimoana SC (2009). Undamaged cotton plants yield more if their neighbour is damaged: implications for pest management. Bulletin of Entomological Research 99: 467-478
- Brier, H.B., Murray, D.A.H., **Wilson, L.J**., Nicholas, A.H., Miles, M.M., Grundy, P.R. and McLennan, A.J. (2008) An overview of integrated pest management (IPM) in north-eastern Australian grain farming systems: past, present and future prospects. Australian Journal of Experimental Agriculture 48: 1574-1593

Invited Reviews

- Peshin R, Bandrai RS, Zhang W, Wilson LJ and Dhawan A. (2009) Chapter 1. Integrated pest management: A global overview of history, programs and adoption. In: Integrated Pest management: Innovation-Development Process. (R Peshin and AK Dhawan, Eds). Springer Science and Business Media BV, pp 1-49.
- Fitt GP, Wilson LJ, Kelly D and Mensah R (2009) Chapter 17. Advances in integrated pest management as a component of sustainable agriculture: The case study of the Australian Cotton Industry. In: Integrated Pest management: Innovation-Development Process. (R Peshin and AK Dhawan, Eds). Springer Science and Business Media BV, pp 507-524

Conference Papers

- Herron, G, and **Wilson, L.** (2010) Aphids: Where to from here? Paper at ACGRA Conference, Broadbeach, August 2010
- Kahn, M. Heimoana, S. and **Wilson, L.J.** (2010) Understanding pale cotton stainer (PCS) damage to Bollgard® II cotton Paper at ACGRA Conference, Broadbeach, August 2010
- **Wilson, L.** and Smith, T. (2010) Green Vegetable Bug in cotton regions: gaining a better understanding. Poster at ACGRA Conference, Broadbeach, August 2010
- **Wilson, L.**, Heimoana, S. and S. Yeates (2010) Is late season thrips or jassid damage worth worrying about? Poster at ACGRA Conference, Broadbeach, August 2010
- Gregg, P. and **Wilson, L.J.** (2008) The changing climate for pest management. In, Proceedings of the 14th ACGRA Cotton Conference, Broadbeach, Qld, August 2008.
- Herron, G.A., McLoon, M.O., & **Wilson, L.J.** (2008) Resistance testing summary for the 2006-2007 and 2007-2008 cotton seasons: cotton aphid Aphis gossypii and two-spotted mite Tetranychus urticae. In, Proceedings of the 14th ACGRA Cotton Conference, Broadbeach, Qld, August 2008.

- **LJ Wilson** (2008) IPM in Bt-cotton: Australia as a case study. International Congress of Entomology, Durban South Africa, July 2008-09-27 (abstract only)

Industry Extension material

- **Wilson, L**., Sequiera, R., Murray, D. and Ludgate, Z. (2010) Whitefly- What happened in 2009-10. CSD Trial Results Booklet. PP 84.
- Zara Ludgate, Hugh Brier, and **Lewis Wilson** (2010) Whitefly – the IPM enforcer – 'How to avoid $100/ha sprays in cotton. GRDC Northern Update Meeting, Narrabri, September 2010
- **Wilson, L.**, Mensah, R., Charleston, K. and Khan, M. (2011) Strategies to manage sucking pests in cotton in a wet season. Cotton CRC Pest Profile, On-Farm series.
- Maas, S., Ludgate, Z, **Wilson, L**., De Barro, P., Sequiera, R., Murray, D. and P. Grundy (2009) Silverleaf whitefly – the IPM enforcer. Australian Cottongrower 30(4): 14-17
- **Wilson, L.**, Heimoana, S., Farrell, T. and Smith, T. (2008) Setting thresholds for aphids in cotton. Australian Cottongrower 29(6): 12-16.
- Leven, T., Mensah, R, Sequiera, R.,**Wilson, L**. and Dillon, M. (2010) Key insect and mite pests of Australian cotton. Cotton Pest Management Guide for 2010-11, pp 4-42. (ed. S. Maas). Australian Cotton CRC Publication.
- **Wilson, L.**, Deutscher, S., Mensah, R., . and Johnson, A (2010) Integrated Pest Management (IPM) guidelines for Australian cotton. Cotton Pest Management Guide for 2010-11, pp 43-56. (ed. S. Maas). Australian Cotton CRC Publication.
- Rossiter, L., Larsen, D., Downes, S., **Wilson, L.**, Murray, D. and Miles, M. (2010) Insecticide resistance management strategy for 2010-11. Cotton Pest Management Guide for 2010-11, pp 61-70. (ed. S. Maas). Australian Cotton CRC Publication..
- **Wilson, L.**, Mensah, R., Khan, M., Dillon, M., Wade, M., Scholz, B., Murray, D., Lloyd, R., Heimoana, V. and Holloway, J. (2010) Impact of insecticides and miticides on beneficials in Australian cotton. Cotton Pest Management Guide for 2010-11, pp 57-59. (ed. S. Maas). Australian Cotton CRC Publication.
- Downes, S., **Wilson, L**., Knight, K., Kauter, G. and Leven, T. (2010) Preamble to the resistance management plan for Bollgard II (2010-11). Cotton Pest Management Guide for 2010-11, pp 70-75 (ed. S. Maas). Australian Cotton CRC Publication.
- Taylor, S., Maas, S., Gambley, C., **Wilson, L.** and Kauter, G. (2010) Cotton industry bio-security plan. Cotton Pest Management Guide for 2010-11, pp 131-135 (ed. S. Maas). Australian Cotton CRC Publication.
- Lewis Wilson, Grant Herron , Tanya Smith, Bernie Franzmann, Simone Heimoana, Rod Gordon, Tracey Farrell, James Hill and David Larsen (2008) Aphid Ecology in Cotton. Australian Cotton CRC On-Farm Series publication.
- Lewis Wilson, Grant Herron, Tanya Smith, Simone Heimoana, Rod Gordon, Tracey Farrell, James Hill and David Larsen (2008) Strategies to manage aphids in cotton. Australian Cotton CRC On-Farm Series publication.

Cotton tales contributed to (July 2008 – July 2010):

4. Aphid resistance to neonicotinoids Southern NSW Cottontales No.1 (27.9.09)
5. Aphid resistance to neonicotinoids Namoi Valley Cottontales No.1 (20.7.09)
6. Aphid resistance to neonicotinoids MacquarieValley and Bourke Cottontales No.1 (20.7.09)
7. Aphid resistance to neonicotinoids Gwydir Valley Cottontales No.1 (20.7.09)
8. Be alert for aphids. Walgett Cottontales No. 4 (13.10.08)
9. Be alert for aphids. Central Queensland Cottontales No. 4 (13.10.08)

10. Be alert for aphids. Gwydir Valley Cottontales No. 4 (14.10.08)
11. Be alert for aphids. Southern NSW Cottontales No. 3 (13.10.08)
12. Be alert for aphids. Macquarie Valley Cottontales No. 5 (15.10.08)
13. Be alert for aphids. Darling Downs Cottontales No. 4 (24.10.08)
14. Be alert for aphids. Border Rivers Cottontales No. 3 (27.10.08)
15. Silverleaf Whitefly. Gwydir Valley Cottontales No. 12 (10.2.09)
16. Silverleaf Whitefly. MacquarieValley and Bourke Cottontales No. 15 (23.3.09)

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

Mite Yield Loss Tool http://cottassist.cottoncrc.org.au/Mites/Default.aspx

Aphid Ecology in Cotton:
http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/AphidsBunchytop.aspx

Strategies to manage aphids in cotton:
http://www.cottoncrc.org.au/content/Industry/Publications/PestsandBeneficials/AphidsBunchytop.aspx

Appendix 1.
Report on efficacy of Biopesticides to NSW I&I.

Report to NSW I&I
Efficacy and non-target effects of BC667 and BC639
Experiments 2008-09 and 2009-10.

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Disclaimer

1. Under no circumstances should the words "CSIRO" or "CSIRO Division of Plant Industry" or the name of Dr. Wilson or Ms Heimoana be associated in any way with the use of the trial results in any publication, brochure, technical document or public forum
2. I&I NSW will not cause or permit to be published or advertised any statement that asserts or implies approval by CSIRO of any particular chemical without written approval from CSIRO.
3. CSIRO reserves the right to publish the information generated in the experiment and to make public the information once the product is registered.

Summary

- **The efficacy and non-target effects of BC639 and BC667 were investigated at ACRI and compared to deltamethrin and an untreated control.**
- **BC639 at 0.5 l/ha had moderate negative effects on predatory beetles, bugs and Hymenoptera, low negative effects on thrips and very low effects on other spp.**
- **At 0.25 l/ha BC639 is even more selective with low negative effects on predatory beetles and Hymenoptera and very low effects on other groups.**
- **BC667 at 0.5 l/ha is relatively selective with low negative effects on predatory beetles and bugs and very low effects on other groups.**
- **BC639 would appear from these experiments to have some potential for control of *Helicoverpa*, jassids, thrips and apple dimpling bugs. BC667 may have some potential for control of mites.**

Introduction

Management of *Helicoverpa* spp. in cotton in Australia is now highly reliant on Bt-cotton containing genes to express the Cry1Ac and Cry2Ab proteins (Bollgard II) (Naranjo *et al.* 2008). This places great selection pressure on *Helicoverpa* spp. for the survival of resistant individuals which is the rationale for the pre-emptive resistance management plan that growers must agree to when using this product. In the event that resistance frequencies to either of these proteins begins to rise, there may be changes in this plan, one of which could be the re-introduction of a cap on the proportion of the crop that can be planted to Bollgard II. In this case the remainder of the crop would be conventional cotton and it would be critical to have access to effective, selective, cost effective and environmentally compatible insecticides to manage *Helicoverpa* spp., especially *H. armigera*. Furthermore, reduced insecticide use in Bollgard II crops has allowed other pests to survive and become problems, such as green mirids, green vegetable bug. Control of these pests with broad-spectrum insecticides reduces beneficial populations and increases risks from secondary pests such as spider mites, aphids and whitefly. More selective options are required to manage these pests in cotton, especially mirids and other emerging pests in Bollgard II crops.

Dr Robert Mensah (NSW I&I), in collaboration with CRDC and Becker Underwood, has been developing two potential biopesticide options for control of *Helicoverpa*, mirids and potentially other pests such as silver leaf whitefly and aphids. These are BC639a (*Aspergillus spp.*, note this was formerly thought to be a Metarrhizium strain) and BC667 (*Beauveria* spp.). In 2008-09 we included BC639 and BC667 both at 500 ml/ha. In 2009-10 the decision had been made by Becker Underwood not to progress BC667 so we dropped it and included two rates of BC639a 500 and 250 mls/ha..
In the experiment reported here we evaluated these two biopesticides for efficacy (see below) and non-target effects. This allows their fit in IPM systems to be determined. Comparisons were made with untreated cotton, which serves as a consistent reference. Due to confidentiality, only these biopesticides, the control and deltamethrin, a commercial standard, are reported here. Note also that the data represent one experiment (first year) only for BC667, whereas normally we would present data from at least two experiments in consecutive years from which to draw conclusions.

Materials and Methods

The experiments were carried out from early December to January in the 2008/09 and 2009/10 seasons when the cotton had a half to full canopy. The experiments were located in an otherwise unsprayed cotton field planted to cultivar ‘Sicot 80 RRFlex’ at the Australian Cotton Research Institute (ACRI) (30° 13’S, 149° 47’E), 25 km west of Narrabri in New

South Wales. Cotton was planted on the 20th October using seed treated with fungicides, but not insecticides.

There were 8 treatments, however, due to confidentiality and the different target range of some of the other compounds only the pertinent treatments are shown in this report. The treatments shown are: (1) untreated control (2) deltamethrin (Decis Options at 4.95 g ai/ha) and (3) the biopesticides.

A randomised block design with four replicates was used. Plots were 12 rows (1 m spacing) by 18 m long. The central 8 rows of the treated plots were sprayed with insecticide. Five sprays were applied at 7 to 14 day intervals. Consecutive applications were used because the often low abundance of beneficial insects relative to pests means that statistically significant results may not be obtained using single applications. The unsprayed rows in each plot helped to minimise insecticide drift between plots. All insecticide formulations were water miscible. They were applied using two hollow cone nozzles (TX4 Spraying Systems Co., Wheaton, IL, USA) per row at 300 kpa and 5 km/h giving a total spray volume of 60 l/ha. Between each block of the experiment a four-row strip of sunflowers was planted at the same time as the cotton. These served as a source of mirids and predators and also as a windbreak to reduce drift of insecticides between plots. To increase the value of the experiments we artificially infested plots with a low density of mites (*Tetranychus urticae* Koch) before the first spray was applied. These mites were mass reared on cotton seedlings in a glasshouse.

The beneficial and pest insects recorded in this study are listed in Tables 1 and 2. Samples of pests and beneficials were taken using suction samples, plant or leaf washes and visual samples, depending upon which technique was most appropriate, as described below. Sampling was conducted 24 h before the first insecticide application and two or three times between consecutive applications. Sampling continued at approximately twice-weekly intervals until about two weeks after the final insecticide had been applied.

Assessment of the abundance of spider mites or thrips, involved sampling 20 leaves per plot from the third or fourth main-stem node below the plant terminal ((Wilson *et al.* 1996). Sampled leaves for each plot were collected in a plastic bag. A weak (20%) bleach solution and two drops of a surfactant were added to each bag which was then agitated by hand for 1 minute after which the fluid was poured through a sieve with mesh fine enough to retain objects as small as mite eggs (200 micron mesh). This process was repeated then the sieve back-flushed onto a 90 mm diameter filter paper in a Buchner funnel. Mites, insects and other invertebrates collected on the filter paper were then transferred to a labelled plastic petri dish. Samples for a date were then bagged together, the bag sealed and placed in a freezer for later counting. Numbers of motile *T. urticae*, adult and immature thrips (see Table 1 for species), cotton aphids (*Aphis gossypii* Glover) and whitefly (predominantly *Bemisia tabaci* Biotype B) extracted from each sample were counted using a binocular microscope.

Predator abundance was assessed using suction samplers developed from garden blower/vacs. On each sample date a complete row was sampled in each plot. To avoid predator depletion in any particular row, sampling was alternated between the spider mite infested rows in each plot. Small plants were sampled with a single pass of the suction sampler along the row, at a speed of about 0.5 m/s. As plants grew taller a zigzag sampling technique, where the suction device was passed along the lower, middle and top strata of the canopy, was adopted to ensure adequate coverage of the canopy. Insects collected were killed using chloroform and the abundance of key pest and predator species (species listed in Table 1) was recorded.

It is important to note that the main emphasis of these experiments was on non-target effects on beneficials. Data on efficacy against pests was also recorded but because

the compounds were not applied according to pest thresholds it is possible (likely) that efficacy against some pests may have been underestimated.

Statistical analysis

Predators were amalgamated into several groups as the abundance of most species was too low for analysis of individual species (see Table 1). 'Phytophagous' thrips species (Table 1), even though they are often regarded as pests, were treated as predators, in this experiment as research shows that thrips are important facultative predators of mite eggs (Wilson *et al.* 1996). In cotton they rarely cause significant reductions in yield or delays in crop maturity (Sadras and Wilson 1998). *Campylomma liebknechti* (Girault) was also classed as a predator as it has been found to be a predator of mites and *Helicoverpa* spp. (Wilson *et al.* 1998), however, in sufficient numbers it can be a pest. Numbers of other predator or parasitoid species were too few to analyse. Statistical analyses were run using Genstat 12 for PC. All beneficial or pest counts were transformed (ln + 1) prior to analysis to stabilise the mean/variance relationship. Analysis of variance was used to test for treatment differences over the duration of the experiment with terms for block, treatment, date and treatment by date interaction included. Where the interaction term was significant the interaction mean square value was used as the error term to test for main effects of treatment over and above its contribution to the interaction effect. Fisher's protected least significant difference test was used to separate means for insecticide treatments from those of the control. For some groups data are presented both as counts from plant washes and suction samples (thrips, mites, aphids and whitefly). Observation of the data for 2009-10 showed that significant numbers of ants were only found on the 5 samples dates after the pre-spray sample; hence for this group we restricted analysis to these dates.

Results

Results are presented in Tables 3 to 13. The terms very low, low, moderate, high and very high used in this document have specific meaning; reductions compared with the untreated control of 0-10%, very low; 11-20%, low; 21-40%, moderate; 41-60%, high; >60%, very high. Where an insecticide had a negative effect on a beneficial group the magnitude is indicated in brackets.

Predators and parasitoids

Predatory beetles (Coleoptera) (Table 3 a and b): BC639a suppressed populations of predatory beetles, significantly in 2008-09. This was mainly due to suppression of coccinellids (Tables 3a and b). Interestingly suppression of coccinellids was higher at the lower rate than the higher rate in 2009-10. For BC639a at both rates the suppression averaged about 28% which would make the ranking for effect on predatory beetles 'moderate'. BC667 significantly suppressed minute two spotted ladybeetle (*Diomus notescens*) and the effect was quite strong. Based on the results for effects on total predatory beetle, which was not significant BC667 would rank as 'low'. Deltamethrin suppressed predatory beetle populations as a whole in both years, including populations of minute two spotted ladybeetle (*Diomus notescens*) and total coccinellids. It would be ranked as 'high'.

Predatory bugs (Hemiptera) (Table 4 a and b). Deltamethrin significantly suppressed total predatory Hemiptera in both years and would be ranked 'very high'. BC639a and BC667 significantly reduced populations of predatory Hemiptera, and strongly reduced populations of brown smudge bug and 'other beneficial Hemiptera' in 2008-09. In 2009-10 BC639a and BC667 showed no significant suppression of any beneficial Hemipteran group, though the trend was still present toward lower populations of brown smudge bug. BC639a and BC667 would be ranked as 'moderate'.

Wasps and ants (Hymenoptera) (Table 5 a and b): Overall BC639 and BC667 had no significant negative effect on Hymenoptera. However, both BC639 and BC 667 significantly suppressed populations of *Microplitis* in 2008-09. Interestingly, both of these biopesticides also had higher abundance of ants than the control – possibly the oil base used for the formulation is attractive to ants. Overall the effect of BC639 or BC 667 on Hymenoptera would be very low (though high for *Microplitis*). Although Deltamethrin did not significantly suppress Hymenoptera overall, it did significantly reduce populations of *Trichogramma*, *Microplitis* and ants in 2008-09, though numbers of *Microplitis* were significantly higher than the control in 2009-10.

Lacewings (Neuroptera) (Table 6 a and b): The abundance of lacewings was very low, meaning no firm conclusions can be drawn.

Spiders (Table 7 a and b): Across the two experiments there was no evidence that either BC639 or BC667 had negative effects on spiders. In contrast, in 2009-10 deltamethrin significantly reduced abundance of spiders (high).

Thrips (Table 11 a and b): In 2008-09 BC639 significantly suppressed thrips populations. In 2019-10 BC639 significantly suppressed thrips larvae populations. Overall the effect on thrips of BC639 would be moderate. BBC667 had no negative effects on thrips. Deltamethrin caused very high reductions in thrips abundance in both years.

Pests

The experiments are focussed on non-target effects and are not designed for the purpose of evaluating the efficacy against key cotton pests. As a result pest thresholds were not used as criteria in a decision to apply treatments.

Mirids (Table 8 a and b): Numbers of mirids were overall low. Neither BC639 nor BC667 provided control of mirids. Deltamethrin provided very strong control of mirids (nymphs, adults and total) which was anticipated at this high rate.

Lepidoptera (Tables 9 a and b, 11 a and b): In the suction samples non-of the compounds tested reduced Helicoverpa populations, and Deltamethrin actually had significantly higher abundance than the control, probably reflecting that the lower mirid rate was used which has suppressed beneficials but not controlled Helicoverpa – hence allowing larval population to establish and build.

In the visual samples in 2008-09 neither BC639 nor BC667 controlled *Helicoverpa*. In 2009-10, the lower rate of BC639 provided significant suppression of *Helicoverpa* larvae in total. This reflected a strong trend of lower numbers of all of the larval stages, which were close to significant.

Jassids (Table 8 a and b): BC639 provided significant suppression of jassids in 2008-09 but not 2009-10. BC667 did not control jassids. Deltamethrin provided strong control (49%).

Apple dimpling bugs (Table 8 a and b): BC639 provided significant suppression of apple dimpling bugs in 2008-09 but not 2009-10. BC667 did not control apple dimpling bugs. Deltamethrin provided strong control.

Aphids (Table 10 a and b): In 2008-09 none of the compounds tested suppressed aphids. In 2009-10 aphid numbers were too low to draw any useful conclusions.

Whitefly: (Table 13): In 2008-09 whitefly numbers were low and no data is presented. In 2009-10, neither BC639 nor BC667 controlled whitefly. Repeated applications of deltamethrin suppressed whitefly populations in leaf wash samples but not suction samples.

Mites (Table 12): In 2008-09 BC667 provided valuable control of mites in suction samples, and BC639 also trended strongly this way and was just off being significant. There was no evidence of mite suppression for either product in the wash samples, which should

be more reliable. In 2009-10 neither BC639 nor BC667 suppressed mites, and in the suction samples number of mite nymphs were higher than the control in the BC669 plots. However, deltamethrin treated plots had 400% more mites than the control plots, showing a very high risk of mite resurgence.

Discussion

This study focused on non-target effects of these two fungal biopesticides on beneficial insects and arachnids. Overall BC639 at the higher rate is relatively selective, with moderate negative effects on predatory beetles and bugs and Hymenoptera, a low negative effect on thrips and very low effects on other groups. At the lower rate it is even more selective with low negative effects on predatory beetles and Hymenoptera and very low effects on other groups. Overall BC667 is relatively selective with low negative effects on predatory beetles and bugs and very low effects on other groups.

In terms of efficacy the results are quite mixed. BC639 would appear from these experiments to have some potential for control of *Helicoverpa*, jassids, thrips and apple dimpling bugs. BC667 may have some potential for control of mites. It is important to note that these experiments are focussed on non-target effects and though they may indicate some potential for control they are not designed for this purpose.

An overall ranking of the beneficial impact of BC639 and BC667 was obtained by assigning a rating for impact on each of the major beneficial groups. This rating, known as the beneficial disruption index (BDI) is calculated by assigning a value of 1 for the 'very low' impact rating through to 5 for the 'very high' impact rating. This score is averaged across the major predator groups but is also weighted toward the more abundant groups (predatory beetles, bugs and spiders). The weighting assigns a higher value to the more abundant beneficial groups or those that are more obligate beneficials (compared with those that are also phytophagous). The weightings total to 100% and are distributed as 18.5 % for predatory beetles, predatory bugs and spiders, 12.5% for total wasps and 6.25% for apple dimpling bugs, lacewings, Trichogrammatids, ants and thrips. This system has been applied consistently across all other insecticides tested. In deriving the values for each of the beneficial groups, we have used data reported here. In cases where there is likely to be a negative effect, such as for predatory bugs, we have used the value from the appropriate table.

BC639 at the low rate has been assigned a BDI of 1.3 and BC667 of 1.4 placing them into the very low negative impact category. BC639 at the high rate had BDI of 2.2 which also falls into the 'low' impact on beneficials category. Note that these values are very preliminary and may change (higher or lower) as more information becomes available. Relative positioning in the 'Impact of insecticides applied as foliar sprays on key beneficial groups in cotton' table that appears in the Cotton Pest Management Guide is indicated tentatively in Table 12.

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Table 1. Predatory groups recorded in this study.

Group	Class and Order	Family / Species	Common name
Predatory Coleoptera (adults)	Insecta, Coleoptera	*Coccinella transversalis* (Fabricius)	transverse ladybeetle
		Coelophora inaequalis (Fabricius)	variable ladybeetle
		Diomus notescens (Bell)	Minute two-spotted ladybird
		Harmonia testudinaria (Fabricius)	three banded ladybeetle
		Stethorus sp	mite-eating ladybeetle
		Harmonia conformis (Boisduval)	common spotted ladybeetle
		Dicranolaius bellulus (Guérin-Méneville)	red and blue beetle
		Carabidae (several species)	
		Anthicus spp.	
Predatory Hemiptera (adults and nymphs)	Insecta, Hemiptera	*Campylomma liebknechti* (Girault)	apple dimpling bug (also a pest)
		Deraeocoris signatus (Distant)	brown smudge bug
		Orius sp.	pirate bug
		Geocoris sp. Kirkaldy	big-eyed bug
		Nabis sp.	damsel bug
		Reduviidae	assassin bugs
		Cermatulus nasalis (Westwood)	glossy shield bug
		Oechelia schellenbergii (Guérin-Méneville)	predatory shield bug
Campylomma (separated into adults and nymphs)	Insecta, Hemiptera	*Campylomma liebknechti* (Girault)	apple dimpling bug
Lacewings (adults)	Insecta, Neuroptera	*Mallada* spp. and *Micromus tasmaniae* Walker	lacewing adults
Hymenoptera	Insecta, Hymenoptera	Trichogrammatids	
		Microplitis demolitor Wilkinson	
		Other Hymenoptera (parasitoids, many families)	
Ants	Insecta, Hymenoptera	Formicidae (mainly *Iridomyrmex* spp. and *Pheidole* spp.)	ants
Thrips (separated into adults and	Insecta, Thysanoptera	*Thrips tabaci* Lindeman, *T. imaginis* Bagnall, *Frankliniella schultzei*	flower thrips

larvae)		(Trybom) and *F. occidentalis* (Pergande)	
Spiders	Arachnida, Araneae	Araneae (many species of Araneomorphae)	Spiders

Table 2. Pest groups recorded in this study.

Group	Class and Order	Family / Species	Common name
Green mirids (separated into adults and nymphs)	Insecta, Hemiptera	*Creontiades dilutus* (Stål)	green mirid
Aphids	Insecta, Hemiptera	*Aphis gossypii* Glover	cotton aphid
Helicoverpa larvae	Insecta, Lepidoptera	*Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren)	*Heliothis*
Cotton tip-worm	Insecta, Lepidoptera	*Crocidosema plebejana* Zeller	Cotton tip-worm
Total Lepidopteran larvae	Insecta, Lepidoptera	*Helicoverpa* spp., *Earias huegeliana* Gaede, *Crocidosema plebejana* Zeller, *Anomis flava* (F.), *Spodoptera exigua* (Hübner).	
Mites	Arachnida, Acarina	*Tetranychus urticae* Koch	Mites

Table 3a. Mean abundance of predatory and pest Coleoptera in each insecticide treatment, ACRI, 2008-2009.

		Predatory Coleoptera											
	Rate	Total Coleoptera Beneficial		Dicranolaius bellulus		*Diomus notescens*		*Total Coccinellids*		Total other predatory beetles		Total Pest Coleoptera	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	0.1982*	-27.47	0.0394	-39.27	0.0345	-23.34	0.1632*	-22.99	0.0023	-50.06	0.1081	-24.90
BC667	500ml/ha	0.2253	-16.39	0.0418	-35.48	0.0241*	-46.78	0.1859	-11.26	0.0044	-2.57	0.1334	-6.12
Decis Options	4.95 g/ha	0.1545*	-44.75	0.0405	-37.56	0.0101*	-77.86	0.1104*	-49.29	0.0087	93.19	0.0876*	-39.78
Control	---	0.2641	0.00	0.0641	0.00	0.0447	0.00	0.2072	0.00	0.0045	0.00	0.1416	0.00
P													
LSD (p = 0.05)		0.0469		n.s.		0.0167		0.0418		n.s.		0.0381	
df		382		382		382		382		382		382	

Table 3b. Mean abundance of predatory and pest Coleoptera in each insecticide treatment, ACRI, 2009-10.

		Predatory Coleoptera											
	Rate	Total Coleoptera Beneficial		Dicranolaius bellulus		*Diomus notescens*		*Total Coccinellids*		Total other predatory beetles		Total Pest Coleoptera	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.193	-10.9	0.117	-4.0	0.008	236.1	0.074	-25.6	0.013	159.2	0.358	15.0
BC639 Low Rate	250ml/ha	0.180	-17.4	0.113	-7.5	0.004	50.1	0.064*	-36.4	0.010	93.5	0.322	1.4
Decis Options	4.95g/ha	0.107 *	-52.5	0.066*	-47.8	0.000	-100.0	0.031*	-69.2	0.014	187.3	0.199*	-41.2
Control	---	0.214	0.0	0.122	0.0	0.002	0.0	0.098	0.0	0.005	0.0	0.318	0.0
P		<0.001		<0.001		0.015		0.001		0.64		<0.001	
LSD (p = 0.05)		0.047		0.038		0.0077		0.030		ns		0.061	
df		349		349		349		349		349		349	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 4a. Mean abundance of predatory Hemiptera in each insecticide treatment, ACRI, 2008-2009.

		Predatory Hemiptera											
	Rate	*Geocoris lubra*		Orius spp.		*Nabis kinbergii*		Deraeocoris signatus		Other Beneficial Hemiptera		Total Predatory Hemiptera	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	0.0066	0.68	0.0078	129.52	0.0167	-5.80	0.0011*	-85.76	0.2077*	-24.32	0.2308*	-22.19
BC667	500ml/ha	0.0045	0.45	0.0090	166.22	0.0155	-12.18	0.0011*	-85.76	0.2326*	-14.17	0.2552	-12.84
Decis Options	4.95 g/ha	0.0089	0.89	0.0034	-0.17	0.0045	-74.69	0.0023*	-71.51	0.0850*	-70.92	0.1030*	-67.49
Control	---	0.0000	0.00	0.0034	-0.17	0.0177	0.00	0.0079	0.00	0.2663	0.00	0.2879	0.00
LSD (p = 0.05)		n.s.		n.s.		n.s.		0.0046		0.0048		0.0494	
df		382		382		382		382		382		382	

Table 4b. Mean abundance of predatory Hemiptera in each insecticide treatment, ACRI, 2009-10.

		Predatory Hemiptera											
	Rate	*Geocoris lubra*		Orius spp.		*Nabis kinbergii*		Deraeocoris signatus		Other Beneficial Hemiptera		Total Predatory Hemiptera	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.011	-40.9	0.001	-67.5	0.021	0.2	0.000	-100.0	0.011	129.5	0.052	-4.0
BC639 Low Rate	250ml/ha	0.005	-73.7	0.001	-67.5	0.023	7.8	0.000	-100.0	0.013	163.6	0.055	2.5
Decis Options	4.95g/ha	0.005	-74.0	0.001	-67.5	0.001*	-94.3	0.001	-2.3	0.008	66.4	0.018*	-67.8
Control	---	0.019	0.0	0.0035	0.0	0.021	0.0	0.001	0.0	0.005	0.0	0.054	0.0
P		0.19		0.002		<0.001		0.52		0.32		<0.001	
LSD (p = 0.05)		n.s.		0..0077		0.0177		n.s.		ns		0.0252	
df		349		349		349		349		349		349	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 5a. Mean abundance of Hymenoptera in each insecticide treatment, ACRI, 2008-2009.

	Rate	Total (Wasp) Hymenoptera		Trichogramma		*Microplitis*		Total Hymenoptera Telenomus		Other wasp spp.		Ants	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	0.3001	-4.31	0.1736	-0.85	0.0056*	-64.05	0.0090	14.46	0.1360	-6.03	0.1134*	94.83
BC667	500ml/ha	0.2977	-5.20	0.1929	11.29	0.0056*	-63.67	0.0079	0.74	0.1165	-20.25	0.0968*	64.91
Decis Options	4.95 g/ha	0.2713	-14.77	0.1293*	-27.78	0.0045*	-70.96	0.0078	0.00	0.1500	4.42	0.0345*	-43.02
Control	---	0.3117	0.00	0.1750	0.00	0.0154	0.00	0.0078	0.00	0.1441	0.00	0.0598	0.00
LSD (p = 0.05)		n.s.		0.0375		0.0065		n.s.		n.s.		0.0051	
df		382		382		382		382		382		382	

Table 5b. Mean abundance of Hymenoptera in each insecticide treatment, ACRI, 2009-10.

	Rate	Total (Wasp) Hymenoptera		Trichogramma		*Microplitis*		Total Hymenoptera Telenomus		Other wasp spp.		Ants	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.149	-23.0	0.031	-12.2	0.020	-33.4	0.007	-17.1	0.098	-23.1	0.282	12.4
BC639 Low Rate	250ml/ha	0.169	-11.7	0.031	-12.3	0.034	10.8	0.011	26.7	0.102	-20.0	0.233	-9.4
Decis Options	4.95g/ha	0.173	-9.5	0.016	-56.4	0.051*	67.8	0.018	109.1	0.099	-22.0	0.182	-31.1
Control	---	0.190	0.0	0.035	0.0	0.031	0.0	0.009	0.0	0.126	0.0	0.254	0.0
P		0.126		0.38		0.006		0.086		0.065		0.033	
LSD (p = 0.05)		n.s.		n.s.		0.0196		n.s.		n.s.		0.13	
df		349		349		349		349		349		157	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 6a. Mean abundance of *Neuroptera* in each insecticide treatment, ACRI, 2008-2009.

		Neuroptera							
	Rate	Total Neuroptera		LW Larvae		Green Adult		Brown Adult	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	0.0109	100.74	0.0023	0.00	0.0011	0.00	0.0077	252.55
BC667	500ml/ha	0.0090	65.20	0.0000	-100.00	0.0011	0.00	0.0079	260.24
Decis Options	4.95 g/ha	0.0079	44.47	0.0023	0.00	0.0023	100.11	0.0034	54.04
Control	---	0.0055	0.00	0.0023	0.00	0.0011	0.00	0.0022	0.00
P									
LSD (p = 0.05)		n.s.		n.s.		n.s.		n.s.	
df		382		382		382		382	

Table 6b. Mean abundance of *Neuroptera* in each insecticide treatment, ACRI, 2009-10.

		Neuroptera							
	Rate	Total Neuroptera		LW Larvae		Green Adult		Brown Adult	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.011	8.7	0.004	-41.4	0.088	85.6	0.000	0.00
BC639 Low Rate	250ml/ha	0.009	-14.6	0.007	17.3	0.016	-68.0	0.000	0.00
Decis Options	4.95g/ha	0.004	-63.5	0.002	-61.0	0.000	-100.0	0.016	0.00
Control	---	0.010	0.0	0.006	0.0	0.048	0.0	0.000	0.00
P		0.49		0.84		0.16		0.43	
LSD (p = 0.05)		n.s.		n.s.		n.s.		n.s.	
df		349		349		349		349	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 7a. Mean abundance Arachnids in each insecticide treatment, ACRI, 2008-2009.

		Arachnids					
		Total Spiders		Tangleweb		Other Spiders	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%
BC639	500ml/ha	0.3207	7.20	0.0898	17.30	0.2503	4.58
BC667	500ml/ha	0.3451	16.82	0.0849	10.58	0.2809	19.25
Decis Options	4.95 g/ha	0.2986	-1.35	0.0723	-6.51	0.2445	1.87
Control	---	0.3021	0.00	0.0771	0.00	0.2405	0.00
P							
LSD (p = 0.05)		n.s.		n.s.		n.s.	
df		382		382		382	

Table 7b. Mean abundance Arachnids in each insecticide treatment, ACRI, 2009-10.

		Arachnids					
		Total Spiders		Tangleweb		Other Spiders	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.375	7.9	0.019	56.9	0.362	6.7
BC639 Low Rate	250ml/ha	0.369	5.8	0.018	48.3	0.356	4.5
Decis Options	4.95g/ha	0.204*	-46.4	0.008	-32.1	0.198*	-46.4
Control	---	0.352	0.0	0.012	0.0	0.343	0.0
P		<0.001		0.193		<0.001	
LSD (p = 0.05)		0.050		n.s.		0.049	
df		349		349		349	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 8a. Mean abundance of *Creontiades dilutus*, *Campylomma liebknechti* and leaf hoppers in each insecticide treatment, ACRI, 2008-2009.

	Rate	Total Hemiptera Pests		*Creontiades dilutus*		*Nezara viridula*		*Campylomma liebknechti*		Jassids		Total Other Hemiptera Pests	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	1.5738*	-18.86	0.0457*	66.75	0.0033	0.33	0.3777*	-26.03	1.1120*	-30.68	0.2403	-0.32
BC667	500ml/ha	1.7766	4.16	0.0383	39.30	0.0032	0.32	0.4456	-9.53	1.4209	6.71	0.2757	16.51
Decis Options	4.95 g/ha	1.4680*	-29.13	0.0144	-48.41	0.0000	0.00	0.3096*	-41.53	0.9825*	-43.22	0.1499*	-40.64
Control	---	1.7429	0.00	0.0277	0.00	0.0000	0.00	0.4827	0.00	1.3720	0.00	0.2410	0.00
P													
LSD (p = 0.05)		0.0994		0.0159		n.s.		0.0601		0.0936		0.0533	
df		382		382		382		382		382		382	

Table 8b. Mean abundance of *Creontiades dilutus*, *Nezara viridula* and *Campylomma liebknechti* in each insecticide treatment, ACRI, 2009-10.

	Rate	Total Hemiptera Pests		*Creontiades dilutus Adult*		*Creontiades dilutus Nymph*		*Total Creontiades dilutus*		*Nezara viridula*		*Campylomma liebknechti*	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	2.330	18.0	0.018	63.3	0.020	17.3	0.038	33.3	0.000	0.00	0.466	-1.3
BC639 Low Rate	250ml/ha	2.287	12.4	0.014	20.3	0.018	3.5	0.031	8.8	0.001	0.00	0.488	4.6
Decis Options	4.95g/ha	1.879*	-29.5	0.001*	-89.1	0.000*	-100.0	0.001*	-95.8	0.000	0.00	0.246*	-53.7
Control	---	2.182	0.0	0.011	0.0	0.017	0.0	0.029	0.0	0.000	0.00	0.471	0.0
P		<0.001		0.012		<0.001		<0.001		0.44		<0.001	
LSD (p = 0.05)		0.145		0.0095		0.013		0.016		n.s.		0.067	
df		349		349		349		349		349		349	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 8b continued. Mean abundance of leafhoppers and other Hemiptera pests in each insecticide treatment, ACRI, 2009-10.

	Rate	Jassids all		Other Hemiptera Pests	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%
BC639 High Rate	500ml/ha	0.670	-8.3	0.011	8.6
BC639 Low Rate	250ml/ha	0.646	-12.9	0.011	10.3
Decis Options	4.95g/ha	0.425*	-49.1	0.009	-4.0
Control	---	0.714	0.0	0.010	0.0
P		<0.001		0.81	
LSD (p = 0.05)		0.087		n.s.	
df		349		349	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 9a. Mean abundance of Lepidoptera and *Helicoverpa* spp. in each insecticide treatment, ACRI, 2008-2009.

	Rate	Total Lepidoptera		*Helicoverpa* Eggs		*Helicoverpa* Larvae	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%
BC639	500ml/ha	0.1289	-10.16	0.0579	30.40	0.0738	-27.87
BC667	500ml/ha	0.1355	-5.29	0.0339	-24.59	0.1037	2.80
Decis Options	4.95 g/ha	0.2005	44.91	0.0523	17.57	0.1537*	56.34
Control	---	0.1425	0.00	0.0447	0.00	0.1010	0.00
P							
LSD (p = 0.05)		n.s.		n.s.		0.0318	
df		382		382		382	

Table 9b. Mean abundance of Lepidoptera and *Helicoverpa* spp. in each insecticide treatment, ACRI, 2009-10.

	Rate	Total Lepidoptera		*Helicoverpa* Eggs		*Helicoverpa* Larvae	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.100	-1.4	0.018	13.5	0.080	-4.7
BC639 Low Rate	250ml/ha	0.103	1.3	0.020	27.0	0.079	-5.7
Decis Options	4.95g/ha	0.114	12.9	0.027	66.5	0.089	7.6
Control	---	0.0942	0.0	0.016	0.0	0.083	0.0
P		0.019		0.236		<0.001	
LSD (p = 0.05)		0.031		n.s.		0.0270	
df		349		349		349	

1. Values are means of transformed data from suction samples, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 10a. Mean abundance of thrips (*Thrips tabaci* and *Frankliniella schultzei*), *Tetranychus urticae* and *Aphis gossypii* in each insecticide treatment, ACRI, 2008-2009.

		Total Thrips				*Tetranychus urticae*				*Aphis gossypii*			
	Rate	Washes		Suction Samples		Washes		Suction Samples		Washes		Suction Samples	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	2.3377*	-26.64	0.9345	-2.06	2.0121	20.92	0.0844	-41.80	0.0028	50.07	0.0271	54.43
BC667	500ml/ha	2.7191	11.06	0.9510	0.62	1.8772	3.31	0.0571*	-61.14	0.0046	148.02	0.0317	80.71
Decis Options	4.95 g/ha	2.1035*	-43.60	0.9056*	-6.66	3.3277*	401.59	0.3755*	201.16	0.0028	50.07	0.0261	48.35
Control	---	2.6215	0.00	0.9472	0.00	1.8497	0.00	0.1409	0.00	0.0019	0.00	0.0176	0.00
P													
LSD (p = 0.05)		0.1990		0.0390		0.2989		0.0661		n.s.		0.0208	
df		415		382		415		382		415		382	

Table 10b. Mean abundance of thrips (*Thrips tabaci* and *Frankliniella schultzei*) in each insecticide treatment, ACRI, 2009-10.

		Total Thrips				Thrips Adults				Thrips Nymphs			
	Rate	Washes		Suction Samples		Washes		Suction Samples		Washes		Suction Samples	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.76	-9.2	0.764	-11.9	0.36	-9.3	0.641*	-17.0	0.57	-9.7	0.254	19.1
BC639 Low Rate	250ml/ha	0.83	4.3	0.857	4.2	0.40	3.6	0.771	7.4	0.62	2.0	0.201	-8.3
Decis Options	4.95g/ha	0.34*	-67.3	0.692*	-23.3	0.15*	-66.9	0.594*	-25.1	0.23*	-69.7	0.203	-7.4
Control	---	0.81	0.0	0.833	0.0	0.39	0.0	0.733	0.0	0.61	0.0	0.217	0.0
P		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
LSD (p = 0.05)		0.1087		0.094		0.0623		0.0868		0.1052		0.0530	
df		383		349		383		349		383		349	

1. Values are means of transformed data from suction samples or washes, ie ln (mean number of insects per m per sample +1).
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 10b continued. Mean abundance of mites (*Tetranychus urticae*) in each insecticide treatment, ACRI, 2009-10.

		Total Mites				Mites Adults				Mites Nymphs			
	Rate	Washes		Suction Samples		Washes		Suction Samples		Washes		Suction Samples	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	1.20	1.8	0.435	29.8	0.79	13.2	0.392	16.3	0.93	-4.5	0.100*	1103.7
BC639 Low Rate	250ml/ha	1.18	-0.9	0.386	12.2	0.77	8.8	0.368	7.7	0.96	0.7	0.027	209.4
Decis Options	4.95g/ha	2.66*	485.2	1.147	411.7	1.94*	460.2	0.990*	310.1	2.24*	426.2	0.552*	8285.8
Control	---	1.19	0.0	0.351	0.0	0.72	0.0	0.346	0.0	0.96	0.0	0.009	0.0
P		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
LSD (p = 0.05)		0.1762		0.177		0.155		0.173		0.1618		0.0822	
df		383		349		383		349		383		349	

Table10b continued. Mean abundance of whitefly and aphids in each insecticide treatment, ACRI, 2009-10.

		Whitefly				Aphids			
	Rate	Washes		Suction Samples		Washes		Suction Samples	
Insecticide	(g ai/ha)	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%
BC639 High Rate	500ml/ha	0.051	15.3	1.184	6.0	0.0000	0.00	0.002	-72.0
BC639 Low Rate	250ml/ha	0.051	15.5	1.153	1.3	0.0012	0.00	0.005	-45.9
Decis Options	4.95g/ha	0.015*	-67.7	1.182	5.6	0.0000	0.00	0.004	-57.9
Control	---	0.045	0.0	1.145	0.0	0.0000	0.00	0.009	0.0
P		<0.001		<0.001		0.53		0.68	
LSD (p = 0.05)		0.021		0.1254		n.s.		n.s.	
df		383		349		383		349	

1. Values are means of transformed data from suction samples or washes, ie ln (mean number of insects per m per sample +1).
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide – back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 11a. Mean abundance of *Helicoverpa* spp. in each insecticide treatment, ACRI, 2008-2009.

	Rate	Total Eggs		VS/S Larvae		ML/L Larvae		Total Larvae	
Insecticide	g ai/ha	Mean	%	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	0.8149	0.29	0.4995	-1.60	0.3227	39.96	0.6711	11.92
BC667	500ml/ha	0.7122	-17.27	0.5244	4.73	0.1808	-27.17	0.6149	-0.58
Control	---	0.8133	0.00	0.5058	0.00	0.2407	0.00	0.6176	0.00
P									
LSD (p = 0.05)		n.s.		n.s.		n.s.		n.s.	
df		155		155		155		155	

Table 11b. Mean abundance of *Helicoverpa* spp. In each insecticide treatment, ACRI, 2009-10.

	Rate	Total Eggs		White Eggs		Brown Eggs	
Insecticide	g ai/ha	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	0.475	-9.53	0.370	-9.20	0.142	-29.34
BC639	250ml/ha	0.585	18.36	0.505	33.19	0.129	-36.29
Control	---	0.514	0.00	0.401	0.00	0.195	0.00
P		0.17		0.057		0.801	
LSD (p = 0.05)		n.s.		ns		n.s.	
df		239		239		239	

Table 11b. Mean abundance of *Helicoverpa* spp. In each insecticide treatment, ACRI, 2009-10.

	Rate	Total Larvae		VS Larvae		S Larvae		M Larvae		L Larvae	
Insecticide	g ai/ha	Mean[1]	%[2]	Mean	%	Mean	%	Mean	%	Mean	%
BC639	500ml/ha	0.625	4.39	0.106	-11.53	0.303	1.97	0.269	13.70	0.093	-40.17
BC639	250ml/ha	0.410*	-39.04	0.047	-61.87	0.207	-33.62	0.167	-33.16	0.088	-43.57
Control	---	0.606	0.00	0.119	0.00	0.298	0.00	0.240	0.00	0.151	0.00
P		<0.001		0.601		0.16		0.002		0.049	
LSD (p = 0.05)		0.196		n.s.		n.s.		0.129		0.0965	
df		239		239		239		239		239	

1. Values are means of transformed data from 1 m visual counts, ie ln (mean number of insects per m per sample +1)
2. Values are percentage change compared to the control treatment using back-transformed means, calculated as; 100x ((back-transformed mean for insecticide - back-transformed mean for control)/back-transformed mean for control)
3. Asterisks in each column indicate treatments significantly different from the control.

Table 12. Impact of insecticides applied as foliar sprays on key beneficial groups in cotton (high = more disruptive).

Insecticides (in increasing rank order of impact on beneficials)	Rate (g ai / ha)	Target Pest(s): Helicoverpa	Mites	Mirids	Aphids	Thrips	Silverleaf whitefly	Persistence[8]	Beneficials: Overall Ranking[10]	Predatory beetles: Total[1]	Red & Blue beetle	Minute 2-spt lady beetle	Other lady beetles	Predatory bugs: Total[2]	Damsel bugs	Big-eyed Bugs	Other Predatory bugs	Apple Dimpling	Lacewing adults	Spiders	Hymenoptera: Total (wasps)	Eretmocerus[19]	Trichogramma	Ants	Thrips	Pest resurgence[12]: Mite	Aphid	Helicoverpa	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	M	VL	L				VL
BC639	0.5 li							short	very low	L	VL	VL	M	VL	VL	H	VL	VL	VL	VL	L	-	L	VL	VL				-
PSO (Canopy)[16]	2%	✓			✓			short	very low	VL	L	L	VL	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
BC667	1 li							short	very low	L	M	H	L	VL	VL	H	VL	VL	VL	VL	L	-	L	VL	VL				-
Pyriproxyfen	50						✓	long	very low	M	-	M	M	VL	-	-	-	-	L	VL	VL	L	VL	VL	VL				L
Etoxazole	38.5		✓					short	low	VL	VL	-	L	VL	VL	VL	VL	VL	VL	M	L	-	VL	VL	L				VL
Indoxacarb (low)	60			✓				medium	low	L	L	H	M	VL	L	-	L	H	M	VL	VL	-	VL	H	VL		+ve		-
Indoxacarb (low+salt)	60			✓				medium	low	L	L	H	M	VL	L	-	L	H	M	VL	L	-	VL	H	VL				-
Indoxacarb (low+Canopy)	60			✓				medium	low	L	L	H	M	VL	L	-	L	H	M	VL	L	-	VL	H	VL		+ve		-
Rynaxypyr®	52.5	✓						long	low	L	M	M	L	VL	VL	VL	L	VL	VH	VL	L	-	L	L	VL		+ve		-
Dicofol[3]	960		✓					long	low	L	-	-	-	L	-	-	-	L	-	L	-	M	-	-	VL				VL
Amorphous silica[17]	2500	✓						short	low	L	L	-	M	M	-	VL	-	L	L	L	L	-	-	M	VL				-
BC639	1 li							short	low	M	M	M	M	M	VL	H	M	M	VL	VL	M	-	M	VL	L				-
Spinosad	96	✓						medium	low	VL	M	L	VL	M	L	H	VL	L	VL	VL	M	H	H	H	H	+ve			H[15]
Diafenthiuron	350		✓		✓		✓	medium	low	M	H	VL	M	L	M	VL	L	H	VL	L	L	H	VL	H	L			+ve	M
Pymetrozine	150				✓			short	low	M	M	M	M	L	L	L	VL	H	M	L	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	M	-	L	M	L	M	L	-	-	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]
Spirotetramat	96				✓		✓	medium	moderate	M	L	H	H	VL	VL	VL	VL	M	VH	M	M	-	M	M	M				-
Abamectin	5.4	✓[6]	✓					medium	moderate	L	M	H	VL	M	L	M	M	H	VL	M	M	H	M	H	M				H
Emamectin	8.4	✓						medium	moderate	L	VL	M	VL	H	H	H	H	H	L	M	M	-	M	VL	M				H
Dimethoate (low)	80		✓[18]	✓	✓[18]	✓		short	moderate	M	L	H	H	M	L	-	H	M	M	L	M	-	M	H	M	+ve	+ve	+ve	H
Dimethoate (low + salt)	80		✓[18]	✓	✓[18]	✓		short	moderate	M	L	H	H	M	L	-	H	M	M	L	M	-	M	H	M	+ve	+ve	+ve	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	H	VL	VL	L	VL	M	M	L	H	H	M			+ve	+ve	L
Acetamiprid	22.5				✓			medium	moderate	M	M	VH	H	M	M	H	M	VH	L	VL	L	VH	H	VH	VH				M[15]
Clothianidin (low)	25			✓				medium	moderate	M	VL	-	H	L	M	VL	VL	H	H	M	M	H	M	VH	VL			+ve	-
Amitraz	400	✓	✓[9]		✓[9]			medium	moderate	H	M	VH	H	L	-	-	-	H	VL	M	M	H	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	H	H	M	VL	M	-	H	VH	H	+ve		+ve	H
Endosulfan (high)	735	✓		✓	✓	✓		medium	moderate	M	VL	VH	M	M	M	M	M	H	L	M	L	H	VH	VH	H				M[15]
Fipronil (high)	25			✓		✓		medium	moderate	L	VL	H	L	M	H	H	L	VH	L	M	M	-	M	VH	VH	+ve		+ve	VH
Imidacloprid	49			✓	✓			medium	moderate	H	L	VH	H	H	M	H	L	VH	L	L	L	VH	M	H	H	+ve		+ve	M
Clothianidin (high)	50			✓				medium	moderate	H	VL	-	VH	M	M	L	VL	H	H	M	M	VH	H	VH	VL	+ve		+ve	-
Methomyl	169	✓						very short	high	H	L	VH	VH	M	L	VH	L	VH	M	M	M	VH	H	H	H	+ve			H[15]
Thiodicarb	750	✓						long	high	VH	M	VH	VH	M	M	L	L	VH	VL	M	M	-	M	M	H	+ve	+ve		M[15]
Dimethoate (high)	200		✓[18]	✓	✓[18]	✓		short	high	M	M	H	H	M	H	-	H	H	VH	M	H	H	H	VH	M	+ve		+ve	H
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	H	H	H	VH	L	M	H	VH	H	VH	H	+ve			H
Carbaryl[3]		✓						short	high	H	-	-	-	H	-	-	-	-	-	-	-	-	-	-	H	-	-	-	H
Pyrethroids[4]		✓	✓[7]	✓[7]			✓[7]	long	very high	VH	-	-	-	VH	-	-	-	VH	VH	VH	VH	VH	VH	VH	VH	+ve	+ve	+ve	H

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

Appendix 2.
ACGRA Cotton Conference paper on Pale Cotton Stainer.

Understanding pale cotton stainer (PCS) damage to Bollgard® II cotton

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Abstract Trials were conducted to understand damage of pale cotton stainer, *Dysdercus sidae* (PCS) to Bollgard® II cotton. Three boll age groups, 5, 10/15 and 30/35 day old bolls were exposed to male and female PCS adults separately for 7 days in three experiments. In the first experiment damages were assessed destructively immediately after termination and in the other experiments damages were assessed after harvest. The damage caused by PCS was similar to damage caused by mirid and green vegetable bug (GVB) characterised by black spot on the boll wall, warty growth inside the boll wall, brown coloured lint and tight lock (in severe case). However, unlike mirid and GVB, PCS caused damage to seed which affected seed germination. For 5 and 15 day old bolls, 32 and 38 per cent of seeds failed to germinate. Younger bolls incurred significantly more damage ($P < 0.05$) than older bolls. Female PCS caused significantly more damage ($P < 0.05$) than the male. Female feeding on younger bolls contributed up to 50% yield loss.

Key words pale cotton stainer, *Dysdercus sidae*, cotton, Bollgard® II, damage, boll age, sucking pest, stinkbugs

Introduction

Pale cotton stainer (PCS), *Dysdercus sidae*, is considered a minor pest of cotton in Australia and rarely warranting any control. However, in the 2007-08 cotton season we received many reports of increased levels of incidence of PCS in Bollgard® II in different locations. Why were they a problem in that particular season? We still do not have a good explanation but a combination of little pesticide use on Bollgard® II cotton and also a relatively mild season may have provided a favourable environment for PCS to develop to higher than normal abundance (Wilson, Khan and Farrell 2008). During this season many consultants reported that a proportion of bolls in field with higher PCS abundance did not open properly and remained 'tight-locked', often with stained lint.

Although PCS are known to feed on developing and mature seed, detail knowledge about their damage to developing cotton bolls was lacking. Such information is important for identifying the risk this pest poses to developing fruit, and is necessary to improve management decisions for this pest as it will help in development of economic thresholds. Here we report results from experiments on PCS damage to different age of boll by male and female separately. We also investigated the impact of PCS damage on seed germination.

Methodology

Observational data

Pale cotton stainers were placed on mature bolls (about 10-15 days before opening) and feeding behaviour was monitored and photographed. Where feeding had occurred, the area was marked and examined under a microscope to look for external and internal damage symptoms. In additional observations we caged male or female PCS onto mature bolls for 7 days then opened the bolls and separated seeds from lint. The seeds were cut in half to check for signs of damage.

Experiments

Three experiments were conducted to understand PCS damage. Experiments 1 and 2 were carried out in a glasshouse at DEEDI, Toowoomba, Tor Street Research Station and Experiment 3 was conducted in the field at ACRI, Narrabri.

Experiments 1 and 2

In Experiment 1 damage was assessed using destructive sampling of bolls to inspect them for signs of damage while in Experiment 2 damage was assessed by examining bolls once they had matured and opened. In Experiment 2 we also investigated the effects of PCS damage on seed germination. Three age group - 5, 10 and 30 day old for Experiment 1 and 5, 15 and 35 day old bolls for Experiment 2 were presented for feeding on by male and female cotton stainer separately for 7 days. Another set of bolls of same age group were assessed without insect feeding, as the untreated control. The experiments were replicated 7 times. Boll age was determined by tagging the first position bolls at flowering. These were usually at 4 or 5 nodes (one that set first) from the bottom of the plant. Cotton stainer adults, 5-7 days old, derived from a culture, were confined on the bolls using a fine mesh bag in Experiment 1 and using foam cup cages in Experiment 2. Insects were checked every day and dead ones replaced with an insect of the same age from the stock culture.

In Experiment 1 bolls were allowed to develop symptoms for another 7 days after the insects were removed before being brought back to the laboratory for assessment. Damage symptoms were recorded and quantitatively the damage was assessed as the number of black spots on the boll and warts inside the boll wall.

In Experiment 2 boll damage parameters such as lock damage, lint yield for individual boll, per cent damaged seed and per cent germination was assessed after harvest. For lock damage, assessment for each lock was made in 5 categories describing the amount of brown coloured lint per lock. Each damage category then assigned a damage score (DS) as DS 1 = spot of brown coloured lint, DS 2 = ¼ lock damage, DS 3 = ½ lock damage, DS 4 = ¾ lock damage and DS 5 = full lock damage. A total boll rating was obtained by adding the ratings for the individual locks.

For Experiment 2 cotton was ginned using a 20 saw hand gin. Both damaged and undamaged seeds from each boll were set up in a petridish lined with moist filter paper for germination testing and results were recorded after 7 days.

Experiment 3

Experiment 3 was similar to Experiment 2, except it was conducted in the field. We caged flowers to prevent damage from other pests and so we knew boll age and could introduce PCS at the correct time. We introduced into the cages either 1 male, 1 female or a mating male/female couple when bolls were 5, 10 or 30 days old. Caged controls were used with no PCS introduced. PCS were left on the bolls for 7 days, during which time they were monitored and dead stainers replaced. After this the stainers were removed. Once the bolls had matured they were collected, scored for damage, ginned and weighed similar to Experiment 2.

Analysis

Data were analysed using ANOVA for each boll age to compare damage by males, females and between boll age group. Data were also subjected to GLM analysis to assess the interaction between boll age and gender.

Results

Observations

PCS feed by initially probing the surface of the boll with their stout proboscis. They then progressively insert the fine stylet, which is sheathed by the proboscis, into the boll coat. The proboscis is then folded back under the PCS's body and only the fine stylet remains in the boll (Figure 1).

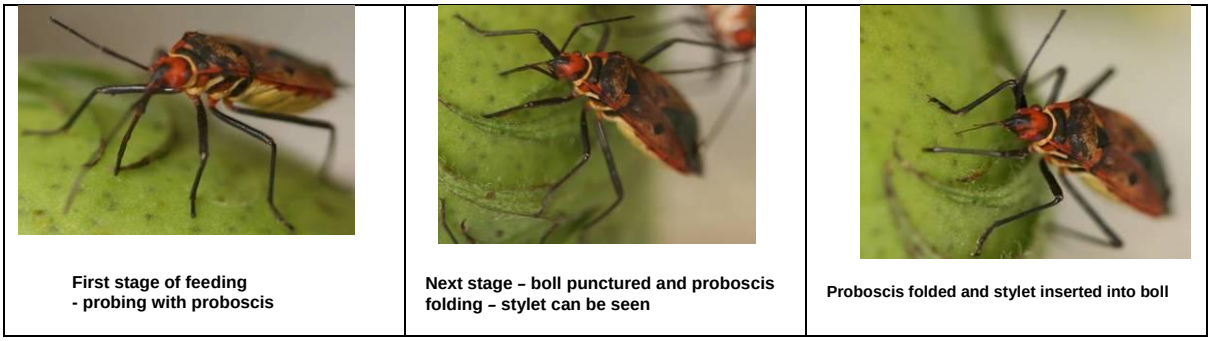

Figure 1. Feeding steps of pale cotton stainer on a mature cotton boll.

The puncture mark on the outside of the mature bolls was very small and would be impossible to see in the field. On the inside boll wall there was only a small black wound – again hard to see. By dissecting across the puncture we were able to show the path through the boll wall (Figure 2).

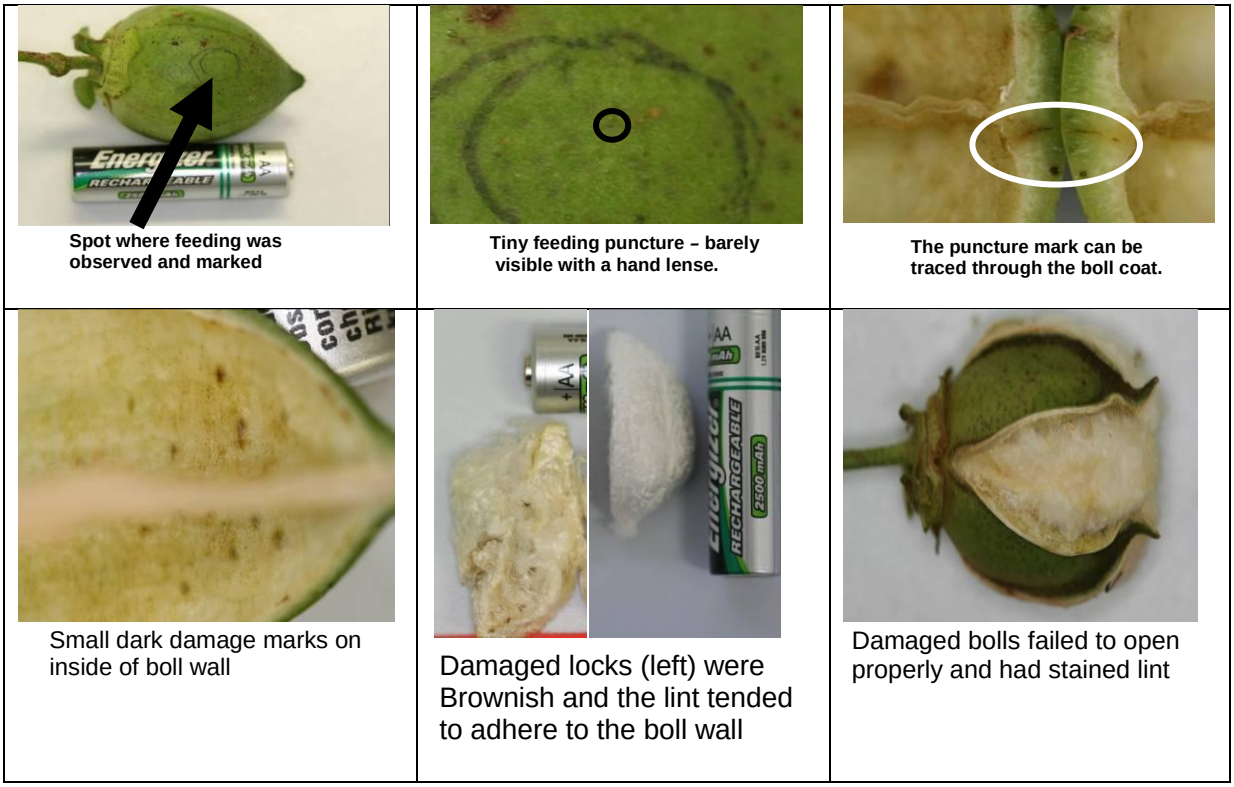

Figure 2. Symptoms of pale cotton stainer damage to mature bolls.

Feeding by male cotton stainers did not produce much evidence of damage to seeds, but feeding by female stainers caused obvious lesions on seeds (Figure 3).

Figure 3. Male pale cotton stainers caused little damage to seeds but females caused brown damaged areas.

Experiment 1

Pale cotton stainers caused similar types of damage as caused by mirids or green vegetable bug (GVB) characterised by small black spots on the outside of the boll wall and small dark spots on the inner ball wall (Figure 4). PCS caused substantial damage to seeds, as seen above, however, the relationship between the damage symptoms is very poor. For example brown coloured lint is not always associated with black spots or warts or seed damage and vice versa. This may be due to the fact that males may cause damage to the boll wall but not much damage to seeds, and that damage spots may be very small.

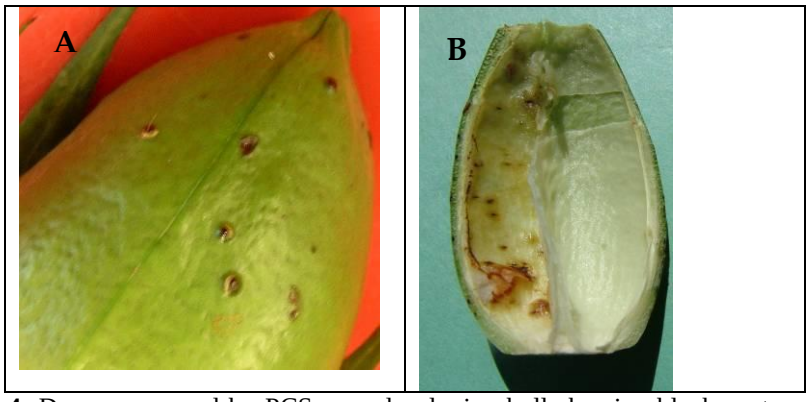

Figure 4. Damages caused by PCS on a developing boll showing black spot on the outer boll wall of young bolls (A) and occasional warty growth inside boll wall (B).

The results showed that females produced more black spots and warts than male except in 5 day old bolls where males produced more black spots than females (Table 1). However, analysis showed that for each boll age group differences between males and females for either black spot or wart was not significant ($P > 0.05$). The analysis also showed that though damage was significantly different ($P < 0.05$) between female and untreated control, the difference between male and untreated control was not significant ($P > 0.05$). GLM analysis showed that there was no significant interaction ($P > 0.05$) between boll age and insect gender.

Table 1. Number of black spots and warts produced by pale cotton stainer to bolls of different ages under glasshouse condition. Means followed by the same letter for each boll age are not significantly different (*P > 0.05*)

Boll age (day)	Treatment	Black spot/boll	Wart/boll
5	Male	2.57a	2.14ab
	Female	1.86a	6.43a
	Control	0.00a	0.00b
10	Male	4.86a	1.71ab
	Female	5.29a	4.00a
	Control	0.00a	0.00b
30	Male	2.14ab	1.57ab
	Female	5.14a	3.43a
	Control	0.00b	0.00b

Experiment 2

Analysis revealed that damage parameters such as lock damage, lint yield and per cent damaged seed was significantly higher (*P< 0.05*) for females than either for male or control for 5 and 15 day old boll (Table 2). For 35 day old boll, however, differences between male and female damage were not significant (*P* > 0.05). Per cent seed germination was significantly lower (*P< 0.05*) for female for all boll age groups. GLM analysis showed that there was significant interaction (*P* < 0.05) between boll age and insect gender for all damage parameter except per cent germination. Further analysis on lint yield data showed that per cent yield loss of male and female was 7.8 and 44.4% for 5 day old boll; 7.6 and 36.8% for 15 day old boll and 1.4 and 6.1% for 35 day old boll.

Table 2. Pale cotton stainer damage to bolls of different ages under glasshouse condition. Means followed by the same letter for each boll age are not significantly different (*P > 0.05*).

Boll age (day)	Treatment	Lock damage	Lint yield (g/boll)	Percent damaged seed	Percent seed germination
5	Male	2.7b	2.3b	2.20b	90.4b
	Female	8.3a	1.4a	15.72a	68.0a
	Control	0.0b	2.6b	0.00b	97.1b
15	Male	0.9b	2.6b	2.95b	92.4b
	Female	7.4a	1.8a	28.83a	62.9a
	Control	0.0b	2.9b	0.00b	94.9b
35	Male	1.1	2.4	2.42	91.6ab
	Female	0.7	2.4	4.41	83.4a
	Control	0.0	2.6	1.43	94.5b

Experiment 3

Female PCS damaged a higher proportion of locks per boll for 5 and 10 day old bolls, than did males which were not significantly different from the control (Table 3). Females placed on 5 day old bolls reduced boll lint weight by about 50%, while males and couples reduced it by about 30%. PCS did not affect lint weight when caged with 10 or 30 day old bolls. Females placed on 5 day old bolls also reduced total seed weight per boll by about 42%, while males and couples reduced it by about 20%. PCS did not affect total seed weight per boll when caged with 10 or 30 day old bolls.

Table 3. Pale cotton stainer damage to bolls of different ages under field condition. Means followed by the same letter for each boll age are not significantly different (*P > 0.05*).

Boll age (day)	Treatment	% Locks damaged	Lint yield (g/boll)	Total Seed weight (g)
5	Male	15.7ab	2.2b	3.2b
	Female	62.7c	1.6c	2.3c
	Couple	24.5ab	2.2b	3.3b
	Control	4.2a	3.1a	4.0a
10	Male	11.8a	2.3	3.1
	Female	17.1a	2.3	3.1
	Couple	53.1b	2.0	2.7
	Control	9.5a	2.3	3.2
30	Male	18.5	2.6	3.6
	Female	12.0	2.2	3.1
	Couple	9.0	2.3	3.2
	Control	0.7	2,6	3.4

Discussions and conclusions

Although there are some similarities in the damages caused by PCS, mirid and GVB with respect to boll age, such as higher damage to younger bolls (Khan and Bauer 2001 and 2002 and Lei, Khan and Wilson 2002). Unlike mirid and GVB, PCS can also cause substantial damage to older (> 30 day old) bolls (Tables 1, 2 and 3).

With their strong piercing/sucking mouthparts, PCS feed on mature seed in older bolls (Wilson, Khan and Farrell 2008). In the field, PCS are seen sitting on open bolls feeding on seed. This may not cause damage to lint but causes damage to seed. The visual observations showed that females caused much greater damage to seed than males, and this is shown in the Trial 2 where more than 15% seed in 35 day old boll fed on by females failed to germinate while germination of seed from bolls fed on by males was unaffected.

Black spots on the external boll surface and the warty growths on the inner boll wall shown in Figure 4 were observed on 10 day old bolls fed on by PCS. This damage was quite different to that on older bolls. We suggest that the boll wall of younger boll is softer, so that probing with the proboscis could probably cause damage in addition to damage caused by the stylet. Further, a young boll is still expanding so the damage becomes more visible as the size of the damage spot grows with the boll. On younger bolls, both boll wall and seed are tender with a high moisture content which during PCS feeding this moisture oozes out, and along with saliva semi solidifies inside boll wall producing soft squishy warts. In contrast the coat of older bolls is hard and growth has ceased, so the feeding spots caused by the fine stylets are barely visible and there is no production of warty growths inside the boll wall.

In all experiments females caused more damage than males. The females used in the experiments were mated and perhaps the dietary requirements of a gravid female encouraged them to feed more, especially on seeds which would provide a good source of protein, essential for egg development.

Cotton fibres usually develop from the epidermal cells of the seed coat (Ritche *et al* 2004). In our experiments PCS feeding on younger bolls (5 and 15 day old) caused damage to developing seeds. As

a result damaged seeds did not grow to full size, producing less fibre which may have resulted in the observed yield loss. Additionally, tight locking as a result of severe damage also contributed to yield loss (Figure 2).

The nature of damage to lock was found different from that observed in the field. In this trial damaged lock did not develop brown coloured lint, instead, the lock either developed partially or fully depending on the severity of damage. An hypothesis for this difference is that in the field PCS might pick up some (unknown) organism and release them with saliva to the damaged boll during feeding; resulting in the development of brown coloured lint. Secondary infections (fungal and bacterial) through the feeding wound also develop brown coloured lint. The cotton stainers used in these trials were 6-7 generation from laboratory culture and may not have that organism in their saliva.

Yield loss found in our experiments was significant, up to 50% loss by female feeding on younger bolls. Extrapolating this result to the field requires care as growing and weather conditions are major influences on yield in the field. Further, PCS tend to be more abundant later in the season, when there are few young bolls. In this situation we found that feeding on older bolls did not reduce boll weight, but did affect boll opening and harvestability, as well as reduce germination success. Also for better understanding of PCS damage in field situation we need to conduct these experiments in the field with PCS collected from fields rather than laboratory culture.

Acknowledgements

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