Australian Government
Cotton Research and
Development Corporation

FINAL REPORT 2013

Part 1 - Summary Details

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

CRDC Project Number: **CRC 1102**

Project Title: IPM for silver leaf whitefly and emerging pests in central regions

Project Commencement Date: 1/7/2010 **Project Completion Date:** 30/6/2013

CRDC Program: 3 Crop Protection

Part 2 – Contact Details

Administrator: Jo Cain
Organisation: CSIRO Plant Industry
Postal Address: Locked Bag 59, Narrabri, NSW, 2390
Ph: 02-67991513 **Fax:** 02-67931186 **E-mail:** jo.cain@csiro.au

Principal Researcher: Lewis Wilson
Organisation: CSIRO Plant Industry
Postal Address: Locked Bag 59, Narrabri, NSW, 2390
Ph: **Fax:** **E-mail:**

Supervisor: As Above
Organisation:
Postal Address:
Ph: **Fax:** **E-mail:**

Signature of Research Provider Representative: Lewis Wilson

Part 4 – Final Report Executive Summary

IPM for silver leaf whitefly and emerging pests in central regions

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

a. Populations of silverleaf whitefly (SLW) were maintained throughout the year on non-cultivated hosts. Key winter hosts for nymphs were sowthistle, bladder ketmia and blackberry nightshade. Sowthistle is an important indicator host of potential SLW abundance.

b. Life history studies with silverleaf whitefly showed that survival of eggs is generally between 50-80% while survival of nymphs declined from 50% in December to 10% in March. A range of SLW predators were identified.

c. System experiments for SLW management found (i) no evidence that SLW are worse on Bt-cotton than non-Bt-cotton (ii) okra leaf shape offers resistance to SLW (iii) broad-spectrum sprays led to much higher populations of SLW

d. Investigation of the fate of honeydew on bolls showed (i) breakdown by sunlight occurs slowly (ii) rainfall substantially reduces contamination (iii) a non-linear relationship was found between % honeydew removed and rainfall.

e. Green vegetable bug (GVB) abundance increased when the drought broke and there were abundant weed hosts. Parasitism by the tachinid, *Trichopoda giacomellii*, also increased probably reflecting higher, consistent availability of GVB as hosts

f. Sequential host use studies showed GVB prefer to feed and oviposit in legume crops such as mungbean, pigeon pea, soybean and lucerne. Some legumes may have potential to draw GVB away from cotton.

g. Results confirm that sorghum is a potential host for GVB but only during the flowering and early seed maturation period.

h. Twelve additional hosts were identified for cotton bunchy top disease (CBT) predominantly from Malvaceae, but also from the Euphorbiaceae, Lamiaceae, Fabaceae and Aizoaceae.

i. Neonicotinoid seed treatments on cotton significantly reduced transmission of CBT by neonicotinoid-susceptible aphids. Foliar applications of an aphicide were only effective at reducing primary transmission if timed just before or just after aphids colonised the crop.

j. The IPM fit of nine new compounds was evaluated. Two recently registered compounds will be added to the ‘Impact of insecticides and miticides on beneficials’ table for 2014/15.

k. Information was provided to industry to assist in management of spur-throated locusts and cluster caterpillars (2010-11) and broad mites (2011-12)

l. Simone Heimoana’s Phd thesis ‘The effects of aphids (*Aphis gossypii*) Glover on photosynthesis in cotton (*Gossypium hirsutum*)’ was awarded.

m. Contributions were made to the TIMS Committee, TIMS Insecticide and Bt Technical Panels, to REFCOM and the Industry Bio-security Committee.

This project provides new information to improve management of emerging pests. Outcomes have been delivered to industry through presentations, published resources and the WWW.

Background

The advent of Bt-cotton led to a dramatic decline in insecticide use against *Helicoverp*a spp. in the Australian cotton industry. Though this had significant benefits to the industry a downside was that some pest species formerly controlled by insecticides applied against *Helicoverpa* spp., and which were not affected by the Bt proteins, were no longer controlled. Several species have increased in significance in Bt-cotton, including the green mirid and green vegetable bug. In addition, silverleaf whitefly (SLW) has gradually achieved pest status in central and southern regions. It became a major problem in the Lower Namoi, Mungindi, and Gwydir regions in 2008-09, and has since reached pest status in cotton in other regions as well (Upper Namoi, Macquarie and Goondiwindi). In combination these emergent pests challenge the IPM systems developed for cotton because many of the control options used are detrimental to beneficial species.

This project built on a series of projects focusing on developing and enhancing integrated pest management (IPM) in cotton, especially in Bt-cotton systems. This was done by providing key information about the ecology and management of pests and beneficials and developing that information into a format suitable for use by industry. The project aimed to maintain capacity to respond to emerging pest problems (e.g. broad mites), to address key pest management challenges, such as management of mirids, GVB and SLW and maintain core entomology skills essential given the changing pest management environment (BGII and the advent of BGIII). The project also provided key support for (1) the project of Dr Grant Herron, NSW DPI (DAN197) investigating resistance to pesticides in mites and aphids (2) the project of Murray Sharman, QDAFF (DAQ1201) which had an objective to understand alternatives hosts for cotton bunchy top disease (CBT), and (3) also interacted with Dr Robert Mensah in evaluation of the efficacy and non-target effects of some novel biopesticides and semiochemicals.

Objectives

a. *Researching SLW ecology (hosts, parasites, survival) in central regions*
This objective has been met when studies initiated to understand seasonal host use and life history in cotton were completed. In addition a range of simple laboratory experiments were used to identify potential predators of SLW.

b. *Researching IPM compatible management strategies for SLW and mirids*
Experiments that aimed to understand the interactions between mirid management options, Bt-cotton, leaf shape and the risk of SLW outbreaks have been completed.

c. Investigating breakdown of honeydew on lint and effect on fibre quality.
A range of experiments investigating the fate of honeydew on cotton lint, including the effects of UV radiation and rainfall have been completed.

d. *Finalising surveys of crop and non-crop hosts for GVB*
A final season of sampling at three sample areas to understand seasonal host use by GVB was completed. In addition a final crop rotation experiment cycle was managed to compare preferences for particular host and movement between hosts in the crop sequence for adults and nymphs of GVB.

e. *Finalising thresholds for late thrips and jassids from existing data*
Analysis of data for crop damage tolerance was completed and tentative thresholds were developed. These were used by industry in recent outbreaks of spur-throated locusts and cluster caterpillars.

f. *Use new techniques to evaluate the presence of cotton bunchy top disease in alternative hosts – to understand its ecology and risks.*
Strong collaboration with Murray Sharman (QDAFF) has allowed identification of a range of new hosts for CBT disease. In addition it is now clear that CBT consists of at least 2 strains. Further, we have shown that seed treatments are at least partially effective in reducing transmission of CBT by cotton aphids that infest seedling cotton crops.

g. *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.*
The efficacy and non-target effects of several new insecticides, a semio-chemical and two fungal bio-pesticides were investigated. Reports were provided to the companies/collaborators. Information has been used to update the 'Effect of insecticides and miticides on beneficials' table in the Cotton Pest Management Guide.

h. *Maintain vigilance for emerging problems and would help respond as necessary.*
During the lifetime of this project there were outbreaks of spur-throated locusts and cluster caterpillars (2010-11) and broad mites (2011-12). Information was provided to industry to assist in management of the pests.

The project team for this research was:

Role	Name	Telephone	Email	Organisation & Street Address	% time
Principal Researcher	Lewis Wilson	02-67991550	lewis.wilson@csiro.au	CSIRO Plant Industry, Narrabri NSW 2390	35
Other Researcher/s	Robert Mensah	02-67991547	robert.mensah@dpi.nsw.gov.au	NSW DPI, Locked Bag 1000, Narrabri, NSW, 2390	<5
Supervisor/s	Lewis Wilson	02-67991550	lewis.wilson	As above	
Technical Staff	Simone Heimoana	02-67992466	simone.heimoana@csiro.au	CSIRO Plant Industry, Narrabri, NSW, 2390	100
	Tanya Smith	02-67992465	tanya.smith@csiro.au	CSIRO Plant Industry, Narrabri, NSW, 2390	100
	Dee Hamilton	02-67992465	dee.hamilton@csiro.au	CSIRO Plant Industry, Narrabri, NSW, 2390	50
	Ammie Kidd	02-67992465	ammie.kid@csiro.au	CSIRO Plant Industry, Narrabri, NSW, 2390	50
Other	Paul De Barro	07-38335720	Paul.DeBarro@csiro.au	CSIRO Ecosystem Sciences, Brisbane Qld	-
	Moazzem Khan	07-46881310	Moazzem.Khan@dpi.qld.gov.au	QLD DPI, Toowoomba, QLD 4610	5

Methods, Results and Discussion

1. Researching SLW ecology (hosts, parasites, survival) in central regions

1a. Host use and seasonal abundance by *Bemisia tabaci*

Methods

This research monitored seasonal abundance and host use by SLW. This was to provide local information of the seasonal pattern of abundance to help explain where populations in cotton originated and to identify key host species that *B. tabaci* used to sustain populations, especially during winter and spring when there is no cotton. This was was addressed by establishing 4 sample sites at ACRI, Mirrabooka (eastern lower Namoi), Glencoe (central lower Namoi) and Sunnyside (western lower Namoi). These sites provided a range of potential native, weed and crop hosts. The abundance of SLW was monitored by making counts of the numbers per unit (usually per leaf or per plant) on each potential host species at each sample site every 3-5 weeks. These leaves/plants were collected and taken back to ACRI where the abundance of nymphal stages was counted. Samples were stored in alcohol to confirm species if necessary.

Results and discussion

Our surveys indicate clear trends in terms of host use. The data is presented for adults and nymphs to show all hosts from which they were recorded (Figures 1 and 3). The total mean density recorded from each host is used as it is an indication of how important that host is. This number combines both host suitability and host abundance – eg. a host that is highly suitable but rarely abundant cannot have a high total mean score. We also divided the year into the four seasons (Summer – December to February, Autumn – March to May, Winter – June to August and Spring – September to November). This allows us to see which weed hosts are more important for maintenance of nymphal and adult SLW populations during each season. For the seasonal breakdown of host use we have only shown the 20 hosts with the highest abundance of SLW. The distinction between nymphal and adult hosts is important because adults may feed on, or be found on, hosts that they do not oviposit on and on which do not develop. Hence, it is critical to also survey nymphal hosts to identify those hosts that are actually contributing to the ongoing population development.

Overall we found that although adults and nymphs were found on a wide range of hosts, only 3-5 hosts had high numbers indicating that they were good hosts and commonly found hosts. For adults this included turnip weed (*Rapistrum rugosum*), sowthistle (*Sonchus oleraceus*) and paddy melon (*Citrullus lanatus*)(Fig. 1). For nymphs the list was similar with sowthislte, turnip weed, bladder ketmia (*Hibiscus trionum*), paddy melon and caustic weed (*Chamaesyce drummondii*) as the top 5 hosts (Fig. 3). However, adults and nymphs were recorded from more than 50 host species and it is possible that the significance of particular hosts will vary between years depending on their abundance in response to seasonal conditions.

When host use is broken down by season some very clear patterns emerge. We are most strongly interested in hosts used in winter and spring – as these species could potentially be targeted for sampling in spring as an indication of population levels that might develop the following summer. The list of species used in winter and spring is much shorter, and only a few hosts appear to be really important. These included sowthistle, turnip weed and marshmallow for adult *Bemisia tabaci* (Fig. 2). Nymphs showed a similar range but a different order: sowthistle, bladder ketmia and blackberry nightshade (Fig. 4). In particular in spring sowthistle seems to be an important indicator host. However, we still need to confirm the bio-type of the whitefly to be sure that they are B-biotype. This will be done through 2014.

Data were also collected on seasonal abundance at the four sites and trends at each site were essentially similar. Hence, though absolute numbers are different, we have combined the data from all sites as an indication of overall *B. tabaci* abundance across the district. For display we have transformed the data (ln(x+1)) so that the wide range in abundances can be accommodated (otherwise small numbers simply merge into the x axis). Data for adults, nymphs and eggs (Fig.5) shows that during the winter months populations of *B. tabaci* are very low. However, it is clear, especially from data for winter 2013, that breeding populations are able to persist at low levels on a range of weed species, as discussed above. Populations began to build on weed hosts in spring in 2011, 2012 and 2013 in each cotton season. From these initial populations in October – November a subsequent population began to build at a higher level through December and early January on weeds. From mid-January populations built virtually exponentially, and there were high numbers on a range of weed species. These populations were maintained until late May and early June.

Populations on crops lagged behind those on weeds in spring. However, populations on the crops began to show up in late December through to mid January depending on the year. From then on population built quickly in the crops but then declined very quickly as crops finished off and were defoliated from March to May. Thereafter populations were sustained on weeds.

Conclusions

In summary this research has identified a wide range of hosts for *B. tabaci* and provides the basis to identify which hosts are the most important in maintaining populations, especially through winter and spring. Secondly, the data also show that populations are maintained through winter and spring on a relatively small subset of potential hosts. With confirmation of the biotype of *B. tabaci* present (almost certainly B-biotype but needs to be confirmed) there is potential to target weed species as an indication of potential populations for summer. This needs to be treated with caution though as *B. tabaci* has a relatively long life cycle at typical spring summer temperatures – 3 to 5 weeks. Hence, though samples of weeds in spring may indicate potential for a SLW problem on cotton, prolonged cool conditions through November and December could substantially delay population development. Conversely a moderate population on weeds in spring could be accelerated into a major problem if conditions in December or early January are hotter than usual. Sampling weeds in mid to late December probably provides the best opportunity to make useful predictions

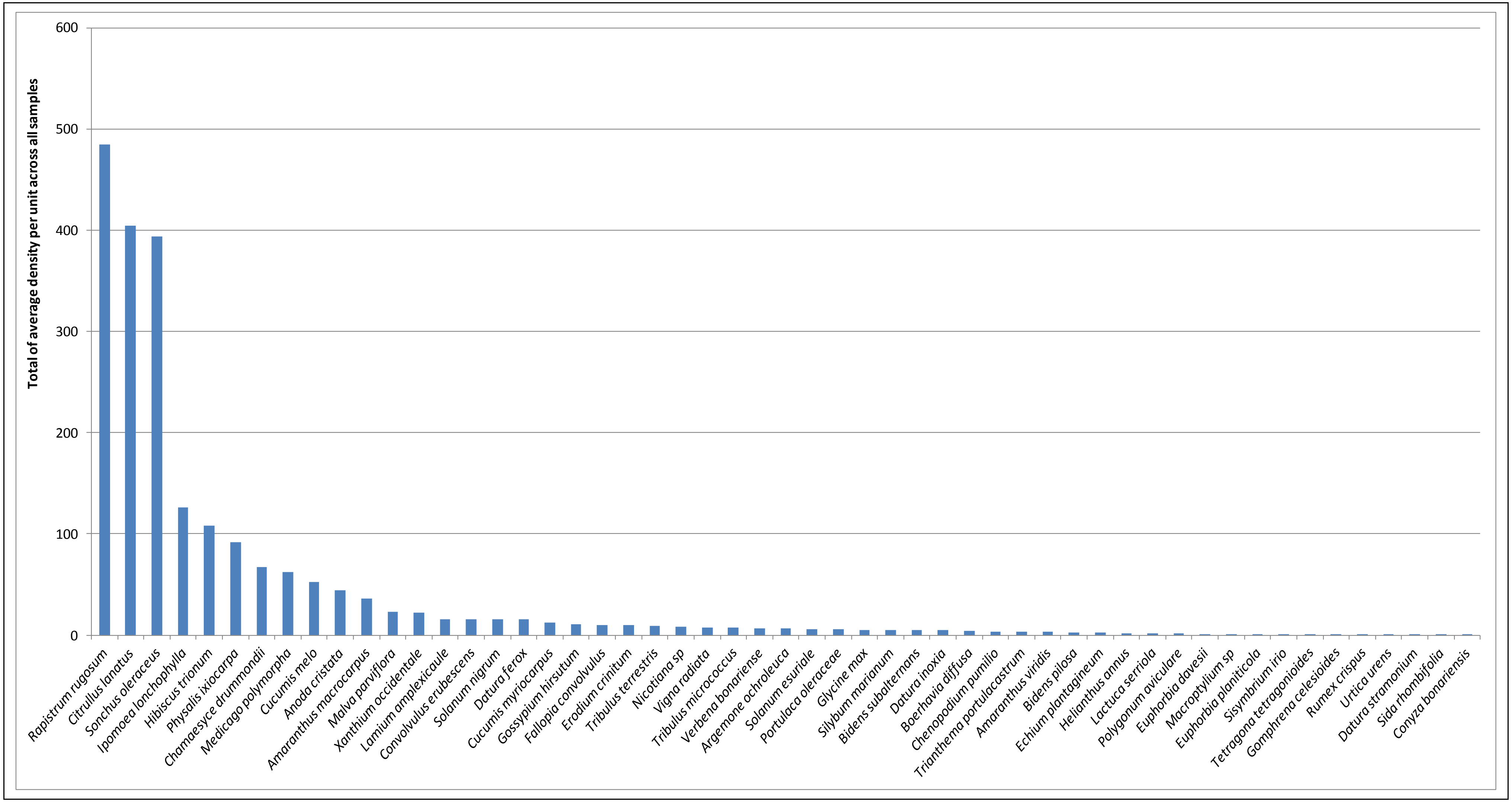

Figure 1. Total mean abundance (mean abundance summed across all dates) of *B. tabaci* adults on non-cultivated hosts in the Namoi Valley, 2011-2013.

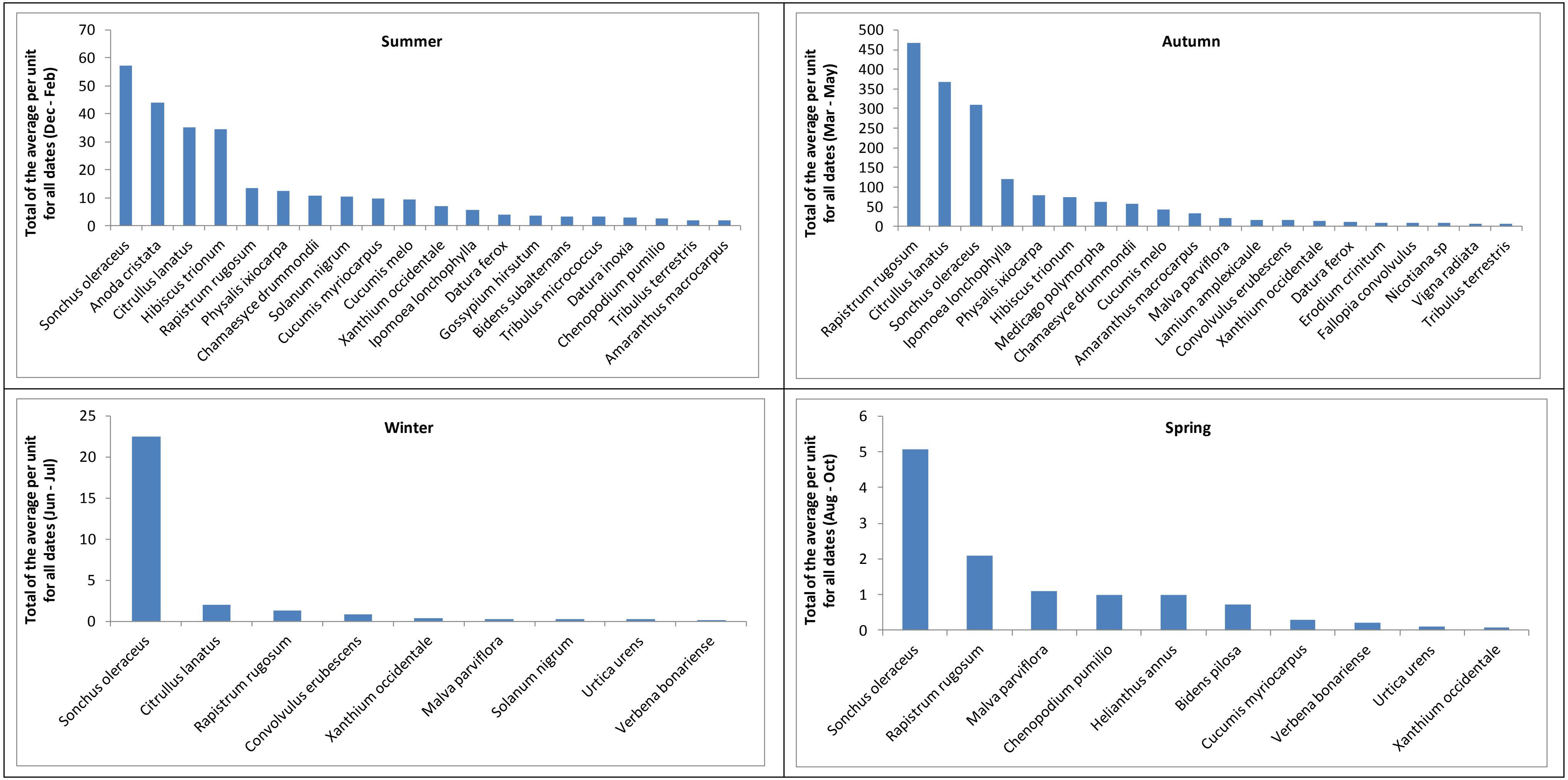

Figure 2. Total mean abundance (mean abundance summed across all dates) of *B. tabaci* adults for the four seasons for non-cultivated hosts sampled at four sites in the Namoi Valley. Only the top twenty species are shown for summer and autumn.

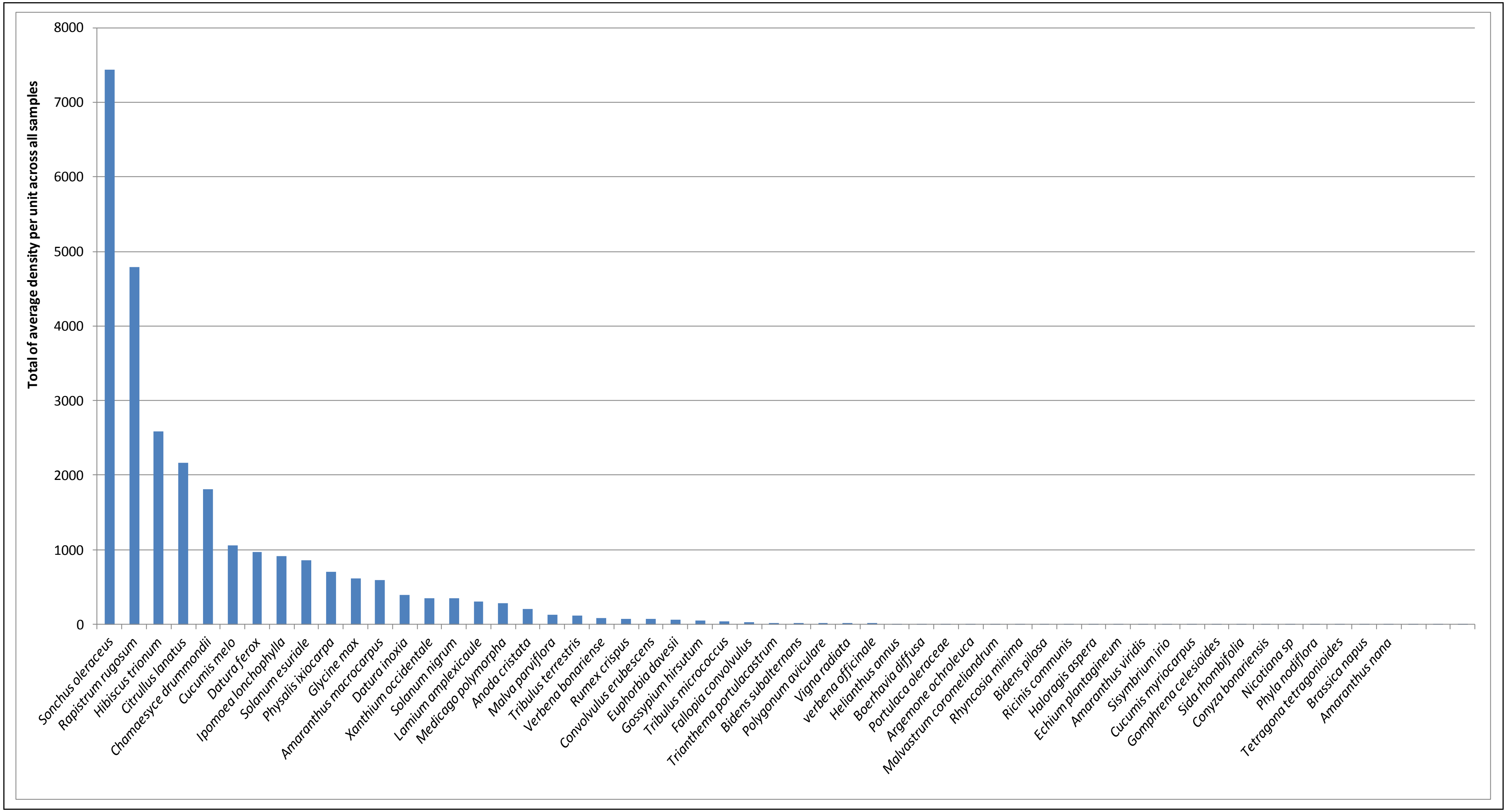

Figure 3. Total mean abundance (mean abundance summed across all dates) of *B. tabaci* nymphs on non-cultivated hosts in the Namoi Valley, 2011-2013.

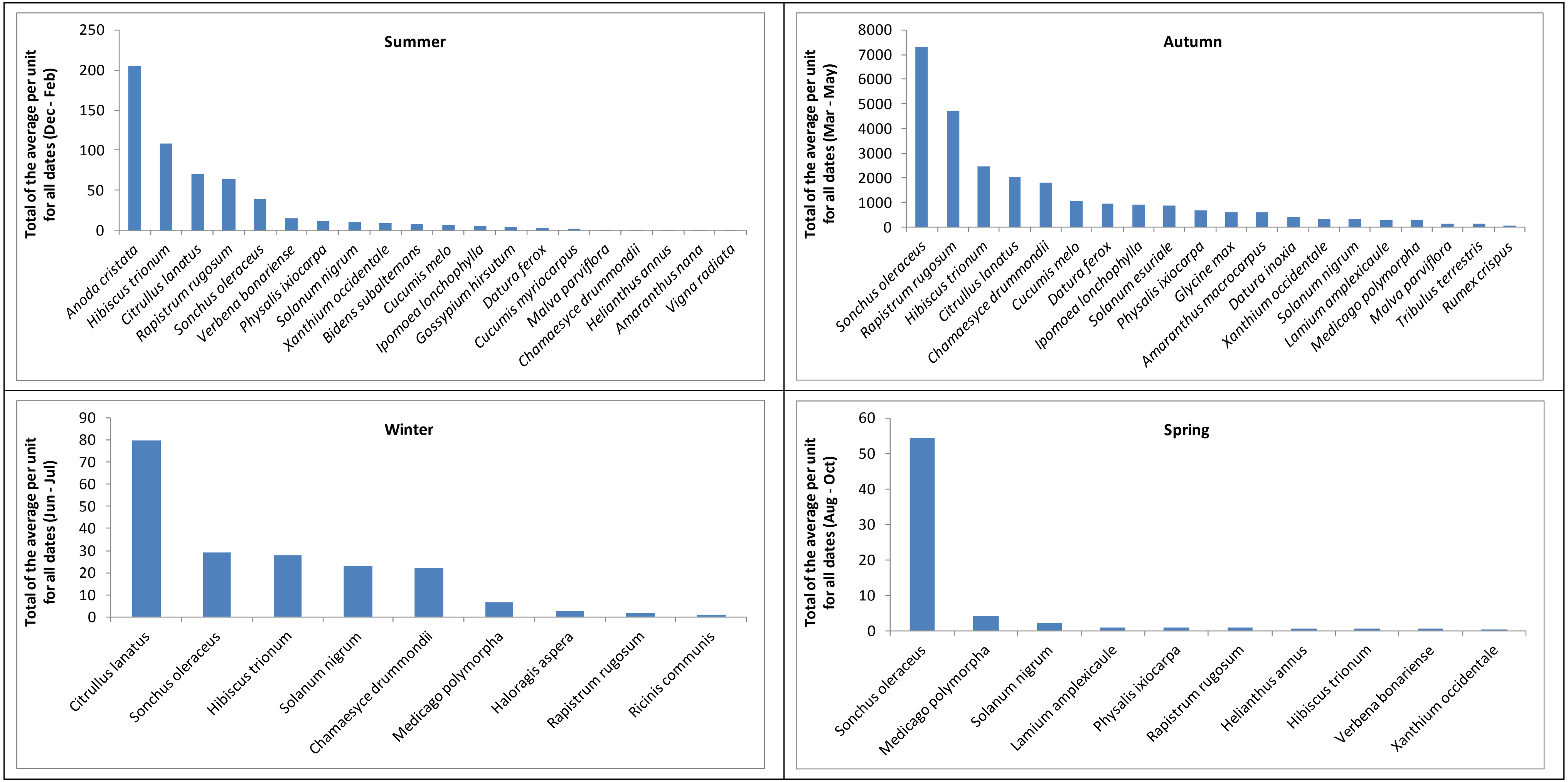

Figure 4. Total mean abundance (mean abundance summed across all dates) of *B. tabaci* nymphs for the four seasons for non-cultivated hosts sampled at four sites in the Namoi Valley. Only the top twenty species are shown for summer and autumn.

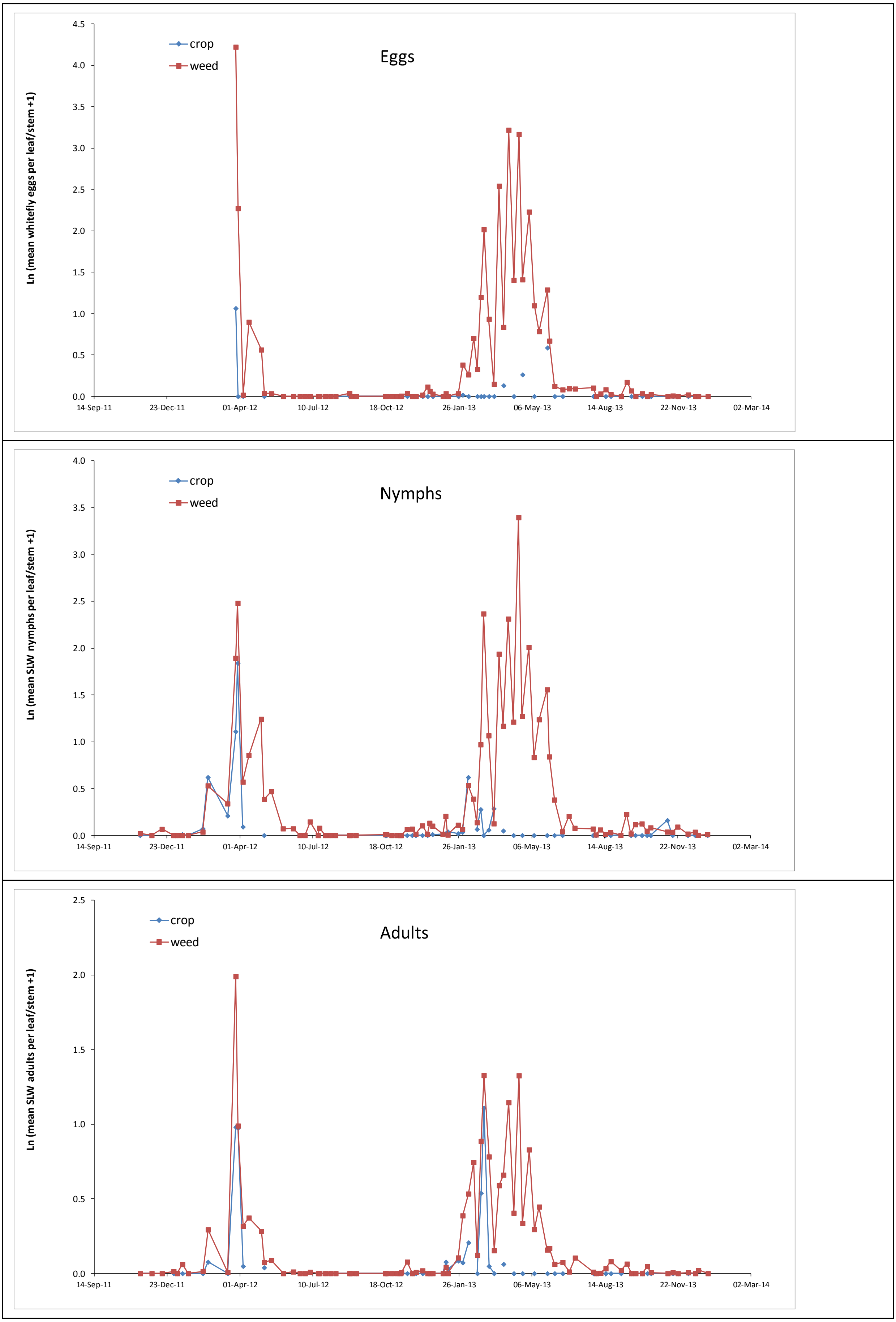

Figure 5. Abundance of *Bemisia tabaci* adults, nymphs and whitefly eggs on weed and crop hosts in the Namoi Valley, 2011 – 2014. Data are derived from 4 sampling sites.

1b. Life history studies.

This research involved detailed field life history studies at several stages through the cotton cycle to understand the effect of mortality factors on survival of SLW. This usually included studies initiated in December, January, February and sometimes March. The methods used have been developed from those observed by Dr Heimoana when she visited the USDA ARS research group at Maricopa, Arizona on a CRDC funded Scientific Exchange and from extensive review of the scientific literature by Ms Tanya Smith.

Preliminary - Experiment 1 in 2010-11

In the first experiment we caged adult SLW onto leaves and after 24 h removed the adults, searched the leaves and recorded the number of eggs deposited. We thinned these back to about 5 eggs per leaf (though we later found out that we had overlooked some eggs as they are very hard to see, even with a 30x hand lens). Each egg was circled in ink and numbered so its survival could be followed every 2-3 days, until it hatched. This proved to be extremely challenging – the eggs are very small and spindle shaped – so from directly above they are very hard to see. The hatched crawlers are tiny, similar in appearance to broad mites, and move slowly across the leaf surface before settling – hence it is difficult to assign a particular crawler as having originated from a particular egg. Further, this meant that we could no longer just search the marked areas but had to scan the whole leaf surface – for the 60 leaves, each with potentially 5 crawlers, this took 3 people about 4 hours (eg, 12 person hours per inspection – using a hand lens about 80% of the time). Nevertheless, we attempted to identify the settling crawlers and followed their survival through to adult. This was again done by drawing a circle in ink around each nymph and writing a number next to the circle – as the settled nymphs don't move we could follow their subsequent development and survival. We included a treatment that was protected from beneficials in sealed fine mesh bags and a treatment that was 'open' where the bags were placed around the leaves but not sealed. The aim of the bags was to attempt to isolate mortality from environmental or plant based causes, from that caused by predation or parasitism.

Eggs took about 7 – 12 days to hatch – though we suspect some of the earlier hatchings may have been eggs already on the leaf that had been overlooked (Fig. 6 – caged data). Total developmental time took between 21-26 days. When the data was expressed as % survival from the initial number of eggs it was possible to crudely allocate the amount of mortality for each stage. This suggests that mortality of eggs was about 40% and that for every stage about 10-16%, totalling 90-95% mortality. The only species that was seen commonly associated with the SLW eggs or nymphs in each treatment were thrips adults and larvae, most other predators species were rare. There was little difference in abundance of potential predators between the cages and uncaged leaves, as thrips were attracted to the white cages and able to wriggle through the mesh, and correspondingly little difference in mortality rates (Fig. 6). Hence, we suspected that thrips might be predators of SLW and this was later confirmed (see below).

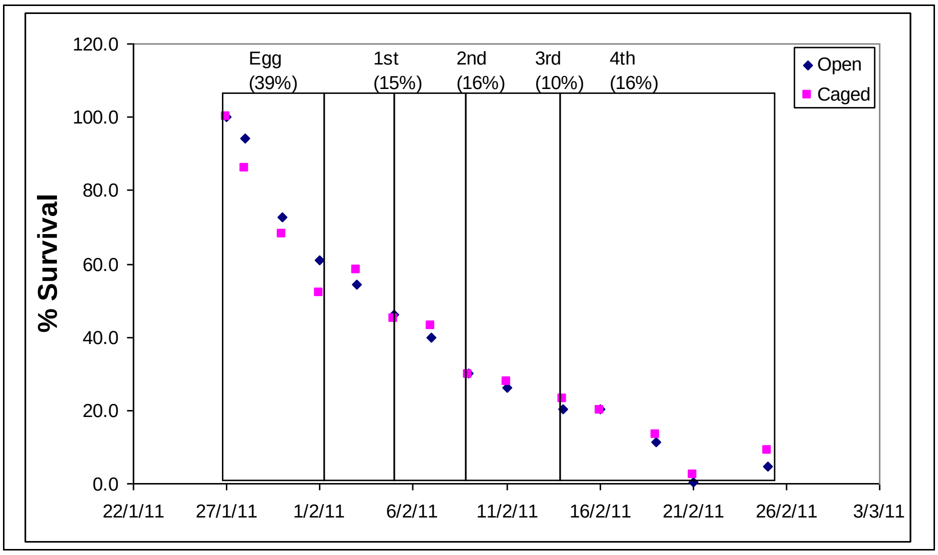

Figure 6. Survival of eggs through each stage for either caged or uncaged leaves in Experiment 1. Approximate % mortality in each stage is indicated.

Experiments 2, 3 and 4 in 2010-11

In the preliminary experiment we found that attempting to follow the crawlers was very difficult and time-consuming – and would probably lead to eye strain and staff burnout due to the high reliance on using a hand lens to search leaves over an extended period in the field under summer conditions. In later experiments we therefore separated egg survival from nymph survival – which is the approach taken by Dr Steve Naranjo in much of his pioneering work (USDA ARS, Maricopa, Arizona).

Egg Survival

This was assessed by caging adult SLW collected from our culture on leaves in the field so they could oviposit. We then marked a given number of eggs on each leaf, usually five, and monitored the survival of the eggs. The leaves were then enclosed in fine mesh bags. At a time close to the date that all eggs should have hatched we collected the leaves and made a final check for survival under the microscope. This experiment was completed twice (Experiment 2 in mid February, and Experiment 3 in late March. In Experiment 3 both protected (closed bag) and unprotected (open bag) treatments were included. Across both experiments and treatments only a small percentage could be categorically identified as eaten (3-6%), and this did not differ between protected and unprotected leaves in Experiment 3 (Table 1). Between 24-38% were missing which may have been due to dislodgement or predation. Combining missing and eaten gave a range of 30-43% egg mortality which was similar to that found in Experiment 1.

Table 1. Numbers and percentages of eggs in each class after 18 days from egg lay (Experiment 2) or 13 days (Experiment 3).

	Protected / unprotected		Hatched	Unhatched	Missing	Eaten	Total
Experiment 2	Protected	Total eggs	96	10	73	12	191
		Percentage	50	5	38	6	100
Experiment 3	Protected	Total eggs	64	8	23	2	97
		Percentage	66	8	24	2	100
	Unprotected	Total eggs	57	10	30	4	101
		Percentage	56	10	30	4	100

We thought that if eggs had been dislodged rather than eaten that the number missing would likely be higher for eggs laid near the leaf edge – which is more likely to rub against other leaves. We therefore classified the location of eggs as either near the base (where the leaf blade joins the petiole, near the leaf edge or centrally on the leaf. As the data for protected and unprotected leaves were so similar for Experiment 3 we combined these treatments. However, the data showed that the percentage of eggs missing was similar for all regions – which suggests that eggs either just fell off or were eaten with no remains left (Table 2).

Table 2. Percentage of eggs in each class for the leaf area near the junction of the petiole and leaf blade, and leaf edge (5mm) or central area for Experiments 2 and 3.

	Leaf Region	Hatched	Unhatched	Missing	Eaten	Total
Experiment 2	Base	60	0	27	13	100
	Central	50	6	39	4	100
	Edge	47	5	39	9	100
Experiment 3	Base	58	10	29	4	100
	Central	64	9	25	2	100
	Edge	56	11	22	11	100

Tanya Smith (Technical Officer), also noticed that thrips often caused damage in close proximity to eggs and wondered if this could be associated with missing eggs. She recorded whether or not there was thrips damage around each egg. The presence of thrips damage did seem to be associated with higher percentages of missing eggs (Table 3). The proportion of successful hatching was also slightly lower for eggs with thrips damage around them. It is possible that the thrips were actually consuming the eggs, leaving no remains or that they were causing the eggs to become dislodged. Predation on SLW eggs by thrips adults and larvae should be investigated further.

Table 3. Percentage of eggs in each class for eggs with thrips damage around them or no damage for Experiments 2 and 3.

	Protected / unprotected	Thrips Damage	Hatched	Unhatched	Missing	Eaten	Total
Experiment 2	Protected	No	52	5	35	8	100
		Yes	43	5	51	0	100
Experiment 3	Protected	No	81	8	8	4	100
		Yes	49	9	42	0	100
	Unprotected	No	60	12	22	6	100
		Yes	50	6	44	0	100

Nymph survival

In late February 2011 adult SLW from our culture were caged onto leaves in the field to oviposit. The resulting eggs were then allowed to hatch. Once the crawlers had settled we circled between 2 and 21 of them individually on each leaf – depending on numbers, totalling 154 nymphs (Experiment 4). These nymphs were then checked every 2-3 days for survival and where possible we also noted the cause of death (parasitism, predation, dead, missing). The leaves were enclosed in a fine mesh cage.

The results at this late stage in the season showed that development from crawler to late fourth instar took almost 4 weeks. Cumulative losses to all sources of mortality built gradually and only about 10% survived to late 4th instar (Figure 7) and emergence as adults. In this experiment mortality was initially greatest for the missing and dead categories.

However, from about the 3rd instar onward parasitism from both *Encarsia* spp. and *Eretmocerus* spp. increased peaking at about 30%. Losses directly attributable to predation (eaten) were about 10%, though it is likely that a proportion of the 'missing' category was also due to predation. Interestingly, in this particular experiment there were quite a few spur-throated locusts present, and death of nymphs due to being consumed with leaf tissue by locusts accounted for about 5% of mortality. We are now confident that we have a good methodology and can use this to investigate SLW survival in the field at different stages of the year.

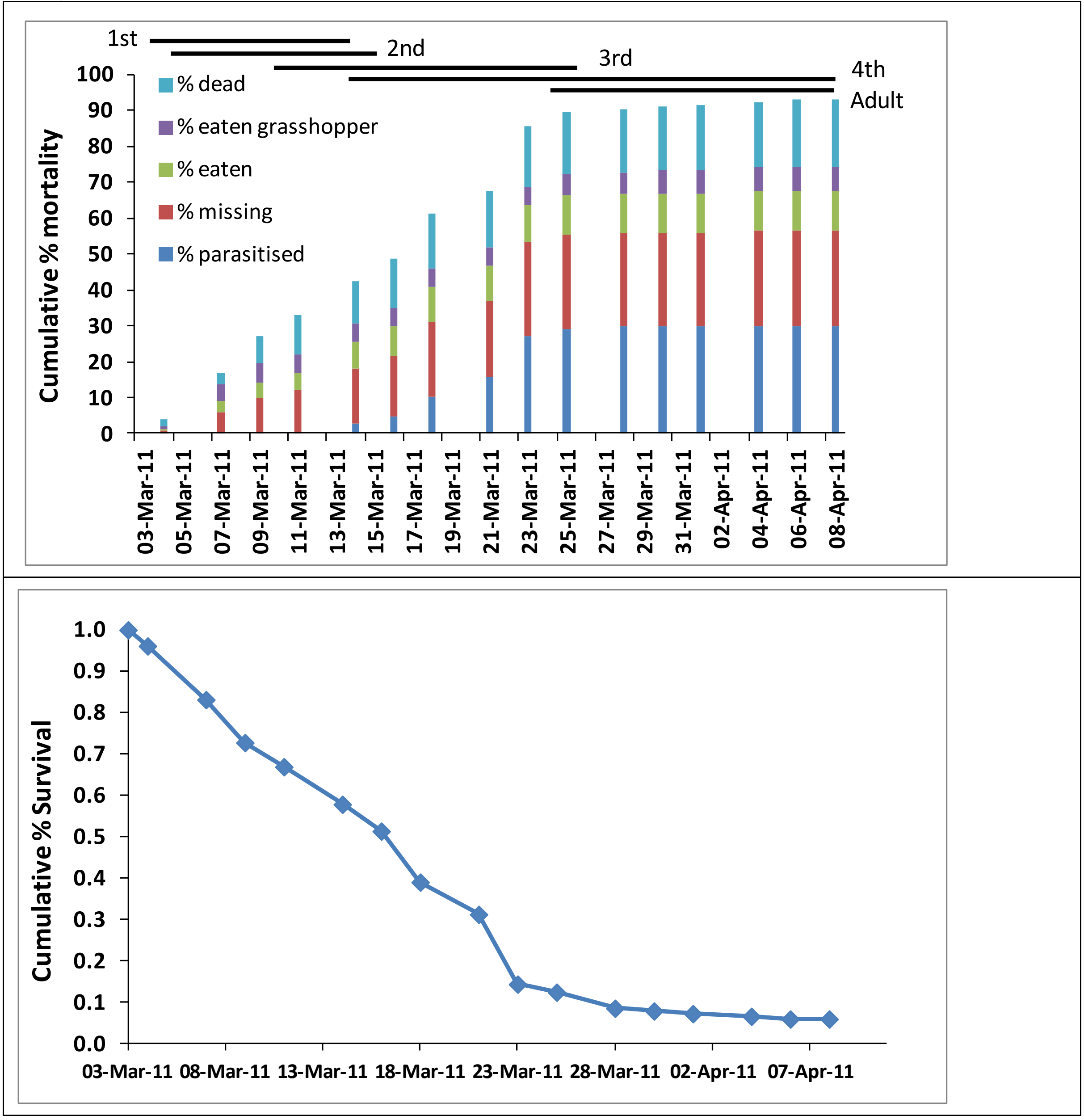

Figure 7. Cumulative survival and partitioned cumulative mortality of nymphal stages of SLW, 2011, Experiment 4.

Experiments 2011-12

Experiments 5,6,7,8 and 9 - egg survival

Egg survival in the field was studied by caging SLW adults on leaves where they laid eggs. Five eggs were circled on each of 20 uncaged and 20 cages leaves (100 eggs for each treatment). The cages were intended to exclude effects of predation. The fate of the eggs was assessed as hatched, unhatched, missing, dead or predated. Five experiments were done, beginning in mid December and ending in mid March. The proportion of eggs successfully hatching declined from 90% or more in mid December for caged or uncaged leaves, to about 60% in late February and early March for caged or uncaged leaves. Losses of eggs in late February and early March, from both the caged and uncaged leaves, increased substantially, mostly either missing (which may well be predated) or predated. This period coincided with the increase in background SLW populations on the crop, which may have resulted in build up/migration of beneficial populations as well. However, about 10% of eggs simply failed to hatch (Fig. 8).

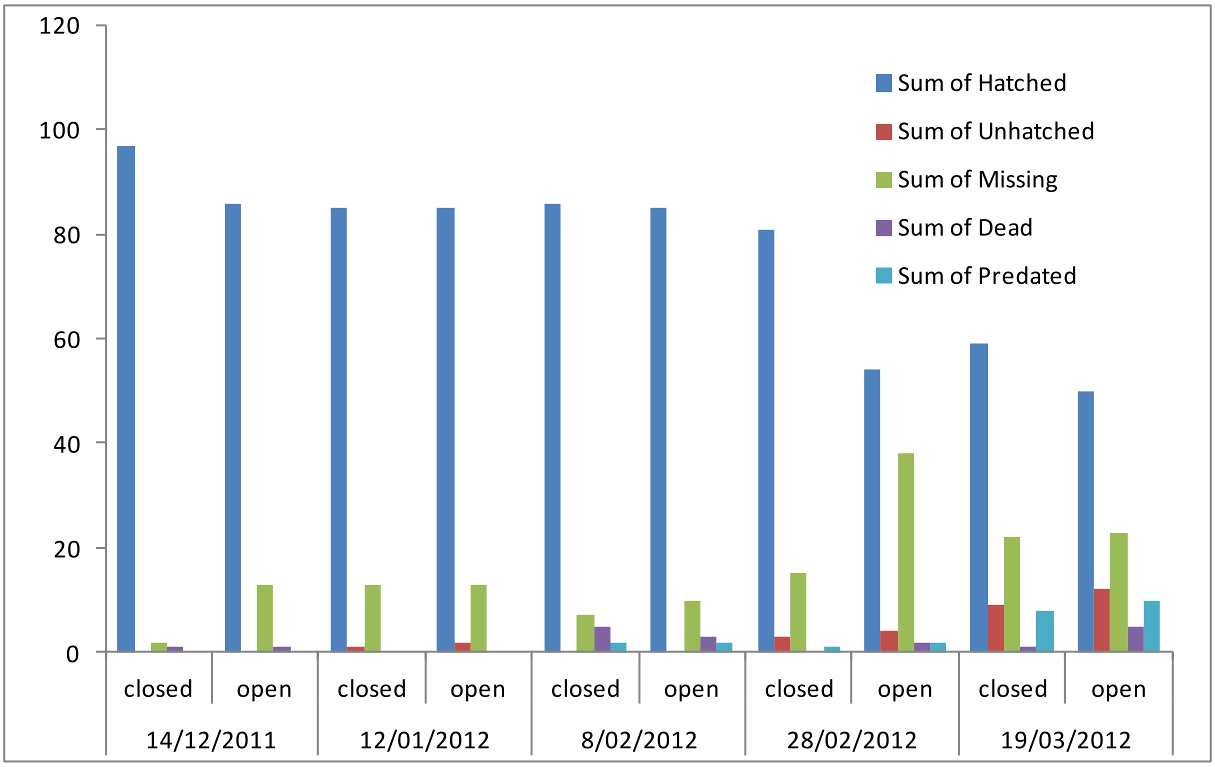

Figure 8. Number of eggs successfully hatched, not hatched, missing, dead or predated (eaten) in Block 17 ACRI, Experiments 5 – 9, 2011-12.

<u>Experiments 10,11,12 - nymph survival</u>

Nymph survival in the field was studied by caging SLW adults on leaves where they laid eggs. Eggs were allowed to hatch and once the crawlers had settled five nymphs were circled on each of 20 uncaged and 20 cages leaves (100 nymphs for each treatment). The cages were intended to exclude effects of predation. The fate of the nymphs was assessed by regular inspection of the leaves – the larvae were easy to locate as they were circled. Nymphs were recorded as healthy, missing, dead, predated or emerged. Three experiments were done, the first Experiment 10 in mid December, the second Experiment 11 in mid-January and the third, Experiment 12 in late February – limited by availability of leaves as each experiment takes more than 3 weeks of observation.

Nymph survival to emergence on closed cage leaves was about 35% in December (Fig 9 and 40% in January (Fig. 10) and February (Fig. 11). There was a shift in the main cause of mortality on the caged leaves, from mostly 'missing' in Experiment 10 to mostly 'dead' in Experiment 12. We are uncertain why this should have occurred but it may reflect declining food quality.

Nymph survival to emergence on open cage leaves was about 50% in December (Fig 9). Survival in the two later experiments was generally lower for uncaged leaves compared with caged leaves and was about 15% in January (Fig. 10) and 25% February (Fig. 11).Nymph survival on the leaves with 'open' bags showed a high proportion of eggs missing, and less dead, in Experiment 10, but a shift to a high proportion of eggs dead, and less missing, in Experiment 11. Only a few percent of nymph deaths were attributed directly to predation in Experiment 10 or 11. However, in Experiment 12 mortality due to parasitism increased to about 20% and to predation to about 10%. In Experiment 12 the proportion missing in the closed and open bags was similar at about 15%, but the proportion 'dead' was higher on leaves with closed bags.

Overall these three experiments suggest increasing losses due to parasitism and predation as the season progresses from December through to March. It is likely that these losses are higher than we have estimated because some of the 'missing' category may have been eaten with no remains left. Higher losses due to poor food quality are also possible later in the season.

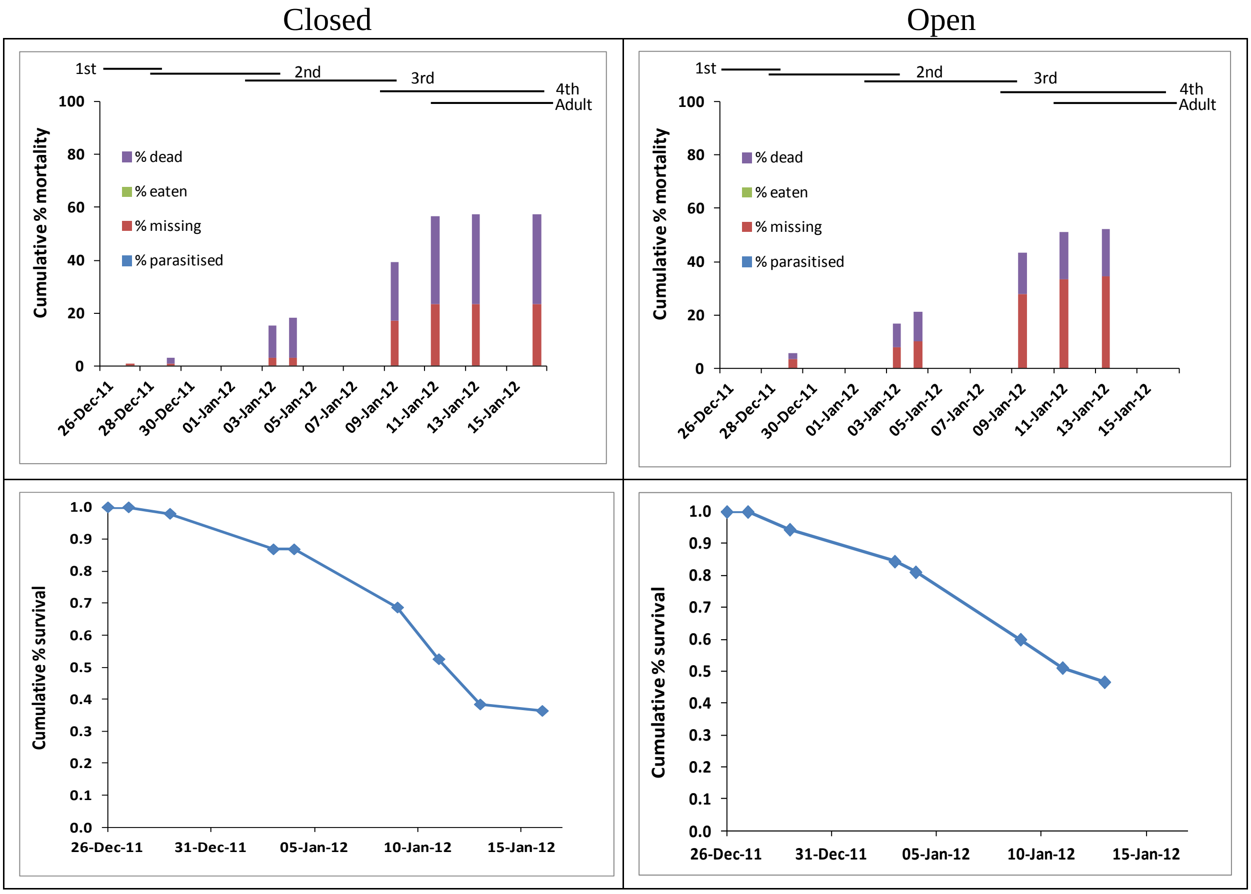

Figure 9. Lower, percentage survival of SLW crawlers on cotton leaves enclosed in mesh cages (closed) or on leaves with 'open' mesh cages (open); Upper, distribution of mortality to parasitism, missing, eaten (predation) or dead (died with no obvious cause) in Experiment 10, ACRI, 2011-12

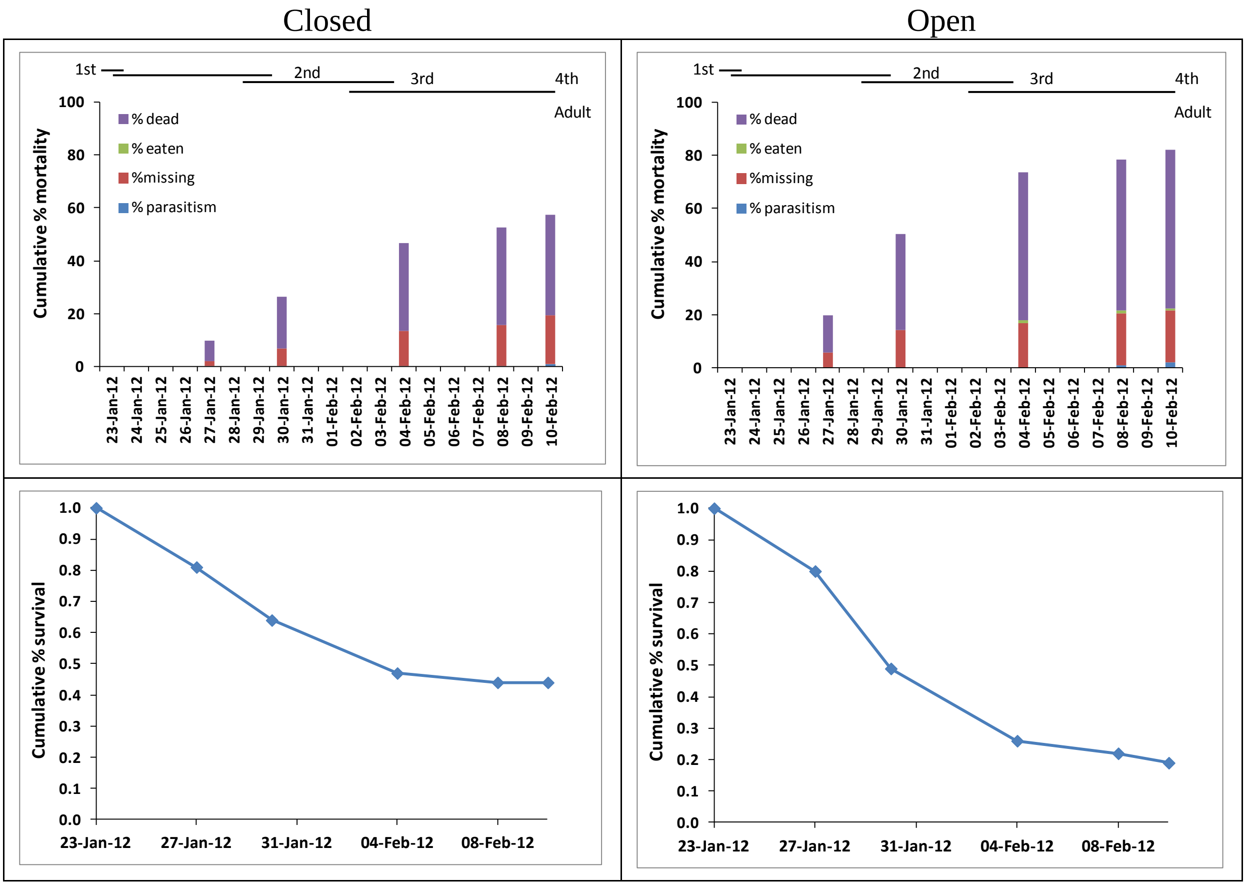

Figure 10. Lower, percentage survival of SLW crawlers on cotton leaves enclosed in mesh cages (closed) or on leaves with ‘open’ mesh cages (open); Upper, distribution of mortality to parasitism, missing, eaten (predation) or dead in Expt 11, ACRI, 2011-12

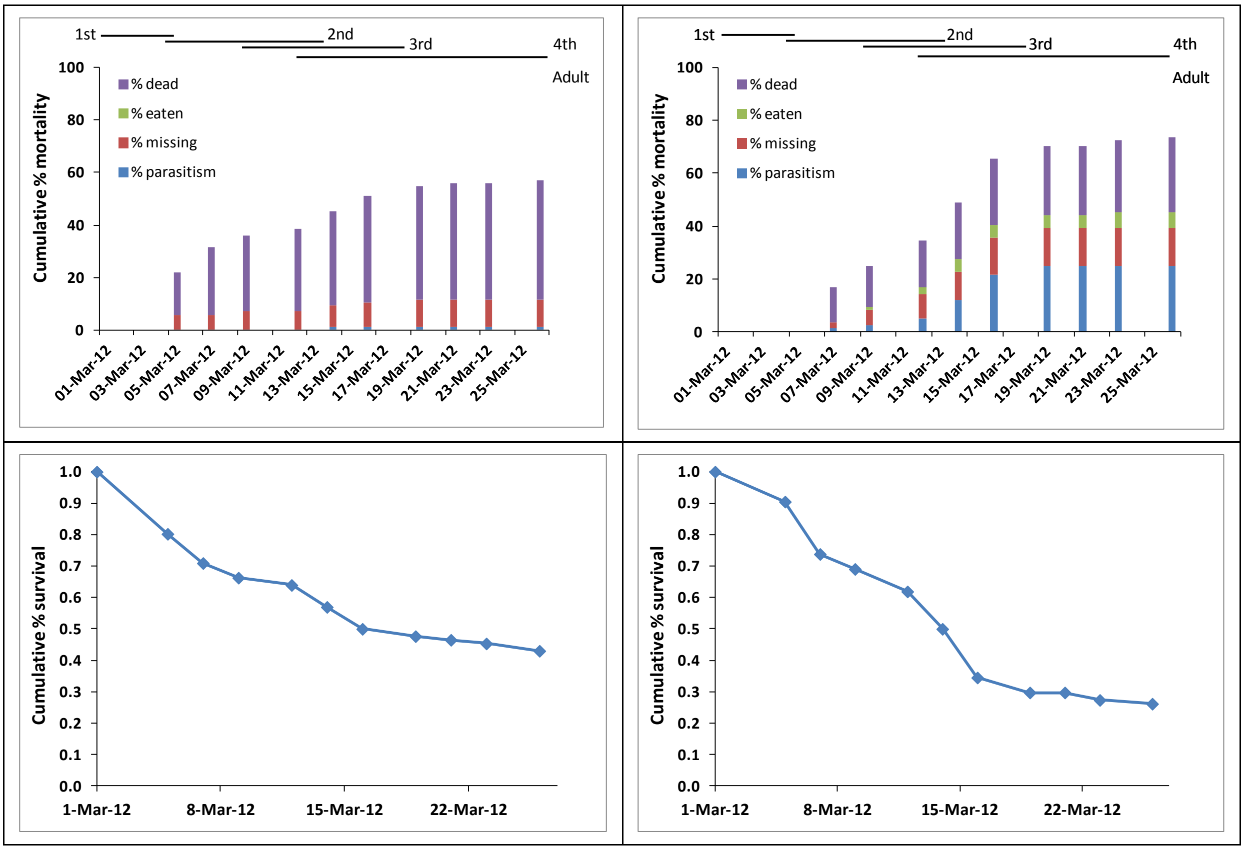

Figure 11. Lower, percentage survival of SLW crawlers on cotton leaves enclosed in mesh cages (closed) or on leaves with ‘open’ mesh cages (open); Upper, distribution of mortality to parasitism, missing, eaten (predation) or dead in Experiment 12, ACRI, 2011-12

Experiments 2011-12

Experiments 13, 14 and 15 - egg survival

Three experiments were completed assessing the survival of SLW eggs, Experiment 13 in January, Experiment 14 in February and Experiment 15 in March. In this season we changed from using white mesh bags to enclose leaves to using green cages. This was because we noticed that the white cages tended to attract thrips, some of which inevitably managed to enter the closed bags. Our studies with predation on SLW by thrips had confirmed that eggs and small nymphs may be eaten (reported below) so we felt it important to try and reduce this risk – and certainly we didn't see obviously large numbers of adult thrips on the green cages. We also included an extra treatment in Experiment 14 and 15 to test if the 'open' cage was still reducing predation – this treatment was a completely open leaf (no cage).

We found that egg survival was high in Experiment 13 in January, at over 95% for the closed cages and about 80% for the open cages (Fig. 12). The small amount of egg mortality in the open cages was mostly due to 'missing' and predation. In Experiment 14, survival of eggs in closed cages was about 75%, and was lower, though similar for both open cages and open leaves at about 45-55%. Losses in the closed cages were mostly due to 'missing'. In the open cages and open leaves losses were also mostly due to 'missing' (20-30%) and predation 5-10%. Results for Experiment 15 were similar to those of Experiment 14, though a higher proportion of eggs were scored as missing.

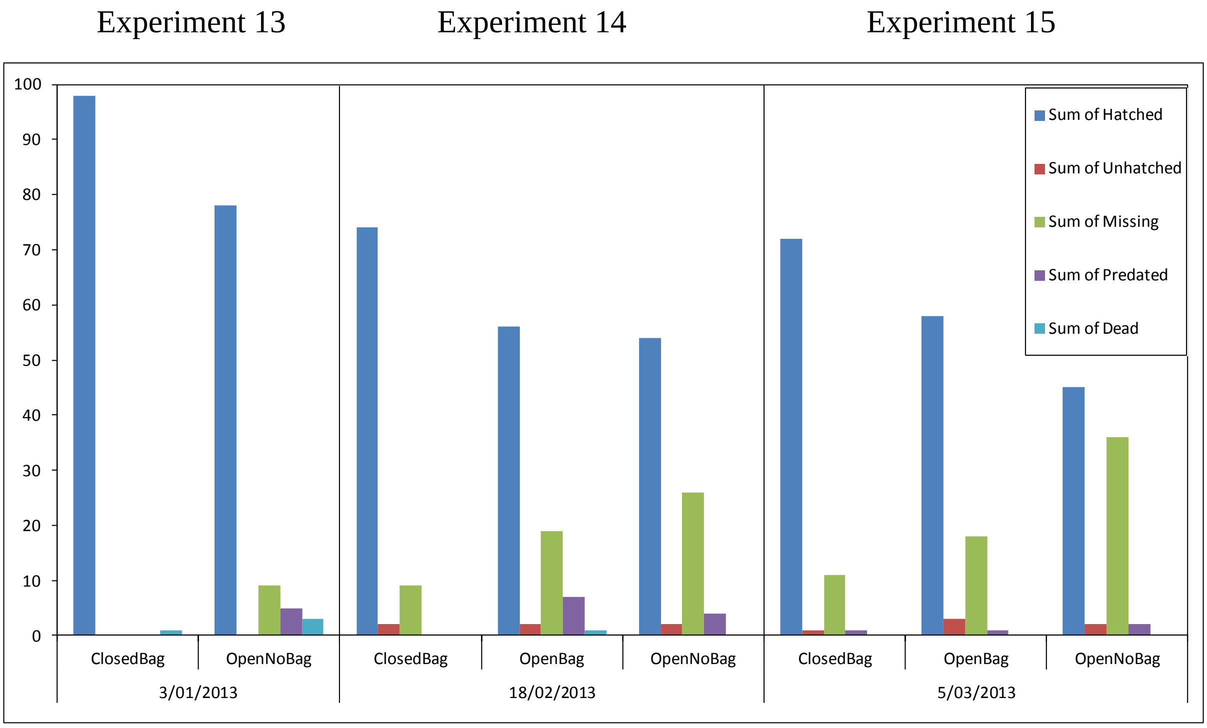

Figure 12. Number of eggs successfully hatched, not hatched, missing, dead or predated (eaten) in Block 18 ACRI, Experiments 13-15, 2011-12.

Experiments 16, 17 and 18 - nymph survival

Nymph survival was assessed in three experiments, Experiment 16 in January, Experiment 17 from mid-February to mid-March and Experiment 18 from mid- March to late- April. Survival on leaves in the closed bags declined as the season progressed from about 85% in January (Fig. 13), to 60% in late February (Fig 14) and 40% in late March (Figure 15). The cause of mortality was equally shared between missing and dead and increased as the season progressed.

In the open cages survival was about 30% in January, but much lower at about 12% in February and 8% in March/April (Figs 13-15). The results for the uncaged leaves were essentially similar to those for the open cage leaves. The main source of mortality was ‘missing’ which accounted for about 50 – 60% in all three experiments. However in the two later experiments there was an increase in morality due to ‘dead’ predation and a small component of parasitism.

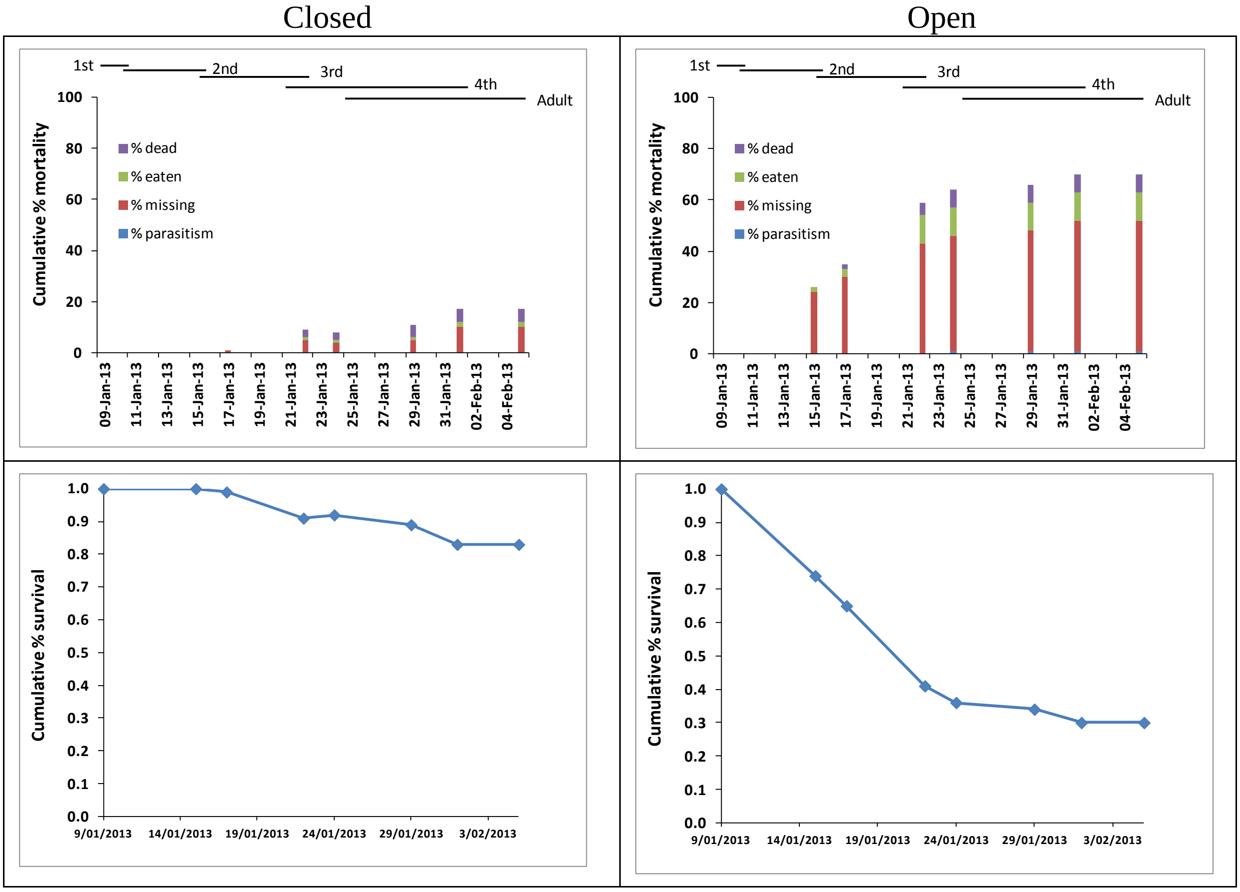

Figure 13. Lower, percentage survival of SLW crawlers on cotton leaves enclosed in mesh cages (closed) or on leaves with ‘open’ mesh cages (open); Upper, distribution of mortality to parasitism, missing, eaten (predation) or dead (died with no obvious cause) in Experiment 16, ACRI, 2012-13.

Conclusions

We have adapted methods developed by others to allow us to study survival of eggs and nymphs of SLW in the field and used these to complete 18 experiments. The results show that survival of eggs on leaves in closed cages declined from about 98% in December to about 60% in March/April (Fig. 16). Survival of eggs on leaves in open cages followed a very similar trend though was generally slightly lower ranging from about 86% in December to about 50% in March April. The main source of mortality was ‘missing’ which increased in the experiments done later in the season. The cause of the increase in the missing category was unclear but may be due to an increase in predation since an unknown proportion of missing eggs were probably due to predation (even in the closed cages). Our later experiments showed that some predators consume the whole egg, leaving no remains. This would match with the general increase in beneficial numbers in the cotton as natural SLW populations built later in the season. Another factor is that older leaves on cut-out plants will be less functional and transpire less, potentially creating a less favourable micro-climate for egg survival.

Nymphs that developed on leaves in closed cages showed variable survival rates ranging from less than 10% to about 85%. There was no consistent trend (the line displayed is not statistically significant) (Fig. 17) across the season. With the exception of one experiment with very high survival (Experiment 16) and one with low survival (Experiment 1) survival in most experiments ranged between about 35-55%. This consistency may be expected as the cages probably buffer environmental effects to some extent and provide some protection from predation and parasitism.

In the open cages there was a very clear trend toward reduced survival as the season progressed, from about 50% for experiments that began in December to less than 10% for experiments that began in March (Fig. 17). The decrease in survival was driven by a trend toward increasing predation and parasitism, and by an increase in the proportion of nymphs missing. It is likely that a proportion of missing nymphs were also eaten as our subsequent predation experiments showed that nymphs were often consumed with no remains left behind. The results strongly emphasize the need to maintain beneficial populations - especially through December to January - as a loss of predation at this stage could significantly increase survival and the risk of later outbreaks. Selection of insecticides against other pest such as mirids is therefore a critical decision. Use of a broad-spectrum product could significantly increase the risk of later outbreaks – as is discussed later in this report.

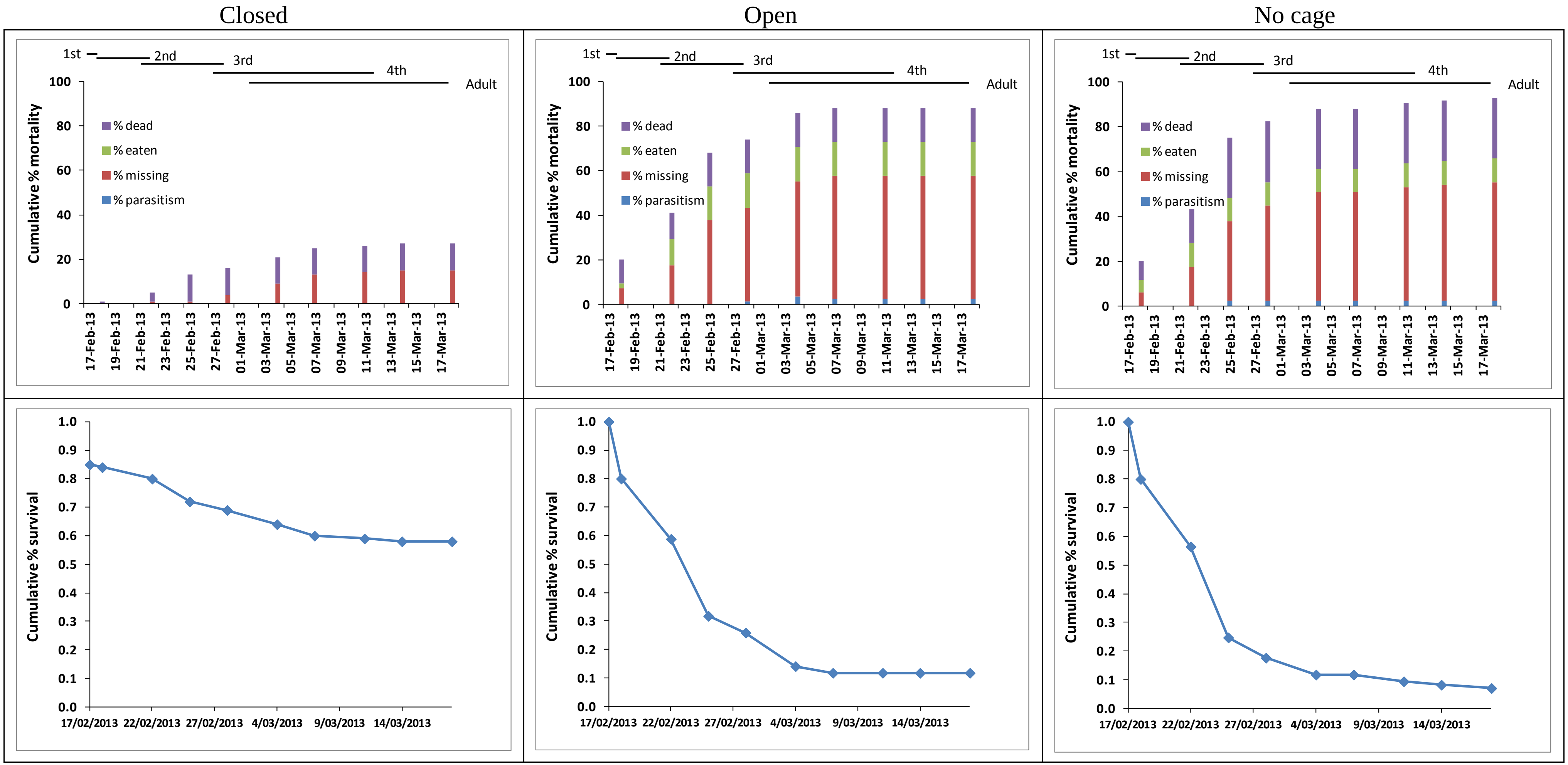

Figure 14. Lower, percentage survival of SLW crawlers on cotton leaves enclosed in mesh cages (closed), on leaves with ‘open’ mesh cages (open) or on uncaged leaves (no cage); Upper, distribution of mortality to parasitism, missing, eaten (predation) or dead (died with no obvious cause) in Experiment 17, ACRI, 2012-13.

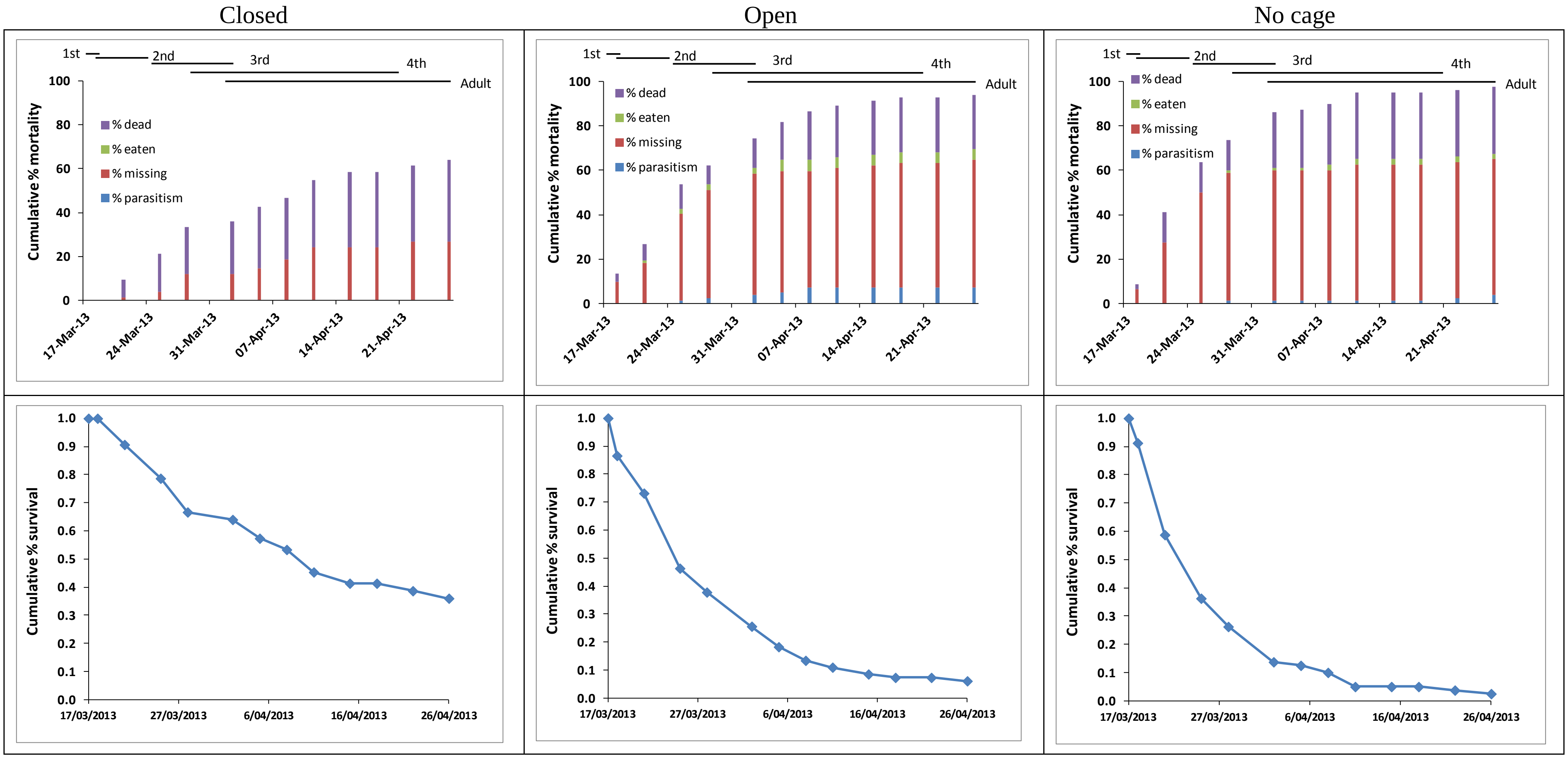

Figure 15. Lower, percentage survival of SLW crawlers on cotton leaves enclosed in mesh cages (closed), on leaves with ‘open’ mesh cages (open) or on uncaged leaves (no cage); Upper, distribution of mortality to parasitism, missing, eaten (predation) or dead (died with no obvious cause) in Experiment 18, ACRI, 2012-13.

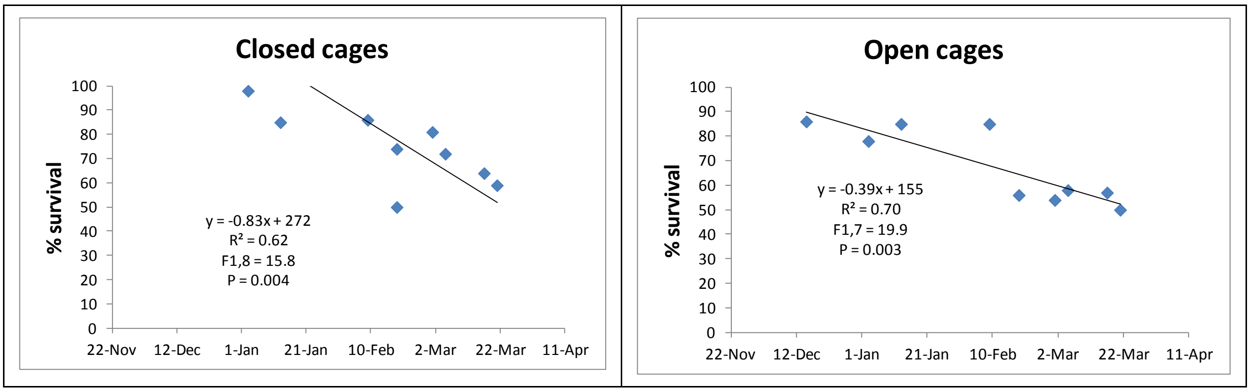

Figure 16. Relationship between survival and the time of season that experiments were done for eggs on leaves in closed or open cages, ACRI, 2010-13.

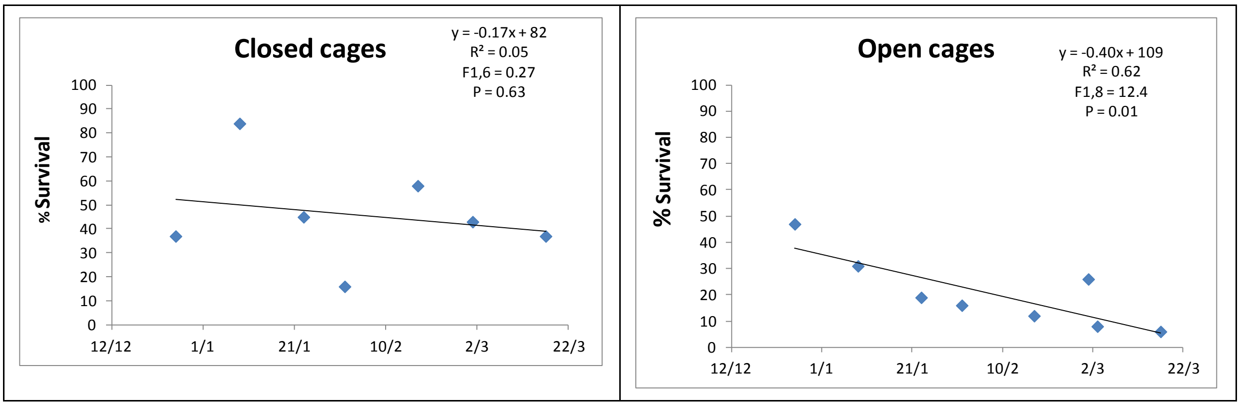

Figure 17. Relationship between survival and the time of season that experiments were done for crawlers to adults of SLW that developed on leaves in open or closed cages, ACRI, 2010-13.

1c. Predation of SLW eggs and nymphs

We also carried out some preliminary studies to identify potential predators of SLW. This was done by taking suitable leaves from our SLW colony and cutting discs with suitable numbers of eggs or nymphs. We usually physically removed extra eggs or nymphs but if numbers were close to what we were aiming for we didn't remove extras to reduce the risk of leaving damaged SLW remains on the leaf as these could affect SLW behaviour. Potential predators were collected from the field and placed with a piece of leaf tissue with a known number of eggs or nymphs on it. Control containers without predators were included. Experiments varied slightly but in general were monitored at 24, 48 and 72 hrs. We did not have the resources to culture predator species to produce adults and larvae of known ages, but instead collected specimens directly from the glasshouse (thrips only) or the field. Hence, though the studies were not as tightly controlled as we would have liked them to be, they nevertheless provide an indication of potential predators species.

The first species tested were thrips – as we often observed adults or larvae active in the vicinity of SLW eggs or nymphs. We collected second instar thrips larvae from plants in the glasshouse. These had been sprayed to control thrips at regular intervals so these survivors were most likely western flower thrips (samples have been retained for identification). SLW were caged on leaves of Sipima 380 and allowed to lay overnight. The following day the adults were removed and leaf discs with a known number of eggs were punched out and placed on moistened cottonwool in small petri dishes. Since the eggs were not deposited uniformly it was not possible to use the same number on each leaf disc, though we aimed to have similar numbers. A single thrips larvae was added to each disc (n = 35). Controls without thrips larvae (n =7) were also set up. The discs were placed in an incubator at 30°C at 14 Light:10 Dark hours and were checked daily for three days after which a final check was made at 6 days. We found that thrips larvae did consume some SLW eggs though there was no clear indication that this increased with increasing numbers of eggs offered (Figure 18). This suggests that thrips larvae will eat SLW eggs opportunistically if they encounter them. Over the six days the larvae ate about 1 egg per day, but some ate as many as 4 eggs per day – however as this is an atypical situation egg consumption in the field could be significantly higher or lower. It is possible that some of the eggs were damaged, though not fully consumed, and would not hatch successfully. Further testing with thrips larvae and adult confirmed consumption of SLW eggs and nymphs (Table 8).

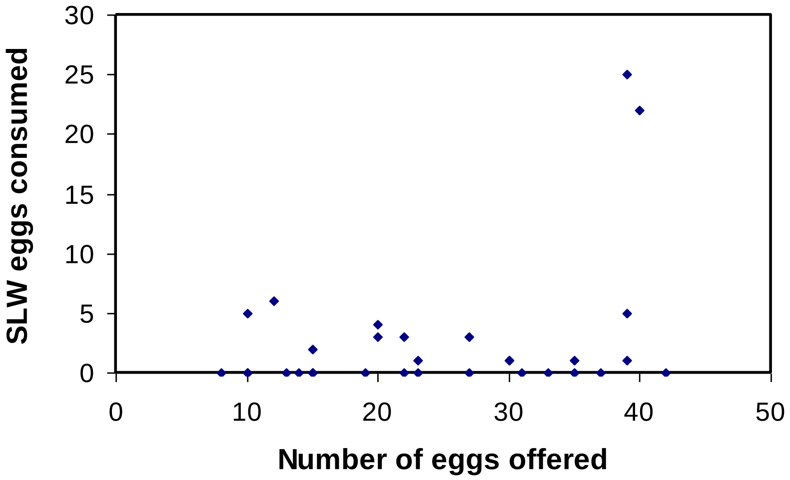

Figure 18. Consumption of thrips eggs by second instar larvae of western flower thrips over a six day period.

We also investigated predation by some other species that we had seen in association with SLW or that we suspected might take small prey items based on knowledge that they were predators of mites or aphids. This included the minute mite eating ladybeetle (*Stethorus* spp.), brown smudge bug (*Deraeocoris signatus*), transverse ladybeetle (*Coccinella transversalis*), spotted amber ladybeetle (*Hippodamia variegata*), 8-spotted ladybeetle (*Harmonia octomaculata*), variable ladybeetle (*Coleophora inequalis*), striped ladybeetle (*Micraspis frenata*), minute two spotted ladybeetle (*Diomus notescens*) and red and blue beetle (*Dicranoliaus bellulus*). We also included further studies with thrips species, and with apple dimpling bugs (*Campylomma liebknechti),* which are known to be plant feeders as well as predators of mites).

Stethorus spp. arevoracious predators of SLW eggs, with both nymphs and adults causing over 90% mortality over a 4 day period (Table 4). Brown smudge bug adults and nymphs were also quite voracious predators of both whitefly eggs and nymphs – though interestingly the small red instar nymphs did not appear to eat eggs. This may be an artefact of the experimental conditions (Table 5) and the stage of the nymphs (e.g. if close to moulting they may not feed).

A range of ladybeetle species were also tested – though only at small numbers. The results confirm that larvae of the variable, spotted amber and 8-spotted ladybeetles, and adults of the spotted amber, minute two spotted and transverse ladybeetles will all consume SLW eggs (Table 6). Similarly adults of the variable, amber spotted, striped and transverse ladybeetles will all consume SLW nymphs (Table 7). It is likely that with larger numbers tested, most of the coccinellid species will take SLW eggs and/or nymphs as prey. Apple-dimpling bug adults consumed SLW eggs and nymphs and the red and blue beetle adults consumed SLW nymphs (Table 8).

Though preliminary, these result show that a range of predatory species, including those that are also phytophagous such as thrips and apple-dimpling bugs, will attack SLW eggs or nymphs. In most cases attacked eggs or nymphs had simply disappeared – reinforcing that some of the ‘missing’ category in the SLW life history studies was due to predation.

Table 4. Percentage mortality of SLW eggs or nymphs caused by adults or nymphs of mite eating ladybeetle (*Stethorus* spp.)

Year	Predator lifestage	Whitefly lifestage	Number of predators tested	Total number of prey offered	Mean % mortality at 0 days after offering	Mean % mortality at 1 day after offering	Mean % mortality at 4 days after offering
2011	Control	Egg	4	64	0	0	0
2011	Adult	Egg	12	244	0	**65**	**92**
2011	Larva	Egg	12	246	0	**53**	**98**

Table 5 . Percentage mortality of SLW eggs or nymphs caused by adults or nymphs of brown smudge bug (*Deraeocoris signatis*).

Year	Predator lifestage	Whitefly lifestage	Number of predators tested	Total number of prey offered	Mean % mortality at 0 days after offering	Mean % mortality at 1 day after offering	Mean % mortality at 2 days after offering	Mean % mortality at 3 days after offering
2011	Control	Nymph	5	171	0	0		0
2013	Control	Nymph	1	8	0	0	0	
2011	Adult	Egg	5	69	0	**19**		**25**
2011	Brown nymph (older)	Egg	5	97	0	0		**60**
2011	Red nymph (younger)	Egg	5	102	0	0		0
2011	Adult	Nymph	5	84	0	0		**24**
2011	Brown nymph	Nymph	5	85	5	0		**68**
2011	Red nymph	Nymph	5	54	0	0		**14**
2013	Nymph	Nymph	7	57	0	**9**	**18**	

Table 6 . Percentage mortality of SLW eggs caused by adults or nymphs of Ladybeetles (Coccinellidae)

Year	Treatment	Predator lifestage	Whitefly lifestage	Number of predators tested	Total number of prey offered	Mean % mortality at x days after offering						
						0	1	2	3	4	6	7
2011	Control	Control	Egg	5	51	0		0				0
2012a	Control	Control	Egg	1	4	0			0			
2012b	Control	Control	Egg	1	5	0	0		0			
2011	*Coelophora inequalis*	Larva	Egg	3	34	0		0				**100**
2011	*Coelophora inequalis*	Adult	Egg	6	113	0		0				0
2012a	*Coelophora inequalis*	Adult	Egg	3	30	0			0			
2011	*Hippodamia variegata*	Larva	Egg	6	62	0		**3**				**24**
2011	*Hippodamia variegata*	Adult	Egg	5	118	0		**10**				**25**
2012a	*Hippodamia variegata*	Adult	Egg	2	39	0			**8**			
2012b	*Hippodamia variegata*	Adult	Egg	10	98	0	**46**		**60**			
2012a	*Diomus notescens*	Adult	Egg	5	39	**2**			**14**			
2012b	*Micraspis frenata*	Adult	Egg	2	32	0			0			
2012a	*Coccinella transversalis*	Adult	Egg	10	113	0	0		**1**	**6**		
2011	*Harmonia octomaculata*	Larva	Egg	1	20	0		**20**				**35**

Table 7 . Percentage mortality of SLW nymphs caused by adults or nymphs of Ladybeetles (Coccinellidae)

Year	Treatment	Predator lifestage	Whitefly lifestage	Number of predators tested	Total number of prey offered	Mean % mortality at x days after offering						
						0	1	2	3	4	6	7
2011	Control	Control	Nymph	5	106	0		0				0
2012	Control	Control	Nymph	1	5	0			0			
2013	Control	Control	Nymph	1	6	0	0	0	0	0		
2011	*Coelophora inequalis*	Adult	Nymph	5	102	0		0				**4**
2011	*Hippodamia variegata*	Adult	Nymph	5	121	0		**35**				**60**
2011	*Hippodamia variegata*	Adult	Nymph	5	121	0		**13**				**31**
2013	*Micraspis frenata*	Adult	Nymph	8	65	0	0	**33**	**34**	**42**		
2012	*Coccinella transversalis*	Adult	Nymph	10	99	0	**1**	**9**	**48**		**72**	

Table 8. Percentage mortality of SLW r nymphs caused by thrips, apple-dimpling bugs or red and blue beetles.

Year	Treatment	Predator lifestage	Whitefly lifestage	Number of predators tested	Total number of prey offered	Mean % mortality at x days after offering				
						0	1	2	3	4
2012a	Control	Control	Egg	3	25	0	0	0	0	
2012b	Control	Control	Egg	1	9			0	0	
2012a	Thrips	Adult	Egg	22	214	0	**5**	**14**	**18**	
2012a	Thrips	Second instar	Egg	9	98	0	**10**	**24**	**54**	
2012b	*Campylomma liebknechti*	Adult	Egg	5	50			**42**	**66**	
2012c	Control	Control	Nymph	1	5	0	0		0	
2013	Control	Control	Nymph	1	6	0	0	0	0	0
2012c	Thrips	Adult	Nymph	10	100	0	0		**32**	
2013	Thrips	Adult	Nymph	8	36	0	0	0		
2012c	*Campylomma liebknechti*	Nymph	Nymph	5	48		**26**	**69**	**92**	
2013	*Dicranolaius bellulus*	Adult	Nymph	8	69	0	0	**31**	**38**	**41**

2. *Researching IPM compatible management strategies for SLW and mirids*

Methods

These experiments were developed to answer several questions related to the development of SLW outbreaks. The rationale for these experiments was:

> i. Consultants reported more issues with SLW on BGII than conventional cotton. We suspected that this was likely to be due to differences in spray regimes, rather than the presence of the transgenes *per se* – but this needed to be tested, so the experiments included Sicot 71 BRF and RRF.
>
> ii. As a further component of (i), sprays applied against mirids or other sucking pests may reduce beneficials and increase risk of outbreaks of SLW which has proven to be important in development of management strategies for SLW in Central Qld. This needed to be tested in central growing regions and if true, is an important message to substantiate for industry.
>
> iii. In small plot experiments with cotton genotypes the okra leaf shaped lines tended to have fewer whitefly. Does this hold true with commercial cultivars and is there any interaction with spraying?

The experiments each had 24 plots (3 varieties x 2 spray treatments x 4 replications). Plots were challenging to manage and involved artificially infesting cotton with SLW. This was achieved by culturing SLW on kale plants in a glasshouse during spring, then transplanting the kale plants into the cotton plots – 1 or two per plot depending on the experiment (Fig. 19). This generally worked well and we were able to generate good SLW numbers in 2 of three experiments. The spray treatments were applied with a 'Spray Mantis' sprayer and included several different sprays in Experiment 1 but only dimethoate in the experiments 2 and 3.

Figure 19. Transplantation of kale heavily infested with SLW to generate infestations in field plots.

The abundance of SLW adults and nymphs (healthy and parasitised) was scored each week. Data were log transformed (ln(x+1)) for analysis. Suction samples to quantify the abundance of beneficials were also taken weekly.

Results and Discussion

Experiment 1.

We found significant effects of spray treatments ($F_{1,267} = 43.1$, $P < 0.001$) and variety ($F_{2,267} = 10.1$, $P < 0.001$) (leaf shape or presence of Bt) and interactions between variety and spray ($F_{2,267} = 3.6$, $P < 0.029$). Sprayed cotton (mean of 1.9 SLW per leaf) had more SLW in total (adults and nymphs) than unsprayed cotton (mean of 1.25 SLW per leaf) (Fig 20A). In part, this was likely due to a reduction in predator species in the sprayed plots (Figure 20B). The varietal comparison (Fig. 20C) shows that (i) the okra leaf shape variety had less whitefly than the two normal leaf varieties (ii) the GM variety had less SLW than the non-GM variety. The latter result was likely due to the ongoing *Helicoverpa* damage to the non-Bt variety, which consequently continued growing and remained with fresh new growth that was attractive to SLW, whereas the Bt-cottons had retained more fruit and were maturing earlier as a result. Mirid sprays resulted in larger SLW populations on the normal leaf varieties than on the okra leaf variety (Fig. 20D).

Aphids were strongly negatively affected by spraying ($F_{1,249} = 11.2$, $P < 0.001$) with populations overall much lower in the sprayed plots, the opposite trend to mites (Fig 20E). This probably reflects the multiple use of dimethoate as the mirid control option. Cotton aphids are susceptible to dimethoate so multiple applications would be expected to dramatically reduce populations.

Mites were also strongly affected by spraying ($F_{1,249} = 132$, $P < 0.001$), by variety ($F_{2,249} = 6.21$, $P < 0.001$) and by the interaction of spray by variety ($F_{2,249} = 9.76$, $P < 0.001$). However, the spray by treatment was most revealing, showing clearly that mites were more abundant in sprayed plots, and that this effect was stronger on the okra leaf variety (Fig. 20F).

Experiment 2.

This experiment was less successful due to difficulty in establishing a strong whitefly population and contamination of plots with high numbers of spider mites and aphids. In addition the non-Bt cotton plots suffered from high populations of loopers which caused heavy defoliation. Nevertheless we persisted with the experiment as it had value for comparing the effects of sprays on Bollgard II and conventional cotton on mites and aphids.

For whitefly, which were a combination of silverleaf whitefly and greenhouse whitefly that season, there were significant effects of date by treatment ($F_{8,120} = 3.53$, $P < 0.001$) and date by variety ($F_{1,120} = 1.76$, $P = 0.042$). The date by treatment interaction showed higher abundance of whitefly in the sprayed plots toward the end of the experiment (Fig. 21A). The date by variety interaction showed slightly lower abundance of SLW on the okra leaf variety on one date and on the Bt-normal leaf on another (Fig 21B).

Aphids showed a strong spray effect ($F_{1,120} = 318$, $P < 0.001$) and spray by variety interaction ($F_{2,120} = 11.5$, $P < 0.001$). The spray effect showed that aphids were less abundant on sprayed plots (0.6 aphids per leaf) than on unsprayed plots (2.3 aphids per leaf) (Fig. 21C) and probably reflects the multiple use of dimethoate as the mirid control option ($F_{1,120} = 318$, $P < 0.001$) as discussed above. The spray by treatment effect also showed that aphids were more abundant on sprayed plots, but this effect was stronger for Sicot 71 RRF (non-Bt, normal leaf), probably because of a more attractive growth late in the season and which was confirmed by a significant variety by date effect ($F_{14,120} = 2.33$, $P = 0.007$) (Fig 21D).

Spider mites similarly showed strong spray effect ($F_{1,120} = 72.9$, $P < 0.001$) (but in the opposite direction to aphids) and variety effects ($F_{2,120} = 7.37$, $P < 0.001$). Mites were significantly more abundant in the sprayed plots than the unsprayed plots (Fig 21E). This probably also reflects use of dimethoate, to which mites are resistant, which would reduce beneficial abundance. Mites were more abundant on the non-Bt normal leaf (Sicot 71RRF), possibly because of its extended growth period due to damage to fruit by Helicoverpa species (Fig 21F). Across these three pest species the strongest effects were therefore due to spraying and there were no consistent effects of variety or transgene.

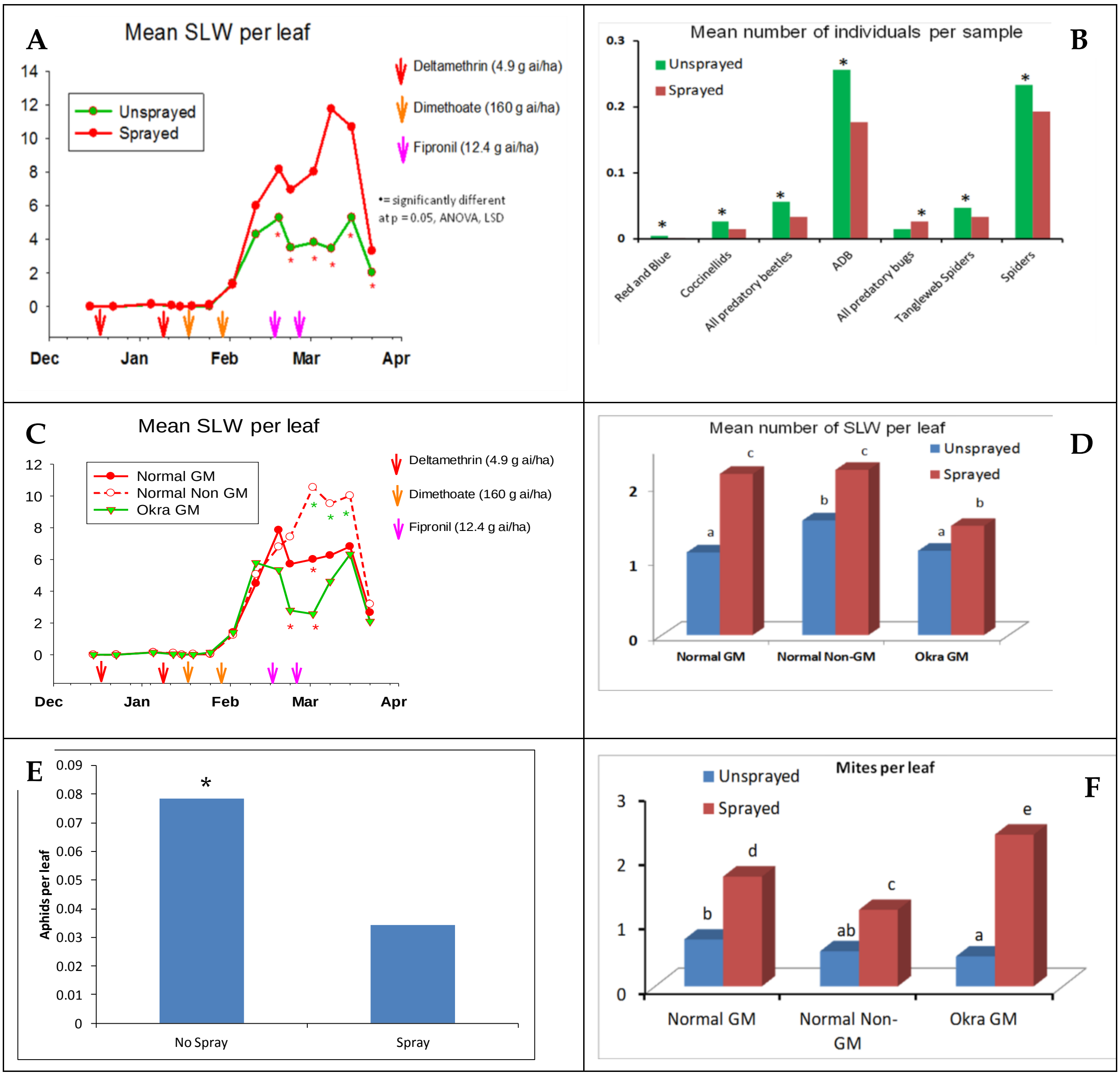

Figure 20. (A) Effect of mirid sprays on development of SLW nymph and adult populations and (B) on beneficial species. (C) Comparison of SLW abundance on three varieties and (D) effect of interaction between spraying and variety on SLW numbers. (E) Effect of mirid sprays on aphid abundance and (F) effect of interaction between spraying and variety on mite abundance in Experiment 1. Asterisks indicate significant differences between treatments (for (C) green = different to both other treatments, red next to red line = difference between the two normal leaf varieties, red next to green line = significant difference between okra leaf and normal leaf GM varieties.). Bars with the same letter are not significantly different at P = 0.05.

Experiment 3.

Experiment 3 was successfully infested with SLW. There were significant effects of spray ($F_{1,267} = 43.1$, $P < 0.001$) and variety ($F_{1,267} = 43.1$, $P < 0.001$) but the interaction term of spray by variety was not significant. Overall, there were more SLW in the sprayed (9.6 SLW per leaf) than the unsprayed plots (4.7 SLW per leaf) (Fig 22A). In part this was likely due to a reduction in predator species in the sprayed plots (Fig. 22C). SLW were also significantly less abundant on okra leaf (4.0 SLW per leaf) than normal leaf (7.3 SLW per leaf) cotton and were more abundant on non- Bt normal leaf cotton (10.4 SLW per leaf) than Bt-normal leaf cotton (7.3 SLW per leaf) (Fig. 22B).

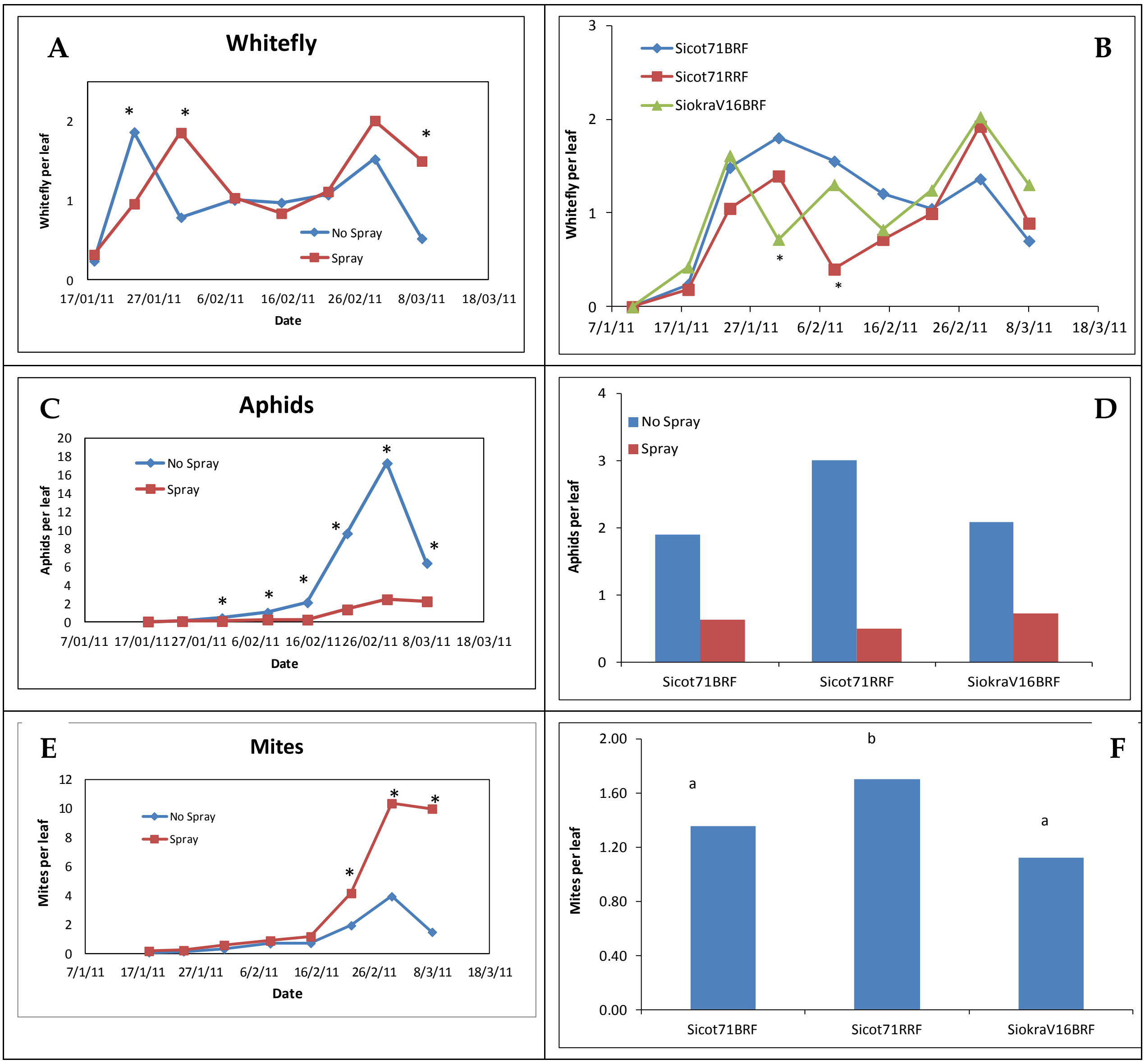

Figure 21. (A) Effect of mirid sprays on development of SLW nymph and adult populations and (B) comparison of SLW abundance on three varieties. (C) Effect of spray on aphid populations and (D) effect of interaction between spraying and variety on aphid numbers. (E) Effect of mirid sprays on mite abundance and (F) effect of variety on mite abundance in Expt 2. Asterisks or letters indicate significant difference between treatments at P=0.05.

The levels of honeydew contamination on bolls followed the patterns of SLW abundance and were lower on unsprayed cotton and okra leaf cotton (sprayed or unsprayed) and highest on non-Bt cotton (Fig. 22D) (spray x variety, $F_{2,51} = 5.22$, $P = 0.009$).

Aphids showed a spray effect and were more abundant in the unsprayed treatment (0.57 aphids per m across all dates) ($F_{1,105} = 6.36$, $P = 0.013$) than the sprayed treatment (0.4 aphids per m across all dates), as observed in the first two experiments. Again this is probably related to use of dimethoate which controlled the aphids.

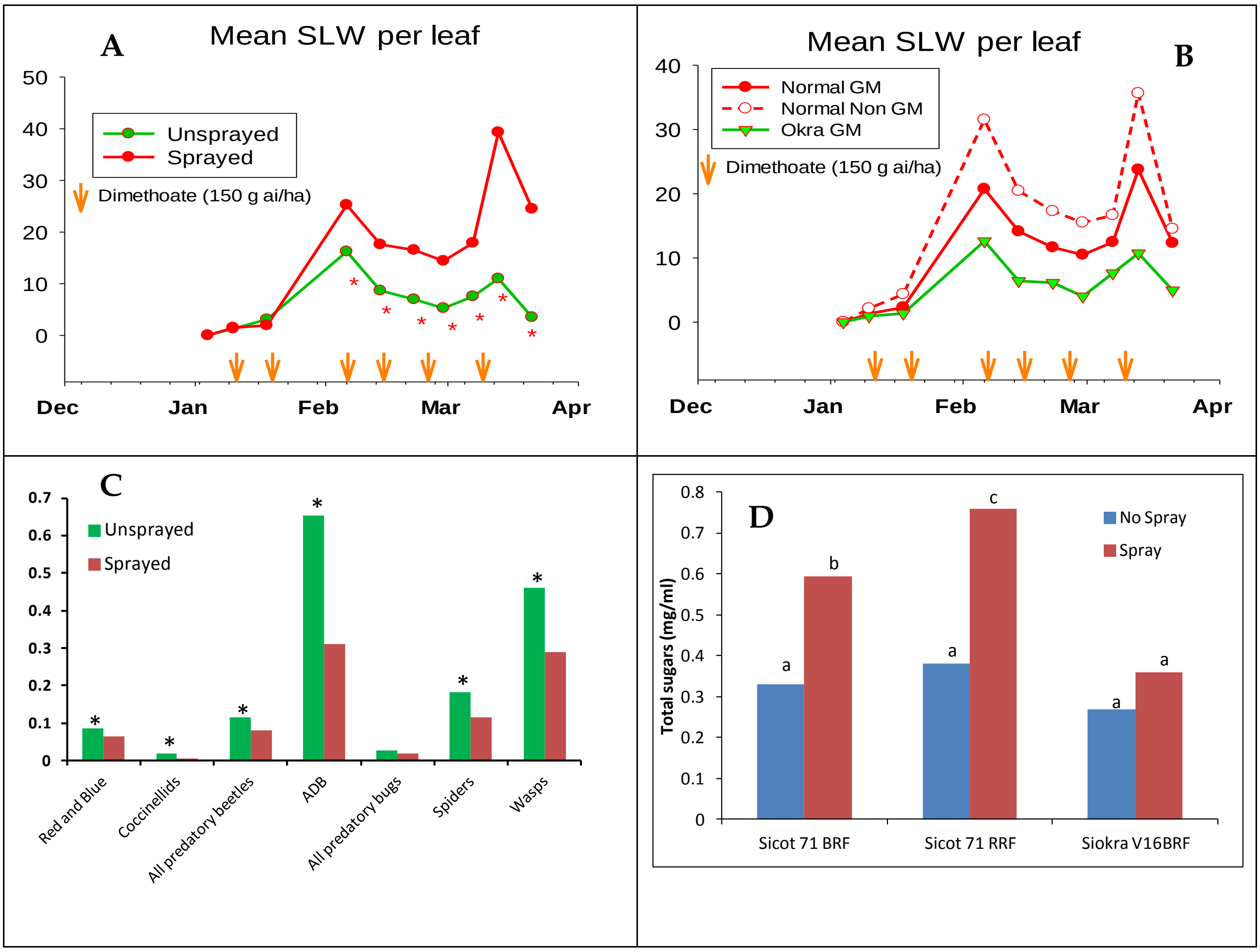

Figure 22. Effect of mirid sprays on (A) SLW and (C) predator populations and (B) effect of variety on development of SLW nymph and adult populations (D) effect of interaction between spraying and variety on total contaminant sugar concentration on bolls in Experiment 3. Asterisks or letters indicate significant difference between treatments at P=0.05.

Conclusions

Our results show:

1. No evidence that SLW are worse on Bt-cotton than non-Bt-cotton. Differences observed in the industry are therefore likely to be due to differences in spray regimes between Bt and non-Bt cotton. Some of the insecticides applied to non-Bt cotton for control of *Helicoverpa* spp. tend to suppress SLW, including emamectin, rynaxapyr and abamectin (and formerly endosulfan). In contrast, those applied to Bt-cotton are targeted against mirids and are often quite detrimental to beneficial populations but do not suppress SLW – which can then build quickly.

2. Okra leaf shape offers considerable resistance to SLW and reduces the risk of the crop suffering severe honeydew contamination.

3. Even though SLW has a long life cycle (21-35 days) compared with mites or aphids (about 7 days), insecticides can still significantly influence the development of populations. Our results show very consistently that broad spectrum sprays applied against mirids can lead to much higher populations of SLW.

4. Confirmed results found in previous projects we also showed that use of broad-spectrum insecticides (dimethoate) against mirids also increased the risk of outbreaks of spider mites, probably due to negative effects against beneficial species.

5. Aphid abundance was lower in the sprayed plots even though there is evidence that beneficial numbers were negatively affected. This is because dimethoate controls the aphids. However, it's use against mirids is also inadvertently selecting for OP/carbamate resistance in aphids, and this should be minimised.

3. *Investigating breakdown of honeydew on lint and effect on fibre quality.*

Methods

A range of experiments has been completed investigating the fate of honeydew on cotton lint, including the effects of UV radiation and rainfall. The experiments are presented chronologically but there is a conclusion at the end to bring the results together. The experiments used either naturally deposited aphid or SLW honeydew, or artificial honeydew created by mixing sugars in ratios (Table 9) similar to the published ratios for SLW honeydew. We collaborated with Dr Paul De Barro (CES) to develop some of the methods and with Dr Michael O'Shea to design experiments and provide analysis of the samples ($40 per sample).

Table 9. Composition of artificial SLW honeydew.

Sugar	%
Glucose	10
Fructose	13
Sucrose	18
Trehalulose	43
Melezitose	16

A technique was developed to apply about 1 ml of honeydew to bolls collected from the field using a perfume atomiser. To ensure we could store treated bolls without further breakdown of honeydew we evaluated the ratios of each of the honeydew constituents in artificial honeydew that had been stored in the freezer, fridge or applied to bolls then stored in the freezer for 72 days. These were evaluated against the original ratios used in the formulation of the honeydew. Freezing or refrigerating prevented honeydew breakdown, even when applied to bolls, but although storage in the fridge prevented breakdown the honeydew became mouldy so all samples were stored frozen (Fig. 23). The slight increase in glucose and fructose in treatments other than the original may be explained by the breakdown of sucrose into these components. Melezitose and trehalulose are relatively stable.

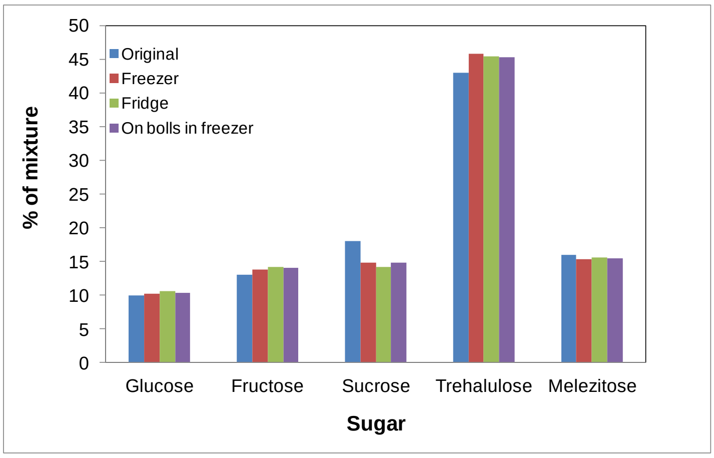

Figure 23. Sugar ratios in artificial honeydew. Original mixing ratios and ratios ofter storage in the freezer, refrigerator or applied to bolls then stored frozen for 72 days.

All experiments used a similar basic formula. Essentially, clean bolls were collected from the field. These bolls were contaminated with artificial honeydew in the laboratory using the perfume atomiser and a repeatable technique. A ‘set’ of bolls was retained in the freezer as a reference for ‘before exposure’. The remaining bolls were placed in the field, usually attached to a short branch in the lower canopy, but in some cases (e.g. testing exposure to sunlight) they were placed in the upper canopy. Bolls were collected after the prescribed periods and taken to the laboratory where they were stored in the freezer until the experiment was complete. At this stage the exposed and the control bolls were washed using a standard procedure and a sample of the rinsate was collected and stored frozen for transport to Michael O’Shea at BSES for analysis.

Results

1. Fate of honeydew deposited by cotton aphid.

The first experiment made use of opportunistic aphid (*Aphis gossypii*) populations present in the field at ACRI. Collections of bolls (10 bolls by 10 replicates; 100 bolls per date) were made on a weekly basis over about a 2 month period. The samples were washed in lots of 10 bolls to extract honeydew, and a sub-sample was taken and frozen. During the sampling period there were aphids in the crop which were secreting honeydew, though aphid numbers declined during the later sample dates. Between the first and second dates, and the third and fourth dates it is clear that aphid honeydew levels had increased due to the ongoing presence of aphids (Fig. 24). Rainfall between the second and third, the fourth and fifth and the fifth and sixth dates is associated with decline in honeydew levels. It is most likely that this was not due to breakdown of the sugars but to the solubility of the sugars, so rainfall essentially washed them away. Each rainfall event significantly reduced honeydew levels ($P < 0.05$ for all sugars). Interestingly this aphid honeydew consisted primarily of glucose and fructose, with only very small amounts of sucrose, melezitose or trehalulose.

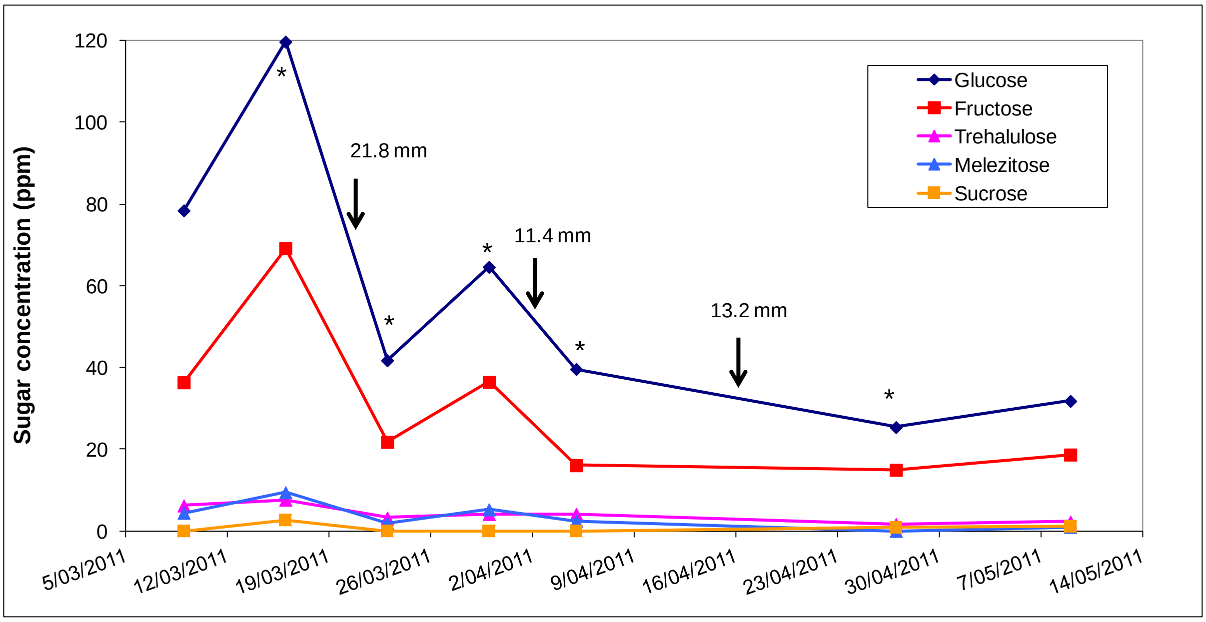

Figure 24. Concentration of sugars from aphid honeydew on open cotton bolls over time. Rainfall events are indicated. Asterisks indicates sugar concentrations on that data significantly different to the previous date using ANOVA / LSD in Genstat 13.

2. Fate of artificial SLW honeydew after single rain event.

We collected bolls of about the same age and size from a field that did not have SLW or aphid populations. These bolls were treated with artificial honeydew using the standard application procedure. A control set of treated bolls (10 bolls by 10 replicates) was frozen. We placed a set of bolls (10 bolls by 10 replicates) out just before a rain event and collected it after 7 days. The control (frozen) and exposed bolls were then all washed out at the same time. The rainfall event occurred over five days and totalled 21.8 mm. The rainfall dramatically reduced honeydew levels, again most likely to the sugars being washed from the bolls rather than by being broken down – given the short time period of exposure (Fig. 25).

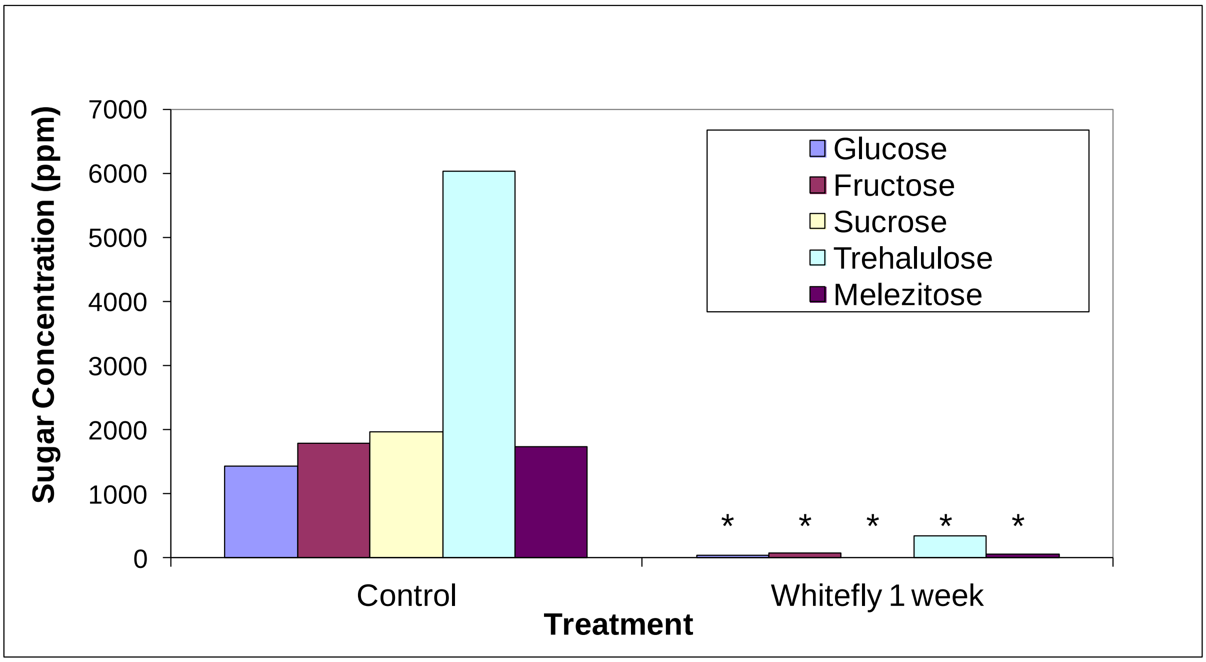

Figure 25. Sugar concentrations in cotton bolls artificially treated with honeydew and stored frozen (control) or exposed in the field for 1 week during a rainfall event totalling 21.8 mm over 5 days. Differences between control and exposure for one week are all highly significant ($P < 0.001$ in all cases using ANOVA/LSD).

3. Fate of artificial honeydew after multiple rain events.

This was an extension of Experiment 2 as bolls were exposed for a prolonged period which included several rainfall events. Bolls were collected and contaminated with artificial honeydew. A control set (10 bolls x 10 replications) was stored frozen for later analysis. The bolls were then all placed in the field (enough for 3 collection dates, each 10 bolls by 10 replicates = 300 bolls) and left there for 8, 22 or 36 days (Fig. 26), after which they were collected and washed to remove the honeydew. At 22 and 36 days additional samples of uncontaminated bolls in the field were also collected and processed to estimate the natural levels of sugars on the bolls.

Rainfall events between the first and second dates and second and third dates both significantly reduced the concentration of sugars – again probably by washing the sugars out (Fig. 26). Between the third and fourth dates, where there was no rain, there did not seem to be any further reduction. Sugar concentrations on uncontaminated field bolls were still substantially lower than on treated bolls exposed for 36 days.

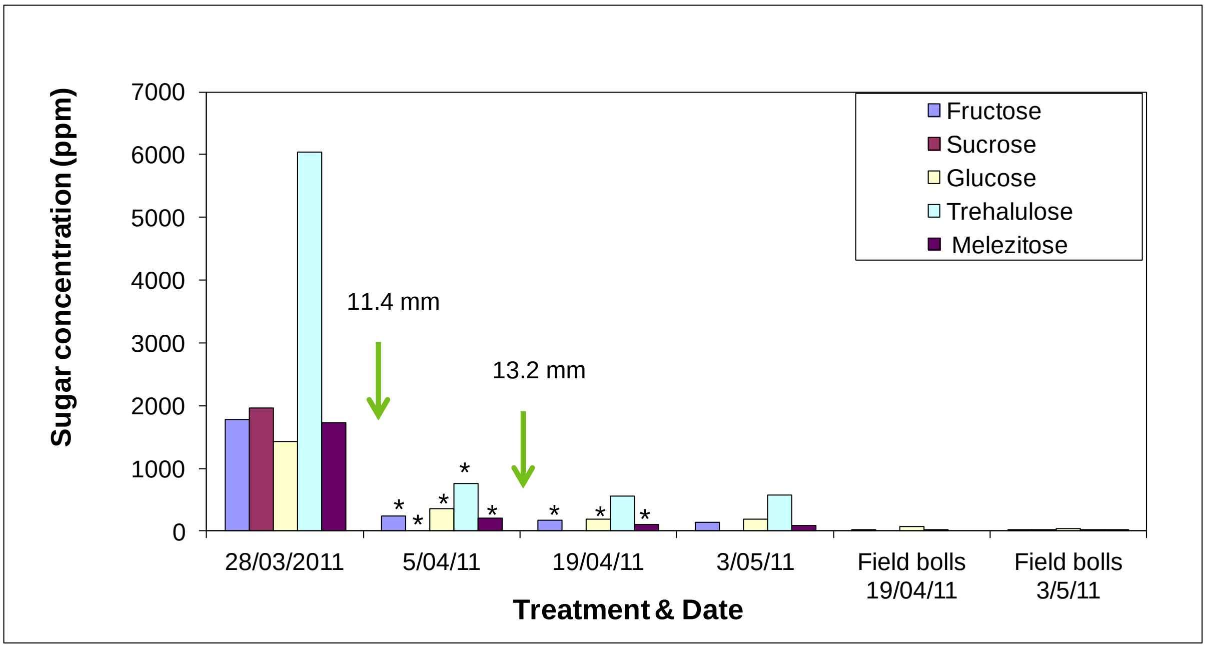

Figure 26. Sugar levels on bolls after initial treatment with artificial honeydew and after exposure for 8 (5/4), 22 (19/4) or 36 (3/5) days. Sugars on uncontaminated bolls from 2 dates are also shown for reference. For the artificial honeydew treatments, sugar levels significantly different from the previous date ($p < 0.05$) are indicated using an asterisk. For the comparison of field and artificial honeydew on the 3/5/11, all sugars were significantly lower in the field bolls ($p < 0.001$ in all cases) except for sucrose whish was virtually zero in both cases. Comparisons between field and artificial honeydew bolls could not be made for 19/4/11 due to only one replication of the field bolls.

4. Effect of sooty mould on honeydew

In this experiment we were seeking to develop a method to analyse the effect of microbial breakdown by sooty mould of honeydew concentrations on cotton fibres. We used bolls collected from the field – one third untreated, one third treated with honeydew and the final third treated with honeydew and inoculated with sooty mould collected from bolls in the field. Bolls of each treatment were sealed into plastic bags and kept in an incubator at 29°C and checked periodically. The sooty mould developed slowly and after 24 days and 42 days a set of bolls from each treatment was washed off to extract the sugars. The samples were frozen prior to analysis and a sample of sooty mould was taken for identification (Fig. 27).

Figure 27. Bolls with no honeydew, honeydew or honey dew plus inoculation with sooty mould after 3 weeks in an incubator.

Despite being incubated for 42 days there was no real change in the sugar concentration over time (Fig. 28). This is surprising and may be due to the experimental design as the bags were sealed and this may have limited oxygen availability. We will need to talk to a pathologist and also a sugar chemist about the design and possible modifications. Sooty mould fungi (generally Ascomycetes) identified from cotton grown at ACRI in 2011 included primarily *Cladosporium cladosporioides* and *Rhizopus stolonifer* (a Zygomycete) as well as traces of *Fusarium oxysporum, Alternaria tenuissima, Aspergillus flavipes* and *Eurotium chevaleri* (Michael Priest, NSW Agriculture, Orange).

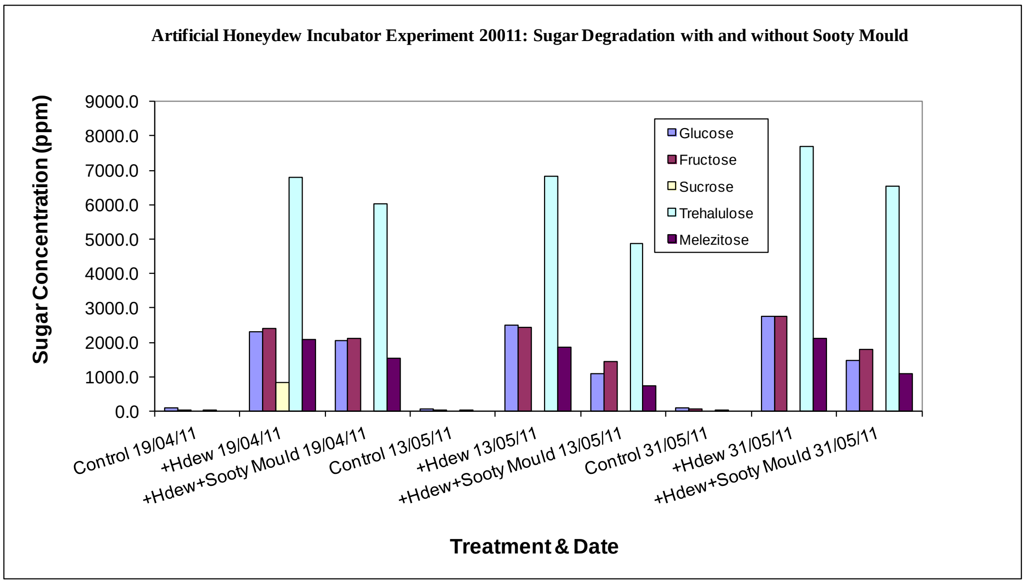

Figure 28. Sugar concentration on bolls with no honeydew, honeydew or honey dew plus inoculation with sooty mould after 3 weeks in an incubator.

5. Quantify amounts and fate of naturally deposited SLW honeydew.

We identified the need to quantify how much honeydew was naturally deposited by SLW or aphids and to monitor the build up and fate of honeydew under natural conditions. This also provided a reference point for future experiments to adjust the amount of artificial honeydew applied to bolls to levels similar to those naturally occurring following outbreaks of SLW.

Bolls were collected weekly from plot 24 in Experiment 3 in the Mirids by Whitefly experiment described in the previous section. The whitefly population had just begun to decline and counting ceased two weeks after the sampling of bolls began (Fig. 29). Nevertheless, honeydew accumulation continued even with a lower SLW population. Two key points emerged (i) firstly the level of honeydew on these bolls was very noticeable but was still only about 1/10th of the level that we typically applied for artificial contamination (ii) secondly, 18.8 mm of rainfall spread over a number of small events was effective at removing most of the sugars, back to negligible amounts (even though bolls still were covered with residual sooty moulds).

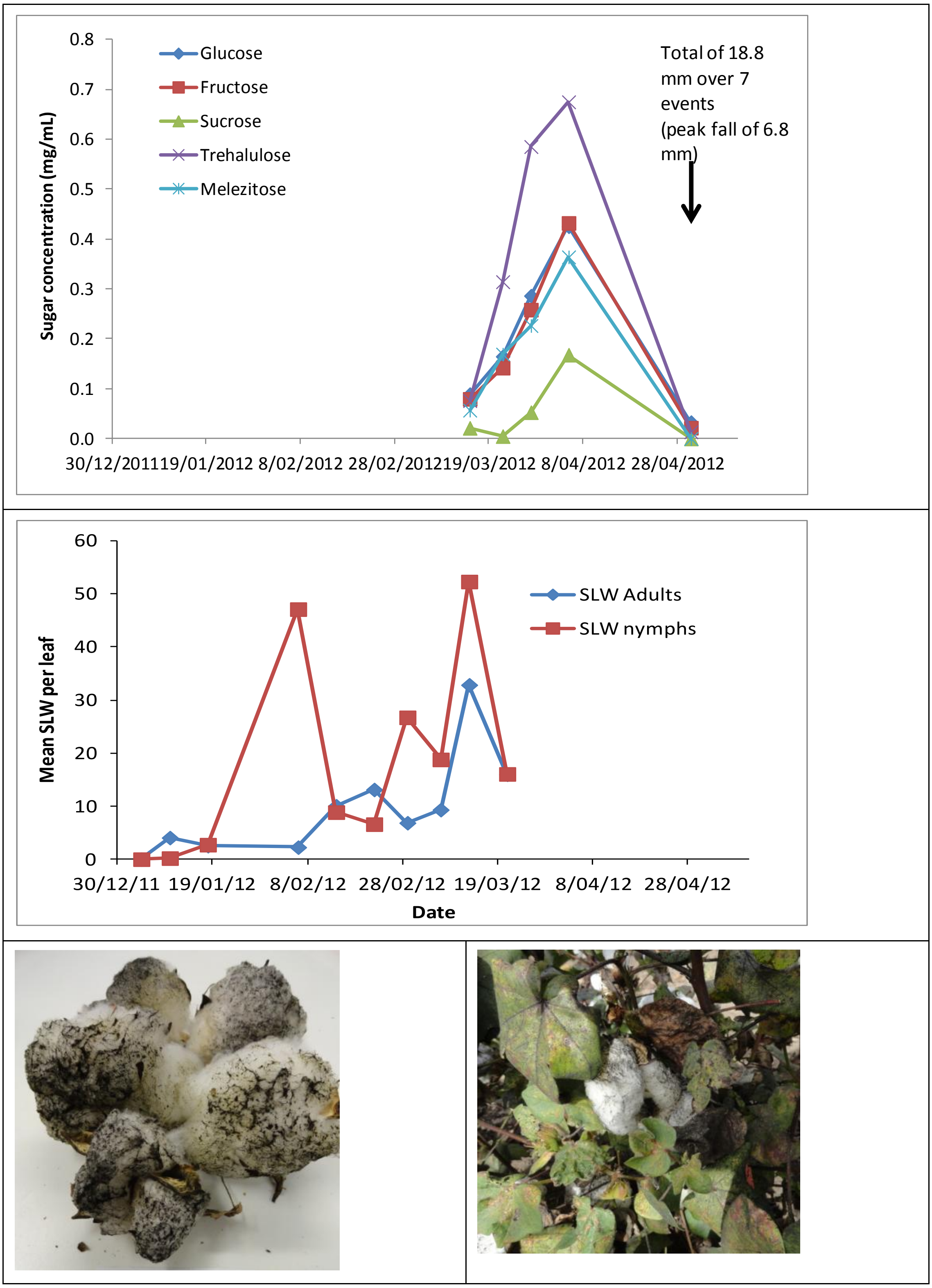

Figure 29. Top, build up of naturally deposited SLW honeydew on bolls. Middle, SLW populations in this plot during the counting period. Bottom, bolls with little honeydew remaining but with residual sooty moulds present.

6. Effect of sunlight (UV) alone on decline in honeydew.

The rapid decline that we found after exposing bolls treated with honeydew in the field (e.g. see Experiments 1-3) could be due to coincident rainfall or could be due to exposure to UV radiation in sunlight. To try to separate these effects we contaminated bolls and placed them in the upper canopy for 4 days in bright sunlight with no rainfall event occurring (see Figure 30A). We also included non-contaminated bolls as controls to account for the potential for honeydew to be deposited during the experiment (perhaps by aphids or SLW in the crop). There was no evidence of contamination of bolls in the field (see bolls labelled ‘Cont’ in Figure 30B).We found no evidence of a decline in sugars over the 4 day period so it is unlikely that short term exposure to sunlight alone would affect honeydew contamination levels on bolls. Glucose showed a slight increase between the 2nd and 3rd dates but we suspect this small variance was just due to natural variation in the contamination of the bolls.

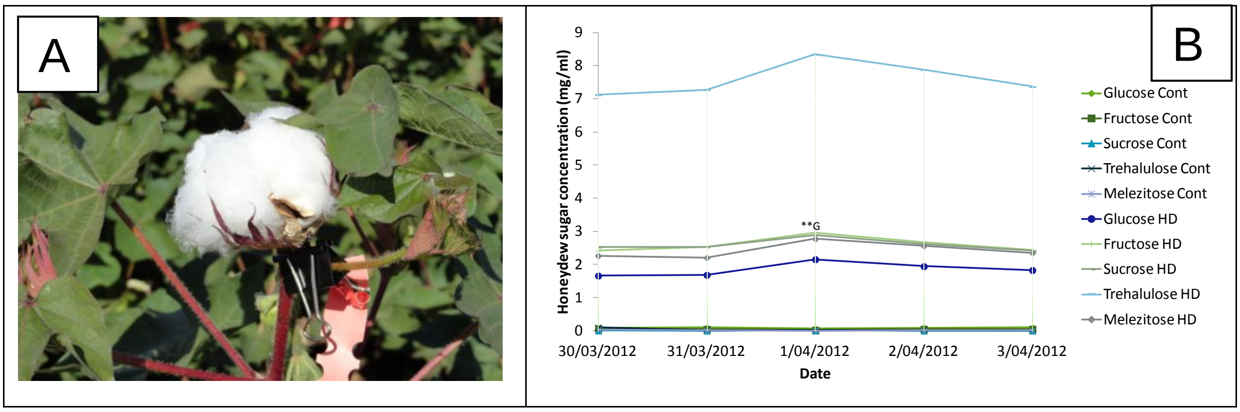

Figure 30. A. Boll exposed in the top of the canopy. B. Sugar types and content of bolls over 4 day exposure period to UV radiation (mean values mg/mL). Note that the bolls labelled HD were contaminated before exposure while those labelled Cont were non contaminated controls. Asterisks indicate a significant difference from the previous date for that sugar.

7. Simulating the effects of rainfall 1 – using a micro-sprinkler system.

One of the project technical officers, Simone Heimoana, developed a simple rainfall simulator using garden irrigation pipe and mister nozzles (Fig. 31A). Contaminated bolls were placed into a funnel sitting on top of a measuring cylinder so that we could measure the amount of rainfall applied to the boll that ran-off the boll (Fig. 31B). We also measured the change in weight of the boll to account for rainfall absorbed by the boll. These two values were combined to estimate the total rainfall intercepted by the boll.

Using this we investigated the effect of increasing periods of rainfall (simulated) on the amount of honeydew remaining on contaminated bolls that were exposed to this rainfall. An experiment was designed with 4 target rainfall rates; 0, 10, 20 and 30 mm rainfall. We did some initial calibrations to ensure this was achievable. The bolls were contaminated with artificial honeydew and placed in the simulator area, exposed for 10 mm, then removed and so on. There were 3 replicates of the entire experiment and in each replicate 10 bolls were exposed to the target rainfall.

We found that as rainfall increased there was a decrease in the concentration of sugar remaining on the bolls, as expected. There was a strong positive relationship between the amount of simulated rainfall and the proportion of honeydew removed (Fig. 31C and D)

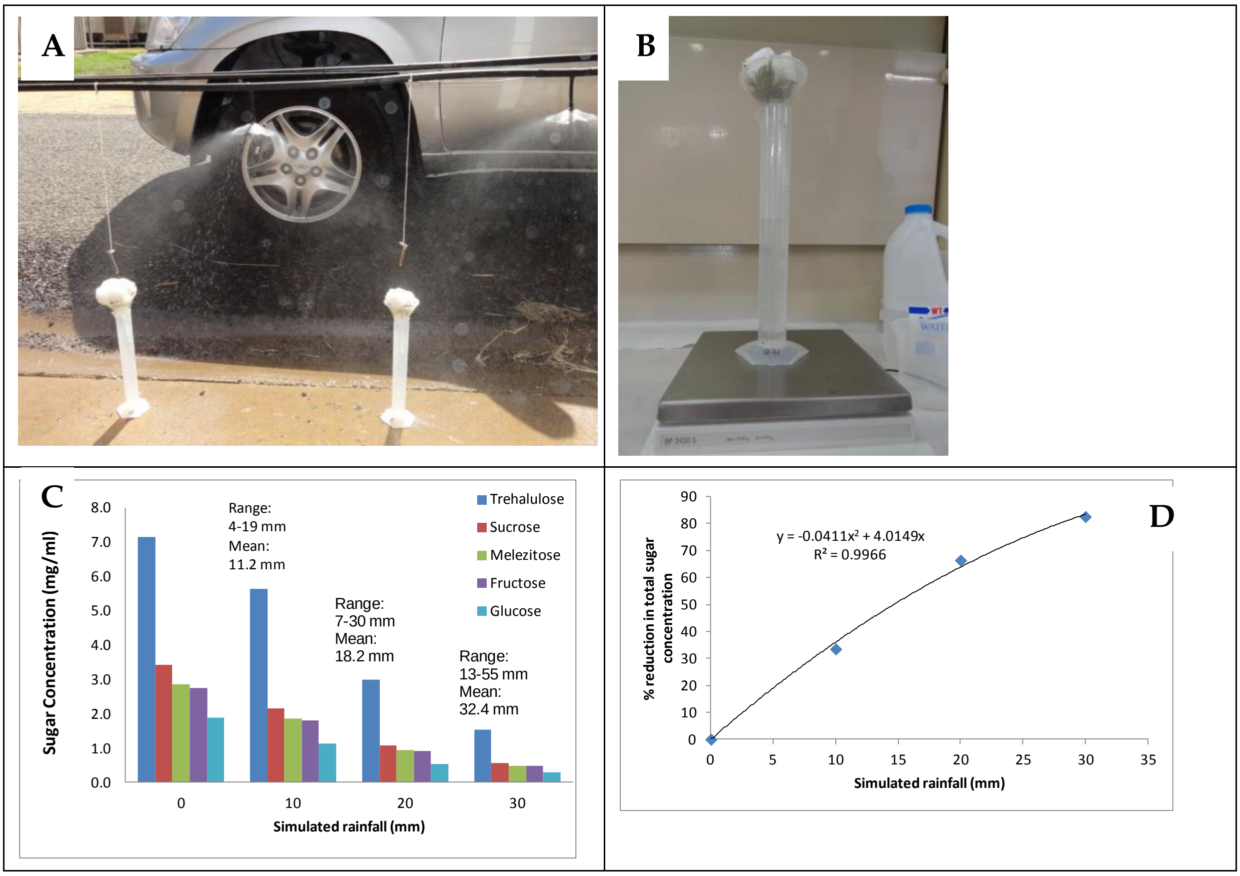

Figure 31. (A) Micro-sprinkler system developed to apply quantifiable amounts of water to bolls and (B) close-up showing boll sitting in small funnel which empties into a measuring cylinder so that water running off the boll could be collected, measured and analysed. A calibration allowed this amount to be converted to mm of ‘rainfall’. (C) Effect of differing amounts of simulated rainfall on remaining concentration of contaminant sugars on bolls and (D) relationship between simulated rainfall and proportion (%) reduction of sugar concentration.

8. Within-plant distribution of honeydew

Logic suggests that bolls in the lower canopy should be more contaminated than upper bolls. Knowledge of the degree of contamination of bolls at different strata in the crop would be valuable in understanding how much contamination is too much as ‘cleaner’ upper bolls are a source of dilution for more contaminated lower bolls. We collected bolls on the 23 March 2012 to consider the contamination of bolls at different node positions. At this stage the plants were heavily infested with SLW and the bolls and leaves even in the upper canopy had some honeydew. We found that bolls in the lower canopy – lower than node 11 below the terminal had the highest levels of sugars (Fig. 32). There were lower levels of honeydew in the upper canopy (nodes 10 and above). However, it would be valuable to extend this work earlier to follow the progression of honeydew deposition within the canopy.

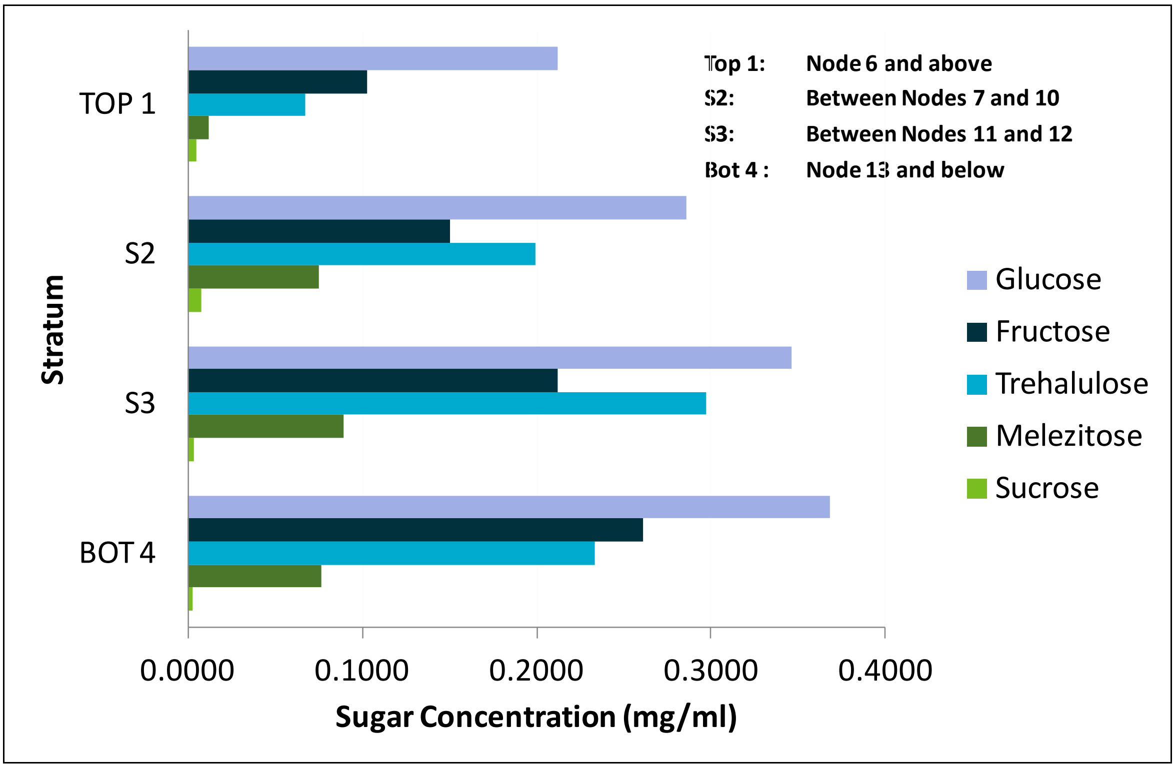

Figure 32. Concentration of sugars on bolls at different strata in the plants. These bolls were collected from cotton heavily affected by honeydew.

9. Natural change in honeydew levels over time.

This experiment was similar to Experiment 3. Clean bolls were collected and allocated to either (i) treatment with artificial honeydew or to (ii) control (untreated). A set of the treated and controls (10 bolls x 10 replications) were stored frozen for later analysis. The bolls were then all placed in the field (4 dates by treatment or control, each 10 bolls by 10 replicates = 800 bolls) and left there for 7, 19, 42 or 56 days (Fig. 33), after which they were collected and washed to remove the honeydew. The control bolls provided a reference for natural levels of honeydew that could be occurring in the field and which could confound results. The results were analysed using Genstat (ANOVA and LSD).

We found that the level of contamination on the control bolls was very low compared to the amounts on the ‘artificially contaminated bolls and did not change significantly during the course of the experiments (bolls labelled ‘Cont’ in Fig. 33). For the artificially contaminated bolls there were small but significant declines in levels of trehalulose and sucrose between the 20th and 27th March and for sucrose and fructose between the 27th March and the 8th April. However, the biggest declines for all sugars occurred between the 8th April and 1st May, during which there was 18.8 mm of rainfall. At 22 and 36 days samples of uncontaminated bolls in the field were also collected and processed to estimate the natural levels of sugars on the bolls. Rainfall events between the first and second dates and second and third dates both significantly reduced the concentration of sugars – again probably by washing the sugars out. Between the third and fourth dates, where there was no rain, there did not seem to be any further reduction. Sugar concentrations on uncontaminated field bolls were still substantially lower than on treated bolls exposed for 36 days.

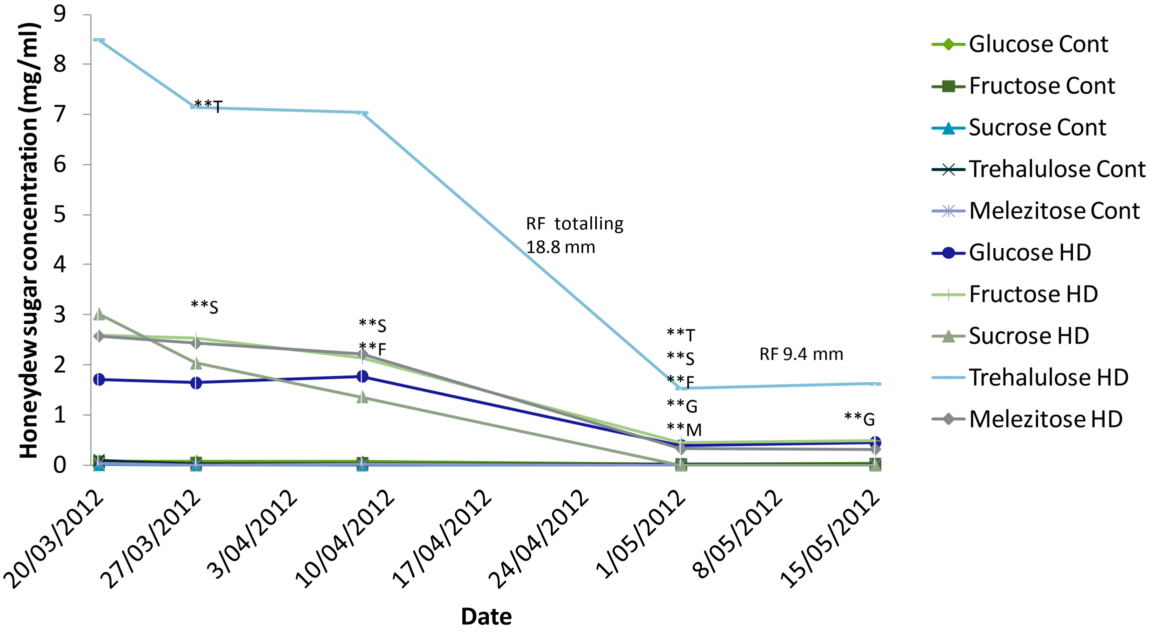

Figure 33. Decline on sugar concentration on bolls artificially contaminated with honeydew then exposed in the field and collected after a range of time periods.

10. Sunlight degradation of artificial SLW honeydew.

This experiment sought to confirm results of Experiment 6 that long term exposure of contaminated bolls to sunlight alone should not result in a decline in sugars. In the 2012/13 season we attempted to repeat Experiment 6 over a longer 4 week exposure time frame. Bolls were placed in the top of the canopy in the field on the 19/03/13 – when there was a prediction of a low chance of rainfall. However, on the 22/03/13 the forecast changed to indicate the risk of isolated localised showers so that day we attempted to protect the experiment by covering each replication, except for Rep 1 (a control), with plastic sheeting to protect the bolls from possible rain (Figure 34). Later that day there was 18.4 mm rainfall.

Figure 34. Left, UV honeydew degradation experiment in B2 covered in plastic sheeting to protect it from impending rain and right, after the storms.

Unfortunately the storm had strong westerly winds (average gusts of 12 km/hr with max recorded at 81.4 km/hr at Narrabri airport) and most of the plastic sheeting was ripped off (Fig. 34). Consequently, most of the honeydew was washed off the bolls by rain resulting in a significant decline in total sugar concentration (levels below 1 mg/ml) on the bolls (data square root transformed for analysis, $F_{4,81} < 0.001$, LSD = 0.1). The results for the first week therefore supported previous rainfall experiments (Fig. 35) as the rainfall washed off most (about 90%) of the artificial honeydew. In addition, there was a further 0.6 mm of rain between the second and third dates which resulted in a slight but significant further reduction in sugar concentrations. There was also a slight but significant increase in honeydew concentrations between the third and fourth dates and a decrease between the fourth and fifth dates. The slight accumulation of HD in the –HD bolls between the first and second dates, probably reflected rebuilding of background SLW populations after the rain. In the –HD treatment there was a slight but not-significant decline in honeydew levels over time. In the absence of rain there was no evidence of a sharp decline in honeydew concentration, implying that breakdown from other causes such as UV is slow.

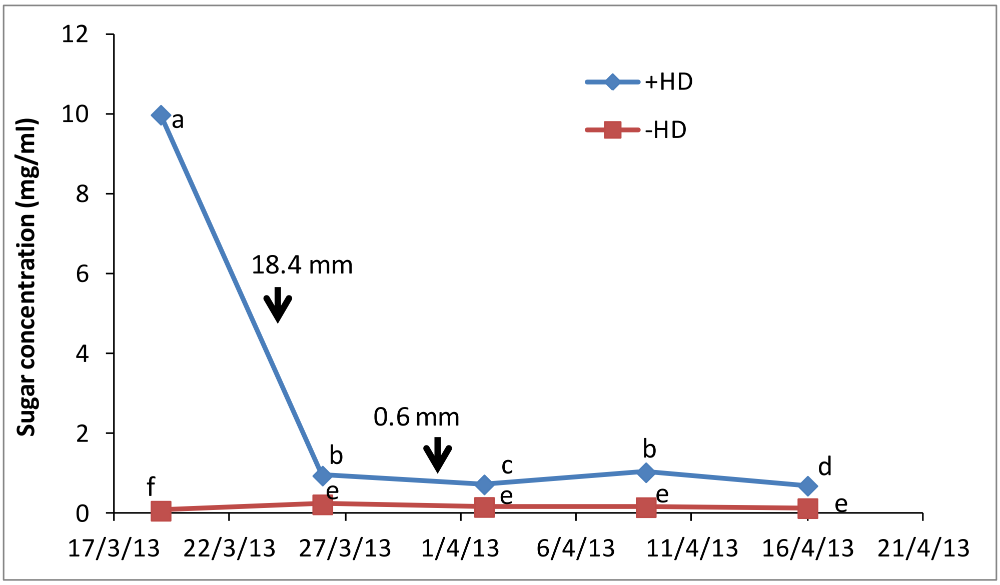

Figure 35. Concentration (mg/ml) of total sugars on honeydew contaminated (+HD) and non-contaminated bolls (-HD) bolls. Means followed by the same letter are not significantly different according to ANOVA/LSD at p = 0.05 (note back-transformed means are displayed)

11. Effects of rainfall simulated by using a lateral move sprayer.

This experiment extended the results of Experiments 2, 3 and 7 to evaluate if there is a predictable relationship between rainfall and the percentage of honeydew removed from bolls. We used the lateral move at Kilmarnock, Boggabri (Andrew Watson) as a rainfall simulator (Fig. 36B and D). Andrew was a terrific collaborator and showed a strong interest in the work. The system applied 60 mm per week (4 runs of 15 mm each) which gave us a good opportunity to see the effect of cumulative irrigation on honeydew wash off. Bolls received 5 puffs of honeydew each weighing between 0.43 and 0.7 g (mean 0.57 g). Bolls were placed into the canopy, half way down from the top of plants (plants were 1-1.2 m tall) (Fig. 36A and C). We used 100 bolls (10 replicates of 10 bolls) for each pass of the irrigator and retained a reference set of 100 uncontaminated and 100 contaminated bolls in the freezer as controls. In addition we also placed 100 contaminated and 100 clean bolls in an adjacent furrow irrigated field to assess how much honeydew would have been removed by rainfall alone.

For each replication in the field with the travelling irrigator a rain gauge was placed into the canopy at the same level as the bolls to estimate the amount of water that bolls at that level would receive (Fig. 36C). However, due to deflection of water drops as they fall through the canopy this was still only an approximation. Sprinklers applied a relatively high water volume but this only fell a short distance, compared with actual rainfall (Fig. 36D). Half-way through the experiment, rain gauges were also placed above the crop in each replication to estimate the actual amount of water being delivered from the system and variability and so that the proportion of irrigation water that would actually reach the bolls in mid canopy could be calculated. Rain gauges were also placed near both the field with the travelling irrigator and the furrow irrigated field so we could estimate rainfall. After each run (once along the field) of the irrigator, 100 bolls were collected and washed. At the beginning of a run, only 7 mm were applied to the end buffer of the field and on the return, at the end of the second run, 23 mm were applied (= 30mm for 2 runs). We captured above and in-canopy water in this area. On the first day of the experiments, we measured 2.1 mm of rainfall, a week later we received an additional 6 mm.

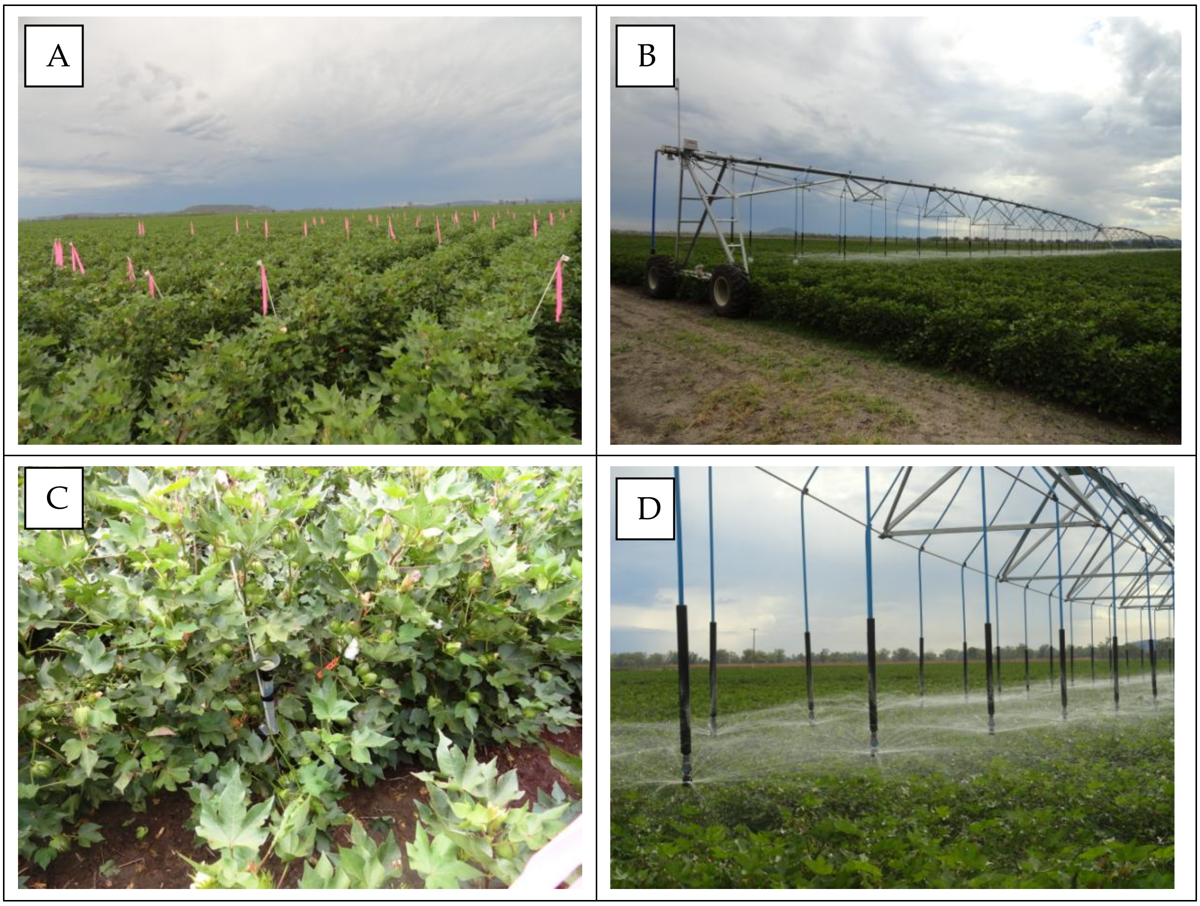

Figure 36. A. Layout of the experiment at Kilmarnock, (B) lateral move in operation, (C) boll placed in canopy and associated rain gauge (D) close-up view of sprinkler distribution.

We used data from the rain gauges to assess the amount of water applied. We assessed several runs of the travelling irrigator using the gauges above the crop to see how close the actual water delivered was to that expected by the grower. After allowing for rainfall, the amount of irrigation applied was quite variable between replicates within each application, which is not unexpected as the distribution of droplets from the irrigated would not be expected to be perfectly even. In terms of the amount of water applied at lower amounts of 7 mls plus 6.04 mm rainfall the actual (6.7 mm) was 52% of the expected (13.04). At 15 mm with no additional rain the actual (12.6) was 84% of the expected, however in a repeat pass at 15 mm where there was an additional 6.04 of rainfall the actual water received (19.5 mm) was 93% on the expected (21.04). A single pass with an expectation of 23 mm was actually slightly higher (123%) than the expected (28.3 mm) (see Fig. 37 left.). The variability within passes and between passes at the same rate made it difficult to accurately quantify or estimate the amount of water reaching the crop. Based on the findings we have compiled the values below (Table 10) which shows that the expected and actual cumulative amounts are surprisingly close (Fig. 37 right). The slope (0.86) is close to 1 but the actual amount is consistently less than expected and this increasingly at higher cumulative amounts of water.

Table 10. Expected and actual amounts (mm) of water applied at Kilmarnock

Pass of the irrigator	Expected (irrigation + rain)	Expected cumulative	Actual	Actual cumulative
1	7 + 2.1	9.1	Estimated 9.9	9.9
	15 +2.1	17.1	12.6 + 2.1 = 14.7	14.7
2	15	32.1	12.6	27.3
3	15 + 6.04	53.1	19.5	46.8
4	15	68.1	12.6	59.4

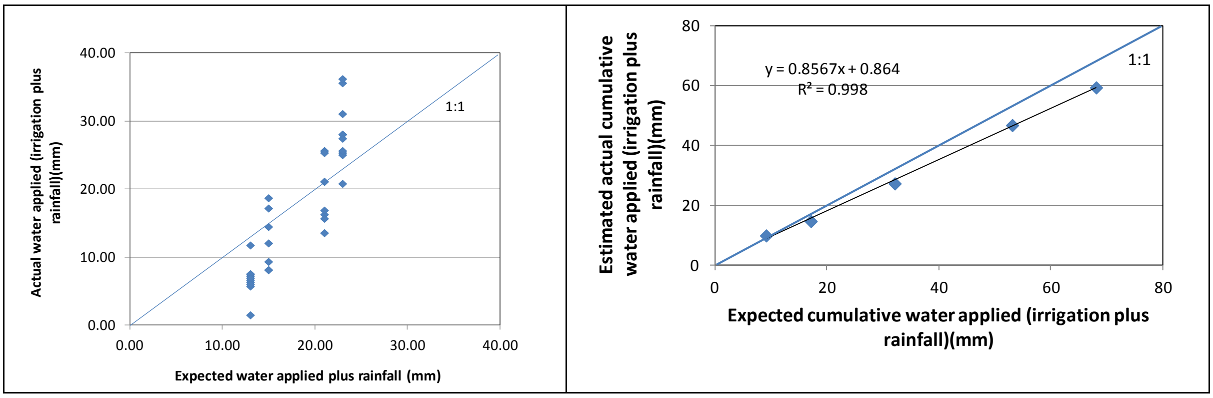

Figure 37. Left, relationship between water applied (irrigation and rainfall) and measured rainfall for 4 irrigation passes and right, relationship between expected cumulative total water applied and actual total water applied.

Comparing the water applied (irrigation and rainfall) above the crop with that received in gauges level with bolls in the mid canopy proved more problematic. Broadly, our data show that slightly less water reaches the bolls than comes in at the top of the crop (Table 11). However, on one date the water reaching bolls was much higher than that applied above the crop. This variability probably reflects droplet dispersal as well as directed flow along leaves and branches which means that some areas receive about the same amount as that applied, but many receive substantially more or substantially less. We also had bolls in an adjacent field that was furrow irrigated, so we were able to measure rainfall coming in and the amount of rainfall reaching the bolls, without the confounding effects of irrigation. For the first event the crop received 1.81 mm of rain and this resulted in 1.45 mm at the boll level (80.1%). In the second event the crop received 6.04 mm and this resulted in 5.95 mm at boll level (98.5%). So even with true rainfall there is variability as expected.

Table 11. Relationship between amount of water applied to the crop and amount intercepted at boll level.

Water above (irrigation + rainfall)	Water at boll level (irrigation + rainfall)	% water applied reaching bolls
6.7	4.9	73%
28.3	25.8	91%
19.5	17.9	92%
12.6	22.6	179%
1.8 (rain only)	1.4	80%
6.04 (rain only)	5.9	98%

The uncontaminated cotton bolls (C4 in Fig. 38) used in the experiment had a very low profile of sugars). The contaminated bolls that were not exposed in the field showed that we had effectively applied high levels of artificial honeydew (C3 in Fig. 38). Control bolls that only received rainfall totalling 8.1 mm had total honeydew sugar levels reduced by 83% (C2 in Fig. 38).

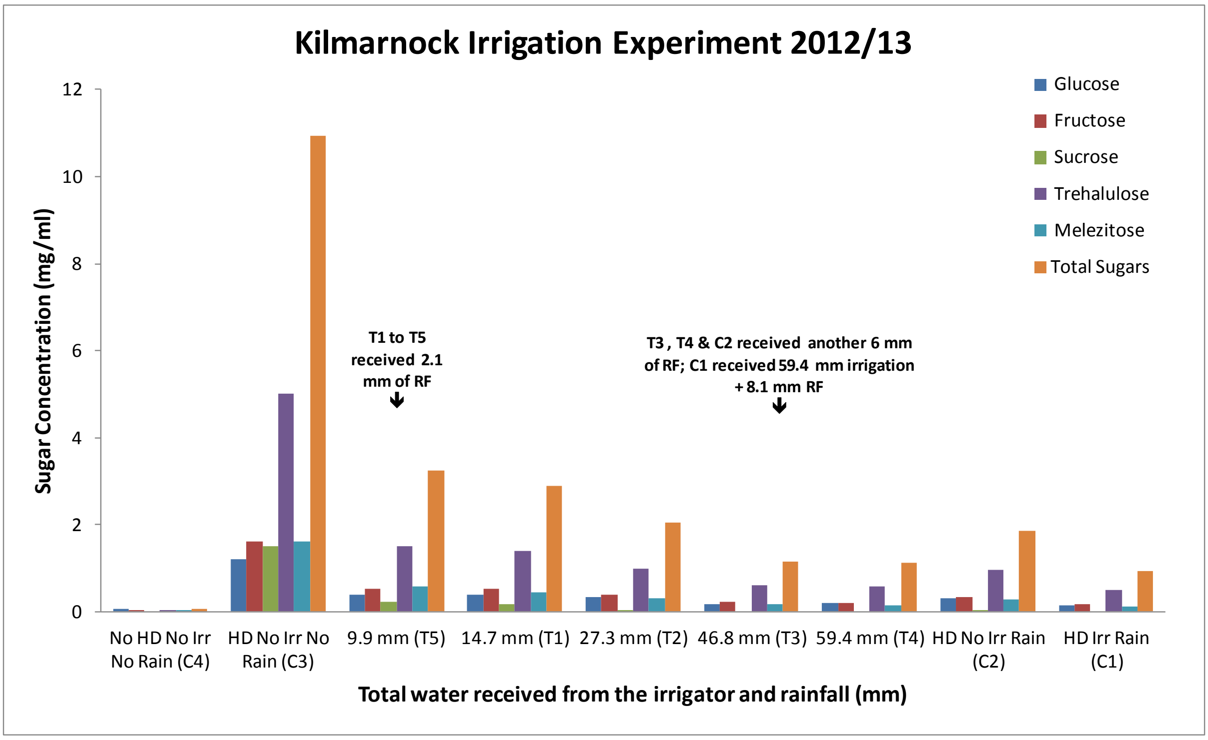

Figure 38. Effect of water applied on honeydew on bolls.

The water applied, the combination of both irrigation and rainfall, washed honeydew from bolls very effectively (Fig. 38). Even the lowest amounts of water applied (9.9 mm) removed 71% of sugars, while the highest amounts washed off over 89% (59.4 mm). There was a strong non-linear relationship between increasing amounts of water applied and the amount of honeydew removed (Fig 39). The initial effect of 9.9 mm was great and the increasing amounts of water applied after that did remove more honeydew but at a lower rate. Note that the amount of honeydew applied to bolls was very high compared to natural HD levels produced in the field by whitefly and aphids. Therefore, the lower levels of naturally occurring HD should be even easier to wash off with rainfall or irrigation.

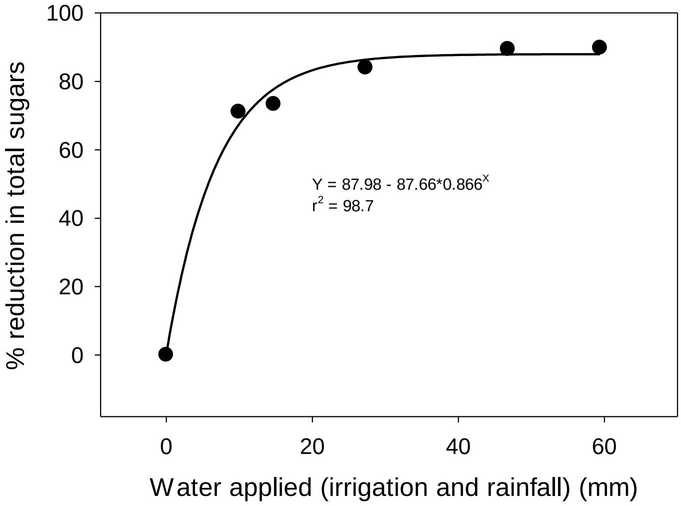

Figure 39. Relationship between total water applied (irrigation and rainfall) and the % reduction in total sugars. (Regression: $F_{2,3} = 195$, $P < 0.001$).

12. Deposition of honeydew within the canopy.

We were interested in monitoring the distribution and amount of naturally deposited honeydew in the crop. To estimate honeydew deposition in the different crop strata we clipped clean bolls at various levels into the canopy (lower, mid, upper) at approximately the same time as bolls were naturally opening in the canopy (Table 12). These clean bolls were then exposed to honeydew contamination. We collected bolls from the different strata weekly.

Table 12. Timing of bolls placed into the field.

12/03/2013	Control (uncontaminated bolls retained in the laboratory)
19/03/2013	Lower Week 1
26/03/2013	Lower Week 2
26/03/2013	Mid Week 2
2/04/2013	Lower Week 3
2/04/2013	Mid Week 3
2/04/2013	Upper Week 3

Analysis of the control bolls shows they were mature and had very low levels of natural plant sugars (<0.05 mg/ml) and essentially no trehaluose or melezitose. Most honeydew was collected from bolls that were exposed in the lower canopy for 1 week (Fig. 40). Individual sugar concentrations were very low (<0.15 mg/ml). This honeydew had lower levels of trehalulose than typical for whiteflies and contained no sucrose. It must be noted that plots had been infested with whiteflies for 2.5 months and that the experiment was carried out late in the season when the quality of cotton leaves had already suffered from high infestations. Honeydew composition and concentration is very variable depending on food quality and insect life stage. Sugar levels dropped sharply after 2 weeks of exposure due to 18.2 mm rainfall which washed off almost all of the honeydew that had collected during the first week in the field. Whitefly populations also reduced to a point where little new honeydew accumulated in the third week of the experiment. Nevertheless, the data was valuable because the rainfall event added to the dataset of rainfall by % honeydew removed that we have been accumulating (discussed later).

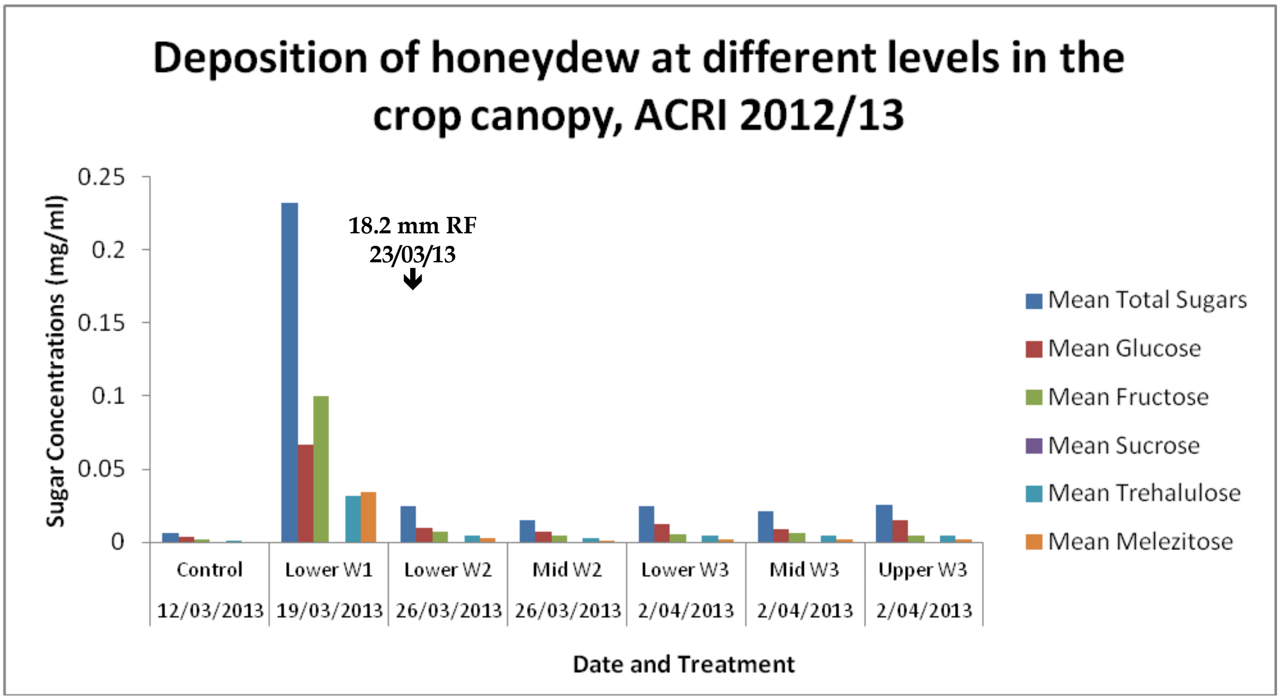

Figure 40. Levels of honeydew on bolls placed in different strata of plants over time, coinciding with bolls opening in these strata, ACRI 2012/13

13. Distribution of honeydew on bolls

We hypothesised that most honeydew is deposited on the surface of bolls and little makes its way to the interior of bolls as cotton is relatively hydrophobic. Water would initially run over the surface of bolls rather than into them. This would mean that initial rainfall would run over the outside of bolls, potentially removing most of the honeydew. We tested this by comparing the levels of contamination on the outer surface (OUT) of bolls by cutting off the outer 5 mm of fibres and comparing this to the remainder (IN) for both naturally and artificially contaminated bolls.

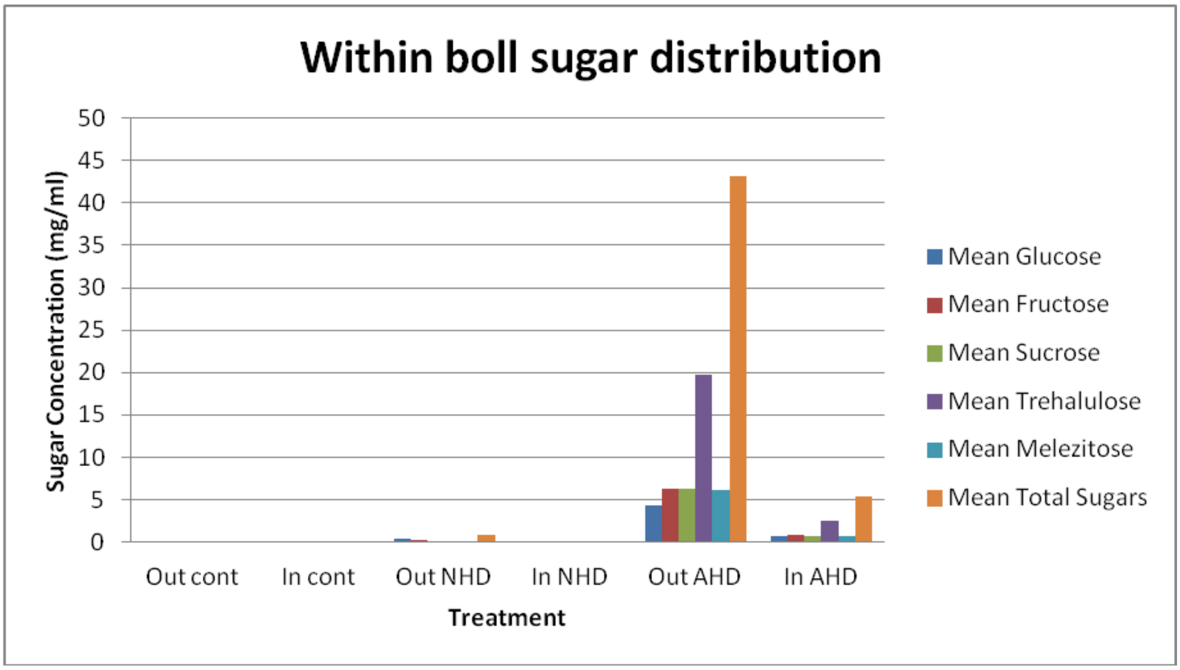

Figure 41: Within boll sugar distribution of control, natural honeydew and artificial honeydew bolls (NHD-Natural honeydew, AHD- Artificial honeydew)

The results show that no sugars were found on mature, uncontaminated control bolls (Fig 41). When bolls were artificially contaminated the majority of the sugars were contained in the outer 5 mm of lint. However, there was evidence of some sugars penetrating past the outer 5 mm. This may reflect several issues that could affect the results. Firstly, contamination on the cutting scissors, even at small amounts, could be passed onto the inner fibres. Secondly, the puffer used to apply the honeydew is shooting droplets out at speed and from a range of different angles (e.g. different angles for each of 5 puffs), which probably increases the chances that droplets may penetrate past the exterior of the bolls. Finally, as the deposition of honeydew is intense there may be some dribbling into the interior. However the most revealing is the naturally deposited honeydew. This shows that at this low level of contamination natural honeydew did not penetrate beyond the outer surface of the lint. Hence, the concentration of naturally deposited honeydew on the outer fibres increases the chance that rainfall will remove it.

14. Evaluation of single versus triple rinse

Our standard boll washing technique involves a single rinse, mainly because the number of samples that would be generated with multiple samples would be prohibitive. However, to understand how effective the single rinse was we prepared some bolls and washed them in single, double and triple rinses. We used bolls containing no honeydew, naturally deposited honeydew (real) and artificial honeydew (AD) and washed each sample separately 3 times, then analysed the sugar concentrations of each wash (Fig. 42).

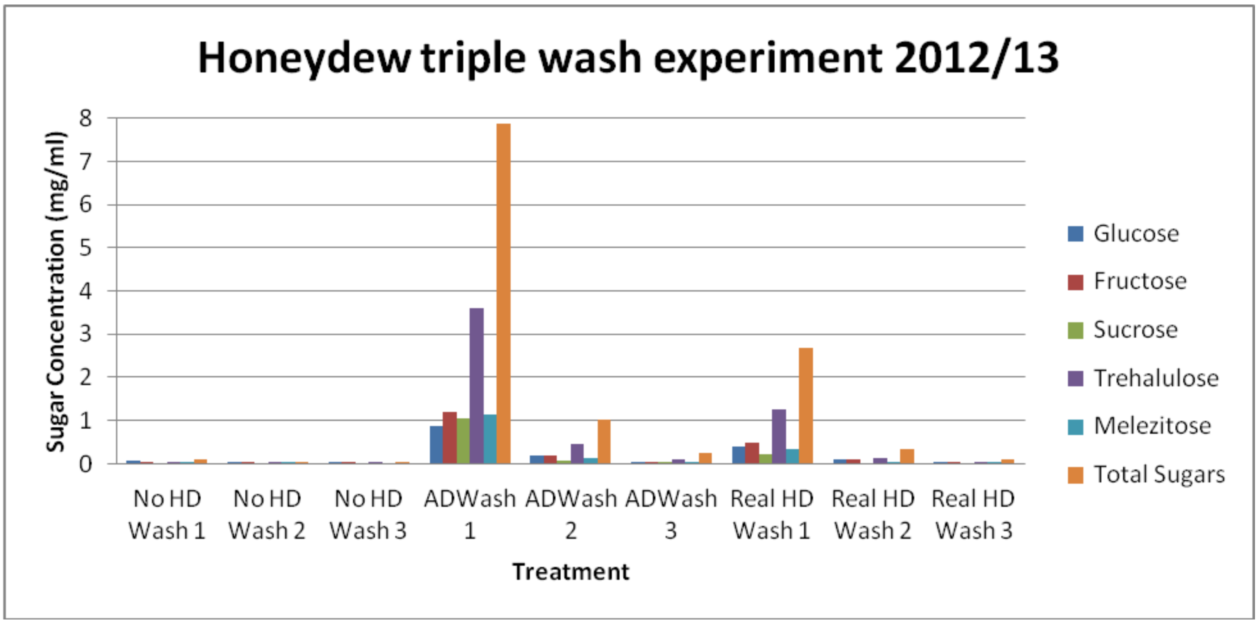

Figure 42. Reduction in boll sugar content after successive washes.

Bolls used were very mature and had minimal amounts of natural plant sugars (No HD). Of the total honeydew that was removed from the artificial and naturally contaminated bolls the first wash removed approximately 86%, the second wash a further 11% and the third wash a further (2-3%) (Table 13). Hence our single wash method is providing a reliable assessment of sugar levels on bolls and is saving significant costs in processing samples.

Table 13: Proportions of total sugars washed out of cotton bolls in three consecutive washes

Treatment	**% HD washed out**		
	No HD	Art. HD	Real HD
Wash 1	59.25	86.02	85.92
Wash 2	28.70	11.28	11.02
Wash 3	12.04	2.71	3.06
Total	100	100	100

Honeydew – Conclusions

Our strategy in the first 3 years has been to establish the factors that reduce the levels of honeydew on bolls. Our data shows that breakdown of honeydew by sunlight alone happens very slowly. However, we don't yet understand if the presence of sooty moulds contributes to this as well and this is a topic for a further project. We have shown very clearly that rainfall has a very significant effect on the level of honeydew, and as little as 10-15 mm can substantially reduce levels of contamination.

We have combined all of the data collected from natural rain events (11 events ranging from 8.1 to 21.8 mm) as well as those simulated by our micro sprinkler system (0 to 32 mm) and by a travelling irrigator (0 to 60 mm) and used this data in a regression analysis. The percentage reduction in total sugars was regressed against cumulative rainfall. A linear regression was marginally significant ($F_{1,15} = 4.71$, $P = 0.046$, $r^2 = 0.19$). The data showed that a high percentage of honeydew was removed by even 10 mm of rainfall, with higher amounts removing more but at a declining rate. Hence a 'growth to an asymptote' model was fitted using the non-linear function in Genstat 13. This showed a strong relationship ($F_{2,18} = 21.7$, $P < 0.001$, $r^2 = 0.67$)(Fig. 43). Comparison of the source of data (actual rainfall, travelling irrigator or micro-sprinkler showed no significant difference ($F_{4,16}$, $F = 2.18$, $P = 0.145$)).

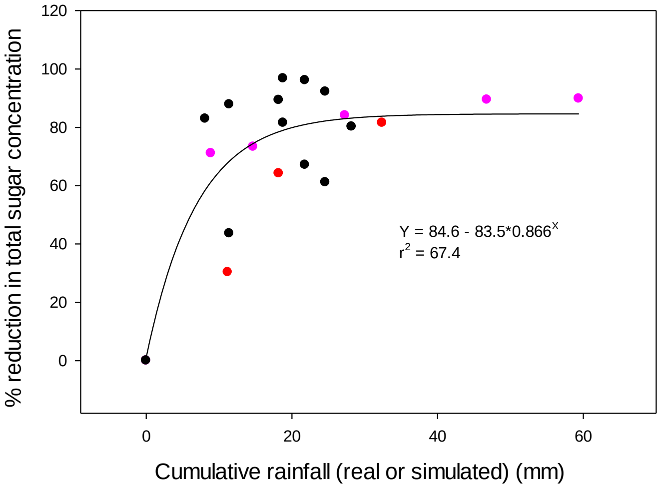

Figure 43. Relationship between the percentage reduction in honeydew concentration and the cumulative rainfall. Data for real rainfall events is shown in black, for the micro-sprinkler experiment in red and for the travelling irrigator experiment in pink.

4. *Finalising surveys of crop and non-crop hosts for GVB*

Methods, results and discussion – Surveys of weed and crop hosts and diapause habitats

Until September 2010 we were sampling green vegetable bug populations at three sites and results of this work were reported as part of the final report for project CRC1.01.62. As sampling three sites was very time consuming and other objectives needed to be addressed we scaled sampling down for a final season to once every 3 weeks at the most productive of the sites (Breeza – though even this site involves several farms) and sampling continued until September 2011.

Data over this period indicated:

(i) Generally higher numbers of GVB, possibly due to widespread rainfall and abundant growth of weed hosts from August 2010 onwards (Fig. 44 A and B)

(ii) Numbers of green adults were low through winter but there were still active populations at this time.

(iii) Populations were found mainly on crops in February- April, mostly soybean, but also cotton, sunflower and sorghum (Table 14). On weed hosts adults were most abundant on stinging nettle (*Urtica incisa*), turnip weed (*Rapistrum rugosum*), sowthistle (*Sonchus oleraceous*), marshmallow (*Malva parviflora*) and wild sunflower (*Helianthus annuus*)

(iv) Nymphal populations broadly followed but lagged behind adult populations as expected, for instance peaks through October to December 2010 followed the transition of bronze adults back to green on weed hosts, contributing to overall adult populations from August to October 2010 (Fig. 44 A, B and D)

(v) Nymphal numbers first built on weed hosts followed by a later population that built on crops (mainly on soybean – see Table 14). No nymphs were found on sorghum and only 2 were found on cotton. Nymphs were mostly found on turnip weed (*Rapistrum rugosum*), cobblers pegs (*Bidens subalternans*), bishops weed (*Ammi majus*) and stinging nettle (*Urtica incisa*). A significant number of nymphs was found on grasses but as grasses were generally poor hosts we suspect that these nymphs were probably on a broad leaf weed host inadvertently sampled with the sweep net while sweeping an area of grasses.

(vi) Parasitism rates by the tachinid fly, *Trichopoda giacomellii*, were very low coming out of the drought period but began to build from January 2011 and reached much higher proportions than through the previous drought years. This may reflect the generally higher abundance of GVB during this period. This provides more continual access to hosts for the parasite. By contract in the drought years there were only very patchy and ephemeral bursts of host plants and hence GVB populations also fluctuated with number dropping to almost zero for periods, making it difficult for parasite populations to be sustained in these patches. Parasitism rates of the bronze adults in diapause habitats were also much higher than in previous years, but did decline through the winter, though this may be an artefact as eggs may be dislodged from the bugs over time.

(vii) Most GVB adults in diapauses were recorded from beneath the bark of river red gums, but significant numbers were also found under other sheltered sites (e.g. under cardboard containers, tyres and wood) (Table 14).

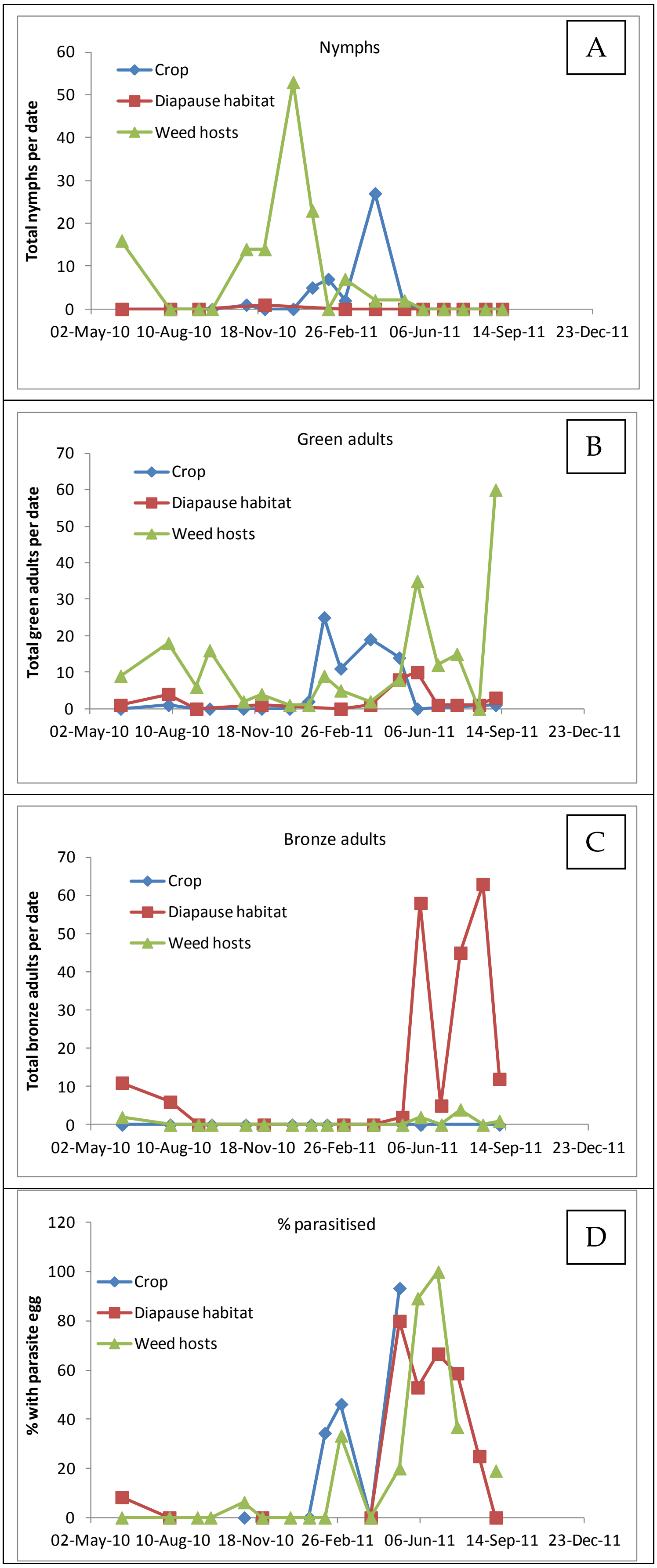

Figure 44. Abundance of (a) nymphs (b) green adults (c) bronze adults and (d) proportion of bugs parasitised for survey at Breeza, 2010-11.

Table 14. Abundance of GVB females, males and nymphs on weeds, crop hosts and in diapause habitats, Breeza, 2010-11.

Type	Species or habitat	Total GVB adult females	Total GVB adult males	Total GVB nymphs
Weed hosts	*Urtica incisa*	22	18	7
	Rapistrum rugosum	16	8	26
	Sonchus oleraceus	16	22	0
	Malva parviflora	13	13	6
	Helianthus annus	6	5	3
	Rumex crispus	4	3	3
	Hibiscus trionum	4	5	0
	Lamium amplexicaule	4	2	0
	Poaceae	3	6	45
	Melilotus indicus	3	4	0
	Rheum sp.	3	2	0
	Vicia sativa	2	2	3
	Asparagus officinalis	2	0	0
	Lycium ferrocissimum	2	0	0
	Vigna sp.	2	2	0
	Bidens subalternans	1	0	13
	Ammi majus	1	1	10
	Gossypium hirsutum	1	4	7
	Lactuca serriola	1	1	2
	Acacia spp	1	0	0
	Avena sp.	1	0	0
	Myoporum montanum	1	0	0
	Phalaris sp.	1	2	0
	Sysimbrium irio	1	0	0
	Triticum aestivum	1	1	0
	Solanum esuriale	0	0	3
	Polygonum aviculare	0	0	2
	Cucurbita pepo	0	0	1
Crops	*Glycine max*	10	5	37
	Gossypium hirsutum	7	10	2
	Helianthus annus	8	16	1
	Sorghum sp.	8	8	0
	Triticum aestivum	0	1	1
	Zea mays	0	0	2
Diapause habitat	Bark-*Eucalyptus camaldulensis*	46	44	0
	Cardboard	35	25	0
	Hide	0	0	0
	Litter	13	8	0
	machinery	0	0	0
	metal	1	1	0
	Onion baskets	1	0	1
	Tarp	7	6	0
	tyres	10	6	0
	wood	15	14	0

Methods, results and discussion – Surveys of crop sequences.

We also investigated crop host use by planting a range of hosts in summer and monitoring GVB abundance. These hosts included potential summer crops such cotton, soybean, mungbean, maize, sunflower and pigeon pea (as refuge crops), potential winter crops such as wheat, faba bean and canola and a perennial crop, lucerne. To show the consistency of pattern I have included the data for the entire experiment, including some that were presented in the Final Report for project CRC 1.10.62. The results show reasonably consistent patterns across years. Across the three years the populations of GVB adults and nymphs increased with each successive year, and were much higher in the last year, probably reflecting the drought breaking rains beginning in August 2010 which would have increased the abundance of hosts for GVB across the district (Fig. 45 and 46).

Adult GVB were present periodically on the perennial lucerne but only in very low numbers, however, numbers on lucerne were high in the final season (Fig 45). Adult populations tended to begin to increase in late December –early January, firstly on mung bean, then on soybean and pigeon pea later in February – March. Populations of adults were found in cotton but only at low densities from January to March – the boll filling period. In winter small populations were found late in the canola period (October) but virtually none were found in faba bean or wheat.

Nymphal populations typically began to build in mid to late December, starting on mungbean or lucerne, followed by soybean (Fig 46). Significant populations developed on pigeon pea in February – March. Few nymphs were found in cotton and few GVB were found on sunflower. In winter no nymphs were found on wheat or faba bean, but late winter populations were found in canola. It is clear that adults prefer to oviposit in crops such as mungbean, soybean, pigeon pea and lucerne rather than cotton. Care should be taken though as bird feeding on sunflower, sorghum and maize damages the flowering heads which may influence the suitability of these crops for GVB.

The results suggest that very high populations of GVB can build in early rotation crops such as mungbean and sometimes soybean. If these crops mature early, adult GVB could be forced into cotton, but in general they are more likely to be attracted to legume crops. This suggests that (1) changes in the cropping system toward earlier production of legume crops could create a greater risk to cotton (2) some legumes have the potential to be trap crops and draw GVB away from cotton, especially if they are attractive during the short boll fill period when cotton is prone to damage from GVB. Careful management of planting date and irrigation could ensure the effectiveness of these trap crops.

Methods, results and discussion – Sorghum as a host

The regular crop rotation experiments ceased in early 2011. However, a remaining question was whether sorghum was a host for GVB, based on reports from some consultants who suspected that influxes into cotton had originated in nearby sorghum crops since they were able to find GVB in these sorghum crops. Surveys carried out by us at the time found few GVB in sorghum – but sampling may have occurred too late in the crop cycle. Conversations with consultants suggested that it was only at the soft dough stage that GVB was found in sorghum. We set up a small experiment to see if GVB could survive in sorghum crops. In Block 17 at ACRI we established two plantings of sorghum (Pioneer G22), one in mid October 2011 and a later planting in late December 2011. These were planted next to 8 rows of Sicot71BRF to encourage natural movement of GVB between the crops. Each crop was marked out to consist of 3 reps of 4 rows, each 30 m in length (9 plots). As each crop came into flower 3 egg rafts from our GVB colony (as available) were transferred to protected areas of fresh leaf, 3 in each plot (about 10 m apart from each other).

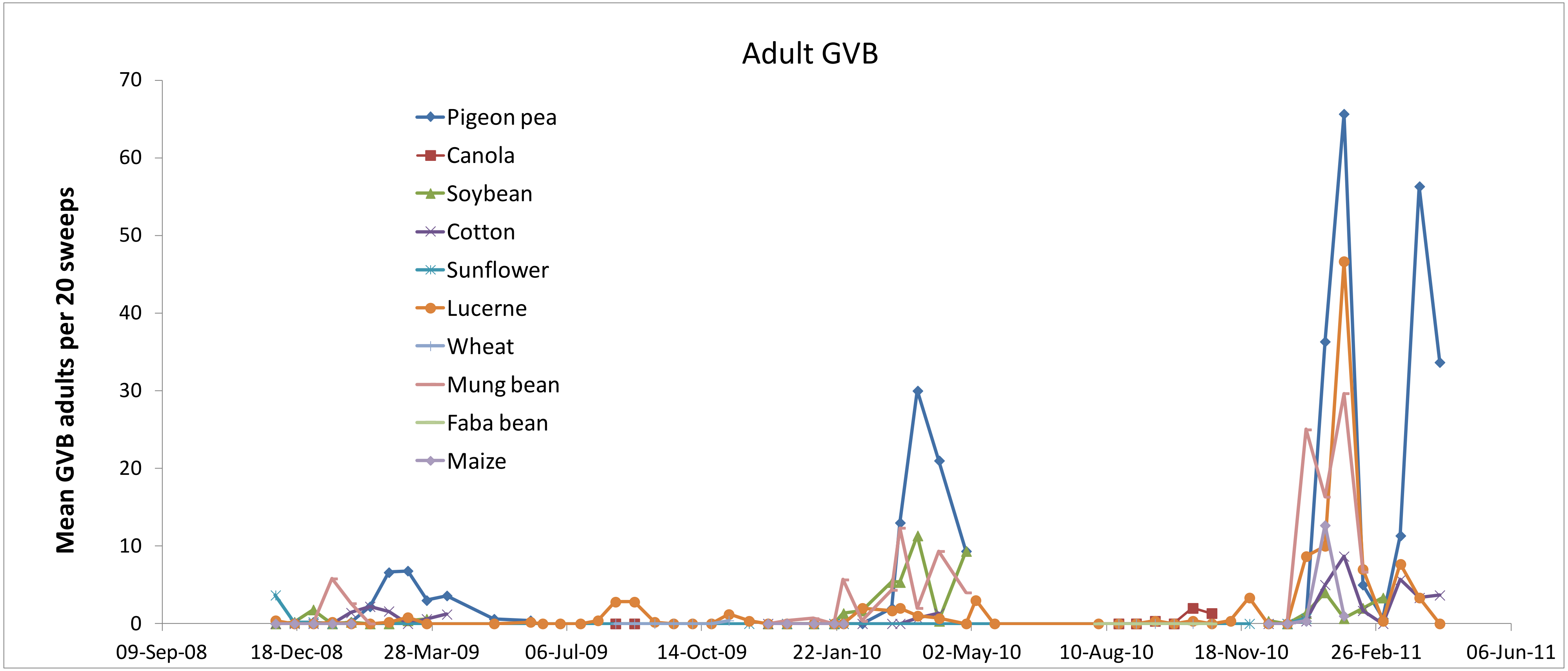

Figure 45. Abundance of adults of GVB on a range of crop hosts, ACRI, 2010-11.

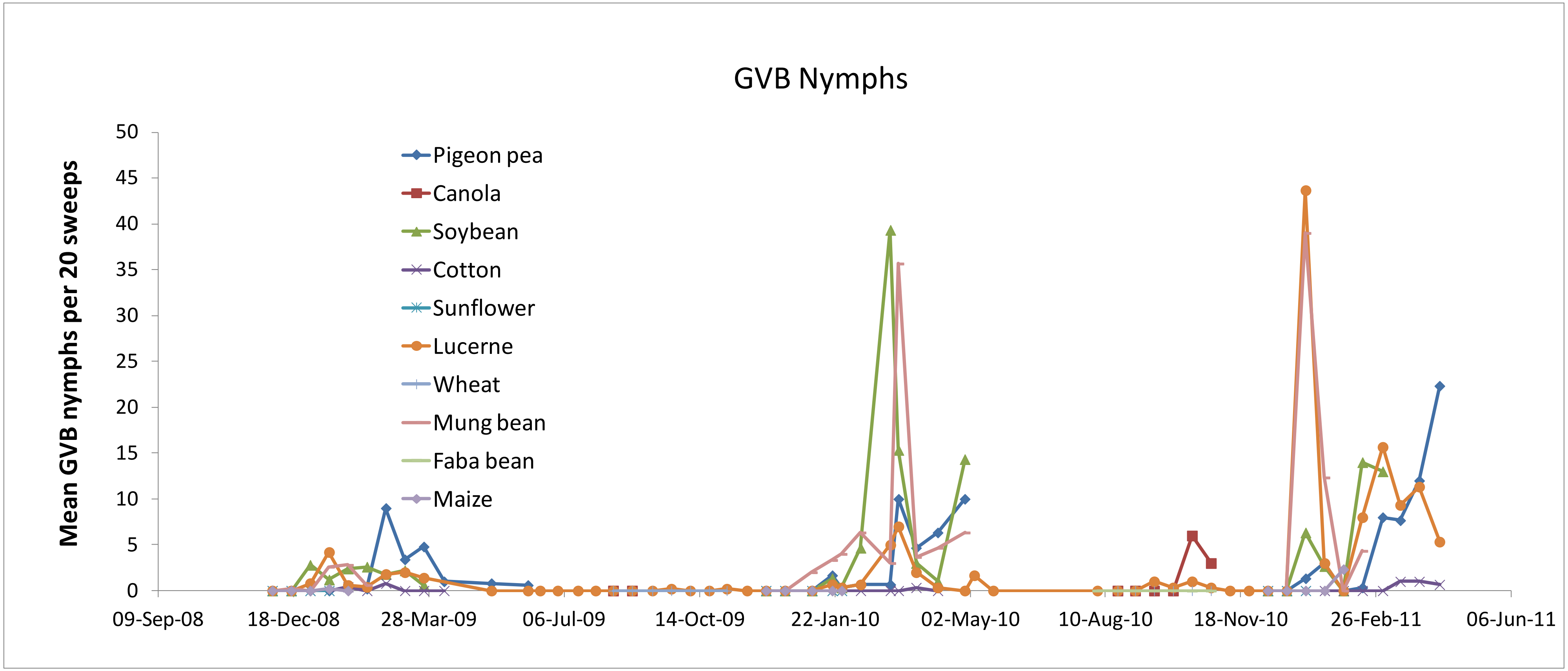

Figure 46. Abundance of nymphs of GVB on a range of crops hosts, ACRI, 2010-11.

The data for GVB estimated in beatsheets or visual samples showed low numbers on the early planted sorghum at the start of the sampling period which coincided with late flowering (Fig. 47 and 48). Through most of January to March, GVB were mostly recorded in cotton, with few specimens in sorghum until mid-February when numbers began to build in the late planted sorghum, coinciding with the start of flowering. There was also a late increase in the early planted sorghum – which was probably related to secondary heads forming after the initial heads had matured. The data suggest that vegetative and mature sorghum were poor hosts for GVB. Cotton through this period had developing bolls that may have been an adequate food source.

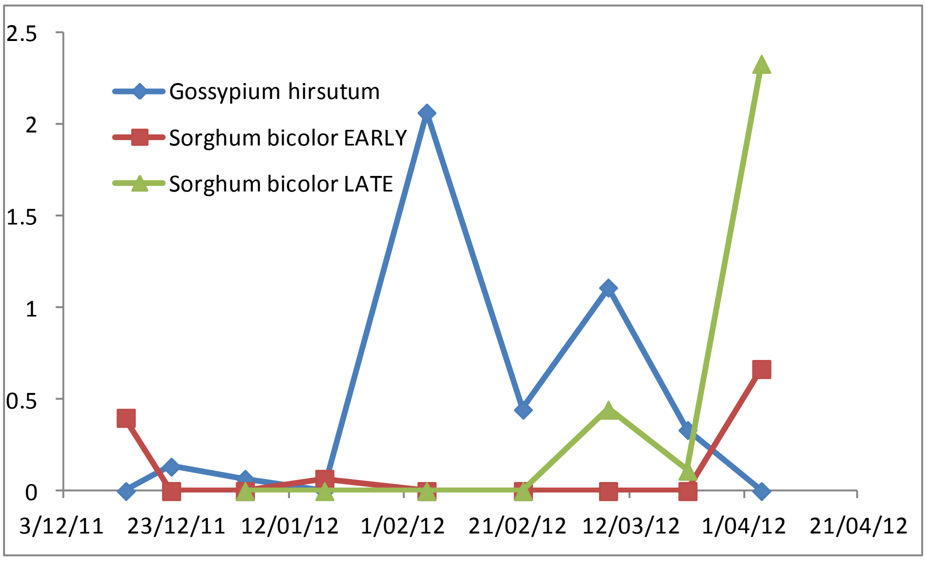

Figure 47. Number of GVB (adults and nymphs) per m in beatsheets for each crop.

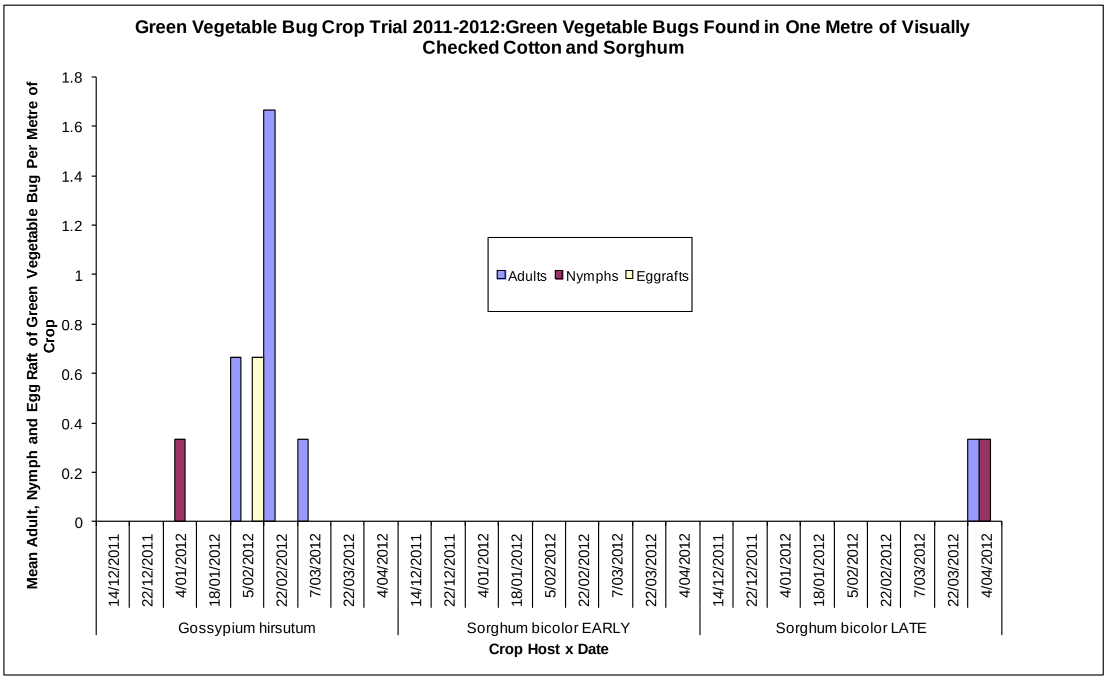

Figure 48. Mean number of adults, nymphs and egg rafts of GVB per m in each crop type in visual checks.

In addition we tested if GVB could develop through to maturation and oviposition in sorghum and cotton. Small cages were used to enclose single stems on plants of sorghum or the mainstem of cotton. There were two infestation periods. The first occurred in January when the early planted sorghum was in the late stage of flowering, the late planted sorghum was just starting to flower and the cotton was vegetative. The first set consisted of five cages per crop which were each infested with two egg rafts The second infestation occurred in mid-February when early planted sorghum was maturing heads, the late planted sorghum was at the end of flowering and the cotton was at the late stage of flowering. This set consisted of four cages in each of cotton and the late sorghum sowing and three in the early sowing of sorghum. They were infested with one egg raft each. The results showed generally poor survival on vegetative sorghum and maturing sorghum and on cotton (early experiment) while sorghum with flowers and young (soft) seeds seemed to be suitable (Figure 49). In both experiments, GVB were able to persist in all crops, especially the late sown sorghum which had flowers and softer maturing seeds. Hence the results broadly confirm that sorghum is a potential host for GVB but only during the flowering and early seed maturation period.

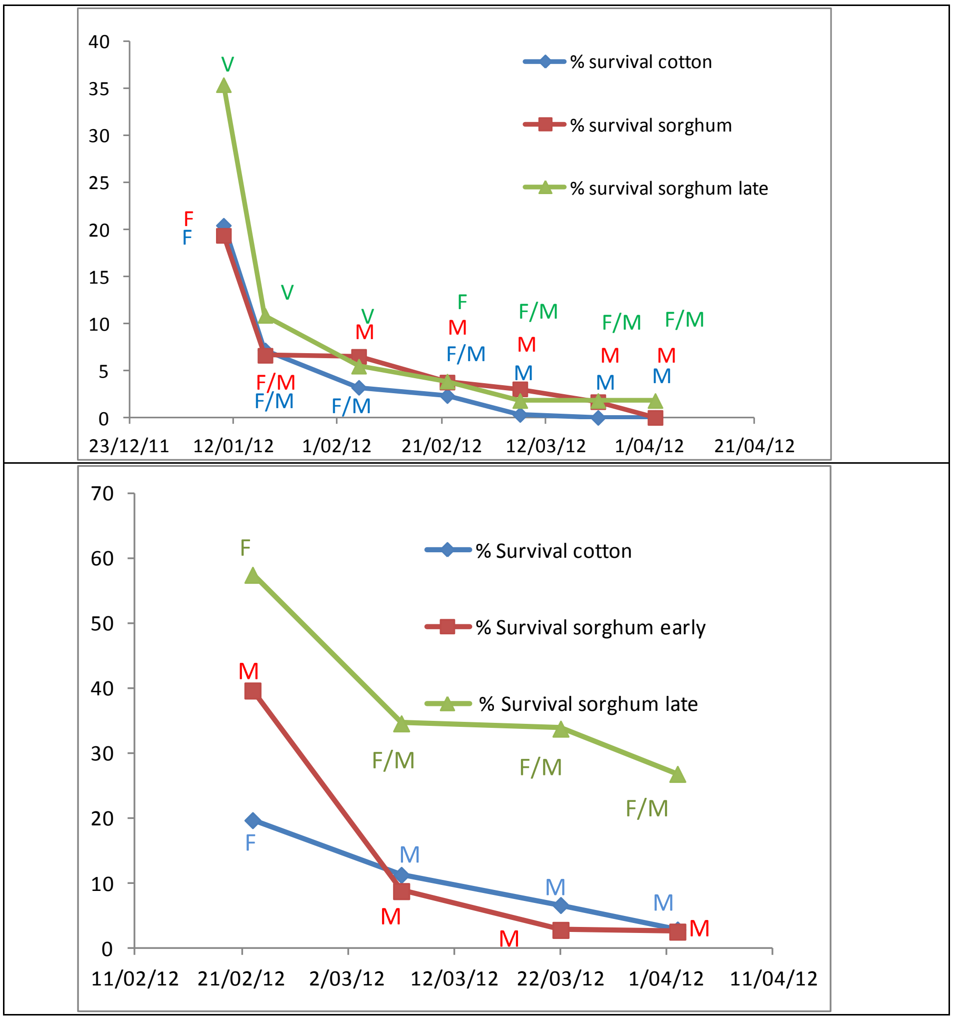

Figure 49. Survival (%) of GVB from egg rafts caged on cotton, early planted and late planted sorghum, for experiment starting in early January (top) or mid February (bottom). Crop growth stage is indicated as v; vegetative, f; flowering, m; maturing.

5. *Finalising thresholds for late thrips and jassids from existing data*

Analysis of the data was completed and tentative thresholds developed and reported in the final report for Project CRC1.01.62. This occurred because that report was written after the submission for this project was prepared – hence – this milestone was completed in this project but actually reported in the last projects final report. Thresholds were used by industry in recent outbreaks of spur-throated locusts and cluster caterpillars. This work needs to be published and written up for the Australian Cottongrower.

6. *Use new techniques to evaluate the presence of cotton bunchy top disease in alternative hosts – to understand its ecology and risks.*

This component of the project has two parts: identification of alternative hosts of CBT and understanding the effect of insecticides on transmission.

Identification of alternative hosts of CBT

This first component used primers that were developed to test for the presence of CBT in plant or insect tissue under a CBA funded project. These primers were made available to QDAFF to facilitate collaboration in identification of hosts of CBT to better understand the ecology of the disease and hence predict outbreaks. Murray Sharman (QDAFF) further refined this method and in doing so demonstrated that there were (at least) two strains of CBT, named A and B. During the course of the project we collected samples of suspect infected hosts including cotton volunteer/ratoon plants and a wide range of weed species.

In addition we used the CBT ratoon area at ACRI to do a more targeted evaluation of the potential of plants to host CBT. Seed was collected from the field or sourced from Graham Charles (NSW DPI) for; *Hibiscus trionum, Sida rhombifolia, Sida corrugata, Abutilon theophrasti, Malvastrum coromeliandrum and Anoda cristata*. We also tested some cultivated Malvaceae such as okra (*Abelmoschus esculentus*) and rosella (*Hibiscus sabdariffa*). We used gaps in the CBT ratoon cotton rows in which to plant these potential malvaceous hosts. We aimed to have about 5 plants of each weed. To facilitate planting we organised irrigation just before we wanted to plant. Once plants had germinated and grown we labelled them.

In December or when the ratoon CBT cotton began to show symptoms of CBT, we infested the ratoon plants with aphids from a glasshouse culture. Once aphids were well established we moved populations from bunchy top cotton to the potential hosts, favouring the most bunchy plants for collecting from, until these hosts had well established aphid colonies. We also placed cages on the plants that excluded beneficial species to ensure aphids had a chance to colonise before beneficials ate them. The plants were monitored weekly for changes in morphology and aphid density. Several weeks after infesting the potential hosts with aphids, we collected several leaves from each plant and freeze dried them for later analysis by Murray Sharman. This approach was repeated over several cotton seasons.

In addition to this work we also made annual and targeted collections of cotton plants and weeds that showed symptoms of CBT. These were also tested for the presence of the disease. Our results have identified 12 additional hosts in addition to cotton and marshmallow identified previously (Table 15). These hosts are predominantly Malvaceae, but there are also hosts from the Euphorbiaceae, Lamiaceae, Fabaceae and Aizoaceae.

Table 15. Number of plants of each species tested and proportion positive for CBT a and/or CBT b for species that were positive for at least one of the CBT strains.

Plant Family	Host species	Common name	Field (F), Glasshouse (GH), Ratoon nursery (RN)	Number +ve for CBT A	Number +ve for CBT B	Number tested
Malvaceae	*Abutilon theophrasti*	Velvet leaf	F, GH	2	5	13
	Anoda cristata	Spurred anoda	F, GH, RN	4	7	7
	Gossypium australe	Desert rose	F	0	1	1
	Hibiscus sabdariffa	Rosella	F (CBT A) GH (CBT B)	1	2	13
	Hibiscus trionum	Bladder ketmia	RN	2	0	15
	Malvastrum coromandelianum	Malvastrum	GH	1	*	20
	Sida rhombifolia	Paddy’s lucerne	F	0	1	15
	Gossypium sturtianum	Sturt’s desert rose	F	2	2	3
Euphorbiaceae	*Chamaesyce hirta*	Asthma plant	F	0	3	18
Lamiaceae	*Lamium amplexicaule*	Deadnettle	RN	1	1	3
Fabaceae	*Medicago polymorpha*	Burr Medic	GH	1	0	4
Aizoaceae	*Trianthema portulacastrum*	Black or Giant pigweed	GH, RN	4	0	12

Widespread collections of both cotton and *Malva parviflora* were also made and these showed that (Table 16):

(i) CBT B is the most commonly collected strain and seems most associated with clear CBT symptoms.

(ii) Many of the examples from cotton were volunteers or ratoons and many of these were positive for at least one CBT strain.

(iii) Marshmallow seems to be the most significant non-cultivated host with over half of the specimens collected positive for at least one CBT strain.

Table 16. Number of plants of each species tested and proportion positive for CBT a and/or CBT b.

Plant	Number of plants tested	Positive CBT A	Positive CBT B	Positive both
Cotton	246	83	168	76
Marshmallow	70	14	31	11

s

Insecticide prevention of CBT

In the second component of the work we designed a simple experiment to evaluate if the common seed treatments, Cruiser and Cruiser Extreme, could reduce the effectiveness of transmission of CBT into cotton seedlings. This could be important in a situation where there is an abundance of a host for CBT and aphids nearby – so aphids could migrate from that host into seedling cotton. We put CBT infected aphids onto Cruiser or Cruiser Extreme treated seedlings at 1 week (1 true leaf) and 4 weeks (4 true leaves) after emergence and compared the proportion of plants that showed disease symptoms with unprotected plants that also received the aphids. The aphids used were from a neonicotinoid susceptible culture (a subsample was sent to Dr Grant Herron, NSW DPI for resistance testing).

The results showed that Cruiser reduced the risk of transmission of CBT by about 50% at both 1 and 4 true leaves. The higher rate Cruiser Extreme reduced transmission to a higher level at 85-95% at 1 and 4 true leaves, respectively. This shows that the insecticide has prevented the aphids from feeding long enough to effectively transmit the disease. However, this was against neonicotinoid susceptible aphids and results may be different with neonicotinoid resistant aphids.

Table 17. Transmission of CBT by aphids into unprotected plants (nil) or plants protected by Cruiser or Cruiser Extreme seed treatment.

Aphid Treatment	**Insecticide treatment**	**Placement**	**Mean plants with CBT (of 5)**
No aphids	Nil	-	0 a
+ CBT aphids	Nil	1 true leaf	2.6 d
	Cruiser	1 true leaf	1.6 c
		4 true leaves	1.0 abc
	Cruiser Extreme	1 true leaf	0.2 ab
		4 true leaves	0.6 ab
	$F_{14,56} = 7.3$, $P < 0.001$		LSD = 0.97

In a second experiment we evaluated the potential to use a new insecticide, highly effective against aphids, to prevent transmission. Application of the insecticide 24 h before aphids were placed on plants was effective and dramatically reduced transmission for both rates (77-93%)(Table 18). Application 10 minutes after the plants were infested also reduced transmission but less effectively (62-85%), while application 24 h after infestation was not effective at all. The results highlight that application of insecticide before a known migration of CBT affected aphids into cotton could effectively prevent infection. For instance, if there are heavily infected ratoon plants near a cotton crop and these are sprayed with herbicide, then pre-treatment of the cotton crop may reduce transmission by aphids forced to migrate off the herbicide treated ‘volunteers’ or ‘ratoons’ (also known as rogue cotton).

If an influx of aphids carrying CBT occurs then application of an insecticide would have to occur very quickly to prevent the initial transmission. If the number of aphids coming into the crop was high enough to result in aphids settling on every plant then, unless the insecticide was very well timed, application would be ineffective. However, if the infestation of aphids was at a lower level, with only a low proportion of the crop infested with aphids then the reduced aphid numbers following insecticide application may not prevent initial transmission but may reduce the risk of secondary transmission (eg from plants that became infected with CBT in the crop to new plants in the same crop).

Table 18. Transmission of CBT by aphids into unprotected plants (nil) or plants protected by an insecticide sprayed onto plants at 1 or 2% concentration.

Aphid Treatment	Insecticide treatment	Timing in relation to aphid infestation	Mean plants with CBT (of 5)
No aphids	Nil	-	0.0 a
+ CBT aphids	Nil	-	2.6 c
	Product 1 (1%)	nil	0.0 a
		24 hr before	0.2 ab
		10 min after	0.4 ab
		24 hr after	2.2 c
	Product 1 (2%)	nil	0.0 a
		24 hr before	0.6 ab
		10 min after	1.0 b
		24 hr after	2.4 c
	$F_{14,56} = 7.3$, $P < 0.001$		LSD = 0.97

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.*

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.

However, recently a range of compounds has become available and we have included them in our research (Table 19). The information summarising effects of the newly registered products is incorporated into the ‘Impact of insecticides and miticides on predators in cotton’ table and provided to Susan Maas (CRDC) for inclusion in the Cotton Pest Management Guide (Figure 52). This table has not been updated as no new products have been registered – but will be updated for 2014/15 with two new compounds.

Table 19. List of compounds and rates tested for target and non-target effect in the period 2010/11 to 2012/13.

Company	**Compound**	**Target species**	**Tested**	**Rates (g ai/ha)**	**Status**
Dupont	Cyantraniliprole	Helicoverpa SLW Aphids	2009/10 2010/11 2011/12	30, 60 60, 120 60	Product registered – will be added to CPMG for 2014-15 following finalisation of position
Dow	Sulfoxaflor	Mirids Aphids GWF	2009/10 2010/11 2011/12 2013/14	24, 96 48, 72 48, 96 48, 72	Product registered – will be added to CPMG for 2014-15 following finalisation of position
ISK	Confidential (IKI-220)	Mirids Aphids	2012/13 2013/14	70, 100 70, 100	Initial report provided to ISK. Second year testing completed.
Agnova Technologies	Confidential (MTI-446)	Mirids SLW GVB	2013/14	18, 75	First year of experiments completed.
Becker-Underwood and NSW DPI	BC639a (*Aspergillus fumigatus*)	Mirids	2009/10	250 & 500 ml/ha	Confidential report provided to NSW DPI. Decision made to cease development.
Growth Agriculture and Cotton CRC	Plant X	Helicoverpa Mirids SLW	2010/11 2011/12 2012/13	1, 2 l/ha 1, 2 l/ha 0.5 l/ha	Initial report provided to CRC and Growth – but formulation now changed – will need to be repeated
Growth Agriculture and NSW DPI	Sero X	Helicoverpa Mirids SLW	2012/13	2 l/ha	Completed but formulation changed again – unable to supply in time.
Becker-Underwood and NSW DPI	*Metarhizium* sp.	Helicoverpa Mirids GVB	2011/12	0.5 l/ha	Not pursued – unable to supply
UQld Gatton	Petir Killat	Unknown	2012/13	1.05 l/ha	Not pursued
NSW DPI	Greenfire	Helicoverpa Mirids	2012/13	75 ml/ha	Not pursued
Becker-Underwood and NSW DPI	*Metarhizium* sp.	Helicoverpa Mirids GVB	2013/14	75 ml/ha	Not pursued

Figure 52. 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)							Beneficials																	Pest resurgence[12]			Toxicity to bees[14]
										Predatory beetles				Predatory bugs							Hymenoptera								
		Helicoverpa	Mites	Mirids	Aphids	Thrips	Silverleaf whitefly	Persistence[8]	Overall Ranking[10]	Total[1]	Red & Blue beetle	Minute 2-spt lady beetle	Other lady beetles	Total[2]	Damsel bugs	Big-eyed Bugs	Other Predatory bugs	Apple Dimpling	Lacewing adults	Spiders	Total (wasps)	Eretmocerus[19]	Trichogramma	Ants	Thrips	Mite	Aphid	Helicoverpa	
Bt[11]		✓						very short	very low	VL	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				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	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		✓[1	✓	✓[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.

Authors
Lewis Wilson and Simone Heimoana – CSIRO Plant Industry*
Robert Mensah – DI&I NSW *
Moazzem Khan – QPI&F*
Martin Dillon – Formerly CSIRO Entomology
Mark Wade – Formerly CSIRO Entomology
Brad Scholz – Formerly QPI&F
Dave Murray - QPI&F *
Viliami Heimoana – Formerly DI&I NSW
Richard Lloyd – QPI&F
Richard Sequeira- QPI&F
Paul DeBarro – CSIRO Entomology
Jonathan Holloway – Formerly DI&I NSW

* Cotton Catchment Communities CRC

8. *Maintain vigilance for emerging problems and help respond as necessary.*

During the lifetime of this project there were outbreaks of spur-throated locusts and cluster caterpillars (2010-11) and broad mites (2011-12). Information was provided to industry via the CottonInfo Team, numerous phone calls and meetings with CCA to assist in management of the pests.

9. *Additional Research*

a. PhD thesis – Simone Heimoana

During the course of this project and the previous two projects one of the Research Projects Officers, Simone Heimoana, was involved in additional studies as the basis of a PhD. Simone took additional measurements in existing experiments and also completed a range of targeted experiments. Supervisors for her research were Doug George (UQld), Greg Constable and Lewis Wilson (CSIRO Plant Industry) (with initial input also from Dr Robert Fletcher, formerly UQld, and Dr Tom Lei, formerly CSIRO Plant Industry). Her thesis was submitted and awarded in 2012 and received high praise from reviewers:

Examiner 1. "I have examined dozens of Ph.D. theses over about 30 years, and have never previously recommended that one be accepted without change. This thesis has changed that. There are always some errors in a thesis, and nothing is perfect. I have included a few comments which the candidate and her supervisors might care to act upon, but in the overall scheme of things they are trivial. They do not detract from the exceptional standard of this thesis, and do not alter my recommendation to accept without change. "

Examiner 2. "Ms. Heimoana is to be congratulated for taking on such a challenging project. Having worked in this research area myself, I can speak first-hand as to the rigors of studying the effects of insect-induced injury on plant physiology. Plants are extremely responsive to "environmental conditions". This requires significant care when trying to document and segregate the effects of biotic stressors (with the needed level of replication) within these daily cycles. The cotton aphid pest system introduces additional challenges in that the insect populations tend to develop fast but can be quite ephemeral. Numerous factors including biological and cultural factors influence the aphid populations. Therefore, in summary this study examined two general factors, plant physiology and cotton aphid populations, both of which are extremely dynamic and variable with the desired outcome of determining the relationship between these parameters – not a simple or trivial task."

The abstract of Dr Heimoana's thesis is provided below and a PDF of her thesis will be provided to CRDC if requested.

Abstract: The effects of aphids (*Aphis gossypii*) Glover on photosynthesis in cotton (*Gossypium hirsutum*).

Simone Charlotte Heimoana. University of Queensland.

Background: Aphids can affect cotton photosynthesis with consequent effects on yield and productivity. However, the extent to which aphid feeding affects photosynthesis and other leaf gas parameters is not well known, especially under Australian conditions. Limited studies in other countries have reported none or negative effects on photosynthesis, however the mechanisms underlying this response are unclear. This study aimed to establish the effect of aphids on cotton photosynthesis and to quantify the relationship between aphid population density and photosynthesis. It also attempted to explain some of the effects found by examining aphid stylet damage to leaf tissue, quantifying plant wound responses that might block phloem elements and hence cause possible feedback effects from leaf sugar build-up. Finally, the secondary effects of honeydew production from aphid infestation on cotton photosynthesis were considered. Honeydew is excreted by aphids as a by-product of feeding. Its build-up on cotton leaves may block stomata and thereby reduce photosynthesis. In the field, sooty mould fungi and dust particles adhering to the sticky leaf surface could additionally reduce photosynthesis through optical effects.

Major findings: The feeding of aphids on cotton plants reduced leaf photosynthesis of infested leaves in two out of six experiments. In large scale field experiments increasing aphid density progressively and negatively affected gas exchange parameters with a maximum reduction in photosynthesis of 27% at a density of 27.6 aphids/cm^2. A 10% decline in photosynthesis occurred at 12.9 aphids/cm^2. Reduced stomatal conductance occurred sooner than photosynthetic decline and required 7.7 aphids/cm^2 for a 10% reduction. Aphid infested leaves were found to have a higher proportion of closed stomata than non-infested leaves. The response was inconsistent at lower aphid densities and photosynthesis was not affected, possibly due to insufficient damage levels, lower rates of callose formation and/or plant compensation. Responses of cultivars with different leaf shapes to aphid infestation were similar.

In stocking density experiments, photosynthesis and stomatal conductance declined within 8 days of infestation with initially 0.7 and 1.5 aphids/cm^2. Measurements were taken repeatedly on the same infested leaves, which established a lower aphid threshold at which stomatal conductance and photosynthesis were first affected (at < 4 aphids/cm^2). The relationships between gas parameters and aphid density were described by negative linear functions and no delays or increases in photosynthesis were detected at low aphid densities. Leaf temperature increased significantly during that period. Leaves did not recover after aphid removal and stomatal conductance remained lower and leaf temperature higher than the controls, indicating that leaf damage by aphids was sufficiently severe and/or that leaf age may have influenced recovery.

Light microscopy confirmed intercellular stylet pathways into phloem but was limited in assessing cellular puncture damage. Callose formation in the interveinal areas of aphid infested leaves was evidence of a plant response to damage and older leaves contained more callose due to longer exposure to aphid feeding. Increases in aphid populations corresponded to significant reductions in photosynthesis but not to simultaneous increases in leaf sugar levels of infested leaves due to callose blockage of sieve elements, and feedback effects due to leaf sugar accumulation did not occur. Requirement of assimilate for boll maturation, leaf age and senescence may have had overriding effects in these experiments. Callose was also found in stomatal guard cells, most likely in response to stylet punctures, though recent literature suggests it may also have a function in the control of stomatal aperture. Closure of stomata due to callose mechanisms could reduce leaf photosynthesis, however, this was not investigated as part of this work.

The effect of natural honeydew was measured in the field and was found to significantly reduce photosynthesis of leaves and stomatal conductance by 41%, however, the effect disappeared soon after honeydew was washed off. To assess the effect of honeydew without the confounding effect of aphid feeding, artificial honeydew was composed and was applied to leaves in several layers in the field and glasshouse. Photosynthesis and stomatal conductance were reduced by 18% and affected by the coverage and thickness of the applied honeydew. Leaves did not always recover when artificial honeydew was washed off, indicating that it may have been washed off incompletely or that honeydew may have been lodged in the sub-stomatal cavity. Application of dust to honeydew further reduced photosynthesis by blocking PAR from reaching the leaf surface. Application of honeydew to the lower leaf surface reduced photosynthesis to a greater degree than its application to the upper leaf surface, implicating stomatal blockage.

This study has shown that aphid feeding reduced photosynthesis through (i) damage to leaves, resulting in stomatal closure and hence reduced conductance, and (ii) the contamination of leaf surfaces with honeydew which both impedes stomatal conductance but also accumulates dust which further reduce photosynthesis by reducing the light reaching the leaf surface

b. Clonal structure of cotton aphid populations and interaction with organophosphate/carbamate (*ACE*1) resistance.

This project also allowed ongoing collaboration with Dr Grant Herron and Dr Yizhou Chen (NSW DPI) and Dr Flavie Vanlerberge-Massutti (INRA – France) in the use of microsatellite markers to characterise the aphid clones in cotton and link with resistance. This research has now been published in Pest Management Science. See below.

c. Assist Dr Grant Herron in sampling aphids and mites for resistance testing

Wilson has worked with Dr Herron to support his resistance management studies. This has included passing reports of spray problems and of aphid or mite outbreaks to Dr Herron to follow up. It has also involved co-ordination of the annual resistance aphid and mite collection trip – usually including the Namoi, St George, Darling Downs, McIntyre, Gwydir and often the Macquarie as well. In addition we have collaborated in the design of experiments to ask specific questions relevant to industry – for instance are OP resistant cotton aphids with ACE 1 resistance also resistant to phorate.

Outcomes

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) Seasonal patterns of abundance of silver leaf whitefly were studied and showed that populations are maintained throughout the year, including through winter, on a range of non-cultivated hosts. This provided a basis to understand differences in abundance of this pest between years.

b) Non-cultivated hosts of silverleaf whitefly were identified. Adults used a wide range of hosts in summer with turnip weed (*Rapistrum rugosum*), sowthistle (*Sonchus oleraceus*) and paddy melon (*Citrullus lanatus*) most significant. Nymphs used sowthistle, turnip weed, bladder ketmia (*Hibiscus trionum*), paddy melon and caustic weed (*Chamaesyce drummondii*). This provided information that will assist in understanding differences in abundance between years and in target strategies for on-farm hygiene.

c) Winter hosts of silverleaf whitefly were identified. For nymphs sowthistle, bladder ketmia and blackberry nightshade were most important. Particular in spring, sowthistle seems to be an important indicator host.

d) Life history studies with silverleaf whitefly showed that survival of eggs is generally between 50-80%. Mortality of eggs was due to their disappearance– probably due to either dislodgement or predation. Survival of nymphs was lower than survival of eggs and nymphs showed reduced survival as the season progressed, -from about 50% for experiments that began in December to less than 10% for experiments that began in March. Mortality was due to an increase in nymphs 'missing' but this also coincided with increasing losses to predation and parasitism and some missing nymphs were likely eaten. This information provided the basis to understand factors that influence the build-up of SLW and strongly implicates the need to maintain beneficial populations - especially through December and January.

e) A range of predator species was evaluated to test if they would consume eggs or nymphs of silverleaf whitefly. They included mite eating ladybeetle, minute two spotted ladybeetle, a range of larger lady beetles, brown smudge bug, apple dimpling but, red and blue beetle and phytophagous thrips. All consumed eggs or nymphs. In most cases attacked eggs or nymphs were simply gone – reinforcing that some of the missing category in the SLW life history studies was due to predation.

f) The interactions of Bt-transgenes vs non-Bt cotton, okra leaf shape vs normal leaf shape and sprayed vs unsprayed cotton was explored to understand factors contributing to or reducing the risk of outbreaks of silverleaf whitefly. Results showed

    i) No evidence that SLW are worse on Bt-cotton than non-Bt cotton. Differences are likely to be due to differences in spray regimes between Bt and non-Bt cotton.

    ii) Okra leaf shape offers considerable resistance to SLW

    iii) Broad-spectrum sprays applied against mirids led to much higher populations of SLW and also increased the levels of honeydew contamination on bolls.

    iv) Confirmation that use of broad-spectrum insecticides (dimethoate, fipronil, pyrethroids) against mirids increased the risk of spider mites outbreaks.

g) A methodology was developed and validated to investigate factors affecting the levels of honeydew sugars on bolls. This included development of artificial honeydew and a realistic application method. We also developed a micro-sprinkler system and indentified the opportunity to use a lateral move irrigator to simulate rainfall. Key outcomes were:

    i) Breakdown of honeydew by sunlight alone occurs very slowly.

    ii) Rainfall has a very significant effect on the level of honeydew on bolls, and as little as 10-15 mm can substantially reduce contamination levels in the field.

    iii) Over 90% of honeydew was found on the outer 5 mm of the boll. This combined with the hydrophobic nature of cotton explains why rainfall running over the boll surface is so effective in removing honeydew.

    iv) A simple but strong non-linear relationship was found between % honeydew removed and rainfall which predicted 67% of the variability.

h) The seasonal abundance and non-cultivated host use of green vegetable bug (GVB) showed that:

    i) GVB abundance was higher from August 2010 onwards, possibly due to widespread rainfall and abundant growth of weed hosts

    ii) Green form adult populations were maintained on weeds in winter, but there were also substantial populations of bronze diapause form in diapausing habitats – especially under bark of river redgums

    iii) Adults were most abundant on stinging nettle *(Urtica incisa*), turnip weed (*Rapistrum rugosum*), sowthistle (*Sonchus oleraceus*), marshmallow (*Malva parviflora*) and wild sunflower (*Helianthus annuus*)

    - iv) Nymphs were mostly found on turnip weed (*Rapistrum rugosum*), greater beggar's ticks (*Bidens subalternans*), bishop weed (*Ammi majus*) and stinging nettle (*Urtica incisa*).
    - v) No nymphs were found in winter. Population in October followed the transition of bronze adults back to green on weed hosts
    - vi) Parasitism rates by the tachinid fly, *Trichopoda giacomellii*, were very low coming out of the drought period but higher following drought breaking rains, probably reflecting the generally higher and more consistent availability of GVB as hosts

- i) Studies of sequential host use by GVB showed that they prefer to feed and oviposit in legume crops such as mungbean, pigeon pea, soybean and lucerne. Changes in the cropping system toward earlier production of legume crops could create a greater risk to cotton, however, some legumes have the potential to be trap crops and draw GVB away from cotton.
- j) Results confirm that sorghum is a potential host for GVB but only during the flowering and early seed maturation period.
- k) Research continued with cotton bunchy top disease:
    - i) We identified 12 additional hosts for the disease in addition to cotton and marshmallow. These hosts are predominantly Malvaceae, but there are also hosts from the Euphorbiaceae, Lamiaceae, Fabaceae and Aizoaceae.
    - ii) Studies with seed treatments effective against aphids showed that the insecticide prevented the aphids from feeding long enough to effectively transmit the disease.
    - iii) Studies with a foliarly applied insecticide showed that application before CBT infected aphids settled on plants reduced transmission effectively. Application just after they settled also reduced transmission but application 24 hours after settling had no effect.
- l) Research on the target and non-target effects of new insecticides continued. Nine new compounds were evaluated. Amongst these were two recently registered compounds that will be added to the 'Impact of insecticides and miticides on beneficials' table for 2014/15. Screening for 2 new synthetic insecticides and several bio-pesticides is underway.
- m) Information was provided to industry to assist in management of outbreaks of spur-throated locusts and cluster caterpillars (2010-11) and broad mites (2011-12)
- n) Simone Heimoana successfully completed her PhD 'The effects of aphids (*Aphis gossypii*) Glover on photosynthesis in cotton (*Gossypium hirsutum*)' and her thesis was examined and the degree awarded. This is important in potential succession in the IPM area for the cotton industry.
- o) The project leader, Lewis Wilson, also contributed as a committee member to the TIMS Committee, the TIMS Insecticide and TIMS BT-Cotton Technical Panels, to REFCOM and the Australian Cotton Industry Bio-security Committee.

Describe any:-

a) **technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.); Nil**

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

We have not developed methods that could be patented or that need to be protected, but we have developed methods that are useful to the cotton industry. These include:

1. Formulation of artificial honeydew and use of an atomiser to apply it to bolls in a manner that closely approximates the way it would happen in the field. This provides a means to generate contaminated bolls and hence to ask specific questions that would be difficult to approach if we were reliant on natural outbreaks of SLW to contaminate bolls.
2. Developed the use of a cotton bunchy top refuge as a way to target questions about potential alternative hosts.
3. Developed a method to mass rear SLW on kale which can be transplanted into the field to generate outbreaks of this pest. This provides flexibility in not having to rely on natural outbreaks to occur – and hence increases the chance of success with manipulative experiments using insecticides or other management variables.

c) **Required changes to the Intellectual Property register. Nil**

Conclusion

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

This project has addressed a range of new and ongoing challenges to integrated pest management in cotton.

a. Providing new information on the seasonal abundance and host use of SLW

b. Providing new information on the survival of SLW in cotton, mortality factors and potential predators

c. Providing new information on the fate of honeydew on cotton bolls

d. Completing system analysis experiments of interactions between transgenes (Bt), leaf shape and mirid sprays and outbreaks of SLW

e. Confirming seasonal abundance patterns and key host use by GVB

f. Evaluating the preferences of GVB adults and nymphs in crop sequences.

g. Identifying new hosts for cotton bunchy top disease

h. Evaluating the effect of seed treatments and a foliar applied insecticide on transmission of cotton bunchy top disease

i. Evaluating target and non-target effects of a range of synthetic and biologically based insecticides.

These achievements provide a solid platform from which to modify and adapt existing guidelines for integrated pest management in cotton to allow for a cotton system that is evolving in many areas. In particular, consistent messages that emerge from this research include firstly, that farm hygiene is important in managing risks from a range of pests and diseases (e.g. CBT) as they will use weed hosts on farm in winter to survive, secondly, that mortality due to beneficials (predators and parasites) is a key component of overall mortality and conservation of beneficials remains a core component for IPM even in the transgenic era, thirdly, management of honeydew contamination of cotton is challenging once it occurs, with fortuitous rainfall the main factor, hence pro-active IPM compatible management of SLW to prevent the problem is a better strategy, fourthly, use of insecticides to reduce risks from CBT is effective though limited in durability as insecticide doses decline and increase risks of selecting for pesticide resistance in aphid populations and finally, ongoing evaluation of compounds for the IPM fit need to be maintained to ensure best positioning of compounds to support IPM.

Extension Opportunities

Steps taken:

(i) The efficacy and IPM fit of new chemistry has been published for industry.
(ii) Wilson has presented to the CCA winter meeting at least once and often twice in each year of the project – discussing issues such as Broad mites, SLW management, Early season pest management, Mite ecology and management, Forecasting pest population, Assessing the IPM fit of new insecticides.
(iii) Information on IPM for modern Bollgard II systems has been published
(iv) Heimoana has provided training in identification of mites, aphids, SLW and thrips to visiting groups of agronomist – including for Auscott each year.
(v) Smith has presented information on pest sampling, in collaboration with Sandra Williams, to consultants and agronomists in the Macquarie twice during the project
(vi) Wilson has answered regular phone calls and email requests for identification of pests or discussions on management of particular situations. These requests come from widely across the industry and at peak times in the early season and in Jan –March can be 6-7 calls each week, and sometimes 2-4 per day.
(v) Wilson has spoken at field days at Griffith, Moree (x3), and has supported the Cottoninfo team with requests to contribute to industry driven meetings or farm visits – for instance two meetings in late 2013 in Moree in response to problems with symphyla.

Steps that need to be taken:

(i) Information on the effect of late damage on yield needs to be packaged up into a Cotton Grower article for industry and published in a scientific journal.
(ii) Information on SLW host use and mortality factors needs to be published for industry
(iii) New information on the interaction between seed treatments and foliar sprays and transmission of CBT needs to be made available to industry but should be repeated first – this is underway.
(iv) Information on the interaction between transgenes, leaf shape and mirids sprays and implications for SLW and other secondary pests need to be packaged for industry and published in a journal.
(v) Outcomes from Dr Heimoana's thesis are being written up for scientific publication but should also be published for industry
(iv) Outcome of studies with the fate of honeydew have been widely disseminated verbally but need to be published for industry and scientifically.

For future research.
This is detailed in a new submission that has been funded by CRDC. Aims for that project are:

1. To improve knowledge of and management of SLW by (i) identifying factors contributing to reductions in honeydew on cotton and implications for cotton fibre quality (ii) identifying seasonal host use for SLW whitefly (iii) to assess mortality on cotton through the cotton season and identify potential causes.

2. To provide tools for IPM by (1) assessing the efficacy and non-target effects of new insecticides, biopesticides and semiochemicals (ii) testing options to manage mirids and GVB with reduced risk of flaring SLW or other secondary pests (iii) exploring options for alternatives to the neonicotinoid seed treatments for control of thrips

3. Manage early season damage by measuring the effect of early season thrips damage on plant growth, yield and maturity in southern regions

4. Understand Cotton Bunchy Top Disease by continuing to identify alternative host species, investigate the effectiveness of insecticide application to prevent spread of CBT and continuing to investigate aspects of epidemiology

5. Identify and Manage Emerging pests by providing flexibility to undertake research to manage emergent/exotic pests, including those arising due to changes in the farming system

In addition to better target the objective to identify the species that eat SLW and their effect on mortality a second project has been funded to work with Dr James Harwood at the University of Kentucky to use DNA markers to identify insects that contain DNA from SLW or green vegetable bug. By combining this with consumption and retention studies it is possible to understand the relative significant of different predator species in SLW control.

Publications arising from the research project and/or a publication plan.

Journal Papers

- Wilson L, Downes S, Khan M, Whitehouse M, Baker G, Grundy P, Maas S (2013) IPM in the transgenic era: A review of the challenges from emerging pests in Australian cotton systems. *Crop and Pasture Science* 64: 737-749
- Chen Y, Vanlerberghe-Masutti F, Wilson LJ, Barchia I, McLoon MO, Smith T and Herron GA (2012) Evidence of superclones in Australian cotton aphid *Aphis gossypii* Glover (Aphididae: Hemiptera). *Pest Management Science* 69: 938-948
- Reddall AA, Sadras VO, Wilson LJ and Gregg PC (2011) 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* 67 :360-369
- Lu B, Downes S, Wilson L, Gregg P, Knight K, Kauter G, McCorkell B (2011) Preferences of field bollworm larvae for cotton plant structures: Impact of Bt and history of survival on Bt crops. *Entomologia Experimentalis et Applicata* 140 :17-27
- Lu B, Downes S, Wilson L, Gregg P, Knight K, Kauter G, McCorkell B (2012) Yield, development and quality response of dual-toxin Bt-cotton to manual simulation of damage by *Helicoverpa* spp. in Australia. *Crop Protection* 41 :24-29
- Lu B, Downes S, Wilson L, Gregg P, Knight K, Kauter G, McCorkell B (2012) Yield, development, and quality response of dual-toxin Bt cotton to *Helicoverpa* spp.

infestations in Australia. *Entomologia Experimentalis et Applicata* 145: 72-81

Book Chapters

- Fitt GP and Wilson LJ (2012) Integrated pest management for sustainable agriculture. In; Integrated pest management principles and practice. (eds Abrol DP and Shankar U) pp 27-40 CABI, Wallingford, UK.

- Gregg, P., Wilson L and Harris, G. (2012) The Farm Program. In, Weaving a Future for Australia's cotton, catchments and communities. Seven years of Co-operative Research. Cotton Catchment Communities CRC. ISBN 978-0-9872308-2-9. pp 54 90

Conference Papers

- Wilson, L., Heimoana, S. and Smith, T. (2012) Other Little pests with big challenges. In, Proceedings of the 16th Australian Cotton Conference 'Growing better all the time'. August 14-16th, 2012. pp 89-93
- Marshall, K., Wilson, L. and Herron, G. (2012) Do the neonicotinoid seed treatments Cruiser and Cruiser Extreme control resistant aphids. Proceedings of the 16th Australian Cotton Conference 'Growing better all the time'. August 14-16th, 2012. pp 102
- Sharman, M., Gambley, C., Maas, S., Wilson, L., Smith, L. and Gibband, M. (2012) Cotton blue disease: Bio-security risk. Proceedings of the 16th Australian Cotton Conference 'Growing better all the time'. August 14-16th, 2012. pp 101
- Heimoana, S., Wilson, L., Constable, G. and George, D. (2012) The effect of honeydew on photosynthesis in cotton. Proceedings of the 16th Australian Cotton Conference 'Growing better all the time'. August 14-16th, 2012. pp 99
- Sharman, M., Gambley, C., Maas, S., Wilson, L. and Smith, L. (2012) Cotton bunchy top disease. Proceedings of the 16th Australian Cotton Conference 'Growing better all the time'. August 14-16th, 2012. pp 95

Industry Extension material

- Lu, B., Downes, S., Wilson, L., Gregg, P., Knight, K., Kauter, G. and McCorkell, B. (2011) How do susceptible *Helicoverpa* larvae behave in Bollgard II? The Australian Cottongrower 32(7): 12-15.
- Williams, S., Wilson, L and Vogel, S. (2011) Pests and beneficials in Australian cotton landscapes. Cotton Catchment Communities CRC. 96 pp http://www.cottoncrc.org.au/industry/Publications/Pests_and_Beneficials
- Smith, R., Williams, S., Wilson, L. and Vogel, S. (2012) Reducing pesticide use – Saves costs.In, The Australian cotton production manual 2012. Cotton Catchment Communities CRC. Pp 101-103
- Wilson, L., Williams, S and Mensah, R. (2012) Insect and mite management. In, The Australian cotton production manual 2012. Cotton Catchment Communities CRC. Pp 108-114
- Lu, B., Downes, S., Wilson, L., Gregg, P., Knight, K., Kauter, G. and McCorkell, B. (2011) Is the threshold for *Helicoverpa* spp. larvae in Bollgard II adequate? Australian Cottongrower 32(6): 40-45.
- Taylor, I., Sequeira, R., Grundy, P. and Wilson, L. (2012) SLW – wet conditions, late crops and displaced populations. The Australian Cottongrower 33(2): 16-18
- Ceeny, S., Baker, G., Whitehouse, M., Gregg, P., Tann, C., Leven, T., Downes, S., and Wilson, L. (2012) Refuge crops – investing in cotton's future. The Australian Cottongrower 33 (7): 14-16
- Wilson, L. (2011) Pest Management. CSD Variety Trial Results Booklet 2011. Pp 109-110.

- Wilson, L. (2012) Pest Management. CSD Variety Trial Results Booklet 2012. Pp 102-103.
- Wilson, L. and Heimoana, S. (2013) Pest Management. CSD Variety Trial Results Booklet 2013. Pp 82-83
- Wilson, L,. Schellhorn, N., Miles, M., Grundy, P., Herron, G., Mensah, R. and Gregg, P. (2013) Integrated pest management in cotton – a commonsense approach. Australian Cottongrower 34(4) 22-27.
- *Leven, T., Mass, S., Mensah, R., Sequeira, R., Wilson, L. and Dillon, M. (2012) Key insect and mite pests of cotton. In, Cotton Pest Management Guide 2012-13, The Australian Cotton Industry Development and Delivery Team pp 5-47 http://www.cottoncrc.org.au/industry/Publications/Pests_and_Beneficials
- *Wilson, L., Williams, S., Mensah, R. And Johnson, A. (2012) Integrated Pest Management guidelines for Australian Cotton. In, Cotton Pest Management Guide 2012-13, The Australian Cotton Industry Development and Delivery Team pp 49-62 http://www.cottoncrc.org.au/industry/Publications/Pests_and_Beneficials
- Mass, S., Williams, S., Wilson, L., Mensah, R. and Leven, T. (2013) Integrated pest management in cotton. In, Cotton Pest Management Guide 2013-14, The Australian Cotton Industry Development and Delivery Team pp 47-58
- *Bird, L., Downes, S., Wilson, L., Miles, M. and Leven, T. (2012) Insecticide Resistance Management Strategy (IRMS) for 2012/13. In, Cotton Pest Management Guide 2012-13, The Australian Cotton Industry Development and Delivery Team pp 63-71 http://www.cottoncrc.org.au/industry/Publications/Pests_and_Beneficials
- *Downes, S., Wilson, L., Knight, K., Kauter, G. and Leven, T. (2012) Preamble to the Resistance Management Plan (RMP) for Bollgard II 2012/13. In, Cotton Pest Management Guide 2012-13, The Australian Cotton Industry Development and Delivery Team pp 72- 77 http://www.cottoncrc.org.au/industry/Publications/Pests_and_Beneficials
- *Wilson, L., Heimoana, S., Mensah, R., Khan, M., Dillon, M., Wade, M., Scholz, B., Murray, D., Lloyd, R., Sequeira, R., De Barro, P., Heimoana, V. and Holloway, J. (2012) Impact of insecticides and miticides on beneficials in Australian cotton. In, Cotton Pest Management Guide 2012-13, The Australian Cotton Industry Development and Delivery Team pp 7-9

* Publications updated and republished for industry each year

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

Nil