FINAL REPORT

(due on completion of project)

Part 1 - Summary Details

Cotton CRC Project Number: 1.01.61 CRC155

Project Title: Mirid and stinkbug management in Bollgard® II

Project Commencement Date: 1 July 2008 **Project Completion Date:** 30 June 2011

Cotton CRC Program: **The Farm**

Part 2 – Contact Details

Principal Researcher: Dr Moazzem Khan (Senior Research Scientist, Entomology)

Organisation: DEEDI

Postal Address: PO Box 102, TOOWOOMBA, QLD 4350

Ph: 07 46881310

Signature of Research Provider Representative: ______________________________

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

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

Background

1. Outline the background to the project.

Mirids and stinkbugs have been a major problem in Bollgard II in recent years, requiring 2-5 sprays every season. Although recent research (DAQ131) has established crop stage wise threshold for mirids (Cotton Pest Management Guide 2010-11) a cotton industry survey revealed that growers are 'insurance' spraying when mirid numbers are below suggested thresholds. The insecticdes currently registered for mirids are mostly broad-spectrum and application of these chemicals at early crop stages can flare secondary pests such as whitefly, mites and aphids. One main reason for insurance sprays is possibly a lack of confidence in the relationship between mirid number and fruit loss. Although recent research on mirids has addressed some aspects of this issue, further effort is needed to refine mirid management by preventing economic loss while still avoiding unnecessary sprays and possible secondary pest problems. A recent US study showed that plant based monitoring is more effective for lygus management decisions than number based monitoring. In addition to mirid number based thresholds, the development of plant based thresholds and plant based monitoring procedures (through detailed studies on mirid number, fruit loss and yield) could increase growers and consultants confidence in mirid management decisions, therefore reducing insurance sprays.

The Australian cotton industry places a large emphasis on IPM. To boost IPM further, along with plant based threshold and monitoring procedures, IPM tools are needed. Salt mixtures are considered to be one such tool to reduce chemical rates and to decrease impact of the chemicals on beneficial insects, without reducing the efficacy to the target pest. Further research is needed to identify more chemicals that can mix with salt against mirids and stinkbugs. As with salt mixtures, biopesticides based on fungal pathogens and plant volatiles may be a useful IPM tool in managing mirids and stinkbugs with minimal disruption.

The aim of this report is to present the results of the studies conducted to develop plant based thresholds and monitoring procedures for mirids and to develop a management strategy to fit with the existing IPM systems.

Objectives

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

This project was supported for three years with limited funding without any technical assistance. Therefore objectives were limited. Throughout the project period some growers and consultants helped to achieve the objectives listed below;

1. Investigate mirid and stinkbug damage in Bollgard® II
2. Investigate IPM options and selective insecticides management
3. Disseminate mirids and stinkbugs ecology, biology, damage and management information to industry and scientific community

All of these objectives have been achieved. Additional trials were conducted to evaluate kaolin particle film technology against whitefly and salt mixture against *Solenopsis* mealybug

Methods

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

Mirid damage in Bollgard® II

Altogether three field trials, one for the verification of a crop stage wise threshold and the other two for a plant based threshold, were conducted in growers fields on the Downs.

Verification of crop stage wise threshold

To verify the mirid crop-stage wise threshold that was determined in the previous project (DAQ131), a replicated trial was conducted in Bollgard II cotton in Macalister in the 2008-09 season. In this trial, the crop-stage wise threshold was compared with the growers/consultants management decision on mirids using RCB design with 3 replications. Each replication was 435 m X 72 rows. Crops were sampled weekly, 3 X 20 m suction samples were taken initially and then 5 X 1 m beat sheet per replication were used later. Once the crop started squaring, plants were mapped weekly. Retention levels were determined by counting 1st position fruit for all nodes, 3 X 1 m per replication was taken. Similar assessments were made in the grower's treatments but management decisions were made by the grower/consultant from their own assessment. For crop stage wise threshold plots, once mirid numbers reached the threshold level at each crop stage, crops were sprayed with low rate chemical plus salt. For the growers/consultants management plot, the spray and choice of chemical decision was made from their assessment. Cotton was machine harvested for yield determination. Data were analysed using the t-test to compare the threshold and grower plots for mirid and beneficial numbers and yield.

Mirid plant based threshold

Two trials were conducted to develop a plant based management strategy. Methodology for Trial 1 was based on pre-determined mirid numbers and retention levels which proved to be difficult to achieve naturally if the mirid numbers ran low. To overcome this problem, the methodology in Trial 2 was modified.

Trial 1

The trial was conducted in Bollgard® II cotton in Dalby during the 2008-09 season. Different levels of mirid densities and fruit retention were assessed to develop a plant based threshold using the split plot design. Treatments were three levels of mirid (1, 2 and 3/m), two crop stages (squaring and early boll) and three retention levels (80, 70 and 60%). Treatments were replicated three times. Each replication was 246 m X 12 rows. Weekly sampling (1 x 20m suction per replication was taken until plants were tall enough to do beat sheet at 3 X 1m per replication) was conducted to determine population level in the trial and the mirid numbers were manipulated using different rates of fipronil. Once the mirid numbers reached the desired level at each cropping stage, 3 X 1m row per replication was assessed to determine retention levels. The selected cotton was monitored weekly until harvest and cotton was hand harvested. There was a sprayed control for each mirid level, crop stage and retention level. The sprayed control cotton was sprayed once the mirid levels reached the desired level and were kept mirid free until harvest.

Throughout the squaring and early boll stage, mirid numbers were very low, less than 0.5/metre. In the 3rd week of January, when plants were already in late boll stage they reached 1-2/metre. Plant mapping data also showed that the retention level was quite high, 80% and above. Therefore, the planned trial protocol to develop a plant based threshold at squaring and early boll stage was discontinued.

However, the trial was continued at the late boll stage with a modified protocol even though previous research showed that mirids do not cause significant damage at the late boll stage. Each replication of 246 m X 12 rows for each treatment (1/m and 2/m mirids) was divided into two management levels, sprayed and unsprayed. Cotton was sprayed immediately after mirids reached the desired level. For each replication, 2 X 1 m row was selected for each 60, 70 and 80% retention level. Instead of assessing all nodes, retention level was determined by assessing first position bolls up to 5 nodes from the first unfolded leaf. Beyond 5 nodes first position bolls are already mature enough to incur any damage from mirid feeding. On several occasions, damage was artificially inflicted to get 60 and 70% retention level. Assessment of retention levels continued until the 2nd week of February. Cotton was hand harvested. Data were analysed using analysis of variance to compare between retention levels. The t-test was used to compare management levels (unsprayed vs. sprayed), mirid levels (1 vs 2/metre) for each retention level. A General Linear Model was used to detect any interaction between treatments.

Trial 2

This trial was conducted over two seasons in irrigated Bollgard II cotton (Sicot 71BRF) across 6 sites during 2009-10 and across 3 sites during 2010-11 on the Darling Downs. Details of these sites are given in Table 1. At each site, the field was divided into three plots to accommodate three treatments (each plot was about 1 to 3 ha).

Three treatments were established as unsprayed, sprayed and commercial (farmers management) to obtain different levels of mirid activity and damage. Cotton was sampled weekly starting from the 4 leaf stage; first with a suction machine (3 X 20 m per plot) and then with a beat sheet (once plants grew tall enough, usually at the 8 leaf stage 7 X 1 m per plot were taken during 2009-10 and 9 X 1 m per plot during 2010-11 seasons). Mirid numbers were recorded as adult, small (1st to 3rd instar) and large (4th & 5th instar) nymphs. Weekly retention levels were recorded once plants started squaring. During the 2009-10 and during 2010-11 season 20 plants (5 groups of 4 plants) and 27 plants (9 groups of 3 plants) per plot respectively were earmarked for retention assessment. The same plants were checked throughout the trial period. Retention was determined in two steps, first by recording 1st and 2nd position fruits for top 5 nodes, then for whole plants.

Table 1. Description of trial sites

Seasons	Trial site	Variety	Plot size (per replication)	Date of planting
2009-10	Tarcoola, Macalister, (Neville Walton)	Sicot 71BRF	400 m X 72 rows	1st wk Nov
	Mirrabooka, Macalister (Mark Bailey)	Sicot 71BRF	800 m X 24 rows	1st wk Nov
	Loch Eiton , Nandi (Paul/Steven McVeigh)	Sicot 71BRF	400 m X 24 rows	3rd wk Oct
	Bogaroo, Daandine	Sicot 71BRF	850 m X 32 rows	1st wk Nov
	Cranbrook, Brookstead (Russell Keeley)	Sicot 71BRF	550 m X 24 rows	3rd wk Oct
	Gebur, Jandowae (Simon Donaldson)	Sicot 71BRF	850 m X 24 rows	3rd wk Oct
2010-11	Mayfield, Nandi (Glen /Shaun Fresser)	Sicot 71BRF	643 m X 24 rows	3rd wk Oct
	Tarcoola, Macalister, (Neville Walton)	Sicot 71BRF	400 m X 72 rows	3rd wk Oct
	Gebur, Jandowae (Simon Donaldson)	Sicot 71BRF	1100 m X 24 rows	3rd wk Oct

Data were analysed using ANOVA for mirid number, retention and yield. Data were also subjected to regression analysis to determine the relationship between mirid numbers and retention, mirid numbers and yield and between retention and yield.

Pale cotton stainer damage assessment in glasshouse

Two trials were conducted to understand cotton stainer damage. In Trial 1, damage was assessed immediately after insects were taken off the plants and in Trial 2 damage was assessed at harvest. Three age groups – 5, 10 and 30 day old bolls in Trial 1 and 5, 15 and 35 day old bolls in Trial 2 were presented for feeding by either a male or female cotton stainer for 7 days. The untreated control bolls of the same age group were assessed without insect feeding. The trial was replicated 7 times. Boll age was determined by tagging at bloom. First position boll at 4 or 5 nodes (ones that set first) from the bottom were used. Tagged bolls were monitored regularly for damage until caged. Only undamaged bolls were included in the trial. Bolls were caged using foam cup cage the day before the cotton stainer adults, 5-7 days old, were confined on the bolls. Insects were checked every day and dead ones were replaced with an insect of the same age from the stock culture. For Trial 1, bolls were allowed another 7 days after insects were removed to develop symptoms and then brought back to the laboratory for assessment. Boll damage was assessed as the number of black spots on the boll; warts inside the boll wall and lock damage. For Trial 2, boll damage was assessed in two stages. Firstly external damage (number of black spots) was assessed before the boll was open. Other damage parameters such as lock damage, lint yield for individual bolls, percent damaged seed and percent germination was assessed after harvest. For lock damage, an assessment of each lock was made for spots of brown coloured lint, ¼, ½, ¾ and full lock damage. For easy analysis, each type of damage was assigned a damage score. A spot of brown coloured lint = 1, ¼ lock damage = 2, ½ lock damage = 3, ¾ lock damage = 4 and full lock damage = 5. A total boll rating was obtained by adding the ratings for the individual locks. Cotton was ginned using a 20 saw hand gin.

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

Population changes in mirids and stinkbugs

Mirid and stinkbug population changes were assessed on cotton, at Nandi and in Macalister during the 2008-09 season, at Macalister, Nandi, Brookstead and Jandowae during the 2009-10 season and at Macalister, Nandi and Jandowae during the 2010-11 season. At each site, population estimates were obtained from November to April by sampling once every week. A twenty metre suction and a single 1 metre beat sheet sample of cotton plants constituted a sample. Suction sampling was continued until the plants grew tall enough to use the beat sheet.

Selective management option for mirids and stinkbugs

Two trials, one with Shield and salt mixture and another with Plant X and fungus, were conducted against mirids. Planned trials against stinkbugs were not possible because of low GVB or pale cotton stainer population. Two additional trials, one with kaolin particle film against whitefly and another with salt mixture against *Solenopsis* mealybug, were conducted in the glasshouse.

Shield® and salt mixture against mirid

To evaluate Shield® 200 SC (clothianidin) against mirids and beneficials a trial was conducted in Bollgard II cotton (var. Sicot 71BR) in Macalister. The trial was conducted at the maximum fruiting stage using randomised block design with 3 replications. Plots were 24 rows by 15 m long. Three rates of Shield® alone and mixed with table salt, NaCl (@ 10 g/L of water) were used. Fipronil 200 SC was used as a standard control. Treatment details are given in Table 2. The chemicals were applied very early in the morning with a ground rig (100 L/ha) using a Spray Coupe with a 24 m boom, fitted with Turbo Tjet (110 x 02'S) nozzles (one from either side) at 24 km/h with 4.5 bar pressure.

Pre-treatment assessments were made the day before treatments were applied. Post-treatment assessments were made at 3, 7 and 20 days after treatment (DAT). Mirids and beneficials were assessed using a beat sheet, 3 x 1 m sections of row per replication.

Table 2. Treatments and rates used in the trial

Treatment	Formulation (g/L)	Rate (mL/ha)
Shield®	Clothianidin (200)	100
Shield®	Clothianidin (200)	125
Shield®	Clothianidin (200)	250
Shield® + salt	Clothianidin (200) + Table Salt	100 + 10 g/L of water
Shield® + Salt	Clothianidin + Table Salt	125 + 10 g/L of water
Regent® + salt	Fipronil (200) + Table Salt	50 + 10 g/L of water
Control	Untreated	-

Data were analysed using ANOVA and means were compared using LSD at P=0.05. For beneficials, data were subjected to analysis of covariance using prespray data as a covariate.

Plant X and fungus against mirids

Plant X, a plant product and a fungus being developed by Dr Robert Mensah of I&I NSW, reportedly has activity against mirids and is considered relatively selective. As part of a broader evaluation of Plant X and fungus, an efficacy trial was conducted on the Darling Downs. The trial was conducted in the farmer's field near Dalby at peak boll set. Eight treatments; two rates each of Plant X (1 and 2% v/v) and fungus BC639 (250 and 500 mL/ha), Regent 40 mL/ha plus Plant X (2% v/v), Regent 40 mL/ha plus salt (10 g/L of water), Regent 125 mL/ha and unsprayed control, were used for the trial. The treatments were replicated 3 times in a RCB design. Each replication measured 15 m X 24 rows. The chemicals were applied with a 24 m ground rig (100 L/ha), fitted with flat nozzles 100/210-02 from the top at 15 km/h with 3.5 bar pressure.

Pre-spray counts for mirids and beneficials were made the day before spraying and post spray counts were made 3, 7 and 14 days after treatment (DAT). For each replication, 3 X 1 m area was assessed using a beat sheet. Data were subjected to ANOVA and means were compared using LSD at P=0.05.

Kaolin against whitefly

A choice test to evaluate kaolin against whitefly was conducted in a glasshouse in July 2009 on squaring plants using a randomized block design with 6 replications. Kaolin used in this trial was Surrround™ WP (SWP) from USA (Englehard Corp., Iselin, NJ). The treatments were SWP 100 g/L, SWP 100 g/L plus canopy oil (1% v/v), canopy oil (1% v/v) and untreated control. Kaolin, 500 mL per treatment, was applied to run off using a hand pressure sprayer. After air drying, plants were arranged randomly on benches inside the glasshouse. Adult whitefly, male and female (about 1000), were released in the middle of the glasshouse to select treated plants. Numbers of whitefly per plant were recorded 1, 3 and 5 hours and then 1, 3 and 7 days after release.

Salt mixture against Solenopsis mealybug

The trial was conducted in a glasshouse to evaluate a salt and Shield® (clothianidin) mixture against *Solenopsis* mealybug. Four treatments; Shield® 250 mL/ha, Shield® 250 mL/ha plus salt (10 g/L), salt (10 g/L) and water treated control with 6 replications were evaluated. *Solenopsis* mealybug, originally collected from Emerald were established on 8/9 leaf stage plants a month before the treatments were applied. Pre-spray counts were made the day before treating, and numbers of small and large nymphs, adults and adults with ovisacs were recorded. The treatments were applied using a Knapsack sprayer (@250 L/ha). The knapsack sprayer was calibrated to discharge each treatment for one minute. Post treatment counts were made at 3, 6 and 15 days after treatment (DAT). Data were analysed using oneway ANOVA and means were separated using Fisher's Least Significant Difference Test at 5%.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Mirid damage in Bollgard® II

Verification of crop stage wise threshold

Bollgard II cotton, Sicot 71BR was planted on the 1st week of November 2008 and sampling commenced from the 2nd week of December at the 6 leaf stage. Assessment of retention level started from the last week of December. Data on mirid numbers are summarised in Figure 1. Mirids moved to cotton at the seedling stage and on the the 24th December mirid population were 1.2-1.3/metre. The first plant mapping on the 30th December showed 85 to 90% retention. On the 28th December, Regent (@60 mL/ha plus salt) was sprayed in the grower plots, although mirid population and retention level were below threshold level. Mirids reached threshold level on the 13th January (3.4 and 4.3/metre in threshold and grower plots respectively) at early boll stage and both treatments were sprayed (Regent @ 50 mL/ha plus salt) on the 15th January. The retention level at this time was 80 to 90%. Thereafter mirid numbers remained low for a few weeks. However at the end of the early boll stage mirid numbers again reached above 3/metre in both the threshold and grower plots and retention level reduced to 65%. Cotton was sprayed with Regent (@80 mL/ha plus salt) on 25th February. For the whole season, the mirid numbers were slightly higher in grower plot than the threshold plot and the difference was not significant ($t = -1.51$; $p = 0.137$; $df = 49$). The major beneficials were ladybird beetles, red and blue beetles and spiders. There was no significant difference ($t = -0.48$; $p = 0.674$; $df = 79$) between beneficial numbers in the threshold plot and the grower plot. Overall yield was low, 6.5 bale/ha in the grower plot and 6.2 bale/ha in the threshold plot and the difference was not significant ($t = 2.32$; $p = 0.146$; $df = 2$). Low yield was mainly due to water shortage at late boll stage. The trial clearly showed the decision to spray at below threshold level at early stage did not contribute to yield.

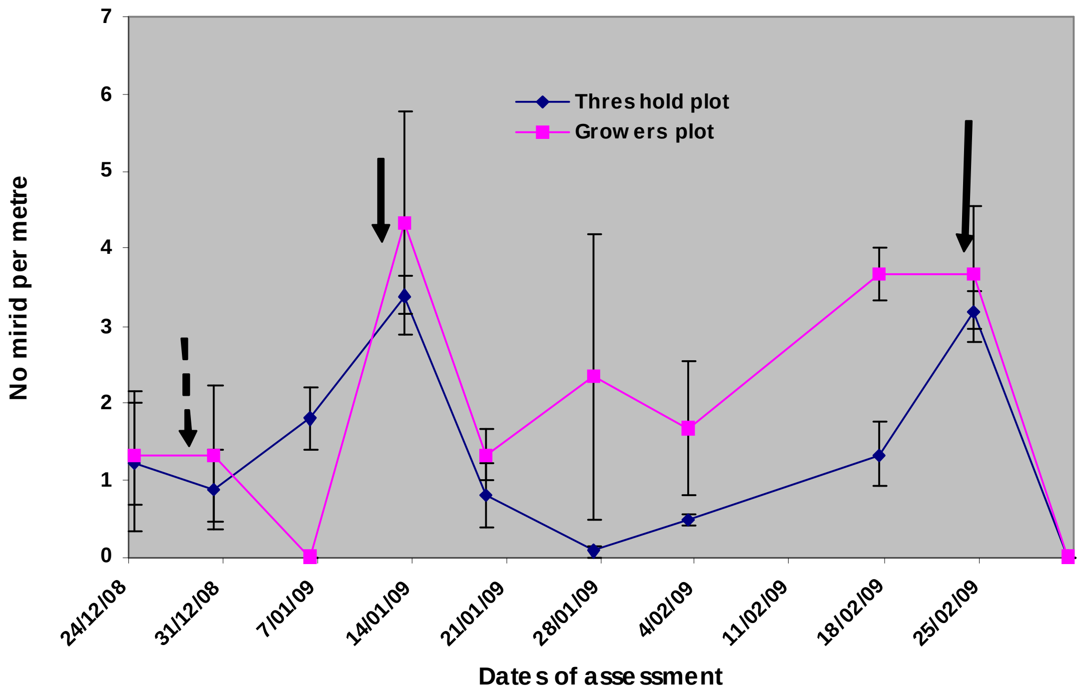

Figure 1. Number of mirids in crop stage wise threshold treated plot and growers treated plot. Solid arrow indicates sprayed at both plots and broken arrow indicates sprayed only at grower plot. Error bars indicate standard error of means

Mirid plant based threshold

Trial 1

Mirid numbers reached the peak, 1.8 and 2.4/metre, in the 1st week of February and thereafter the population reduced to zero. The subsequent retention level for the top

5 nodes also reduced even when there were no mirids, suggesting physiological loss. The grower decided to finish the cotton quickly by not irrigating further, which possibly contributed to the physiological drop. Analysis showed that there were no significant differences (*P > 0.05*) between neither management nor mirid levels for yield in relation to different retention levels and interaction between treatments was not significant (*P > 0.05*) (Table 3). This result suggests that fruit loss at this very late stage did not contribute in yield performance, reinforcing the previous finding for late boll stage. However, if the grower had decided to irrigate further to prolong cotton growth, the result on yield could be different.

Table 3. Yield (bale/ha) at different mirid, retention and management level

Pre-determined mirid and retention level	Yield (bale/ha)	
	Unsprayed	Sprayed
Mirid 1/m		
60%	8.5a	9.4a
70%	8.5a	9.3a
80%	9.1a	8.1a
Mirid 2/m		
60%	8.4a	8.6a
70%	8.3a	9.3a
80%	8.6a	9.2a

Means in a column followed by same letter are not significantly different at *P>0.05*

Trials 2

Mirid numbers at different assessment dates for 9 sites are given in Figure 2. During the 2009-10 season in the unsprayed plots, average mirid populations for the whole year was between 0.6 – 2.6/m and reached 6.3/m on one occasion. In the commercial treatment, the highest population was 5.4/m, with the average population of 0.2 – 1.4/m. In the sprayed plots, the whole year average mirid population was 0.3 – 1.5/m. In at least one occasion in every site, mirid numbers reached 2+ per metre because the field could not be sprayed at the intended time due to rain. During the 2010-11 season, the unsprayed plots average mirid number for whole year was 0.17 – 1.49/m, in sprayed plots 0 .12 – 1.06/m and in commercial plots 0.18 – 1.26/m. Only one site reached above the mirid threshold level during that season.

Yield (bale/ha) for each site is summarised in Table 4. The analysis for all sites together showed that there was no significant difference between treatments (P > 0.05).

In all sites fruit loss was low around 10% at early squaring stage which later increased up to 60%, although the mirid number reduced, indicating some of the fruit loss at later stage was due to reasons other than mirids. During the 2009-10 seasons, two sites were under water stress. The three other sites were affected by stagnant water because of heavy rain. During the 2010-11 season, crops in all sites were affected by the flood. Analysis showed that there was no significant difference between treatments (P > 0.05) either for 5 nodes or all nodes and the relationship between mirid number and fruit loss was poor (Figure 3).

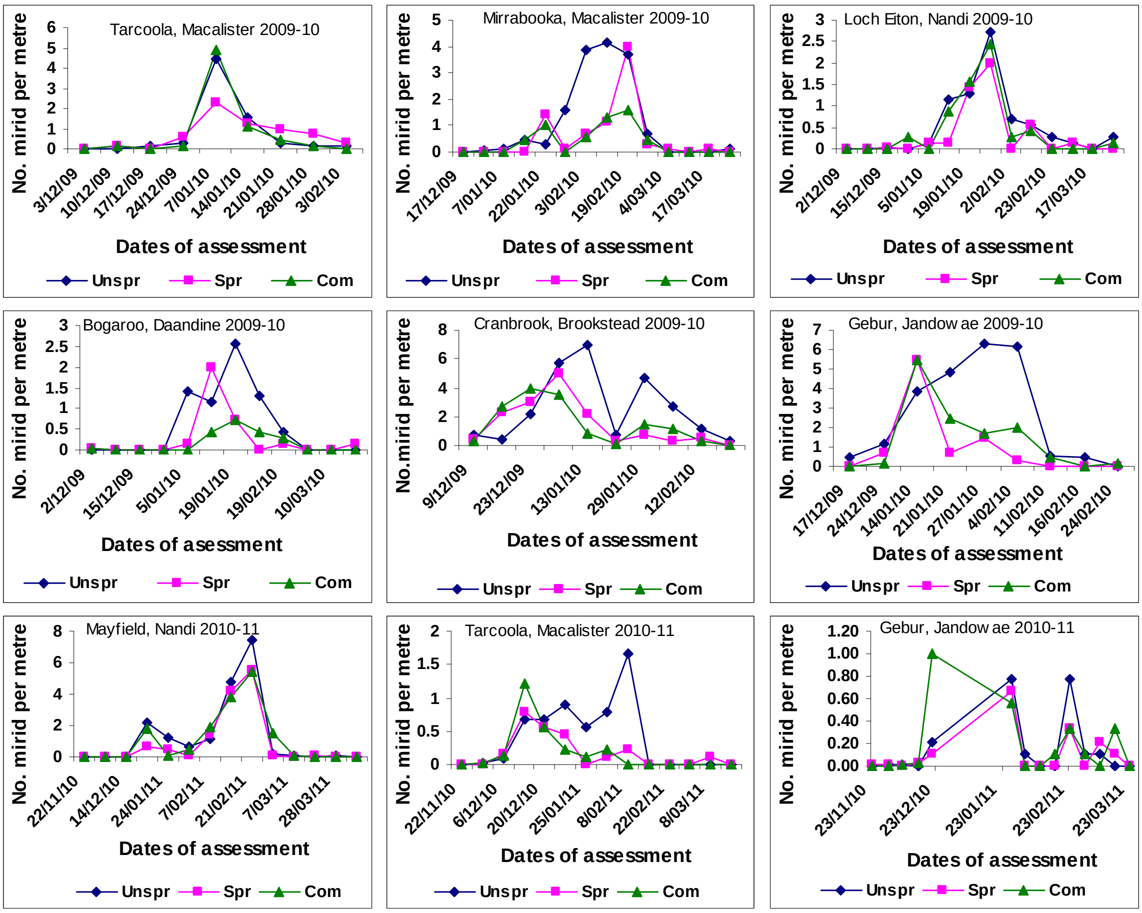

Figure 2. Number of mirids (per metre per date) in different treatments at different sites

Table 4. Yield (bale/ha) in different treatments at different sites

Year	Site	Unsprayed	Sprayed	Commercial
2009-10	Tarcoola, Macalister,	8.2	6.9	6.5
	Mirrabooka, Macalister	10	10.6	10.3
	Loch Eiton , Nandi	9.40	9.70	10.50
	Bogaroo, Daandine	9.5	9	10.2
	Cranbrook, Brookstead	7.9	8.2	8.1
	Gebur, Jandowae	10.35	10.46	10.08
2010-11	Mayfield, Nandi	8.65	8.76	8.65
	Tarcoola, Macalister,	7.1	7.7	7.5
	Gebur, Jandowae	8.28	8.23	8.29

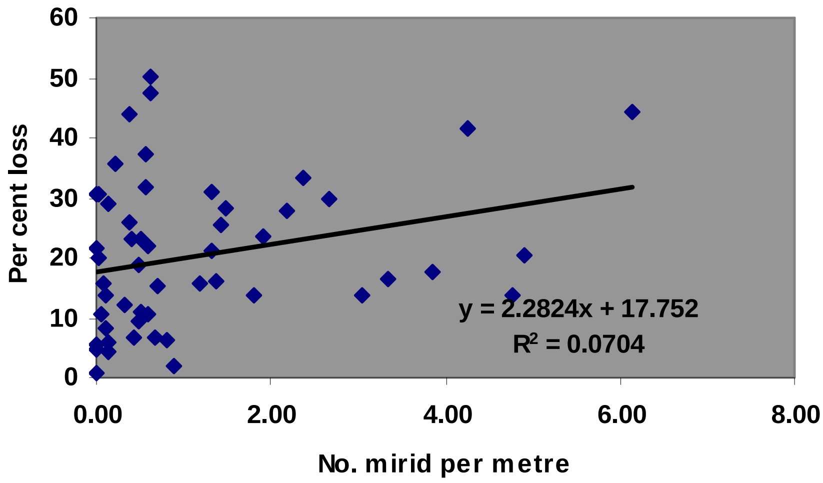

Figure 3. Relationship between mirid number and per cent fruit loss for all sites

When per cent fruit loss for mirid damage was compared between the top 5 and all nodes, for both the 1st and 2nd position fruits, it was found that assessing all nodes for fruit retention was a better reflection of the actual retention, particularly up to 14/15 nodes (about the 4th week of January in late October to early November planting) (Figure 4). Therefore, for better mirid management decision growers and consultants need to assess 1st and 2nd position fruits of all nodes up to 14/15 node stage. However, some of the fruit loss at this stage was due to factors other than mirids such as weather conditions, nutrition levels and for physiological reasons, which was difficult to separate from mirid damage. Thereafter, per cent fruit loss increased substantially even though mirid number was very low, indicating most of the fruit loss at this stage was due to factors other than mirids and most of the bolls below 7/8 nodes were large enough to tolerate mirid damage. Therefore from this stage an assessment of the top 5 nodes for fruit retention may be a suitable way to make a mirid management decision.

The regression analysis between fruit loss and yield showed that the relationship between these two parameters, up to 14/15 node stage, was significant ($P < 0.05$) (Figure 5). Regression analysis also showed that for a higher yield (≥ 10 bale/ha), a retention level of ≥ 80 per cent needed to be maintained up to 14/15 node stage. Given the fact that under favourable conditions Bollgard® II will yield ≥ 10 bale per hectare, it is proposed that a plant based threshold for mirids is 80% retention level up to 14/15 node stage. It should be considered that two per cent fruit loss per week will occur for every mirid above the base population (see Figure 3) and the retention levels should be assessed weekly for all nodes up to the 14/15 node stage. After the 14/15 node stage, retention levels should be maintained at 65 – 70% until harvest to achieve better yield.

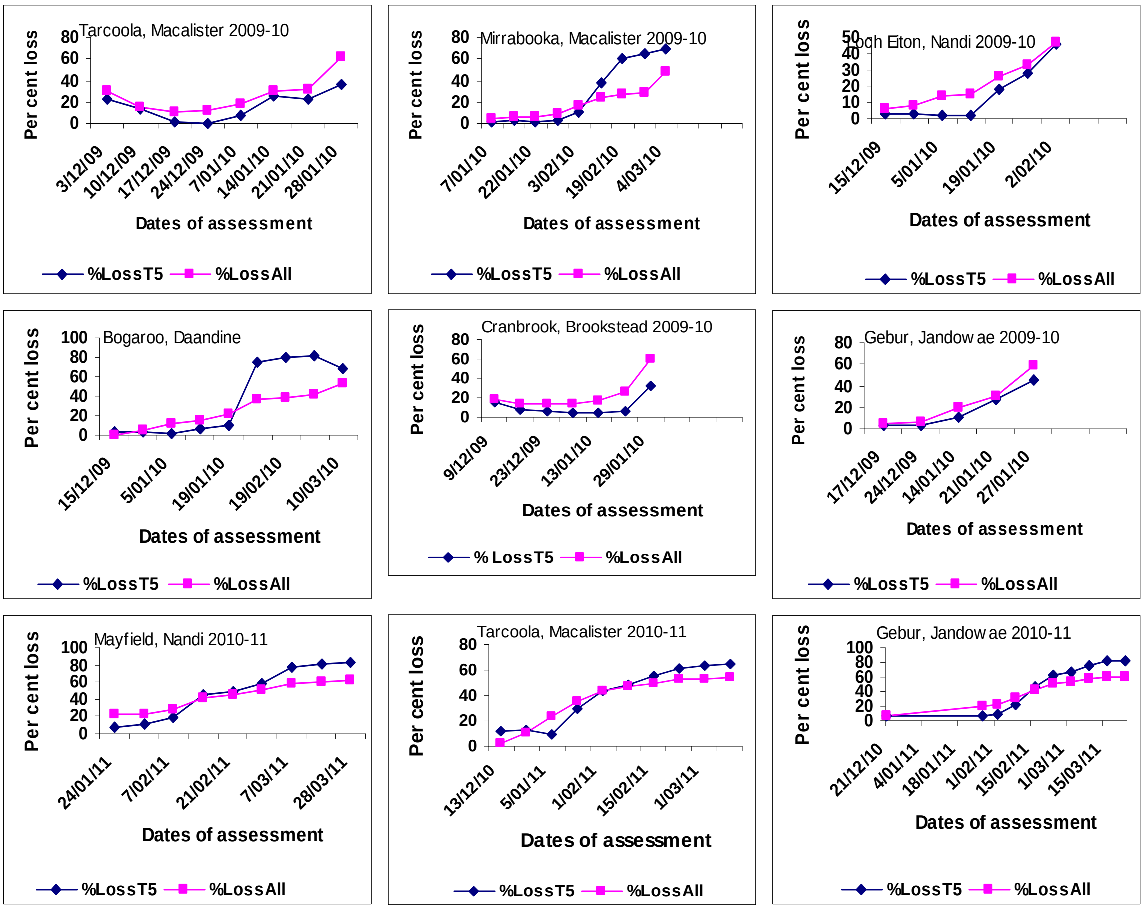

Figure 4. Per cent loss at different dates for the top 5 nodes and all nodes

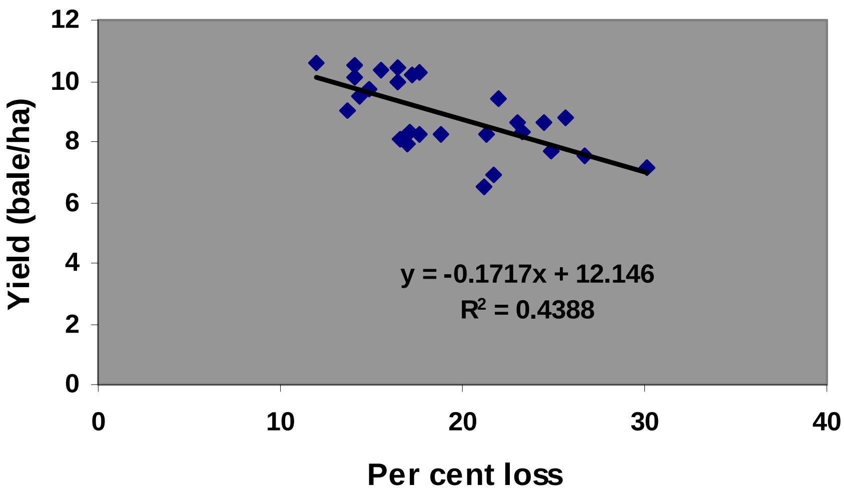

Figure 5. Relationship between per cent fruit loss and yield for all sites

Pale cotton stainer damage assessment in the glasshouse

Trial 1

The results are summarised in Figure 6. Irrespective of boll age, females caused more damage than males for all damage types. This is in agreement with previous findings. The females used in the trial were mated and perhaps the gravid female's dietary requirements encouraged them to feed more. However, analysis showed that differences between damage caused by male and female was not significant ($P > 0.05$), though damage caused by females was significantly different ($P < 0.05$) from the untreated control. There was no significant difference ($P > 0.05$) between the damage for boll age caused by either males or females. GLM analysis also showed that there was no significant interaction ($P > 0.05$) between boll age and insect gender. However, 30 day old bolls incurred relatively less damage from either males or females than younger bolls, but these differences were not significant.

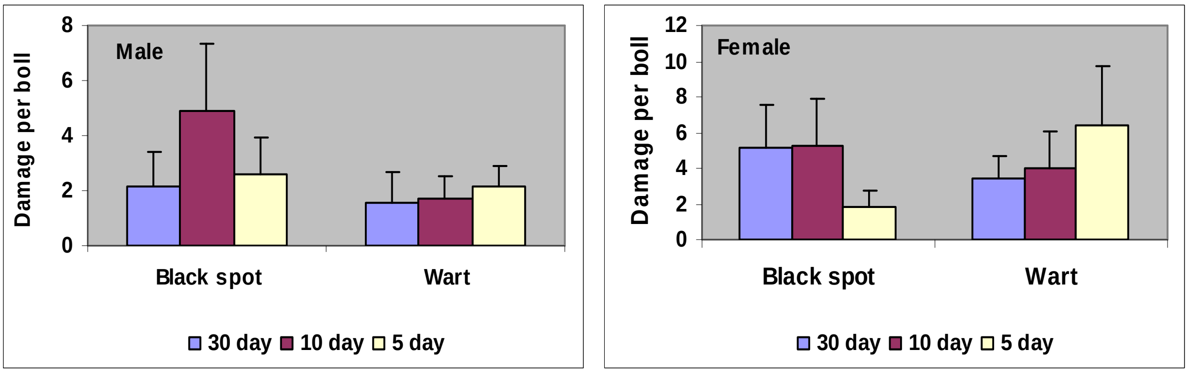

Figure 6. Pale cotton stainer damage to different aged bolls in the glasshouse. Error bars indicate standard error of means

Trial 2

No black spot (external damage) developed on the 35 day old bolls exposed to feeding by either the males or females. The nature of the damage to the lock was found to be different from the field observations. In this trial, damaged locks did not develop brown coloured lint, instead, the lock either developed partially or fully depending on the severity of damage. This was perhaps due to the fact that the cotton stainer in the field during feeding picked up some (unknown) organism and then released them through their saliva to the boll which ultimately developed brown coloured lint. The cotton stainers used in the trial were 6-7th generation from a laboratory culture and over the generations they may have lost that organism from their saliva. However, this hypothesis needs to be examined in collaboration with a plant pathologist.

The results showed that similar to Trial 1 irrespective of boll age females caused more damage than males and the damage varied with boll age. Analysis revealed that for female per cent yield loss and seed damage, compared to control, was significantly higher ($P< 0.05$) for 5 and 15 day old bolls than for 35 day old bolls (Figure 7). Per cent seed germination was significantly lower ($P< 0.05$) for females at these boll ages (Figure 7). Analysis also revealed that for all damage parameters there was no significant difference ($P > 0.05$) between 5 and 15 day old bolls for females indicating bolls up to 15 day old may be susceptible to cotton stainer damage. On the other hand, all damage parameter for 35 day old bolls were not significant ($P > 0.05$) indicating the pale cotton stainer inability to cause damage to very old bolls. GLM analysis also showed that there were significant interactions ($P < 0.05$) between boll age and insect gender for all damage parameters except percent germination.

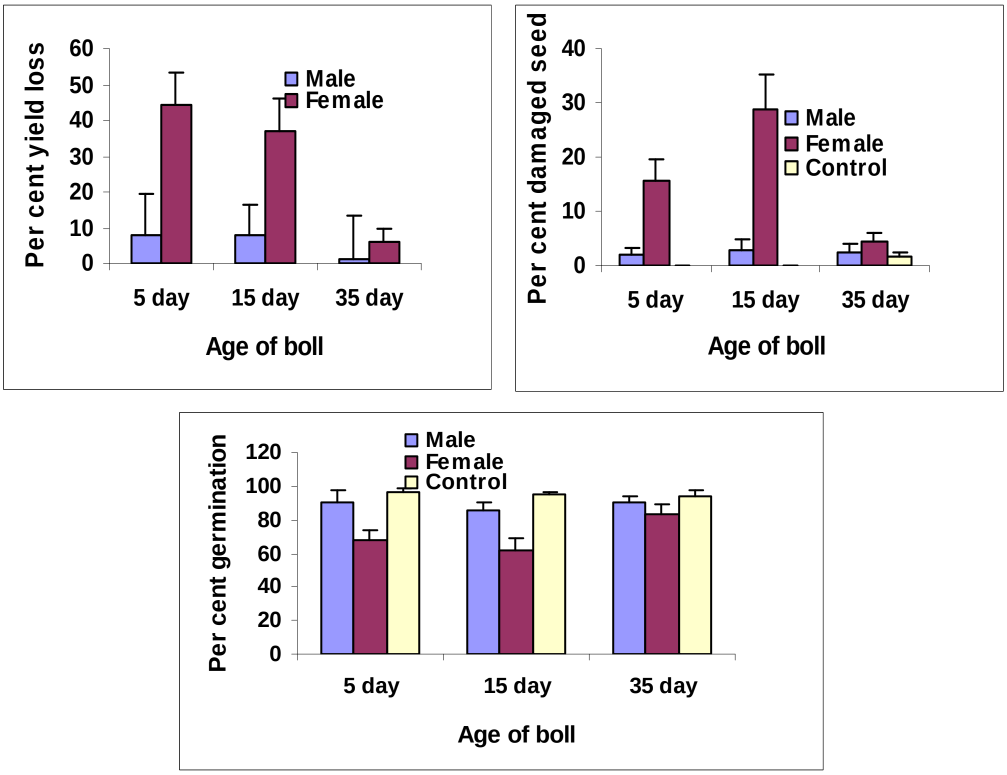

Figure 7. Per cent yield loss (compared to control), damaged seed and germination caused by pale cotton stainer. Error bars indicate standard error of means

Population changes in mirids and stinkbugs

Mirids

Results are summarised in Figure 8. The highest mirid population was recorded in the 2009-10 season. This was perhaps due to the relatively wet winter which contributed large numbers of wild hosts to support an initial build up of the mirid population. Favourable summer temperatures, 31.4°C (average of October 2009 to March 2010, Bureau of Meteorology), also contributed to the rapid build up of mirid numbers on cotton. In the 2008-09 and 2010-11 season, mirid numbers were more or less similar, (1 - 3/m), except at one site in 2010-11 season where on one occasion the mirid numbers reached 7/m. In the 2010-11 season, summer temperatures were mild but heavy rain and floods kept mirid populations low. Irrespective of sites and year, figures shows that mirids moved into the cotton by the 2nd week of December and reached their peak between late January and the 2nd week of February, the square and boll setting stage. This agreed with the previous findings that mirids prefer to feed on squares and bolls. By the end of February to early March the population died down.

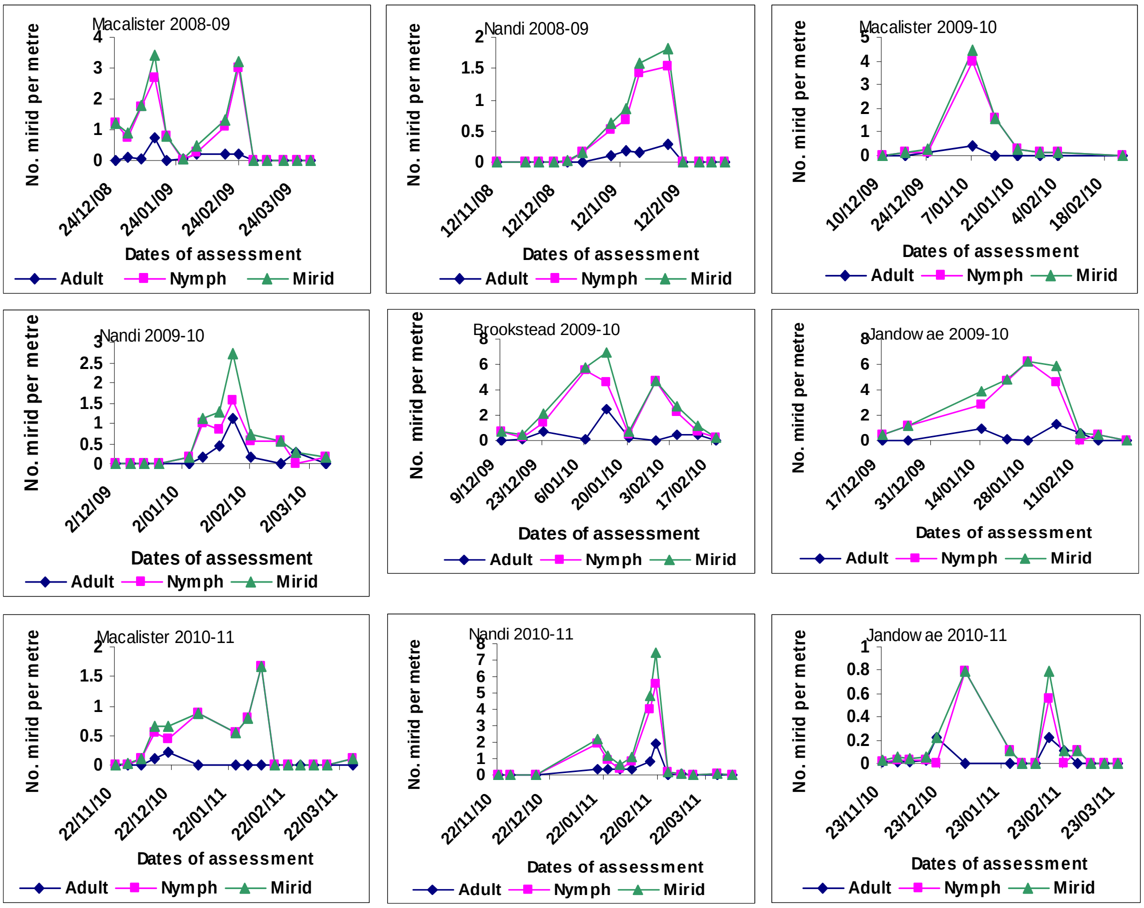

Figure 8. Seasonal abundance of mirids in irrigated Bollgard® II cotton on the Darling Downs

Stinkbugs

Green vegetable bug (GVB) was the predominant stinkbug species at every site in every year followed by cotton stainer. Cotton stainer never reached threshold level. GVB on the other hand reached threshold levels at 2 sites each during 2008-09 (Macalister and Nandi) and 2010-11 (Nandi and Jandowae). They reached peak (> 1 < 2) from mid February to the 1st week of March. Overall parasitism for GVB was very low (about 30%); the highest level was found in 2010-11 season. Only on one occasion in one site (Macalsiter) parasitism level reached 50%.

Selective management option for mirids and stinkbugs

Shield® and salt mixture against mirid

The pre-treatment population structure of mirids and different beneficials are given in Figure 9. The mirid population was 3-4/metre, of which 88% were nymphs. Of the nymphs, 74% were 1st and 2nd instar and 24% were 3rd instar. There were 2 to 7 beneficial insects/m, with spiders the most abundant beneficial group (2–3/metre) followed by red and blue beetles (0.3–1.4/metre) and ladybird beetles (0.1–0.7/metre).

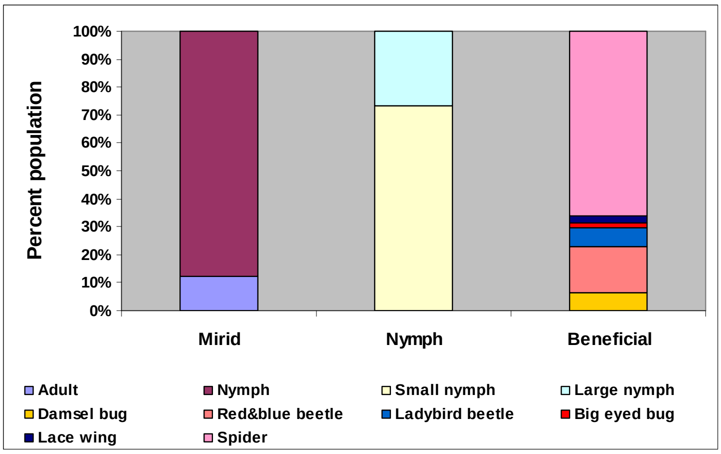

Figure 9. Population structure of mirids and beneficials before treatment (small nymphs = 1st and 2nd instar and large nymphs = 3rd, 4th and 5th instar)

Shield® plus salt performed better than Shield® alone or the standard control (Regent® + salt) at both 3 and 7 DAT (Figure 10). At 3 DAT, Shield® 100, 125 and 250 mL/ha reduced the mirid population by 71, 69 and 70% while Shield® 100 and 125 mL/ha plus salt reduced the mirid population by 94 and 96% respectively, increasing effectiveness by 25%. At 3 DAT the standard control reduced mirid numbers by 82%. At 7 DAT, the effectiveness of Shield® alone, Shield® plus salt and the standard control increased further, reducing the population by 90 to 100%. At 20 DAT, the effectiveness of Shield® alone and Shield® plus salt diminished compared to 7 DAT (Figure 8), indicating a short residual effect of Shield®. However, at 20 DAT the standard control still was found to be reasonably effective, reducing the population by 64% and indicating the superior residual effect of fipronil.

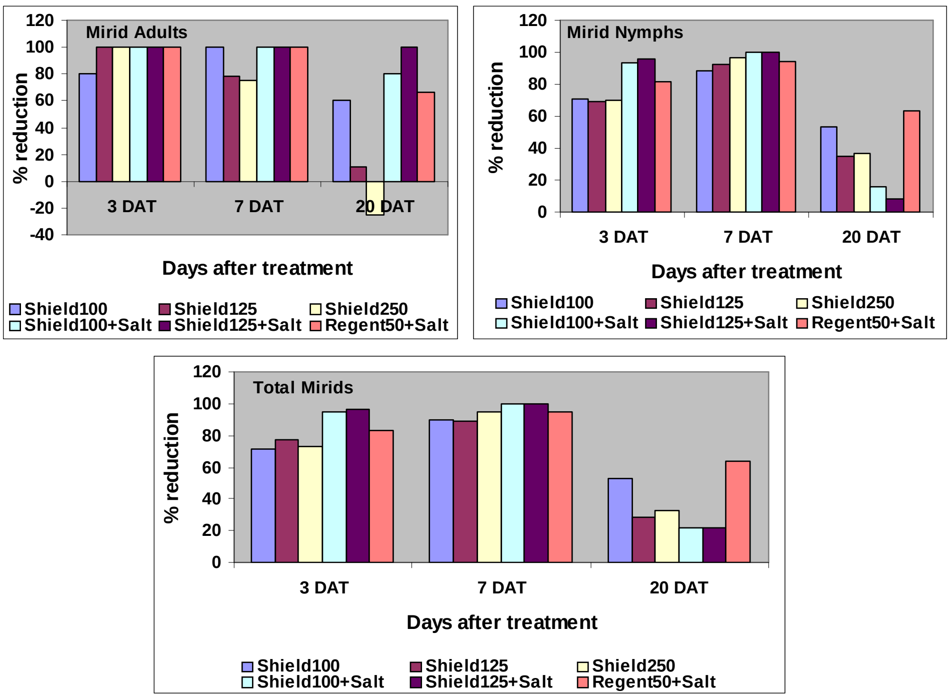

Figure 10. Effect of Shield® with or without salt against mirids in Bollgard®II (Percentage of reduction was calculated in relation to mirid number in untreated control before spray.)

Analysis showed that both at 3 and 7 DAT mirid number reduced significantly ($P < 0.05$) in all treatments including the standard control (Regent® + salt) compared to the untreated control. However, at 20 DAT differences were not significant ($P > 0.05$).

The impact of Shield® varied with predator species (Figure 11). The impact of Shield® alone (all rates) on red and blue beetle both at 3 and 7 DAT was moderate to high (25 – 60% reduction). However, when Shield® 100 and 125 mL/ha was mixed with salt, the impact was very low (<10% reduction). The impact of the standard control on red and blue beetle was low and high at 3 and 7 DAT, respectively. At 20 DAT, impact was very low except in Shield® 100 mL/ha and standard control. The impact of Shield® 100 mL/ha was high (60% reduction) and that of standard control was moderate (30% reduction). The impact of Shield® alone or salt mixture on ladybird beetles was inconsistent, and might be due to the low initial population (<1/m). The impact of all treatments on spiders was very low.

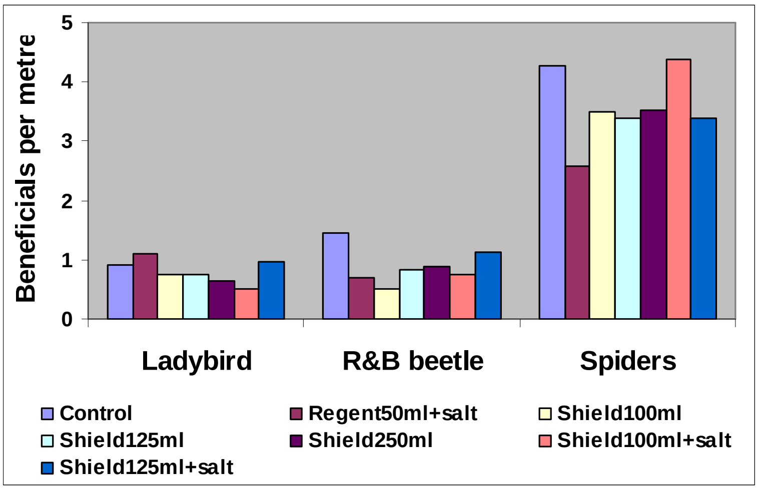

Figure 11. Effect of Shield® with or without salt against beneficials in Bollgard®II (Value on Y-axis was means for beneficials at different treatments from covariance analysis using the prespray data as a covariate)

Plant X and fungus against mirids

The pre-spray assessment showed that mirid numbers were 2 – 4 per metre across the treatments, of them 92% was nymphs. Of the nymphs, 53% were 1st and 2nd instars followed by 4th and 5th instar nymphs (24%). The rest (23%) were 3rd instar nymphs.

The results are summarised in Figures 12 & 13. Analysis showed that at 3 DAT in 2% Plant X and fungus treated plots, mirid numbers reduced by 56 and 68% compared to pre-spray population. In the 1% Plant X treatment plots, mirid numbers increased by 6%. At 3 DAT, mirid numbers in Regent plus salt and full rate Regent treated plots reduced by 94 and 84%. At 7 DAT, mirid numbers increased compared to pre-spray counts, except in Regent plus salt and full rate Regent treated plots where mirid number reduced by 57 and 61%. Analysis of small (1st and 2nd instar), medium (3rd instar) and large (4th and 5th instar) nymphs separately showed that the effect of Plant X and fungus at 7 DAT was around 60% on medium and large nymphs while there was no effect of these treatments on 1st instar nymphs. These 1st instar nymphs probably just hatched out from the eggs laid a day or two before the spray and therefore were not exposed to the treatments. At 14 DAT, the overall effect of Plant X and fungus on all stages of nymphs was low, 0 to 45%, indicating diminishing residual effectiveness of the products. On the other hand, the effect of Regent plus salt and full rate Regent was 76 to 100%. Adult numbers were too low (0 – 0.5/m) to adequately evaluate the efficacy of the products.

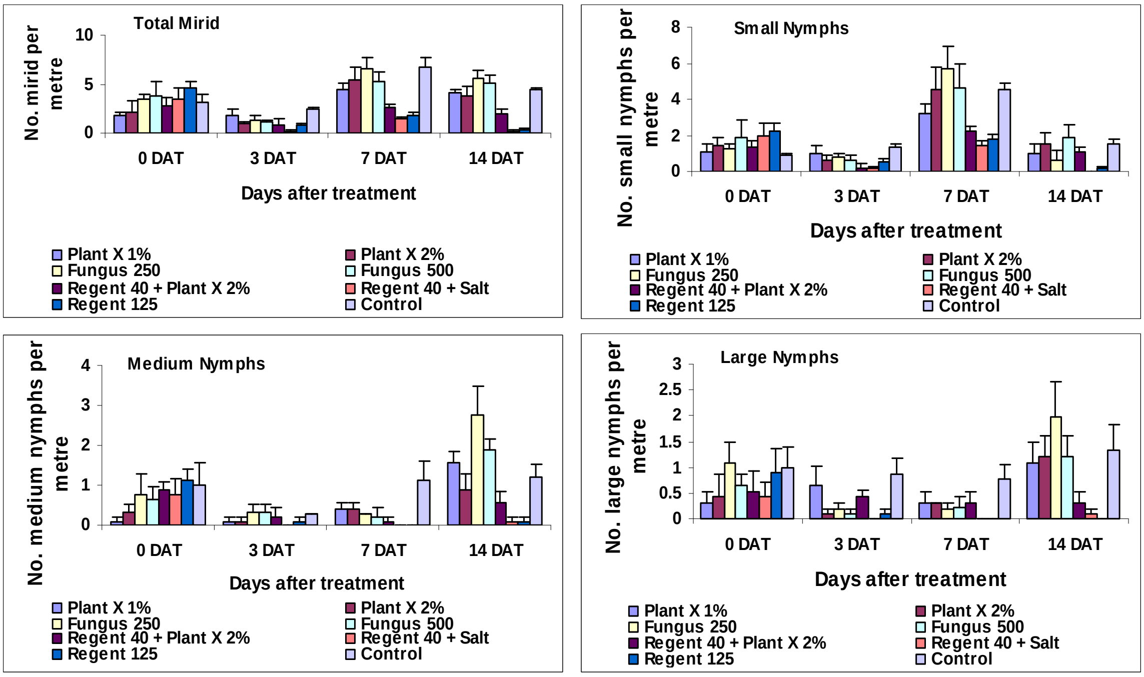

Figure 12. Effect of Plant X and fungus against mirids in Bollgard®II. Error bars indicate standard error of mean

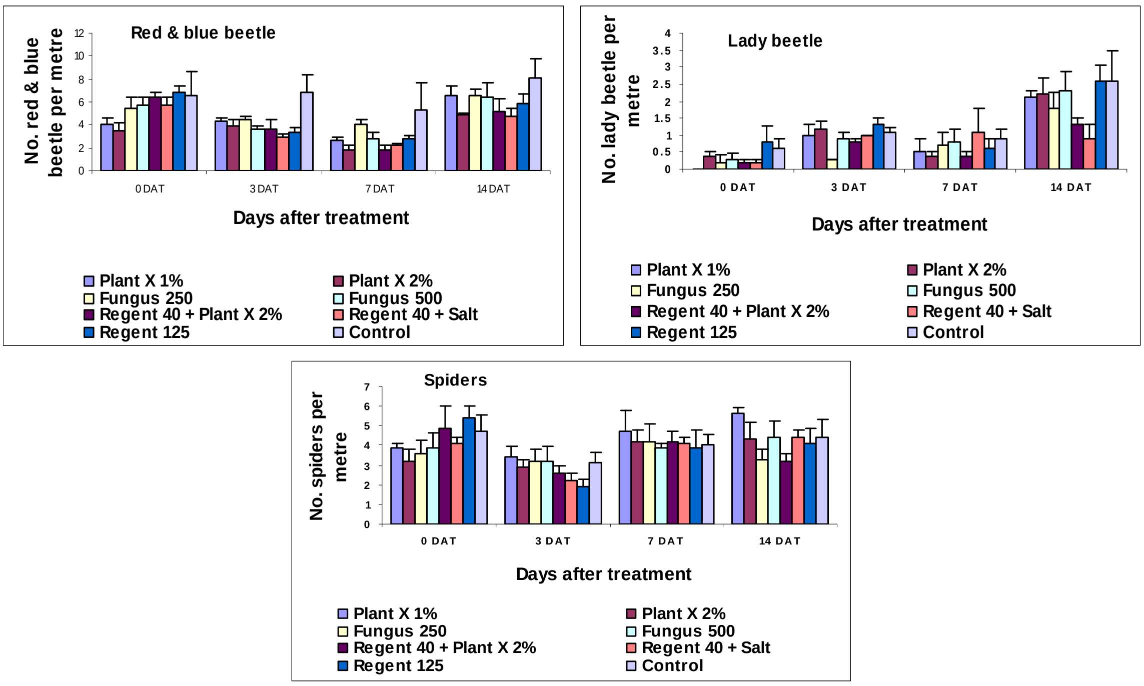

Figure 13. Effect of Plant X and fungus against beneficials in Bollgard®II. Error bars indicate standard error of mean

Red and blue beetle, lady birds and spiders were the major beneficial group in the trial. The effect of Plant X and Fungus on these beneficials were low except for red and blue beetle at 7 DAT where the population reduced by 25 to 50%. On the other hand, the effect of Regent plus salt and full rate Regent on red and blue beetle was low to high (15 to 60%).

The low impact of Plant X and fungus on beneficials, compared with the current standard industry treatments, warrants them being evaluated differently to conventional insecticides. Further work to evaluate their efficacy as population suppressants, rather than knockdowns may be warranted – perhaps with earlier intervention and multiple applications.

Kaolin against whitefly

Upon release, most of the whitefly flew to glasshouse ceiling and later escaped. About 20% of the insects were recovered. Within 1 hour preferred plants were selected and trends remained similar throughout the trial period (Figure 14). Untreated control plants accounted for 53 - 69% of the insects followed by plants with canopy oil (15 – 22%), SWP (8 – 15%) and SWP plus canopy oil (4 – 11%). Analysis showed that in every occasion significantly ($P < 0.05$) more insects selected untreated plants than SWP and canopy oil treated plants. However, there was no significant difference ($P > 0.05$) between SWP, SWP plus canopy oil and canopy oil. Further trials are needed with a modified methodology (eg using a large walk-in cage to avoid insects' escaping) to confirm this result.

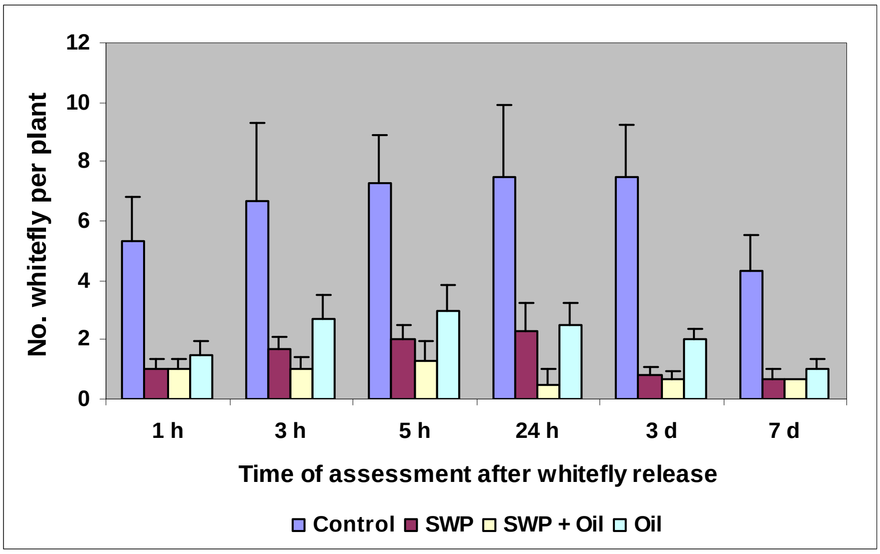

Figure 14. Effect of kaolin particle (SWP) against whitefly in glasshouse

Salt mixture against Solenopsis mealybug

The results showed that both Shield® alone and Shield® plus salt were quite effective against the mealybug (Figure 15), with 67 – 73 % mortality, at 3 DAT which increased up to 92 – 95% at 15 DAT. Salt mixture increased mortality by 5 to 12% for both nymphs and adults at 3 and 6 DAT but at 15 DAT efficacy of salt mixture was reduced. Analysis revealed that there was no significant difference (*P* > *0.05*) between Shield® alone and salt mixture for both nymphs and adults at any dates. This was a preliminary test and needs to be tested in the field as the performance of the chemical could be influenced by different factors such as spray coverage, crop canopy.

A modified rearing method for *Solenopsis* mealybug has been developed through this trial which is easy and effective and is being used to culture mealybug in the glasshouse.

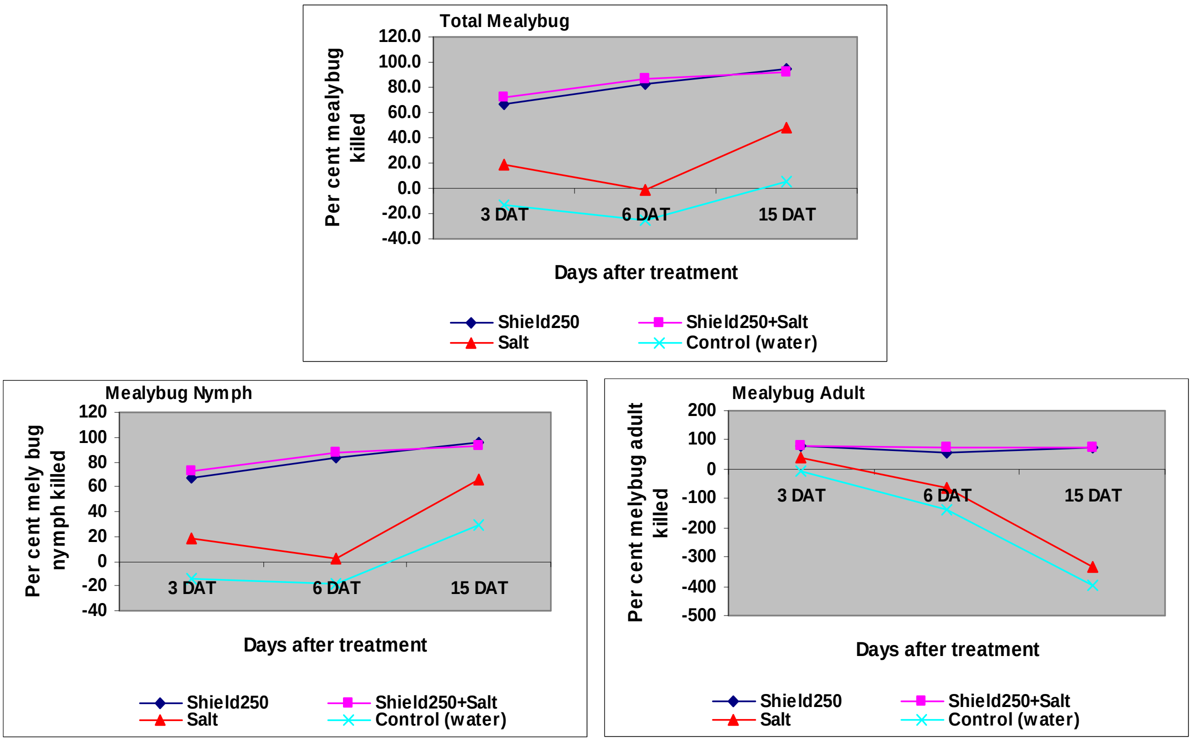

Figure 15. Effect of salt mixture against *Solenopsis* mealybug in the glasshouse

Outcomes

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

All the outcomes identified in the project application (listed below) have been achieved.

Expected Outputs *eg A number of workshops are organised, asking entomologists to present & discuss findings with growers.*	**Expected Science Outcomes** (NB: A direct science outcome might not be applicable for all extension outputs.)	**Expected Industry/ Applied Outcomes** *Eg These growers gain knowledge and change practices in pesticide application.*
Verify and refine thresholds for mirids	Researchers gain knowledge and adoption of their research	Judicious and timely application of chemicals
Identify environmentally friendly management tools for the pests	New IPM tools identified	Reduce chemical use
Identify less disruptive chemicals for managing the pests	Less disruptive chemicals against the pests identified	Reduce broad-spectrum chemical use

The major outputs that were generated through this project including a plant based management strategy for mirids, identifying the possibility of using Plant X and fungus against mirids for suppression and identifying an insecticide (Shield®) that can be mixed with salt to increase the efficacy of the chemical against mirids and reduce its impact on beneficials. All these outputs will substantially contribute to the planned outcomes. For example, a plant based management strategy (≥ 80% retention and weekly assessment of retention levels for all nodes up to 14/15 node stage, information on per mirid per week fruit loss) along with a crop stage wise threshold (developed in the previous project) will further improve grower and consultant decision making, thereby facilitating more judicious and timely application of chemical. Use of Plant X, fungus and salt mixtures will allow for a further reduction in chemical use for mirid control. There will be follow on effects from this management approach. As a reduced rate chemical plus salt is comparatively softer than a broad-spectrum chemical, it will encourage higher survival of beneficials and reduce the likelihood of secondary pest outbreaks of whitefly, aphids and mites.

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

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
c) required changes to the Intellectual Property register.

No IP or patents are involved.

Conclusion

7. 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?

The results of this project, such as a plant based threshold and monitoring procedure for mirids, will further improve the grower and consultant decision making process. This information will help them to further increase cotton yield. This will also enable them to make a decision about when to use chemical control to obtain higher yield. The information on Shield® and salt mixtures will give growers and consultants another selective option to manage mirids with minimal impact on beneficial thereby avoiding flare up of whitefly. The information on the damage of pale cotton stainer will further increase confidence in managing this pest.

The take home messages are outlined below;

- A plant based management strategy for mirids which considers mirid damage rate (per cent fruit loss per mirid per week) and retention levels will provide a higher yield (≥ 10 bale/ha) if the crop dose not suffer from any other stresses such as water, nutrient and disease.
- The plant based management strategy action threshold is 80% retention at up to the 14/15 node stage. Thereafter, threshold is 60 – 70% retention.
- Retention levels should be determined by assessing the 1st and 2nd position bolls of all nodes up to the 14/15 node stage. Thereafter retention can be assessed for the top 5 nodes.
- A plant based threshold should be considered in association with per cent fruit loss for each mirid. It should be considered that two per cent fruit loss per week will occur for every mirid above the base population.
- Younger bolls of up to 15 days old are most susceptible to pale cotton stainer damage and can cause up to 40% yield loss.
- Seed germination for up to 15 day old bolls is significantly reduced by up 30% compared to the control. Thirty five day old bolls incur negligible damage.
- Salt mixture allows a reduced Shield® rate by > 50% without reducing efficacy.
- Low rate of Shield® plus salt increased mortality by 25% compared to low rate Shield® alone.
- Shield® and salt mixtures can reduce the impact on beneficial.
- Plant X and fungus can be used for suppression when low mirid populations are present. However, more research needs to be done to confirm this finding.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.
(b) for the future presentation and dissemination of the project outcomes.
(c) for future research.

The proposed plant based management strategy need to be followed up. The plant based threshold and retention level assessment procedure need to verified using large scale trials involving growers and consultants. This activity will further boost their confidence in mirid management decisions. Industry needs to promote the assessment of all nodes up to 14/15 node stage to determine retention levels. This can be done through farm walks and other extension activities.

It is planned to publish the information generated from this project into the cotton grower magazine and placed on the Cotton CRC website. The project outcomes will further disseminate through growers meetings, field days, CCA meetings, farm walk sand conferences. The principal researcher will also write scientific journal articles on research outcomes.

The research on salt mixtures only reports the impact on predators, not on parasitoid, particularly the parasitoid of whitefly. One of the claimed benefits of using reduced rate chemical plus salt is that salt mixtures prevent the flaring of whitefly. Along with weather conditions, parasitoids play important rule in regulating whietfly population. Therefore, it is necessary to conduct further research on the impact of selective management options on parasitoids. There is a concern in the industry that the use of low rate insecticides for mirids requires consecutive sprays which may outweigh the benefit of using low rates, particularly from an IPM perspective. This idea has never been experimentally tested. Further research is needed to address this issue.

Publications

9. A. List the publications arising from the research project and/or a publication plan.
   (NB: Where possible, please provide a copy of any publication/s)

1. **Khan, M.** (2011). Exploring an IPM option for sucking pest management in Bollgard® II cotton in Australia. The World Cotton Research Conference 5, Mumbai, India (in press).
2. McColl, S., **Khan, M.** and Umina, P. (2011). Review of the biology and control of Creontiades dilutus (Stål) (Hemiptera: Miridae). *Australian Journal of Entomology* 50, 107-117.
3. **Khan, M.**, Heimoana, S. and Wilson, L. (2010). Understanding pale cotton stainer (PCS) damage to Bollgard® II cotton. 15th Australian Cotton Conference, Gold Coast.
4. **Khan, M**. (2010). Mirid in Bollgard® II cotton- a challenge for IPM. Cotton CRC Science Forum, Narrabri.
5. **Khan, M**. (2010). Brown stinkbug in cotton. DEEDI Entomology IPM Blog website.

6. **Khan, M.**, Gregg, P. and Mensah, R. (2009). Effect of temperature on the biology of *Creontiades dilutus* (Stål) (Heteroptera: Miridae). *Australian Journal of Entomology* 48(3), 210-216.
7. **Khan, M**. (2009). Further trial of salt mixture against mirids. *The Australian Cottongrower* 30(4), 37-38.
8. **Khan, M.**, Quade, A. and Murray, D. (2008). Damage assessment and action threshold for mirids, *Creontiades spp*. in Bollgard® II cotton in Australia. *Journal of Insect Science* (online journal) 8(49). www.insectscience.org/papres P. B. Goodell and P. C. Ellsworth (ed). Second International Lygus Symposium, Asilomar Conference Center, Pacific Grove, CA USA.
9. **Khan, M.**, Quade, A. and Murray, D. (2008). Reduced rate of chemical plus additive- and effective IPM tool for managing mirids, *Creontiades spp*. in Australian cotton. Journal of Insect Science (online journal) 8/49). www.insectscienec.org/papers P. B. Goodell and P. C. Ellsworth (ed). Second International Lygus Symposium, Asilomar Conference Center, Pacific Grove, CA USA.
10. **Khan, M.**, Quade, A. and Boshammer, M. (2008). Insecticides for use against pale cotton stainer bug. *The Australian Cottongrower* 29(2), 41-42.
11. Wilson, L., **Khan, M**. and Farrell, T. (2008). Pale Cotton Stainers, *Dysdercus sidae.* Pest Profile. Australian Cotton Catchment Communities CRC.
12. **Khan, M**. and Quade, A. (2008). Biology and pictorial identification of mirids. *The Australian Cottongrower* 29(1): 24-27.

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

No

Part 4 – Final Report Executive Summary

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

In Bollgard® II cotton 2-5 sprays are required to manage mirid and stinkbugs every season. An industry survey revealed that growers are 'insurance' spraying when mirid numbers are below suggested thresholds. One main reason for insurance sprays is possibly a lack of confidence in the relationship between mirid numbers and fruit loss. The development of a plant based management strategy (through detailed studies on mirid numbers, fruit loss and yield) could increase growers and consultant's confidence in making decisions on mirid management, therefore reducing insurance sprays. The main objectives of this project was to develop a plant based management strategy for mirids and to develop selective management options for mirids and stinkbugs to fit with the existing IPM systems.

Through this project, a plant based management strategy for mirids has been developed. This strategy will consider two major factors; mirid damage rates (per cent fruit loss per mirid per week) and retention levels. The action threshold for a plant based management strategy is determined as ≥ 80% retention at up to the 14/15 node stage. Thereafter, the threshold is 60 – 70% retention. Retention levels should be determined by assessing the 1st and 2nd position bolls of all nodes up to the 14/15 node stage. Thereafter, retention levels can be assessed for the top 5 nodes. A plant based threshold should be considered in association with per cent fruit loss for each mirid. It should be considered that two per cent fruit loss per week will occur for every mirid above the base population.

When pale cotton stainer feed on young bolls (up to 15 days old), they can cause up to 40% yield loss. The germination rate of seed from damaged bolls is significantly reduced, by up to 30% compared to the control. This finding from the glasshouse trials needs further research in field conditions to confirm the result.

When salt is mixed with low rates (100 mL/ha) of Shield® (clothianidin), mortality is increased by 25% compared to the low rates of Shield® alone, which is similar to the full rates (250 mL/ha). Shield® and salt mixtures also reduced the impact on benficials significantly. However, caution needs to be exercised when using Shield® against mirids if aphids are present in the field, as aphids are capable of developing resistance against Shield®.

Futher information

Moazzem Khan
DEEDI, Toowoomba
07 46881310 or 0428600705